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ВАВИЛОВСКИЙ ЖУРНАЛ ГЕНЕТИКИ И СЕЛЕКЦИИ

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Биоресурсные коллекции

VAVILOV JOURNAL OF GENETICS AND BREEDING

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Bioresource collections

К истории создания ВИР

Уважаемые коллеги! Текущий выпуск журнала посвящен 125-летию Федерального исследовательского центра Всероссийский институт генетических ресурсов растений имени Н.И. Вавилова (ВИР).

История института началась в 1894 г., когда было основано Бюро по прикладной ботанике при Ученом комитете Министерства земледелия и государственных имуществ Российской Империи. Цель создания Бюро – систематические исследования важных для земледелия сельскохозяйственных культур. Первые исследования были посвящены описанию ботанических особенностей и физиологических свойств местных сортов, собираемых не только со всех уголков империи, но и из-за рубежа. Получили развитие работы по таксономии культурной флоры, предселекционные исследования и селекция. Результаты деятельности Бюро в первые два десятилетия были впечатляющими: за этот период проведено 80 широкомасштабных полевых экспериментов, в которых только злаковых растений изучено около 20 тыс. образцов. Роберт Регель, занимавший пост директора с 1905 по 1920 г., в 1906 г. доложил результаты работы Бюро на Всемирной выставке в Милане, где представленная коллекция ячменя была удостоена высшей награды.

Развитие генетических исследований в России также тесно связано с Бюро по прикладной ботанике. В 1910 г. сотрудник Бюро Константин Флаксбергер перевел на русский язык работы Григория Менделя о законах наследственности, а в 1912 г. Николай Вавилов впервые обосновал глубокую связь между селекцией и генетикой и спрогнозировал существенное влияние последней на селекционный процесс. Широкое развитие генетические исследования получили с 1925 г., когда Н.И. Вавилов, ставший в 1920 г. директором Бюро, основал Отдел генетики, пригласив возглавить его талантливого молодого ученого Георгия Карпеченко. Масштабные работы были начаты на разных группах сельскохозяйственных культур. Только на злаках в течение двух лет (1928–1929) исследованиями было охвачено 70 тыс. растений ячменя и более 2 тыс. межвидовых гибридов пшеницы.

С 1917 по 1930 г. организация несколько раз переименовывалась, пока не получила в 1930 г. название «Всесоюзный научно-исследовательский институт растениеводства». Н.И. Вавилов, выдающийся ботаник, генетик, селекционер, возглавлявший институт более двадцати лет, организовал свыше 180 экспедиций по всем континентам, кроме Австралии, в результате чего была создана богатейшая коллекция культурных растений, не имеющая аналогов в мире. Основными научными и организационными достижениями Н.И. Вавилова являются: открытие закона гомологических рядов в наследственной изменчивости, создание учений о центрах происхождения культурных растений и иммунитете растений, масштабное изучение коллекций культурных растений более чем в 70 эколого-географических точках Советского Союза, создание системы государственных сортоиспытаний.

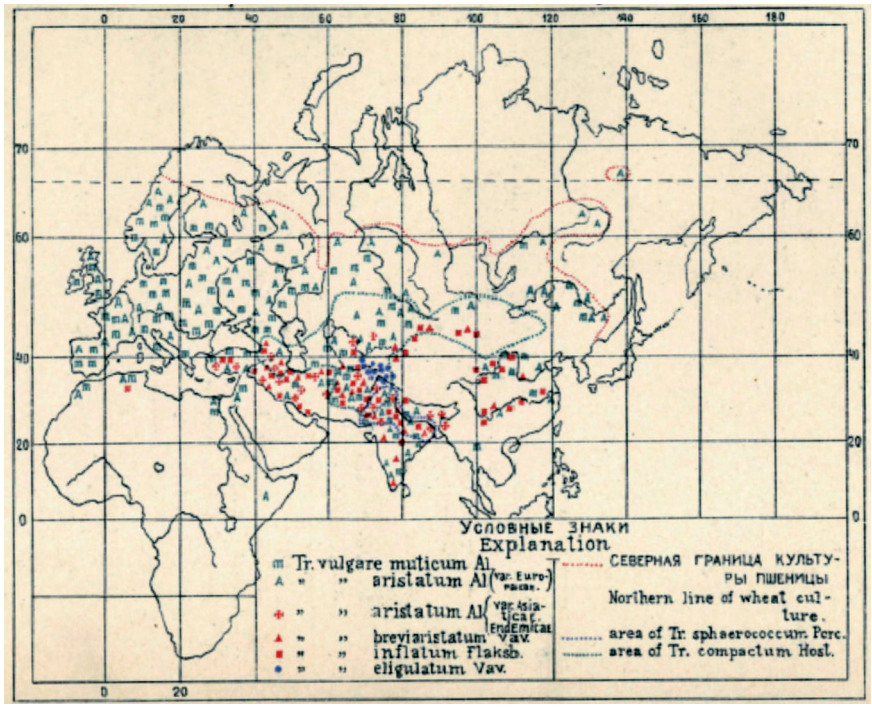
В Великую Отечественную войну, во время блокады Ленинграда, погибавшие от голода сотрудники ВИР не притронулись к семенному фонду и сберегли коллекцию, сохранив уникальное генетическое разнообразие культурных видов растений и их диких родичей для потомков, для будущей селекции. Сегодня коллекция генетических ресурсов ВИР насчитывает более 320 тыс. образцов. Исследования ведутся не только по направлениям, заложенным в институте со дня его основания, но и по новым, с применением современных методов. Результаты изучения коллекции публикуются в каталогах ВИР и рецензируемых международных и отечественных журналах. Ежегодно тысячи образцов из коллекции передаются на безвозмездной основе по запросам государственных научных учреждений.

Статьи этого выпуска посвящены результатам изучения генетических ресурсов растений по направлениям

Р.Э. Регель

Н.И. Вавилов

Здание Министерства земледелия и государственных имуществ Российской Империи, фрагмент фото конца XIX в.



Центры происхождения мягких пшениц, из книги Н.И. Вавилова «Центры происхождения культурных растений» (1926)

«Иммунитет растений к болезням», «Селекция на продуктивность и качество», «Биология развития растений», «Актуальные технологии генетики растений» и «Биоресурсные коллекции». Следуя традициям Н.И. Вавилова, исследователи применяют новые методики для изучения иммунитета растений; эта тема рассмотрена в обзоре по молекулярным маркерам устойчивости капусты (*Brassica* ssp.) к патогенам. Осваиваются новые для Российской Федерации культуры. Так, авторы одной из статей обсуждают альтернариозные пятнистости гуара (*Cyamopsis tetragonoloba* L.). Работа по локализации генов устойчивости к ржавчине у российских сортов льна свидетельствует о возвращении интереса к генофонду староместных сортов как источнику генов устойчивости.

По направлению «Селекция на продуктивность и качество» сохраняются подходы, определенные еще Р.Э. Регелем и развиваемые несколькими поколениями ученых ВИР, в ряду которых особое место занимает выдающийся специалист в области биохимии растений В.Г. Конарев. Коллективом исследователей его научной школы представлены результаты метаболомного анализа диких и культурных видов чины (*Lathyrus* L.). Также в этом разделе опубликованы данные анализа биохимического состава ягод сортов земляники садовой (*Fragaria* × *ananassa*) из коллекции ВИР и сортов овса (*Avena sativa* L.) различного географического происхождения.

Значительная роль при исследовании генетических ресурсов растений всегда отводится их морфогенезу и онтогенезу, так как учет факторов, влияющих на тип развития, тип роста, сроки цветения и архитектуру растений, исключительно важен для раскрытия потенциала урожайности будущих сортов. В рубрику «Биология развития растений» вошла работа по транскриптомному анализу эмбриогенных каллусов люцерны *Medicago truncatula.* Модели развития соцветий и результаты изучения сроков цветения представлены для таких видов, как люцерна посевная и соя. Диапазон фотопериодической реакции у растений рассмотрен на примере гуара и овса посевного.

В статьях раздела «Актуальные технологии генетики растений» продолжены заложенные Г.Д. Карпеченко традиции по расширению генетического разнообразия и созданию новых форм путем отдаленной гибридизации. Приведены результаты изучения генетического разнообразия и селекционной ценности синтетической гексаплоидной пшеницы, привлеченной в коллекцию ВИР из CIMMYT, и данные всестороннего исследования вновь синтезированного тетраплоида с геномной формулой DDA^UA^U. Обсуждаются важные для развития гибридной селекции картофеля сведения о типах цитоплазм и генетическом разнообразии российских сортов и сортов из стран ближнего зарубежья. О критическом значении методов биотехнологии для ускорения селекционного процесса свидетельствуют результаты культивирования *in vitro* раносозревающих сортов черешни.

Для эффективного сохранения и использования огромных массивов фенотипических характеристик и генотипических данных, полученных при изучении коллекций генетических ресурсов растений, осуществляются структурирование и анализ этих данных. В раздел «Биоресурсные коллекции» вошли статьи, посвященные коллекциям винограда, ржи и представителям семейства Капустные.

Таким образом, ВИР, сохраняя более чем вековые традиции, заложенные его основателями и выдающимися учеными, в настоящее время активно привлекает к изучению генетических ресурсов растений самые современные методы. Его сотрудники ведут объемную и сложную работу по гарантированному сохранению генетических ресурсов на высоком уровне, обеспечивают на регулярной основе отечественные селекционные центры исходным материалом для создания новых высокопродуктивных форм культурных растений, устойчивых к неблагоприятным абиотическим и биотическим стрессовым факторам и обладающих хозяйственно ценными характеристиками.

Редакция «Вавиловского журнала генетики и селекции» поздравляет Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений имени Н.И. Вавилова со 125-летием и желает его сотрудникам успехов в научных исследованиях на благо российской науки.

Научные редакторы выпуска:
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Врио директора ВИР проф. РАН Е.К. Хлесткина,
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Альтернариозные пятнистости гуара

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Однолетняя бобовая культура гуар (*Cyamopsis tetragonoloba* (L.) Taub.) перспективна для выращивания на юге России. В 2018 г. были проведены фитосанитарные обследования посевов гуара (13 коллекционных образцов) в пяти филиалах ВИР (Краснодарский край, Дагестан, Астраханская и Волгоградская области). Во всех пунктах на листьях гуара отмечено несколько типов листовых пятнистостей, среди которых преобладали симптомы поражения растений грибами рода *Alternaria* Nees. Видовой состав микромицетов идентифицировали с помощью микробиологических методов и высокопроизводительного секвенирования последовательности межгенного спейсера гена ядерной рибосомальной РНК (ITS2). Установлено, что грибы *Alternaria* spp. в различных эколого-географических условиях юга России вызывают два основных типа пятнистостей листьев: типичную (бежевые и бурые округлые пятна, обычно сопровождающиеся концентрической зональностью) и коричневую пятнистость (мелкие бурые выпуклые сливающиеся пятна). В большинстве случаев поражение тканей листа вызвано грибом *A. tenuissima* (Nees & T. Nees : Fr.) Wiltshire. Выявлена также сопутствующая микофлора (прежде всего, грибы рода *Fusarium* Link). Один из самых вредоносных патогенов гуара в странах, где сосредоточены основные посевные площади (Индия, Пакистан, США), – специализированный микромицет *A. cyamopsidis* Rangaswami & A.V. Rao на посевах в России не обнаружен. Образцы гуара различаются по степени поражения возбудителем альтернариоза *A. tenuissima*. Показано дифференциальное взаимодействие паразита и хозяина. Соответственно, для предотвращения эпифитотий следует выращивать сорта, защищенные нетождественными генами устойчивости. Во всех пунктах изучения слабо поражались альтернариозом образцы к-52568 (Аргентина) и к-52569 (Пакистан), ряд образцов был устойчив только в условиях одной или двух опытных станций. Изученные формы гетерогенны по устойчивости к патогену, что предоставляет возможность отбора резистентных к болезни линий из большей части коллекционных образцов. Так, в различных филиалах ВИР из образцов к-52571, к-52573 и к-52580 отобраны растения без симптомов поражения, собран семенной материал, который может быть использован для создания новых доноров устойчивости к болезни.
Ключевые слова: гуар; фитосанитарный мониторинг; альтернариоз; высокопроизводительное секвенирование (NGS); устойчивость; взаимодействие паразит–растение-хозяин.

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Alternaria leaf blight of clusterbean

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The annual legume crop clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) is a promising crop for cultivation in the south of Russia. In 2018, phytosanitary examinations of clusterbean fields (13 collection accessions) were conducted in five VIR branches (Krasnodar Territory, Dagestan, Astrakhan and Volgograd Regions). At all points, several types of leaf spots were observed on clusterbean leaves and symptoms of plant damage by fungi of the genus *Alternaria* Nees prevailed. Using microbiological methods and Next-Generation Sequencing (NGS) of the nuclear ribosomal internal transcribed spacer two (ITS2), the species composition of micromycetes was identified. It was found that the micromycetes *Alternaria* spp. in different ecological and geographical conditions of the south of Russia cause two main types of leaf spots: the typical (beige and brown round spots, usually accompanied by concentric zonality) and brown spot (small brown bulging merging spots). Overwhelmingly the damage to leaf tissues is caused by the fungus *A. tenuissima* (Nees & T. Nees : Fr.) Wiltshire. A quite numerous accompanying mycoflora (first of all, fungi of the genus *Fusarium* Link) was also detected. *A. cyamopsidis* Rangaswami & A.V. Rao, one of the most harmful guar pathogens in the countries where the main acreage is located (India, Pakistan, USA), was not found on clusterbean fields in Russia. The accessions of clusterbean differ in degree of damage by *A. tenuissima* that causes Alternaria leaf

blight. Differential interaction of parasite and plant host was revealed. Therefore, to prevent epiphytotics, varieties protected by non-identical resistance genes should be grown. At all VIR branches, accessions k-52568 (Argentina) and k-52569 (Pakistan) were weakly damaged by Alternaria leaf blight, and some accessions were resistant only in the environmental conditions of one or two experimental stations. The accessions studied were heterogeneous in pathogen resistance, which allows selecting disease-resistant lines from most of the collection accessions. Thus, in various VIR branches, plants without symptoms of disease were selected from accessions k-52571, k-52573 and k-52580, and seeds were collected to create new donors of disease resistance.
Key words: clusterbean; phytosanitary monitoring; Alternaria leaf blight; next-generation sequencing (NGS); resistance; parasite – host-plant interaction.

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Введение

Гуар *Cyamopsis tetragonoloba* (L.) Taub. – новая для России однолетняя бобовая культура многоцелевого использования. Его используют в пищу и на корм скоту, однако наиболее ценный продукт переработки семян – гуаровая камедь, которую применяют в качестве стабилизатора консистенции, увеличивающего вязкость и желирующие свойства, в косметологии, пищевой, бумажной, текстильной, угольной и нефтебуровой промышленности (Pathak, 2015). Актуальность проблемы импортозамещения гуаровой камеди промышленного назначения обусловила необходимость изучения перспектив выращивания тропической культуры в условиях России.

Основные направления селекционно-генетических работ – улучшение продуктивности, качества и устойчивости к вредным организмам. Гуар характеризуется широкой вариацией морфологических признаков (Malaghan et al., 2013; Boghara et al., 2016), однако разнообразие возделываемых сортов по генам устойчивости к фитопатогенам невысоко (Kumar et al., 2017).

На гуаре питаются насекомые и клещи; выявлены многочисленные бактериальные, вирусные, грибные и нематодные заболевания, среди которых наиболее широко распространены и вредоносны бактериоз (возбудитель – *Xanthomonas axonopodis* pv. *cyamopsidis* (Patel) Vauterin) и альтернариозная пятнистость (возбудитель – *Alternaria cyamopsidis* Rangaswami & A.V. Rao) (Gandhi, Chand, 1985; Woudenberg et al., 2014). Повсеместному и быстрому распространению болезней способствует сохранение инфекции в семенах.

Всероссийским институтом генетических ресурсов растений имени Н.И. Вавилова (ВИР) в 2017–2018 гг. организовано экологическое испытание коллекционных образцов гуара на юге России. В результате фитосанитарного обследования посевов в 2017 г. на Кубанской опытной станции ВИР (Гулькевичский район Краснодарского края), а также лабораторного анализа инфицированного растительного материала выявлены два наиболее распространенных заболевания: бактериоз и альтернариоз. Для бактериоза было характерно эпифитотийное развитие, отмечали массовое усыхание и даже гибель растений некоторых образцов. С помощью балловой шкалы была оценена степень поражения растений возбудителем заболевания и выделены устойчивые формы (к-52569, к-52575 и к-52580) (Радченко, Соколова, 2018).

В августе 2018 г. фитосанитарные обследования посевов гуара проведены в пяти южных филиалах ВИР. В отличие от предыдущего полевого сезона, в условиях повсеместной засухи обнаружены лишь единичные случаи поражения растений возбудителем бактериоза. Во всех пунктах на листьях гуара отмечено несколько типов листовых пятнистостей, среди которых преобладали симптомы поражения растений грибами рода *Alternaria* Nees (бежевые и бурые округлые пятна, часто с концентрической зональностью). В ряде случаев альтернариозные пятнистости напоминали солнечные ожоги, а также сопровождались деформацией листьев. Наблюдали и «коричневую пятнистость» (мелкие бурые выпуклые пятна) неясной этиологии.

Цель настоящей работы – с помощью микологических и молекулярно-генетических методов идентифицировать видовой состав грибов рода *Alternaria* на гуаре и оценить степень поражения этими патогенами коллекционных образцов, испытываемых в различных эколого-географических условиях юга России.

Материалы и методы

В 2018 г. были обследованы посевы экологического испытания 13 образцов гуара различного происхождения на Крымской опытно-селекционной (КОСС ВИР), Кубанской (КОС ВИР), Дагестанской (ДОС ВИР), Волгоградской (ВОС ВИР) и Астраханской (АОС ВИР) опытных станциях ВИР. Целесообразность промышленного возделывания гуара определяет, прежде всего, сумма эффективных температур воздуха выше 10°, которая должна составлять около 3400–3500 °С (Лебедь и др., 2017). Климатические условия южных филиалов ВИР вполне отвечают данному требованию. ДОС ВИР расположена в южной плоскостной зоне Дагестана у Каспийского моря, в 10 км от г. Дербента. Почвы каштановые, тяжелосуглинистые. Близость моря, искусственное орошение обусловливают постоянно высокую относительную влажность воздуха, которая в июле (средняя температура 24.9 °С), как правило, не ниже 67 %. КОС ВИР расположена в степной части Прикубанской равнины. Почвенный покров представлен мощным предкавказским черноземом. Климат умеренно континентальный, с жарким летом. Среднегодовая температура воздуха 10.6 °С, среднемесячная температура июля 23.3 °С. Среднемноголетняя годовая сумма осадков составляет 545 мм, однако в разные годы колеблется от 400 до 770 мм. КОСС ВИР находится в г. Крымске, в западной части предгорной зоны Краснодарского края. Почвы – слитые и деградированные черноземы глинистого механического состава. Среднегодовая температура

воздуха 10.6 °С. Летом обычны засушливые периоды, которые прерываются ливнями. ВОС ВИР расположена на землях Волго-Ахтубинской поймы. Почвы – аллювиальные суглинки. Климат резко континентальный. Весна сухая, с быстрым нарастанием дневных температур и частыми ветрами. Лето сухое, знойное. В дельте Волги, недалеко от г. Астрахани находится АОС ВИР. Климат здесь самый континентальный и засушливый на всей европейской части России. Средняя многолетняя температура воздуха составляет 10.5 °С, среднесуточная температура июля – 25.6 °С.

Образцы высевали во второй половине мая на делянках длиной 3–3.5 м в двух повторностях. В 2018 г. на юге России повсеместно наблюдалась засуха, после посева вплоть до периода проведения фитосанитарного мониторинга (конец июля – начало августа) осадков практически не было. В условиях ДОС ВИР растения поливали дважды, на ВОС ВИР и АОС ВИР использовали систему капельного орошения растений. Для проведения лабораторной экспертизы пораженные листья собирали в бумажные пакеты, подсушивали и хранили в бытовом холодильнике.

На питомниках экологического испытания филиалов ВИР изучали перспективные для выращивания в России образцы гуара различного происхождения. В этот набор вошли местные образцы без названий из Аргентины (к-52568) и Пакистана (к-52569), отбор из пакистанского местного образца, сделанный в Крыму АО «Таврида» (к-52571), селекционные сорта из США Santa Cruz (к-52584), Kinman (к-52585) и Lewis (к-52586), индийские образцы, полученные из Ростовского университета (к-52580 и к-52581), сорта селекции КОС ВИР Вавиловский 130 (к-52572, отбор из к-52568), Кубанский (к-52573, отбор из к-52571), Кубанский юбилейный (к-52742, отбор из к-52581), сорта Вектор (к-52574) и Синус (к-52575).

Проведен микологический анализ образцов растений гуара с разными симптомами поражения фитопатогенами. Фрагменты пораженных тканей помещали в марлевые мешочки, промывали 2 ч под струей водопроводной воды, поверхностно дезинфицировали в течение 1 мин 0.1 % раствором нитрата серебра, промывали несколько раз стерильной водой со стрептомицином и раскладывали в чашки Петри на поверхность агаризованной картофельно-сахарозной среды. Чашки инкубировали в термостате при 24 °С в течение 7 сут, а затем при эритемном освещении. После появления мицелия на или вокруг растительной ткани его переносили на свежую питательную среду и получали колонии (изоляты) фитопатогенных грибов. После количественного учета видового разнообразия микромицетов выделяли группу грибов рода *Alternaria*.

Для идентификации грибов рода *Alternaria* по морфологическим признакам моноспоровые изоляты выращивали на картофельно-морковной питательной среде при 24 °С и переменном освещении (16 ч свет : 8 ч темнота). Идентификацию изолятов осуществляли на 5–7-е сутки, просматривая спороношение на поверхности колоний под бинокуляром, с использованием определителя (Simmons, 2007).

Грибы рода *Alternaria* в листьях гуара идентифицировали также с помощью высокопроизводительного секвенирования последовательности межгенного спейсера гена ядерной рибосомальной РНК (ITS2). При этом был выбран вариабельный участок ядерного генома 5.8SrRNA – ITS2 – 28SrRNA, позволяющий, согласно литературным данным, дифференцировать виды *Alternaria* из секции *Porri*, к которой относится опасный специализированный патоген гуара *A. cyamopsidis*, от других представителей рода (Pavón et al., 2011; Woudenberg et al., 2013).

Анализировали листья гуара с различными типами пятнистостей, которые были собраны в пяти филиалах ВИР. Листья высушивали в естественных условиях и растирали в жидком азоте. ДНК выделяли из 10–20 пораженных листьев с помощью набора реактивов NucleoSpin Soil (MACHEREY-NAGEL, Германия) согласно инструкции производителя. Качество и концентрацию ДНК оценивали на спектрофотометре NanoDrop 2000C. Из листьев, собранных на ДОС ВИР, не удалось выделить ДНК удовлетворительного качества, поэтому образец был исключен из анализа.

В исследуемых образцах была проведена амплификация фрагментов рибосомальных оперонов грибов (ITS2) методом ПЦР с использованием праймеров *ITS1F*/*ITS2* (GCATCGATGAAGAACGCAGC/TCCTCCGCTTATTG ATATGC) (White et al., 1990). Секвенирование выполняли на платформе MiSeq с помощью набора реактивов MiSeq® ReagentKit v3 (600 cycle) с двусторонним чтением (Illumina, США). Полученные последовательности обрабатывали с помощью программного пакета PIPITS для библиотек ITS (Gweon et al., 2015). Работу проводили с использованием оборудования ЦКП «Геномные технологии, протеомика и клеточная биология» ФГБНУ ВНИИСХМ. Идентификацию организмов осуществили с помощью информационно-поисковой системы BLAST базы данных GenBank NCBI путем поиска последовательностей по подобию (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Редкие сайты, замены в которых встречались в одной-пяти последовательностях, были исключены из анализа.

Во всех зонах изучения на гуаре наблюдали умеренное развитие альтернариозных пятнистостей. Поражение растений на естественном инфекционном фоне оценивали по шкале (Вишнякова и др., 2010): 1 – поражено до 10 % листовой поверхности (очень слабое); 2 – поражено 11–25 % (слабое); 3 – поражено 26–50 % (среднее); 4 – поражено 51–75 % (сильное); 5 – поражено 76–100 % (очень сильное).

На КОСС ВИР осматривали и оценивали по 15 растений каждого из тринадцати образцов, на ДОС ВИР и АОС ВИР – по 20, на ВОС ВИР – по 40 и на КОС ВИР – по 60 растений. Учет развития коричневой (предположительно альтернариозной) пятнистости выполняли на трех станциях: КОСС ВИР, АОС ВИР и ВОС ВИР. Учет степени поражения растений восьми образцов на КОСС ВИР с интервалом 10 дней продолжили до 20 сентября.

Результаты

На листьях гуара выявлены различные пятнистости, в большинстве случаев без развитого спороношения (табл. 1). В образце из Астрахани на одном пятне отмечено очень скудное спороношение грибов рода *Ascochyta* Lib. На нескольких образцах обнаружен налет сапротрофных видов родов *Cladosporium* Link и *Alternaria*. Из пора-

Table 1. Micromycetes detected on clusterbean plants

Experimental station	Description of the sample	Micromycetes	
		detected by microscopic examination of herbarium material	solated from damaged tissue
Dagestan Experimental Station of VIR	Bordered spots	–	*Alternaria* sect. *Infectoriae*, *Alternaria* sect. *Alternaria*
	Sunburn-like fringing spots	–	*Alternaria* sect. *Alternaria*, *Trichoderma* sp.
	Small convex brown spots	–	*Alternaria tenuissima*, *Alternaria* sect. *Alternaria*, *Stemphylium* sp.
Kuban Experimental Station of VIR	Brown spots	*Alternaria* sp., *Cladosporium* sp.	*Alternaria* sect. *Alternaria* (*A. tenuissima*)
	Small convex brown spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*), *Alternaria* sect. *Infectoriae*
	Large fringing brown spots	–	*Alternaria* sect. *Alternaria*, *Fusarium equiseti*, *F. acuminatum*
Krymsk Experimental and Breeding Station of VIR	Bordered spots	–	*Alternaria* sect. *Alternaria*, *Fusarium equiseti*
	Small convex brown spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*, *A. arborescens*), *Fusarium equiseti*, *Phoma* sp.
Astrakhan Experimental Station of VIR	Bordered and albescent spots	*Alternaria* sp., *Ascochyta* sp.	*Alternaria* sp., *Rhizopus* sp.
	Small convex brown spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*), *Alternaria* sect. *Infectoriae*, *Ulocladium* sp.
Volgograd Experimental Station of VIR	Bordered spots	–	*Alternaria* sect. *Alternaria*, *Fusarium equiseti*
	Small convex brown spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*), *Fusarium equiseti*
	Rounded fawn spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*), *Fusarium equiseti*
	Fringing brown spots	–	*Alternaria* sect. *Alternaria* (*A. tenuissima*), *Ulocladium* sp., *Fusarium equiseti*, *F. sporotrichioides*, *F. acuminatum*

женных тканей листьев были выделены микромицеты, являющиеся в основном сапротрофами или слабыми патогенами: *Alternaria* sect. *Infectoriae*, *Alternaria* sect. *Alternaria* (*A. tenuissima* (Nees & T. Nees : Fr.) Wiltshire, *A. arborescens* E.G. Simmons, *Alternaria* sp.), *Stemphylium* sp., *Trichoderma* sp., *Ulocladium* sp., *Fusarium equiseti* (Corda) Sacc., *F. acuminatum* Ellis & Everh., *F. sporotrichioides* Sherb., *Phoma* sp., *Rhizopus* sp.

Следует особо отметить, что грибы рода *Alternaria* (преимущественно *A. tenuissima*) были обнаружены и доминировали во всех анализированных образцах, в том числе с нетипичным симптомом «коричневая пятнистость» (мелкие выпуклые бурые пятна). Так, при анализе 97 фрагментов пораженных тканей с типичными симптомами альтернариоза и краевыми пятнами типа солнечных ожогов выделено 89 изолятов микромицетов, среди которых 71 – грибы рода *Alternaria*, 16 – *Fusarium*, 1 – *Trichoderma*, 1 – *Ulocladium*. При экспертизе 62 фрагментов тканей с коричневой пятнистостью среди 43 выделенных колоний идентифицирован 31 изолят грибов рода *Alternaria*, 8 – *Fusarium*, 2 – *Phoma*, 1 – *Ulocladium*, 1 – *Stemphylium*.

С помощью микологической экспертизы из пораженных тканей листьев, собранных во всех филиалах ВИР, были выявлены лишь мелкоспоровые грибы *Alternaria* и не найден крупноспоровый специализированный вид *A. cyamopsidis*. Этот патоген из секции *Porri* хорошо отличается от мелкоспоровых видов по морфологическим признакам, однако в силу случайных причин (например, слабая представленность в исследованных образцах) мог быть не детектирован.

С целью идентификации *A. cyamopsidis* проанализированы четыре ампликонные библиотеки фрагментов рибосомальных оперонов грибов (ITS2). В качестве матрицы использовали очищенный препарат суммарной ДНК, которая была выделена из пораженных листьев гуара, собранных на посевах четырех филиалов ВИР. Метод высокопроизводительного секвенирования (NGS – New Generation Sequences), в отличие от традиционного секвенирования по Сэнгеру, позволяет получить и проанализировать тысячи последовательностей и выявить варианты, представленные даже в следовых количествах. Известно, что SNP и делеции в исследуемом фрагменте дают возможность дифференцировать виды из секции *Porri* от других представителей рода *Alternaria* (Pavón et al., 2011; Woudenberg et al., 2013, 2014).

Для каждой из четырех проб ДНК было получено и проанализировано около 50 000 нуклеотидных последовательностей. Доля *Alternaria* spp. достигала 53.6 % от общего числа выявленных организмов. Ни в одном из

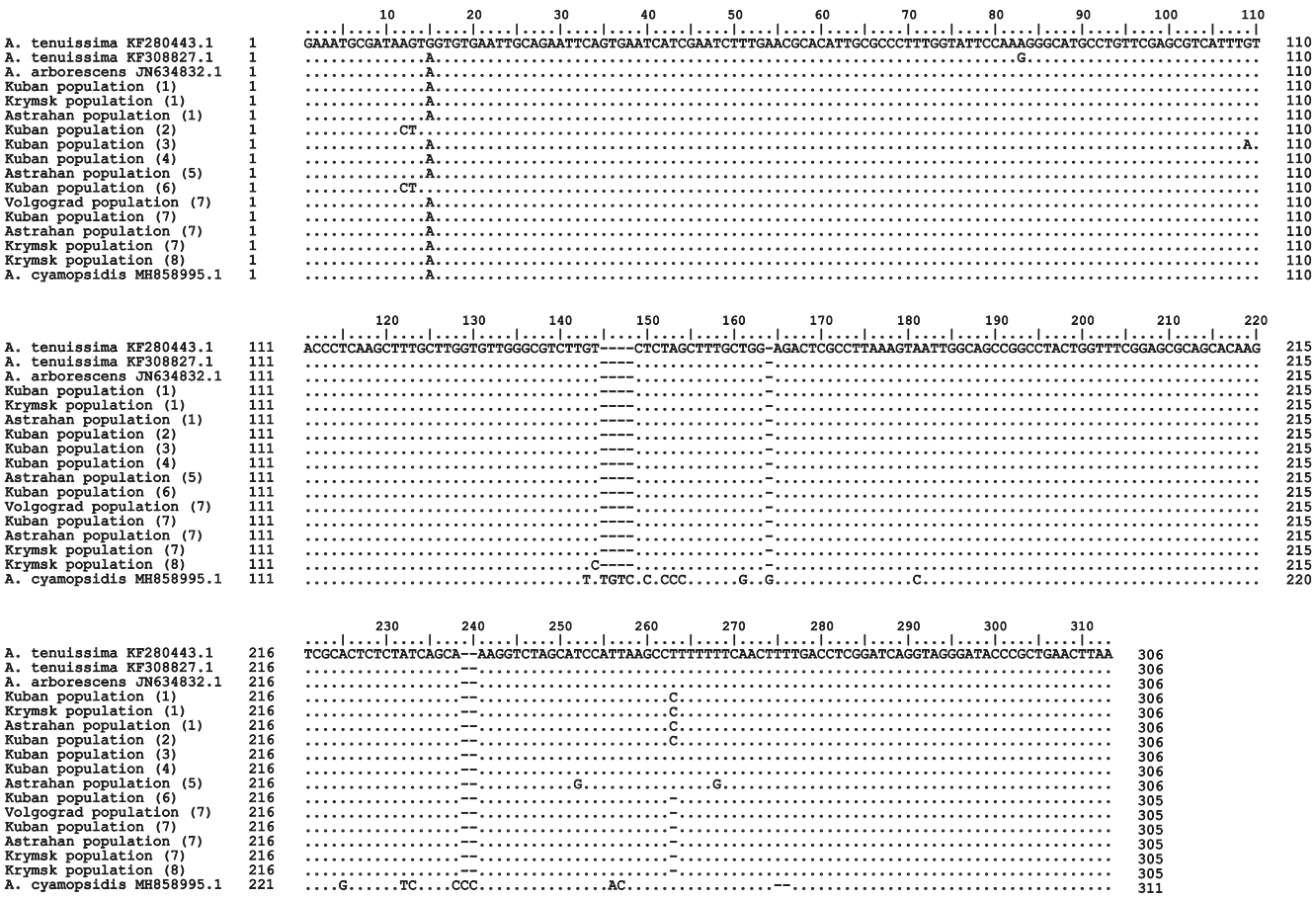

Fig. 1. Sequence alignment of the *Alternaria* spp. nuclear rRNA intergenic spacer (ITS2) 5.8SrRNA – ITS2 – 28SrRNA.
Kuban, Krymsk, Astrakhan, and Volgograd designate micromycetes revealed in leaf samples collected in VIR branches KES VIR, KEBS VIR, AES VIR, and VES VIR, respectively.

Genbank accessions KF280443.1 and KF308827.1 (*A. tenuissima*), JN634832.1 (*A. arborescens*) and MH858995.1 (*A. cyamopsidis*) are chosen as reference sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). 1–8: sequence variants found in the samples examined.

исследованных образцов не обнаружен специализированный патоген *A. cyamopsidis*. Все найденные последовательности *Alternaria* spp. оказались на 98–100 % сходны с мелкоспоровыми видами *A. tenuissima* и *A. arborescens* из секции *Alternaria* (рис. 1).

Помимо *Alternaria* были обнаружены последовательности *Cladosporium* Link, *Toxicocladosporium* Crous & U. Braun, *Phoma* Saccardo, *Stemphylium* Wallr, *Botrytis* P. Micheli ex Pers, *Fusarium* Link, *Rachicladosporium* Crous, U. Braun & C.F. Hill, *Pyrenochaeta* De Not, *Sclerostagonospora* Höhn, *Chalara* Rabenh, *Phacidium* Fries, *Typhula* (Pers.) Fr. и *Peyronellaea* Goid. ex Toglian, emend. Aveskamp, Gruyter & Verkley, т. е. 13 родов, которые могут содержать слабопатогенные и сапротрофные грибы. Присутствие ДНК микромицетов разных типов связано с тем, что ДНК выделяли из суммарной навески 10–20 листьев, характеризующихся максимальным разнообразием типов листовых пятнистостей.

В пяти филиалах ВИР оценили пораженность 13 образцов гуара «типичной» альтернариозной пятнистостью. Образцы к-52568 (Аргентина) и к-52569 (Пакистан) характеризовались устойчивостью к альтернариозу во всех пунктах изучения (табл. 2). Остальные формы, проявившие устойчивость в одном или двух пунктах, оказались восприимчивы на других опытных станциях. Так, наиболее устойчивый на АОС ВИР образец к-52584 проявил резистентность и в условиях КОСС ВИР и ВОС ВИР, однако был восприимчив в Дагестане и на КОС ВИР.

Обнаружены различия между образцами и по устойчивости к коричневой альтернариозной пятнистости (табл. 3). В условиях КОСС ВИР наиболее высокий уровень резистентности выявлен у образца к-52568, который в средней степени поражался в Астрахани и Волгограде. Наиболее восприимчивыми во всех эколого-географических зонах оказались образцы гуара из США.

Динамика развития двух типов альтернариозных пятнистостей (рис. 2, *а*, *б*) различалась: наблюдали существенное усиление симптомов «типичной» альтернариозной пятнистости, незначительное и плавное – коричневой пятнистости. Тем не менее уровень поражения растений к концу сезона в обоих случаях оказался схож: 1.6–2.8 балла.

Результаты мониторинга степени поражения образцов гуара свидетельствуют о возможном дифференциальном взаимодействии генотипов возбудителей альтернариоза и растений. Для проверки этого предположения полученные

Table 2. Damage score of clusterbean accessions by typical Alternaria leaf blight in five VIR branches

VIR accession number	Name	Origin	Mean damage score				
			DES VIR	KES VIR	KEBS VIR	VES VIR	AES VIR
52568	No	Argentina	0.60 a*	0.73 abc	0.40 ab	1.20 b	0.65 bc
52569	No	Pakistan	0.65 ab	0.72 abc	0.67 ab	1.08 ab	0.80 cde
52571	No	Crimea	0.75 abc	0.87 bcd	0.80 б	2.08 e	0.80 cde
52572	Vavilovskiy 130	Krasnodar Kray	1.30 d	0.77 abcd	0.67 ab	1.60 cd	0.85 cde
52573	Kubanskiy		1.10 cd	0.63 a	0.67 ab	1.05 ab	0.95 de
52574	Vector		1.00 bcd	0.63 a	0.47 ab	1.43 c	0.95 de
52575	Sinus		0.80 abc	0.80 abcd	0.60 ab	1.00 ab	0.70 bcd
52580	No	Rostov oblast	1.00 bcd	0.72 abc	0.47 ab	1.45 c	0.90 cde
52581	No		1.05 cd	0.67 ab	0.33 a	1.08 ab	1.05 e
52584	Santa Cruz	United States	1.35 d	0.90 cd	0.47 ab	1.03 ab	0.30 a
52585	Kinman		1.25 d	0.97 d	0.40 ab	0.98 a	0.50 ab
52586	Lewis		1.30 d	0.83 abcd	0.47 ab	1.78 d	0.65 bc
52742	Kubanskiy Yubileynyy	Krasnodar Kray	1.05 cd	0.72 abc	0.53 ab	0.98 a	1.0 e

* Here and below, the differences between the variants marked with identical letters within the columns are nonsignificant (Duncan's multiple range test, $p < 0.05$).

Table 3. Damage score of clusterbean accessions by brown Alternaria leaf blight in three VIR branches

VIR accession number	Name	Origin	Mean damage score		
			KEBS VIR	VES VIR	AES VIR
52568	No	Argentina	1.13 a	2.35 c	1.15 cde
52569	No	Pakistan	2.40 c	1.85 ab	1.40 ef
52571	No	Crimea	2.00 bc	1.75 ab	0.75 abc
52572	Vavilovskiy 130	Krasnodar Kray	1.87 bc	1.80 ab	1.25 def
52573	Kubanskiy		1.07 a	2.33 c	0.50 ab
52574	Vector		1.47 ab	2.90 e	1.70 f
52575	Sinus		1.80 bc	1.95 b	1.50 ef
52580	No	Rostov oblast	2.07 bc	2.65 de	0.40 a
52581	No		1.93 bc	2.28 c	1.10 cde
52584	Santa Cruz	United States	1.93 bc	2.75 e	0.90 bcd
52585	Kinman		2.47 c	2.50 cd	1.75 f
52586	Lewis		2.13 bc	2.88 e	2.50 g
52742	Kubanskiy Yubileynyy	Krasnodar Kray	2.00 bc	1.68 a	1.55 ef

данные были обработаны с помощью дисперсионного анализа. Анализировали двухфакторный комплекс: варьирование по генотипам патогенов (пять пунктов изучения) и по генотипам растений (13 образцов). Наиболее интересный результат дисперсионного анализа – дифференциальное взаимодействие генотипов растений и грибов (табл. 4). Дисперсия для взаимодействия в нашем случае в 5.41 раза превышает случайную. Попарное сравнение степени поражения образцов гуара в филиалах ВИР выявило аналогичные различия в четырех случаях из пяти: $F_{факт.}$ = 3.76–13.86, $F_{0.5}$ = 1.78. Не различались по вирулентности лишь патогены на КОС ВИР и КОСС ВИР ($F_{факт.}$ = 1.32, $F_{0.5}$ = 1.78).

Предположили также различия по вирулентности между грибами, которые вызывают коричневую альтернариозную пятнистость, в трех филиалах ВИР. В данном случае анализировали варьирование по генотипам растений (13 образцов) и по генотипам патогенов из трех пунктов изучения. Дисперсия для взаимодействия в 8.14 раза превышает случайную, что убедительно доказывает специфичность отношений паразит–растение-хозяин (табл. 5). Дифференциальное взаимодействие выявлено и при

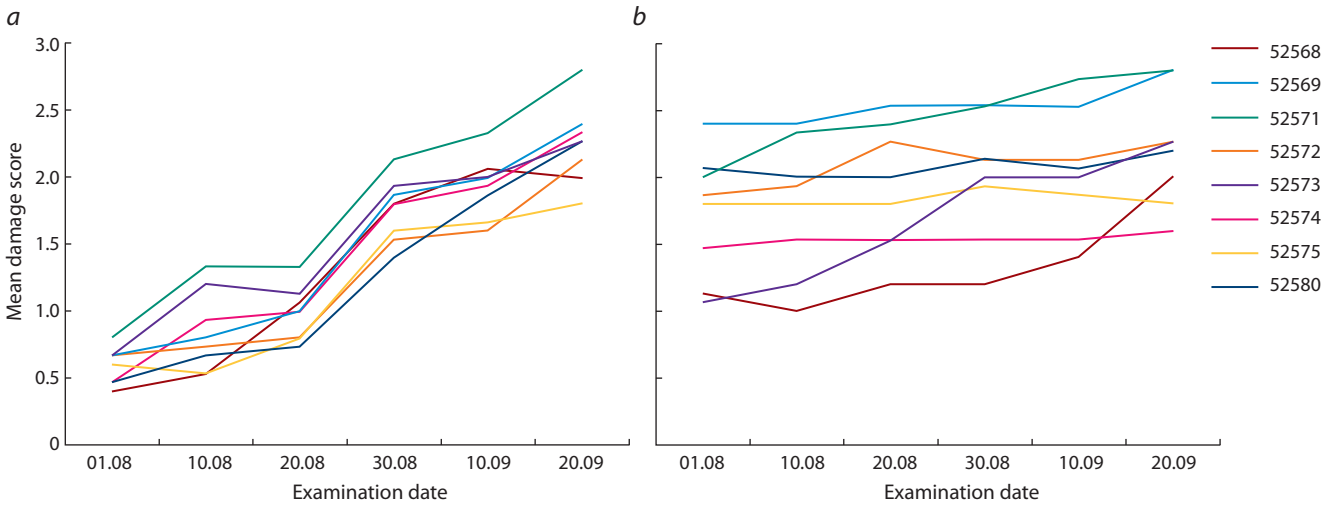

Fig. 2. Dynamics of clusterbean accession damage by (*a*) typical and (*b*) brown Alternaria leaf blight in Krymsk.

Table 4. Analysis of variance for damage of clusterbean accessions by typical Alternaria leaf blight in five VIR branches

Variation	Sum of squares	Degrees of freedom	Dispersion	F_{fakt}	$F_{0.5}$
In plant genotype	14.98	12	1.25	5.47	1.76
In pathogen genotype	59.88	4	14.97	65.62	2.38
In the interaction between plant and pathogen genotypes	59.29	48	1.24	5.41	1.37
Residual	207.60	910	0.23	–	–
Total	341.75	974	–	–	–

Table 5. Analysis of variance for damage of clusterbean accessions by brown Alternaria leaf blight in three VIR branches

Variation	Sum of squares	Degrees of freedom	Dispersion	F_{fakt}	$F_{0.5}$
In plant genotype	56.59	12	4.72	9.77	1.77
In pathogen genotype	109.58	2	54.79	113.49	3.01
In the interaction between plant and pathogen genotypes	94.29	24	3.93	8.14	1.54
Residual	263.60	546	0.48	–	–
Total	524.06	584	–	–	–

попарном сравнении степени поражения образцов гуара в филиалах ВИР во всех анализировавшихся случаях ($F_{факт.}$ = 4.47–12.14, $F_{0.5}$ = 1.78).

Обсуждение

На листьях гуара во всех пунктах изучения отмечено умеренное развитие пятнистостей. В большинстве случаев симптомы поражения были характерны для альтернариоза (округлые бежевые пятна с концентрической зональностью), выявлены случаи поражения растений грибами из родов *Ascochyta*, *Cladosporium* и др. Повсеместно была распространена «коричневая пятнистость» (мелкие выпуклые бурые сливающиеся пятна), у ряда образцов поражение достигало трех баллов (до 50 % площади листовой поверхности). Результаты микологической экспертизы «проблемных» образцов листьев, собранных в филиалах ВИР, несколько неожиданно показали, что в основном поражение тканей листа обусловлено грибом *A. tenuissima* (см. табл. 1). Выявлена и сопутствующая микофлора (прежде всего, грибы рода *Fusarium*), причем фитопатогенный ландшафт во всех зонах выращивания гуара весьма схож. В полевых условиях степень поражения растений коричневой пятнистостью в начале августа превышала пораженность гуара «типичным» альтернариозом, однако к концу сезона эти различия нивелировались.

Известно, что виды комплекса *A. alternata*, к которому относится и наиболее распространенный вид *A. tenuissima*, существуют в природе преимущественно как са-

протрофы и часто бессимптомно присутствуют в семенах растений. Однако иногда они вызывают сильные массовые заболевания различных растений, в том числе пшеницы, подсолнечника и др. Вероятно, такие заболевания связаны с определенным сочетанием погодных, эдафических и агротехнических факторов, снижающих иммунитет растений и благоприятствующих развитию патогенов (Ганнибал, 2011). Видимо, в нашем случае повсеместное развитие *A. tenuissima* было спровоцировано засушливыми условиями.

С помощью высокопроизводительного секвенирования, которое позволяет идентифицировать организмы, представленные даже в следовых количествах, удалось идентифицировать патогенные грибы, относящиеся к 14 родам. Все найденные последовательности *Alternaria* оказались на 98–100 % сходны с мелкоспоровыми видами *A. tenuissima* и *A. arborescens*. К сожалению, анализировавшийся фрагмент ядерного генома 5.8SrRNA – ITS2 – 28SrRNA не позволяет дифференцировать эти виды.

Интересно, что последовательности крупноспорового вида *A. cyamopsidis* нами не обнаружены. В то же время имеются многочисленные публикации, где обсуждается взаимодействие гуара только с этим специализированным патогеном (Saharan et al., 2001; Joshi et al., 2004; Meena et al., 2012), вредоносность которого высока во всех зонах выращивания (Kumar, 2005; Woudenberg et al., 2014). Результаты нашей работы свидетельствуют об отсутствии этого патогена в России, однако можно ожидать заноса инфекции с интродуцируемым семенным материалом.

Заключение

Эксперименты показали, что образцы гуара различаются по устойчивости к альтернариозным пятнистостям, причем эта устойчивость специфична. Это означает, что для предотвращения эпифитотий следует создавать сорта, защищенные разными генами устойчивости. Очевидно, выявленное нами дифференциальное взаимодействие с растением-хозяином характерно для доминировавшего вида *A. tenuissima*. В литературе есть сведения лишь о специфичности отношений гуара и возбудителя бактериоза *X. axonopodis* pv. *cyamopsidis* (Vijayanand et al., 1999; Kaur et al., 2005), однако дифференциальное взаимодействие гуара и *A. cyamopsidis* не обсуждается.

Образцы к-52568 (Аргентина) и к-52569 (Пакистан) характеризовались более или менее выраженной устойчивостью к альтернариозу во всех пунктах изучения. В условиях КОСС ВИР наиболее высоким уровнем резистентности к коричневой альтернариозной пятнистости отличался образец к-52568, который в средней степени поражался в Астрахани и Волгограде.

Практически все изученные формы гетерогенны по устойчивости к альтернариозу, и резистентные к болезни линии могут быть отобраны из большей части коллекционных образцов. Так, нами собраны семена растений образца к-52573, которые не имели симптомов поражения на КОС ВИР, АОС ВИР (к-52580) и ВОС ВИР (к-52571). Полученные линии будут испытаны на юге России. К сожалению, срок «полезной жизни» источников устойчивости ограничен в силу специфичности отношений паразит–хозяин. Необходимо вовлечение в селекцию возможно большего числа генетически разнородных образцов и рациональное их использование.

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Localization of rust resistance genes in old local Russian flaxes by methods of classical genetics

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Flax rust, a disease that destroyed a significant portion of the yield before the creation of resistant varieties, is currently defeated, but it can cause new outbreaks as identical resistance genes are used in breeding. Since only one of the allelic genes can be introduced into a variety, the aim of this work is to identify genes for resistance to the disease in lines selected during the evaluation of old Russian flaxes from the VIR collection. The original accessions were added to the collection in 1922, that is, before the release of breeding varieties, so their genes are of natural origin. The analysis was performed on an artificial infectious background by methods of classical genetics, including the test for allelism. Nine monogenic lines with the original *R* genes were crossed to tester varieties for six loci: *K*, *L*, *M*, *N*, *P*, and *Q*. F_2 hybrids in the phase of cotyledon leaves were inoculated with monopustule clones of the fungus, not virulent to any of evaluated genes. Gene allelism was checked by the absence of the segregation. It was exactly proven that *R* genes of the k-716 line from the Pskov kryazh (gc-32) and the k-780 accession from the Minsk oblast (gc-33) were located in the *P* locus, the gene of the k-846 line from the Ivanovo-Voznesensk oblast (gc-39) was in the *M* locus, and the gene of the k-834 line from the Vladimir oblast (gc-38) probably belonged to the *K* locus. The segregation in the crosses of all testers to the k-630 line from the Simbirsk oblast (gc-25) showed that its gene was not allelic to any of the known loci. Probably, there was a formerly unknown locus. The location of the other genes failed to be identified due to the linkage between loci *N* and *P* and the presence of several resistance genes in some lines. The gene in gc-9 was in either *M* or *K* locus; and the genes of gc-34, gc-40, and gc-46 were located in *P* or *K*. Since all the evaluated genes were original, the genes of these lines were different alleles of the identified loci.

Key words: flax rust; resistance genes; localization of genes; linkage; allelism.

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Локализация генов устойчивости к ржавчине у староместных российских льнов методами классической генетики

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Ржавчина льна – болезнь, которая уносила значительную часть урожая до создания устойчивых сортов, в настоящее время побеждена, однако при использовании в селекции идентичных генов устойчивости могут возникать новые эпифитотии. Поскольку в сорт может быть введен только один из аллельных генов, целью настоящей работы была попытка идентификации генов устойчивости к болезни у линий, выделенных при оценке коллекции ВИР, из староместных российских льнов. Исходные образцы поступили в коллекцию в 1922 г., т.е. до начала распространения селекционных сортов, поэтому их гены имеют естественное происхождение. Анализ проводили на искусственном инфекционном фоне методами классической генетики, используя тест на аллелизм. Девять моногенных линий, обладающих оригинальными *R*-генами, были скрещены с сортами-тестерами шести локусов *K*, *L*, *M*, *N*, *P* и *Q*. Гибриды F_2 в фазу семядольных листьев были инокулированы монопустульным клоном гриба, авирулентным ко всем изучавшимся генам. Об аллельности генов судили по отсутствию расщепления. Точно определено, что *R*-гены у линий из Псковского кряжа к-716 (гк-32) и образца из Минской области к-780 (гк-33) расположены в локусе *P*, ген линии из Иваново-Вознесенской области к-846 (гк-39) – в локусе *M*, а ген линии из Владимирской области к-834 (гк-38), вероятно, относится к локусу *K*. При скрещивании линии из Симбирской области к-630 (гк-25) со всеми тестерами локусов получено расщепление, означающее, что этот ген не аллелен ни одному из известных локусов. Вероятно, существует еще один неизвестный локус. Расположение других генов точно установить не уда-

лось из-за сцепления между локусами *N* и *P*, а также присутствия в некоторых линиях по нескольку генов устойчивости. Ген устойчивости гк-9 расположен либо в локусе *M*, либо в *K*, гены гк-34, гк-40 и гк-46 – в *P* или *K*. Поскольку все изучаемые гены оригинальны, гены этих линий являются разными аллелями установленных локусов.

Ключевые слова: ржавчина льна; гены устойчивости; локализация генов; сцепление; аллельность.

Introduction

Flax rust is a serious disease that destroyed a significant part of the crop in Soviet Union in the last century. Its most destructive epiphytotics occurred on the American continent (Flor, 1964) and gave an impetus to the intensification of its genetic investigation, search for immune forms, and breeding of resistant varieties (Flor, 1946, 1956). By now, it has been discovered that the resistance of cultivated flax *Linum usitatissimum* L. to rust *Melampsora lini* (Pers.) Lev. is controlled by 32 genes located in six loci (*K*, *L*, *M*, *N*, *P*, and *Q*) consisting of closely linked or allelic genes (Islam, Mayo, 1990). Genes of locus *L* in variety Ottawa 770B and *M* in variety Dakota were discovered by W.M. Myers in 1937; genes *N* in Bombay; *P* in Koto, and *K* in variety Klay were found by H.H. Flor (Flor, 1947, 1955). Gene *Q* was discovered by us (Kutuzova, Kulikova, 1989) in Russia, and identified in variety Natasja (Kutuzova, 1994).

Flor found out that resistance genes are characterized by multiple allelism (Flor, 1941, 1947, 1954, 1955; Flor, Comstock, 1972). Among the varieties bred in America, 11 alleles of the *L* gene (locus *L*) have been found: *L1–L8*, *L10*, and *L11*. Allele *L9* was identified in Australia, in the generally susceptible American variety Bison (Kerr, 1960).

Six alleles were found in locus *M*. Flor (1947, 1954) identified five alleles of gene *M* in North America: *M1* through *M5*. Allele *M6* was found in Argentina (Zimmer, Comstock, 1973).

Two alleles were found in the *N* locus: *N1* and *N2* (Flor, 1947, 1955).

Six alleles are known for locus *P*: genes *P1–P3* were identified by H.H. Flor (1955); gene *P4*, by D.E. Zimmer et al. (Zimmer, Compstock, 1973) in North America; genes *P5* and *P6*, by G.B. Kerr (1960) in Australia.

Two alleles are known in locus *K*: gene *K* was identified by H.H. Flor (1955) in North America and allele *K1* was found in Canada (Hoes, Kenaschuk, 1986).

The *Q* gene, which is not identical to genes *L*, *M*, *N*, *P*, or *K*, was mapped to the new *Q* locus. It is effective against all races of the Russian fungus population (Kutuzova, 2014), as well as against the tested races of Australia and North America (Islam, Kutuzova, 1990).

As early as the middle of the 20th century, it was found that all genes in locus *L* were linked rather than allelic (Flor, 1947). In addition, it was shown that loci *K*, *L*, *M* are inherited independently and loci *N* and *P* are linked (Flor, 1962, 1965). Kerr (1960) found that the distance between loci *N* and *P* was 9.5 cM. Flor (1962) determined the distance between genes *N* and *P3* to be 15 cM. However, later it was shown that gene *K1* was located on the same chromosome as the *N* and *P* genes, and the recombination rate was 25 % (Hoes, Kenaschuk, 1986).

Evaluation of the resistance of varieties carrying these genes in different countries showed that some of them had additional genes. In particular, the *P5* gene was found in variety Ottawa 770B in Australia (Kerr, 1960) and India (Misra, Prasada, 1966) in addition to the *L* gene. Gene *P6* was identified in variety Kenya (gene *L4*) in Australia; the same gene was found in variety Bolley Golden (*L10*), and the *L9* gene (*P*) was discovered in variety Koto (Kerr, 1960). In India, an additional *L9* gene was found in already tested varieties: Dakota (*M*), Ward (*M2*), Cass (*M3*),Victory A (*M4*), Polk (*N1*), Koto (*P*) and C. I. 1888-8 (*P4*) (Misra, Prasada, 1966). In Russia, the *Q* gene is allelic to the resistance genes of varieties Kenya (*L4*), Bombay (*N*), Polk (*N1*), and Klaus (*K*) (Kutuzova, Kulikova, 1989).

Around that time, the complicated structure of rust resistance loci was revealed. It was discovered that in locus *L* genes *L*, *L2*, *L5*, and *L6* were closely linked or allelic (Flor, 1962). In the opinion of M.R. Islam and K.W. Shepherd, genes in locus *L* are allelic and their products may differ by one amino acid. Also, in their experiments the relationship between genes and expression of many of them depended on temperature and the presence of inhibitor genes in some lines (Islam, Shepherd, 1991). Linkages were also found in the *M* locus between alleles *M1* and *M4* (Lawrence et al., 1981) and between *M* and *M3* (Hausner et al., 1999b). According to M.R. Islam, all genes in this locus are closely linked, and gene *M* is influenced by inhibitor genes *I-1* and *I-2* (Islam et al., 1989).

Modern methods of molecular genetics allowed discovering the structure and features of *R* gene expression. It was found that the *L* locus is a single gene with 13 allelic variants (*L*, *L1–L11*, and *LH*), which can be distinguished by the response to different races of the pathogen. Loci *N*, *M*, and *P* have a more complicated structure and consist of 4–15 or 6–8 tandem paralogs (Ellis et al., 1999; Dodds et al., 2001a, b; Lawrence et al., 2010). Unique DNA fragments marking genes *L2*, *L6*, *L9*, *L11* (Hausner et al., 1999a); *P* and *P2* (Dodds et al., 2001b); gene *M3*, effective in Canadian environment (Hausner et al., 1999b), and gene *M4*, effective against the majority of rust pathogen races in China (Bo et al., 2008) were discovered. Currently, 19 genes have been sequenced (11 in locus *L*, 3 in *M*, 3 in *N*, and 2 in *P*), and a partial homology between sequenced genes located in different loci has been proven (Ravensdale et al., 2011). The size of these genes is about 4500 bp, and their products are about 1200 aa (Lawrence et al., 1995). Each gene consists of four exons and three introns (Lawrence et al., 1995; Anderson et al., 1997; Dodds et al., 2001a, b). Thus, despite of the extensive study of the genes responsible for flax resistance to rust, there is no clear view of their structure and location in the genome.

The proteins encoded by rust resistance genes, which control the signal transmission about the infection within the cell, belong to the class of TIR-NBS-LRR proteins. Their amino terminal regions contain the LZ domain, leucine zipper, in most cases belonging to the TIR family (Toll/Interleukin-1 Resistance), and the nucleotide-binding site (NBS). The carboxy terminal region is enriched with imperfect leucine-rich repeats (LRRs) (Hammond-Kosack, Jones, 1997). The LRR

domain of a protein molecule is horseshoe-shaped. It consists of leucine-enriched repeats (xxLxLxx motifs, where L is leucine and x is any amino acid) of 24–30 aa each, and is responsible for R-Avr interaction (Kobe, Deisenhofer, 1995; cit. ex: Ravensdale et al., 2011). The differences between alleles of one gene relate mostly to leucine-rich domains. In particular, the products of genes *P2* and *P* differ in the replacement of 10 amino acids in four xxLxLxx motifs of the LRR domain. Differences among products *P*, *P1*, *P2*, *P3*, and *P4* are also related to the LRR domain (Dodds et al., 2001b). Protein products of genes *L6* and *L11* differ in 32 amino acids in the LRR domain (Ellis et al., 2007; cit. ex: Ravensdale et al., 2011).

Products of the flax *R* genes can be divided into two subclasses, differing in the presence of a domain at the C-end of CNL, which is not enriched with leucine, and the homology of the LZ domain to TIR. The first subclass includes the *L*, *M*, and, possibly, *N* loci, which have the TIR-NBS-CNL structure (Dodds et al., 2001a). Products of genes *L* and *M* also have an N-terminal hydrophobic site, probably responsible for anchoring to the membrane, which supposes their location inside the cell (Ellis et al., 2007; cit. ex: Ravensdale et al., 2011).

Another feature of genes *L6*, *M*, *N*, but not *P*, is alternative splicing, which results in the formation of two products: full-size and truncated (Dodds et al., 2001a, b). For example, the most part of the LRR domain is missing from the truncated product of the *L6* gene, and there is a short C-terminal end. This situation is explained by the translation of only part of the third intron and the termination of exon 3 and 4 translation (Lawrence et al., 1995; Dodds et al., 2001a, b).

Thus, the specificity of rust resistance genes may be caused by changes in different parts of their sequences and the accumulation of various mutations. The accumulation of both "neutral" mutations and ineffective alleles of these genes is indicative of long coevolution of flax and rust. The high polymorphism of the *R* genes at sites of "specificity" may point to a high frequency of mutations, which holds the promise of finding new genes (alleles) not described in the literature thus far.

Methods of molecular genetics allowed description of the structural features of *R* genes, but the effectiveness of genes against the pathogenic fungus and their breeding value can be determined only by genetic and phytopathological methods. That is why another aspect of the work with disease resistance in plants is the analysis of the diversity of the pathogen races. It was previously found that the standard set of differentiator lines developed by Flor (1955), ambiguously distinguished local races of the pathogen from different regions of Russia. For instance, with this set T.V. Krylova identified 5 races among 45 monopustule isolates of the fungus collected in 18 flax-cultivating regions of Russia. Addition of four extra differentiators from local varieties allowed identification of 13 more races (Krylova, 1981). In our experiments with Flor's differentiators we managed to identify 6 races among 50 clones. Addition of three monogenic lines isolated from local Russian flax to the standard set allowed identification of five more races (Kutuzova, Kulikova, 1985).

The existence of six loci for resistance to flax rust allows the inclusion of six or more resistance genes in the variety due to the complex structure of some loci and the linkage of genes *N* and *P*. This result can be achieved by step hybridization between monogenic lines with resistance genes located at different loci or by crossing of two lines having two or three resistance genes. For example, the resistance of variety Rio C. I. 280 is controlled by four genes: *L6*, *M*, *N1*, and *P* (Flor, Comstock, 1972).

With regard to the fact that in the course of breeding only one of the allelic rust resistance genes can be introduced to a variety, the mapping of the genes is necessary to avoid waste of time and money and eliminate the risk of new epiphytotic provocation.

The objectives of the current experiment included an attempt to apply classical genetic methods to map rust resistance genes found in old Russian hill kryazhes and local accessions, which are part of the oldest group of flaxes in the VIR world collection gathered in 1922–1923, before the release of breeding varieties. Lines isolated from them have genes controlling not complete but satisfactorily high resistance and can be successfully used in the creation of convergent and multilinear varieties more resistant than monogenic ones. Currently, genes *L1*, *L3*, *L4*, *L6*, *L8*, *L10*, *M2–M6*, *N*, *N1*, *P*, *P1*, *P3*, *P4*, *P6*, and *K* (identified in oilseed flax on the American continent) and gene *Q* (identified in Russia) are highly effective against rust in Russia (Kutuzova, 2014). Genes of old local Russian varieties are likely to be the primary sources of flax resistance to rust throughout the world, because Europe and America purchased the seed material from Russia for centuries.

Materials and methods

Experiments were conducted with 19 relatively rust-resistant inbred lines from the VIR flax genetic collection. The lines had been selected from heterogeneous and somewhat susceptible accessions of old Russian flaxes that included few resistant plants. They were raised and maintained at artificial isolation. Test crosses of the selected lines showed that each of them had one dominant gene of resistance with rather high efficiency: resistance against 70–100 % of virulent clones isolated from local populations of the fungus (see the Table). This was inferred from the results of infection with 50 monopustule fungus clones. Tests of the genes for their response to infection with five clones of *M. lini* showed that they all were unrelated (Kutuzova, 1981).

Identification of *R* genes was carried out by classical genetics methods including the allelism test. Evaluated lines were crossed to tester lines for each of the six known loci (genes) of resistance (*L*, *M*, *N*, *P*, *K*, and *Q*). The F_1 plants were grown in a greenhouse in winter. For segregation account, F_2 seeds of each hybrid combination, its parental lines, and the universally susceptible variety were sown in rows in boxes. The numbers of required F_2 seeds were calculated with regard to the expected number of *R* genes and possible allelism of *N* and *K* to *Q*. Plants were inoculated in the cotyledon leaf phase with a fungus clone avirulent to all tested genes except *L*, which is practically ineffective against the local Russian population of the fungus. Plants were sprayed with water from a spray gun, spores of the fungus mixed with talc were applied to each plant with a brush, and a wet chamber was arranged for a day. The results of infection were assessed after 8–10 days in case of well-developed mycelium on the susceptible standard variety. The absence of segregation from the hybrid meant allelism of genes between the analyzed and tester lines.

F_2 segregation for resistance against *Melampsora lini* in hybrids of analyzed monogenic lines with tester varieties for loci *K*, *L*, *M*, *N*, *P*, and *Q*

Line, putative locus	Percentage of non-virulent clones	Varieties differentiating rust races, genes					
		Ottawa 770B, *L*	Bombay, *N*, *Q*	Dakota, *M*	Koto, *P*	Clay, *K*, *Q*	Natasja, *Q*
gc-9 line 1-1-1 from k-467 Vologda oblast, *M* or *K*	100 ± 2	35 : 6 $\chi^2 = 2.35$ 1 gene	325 : 5 $\chi^2 = 0.00$ 3 genes	268 : 0	73 : 2 $\chi^2 = 0.59$ 3 genes	359 : 0	74 : 0
gc-25 line 4-1 from k-630 Simbirsk oblast, *X* or *L*	98 ± 2	40 : 6 $\chi^2 = 3.51$ 1 gene	302 : 7 $\chi^2 = 0.99$ 3 genes	97 : 2 $\chi^2 = 3.02$ 2 genes	185 : 2 $\chi^2 = 0.3$ 3 genes	319 : 2 $\chi^2 = 1.84$ 3 genes	103 : 6 $\chi^2 = 2.28$ 2 genes
gc-32 line 2-1 from k-716 Pskov kryazh, *P*	78 ± 5.9	45 : 7 $\chi^2 = 3.69$ 1 gene	346 : 7 $\chi^2 = 0.41$ 3 genes	77 : 7 $\chi^2 = 0.62$ 2 genes	110 : 0	236 : 6 $\chi^2 = 1.32$ 3 genes	76 : 5 $\chi^2 = 0.00$ 2 genes
gc-33 line 2-1 from k-780 Minsk oblast, *P*	84 ± 5.2	41 : 7 $\chi^2 = 2.78$ 1 gene	331 : 4 $\chi^2 = 0.30$ 3 genes	118 : 4 $\chi^2 = 1.84$ 2 genes	159 : 1 $\chi^2 = 0.91$ 3 genes linkage	340 : 7 $\chi^2 = 0.47$ 3 genes	75 : 3 $\chi^2 = 0.77$ 2 genes
gc-34 line 2-1 from k-791 Gomel oblast, *P* or *K*	98 ± 2.0	35 : 8 $\chi^2 = 0.94$ 1 gene	279 : 13 $\chi^2 = 1.61$ 2 genes	74 : 8 $\chi^2 = 1.72$ 2 genes	103 : 0	350 : 0	83 : 8 $\chi^2 = 1.00$ 2 genes
gc-38 line 2-1-1 from k-834 Vladimir oblast, *K*	96 ± 2.7	35 : 5 $\chi^2 = 3.33$ 1 gene	279 : 6 $\chi^2 = 0.55$ 3 genes	80 : 9 $\chi^2 = 2.27$ 2 genes	79 : 6 $\chi^2 = 0.09$ 2 genes	359 : 0	92 : 0
gc-39 line 2-1-1 from k-846 Ivanovo-Voznesensk oblast, *M*	94 ± 3.4	43 : 8 $\chi^2 = 2.36$ 1 gene	355 : 9 $\chi^2 = 1.96$ 3 genes	124 : 0	114 : 4 $\chi^2 = 1.65$ 2 genes	335 : 8 $\chi^2 = 1.32$ 3 genes	83 : 5 $\chi^2 = 0.05$ 2 genes
gc-40 line 1-1 from k-867 Votskiy kryazh, *P* or *K*	86 ± 4.9	35 : 6 $\chi^2 = 2.35$ 1 gene	312 : 5 $\chi^2 = 0.00$ 3 genes	86 : 3 $\chi^2 = 1.26$ $\chi^2 = 1.83$ 2 or 3 genes	206 : 0	321 : 0	86 : 0
gc-46 line 5-1-3 from k-944 Tyumen, *P* or *K*	96 ± 2.7	37 : 8 $\chi^2 = 1.25$ 1 gene	371 : 2 $\chi^2 = 1.87$ 3 genes	77 : 4 $\chi^2 = 0.24$ 2 genes	106 : 0	270 : 0	101 : 0

Results

Since variety Ottawa 770B, which tests locus *L*, is susceptible to almost all races of the Russian rust populations, the fungus clone that we used in this study revealed one dominant gene in the tested flax line in each hybrid combination. None of the identified genes is an allele of locus *L* (see the Table).

In crosses of the line selected from accession k-467, Vologda oblast (gc-9), to variety Bombay (genes *N* and *Q*), segregation of three genes was noted. This indicates the absence of allelism between the gene of the tested line and loci *N* and *Q* and the presence of gene *P*, linked to *N*. The segregation of three genes in the hybrid of the same line to variety Koto confirms the presence of gene *P*, linked to *N* gene, and another gene specific for the line itself. The absence of segregation in F_2 hybrid populations of this line with varieties Dakota (locus *M*) and Clay (loci *K*, *Q*) can be explained only by the fact that the resistance gene present in line gc-9 is located either in locus *M* or in locus *K*, or both of them are present. The absence of segregation in hybrid populations from crosses of line gc-9 to varieties Clay (loci *K*, *Q*) and Natasja (gene *Q*) suggests the probability of the location of the tested line gene in locus *Q*, but the segregation in its hybrid to Bombay (loci *N*, *Q*) refutes this suggestion.

Segregation was observed in crosses of gc-25, from k-630, originating from the Simbirsk oblast, to the testers of all known loci. With all this, hybrids to varieties Koto (locus *P*) and Bombay (*N*, *Q*) showed a linkage between genes *N* and *P*. This result can be explained by assumptions that the gene of this line is an effective allele of locus *L* or there is a previously unknown locus. According to M.M. Levitin and I.V. Fedorova (1972), flax has at least eight loci responsible for resistance to rust agent races. Experiments conducted in the Soviet Union (Krylova, 1981; Kutuzova, Kulikova, 1985) showed that the set of differentiator lines created 35 years ago could not convincingly discriminate the races of the fungus. Fiber flax is a crop that since ancient times is widespread in Russia from the western border to the Pacific coast. It was brought from Russia to Europe and America later, and it is reasonable to expect that in Russia there should be the greatest diversity of resistance genes to this pathogen, more than the known set of loci identified in linseed in other countries can house. Flax rust is known in Russia since 1885. It became widespread in the early twentieth century (Yachevsky, 1911), which boosted the search for highly efficient resistance genes.

The F_2 hybrid of gc-32 selected from k-716 (Pskov oblast) to variety Koto (locus *P*) showed no segregation. It means that

the *R* gene of this line is located in locus *P*. This suggestion was supported by the expected segregation in crosses to testers for loci *N*, *M*, *K*, and *Q*.

In the hybrid population obtained by crossing the gc-33 line selected from k-780 (Minsk oblast) to variety Koto (gene *P*), only one rust-susceptible plant was found. This fact, most likely caused by a crossing over, indicates that the gene of the tested line is linked to locus *P* (16 cM), and, correspondingly, is also linked to gene *N*. This was proven by one-gene segregation in the hybrid to variety Ottawa 770B (locus *L*). In F_2 hybrids to tester varieties for loci *NQ*, *M*, *KQ*, and *Q*, the expected segregation was noted.

Line gc-34, selected from accession k-791 (Gomel oblast), gave an expected segregation for two genes when crossed to tester varieties for loci *NQ*, *M*, and *Q*. With testers for loci *P* and *KQ*, no segregation was found. This can be explained by the fact that the *K1* gene is linked to genes *N* and *P*, as found by Hoes and Kenaschuk (1986). Perhaps the gene of this line is located in the *P* or *K* loci and is allelic to *K1*.

In crosses of line gc-38, selected from k-834 (Vladimir oblast), to the testers for loci *NQ*, *M*, and *P*, the expected segregation was obtained. There was no segregation in hybrids to *KQ* and *Q* testers. But since the hybrid with Bombay, having genes *N* and *Q*, demonstrated the absence of allelism between the resistance gene of the evaluated line and locus *Q*, we have but to suppose that the size of the hybrid population with variety Natasja was insufficient for analysis, and the gene of gc-38 was located in locus *K* and was an allele other than that in gc-34.

The segregation of hybrids between line gc-39, selected from k-846 (Ivanovo-Voznesensk oblast), and testers for all resistance loci, except for variety Dakota, indicated that the gene of this line is located in the *M* locus.

As shown by previous analysis, the gene of line gc-40, selected from k-867 (Votskiy kryazh), is not located in loci *N* or *M*. There was no segregation in F_2 hybrids between this line and testers for loci *P*, *KQ* and *Q*. As already shown, the resistance gene of this line cannot be located in locus *Q*. The possibility of the location of *N* and *P* on the same chromosome as locus *K* (Hoes, Kenaschuk, 1986), may explain the absence of segregation in crosses to varieties Koto and Clay. It suggests that the resistance gene of gc-40 is located either in locus *K* or *P*. The rust resistance gene of this line was repeatedly used by breeders to create rust-resistant varieties. As our research shows, varieties Belorusskiy 1 (k-6601), Uspekh (k-6818), and some other modern varieties are protected by an identical gene (Kutuzova, 2012). A significant part of rust resistance donors created at VNIIL and available for breeding are also protected by an identical gene, inherited from variety Uspekh (Rozhmina, 1988), and this fact should be taken into consideration when using them.

The segregation in the cross of line gc-46, selected from k-944 (Tyumen), is very similar to the results of the previous line. Probably, the gene of k-944 belongs to another allele of the same locus: *K* or *P*.

Thus, classical genetic methods are insufficient for unambiguous mapping of rust resistance genes in all old Russian flaxes. The work is also hindered by the linkage between loci *N* and *P*.

Conclusion

In this work, we discovered that most of the resistance genes in evaluated lines are located in loci *P* and/or *K*. It was exactly determined that the *R* gene of line gc-32, selected from k-716 (Pskov oblast), is located in locus *P*, linked to *N*. The gene for rust resistance in line gc-33, selected from k-780 (Minsk oblast), also belongs to locus *P* (linkage was confirmed in our experiment), and the resistance gene in line gc-39 from k-846 (Ivanovo-Voznesensk oblast), being effective against 94 % of the fungus races, belongs to locus *M*. The resistance gene of line gc-38 from k-834 (Vladimir oblast), is probably located in locus *K*. The positions of other genes could not be clearly identified. Perhaps, the use of molecular methods would clarify their identity.

The lines with genes *P* and *K* should be used in breeding with caution, because it is unknown which of these genes is already quite widespread in the varieties bred in Russia. However, with regard to the linkage of genes *N* and *P*, as well as the association of gene *Q* with loci *N* and *K*, it is difficult to predict which genes (or gene) may be inherited by the hybrid.

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Molecular-genetic marking of *Brassica* L. species for resistance against various pathogens: achievements and prospects

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Cruciferous plants belonging to the genus *Brassica* of the Cabbage family (Brassicaceae) are cultivated as vegetables, oilseeds and forage crops; they occupy one of the first places in Russia in the gross yield of vegetables. The yield of cabbage crops is adversely affected by various pathogens, including bacterial, viral and fungal infections. The diseases such as black rot of cabbage (caused by the bacterium *Xanthomonas campestris* pv. *campestris*), downy mildew (caused by *Hyaloperonospora parasitica*), Turnip Mosaic Virus (TuMV) are not included in the list of quarantine diseases in the territory of the Russian Federation and Eurasian Economic Union (EAEU), but they can affect a part of the sown area and lead to significant (up to 100 %) crop losses. The development of cultivars resistant to these pathogens is an important trend in *Brassica* crop breeding in addition to existing methods of agrotechnical and chemical protection. The development of molecular marker techniques and marker-assisted selection (MAS) methods makes it possible to significantly increase the efficiency of breeding resistant cabbage cultivars. The review contains information on the currently known genes and quantitative trait loci (QTLs) associated with resistance to black rot, downy mildew, and TuMV. Molecular mapping data for resistance genes of *Brassica* species are shown. The molecular markers (RFLP, AFLP, SSR, EST, SNP, InDel, SLAF and others) closely linked to the resistance loci and SCAR-, STS- and dCAPS-markers derived from them for molecular screening are listed. The use of the markers reviewed to assess the *Brassica* accessions and lines can help the researchers in finding sources and donors of pathogen resistance of cabbage crops.
Key words: *Brassica*; resistance; *Xanthomonas campestris*; *Hyaloperonospora parasitica*; TuMV; MAS; QTL.

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Достижения и перспективы молекулярно-генетического маркирования устойчивости к некоторым патогенам у видов рода *Brassica* L.

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Крестоцветные растения, относящиеся к роду *Brassica* семейства Капустные (Brassicaceae), возделываются как овощные, масличные и кормовые культуры. В Российской Федерации они занимают одно из первых мест по валовому сбору овощей. На урожайность капустных культур негативно влияют различные патогены, в том числе бактериальные, вирусные и грибные инфекции. Такие заболевания, как сосудистый бактериоз (возбудитель *Xanthomonas campestris* pv. *campestris*), ложная мучнистая роса, или пероноспороз (*Hyaloperonospora parasitica*), вирус мозаики турнепса (Turnip Mosaic Virus – TuMV), хотя и не входят в список карантинных болезней на территории Российской Федерации и Евразийского экономического союза (ЕАЭС), но могут поражать часть посевных площадей и приводить к значительным (вплоть до 100 %) потерям товарной продукции. Создание устойчивых к этим патогенам сортов является важным направлением в селекции культур *Brassica*, дополняющим существующие методы агротехнической и химической защиты. Развитие методов молекулярного маркирования и маркер-вспомогательной селекции (MAS) позволяет намного повысить эффективность отбора устойчивых генотипов. В обзоре рассмотрены актуальные сведения об известных генах и локусах количественных признаков (QTL), ассоциированных с устойчивостью к сосудистому бактериозу, пероноспорозу капусты и вирусу TuMV. Приведены данные о локализации генов устойчивости на молекулярных картах геномов видов рода *Brassica* (*B. rapa*, *B. oleracea*, *B. napus*, *B.carinata*), разработанных с использованием разных типов молекулярных маркеров (RFLP, AFLP, SSR, EST, SNP, InDel, SLAF и др.). Систематизирована информация

о молекулярных маркерах, тесно сцепленных с локусами устойчивости, часть из которых конвертирована в SCAR-, STS- и dCAPS-маркеры для молекулярного скрининга, пригодные для непосредственного применения в практической селекции. Использование приведенных данных для оценки образцов культур рода *Brassica* может помочь исследователям в поиске источников и доноров генетической устойчивости к рассматриваемым заболеваниям выращиваемых капустных культур.
Ключевые слова: *Brassica*; resistance; *Xanthomonas campestris*; *Hyaloperonospora parasitica*; TuMV; MAS; QTL.

Introduction

Meeting the people's ever growing demand for food, feed and industrial plant products in the world and in the Russian Federation requires a considerable increase of crop yield and expansion of crop areas. One of the main ways to increase yields is searching for methods that could minimize losses from diseases caused by various pathogens. Biological and chemical plant protection techniques (fungicides and bactericides) are costly, often show unstable results in disease control, and in time induce tolerance in their objects. Besides, chemical means of disease control are extremely detrimental to the environment. The most effective and economically justified tool to resist causative organisms is the use of genetically resistant cultivars or genotypes with such morphological and physiological features that preclude infection.

Plants possess efficient mechanisms that enable them to avoid infections or produce protective responses, thus making them resistant to a pathogen's attack. Barriers against infection may be set up by anatomic and morphological features (plant habit, leaf pubescence, waxy bloom, arrangement and structure of stomata, or structure of internal tissues), biochemical factors (for example, phytoncides, phenolic compounds, or glucosinolates), and some specific proteins (PR proteins, plant defensins, thionines, etc.) (Dyakova, 2017). The most effective protection, however, is provided by genetic resistance to pathogens. According to H.H. Flor's popular 'gene-for-gene' hypothesis (Flor, 1971), resistance occurs when a resistance (*R*) gene in the plant recognizes a corresponding specific avirulence (*Avr*) gene in the pathogen (McDowell, Simon, 2006).

There are 37 species within the genus *Brassica* L. Six of them are cultivated species of vital economic significance; they are combined in the classical 'triangle of U' (Nagaharu, 1935), which includes 3 diploid species: *B. rapa* L. (AA, $n = 10$), *B. nigra* L. (BB, $n = 8$), and *B. oleracea* L. (CC, $n = 9$) as well as 3 amphidiploid ones: *B. juncea* Czern. (AABB, $n = 18$), *B. carinata* A. Braun (BBCC, $n = 17$), and *B. napus* L. (AACC, $n = 19$). Diverse forms of these species are cultivated as vegetable, forage, oil and ornamental crops. It has been observed that "in the course of their evolution, diploid *Brassica* spp. developed independently, therefore their genomes underwent various qualitative and quantitative changes, leading to accumulation and combination of preferable alleles in their genes, which ensured their survival in the process of natural selection and shaped their economically useful traits in the process of artificial selection" (Fadina, 2014, p. 24). Broad genetic diversity within and between different crops of the genus *Brassica* and species of other genera in the family Brassicaceae secures a rich resource of resistance genes against major pathogens (Walsh, Jenner, 2002; Neik et al., 2017).

The main diseases that inflict mass damage upon the *Brassica* crops in Russia are: (1) bacterial diseases, such as black rot or vascular bacteriosis (caused by *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *raphani*, and *X. arboricola*) and slimy bacteriosis (caused by *Erwinia carotovora* subsp. *carotovora* (Jones) Berqey); (2) fungal diseases, such as clubroot disease of crucifers (caused by *Plasmodiophora brassicae* Woron.), dark spot (caused by *Alternaria brassicae*, *A. brassicicola* and other *Alternaria* spp.), downy mildew (caused by *Hyaloperonospora parasitica*), powdery mildew (caused by *Erysiphe cruciferarum*), and blackleg disease (caused by *Leptosphaeria maculans* and *L. biglobosa*); and (3) viral infections, such as us turnip mosaic virus (TuMV), cauliflower mosaic virus (CaMV), and turnip yellows virus (TuYV). It should be mentioned that none of the above-mentioned diseases is included in the Common List of Plant Quarantine Objects of the Eurasian Economic Union of February 2018, but they are capable of damaging up to 100 % of crop areas under crucifers.

In this review, we are discussing three important pathogens of crucifers, causing a destructive effect on *Brassica* crops cultivated in Russia: vascular bacteriosis or black rot, downy mildew, and turnip mosaic virus. We have chosen the most harmful diseases among bacterial, fungal and viral infections, against which biochemical control methods do not bring about stable positive results, but there are molecular marker systems developed for relevant resistance genes.

Black rot (vascular bacteriosis)

Black rot is a disease of cruciferous crops caused most frequently by the gram-negative bacterium *Xanthomonas campestris* pv. *campestris* (Pam). Dowson (hereinafter referred to as *Xcc*). Cabbage may also be affected by other *Xanthomonas* species and pathovars (Ignatov, 2014), differing mostly in the symptoms of infection and the set of susceptible host plants. This disease is one of the most harmful for crucifers in the world. All cultivated *Brassica* crops are subject to black rot: turnip, radish, rapeseed, swede, and numerous cruciferous weeds. Within the species *B. rapa*, the disease is most dangerous for both root turnips and leafy forms, including Chinese cabbage (*B. rapa* ssp. *pekinensis*) (Artemyeva et al., 2018).

The pathogen is disseminated by seeds and in recent years have been inducing epiphytotics in all areas where cruciferous crops are grown, inflicting losses on the harvested plant produce from 10 to 100 % (Ignatov, 2014). A number of chemicals have been recommended to protect crops from black rot (Lazarev et al., 2017), but they are not always as effective as expected.

Table 1. Characterization of molecular gene markers and QTL associated with resistance to black rot in *Brassica* spp.

QTL/ resistance gene	Chromosome/linkage group	Marker	Marker type	Reference
		B. oleracea L.		
Xcc Resistant gene	–	C-111000	RAPD	Kaur et al., 2009
QTL-1	C02	BoCL5989 s	dot-blot-SNP	Kifuji et al., 2013
		BoCL5545 s		
XccBo (*Reiho*)*1*	C05	BoGMS1330	SSR	Tonu et al., 2013
	C02	BoCL6200s	SNP	
	C02	BoCL5584		
	C05	BoCL5860		
	C05	BoCL1135		
XccBo (*Reiho*)*2*	C08	BoGMS0971	SSR	
	C08	OL12D05		
XccBo (*GC*)*1*	C09	CB10459	SSR	
	C05	pW114	CAPS	
	C05	pW164		
	C09	pX117		
	C09	pW143		
	C03	pW188		
Xca1bo	III	RAPD04	RAPD	Saha et al., 2016
		ISSR11	ISSR	
BRQTL-C1_1	C01	BnGMS301	dCAPS	Lee et al., 2015
BRQTL-C1_2	C01	BoESSR089	SSR	
BRQTL-C3	C03	B041F06-2	SSR	
RQTL-C6	C06	OI10-G06	SSR	
–	C03	BoESSR291	SSR	Afrin et al., 2018a
–	C01	BoESSR726	SSR	
–	C01	BnGMS301	SSR	
–	C08	BoGMS0971	SSR	
–	C06	OI10G06	SSR	
		B. rapa L.		
R4	–	WE22	RAPD	Ignatov et al., 2000
		WE49		
XccR1d-1, *XccR1i-1*	A06	E11M50_280b	AFLP	Soengas et al., 2007
		E12M48_171r		
XccR4d-1, *XccR4i-1*		E12M61_215b		
		E12M61_215b		
XccR4i-2	A02	E11M59_178r	AFLP	
XccR4i-3	A09	E12M48_1330b	AFLP	
QTL	A03	BRMS-043	SSR	Artemyeva et al., 2016, 2018
	A03	BRMS-050		
	A09	BRMS-051		
	A06	SSR-089		
	A03	Na12E02		
		B. carinata L.		
Xca1bc	B07	At1g70610	ILP	Sharma et al., 2016
		At1g71865		
		Na14-G02	SSR	

Initially five races of the pathogen were identified; afterwards their number grew to eleven (Cruz et al., 2017). *Xcc* races 1 and 4 are considered the most harmful among them. In Russia, before 2012, the most widespread were races 1, 3 and 4, but later, for white cabbage and rapeseed, races 5 and 6 became quite threatening (Lazarev et al., 2017). Resistance to different *Xcc* races is supposed to be controlled by different *R*-genes and QTL. Sources of resistance to major harmful *Xcc* races (1 and 4) are mainly associated with the A and B genomes (*B. rapa* and *B. nigra*) and rarely occur in the C genome (*B. oleracea*) (Taylor et al., 2002; Vicente, Holub, 2013). At the same time, forms resistant to less pathogenic races 2, 3 and 6 are frequent enough (Soengas et al., 2007).

***B. oleracea* (C genome).** There are few sources of genetic resistance to the most pathogenic *Xcc* races (1 and 4) in *B. oleracea*. Nevertheless, research efforts to identify and map *R*-genes and QTL for *B. oleracea* are quite intensive. Molecular maps of this crop's genome have been developed independently by several groups of researchers (Kaur et al., 2009; Kifuji et al., 2013; Tonu et al., 2013; Lee et al., 2015; Saha et al., 2016; Iglesias-Bernabe et al., 2019) using different types of markers (RAPD, SSR, ISSR, dCAPS or SNP). Genes and QTL of resistance to most pathogenic races 1 and 4 were found on different chromosomes/linkage groups (Table 1). For example, Lee et al. (2015) mapped altogether 14 QTL associated with resistance to *Xcc* on eight chromosomes out of nine in *B. oleracea*, and four of them represented the main loci affecting resistance in plants. Thus, the control of this trait in *B. oleracea* is polygenic by nature. For a number of resistance genes and QTL, molecular markers, securely linked with them and associated with resistance, were identified (see Table 1). Afrin et al. (2018a) tested some of those markers (9 SSR and 1 InDel) on 27 inbred cabbage lines resistant to different races of the pathogen. Comparing the results of molecular screening and phytopathology tests helped to select five markers capable of distinguishing resistant forms from susceptible ones. It is noteworthy that those markers were also distributed among different chromosomes: BnGMS301 and BoESSR726 were localized on C01, BoESSR291 on C03, OI10G06 on C06, and BoGMS0971 on C08 (Afrin et al., 2018a).

Xcc resistance genes have also been identified through a search in the genome of *B. oleracea* for sequences containing characteristic domains (LRR, NBS, TIR, etc.). For example, Afrin et al. (2018b) sought NBS-containing sequences in the Gene Expression Omnibus (GEO) database and studied their expression in various plant tissues (leaves, roots, xylems and stems) of *Xcc*-resistant and *Xcc*-susceptible lines. As a result, they selected 7 loci whose expression was associated with resistance for the line SCNU-C-4118, and two more loci for the line SCNU-C-3273. A comparison between the sequences of those loci in resistant and susceptible forms disclosed several InDels and SNP variants which may be used in the development of markers for molecular screening (Afrin et al., 2018b).

***B. rapa* (A genome).** Studying resistance to race 4 in the *B. rapa* line G011 with RAPD analysis resulted in identifying the marker $WE22_{980}$ associated with resistance to *Xcc*. The marker was present in 100 % of resistant dihaploid lines and genotypes of F_2, but was also found in 18 % of susceptible dihaploids. The gene of resistance to *Xcc* (race 4) in *B. rapa* was observed at a distance of approximately 3 cM from the QTL responsible for clubroot resistance (Ignatov et al., 2000).

Soengas et al. (2007) employed AFLP and SSR analyses to study a splitting population of F_2 hybrids from crossing the *Xcc*-resistant line B162 with the susceptible Ro-18. As a result, they developed a molecular map of the *B. rapa* genome, which included ten linkage groups with the total coverage of 664 cM. A cluster containing two main QTL associated with resistance to races 1 and 4 was found on chromosome A06, while linkage groups A02 and A09 carried additional QTL controlling resistance to race 4.

Microsatellite analysis of two mapping populations of the doubled haploid *B. rapa* lines (DH30 and DH38) made it possible to make a map full of SSR markers (Artemyeva et al., 2016) and isolate several markers associated with *Xcc* resistance (see Table 1). Later, the loci linked with resistance to different races in the line DH30 were mapped to linkage groups A01, A03 and A07, and in the line DH38 to groups A03, A06 and A08 (Artemyeva et al., 2018).

***B. carinata* (BC genome).** Using mapping populations of F_2 hybrids obtained from the resistant line NPC-9 and the susceptible line NPC-17, a bulked segregant analysis was carried out with 41 polymorphic markers (ILP and SSR) (Sharma et al., 2016). Only three of them (ILP-At1g70610, ILP-At1g71865 and SSR-Na14-G02) were able to generate polymorphic fragments between resistant and susceptible lines. The resistant locus *Xca1bc* was mapped at a distance of 30.1 cM from the microsatellite marker Na14-G02, which had earlier been found on chromosome B07. On this basis, the authors concluded that the *Xca1bc* locus was also situated on chromosome B07.

Thus, plenty of black rot resistance gene markers have been developed on the *B. oleracea* crops, which provides a possibility to screen collections in search of new donors. For the species *B. rapa* and *B. carinata*, the information on mapped genes and resistance QTL is still limited by now. Further research efforts are needed in this field.

Downy mildew

Downy mildew is a destructive disease caused by the oomycete *Peronospora brassicae* Gaum. from the family Peronosporaceae. Initially, on the basis of morphological descriptions and cross-inoculation tests, 52 *Peronospora* spp. were identified in crucifers; however, following the results of more recent studies, all downy mildew causative agents in *Brassica* crops were grouped into the single aggregate species *Peronospora parasitica* (Pers. ex Fr.) Fr. The modern classification implies that the name *P. parasitica* should be reclassified and enter the genus *Hyaloperonospora*. The taxonomically correct name for this causative agent in *Brassica* spp. is *Hyaloperonospora brassicae* (Goker et al., 2009). Under favorable conditions, *H. brassicae* can infect up to 50–60 % of cabbage seeds and reduce the harvest by 16–20 % (Saharan et al., 2017).

By now, different research teams have identified 5 genes and 4 QTL responsible for the resistance of cruciferous crops to *H. brassicae*, which will be discussed below.

***B. oleracea* (C genome).** Downy mildew resistance in cauliflower was shown to be controlled by a dominant allele of the gene called *Ppa3* (Mahajan et al., 1995). The *Ppa3* gene was later mapped using a splitting population of backcrosses

Table 2. Characterization of molecular gene markers and QTL associated with resistance to downy mildew in *Brassica* spp.

QTL/ resistance gene	Chromosome/linkage group	Marker	Marker type	Reference
		B. oleracea L.		
QTL	–	UBC359620	SCAR	Giovannelli et al., 2002
		OPM16750		
Pp523	C08	OPK17980	RAPD	Farinhó et al., 2004
		OPR15920		
		OPJ19550		
		AT.CTA_133/134		
Pp523	C08	SCJ19443	SCAR	Farinhó et al., 2007
		SCR15920		
		SCAFB1216		
Pp523	C08	CB10139	SSR	Carlier et al., 2012
		CB10028		
Ppa3	–	OPC141189	RAPD	Singh et al., 2012
		OPE141881		
		ISSR-231103	ISSR	
		B. rapa L.		
BraDM	A08	K14-1030	RAPD	Yu et al., 2009
		OI12-G04	SSR	
BraDM	A08	SCK14-825	SCAR	Yu et al., 2011
		kbrb006c05-2	SSR	
		kbrb058m10-1		
BrDW	A08	Brb062-Indel230	Indel	Li et al., 2011
		Brb094-DraI787	CAPS	
		Brb094-AatII666		
		Brb043-Bg1II715		
		Brh019-SNP137	SNP	
BrRPHP1	A01	OPA08650	RAPD	Kim et al., 2011
		BrPEK15B	SCAR	
sBrDM	A08	A08- 028	SNP	Yu et al., 2016
		A08-018		
QTL	A01	A0124655323	SLAF	Zhi et al., 2016

from the resistant line BR-2. The gene was localized between the flanking RAPD markers $OPC14_{1189}$ and $OPE14_{1881}$, not far (26.4 cM) from the marker $ISSR\text{-}23_{1103}$ (Singh et al., 2012).

Another team of researchers, while studying a splitting population of doubled haploids and F_2 hybrids obtained from the line USVL089 of *B. oleracea* (Italica Group), identified one more dominant locus of resistance to downy mildew (Farnham et al., 2002). RAPD analysis of the same population revealed two markers ($UBC359_{620}$ and $OPM16_{750}$) linked with the resistance locus (Giovannelli et al., 2002). To ensure more stable results, RAPD sequences were converted into SCAR markers (Table 2).

One more research team identified the dominantly inherited *Pp523* gene in the resistant broccoli accession OL87125 (Farinhó et al., 2007). The gene was mapped in a population of F_2 hybrids using AFLP, RAPD, ISSR and SSR markers with the LG.3 linkage group. Bulked analysis (19 resistant and 17 susceptible genotypes per each accession) was em-

ployed to isolate the markers $OPK17_{980}$, $OPJ19_{550}$, $OPR15_{920}$ and AT.CTA_133/134 flanking the *Pp523* gene. The markers $OPJ.19_{550}$ and $OPR.15_{920}$ were sequenced and converted into SCAR and CAPS markers (see Table 2).

On the basis of the $OPJ19_{550}$ and $OPR15_{920}$ marker sequences linked with *Pp523*, probes (BoT01 and BoCig) were developed for screening genomic libraries of *B. oleracea*, with isolation of sites containing sequences complementary to the probes. DNA fragments in the isolated BAC clones were shown to be located in three different parts of the genome of *B. oleracea*: 83 clones were mapped to chromosome C08 near *Pp523*, 33 clones also to chromosome C08 at a distance of 60 cM from the resistance gene, and 63 more to chromosome C05 (Carlier et al., 2011). The presence of such triplication supports the hypothesis concerning the existence of *Brassica's* hexaploid ancestor some 14–24 million years ago and the formation of the *B. oleracea* genome by rearrangements and translocations (Carlier et al., 2011).

The authors later supplemented the obtained map for *B. oleracea* by adding 44 SSR markers with a known localization on chromosomes of the C genome. It enabled them to correlate the earlier identified nine linkage groups with nine chromosomes in the *B. oleracea* genome. As a result, the *Pp523* gene was localized in chromosome C08 and correlated with two SSR markers – CB10139 and CB10028 (Carlier et al., 2012).

***B. rapa* (A genome).** Yu et al. (2009) isolated and mapped the main locus (QTL) of downy mildew resistance in Chinese cabbage (*B. rapa* ssp. *pekinensis*) plants in the sprouting stage, and it was named *BraDM*. Using the molecular map developed by Zhang et al. (2008) and supplemented in their work with new SSR, SCAR, STS, SRAP and isoenzyme markers, the authors localized the QTL *BraDM* on chromosome A08 of the *B. rapa* genome. *BraDM* was shown to be located within a site of 2.9 cM, which was flanked by isoenzyme and RAPD markers, PGM (phosphoglucomutase) and K14-1030, respectively. Besides, the SSR marker Ol12G04 was found to be linked to *BraDM* at a distance of 4.36 cM (Yu et al., 2009).

K14-1030 was later sequenced and remapped as a SCAR marker – SCK14-825 (Yu et al., 2011). In addition, using K14-1030 as a probe, Li et al. (2011) isolated from the library the BAC clone KBrB058M10, which was in association with the resistance QTL. On the basis of the obtained information, markers for MAS were developed: the InDel marker $Brb062\text{-}InDel_{230}$, CAPS markers $Brb094\text{-}DraI_{787}$, $Brb094\text{-}AatII_{666}$ and $Brb043\text{-}BglII_{715}$, and SNP marker $Brh019\text{-}SNP_{137}$. All markers, except $Brh019\text{-}SNP_{137}$, had sufficiently high correlation with resistance (69.7–74.2 %) (Li et al., 2011).

Besides, the additional SSR markers kbrb058m10-1 and kbrb006c05-2 flanking the target resistance gene were developed. Interval mapping showed that SCK14-825, kbrb058m10-1 and kbrb006c05-2 had high LOD values (23.2, 19.5 and 15.5) (Yu et al., 2011).

The same population as for mapping the main QTL *BraDM* was used to isolate six additional QTL affecting downy mildew resistance: 4 major (sBrDM8, yBrDM8, rBrDM8 and hBrDM8) and 2 minor (rBrDM6 и hBrDM4) loci (Yu et al., 2016). The locus sBrDM8 responsible for seedling resistance was mapped to chromosome A08 and appeared identical to *BraDM*. The loci yBrDM8, rBrDM8 and hBrDM8 controlled resistance at the young plant, rosette and heading stages; they were also mapped to chromosome A08 in the *BraDM* region. The authors arrived at the conclusion that all those loci could aggregately represent a new dominant gene, *BraDM8*.

A dominant downy mildew resistance gene, *BrRHP1*, was also identified by Kim et al. (2011) in Chinese cabbage (*B. rapa* ssp. *pekinensis*). Using bulked analysis of mapping populations, this gene was localized in the A01 linkage group close to the RAPD marker $OPA08_{650}$. After sequencing a 650 bp DNA fragment generated by the primer OPA08, the SCAR markers BrPERK15A and BrPERK15B were developed. Of these, BrPERK15B revealed polymorphism between resistant and susceptible parental lines. Besides, six SSR markers were developed for identification of the *BrRHP1* gene (Kim et al., 2011).

One more locus of downy mildew resistance was found on chromosome A01 using GWAS analysis, when 960 polymorphic SLAF markers were employed to study 202 inbred lines. The new locus, named SLAFMarker A0124655323, was reliably linked with resistance. Comparing sequences of the locus SLAFMarker A0124655323 in resistant and susceptible lines helped to isolate SNP variants and develop KASP markers for their identification. Their linkage with resistance was higher than 80 % (Zhi et al., 2016). Thus, the main genes controlling resistance to downy mildew in both *B. rapa* and *B. oleracea* concentrated in linkage group 8, and additionally, for *B. rapa* alone, in group 1. For all identified genes, effective molecular markers (SCAR and CAPS) were developed for screening. For *B. carinata,* as far as we know, no downy mildew resistance genes were identified or mapped.

Turnip mosaic virus (TuMV)

TuMV was for the first time described in 1921 in the United States on *B. rapa* plants, and later, in 1935, in the UK on *B. oleracea* (Walsh, Jenner, 2002). Presently, TuMV incidence is registered in all regions of the world. This virus can afflict all crucifers; it is dispersed by polyphagous aphids, by seeds, and via infected plant material. The TuMV incidence in the open field is rated second after cucumber mosaic virus (Gibbs et al., 2015).

TuMV is one of over 100 species in the genus *Potyvirus*; it emerged from a lineage of monocotyledon-infecting potyviruses about 1000 years ago. Potyvirus virions contain a single copy of the genome, a single-stranded RNA molecule of about 10,000 nucleotides (Gibbs et al., 2015). A rich diversity of pathotypes has been found within the virus, and the most widespread among them are pathotypes 1, 3 and 4 (Jenner et al., 2002).

Hypersensitive resistance to viruses in *Brassica* crops is mainly characterized by monogenic dominant inheritance (Fraser, 1992). Extreme and other types of resistance are controlled by both dominant and recessive genes, the share of the latter being unusually large (up to 40 %) (Walsh, Jenner, 2002). The resistance controlled through a combined effect of recessive and dominant genes was described for Chinese cabbage (Rusholme et al., 2007). Below is the information on the identified (described) loci and genes of resistance and on the markers associated with them for different *Brassica* spp.

***B. oleracea* (C genome).** Screening of *B. oleracea* cultivated types failed to identify any sources of resistance (Walsh, Jenner, 2002).

***B. napus* (AC genome).** The dominant *TuRB01* gene was the first to be mapped in *Brassica*; it conveys extreme resistance to some isolates of TuMV pathotype 1 (Walsh et al., 1999). The work was done on a mapping population of dihaploid lines (DH) employing RFLP markers. The *TuRB01* gene was localized on chromosome N6 of the A genome, near the pO120b cluster. The location of *TuRB01* in linkage group 6 within the A genome of *B. napus* indicates that *B. rapa* may possibly be the source of this gene. The second locus, *TuRB02*, which seems likely to quantitatively control the level of susceptibility to the CHN1 isolate, was identified in linkage group N14 of the C genome (Walsh et al., 1999).

Later the same chromosome N6 of the A genome was used to map with AFLP and SSR markers the *TuRB03* gene whose dominant allele ensures resistance to the CDN1 isolate (pathotype 4) and some isolates of pathotype 3 (Hughes et al., 2003). One AFLP and two SSR markers tightly linked with the *TuRB03* gene were offered for its screening (Table 3).

Other single dominant genes, *TuRB04* and *TuRB05*, were found in the differential rapeseed line 165 (Jenner et al., 2002). The *TuRB04* gene controls extreme resistance to some TuMV isolates, while *TuRB05* is responsible for a hypersensitive or necrotic response (HR), which restrains systemic dissemination of the virus. Interaction between *TuRB04* and *TuRB05* ensures extreme resistance (possibly, immunity) of *B. napus* plants to the TuMV isolates of pathotypes 1 and 3.

***B. rapa* (A genome).** Analyzing a splitting population of B1S1 backcrosses – descendants of the resistant RLR22 line – with the use of RFLP and SSR markers resulted in identification and mapping of two genes jointly controlling resistance to pathotypes 4 (CDN1 isolate) and 3 (CZE1 isolate). The *retr01* gene, with its recessive expression, is located on chromosome A04 and linked with the pN202e1 marker. The second gene, dominant *ConTR01*, was mapped to chromosome A08 between the markers pO85e1 and pO85e2. The *retr01* gene is the first registered example of a recessive resistance gene mapped in *Brassica* plants (Rusholme et al., 2007).

Chinese researchers (Zhang et al., 2008) identified two more QTL, *Tu1* and *Tu2*, associated with TuMV resistance at the seedling stage. The *Tu1* locus was mapped between the RAPD marker A04-850 and AFLP marker CA_TG270 in linkage group LG5, and *Tu2* in group LG10. Two additional QTL, linked with TuMV resistance in adult plants under field conditions, were localized in groups LG3 and LG4, respectively. Flanking markers for these loci are presented in Table 3.

For bok choi (*B. rapa* ssp. *chinensis*), TuMV resistance genes were mapped by Xinhua et al. (2009, 2011). After 180 genotypes of the F_2 population from the resistant line Q048 had been evaluated, the bulk analysis of resistant and susceptible backcrosses (10 genotypes in each bulked sample) was performed using AFLP markers (36 polymorphic pairs of primers earlier selected from 240). Monogenic control of resistance was shown, and the dominant *TuRBCH01* gene was mapped to linkage group R6 between the markers EaccMctt3 (7.8 cM) and EatcMcac1 (20.3 cM) (Xinhua et al., 2009). Afterwards, the map was saturated with AFLP and SSR markers, with a total coverage of 1123 cM and an average interval between markers of 5.43 cM. The *TuRBCH01* gene was associated with linkage group R06 flanked by the AFLP markers E36M62-3 and E44M48-1 (Xinhua et al., 2011).

One more recessive resistance gene, *retr02*, was isolated by Qian et al. (2013) in the F_2 population obtained from the TuMV (pathotype C4) resistant line BP8407. At the initial stage, the authors conducted a bulked segregant analysis using SSR markers. Parent forms, F_1 hybrids, and two bulked samples were examined, involving 10 resistant and 10 susceptible F_2 genotypes in each sample. The SSR marker BC84, localized in scaffold000048, was associated with resistance to TuMV-C4. To make gene localization more precise, 145 InDel markers were developed and tested. As a result, 4 polymorphic ones were selected and put to use in the individual screening of 239 F_2 representatives. The *retr02* gene was mapped between the markers BrID10694 (scaffold000060) and BrID101309 (scaffold000104) on chromosome A04. In scaffold000104, the authors identified by BLAST analysis the *Bra035393* sequence, homologous to the sequence AT5G35620 in *Arabidopsis* (recessive resistance gene *lsp*). After the *Bra035393* locus had been sequenced in 52 resistance and 13 susceptive genotypes, the authors identified SNP variants associated with resistance (in exon 3, homozygous resistance forms all had A at position 455 bp, susceptible homozygotes had G at this position, and heterozygotes had A and G) (Qian et al., 2013).

Later, a single-nucleotide insertion (G) was detected at the exon/intron boundary in resistant forms; it was found to be responsible for missplicing and non-functional protein formation (Li et al., 2016). To identify this insertion, dCAPS and KASP markers were developed, which makes the use of MAS methods applicable in the selection of resistant forms, homozygous in the recessive allele of the *retr02* gene.

A dominant resistance gene, *TuRB01b*, that confers immunity to the virus isolate UK 1 (pathotype 1 of TuMV) was identified in the Chinese cabbage cultivar Tropical Delight. The *TuRB01b* locus was mapped using RFLP techniques on chromosome A06 that was flanked by RFLP markers pN101e1 and pW137e1 (Lydiate et al., 2014). Comparative mapping confirmed that chromosome A06 of *B. rapa* was equivalent to chromosome 6 of the *B. napus* genome and that the map position of *TuRB01b* is similar to that of *TuRB01* or *TuRB03* in *B. napus* (Walsh et al., 1999; Lydiate et al., 2014). Hence, an assumption was made that *TuRB01*, *TuRB01b* and *TuRB03* represent the same cluster of resistance genes and may even be allelic (Lydiate et al., 2014).

On the shorter arm of the same chromosome (A06), in the region flanked by SSR markers H132A24-s1 and KS10960, one more gene of resistance to TuMV (isolate C4), *TuRB07*, was localized while studying the resistant *B. rapa* line VC1 (Jin et al., 2014). Comparing it with the reference genome of cv. 'Chiifu' revealed that *TuRB07* was associated with the *Bra018863* gene of the CC-NBS-LRR class.

Thus, TuMV resistance in *B. napus* was under monogenic control mainly by isolate- or pathotype-specific dominant genes (Walsh et al., 1999; Jenner et al., 2002; Hughes et al., 2003). Contrariwise, *B. rapa* demonstrated multigenic control of resistance (additionally to the monogenic one) with broad-spectrum effect, and genes in that case often had recessive inheritance (Rusholme et al., 2007; Qian et al., 2013). Prevailing monogenic resistance in *B. napus* may be explained by possible emergence of this species from one of infrequent interspecific crosses between *B. rapa* and *B. oleracea*. Since

Table 3. Characterization of markers associated with resistance to turnip mosaic virus in *Brassica* spp.

QTL/ resistance gene	Chromosome/linkage group	Marker	Marker type	Reference
		B. napus L.		
TuRB01	N6	pO120b	RFLP	Walsh et al., 1999
TuRB02	N14	pW133a	RFLP	
	N14	pR113bNM		
TuRB03	N6	sNRB93	SSR	Hughes et al., 2003
	N6	sS1949		
	N6	EtcMcac1	AFLP	
retr01	R4	pN202e1	RFLP	Rusholme et al., 2007
ConTR01	R8	pO82e2	RFLP	Rusholme et al., 2007
		pO85e1		
		B. rapa L.		
Tu1	LG5	A04-850	RAPD	Zhang et al., 2008
		CA_TG270	AFLP	
		E31M48470		
		STS3-e32m50-447-320	STS	
		STS1-e31m48-437		
TuRBCH01	R6	E36M62-3	AFLP	Xinxua et al., 2011
	R6	E44M48-1		
	R6	EatcMcac1		
	R6	EaccMctt3		
retr02	A04	BrID10694	InDel	Qian et al., 2013
		BrID101309		
		BC84	SSR	
TuRB01b	A06	pN101e1	RFLP	Lydiate et al., 2014
		pW137e1		
TuRBCS01	A04	BrID10723	InDel	Li et al., 2014
		SAAS_mBr4055_194	SSR	
TuRB07	A06	H132A24-s1	SSR	Jin et al., 2014
	A06	KS10960		
retr02	A04	CAPS-BslI	CAPS	Li et al., 2016
		KASP_retr02	KASP	
		CA_TG270	AFLP	
		E31M48470		
		STS3-e32m50-447-320	STS	
		STS1-e31m48-437		

B. oleracea failed to manifest extreme forms of resistance, *B. napus* could inherit from *B. rapa* only single resistance genes (Walsh, Jenner, 2002).

Conclusion

The pathogens discussed in this review seriously affect the yield of cultivated *Brassica* crops. Growing resistant cultivars is an effective way of infection control. In the past two decades, application of molecular genetic methods enabled researchers to identify and map resistance genes in cruciferous crops, understand principles of their functioning, and develop molecular markers for their identification.

The size of available information on marker-aided identification of the genes controlling resistance to pathogens is not the same for different *Brassica* spp. In the cases of *B. napus* and *B. carinata*, resistance genes have been mapped only for individual pathogens (TuMV and black rot, respectively). Genes of resistance to black rot and downy mildew are studied well enough for *B. oleraceae*, and to all three pathogens for *B. rapa*. In view of this, there is a need to go on with research efforts aimed at identification of new resistance-controlling genes and QTL and development of new markers to expand the applicability of MAS methods and introduce them into *Brassica* crop breeding practice.

On the whole, however, the number of markers developed to search for genes responsible for various types of resistance and efficient for molecular screening is sufficiently large, as far as the discussed *Brassica* spp. are concerned. Their introduction into breeding practice could accelerate the selection of resistant genotypes manifold and ensure pyramiding genes for resistance.

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Comparative analysis of wild and cultivated *Lathyrus* L. spp. according to their primary and secondary metabolite contents

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Species of the genus *Lathyrus* L. are known as forage and medicinal plants, widely used in traditional medicine and homeopathy. The content of protein, essential amino acids and carotene in their green biomass is higher than in other annual leguminous plants traditionally cultivated in Russia. Until now, the requirements for the crop's quality were reduced to a high content of protein and dry matter in seeds and herbage. In-depth biochemical analysis of accessions from the collection of plant genetic resources will significantly improve selection of source materials for breeding. Such tasks can be solved using gas chromatography with mass spectrometry in plant diversity studies. In view of the above, our goal was to analyze organic acids, free amino acids and secondary metabolites in green biomass of *Lathyrus* to facilitate comprehensive assessment of its forage and pharmacological value. We analyzed 32 accessions of *Lathyrus sativus* L., *L. tuberosus* L., *L. sylvestris* L., *L. vernus* (L.) Bernh., *L. latifolius* L. and *L. linifolius* (Reichard) Bassler from the collection of the Vavilov Institute (VIR). The studied *Lathyrus* accessions had significant interspecific and intraspecific variability both in the composition (presence) and number of the identified compounds. The analysis of plants across different years confirmed that biochemical parameters depended on weather conditions. The colder and drier conditions of 2012 contributed to the accumulation of organic acids (mean: 890 mg/100 g), free amino acids (mean: 201.59 mg/100 g), and secondary metabolites (mean: 84.14 mg/100 g). The range of variability for organic acids ranged from 140 to 2140, for free amino acids from 11.8 to 610, and for secondary metabolites from 4.4 to 224.6 mg/100 g. Grass pea accessions with high organic acid, free amino acid and secondary metabolite contents were identified: k-900 (Colombia) for organic acids (2140, 610 and 178 mg/100 g); k-51 (Georgia) and k-959 (Afghanistan) for free amino acids (401.29 and 540.63 mg/100 g); k-893 (Eritrea) for secondary metabolites (199.39 mg/100 g), etc. They can serve as source material for the development of cultivars for different uses (forage and medicinal).
Key words: *Lathyrus* L.; wild species; varieties; green mass; gas chromatography; genetic resource; polymorphism of characters.

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Сравнительный анализ диких и культурных видов чины (*Lathyrus* L.) по содержанию веществ первичного и вторичного метаболизма

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Виды рода *Lathyrus* L. известны как кормовые и лекарственные растения, используемые в народной медицине и гомеопатии. Содержание белка, незаменимых аминокислот и каротина в зеленой массе чины выше, чем у других однолетних зернобобовых растений, традиционно культивируемых в России. До настоящего времени требования к качеству культуры сводились к высокому содержанию белка и сухого вещества в семенах и вегетативной массе. Углубленный биохимический анализ образцов из коллекции генетических ресурсов растений существенно улучшит отбор исходного материала для селекции. Изучение растительных ресурсов с использованием метода газовой хроматографии с масс-спектрометрией позволяет решить подобные задачи. В связи с вышесказанным нашей целью было исследование органических кислот, свободных аминокислот и соединений вторичного метаболизма в зеленой массе чины для всесторонней оценки ее кормовой и фармакологической ценности. Анализировали 32 образца *Lathyrus sativus* L., *L. tuberosus* L., *L. sylvestris* L., *L. vernus* (L.) Bernh., *L. latifolius* L., *L. linifolius* (Reichard) Bassler из коллекции Всероссийского института генетических ресурсов рас-

тений им. Н.И. Вавилова. Изученные образцы *Lathyrus* обладали значительной межвидовой и внутривидовой изменчивостью как по составу (наличию), так и по количеству идентифицированных веществ. Анализ растений в разные годы подтвердил зависимость биохимических показателей от погодных условий. Более холодные и сухие условия 2012 г. способствовали накоплению органических кислот (среднее – 890 мг/100 г), свободных аминокислот (среднее – 201.59 мг/100 г) и соединений вторичного метаболизма (среднее – 84.14 мг/100 г). Диапазон изменчивости органических кислот составил от 140 до 2140, свободных аминокислот – от 11.8 до 610, соединений вторичного метаболизма – от 4.4 до 224.6 мг/100 г. Выделены образцы чины посевной: с повышенным содержанием органических кислот, свободных аминокислот и соединений вторичного метаболизма – к-900 (Колумбия) (2140, 610 и 178 мг/100 г), свободных аминокислот – к-51 (Грузия) и к-959 (Афганистан) (401.29 и 540.63 мг/100 г), соединений вторичного метаболизма – к-893 (Эритрея) (199.39 мг/100 г) и другие, которые могут служить исходным материалом для создания сортов разного направления использования: кормового и лекарственного.
Ключевые слова: *Lathyrus* L.; дикие виды; сорта; зеленая масса; газовая хроматография; генетические ресурсы; полиморфизм признаков.

Introduction

Researching the chemical composition of cultivated plants and their wild relatives is of crucial importance in both theoretical and practical contexts. Still vital is the question of seeking new plants that may serve as sources of bioactive compounds with remedial properties, and introducing them into agricultural practice. *Lathyrus sativus* L., *L. pratensis* L. and *L. tuberosus* L. have been most comprehensively studied with regard to their chemical composition and pharmacological characteristics. Biological activity of several chemical components has been analyzed in *L. sylvestris* L., *L. vernus* (L.) Bernh. and *L. niger* (L.) Bernh. Wide pharmacological demand for peavines is induced by their macro- and microelement composition as well as the presence of flavonoids and a complex set of essential amino acids (Plant Resources of the USSR, 1987, 2011; Zaichikova, 2002a, b). *Lathyrus* spp. are also known as forage plants, outstanding for their high protein content in seed and herbage (Pavlova, 2001; Burlyaeva et al., 2012).

When peavine cultivars are developed, primary attention is given to yield, resistance to biotic and abiotic environmental factors, and the traits that secure their value as animal feed. Of late, the in-depth biochemical analysis has been used to solve numerous problems, such as the assessment of cultivar specificity of seeds (Smolikova et al., 2015; Loskutov et al., 2016) and study of the effects of domestication processes and environmental stressors (Konarev et al., 2015; Puzanskiy et al., 2015), etc. Modern gas chromatography (GC) with mass selective detection (MSD) techniques make it possible to examine plant biochemical composition varying with time and growing conditions (Konarev et al., 2015; Puzanskiy et al., 2015).

The collection of *Lathyrus* spp. at VIR contains 2055 accessions; they belong to more than 50 species. Widely represented in the collection are landraces, cultivars bred domestically and abroad, and their wild relatives from European Russia, Europe, Asia, Africa, Australia, etc. For many years, accessions from the collection have been studied mostly for protein content in seed and herbage. No in-depth biochemical research has been undertaken until now to study wild relatives and cultivated forms of *Lathyrus* spp. We have also failed to find any scientific publications where variations in the biochemical composition of peavine green biomass were analyzed under different weather conditions.

When peavine cultivars are developed for feed, food and medicinal purposes, emphasis is placed by breeders on raising the content of nutrients and secondary metabolites in green biomass. In view of this, the goal of this research was to study the biochemical composition of *Lathyrus* green biomass for comprehensive assessment of its forage and pharmacological value. Research objectives included analyzing inter- and intraspecific polymorphism of biochemical characters in *Lathyrus* spp., assessing the effect of weather conditions on the analyzed parameters, and identifying most promising accessions for breeding practice.

Materials and methods

The experiment employed 32 accessions of six *Lathyrus* species from the VIR collection: grass pea (*L. sativus*), flat pea (*L. sylvestris*), spring pea (*L. vernus*), heath pea (*L. linifolius*), everlasting pea (*L. latifolius*), and tuberous pea (*L. tuberosus*), grown in 2012 and 2013 in fields of the Pushkin Laboratories of VIR in the vicinities of St. Petersburg. Plants were grown on one-meter plots (1 m^2) in two replications (the plots were situated in one and the same place), on naturally irrigated soddy-podzolic soil. Standard agricultural practices for row crops adopted at VIR (Vishnyakova et al., 2010) were applied. Weather conditions in the growing seasons were contrasting. In 2012, the total of active temperatures was 1885.0 °C, with total precipitation of 340.7 mm. In 2013, the total of active temperatures was observed to rise to 2474.3 °C, with total precipitation going up to reach 646.4 mm.

The plants were harvested in the early pod ripening phase. Fresh green biomass of plants was analyzed (five plants from each accession: stems, leaves and pods; in three analytical replications).

A sample of 10 g was weighed, homogenized with an adequate amount of ethanol, and infused for 30 days at 5–6 °C. The extract (200 μL) was vaporized to dry residues on a CentriVapConcentrator (Labconco, USA). The solid residue was silylated with bis(trimethylsilyl)trifluoroacetamide. The silylated compounds were separated on an HP-5MS capillary column (5 % phenyl methylpolysiloxane; 30.0 m, 250.00 μm, 0.25 μm) using the Agilent 6850 chromatography system with a quadrupole mass spectrometry detector Agilent 5975B VL MSD (Agilent Technologies, USA). Conditions of chromatographic analysis: helium flow rate through the column 1.5 mL/min, column heating program +70 °C to +320 °C with the heating rate 4 °C/min, MSD temperature + 250 °C, injector temperature + 300 °C, sample volume 1 μL, internal standard: tricosane in pyridine (1 μg/μL).

The spectra were processed using UniChrom and AMDIS software, NIST 2010 mass spectra libraries, and Science parks of the St. Petersburg State University and the Komarov Botanical Institute. The results were evaluated with MS Excel 2007 and Statistica 7.0 programs. The effect of environmental conditions on the expression of biochemical characters was assessed using one-way analysis of variance (ANOVA) with Fisher's LSD-test. The effect size of the factor's influence (η^2, %) according to Fisher was calculated by Equation (1) (Ivanter, Korosov, 2003):

$$\eta^2 = \frac{SS_{factor}}{SS_{total}} \times 100\ \%,$$

where η^2, % is the effect size of the factor influence; SS_{factor} is the sum of squared deviations for the factor; SS_{total} is the total sum of squared deviations.

Results

The biochemical composition analysis of the *Lathyrus* green biomass samples revealed about 300 components. This paper discusses part of the data obtained (Table 1, Suppl. material 1[1]).

Organic acids. In 2012, the content of organic acids in the green biomass samples of *Lathyrus* averaged 844.72 mg/100 g; depending on the genotype, this parameter varied from 136.27 to 2137.37 mg/100 g. In 2013, the acid content went down to 333.77 mg/100 g, and different accessions varied within 215.37–544.24 mg/100 g (Fig. 1). For grass pea accessions, the mean content of organic acids in 2012 was 890 mg/100 g, with the range of variation from 300 to 2140 mg/100 g. In 2013, a decrease to 3120 mg/100 g was observed in the mean values, and the range narrowed to 220–430 mg/100 g. In the flat pea group, the mean content of organic acids was 590 mg/100 g in 2012, and 480 mg/100 g in 2013. For *L. sylvestris*, this parameter was relatively stable in different years, unlike *L. sativus*, which demonstrated a drop of the organic acid content to 570 mg/100 g in 2013. The organic acid contents in heath, everlasting, and spring pea accessions were somewhat higher: 610, 670, and 640 mg/100 g, respectively.

The lowest level of organic acids was recorded for tuberous pea accessions (140 mg/100 g). The highest acid content in 2012 was observed in grass pea accession k-900 (Colombia): 2140 mg/100 g; and in 2013, in flat pea accession k-591293 (Germany): 540 mg/100 g.

Organic acids were represented mostly by malic (Krebs cycle) and threonic (ascorbic acid oxidation product) acids; their respective contents were 156.22 and 120.52 mg/100 g. Glyceric and citric acids ranked next: their contents were 90.63 and 61.32 mg/100 g, respectively. Dehydroabietic, phosphoric, oxalic, lactic, and fumaric acids respectively averaged 30.42, 27.14, 25.90, 14.61, and 10.65 mg/100 g. The contents of succinic, mesoxalic, quinic, erythronic and gluconic acids did not exceed 10 mg/100 g (9.17, 6.87, 6.58, 6.34, and 5.82, respectively). Respective amounts of tartaric, ribonic and pipecolic acids were 2.77, 2.04, and 1.85. Accumulation of other acids (benzoic, nicotinic, maleic, 4-hydroxybenzoic, azelaic, saccharic, protocatechuic, shikimic, galacturonic, caffeic, sinapic, abietic and neochlorogenic) never exceeded 0.7 mg/100 g, while the respective amounts of galacturonic and saccharic acids were 0.29 and 0.06 mg/100 g.

Table 1. The contents of amino acids, organic acids, and secondary metabolites in the green biomass of some *Lathyrus* species (mg/100 g wet weight)

Species	Organic acids	Amino acids	Secondary metabolites
L. sativus	610.0 ± 64.0*	208.6 ± 16.8	59.2 ± 8.0
	220.0–2140.0**	41.0–610.0	4.4–199.4
L. sylvestris	520.0 ± 84.6	205.1 ± 45.9	43.1 ± 14.3
	340.0–830.0	67.5–340.3	15.2–79.8
L. vernus	640.0 ± 94.9	28.0 ± 2.9	28.1 ± 6.9
	540.0–730.0	25.1–31.0	21.8–35.7
L. linifolius	830.0 ± 89.5	11.8 ± 6.2	71.6 ± 29.7
	250.0–1050.0	3.1–14.8	32.2–100.1
L. latifolius	670.0 ± 75.6	136.3 ± 38.3	132.4 ± 52.1
	100.0–850.0	42.3–157.9	81.5–224.6
L. tuberosus	140.0 ± 98.9	72.9 ± 25.3	6.4 ± 9.8
	2.0–380.0	24.8–95.4	1.1–19.5

* Arithmetic mean ± standard error of the mean;
** Range (min–max).

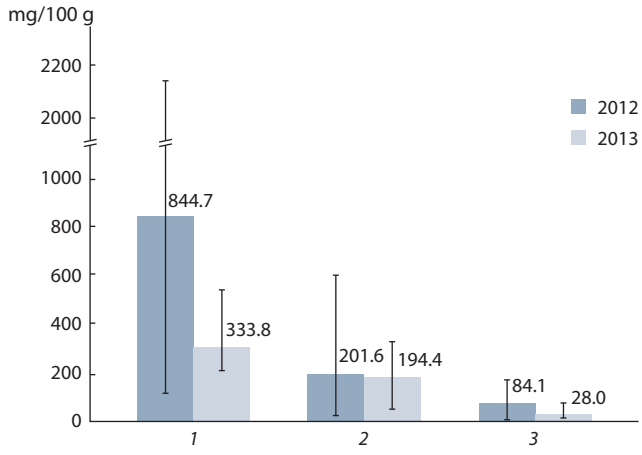

Fig. 1. The contents of organic acids, free amino acids, and phenolic compounds in the green biomass of *Lathyrus* L. in different years of cultivation (mean values, min–max, mg/100 g wet weight).

1 – organic acids; *2* – free amino acids; *3* – secondary metabolites.

Amino acids. The green biomass of *Lathyrus* accessions was found to contain 20 free amino acids, including eight essential ones (see Suppl. material 1). The mean content of free amino acids in 2012 was 201.59 mg/100 g (see Fig. 1); the variation being from 11.75 to 610.00 mg/100 g. In 2013, the amino acid content was slightly lower (194.42 mg/100 g), while the range of variability for this character in different genotypes was within the limits from 40.97 to 340.30 mg/100 g. In 2012, the highest free amino acid contents were registered in grass pea accessions (230.16 mg/100 g), and the lowest in heath pea (11.75 mg/100 g). The same parameters measured

[1] Supplementary Materials 1–2 are available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx12.pdf

in other species were as follows: 114.93 mg/100 g in flat pea, 136.28 in everlasting pea, 72.90 in spring pea, and 28.02 in tuberous pea. In 2013, the mean amino acid content in the green biomass of flat pea was higher (265.22 mg/100 g). Grass pea, however, showed the opposite tendency, as this parameter dropped to 185.92 mg/100 g in 2013. Other *Lathyrus* groups showed no significant variations across the two years.

Secondary metabolic compounds. One of the key indicators that reflect antioxidant activity and resistance to the impact of environmental stressors is the content of secondary metabolites, including phenol-containing compounds. The effect of secondary metabolites is directly connected with their concentrations; in this regard, their quantitative analysis is no less important than qualitative one (Spanou et al., 2010).

In the tested green biomass of *Lathyrus* accessions, secondary metabolites were represented by free phenolcarboxylic (PC) acids (mean content 52.52), quinones (0.38), flavonoids (3.49), phenylpropanoids (0.69), and iridoids (0.11), including the identified α-tocopherol (2.14 mg/100 g) (see Suppl. material 1). The mean content of secondary metabolites in the green biomass of *Lathyrus* accessions in 2012 was 84.14 mg/100 g (from 4.45 to 199.39) (see Fig. 1). In 2013, the mean decreased to 28.04 mg/100 g (from 13.23 to 53.45). In 2012, the highest contents of secondary metabolites were recorded in everlasting pea accessions (132.44 mg/100 g), and the lowest in tuberous pea (6.42 mg/100 g). In the green biomass of grass pea, flat pea, heath pea, and spring pea, the mean values of secondary metabolites (89.32, 78.01, 71.59, and 28.74 mg/100 g, respectively) were lower than in everlasting pea. In 2013, the mean secondary metabolite contents in grass pea and flat pea dropped to 29.03 and 19.80 mg/100 g, respectively.

One-way ANOVA was used to ascertain the significance of the impact of weather conditions on the content of the analyzed biochemical characters. The analysis allowed identification of 79 compounds significantly affected by growing conditions (Table 2), including total organic acids and secondary metabolites. The effect size (percentage) for the influence of environmental conditions (η^2) on the content of organic acids was 67.9 % for lactic acid, 39.0 % for glyceric acid, 37.5 % for threonic acid, 33.4 % for fumaric acid, and 34.5 % for the total organic acid content (Fig. 2). Of free amino acids, including aminoalcohols and amines, the greatest weather impact was registered for the contents of ethanolamine ($\eta^2 = 72.9$ %), leucine (51.6), GABA (48.9), methionine (47.9), putrescine (43.1), adenosine (41.2), glycine (34.6), asparaginic acid (29.9), and asparagine (29.0). For weather impact on the variability of secondary metabolites, the highest effect size was recorded for quercetin ($\eta^2 = 39.8$ %) and dehydroabietic acid (30.4). For the total content of secondary metabolites, it was 26.1 %.

Despite the large number of compounds identified in the green biomass of *Lathyrus* spp., only quantitative indicators in some of them accounted for statistically significant differences between wild species: catechin ($F(5; 54) = 10.47$, $p = 0.0000$), α-alanine ($F(5; 54) = 2.52$, $p = 0.039$), asparagine ($F(5; 54) = 3.32$, $p = 0.011$), glycine ($F(5; 54) = 3.25$, $p = 0.012$), and shikimic acid ($F(5; 54) = 31.66$, $p = 0.0000$). Asparagine, glycine, α-alanine and catechin contents were the highest in flat pea (*L. sylvestris*), and shikimic acid content, in everlasting pea (*L. latifolius*) (Suppl. material 2).

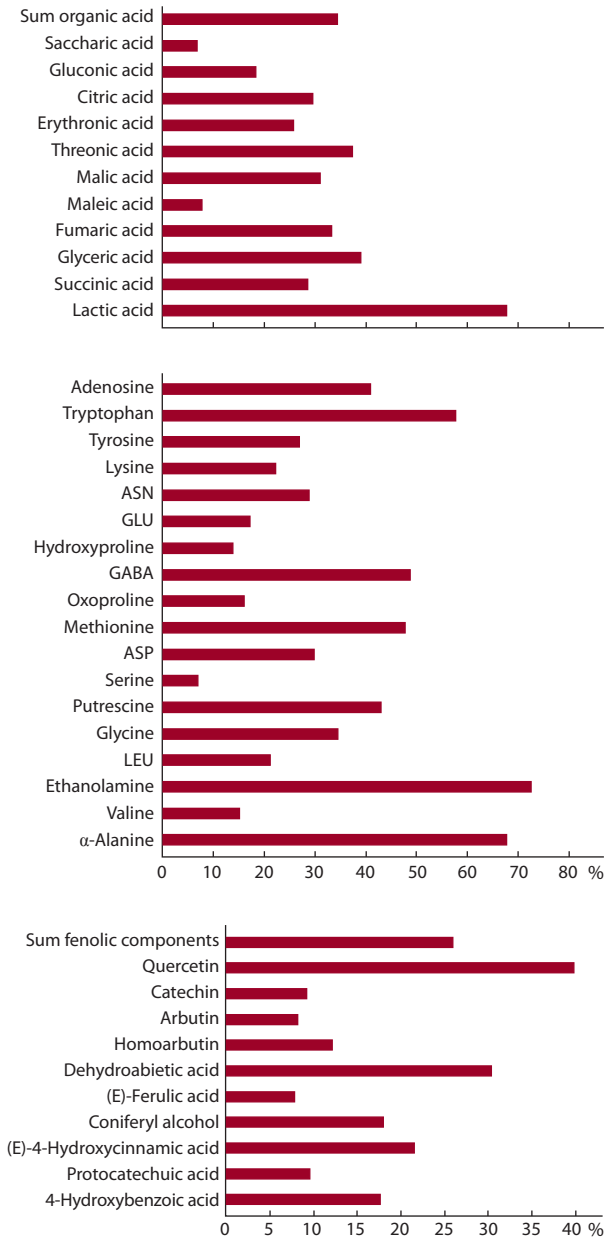

Fig. 2. The effect size (η^2, %) for the influence of weather conditions on the variability of biochemical characteristics.

Discussion

The studied *Lathyrus* accessions demonstrated broad polymorphism in the biochemical composition of green biomass. Significant interspecific and intraspecific variability was observed both in the composition (presence) and the amount of the identified compounds. The highest content of organic acids was found in *L. sativus*: 2140 mg/100 g (k-900, Colombia); free amino acids in *L. sylvestris*: 265.22 mg/100 g (k-2017, Germany); and total secondary metabolites in *L. latifolius*: 132.44 mg/100 g (i-594176, Germany). The analysis showed

Table 2. The results of one-way analysis of variance (ANOVA) to identify the association between the variability of biochemical parameters and weather conditions in the year of reproduction

Effect	df	Lactic acid			Succinic acid			Glyceric acid			Fumaric acid			Maleic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	6515.0	122.9	0.00	1194.8	23.1	0.00	249259.9	37.0	0.00	5736.3	29.1	0.00	18.8	4.9	0.03
Error	58	3074.5			3004.4			390349.6			11439.2			222.2		
Total	59	9589.5			4199.2			639609.5			17175.5			241.0		
η^2		67.9			28.5			39.0			33.4			7.8		
Effect	df	Malic acid			Threonic acid			Erythronic acid			Citric acid			Saccharic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	319326.9	26.2	0.00	340838.3	34.8	0.00	1664.9	20.2	0.00	92898.4	24.4	0.00	0.3	4.1	0.04
Error	58	705960.5			567394.8			4780.5			221251.6			3.9		
Total	59	1025287.3			908233.0			6445.4			314150.1			4.2		
η^2		31.1			37.5			25.8			29.6			6.7		
Effect	df	Gluconic acid			Total organic acid			4-Hydroxybenzoic acid			Protocatechuic acid			(E)-4-Hydroxycinnamic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	1083.8	13.0	0.00	3624902.5	30.6	0.00	0.2	12.4	0.00	0.2	6.2	0.02	853.8	15.9	0.00
Error	58	4819.6			6880021.4			0.7			1.4			3119.8		
Total	59	5903.3			10504923.9			0.9			1.6			3973.6		
η^2		18.4			34.5			17.6			9.6			21.5		
Effect	df	Coniferol			(E)-Ferulic acid			Catechin			Arbutin			Quercetin		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	32.9	12.7	0.00	134.5	4.9	0.03	3.3	5.9	0.02	0.9	5.1	0.03	1.1	38.3	0.00
Error	58	149.9			1576.1			32.8			10.6			1.6		
Total	59	182.9			1710.6			36.1			11.5			2.7		
η^2		18.0			7.9			9.2			8.1			39.8		
Effect	df	Quinic acid			Homoarbutin			Dehydroabietic acid			Total phenolic compounds			Ethanolamine		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	2414.1	96.8	0.00	1.2	8.0	0.01	48067.3	25.3	0.00	45145.9	20.5	0.00	16.3	156.3	0.00
Error	58	1447.1			8.4			110250.0			127578.5			6.0		
Total	59	3861.1			9.6			158317.3			172724.4			22.3		
η^2		62.5			12.1			30.4			26.1			72.9		
Effect	df	α-Alanine			Valine			Serine			Leucine			Adenosine		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	937.3	9.8	0.00	146.9	10.4	0.00	2088.8	4.4	0.04	62.5	15.7	0.00	103.6	40.7	0.00
Error	58	5546.7			817.2			27385.9			231.1			147.7		
Total	59	6484.0			964.2			29474.7			293.6			251.3		
η^2		14.5			15.2			7.1			21.3			41.2		

Table 2. (End)

Effect	df	Glycine			Putrescine			Aspartic acid			GABA		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	108.2	30.7	0.00	12574.3	43.9	0.00	16051.4	24.8	0.00	763.8	55.4	0.00
Error	58	204.2			16604.5			37579.3			798.9		
Total	59	312.4			29178.8			53630.7			1562.7		
η^2		34.6			43.1			29.9			48.9		
Effect	df	Methionine			Oxoproline			Tyrosine			Hydroxyproline		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	1.4	53.4	0.00	908.1	11.3	0.00	31.1	21.6	0.00	0.1	9.4	0.00
Error	58	1.5			4662.5			83.6			0.7		
Total	59	2.9			5570.6			114.6			0.8		
η^2		47.9			16.3			27.1			13.9		
Effect	df	Glutamine			Asparagine			Lysine			Tryptophan		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	8323.9	12.2	0.00	4368.6	23.7	0.00	149.2	16.8	0.00	168.1	79.6	0.00
Error	58	39610.6			10688.9			513.9			122.5		
Total	59	47934.4			15057.5			663.2			290.6		
η^2		17.4			29.0			22.5			57.8		

Note: SS – sum of squares; F – Fisher's criterion value; *p* – level of significance; df – degrees of freedom; η^2, % – the effect size of the factor's influence; year, weather conditions.

sizeable variation of biochemical parameters under different weather conditions. The colder and drier season in 2012 was conducive to the accumulation of organic acids, amino acids, and secondary metabolites.

There is no published information on the content of organic acids in the green biomass of *Lathyrus*. The analyzed accessions contained mostly acids participating in cell respiration, ascorbic acid oxidation products (threonic acid), natural antiseptics and antioxidants (azelaic acid), and anti-stress factors of plant cells (pipecolic and maleic acids) (Yao et al., 1998; Mahmud et al., 2017). The highest contents of individual organic acids were found in the following grass pea accessions: malic acid in k-893 (Eritrea) and k-900 (Colombia) (495 and 505 mg/100 g); threonic acid in k-900 (Colombia) (522 mg/100 g); azelaic acid in k-275 (Azerbaijan) (8.20 mg/100 g); maleic acid in k-889 (Abyssinia) and k-34 (Russia) (8.78 and 8.92 mg/100g). The everlasting pea accession k-51 (Germany) showed a high content of pipecolic acid (59.18 mg/100 g).

Our data on the free amino acid content differ from those published by S.G. Zaichikova et al. (2001), who identified 18 amino acids in *Lathyrus* herbage, including 7 essential ones, plus histidine and arginine not detected by us. In addition to the above-mentioned free amino acids, our experiment revealed tryptophan, GABA, asparagine, glycine, etc. In the said publication by S.G. Zaichikova et al., the main amino acids in peavine green biomass were glutamine and histidine: they respectively accounted for 15 and 11.6 % of the total amount of identified amino acids. E. Pastor-Cavada et al. (2010) identified 17 amino acids, including 9 essential ones, in *Lathyrus* seeds. Our research showed that the green biomass of *Lathyrus* spp. contained 20 amino acids (8 essential ones). The main amino acids in seeds were glutamic and aspartic acids, and in green biomass, according to our data, serine, glutamic and aspartic acids (16.4, 16.6, and 13.5 % of the total amount of identified amino acids, respectively). The major essential amino acids were arginine, leucine, and lysine in seeds and threonine (much more abundant than other amino acids) in green biomass. We singled out the grass pea accession k-842 (Tajikistan) for its high content of glycine (3.26 mg/100 g), an indicator of resistance to environmental stressors (Loskutov et al., 2016).

According to published data, flavonoids are the main phenol-containing compounds in the *Lathyrus* green biomass, their amount reaching 50 % of the total phenolic content. Our research has shown that the main components of phenol-containing compounds are free PC acids: they possess the highest antioxidant capacity (Shetty et al., 2002). Extracts of grass pea and flat pea in relevant concentrations are known to produce stimulating effect on the phagocytic and antibacterial activity of human neutrophils, which is associated with the presence of free PC acids in them (Zaichikova, 2002a, b).

The qualitative and quantitative compositions of secondary metabolites identified by us differed from the data published by F. Sibul et al. (2016), because different research

methods were applied. Sibul et al. used HPLC to identify a wide spectrum of hydroxybenzoic and hydroxycinnamic acids in *Lathyrus* leaves, as well as a high content of quinic acid (30.4–35.0 mg/100 g), with the total secondary metabolite content being 58.1 mg/100g dry weight (DW). In our study, the total content of secondary metabolites was much higher (246.85 mg/100 g), with ferulic and quinic acids being the most abundant (40.63 and 28.30 mg/100 g DW). We managed to identify only four hydroxybenzoic acids (pyrogallol, 4-hydroxybenzoic, protocatechuic and benzoic acids) against six identified by F. Sibul et al. (2016). The content of protocatechuic acid in our experiment was somewhat lower than those reported by other researchers (0.2 and 0.9 mg/100 g DW). Of hydroxycinnamic acids, we identified ferulic, sinapic, and caffeic acids (19.09, 0.03, and 2.09 mg/100 g DW), while our colleagues found ferulic, *p*-coumaric, and caffeic acids (1.38, 1.42, and 1.02 mg/100 g DW). The content of ferulic acid in our accessions was much higher, and that of caffeic acid slightly lower than the values reported by scientists outside Russia. The content of chlorogenic acid was low in the accessions analyzed by us and in the plants tested by F. Sibul et al. (2016): 0.8 mg/100 g DW. The content of luteolin in our genotypes was lower than the same parameter reported by other authors (1.21 and 4 mg/100 g DW). Our accessions contained more catechin and quercetin (0.95 and 4.00) than the plants tested by F. Sibul et al. (2016): 0.04 and 1.60 mg/100 g DW. The levels of kaempferol were practically identical (1.78 and 1.60 mg/100 g DW, respectively), but the content of isorhamnetin in our *Lathyrus* accessions (0.02 mg/100 g DW) was considerably lower than the value published by non-Russian researchers (0.53 mg/100 g DW). F. Sibul et al. (2016) identified a wider spectrum of flavones and glycosides. We did not identify isoflavones, coumarins, or several glycosides. However, our research efforts yielded data on other secondary metabolites (hydroquinone, shikimic acid, and coniferol).

U.D. Chavan (1998) reported the total content of secondary metabolites in sea pea (*L. maritimus* L.), which was beyond the scope of our research; those levels varied from 0.5 to 3.0 %, being roughly close to our results (0.3–0.9 %).

We selected the grass pea accessions k-893 (Eritrea) and k-900 (Colombia) for their high content of secondary metabolites (199.39 and 177.82 mg/100 g) as potential sources of resistance and pharmacological value.

Our research confirmed the impact of weather conditions (temperature and precipitation amount) on the accumulation of organic acids, free amino acids, and major secondary metabolites (Popov et al., 2016). The analysis helped us identify accessions with high contents of substances responsible for protection against adverse environmental factors (maleic and pipecolic acids, glycine, and the aggregate content of secondary metabolites) and compounds of value for pharmacology (azelaic acid), which hold promise in the development of new nutritious, resistant, or medicinal cultivars of *Lathyrus*.

Conclusion

Thus, our research has brought forth new data on the biochemical composition of peavine green biomass. Its results confirm that *Lathyrus* is a promising forage and medicinal crop with a potential for various branches of economy.

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Методический подход к оценке изменчивости признаков продуктивности и качества ягод в генетических коллекциях земляники садовой (*Fragaria* × *ananassa* Duch.)

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Для земляники садовой *Fragaria* × *ananassa* Duch. (2*n* = 8*x* = 56) – ведущей ягодной культуры в мире – актуально изучение взаимодействия генотип–среда. Сложный геномный состав, разнообразие систем генетического контроля, а также сильное модифицирующее влияние условий выращивания на проявление количественных признаков обусловливают необходимость совершенствования методов анализа генотипической изменчивости хозяйственно ценных признаков, направленных на установление генотипов, характеризующихся стабильностью и адаптивными качествами в широком экологическим диапазоне условий выращивания. В 2016–2018 гг. было изучено 27 сортов земляники садовой в коллекциях Северо-Кавказского федерального научного центра садоводства, виноградарства, виноделия и Крымской опытно-селекционной станции – филиала ВИР. Полевые опыты и учеты поставлены и проведены по единой схеме. Изучали следующие признаки: число цветоносов (шт./куст), число ягод (шт./куст), средняя масса ягоды и ягоды первого порядка (г), общий и товарный урожай (г/куст), плотность мякоти ягоды (г), содержание сахаров в ягодах по шкале Брикса (°Вх), сахарокислотный индекс. Цель настоящей работы – разработка методического подхода к оценке вклада взаимодействия генотип–среда в изменчивость признаков продуктивности и качества ягод земляники и определение сортов земляники со стабильным генотипом. Для решения поставленной задачи использованы математические модели двух- и трехфакторного дисперсионного и кластерного анализов по методу Уорда. По результатам проведенной работы установлено, что сорта земляники, выращенные в разных климатических условиях, показывают различия в структуре изменчивости признаков продуктивности и качества ягод. Для условий г. Крымска преобладающим оказалось влияние генотипа сорта, а для условий г. Краснодара, кроме влияния генотипа сорта, существенной является и средовая компонента в виде взаимодействия генотип–среда. Статистически достоверное влияние зоны выращивания установлено для признаков продуктивности и качества ягод, за исключением средней массы ягоды. При этом различия средних значений признаков у сортов могут быть как существенными, так и частично или полностью отсутствовать. Для определения перспективных сортов при выращивании в изучаемых зонах рекомендуется использовать кластерный анализ по информативному комплексу признаков с вычислением евклидовых расстояний для сортов, выращенных в разных условиях. Величина евклидова расстояния будет мерой влияния конкретной среды на генотип растений. Чем меньше значение евклидового расстояния у сорта, согласно комплексу изученных признаков, тем большей стабильностью характеризуется этот сорт.

Ключевые слова: земляника; генотип; среда; сорта; изменчивость; стабильность; признаки; статистические методы; кластерный анализ; евклидово расстояние.

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A methodical approach for evaluating the variability of productivity and fruit quality in the genetic collections of strawberry (*Fragaria* × *ananassa* Duch.)

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For strawberry (*Fragaria* × *ananassa* Duch., 2*n* = 8*x* = 56), which is the leading berry crop in the world, research into the genotype × environment interaction is important. A complicated genomic composition, the diversity of genetic control systems, and a strong modifying effect of growing conditions on the implementation of quantitative traits make it necessary to improve methods for analysis of the genotypic variability of economically valuable traits with the aim of identifying

genotypes that are characterized by stability and adaptive qualities in a wide ecological range of growing conditions. In 2016–2018, twenty-seven strawberry varieties were studied in the collections of North Caucasian Federal Scientific Center of Horticulture, Viticulture and Krymsk Experiment Breeding Station, VIR Branch. Field experiments and data counts were set and carried out according to a single scheme. The following characteristics were studied: the number of inflorescences (units per plant), the number of berries (units per plant), the average weight of berry and berry of the first order (g), total and marketable yield (g per plant), firmness of fruit (g), sugar content in berries on Degrees Brix (°Bx), sugar-acid index. The purpose of this work was the development of a methodical approach to assessing the contribution of the genotype–environment interaction to the variability of the traits of productivity and fruit quality and the determination of strawberry varieties with a stable genotype. To this end, the mathematical models of two- and three-factor analysis of variance and cluster analysis using Ward's method were employed. According to the results of this work, strawberry varieties grown in different climatic conditions show differences in the structure of the variability of the traits of productivity and fruit quality. For the conditions of the city of Krymsk, the influence of the genotype of the variety was predominant, and for the conditions of the city of Krasnodar, in addition to the influence of the genotype of the variety, the environmental component in the form of the genotype–environment interaction is also significant. A statistically significant influence of the growing zone has been established for the traits of productivity and fruit quality, with the exception of the average weight of fruit. At the same time, differences in the mean values of the traits of varieties can be both significant and partially or completely absent. To identify varieties with promise for cultivation in the areas studied, it is recommended to use cluster analysis on the informative complex of traits with the calculation of the Euclidean distances for varieties that were grown under different conditions. The magnitude of the Euclidean distance will be the measure of the influence of a particular environment on the genotype of plants. The smaller the value of the Euclidean distance in a variety, according to the complex of the traits studied, the more stable this variety is.
Key words: strawberry; genotype; environment; varieties; variability; stability; traits; statistical methods; cluster analysis; euclidian distance.

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Введение

Земляника садовая *Fragaria* × *ananassa* Duch. – ведущая ягодная культура в мире. Количество ее сортов, по разным источникам, составляет от 3 тыс. (Зубов, 2004) до 20 тыс. (Копылов, 2007). По данным Global Conservation Strategy for *Fragaria* (Strawberry) (2008), мировая коллекция земляники насчитывает примерно 15 тыс. образцов, из которых около 12 тыс. – сорта и 3 тыс. – элитные гибриды. В ряде работ исследователей этой культуры (Lopez-Medina et al., 2001; Киртбая, Щеглов, 2003; Матала, 2003; Копылов, 2007; Fontana et al., 2016) отмечено, что для земляники садовой важным критерием ее успешного возделывания является правильный подбор сортов, адаптированных к местным условиям возделывания и системам выращивания. Взаимодействие генотип–среда часто затрудняет определение лучших генотипов в различных условиях выращивания.

Земляника садовая – сложная в изучении культура, что объясняется ее сложным геномным составом, полигенным характером наследования признаков, а также сильным модифицирующим влиянием условий выращивания на проявление количественных признаков. Характер развития растений, выражаемый нормой реакции генотипа на климатические и почвенные условия, различен и изменяется в зависимости от сорта. Поэтому работы по изучению нормы реакции сортов земляники в различных эколого-географических условиях актуальны и в настоящее время (Sieczko et al., 2015; Mathey et al., 2017; Gabriel et al., 2018; Singh et al., 2018).

Считается, что если сорт обеспечивает высокую стабильную продуктивность в одном регионе, то он обладает специфической адаптацией. Если же сорт показывает хорошую продуктивность в нескольких различающихся по условиям регионах, имеет большой ареал выращивания, то у него общая адаптация. Сорта такого типа, как правило, слабо реагируют на изменения окружающей среды и сохраняют основные сортовые признаки, стабильный урожай с типичным качеством плодов, несмотря на ухудшение условий выращивания (Фадеева, 1975).

Современное ягодоводство предъявляет повышенные требования к адаптивности сортов для получения стабильных урожаев. R.W. Allard и A.D. Bradshaw (1964) предложили два пути учета стабильности сортов: индивидуальную буферность – сорт представлен генотипами, каждый из которых приспособлен к ряду сред, и популяционную буферность – каждая особь в сорте приспособлена к определенной среде. В генетически гомогенной популяции земляники садовой (вегетативное потомство одной отборной гибридной формы F_1) преобладает популяционная буферность. Проявление взаимодействия генотип–среда, по мнению этих авторов, значительно осложняет селекцию на адаптивность к непредсказуемым изменениям внешних условий и отбор наиболее перспективных сортов для возделывания в производстве.

Традиционная методология определения ценных генотипов растений строится на трех основных подходах, предусматривающих распознавание генотипов сортов по фенотипу, гибридологический анализ, а также методы молекулярно-генетического анализа специфических полинуклеотидных последовательностей. Однако ни один из указанных подходов не отличается в должной степени сочетанием необходимой адекватности и чувствительности для корректного описания изменчивости исследуемой культуры и установления вкладов генетических эффектов в реализацию изучаемых признаков.

Для определения лучших генотипов земляники в различных условиях выращивания предложено несколько генетико-статистических моделей. По мнению R.W. Zobel

с коллегами (1988), M.M. Nachit с коллегами (1992) и P. Annicchiarico (1997), взаимодействие генотип–среда можно исследовать с разных сторон, но так как индивидуальные реакции сортов находят свое отражение в изменчивости комплекса признаков, то для изучения указанного взаимодействия более предпочтительны многомерные статистические методы, а не одномерные регрессионные модели. Другая модель была предложена H.G. Gauch (1988), H.G. Gauch и R.W. Zobel (1988) и предусматривала анализ взаимодействия генотип–среда методом главных компонент.

В настоящее время ни один из перечисленных методических подходов в полной мере не является корректным отражением генетической структуры изменчивости земляники в рамках взаимодействия генотип–среда, поскольку не существует общепринятой стандартной модели, учитывающей весь комплекс аспектов анализа в многофакторных экспериментах с более чем одним фактором. Разработка новых методических подходов к оценке взаимодействия генотип–среда – логическое продолжение имеющихся работ по культуре земляники, выполненных с применением методов многомерной статистики. Целью нашей работы было создание методического подхода к оценке вклада взаимодействия генотип–среда в изменчивость признаков продуктивности и качества ягод земляники и определение сортов земляники со стабильным генотипом.

Материалы и методы

В работе использованы данные, полученные в 2016–2018 гг. на экспериментальной базе Северо-Кавказского федерального научного центра садоводства, виноградарства, виноделия (СКФНЦСВВ) – ЗАО Опытно-производственном хозяйстве «Центральное», расположенном в Прикубанской зоне Краснодарского края (окрестности г. Краснодара), и на участке сортоизучения – Крымской опытно-селекционной станции – филиале ВИР (КОСС ВИР), в предгорной зоне Краснодарского края (окрестности г. Крымска).

Краснодар находится в южной части Восточно-Европейской равнины на Прикубанской низменности, практически в центре Краснодарского края, в южной части Прикубанской равнины. Рельеф Краснодара спокойный, ровный, имеет ровный уклон к северо-западу. Высота над уровнем моря колеблется от 19 до 32 м. Краснодар расположен на южной границе умеренных широт и имеет мягкий континентальный климат. Очень редко наблюдаются вторжения арктического воздуха. Для лета характерно вторжение тропического воздуха. Средняя температура воздуха в июле достигает +25.8 °С. Так как Краснодар, находящийся на границе между теплыми морями и холодным континентом, открыт для вторжения различных (и теплых, и холодных) воздушных масс, то для него характерны резкие погодные изменения, особенно температуры воздуха.

Крымск расположен на берегу р. Адагум в предгорьях северо-западной части Главного Кавказского хребта, в 102 км к юго-западу от Краснодара и 53 км к северо-востоку от Новороссийска. Климат в Крымске умеренно континентальный влажный. Средняя температура воздуха в июле составляет +30 °С. Несмотря на то что средние температуры зимних месяцев в городе положительные, зимой при прохождении холодных атмосферных фронтов нередко температура может опускаться ниже –20 °С.

Объектом исследования стали 27 сортов земляники садовой различного эколого-географического происхождения из коллекции СКФНЦСВВ: Альба, Клери, Сирия, Нелли, Белруби, Азия, Алина, Вима Кимберли, Элегия, Галлия, Роксана, Хоней, Онда, Ирма, Вима Тарда, Тарда Викода, Мармолада, Эльсанта, Елизавета II, Флоренс, Джени, Вима Ксима, Богота, Таира, Кемия, Моллинг Пандора, Вима Занта и 24 сорта коллекции КОСС ВИР: Альба, Клери, Алина, Хоней, Роксана, Дарселект, Камароза, Азия, Нелли, Онда, Майя, Эльсанта, Моллинг Пандора, Флоренс, Сирия, Зенга Зенгана, Аромас, Ирма, Эвью-2, Сискейп, Луиза, Пелагея, Тельма, Елизавета II. Полевые опыты в двух зонах были поставлены по единой схеме. При выращивании сортов в коллекциях применяли 2-строчную посадку растений, схему размещения 130 + 35 × 30 см, одну линию капельного орошения с фертигацией, в качестве мульчи использовали солому.

Изучали следующие признаки: число цветоносов (шт./куст), число ягод (шт./куст), средняя масса ягоды и ягоды первого порядка (г), общий и товарный урожай (г/куст), плотность мякоти ягоды (г), содержание сахаров в ягодах по шкале Брикса (°Вх), сахарокислотный индекс.

Лабораторные исследования осуществляли с использованием лабораторного оборудования: электронные весы Acom JW-1C, рефрактометр ATAGO Pocket Refractometer PAL-α, пенетрометр FT 011 (наконечник Ø 0.50 см^2).

Количественная оценка влияния генотипа сорта, условий года выращивания и их совместного действия на изученные признаки в наших исследованиях выполнена с помощью дисперсионного анализа. Влияние среды выращивания на реализацию хозяйственно-биологических признаков генотипов земляники предусматривает воздействие нескольких факторов, в связи с чем в рамках взаимодействия генотип–среда изучали эффекты сорт × год, а также сорт × год × зона выращивания. Наблюдения и учеты проводили по общепринятой в Российской Федерации Программе и методике сортоизучения плодовых, ягодных и орехоплодных культур (1999). Для математической обработки полученных данных использовали ряд специализированных пособий (Мандель, 1988; Лакин, 1990), а также программный пакет Statistica 10.

Результаты

Достижение поставленной цели первоначально предусматривало оценку уровня межсортовой изменчивости в каждом районе возделывания отдельно, с учетом условий года выращивания.

Результаты двухфакторного дисперсионного анализа по факторам «сорт», «год» и взаимодействию сорт–год в условиях опытного участка в окрестностях Краснодара за 2016–2018 гг. показали достоверные различия между изученными сортами по всем признакам для 5 % уровня значимости. По фактору «сорт» полученные значения F составили 8.10–38.30 при стандартном значении критерия Фишера $F_{ст.}$ 1.49; по фактору «год» – 6.90–156.50 при стандартном $F_{ст.}$ – 2.99; по взаимодействию сорт–год – 1.40–10.50 при стандартном $F_{ст.}$ – 1.35.

Table 1. The contributions of factors variety and year to the traits of strawberry varietie

Traits	Contribution, %		
	variety	year	interactions variety × year
CJSC EPE "Central'noe", NCFSCHVW, Krasnodar			
Number of inflorescences	18.6	13.3	21.5
Number of flowers	31.5	20.4	12.9
Number of berries	27.6	6.6	21.1
Mean fruit weight	31.6	10.8	27.9
Yield per plant	20.5	9.3	23.5
Firmness of fruit	35.3	10.3	26.0
Sugar content in berries	46.9	1.1	2.3
Sugar-acid ratio in berries	14.8	8.4	14.4
KEBS ARIPGR, Krymsk			
Number of berries	93.7	1.5	1.1
Mean fruit weight	95.0	1.5	1.4
Mean weight of first-order berry	96.6	1.0	0.3
Total yield	85.3	6.7	3.7
Marketable yield	88.8	4.3	1.7
Firmness of fruit	72.1	2.3	0.0
Sugar-acid ratio in berries	46.3	3.1	0.0

В условиях опытного участка в окрестностях Крымска за 2016–2017 гг. между изученными сортами, согласно обоим факторам, при 5 % уровне значимости установлены достоверные различия по всем учтенным признакам. По взаимодействию сорт–год для плотности мякоти ягод и сахарокислотного индекса в ягодах достоверных различий между сортами не установлено. По фактору «сорт» получены значения F 10.10–458.40 при стандартном $F_{ст.}$ 1/57; по фактору «год» – 8.20–130.20 при стандартном $F_{ст.}$ – 3.89. По взаимодействию сорт–год значения F для всех признаков, кроме плотности мякоти ягод и сахарокислотного индекса в ягодах, были 1.70–4.30 при стандартном $F_{ст.}$ – 1.57. Вычисленные в ходе дисперсионного анализа доли влияния факторов приведены в табл. 1.

Дисперсионный анализ сортов земляники, выращенных в условиях опытного участка СКФНЦСВВ (окрестности Краснодара), позволил установить, что влияние генотипа сорта всегда выше, чем влияние условий года выращивания. Генотип сорта оказывает максимальное влияние на содержание сахара в ягодах и минимальное – на сахарокислотный индекс ягод; влияние условий года выращивания оказывается максимальным для количества цветоносов и минимальным для содержания сахара в ягодах; максимальное одновременное влияние двух факторов определено для количества цветков. Установлено, что совместное влияние на признаки генотипа сорта и условий года выращивания иногда бывает больше, чем у отдельных факторов. Это дает возможность использовать анализируемый материал для оценки взаимодействия генотип–среда.

Результаты дисперсионного анализа сортов земляники, выращенных на опытном участке ВИР (окрестности Крымска), указывают на решающий вклад генотипа сорта в общую изменчивость по всем признакам, значительно превышающий, в отличие от данных по Краснодару, суммарный вклад фактора «год» и взаимодействия сорт–год.

Реализация признаков продуктивности и качества ягод растений земляники, выражаемая нормой реакции генотипа на климатические и почвенные условия, различна и изменяется в зависимости от сорта. Поэтому изучение нормы реакции в различных эколого-географических условиях представляется следующим этапом в установлении генотипически обусловленной стабильности и адаптивных свойств сортов земляники.

Взаимодействие генотип–среда определялось изменчивостью комплекса хозяйственно-биологических признаков по изученным факторам для исследуемых объектов. Анализ данных по признакам и годам, совпадающим в изучаемых выборках, полученных на опытных участках в окрестностях Краснодара и Крымска, выполнен с помощью трехфакторного дисперсионного анализа, учитывающего влияние генотипа сорта, климатической зоны, условий года выращивания и их совместного действия (табл. 2).

Данные табл. 2 демонстрируют, что вклад генотипа сорта максимально проявляется по средней массе и плотности мякоти ягоды (доля влияния фактора 29.0 и 26.3 % соответственно). В то же время для изученных сортов влияния климатической зоны выращивания на среднюю массу ягоды не обнаружено. Общий урожай не зависит от года выращивания и взаимодействия сорт–год–зона выращивания. Наибольшее влияние на него оказывают факторы генотипа сорта, зоны выращивания и их взаимодействие. Сахарокислотный индекс ягод также не зависит от эффекта взаимодействия трех факторов.

Полученные результаты согласуются с данными исследований K.W. Finlay и G.N. Wilkinson (1963), установивших, что сорта, специфически приспособленные к определенной среде, имеют много общих морфологических и физиологических признаков.

Так как влияние года выращивания на сорта земляники оказалось невелико, а на общий урожай – вообще отсутствовало, представлялось возможным получить среднегодовые значения признаков и использовать их в дальнейшем анализе для определения наиболее стабильных генотипов по двум зонам выращивания. Выделено 14 сортов, у которых есть совпадение по признакам, измеренным в условиях опытных участков в окрестностях обоих городов. Сравнение средних значений этих признаков с помощью *t*-критерия Стьюдента для определения реакции сортов на условия климатической зоны приведено в табл. 3.

Различия средних значений признаков у сортов, выращенных в разных климатических зонах, могут быть весьма существенными или частично либо полностью отсутствовать, например, как у сорта Альба. Такая ситуация существенно осложняет анализ изменчивости признаков продуктивности и качества ягод. Для решения возникше-

Table 2. Analysis of variance of studied traits in strawberry varieties grown in experimental plots in the Krasnodar and Krymsk localities

Variability	df	Number of berries				Mean fruit weight			
		mS	F	σ^2	Contribution, %	mS	F	σ^2	Contribution, %
Among varieties	13	2359.00	10.9**	71.43	11.6	142.12	31.5**	4.59	29.0
Among zones	1	17556.00	81.4**	82.57	13.4	9.96	2.2	0.00	0.0
Over years	1	2520.00	11.6**	10.97	1.8	98.57	21.9**	0.45	2.8
Variety × zone	13	1387.00	6.4**	78.07	12.7	52.90	11.7**	3.23	20.4
Variety × year	13	752.00	3.5**	35.73	5.8	16.11	3.6**	0.77	4.9
Zone × year	1	5974.00	27.7**	54.84	8.9	48.98	10.9**	0.42	2.7
Variety × year × zone	13	711.00	3.3**	66.00	10.7	18.38	4.1**	1.85	11.7
Residual	364	216.00	–	216.00	35.1	4.50	–	4.50	28.5
Variability	df	Total yield				Firmness of fruit			
		mS	F	σ^2	Contribution, %	mS	F	σ^2	Contribution, %
Among varieties	13	434030.00	7.6**	12569.57	11.6	93287.00	37.4**	3026.50	26.3
Among zones	1	3164835.00	55.6**	14799.49	13.7	286524.00	114.9**	1352.53	11.8
Over years	1	17798.00	0.31	0.00	0.0	111846.00	44.9**	520.73	4.5
Variety × zone	13	297261.00	5.2**	16021.20	14.8	21585.00	8.7**	1272.87	11.1
Variety × year	13	124834.00	2.3**	4526.07	4.2	15913.00	6.4**	894.73	7.8
Zone × year	1	396694.00	7.0**	3235.72	3.0	41738.00	16.7**	373.77	3.3
Variety × year × zone	13	98839.00	1.7	0.00	0.0	14206.00	5.7**	1561.87	13.6
Residual	364	56943.00	–	56943.00	52.7	2492.0	–	2492.00	21.7

Variability	df	Sugar-acid ratio in berries			
		mS	F	σ^2	Contribution, %
Among varieties	13	20.01	8.2**	0.59	9.6
Among zones	1	250.48	102.0**	1.18	19.3
Over years	1	168.35	68.6**	0.79	12.9
Variety × zone	13	9.13	3.7**	0.44	7.3
Variety × year	13	1.75	0.7	0.00	0.0
Zone × year	1	72.76	29.6**	0.67	10.9
Variety × year × zone	13	3.21	1.3	0.00	0.0
Residual	364	2.45	–	2.46	40.1

Notes: df, degrees of freedom; mS, mean square; F, Fisher-test (F-test); σ^2, variance; $^{**}p < 0.01$, Fisher's test.

го затруднения применен многомерный статистический метод – кластерный анализ, адекватный решаемой задаче.

Полученные среднегодовые значения пяти признаков – количества ягод, средней массы ягоды, общего урожая, плотности мякоти ягоды, сахарокислотного индекса ягод – составили информативный комплекс признаков для каждого из сортов, учтенных в разных городах, проанализированный при помощи кластерного анализа по методу Уорда (рисунок). Это метод кластеризации предусматривает группировку объектов по критерию максимума межгрупповой и минимума внутригрупповой дисперсии. Такой подход позволяет надеяться на выделение наиболее различающихся групп сортов.

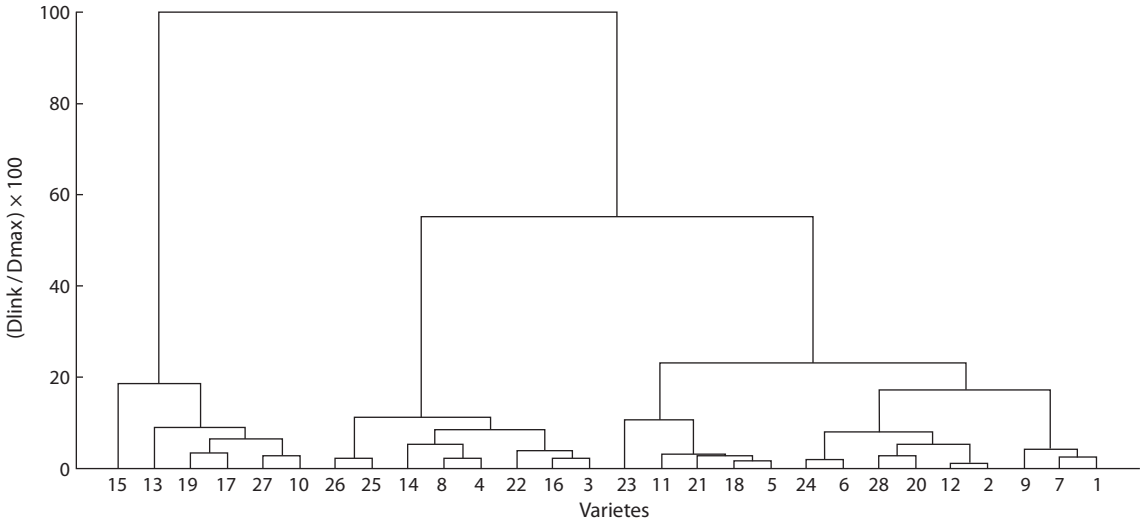
Результаты кластеризации показывают, что на уровне 40 усл. ед. выделяются три группы сортов (в первой – 6, во второй – 8, в третьей – 14). Средние значения признаков для каждой из выделенных групп сортов собраны в табл. 4.

Средние значения признаков в разных кластерах были подвергнуты попарному сравнению с помощью *t*-критерия Стьюдента. Оказалось, что значения таких признаков, как средняя масса ягоды, плотность мякоти ягоды и сахарокислотный индекс, не имеют статистически значимых различий между кластерами. Признак количество ягод различается между первым и вторым кластерами ($t = 4.34$ при $p < 0.01$), а также между первым и третьим кластерами ($t = 4.01$ при $p < 0.01$). Общий урожай имеет статистически значимые различия между первым и вторым кластерами ($t = 10.99$ при $p < 0.01$), первым и третьим кластерами ($t = 6.91$ при $p < 0.01$), вторым и третьим кластерами ($t = 6.70$ при $p < 0.01$).

Table 3. Comparison of mean traits of strawberry varieties grown in different climatic zones

Variety (zone)	Traits					Variety (zone)	Traits				
	1	2	3	4	5		1	2	3	4	5
Asia (a)	55.15*	14.91*	827.59	281.00*	6.80*	Nelli (a)	75.95*	16.41	1241.21*	468.50	5.40
Asia (b)	41.60*	18.22*	758.53	317.00*	5.25*	Nelli (b)	39.90*	15.19	606.23*	388.00	4.05
Alina (a)	44.20*	12.72*	558.71	391.50*	6.74*	Onda (a)	78.05*	12.97*	1019.21*	391.00	8.44
Alina (b)	35.50*	17.06*	606.54	308.00*	4.20*	Onda (b)	43.50*	17.24*	749.35*	402.00	7.10
Alba (a)	48.00	15.83	747.36	438.00	5.66	Roxana (a)	56.30*	17.02*	950.93*	382.50*	6.33*
Alba (b)	47.10	14.72	693.08	378.00	5.25	Roxana (b)	34.30*	20.05*	687.70*	290.00*	4.35*
Elizabeth II (a)	69.00	12.07*	818.79*	328.42	8.92*	Syria (a)	62.95*	12.40	782.44*	450.50*	7.34
Elizabeth II (b)	70.90	8.87*	629.06*	320.90	5.22*	Syria (b)	43.40*	12.50	546.08*	328.00*	6.20
Irma (a)	58.15	15.45	895.62	300.50	7.00*	Florence (a)	66.00	13.69*	898.45*	468.50*	7.57*
Irma (b)	65.90	14.63	979.40	323.00	5.20*	Florence (b)	58.00	11.54*	669.87*	344.00*	4.65*
Clery (a)	54.10	12.76	706.70	446.00*	8.34*	Honeoye (a)	53.35*	9.40*	498.56	291.50*	5.98
Clery (b)	59.60	12.56	748.20	304.00*	6.35*	Honeoye (b)	42.70*	11.46*	489.05	248.00*	7.15
Malling Pandora (a)	79.30*	16.00*	1117.47*	288.00*	5.92	Elsanta (a)	82.80*	12.02	991.02*	267.00	7.57*
Malling Pandora (b)	53.80*	11.13*	598.53*	223.00*	4.85	Elsanta (b)	54.90*	12.98	712.55*	245.00	5.35*

Notes: Trait designations: 1, number of berries (pc); 2, mean fruit weight (g); 3, total yield (g per plant); 4, firmness of fruit (g); 5, sugar-acid index of berries. Experimental plots: a, outskirts of Krasnodar, b, outskirts of Krymsk; * Differences between varieties planted in different climatic zones significant at $p < 0.05$.



Cluster analysis of strawberry varieites in experimental plots in the vicinity of Krasnodar and Krymsk.

Experimental plots: a, outskirts of Krasnodar, b, outskirts of Krymsk. Varieties: 1, Asia (a); 2, Asia (b); 3, Alina (a); 4, Alina (b); 5, Alba (a); 6, Alba (b); 7, Elizabeth II (a); 8, Elizabeth II (b); 9, Irma (a); 10, Irma (b); 11, Clery (a); 12, Clery (b); 13, Malling Pandora (a); 14, Malling Pandora (b); 15, Nelli (a); 16, Nelli (b); 17, Onda (a); 18, Onda (b); 19, Roxana (a); 20, Roxana (b); 21, Syria (a); 22, Syria (b); 23, Florence (a); 24, Florence (b); 25, Honeoye (a); 26, Honeoye (b); 27, Elsanta (a); 28, Elsanta (b).

Наиболее высоким урожаем обладают сорта, вошедшие в первый кластер: Нелли (Краснодар), Моллинг Пандора (Краснодар), Роксана (Краснодар), Онда (Краснодар), Эльсанта (Краснодар), Ирма (Крымск). Наименьший урожай показали сорта из второго кластера: Хоней (Крымск), Хоней (Краснодар), Моллинг Пандора (Крымск), Елизавета II (Крымск), Алина (Крымск), Сирия (Крымск), Нелли (Крымск), Алина (Краснодар). Средний урожай имели сорта из третьего кластера: Флоренс (a), Клери (a), Сирия (a), Онда (b), Альба (a), Флоренс (b), Альба (b), Эльсанта (b), Роксана (b), Клери (b), Азия (b), Ирма (a), Елизавета II (a), Азия (a).

Полученные данные свидетельствуют о том, что сорта Нелли, Моллинг Пандора, Роксана, Онда, Эльсанта дают максимальный урожай в климатических условиях Краснодара, а сорт Ирма – в условиях Крымска. Сорта Хоней

Table 4. Mean values of traits in the recognized clusters of varieties

Traits	Cluster		
	1	2	3
Number of berries, pc	73.05	47.96	53.73
Mean fruit weight, g	14.84	12.30	14.60
Total yield, g per plant	1049.87	566.59	764.01
Firmness of fruit, g	353.33	312.36	356.63
Sugar-acid ratio in berries	6.47	5.55	6.42

и Алина имели невысокий урожай в обеих климатических зонах. В климатических условиях Крымска не смогли полностью реализовать свой потенциал урожая такие сорта, как Моллинг Пандора, Елизавета II, Сирия и Нелли.

Один из способов оценки генотипических различий сортов, выращенных в разных климатических зонах, – измерение евклидова расстояния между ними по результатам проведенного кластерного анализа. Информативный комплекс, который в нашем случае состоял из пяти признаков, позволяет получить интегральную характеристику изучаемых сортов. Так как кластерный анализ проводится с учетом евклидовых расстояний между объектами, по этой величине можно судить о потенциальных генетически обусловленных различиях между сортами, отражающихся во взаимодействии генотип–среда. Чем больше вклад этого взаимодействия в общую изменчивость, тем больше различия между сортами.

При реализации этого алгоритма мы получили следующие евклидовы расстояния для сортов, выращенных в разных климатических условиях, в порядке возрастания: Хоней – 46, Азия – 79, Альба – 81, Ирма – 87, Алина – 97, Клери – 148, Елизавета II – 190, Флоренс – 260, Сирия – 267, Онда – 272, Роксана – 280, Эльсанта – 281, Моллинг Пандора – 524, Нелли – 641. Отмечаются значительные различия для сортов Нелли и Моллинг Пандора, реализующих свой потенциал урожайности в разных климатических условиях. Евклидово расстояние для сортов Хоней и Алина, не реализовавших свой потенциал в обоих изученных климатических зонах, оказалось небольшим. Довольно значительными были евклидовы расстояния для сортов Флоренс, Сирия, Онда, Роксана, Эльсанта, выращенных в условиях Краснодара и Крымска.

Результаты изучения сортов земляники, выращенных в разных климатических условиях, свидетельствуют, что им свойственны различия в структуре изменчивости признаков продуктивности и качества ягод. Для условий Крымска преобладающим оказалось влияние генотипа сорта, а для условий Краснодара, кроме влияния генотипа сорта, оказывается существенной и средовая компонента в виде взаимодействия генотип–среда. Статистически достоверное влияние зоны выращивания установлено для признаков продуктивности и качества ягод, за исключением средней массы ягоды. При этом различия средних значений признаков у сортов могут быть как существенными, так и частично или полностью отсутствующими.

Для выявления перспективных сортов при выращивании в изучаемых зонах рекомендуется использовать кластерный анализ по информативному комплексу признаков с вычислением евклидовых расстояний между сортами, выращенными в разных условиях. Величина евклидова расстояния будет являться мерой влияния конкретной среды на генотип растений. Чем меньше значение евклидового расстояния у сорта, согласно комплексу изученных признаков, тем большей стабильностью характеризуется этот сорт.

Заключение

Предложенный методический подход, основанный на алгоритме многомерной математической статистики, можно использовать в качестве инструмента оценки влияния взаимодействия генотип–среда на комплекс хозяйственно-биологических признаков, определяющих продуктивность и качество ягод сортов земляники садовой в разнообразных экологических условиях выращивания с учетом их стабильности. Он позволит повысить эффективность методов оценки адаптивного и продукционного потенциала сортов земляники и может быть использован для определения генотипов земляники с высокими биометрическими характеристиками хозяйственно ценных признаков для селекционно-генетических исследований и промышленного производства.

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Изучение сортов овса (*Avena sativa* L.) различного географического происхождения по качеству зерна и продуктивности

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С целью выявления образцов с минимальным и максимальным содержанием β-глюканов в зерне проведен скрининг сортов овса, выращенного в условиях Восточной Сибири в течение трех лет. Для определения перспективности дальнейшего использования образцов овса параллельно измеряли другие химические, физические и продукционные характеристики: содержание белка и масла в зерне, пленчатость зерна, натуру, массу 1000 зерен, продолжительность вегетационного периода и величину урожайности. Объектом комплексной оценки служили 14 пленчатых и 5 голозерных образцов овса коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР) различного географического происхождения. Пленчатые образцы формировали зерно с содержанием β-глюканов от 2.9 до 5.2 %, голозерные – от 3.7 до 4.8 %. По минимальным значениям этого показателя выделились сорта красноярской селекции – Тубинский, Казыр, Саян (около 3 %), по максимальному – зарубежный образец Местный Тунис 1 (5.2 %). Наибольшее содержание масла в зерне имеют возделываемые в настоящее время в Красноярском крае сорта Тубинский, Казыр и Саян. Повышенное накопление белка в зерне обнаружено у пленчатого сорта Местный Тунис 1 и голозерного образца Вятский. По содержанию β-глюканов в зерне с учетом других его характеристик и величины урожайности лучшие образцы для крупяного направления (максимальный уровень этих веществ) – Местный Тунис 1, Медведь и Тайдон, а для кормового использования (минимальный уровень) – Тубинский, Вятский и Голец. Не обнаружено заметного преимущества голозерных образцов по сравнению с пленчатыми по содержанию β-глюканов в зерне. У пленчатых образцов отмечена высокая сила положительной связи между содержанием масла либо β-глюканов в зерне и годом выращивания овса. У голозерных форм четкой связи между содержанием рассматриваемых химических веществ в зерне разных образцов овса и годом их выращивания не установлено.
Ключевые слова: овес посевной; оценка; зерно; вегетационный период; масса 1000 зерен; урожайность; натура; пленчатость; масло; белок; β-глюканы.

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The study of oat varieties (*Avena sativa* L.) of various geographical origin for grain quality and productivity

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In order to identify samples with a minimum and maximum content of β-glucans in the grain, screening of oats grown in Eastern Siberia for three years was performed. To determine the prospects for further use of oat samples, other chemical, physical and production characteristics were measured in parallel: the protein and oil content in the grain, its film content, test weight, 1000 grains weight, the vegetation period and the yield. The object of a comprehensive evaluation was 14 hulled and naked 5 VIR (N.I. Vavilov All-Russian Institute of Plant Genetic Resources) oats samples of different origin, mainly from Siberia. The hulled samples formed grains with β-glucan content from 2.9 to 5.2 %, while the naked ones, from 3.7 to 4.8 %. The lowest values were in the Krasnoyarsk varieties Tubinskiy, Kazyr, Sayan (about 3 %); the highest, in the foreign accession Local Tunisia 1 (5.2 %). The highest oil content was shown by

Tubinsky, Kazyr and Sayan, all currently cultivated in the Krasnoyarsk region. An increased accumulation of protein in grain was observed in the hulled variety Local Tunisia 1 and the naked accession of Vyatskiy. According to the content of β-glucans in the grain, taking into account its other characteristics and yield values, the best samples for the food direction (the maximum level of these substances) are Local Tunisia 1, Medved and Taidon, and for feed use (the minimum level) are Tubinskiy, Vyatskiy and Golets. There was no noticeable advantage of naked samples in comparison with hulled ones in the content of β-glucans in the grain. A high strength of the positive relationship between the content of oil or β-glucans in the grain and the year of oat cultivation was observed in the hulled samples. In naked forms, a clear link between the concentrations of chemicals in the different grain samples of oats and the year of cultivation has not been established.
Key words: oats; evaluation; grain; growing season; 1000 grain weight; yield; nature; test weight; film; oil; protein; β-glucans.

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Введение

Овес посевной (*Avena sativa* L.) – древняя культура, известная человечеству не менее 4000 лет. Питательная ценность зерна овса определяется содержанием в нем углеводов, белков, липидов, витаминов и других биологически активных веществ. По сравнению с ячменем и другими зерновыми культурами белки зерна овса имеют более высокую биологическую ценность (Солоненко, Омельянчук, 1991). Зерно этой культуры ценится своими вкусовыми и диетическими качествами, содержит ненасыщенные жирные кислоты, основные минеральные элементы, глобулярные белки и высокое количество β-глюканов, характеризуется наличием разнообразных веществ с антиоксидантными свойствами (Shewry et al., 2008; Zute et al., 2016). Зерно используют на корм животным, в питании человека, а также в качестве пищевого и промышленного сырья (Arendt, Zannini, 2013; Loskutov, Polonskiy, 2017).

Среди особых свойств зерна овса можно отметить наличие специфических водорастворимых пищевых волокон – β-глюканов. Они представляют собой растворимые в воде линейные гомополисахариды, молекулы которых состоят примерно из 2500 остатков β-(1.3)- и β-(1.4)-D-глюкопиранозы. Известно, что, с одной стороны, β-глюканы оказывают профилактическое и лечебное воздействие на организм человека (Brownlee, 2011; Абугалиева, Савин, 2013; Harland, 2014), поддерживая или уменьшая количество холестерина в крови (Harland, 2014) и способствуя снижению риска сердечно-сосудистых заболеваний. С другой стороны, повышенная концентрация β-глюканов в зерне отрицательно коррелирует с питательной ценностью корма, используемого для нежвачных животных, так как создает трудности для его эффективного усвоения в желудочно-кишечном тракте (Svihus, Gullord, 2002).

Таким образом, для создания коммерческих сортов овса кормового и пищевого направлений необходимо проведение селекции, соответственно, на минимальное и максимальное содержание β-глюканов в зерне (Zhu et al., 2016). Информация о величине этих химических соединений в зерне разных образцов овса чрезвычайно скудна (Loskutov, Polonskiy, 2017), а для сортов, выращиваемых в условиях Сибири, полностью отсутствует. Поэтому целесообразно выполнение измерений содержания β-глюканов в зерне у существующего разнообразия образцов овса.

Цель работы состояла в оценке образцов овса на содержание β-глюканов в зерне. Для определения перспективности дальнейшего использования образцов овса в задачу исследований входило параллельное измерение содержания белка и масла в зерне, его физических характеристик, а также величины урожайности.

Материалы и методы

Объектами изучения были 14 пленчатых и 5 голозерных сортов овса из коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР), 5 из которых имели зарубежное происхождение, остальные были сибирской селекции (табл. 1).

Исследования проводили в 2015–2017 гг. на опытных полях Красноярского научно-исследовательского института сельского хозяйства (КрасНИИСХ ФИЦ КНЦ СО РАН), расположенных в лесостепной зоне Емельяновского района Красноярского края Восточной Сибири. Почва опытного участка представлена черноземом обыкновенным маломощным, характеризующимся агрохимическими показателями: содержание гумуса (по Тюрину) – 6.00 %, $N\text{-}NO_3$ (ионометрический экспресс-метод) – 31.3 мг/кг почвы, P_2O_5 (по Мачигину) – 5.00 мг/100 г почвы, K_2O (по Мачигину) – 21.9 мг/100 г почвы; реакция почвенного раствора близка к нейтральной (pH – 6.2). Предшественник – чистый пар. Площадь делянки – 1.8 м^2. Посев проведен в оптимальные для культуры сроки, во вторую декаду мая, уборку образцов осуществляли по мере их созревания. Погодные условия в Красноярской лесостепи в годы исследования были контрастными: 2015 г. – засушливый (ГТК – 0.95); 2016 и 2017 гг. – влажные (ГТК – 1.59 и 1.47).

В полевых условиях регистрировали длину вегетационного периода растений. После уборки зерна определяли его физические и химические характеристики в каждом образце: массу 1000 зерен по методике ВИР (Лоскутов и др., 2012), пленчатость – в соответствии со стандартной методикой (ГОСТ-10843-76, 2001), натуру – известным микрометодом (Walker, Panozzo, 2011), содержание белка – по Къельдалю (ГОСТ-10846-91, 2009), содержание масла и β-глюканов – на автоматическом зерновом анализаторе Infratec Analyzer1241 (Munck, 2005) с использованием 50 мл кюветы. Компания ООО EIRA (официальный представитель FOSS Analytical Ltd. в Латвии) разработала калибровочную модель для определения β-глюканов в зерне. Данные из 150 образцов зерна зерновых культур,

Table 1. Oat accessions used in the work

VIR accession no.	Name	Variety	Origin
		Hulled	
15008	Tubinskiy	mutica	Krasnoyarsk region
–	Kazyr	mutica	
14043	Sayan	aurea	
15114	Pegasus	aristata	Altay region
15113	Korifey	aristata	
15185	Altair	mutica	Kemerovo region
15444	Sapsan	mutica	Kirov region
15443	Avatar	mutica	
15243	Envis	byzantina	England
15259	PA 7836-9687	byzantina	United States
15324	Local Tunisia 1	byzantina	Tunisia
15127	SW Betania	aristata	Sweden
–	Medved	mutica	Kirov region
14857	Krechet	mutica	
		Naked	
15067	Golets	inermis	Krasnoyarsk region
15115	Aldan	inermis	Kemerovo region
15183	Taydon	inermis	
14960	Vyatskiy	inermis	Kirov region
15120	Gosha	inermis	Belarus

проанализированные методами AOAC 995.16 и ICC № 168 для β-глюкана (Megasyme), были использованы для разработки модели калибровки. Калибровочная модель регулируется ежегодно с дополнительными 20–30 данными по эталонному методу. Стандартная ошибка измерения на приборе составляла 0.3 %. Повторность определения каждого показателя двукратная.

Статистическую обработку данных проводили с помощью стандартных компьютерных программ Microsoft Excel. Достоверность результатов оценивали при $p \leq 0.05$.

Результаты

Впервые представлены данные по содержанию β-глюканов в зерне сортов овса, выращенных в условиях Восточной Сибири. Сорта были преимущественно сибирской селекции, в том числе широко распространенные местные сорта – Тубинский и Саян. Содержание β-глюканов в зерне изменялось в зависимости от сортовой принадлежности и года выращивания (табл. 2). Пленчатые образцы формировали зерно с содержанием β-глюканов от 2.9 до 5.2 %, голозерные – от 3.7 до 4.8 %. По минимальным значениям этого показателя выделились сорта красноярской селекции – Тубинский, Казыр, Саян (около 3 %), по максимальному – зарубежный образец Местный Тунис 1 (5.2 %). Следует отметить, что, несмотря на сильное варьирование значения указанного биохимического признака у сорта Местный Тунис 1 по годам (26.4 %), последний характеризовался высоким содержанием β-глюканов во все годы испытания. Наименьшим варьированием рассматриваемого признака за три года изучения отличались пленчатые образцы Сапсан, Кречет, SW Betania и голозерный сорт Алдан.

В ходе исследований сортов овса определено содержание двух других важных химических веществ в зерне (см. табл. 2). В среднем за три года размах варьирования содержания масла в зерне соответствовал 4.4–7.2 % у пленчатых сортов и 7.3–9.0 % у голозерных форм. Самые высокие значения отмечены у пленчатого сорта Местный Тунис 1 и голозерного образца Вятский. Наиболее стабильное содержание масла во все годы было у возделываемых в Красноярском крае сортов Тубинский, Казыр и Саян. Однако при этом они характеризовались относительно низким его содержанием: в пределах 4.6 %. Особый интерес представляет голозерный сорт Вятский, сочетающий высокое содержание масла в зерне с низким варьированием этого показателя в различные по условиям годы выращивания.

Содержание белка в зерне как критерий его качества зависит от сорта, погодных условий и уровня агротехники (Tamm, 2003). В наших опытах этот показатель изменялся от 11.7 до 15.1 % у пленчатых и от 14.8 до 16.8 % у голозерных образцов. Повышенное накопление белка в зерне отмечено у пленчатого сорта Местный Тунис 1 и голозерного образца Вятский. Стабильное по годам содержание белка при среднем его значении 12.6–13.0 % установлено у сортов Саян, Альтаир, Медведь, SW Betania.

Table 2. Biochemical parameters of oat grain samples averaged over three years

Accession name	Content, %					
	oil		protein		β-glucan	
	$\bar{x}$	Cv	$\bar{x}$	Cv	$\bar{x}$	Cv
Hulled						
Tubinskiy	4.63	4.5	12.46	7.6	2.90	10.3
Kazyr	4.63	5.4	11.69	6.7	3.03	6.7
Sayan	4.57	2.5	12.66	2.7	3.04	6.5
Pegasus	7.00	17.4	12.39	5.0	4.50	23.5
Korifey	5.76	9.7	12.96	5.2	4.27	13.4
Altair	6.50	14.0	12.59	4.6	4.28	21.6
Sapsan	6.10	21.8	13.25	6.9	3.83	1.5
Avatar	5.89	19.3	12.11	11.4	4.30	6.2
Medved	5.75	19.5	13.01	4.7	4.50	14.6
Krechet	4.96	9.2	12.99	11.0	3.37	4.5
Envis	4.35	13.3	12.21	5.4	3.60	12.1
PA 7836-9687	6.43	18.2	12.82	8.6	4.20	10.4
Local Tunisia 1	7.25	25.1	15.10	8.4	5.17	26.4
SW Betania	5.54	5.5	12.73	3.6	4.45	1.6
LSD_{05}	1.00		1.17		0.85	
Naked						
Golets	7.28	8.5	16.71	9.0	4.06	13.7
Aldan	8.79	8.5	16.17	14.5	4.14	1.7
Taydon	8.27	20.6	16.48	5.2	4.77	9.9
Vyatskiy	9.01	5.6	16.88	10.3	3.73	8.6
Gosha	8.77	15.5	14.86	7.8	4.37	16.8
LSD_{05}	1.60		2.60		0.88	

Физические характеристики зерна, а также показатели продуктивности образцов овса приведены в табл. 3. Масса 1000 зерен – важный элемент структуры урожая овса, определяющий наряду с натурой технологические и биохимические свойства зерна (Couvreur, 1985). Существенное преимущество по этому показателю имели образцы Пегас, Корифей, Альтаир, Медведь и SW Betania. Варьирование значений массы 1000 зерен у образцов было небольшим, кроме сортов Тубинский, Саян, Пегас (пленчатая группа) и голозерного образца Алдан.

Показатель натуры зерна овса характеризует возможность его использования на крупу и другие продукты питания (Свиркова и др., 2016). Важное практическое значение для переработки имеют сорта с натурой не ниже 550 г/л (ГОСТ 28673-90…, 2010). В среднем размах варьирования этого показателя составил у пленчатых образцов 484–609 г/л, а у голозерных – 588–815 г/л в зависимости от сорта и года выращивания. Наибольшую натуру зерна имели пленчатый сорт Корифей и голозерный Голец. Как и в случае с показателем «масса 1000 зерен», коэффициенты вариации натуры были наибольшими у образцов Тубинский и Алдан.

Известно, что значение пленчатости зерновки овса варьирует в широких пределах и зависит от сорта, условий произрастания растений, степени зрелости зерна и его крупности. Внешние пленки имеют низкую питательную ценность, поэтому их массовую долю у овса целесообразно снижать (Баталова, 2014). Согласно данным табл. 3, значение пленчатости зерна овса изменялось в довольно широких пределах: от 18.5 до 29.0 %. Среди изучаемых образцов минимальная величина этого показателя отмечена у сортов Альтаир, Пегас, Медведь и Envis. К раннеспелым можно отнести сорта Сапсан, Аватар, Медведь, Кречет, PA 7836-9687 и SW Betania (см. табл. 3). Продолжительность вегетационного периода у них меньше, чем у стандарта Тубинский (76 сут). По длине вегетации все голозерные образцы существенно не отличались от стандарта Голец.

Одна из важнейших характеристик любого сорта – его урожайность (Creissen et al., 2016). В среднем за три года выращивания практически такую же величину урожая, как у стандартного сорта Тубинский, сформировали пленчатые образцы Казыр, Медведь и Местный Тунис 1 (см. табл. 3). Среди голозерных образцов следует отметить сорт Алдан, у которого зарегистрировано значение урожайности почти на уровне стандарта Голец. Голозерные образцы по величине урожайности существенно уступали пленчатым.

Результаты вычисления коэффициентов корреляции между химическими и физическими показателями зерна у образцов овса приведены в табл. 4. Показано наличие существенных положительных связей между содержанием β-глюканов и массой 1000 зерен, содержанием β-глюканов и содержанием масла в зерне; содержанием масла и массой 1000 зерен. Установлена значимая отрицательная корреляционная связь между содержанием масла в зерне и его натурой.

Полученные в ходе изучения данные по оценке сортов овса послужили основой для их ранжирования по всем изученным показателям. По содержанию β-глюканов, белка в зерне, урожайности лучшими для крупяного направления в селекции оказались сорта Местный Тунис 1, Медведь и Тайдон, а для кормового использования – Тубинский, Вятский и Голец (табл. 5).

Результаты анализа корреляционных связей между содержанием каждого изучаемого химического вещества у сортов овса по годам представлены в табл. 6. Расчет коэффициентов корреляции выполняли для сортов, контрастных по содержанию, соответственно, масла, белка или β-глюканов в зерне, а именно: шесть пленчатых (три с максимальным и три с минимальным содержанием каждого вещества) и четыре голозерных (два с максимальным и два с минимальным содержанием). Для пленчатых сортов характерны высокий уровень (значение) положительной связи между содержанием масла или β-глюканов в зерне и годом выращивания, а также сильная и средняя связь между содержанием белка и годом выращивания. Что касается голозерных сортов, то для них четкой связи между содержанием рассматриваемых веществ в зерне разных образцов и годом выращивания овса не найдено.

Table 3. Physical parameters of grain and productivity of oat accessions averaged over three years

Accession name	1000 grain weight, g		Est weight, g/L		Hull content, %		Vegetation period, days		Yield, g/m^2	
	$\bar{x}$	Cv, %	$\bar{x}$	Cv, %	$\bar{x}$	Cv	$\bar{x}$	Cv, %	$\bar{x}$	Cv, %
					Hulled					
Tubinskiy	34.5	10.3	547	10.7	26.6	8.0	76	4.7	1059	60.0
Kazyr	35.0	1.4	557	6.2	22.7	12.4	74	9.5	1003	42.0
Sayan	36.8	10.5	537	4.5	26.5	4.4	73	8.2	943	31.0
Pegasus	43.4	10.6	537	4.4	21.8	11.6	79	5.9	860	43.6
Korifey	43.5	1.3	565	2.0	23.7	3.6	76	4.7	926	34.1
Altair	45.4	5.2	539	8.5	20.6	8.8	73	10.2	814	46.7
Sapsan	38.3	6.4	554	8.8	23.7	0.8	70	6.2	965	35.1
Avatar	35.2	4.6	538	8.8	23.9	3.6	70	6.6	921	38.7
Medved	38.1	3.9	549	7.0	21.8	8.9	71	11.4	1031	32.9
Krechet	36.8	2.6	548	5.5	23.8	9.8	70	9.3	771	22.6
Envis	32.1	7.3	528	4.3	22.5	7.1	74	4.7	950	74.4
PA 7836-9687	41.4	3.3	558	3.5	23.0	6.4	72	3.5	880	23.8
Local Tunisia 1	44.4	4.1	526	4.6	22.6	3.1	73	7.2	1058	42.7
SW Betania	37.4	1.7	533	8.6	23.7	10.4	72	4.4	872	39.7
HCP_{05}	3.9		60		2.9		4.0		286	
					Naked					
Golets	28.1	4.6	746	5.5	–	–	73	6.0	671	59.8
Aldan	25.0	10.6	699	16.2	–	–	75	8.2	593	60.2
Taydon	30.5	2.1	703	4.4	–	–	73	5.6	619	41.0
Vyatskiy	26.8	5.2	693	14.0	–	–	72	8.4	596	57.0
Gosha	27.8	5.9	698	4.4	–	–	71	7.3	493	17.5
LSD_{05}	3.0		131		–		4.0		291	

Table 4. Correlation coefficients between physical and chemical parameters of grain in oat accessions; data averaged over three years

Grain parameter	1000 grain weight	Test weight	Hull content	Content		
				oil	protein	β-glucan
1000 grain weight	–					
Test weight	0.075 0.218	–				
Hull content	–0.483 –	0.094 –	–			
Oil content	0.843* –0.425	–0.105 –0.968*	–0.547 –	–		
Protein content	0.520 –0.425	–0.229 0.308	–0.075 –	0.556 –0.314	–	
β-glucan content	0.672* 0.692	–0.276 –0.068	–0.604 –	0.856* –0.155	0.571 –0.369	–

* Statistically significant. Numerator: hulled accessions; denominator: naked accessions.

Table 5. Ranking of oat varieties according to the content of β-glucans and other parameters important in industrial and breeding practice

M 1000 grain weight*	M Test weight	m Hull content	Content				M Yield
			M oil	M protein	M β-glucan content	m β-glucan content	
				Hulled			
Altair	Korifey	Altair	Local Tunisia 1	Local Tunisia 1	Local Tunisia 1	Tubinskiy	Tubinskiy
Local Tunisia 1	PA 7836-9687	Pegasus	Pegasus	Sapsan	Pegasus	Kazyr	Local Tunisia 1
Korifey	Kazyr	Medved	Altair	Medved	Medved	Sayan	Medved
				Naked			
Taydon	Golets	–	Vyatskiy	Vyatskiy	Taydon	Vyatskiy	Golets
Golets	Taydon	–	Aldan	Golets	Gosha	Golets	Taydon
Gosha	Aldan	–	Gosha	Taydon	Aldan		Vyatskiy

* M, maximum; m, minimum.

Table 6. Correlation coefficients between the contents of fat, protein, or β-glucans in the grain of contrasting oat accessions grown in different years

Grain parameter	Correlation coefficients in samples grown in years:		
	2015 vs 2016	2016 vs 2017	2015 vs 2017
Content			
oil	0.981*	0.798	0.881*
	0.524	0.722	–0.077
protein	0.970*	0.679	0.578
	0.942*	–0.365	–0.647
β-glucan	0.974*	0.834*	0.756
	0.965*	–0.087	0.120

Notes: Accessions contrasting in the contents of oil, protein, or β-glucans in grain: six hulled (three with maximum and three with minimum contents of each substance) and four naked (two with maximum and two with minimum contents of each substance). Numerator: hulled accessions; denominator: naked accessions.

Обсуждение

Анализ диапазона изменчивости ряда важных биохимических признаков качества для сортов овса, выращиваемых в условиях Восточной Сибири в течение трех лет, показал, что сортовые различия по содержанию β-глюканов в зерне составили в среднем от 2.9 до 5.2 %. Близкий диапазон изменчивости содержания β-глюканов (от 3.3 до 6.2 %) определен в ходе выполнения российско-шведских совместных исследований образцов овса, культивируемых в Европе (Loskutov, Rines, 2011). В нашей работе по минимальным значениям этого признака выделены сорта красноярской селекции – Тубинский, Казыр, Саян (около 3 %), по максимальному – зарубежный образец Местный Тунис 1 (5.2 %).

В течение трех лет выращивания значение содержания масла в зерне колебалось от 4.4 до 7.2 % у пленчатых и от 7.3 до 9.0 % у голозерных образцов овса соответственно. Наиболее высокое содержание масла определено для пленчатого сорта Местный Тунис 1 и голозерного образца Вятский. На фоне практически равного содержания белка в зерне для большинства изученных образцов повышенное его содержание имели пленчатый сорт Местный Тунис 1 и голозерный Вятский. Следует отметить интересный факт: по высоким значениям всех трех рассмотренных выше биохимических показателей качества выделился африканский сорт Местный Тунис 1 отдаленного происхождения. Среди лучших по массе 1000 зерен, натуре и пленчатости не оказалось ни одного сорта, который выделился хотя бы по одному из анализируемых в работе химических веществ.

Один из известных приемов повышения адаптивности сортов овса к неблагоприятным условиям Сибири – создание форм с коротким вегетационным периодом растений (Svirkova et al., 2016). Вероятно, поэтому большинство взятых нами в исследование пленчатых и голозерных сортов по длине вегетационного периода являются раннеспелыми. В среднем за три года выращивания практически такую же урожайность, как у стандартного сорта Тубинский, имели пленчатые сорта Казыр, Медведь и Местный Тунис 1. Голозерный образец Алдан показал урожайность на уровне стандарта Голец. Все голозерные сорта по урожайности существенно уступали пленчатым. При этом отставание голозерных сортов не связано лишь с меньшей массой зерновки вследствие отсутствия у них пленок. Основная причина невысокой массы 1000 зерен – щуплость эндосперма зерна. Так, средняя масса 1000 зерен всех пленчатых образцов превышает этот показатель у голозерных на 40.2 %, при этом средняя доля пленок составляет только 23.4 %.

В нашей работе у пленчатых образцов овса показано наличие значимых положительных связей между массой 1000 зерен, с одной стороны, и содержанием β-глюканов либо содержанием масла в зерне, с другой, а также между содержанием β-глюканов и содержанием масла. У голозерных образцов установлена существенная отрицательная корреляционная связь между содержанием масла в зерне и его натурой. О факте выявления значимой положи-

тельной корреляции между содержанием β-глюканов и урожайностью, натурой и массой 1000 зерен, а также существенной отрицательной корреляции рассматриваемого химического показателя со степенью пленчатости зерновки имеются сведения в литературе (Saastamoinen et al., 1992; Martinez et al., 2010).

На контрастных по содержанию химических веществ в зерне пленчатых образцах в работе зарегистрирована высокая сила положительной связи между содержанием масла или β-глюканов в зерне по годам выращивания овса. Кроме того, аналогичная тенденция отмечена для содержания белка. Это может означать, что при возделывании овса в разные годы содержание масла и β-глюканов (и отчасти белка) в зерне у пленчатых образцов изменяется почти синхронно, т. е. ранжирование сортов по уровню этих химических соединений в зерне год от года практически не нарушается. У голозерных образцов четкой связи между содержанием рассматриваемых веществ в зерне разных образцов и годом выращивания не обнаружено.

Существенная значимая корреляция между содержанием β-глюканов в зерне пленчатых образцов по годам исследования указывает на высокую зависимость этого химического показателя от генотипа. Последнее означает большую вероятность успешной селекции на этот качественный признак овса. Опубликованные недавно результаты выполнения Европейского проекта по изучению генетических ресурсов овса также продемонстрировали заметный вклад генетической составляющей в формирование рассматриваемого признака (Redaelli et al., 2013).

В зерне пленчатых образцов овса, выращиваемого в Восточной Сибири, установлена положительная корреляция между содержанием β-глюканов и масла. Этим результатом подтвержден недавно обнаруженный одним из наших соавторов аналогичный эффект при культивировании различных сортов овса в условиях Европы (Zute et al., 2016). По-видимому, содержание масла может служить косвенным индикатором содержания β-глюканов в зерне, несмотря на то, что оба соединения имеют разный механизм биосинтеза накопления (Kibite, Edney, 1996), а динамика накопления β-глюканов в зерновке отличается от таковой для других биохимических компонентов (Cox, Frey, 1985). Нами не найдено корреляционной связи между содержанием β-глюканов и белка в зерне овса разных образцов. По этому вопросу в литературе приводятся противоречивые результаты: описана как положительная (Havrlentová et al., 2008), так и отрицательная корреляция (Miller et al., 1993).

При выполнении работы не обнаружено заметного преимущества голозерных образцов по сравнению с пленчатыми в содержании β-глюканов в зерне, на что указывали другие ученые (Havrlentová et al., 2008; Biel et al., 2009). Более того, у голозерных образцов не найдено четкой связи между содержанием β-глюканов, жира и белка в зерне по годам исследования.

Заключение

Таким образом, выполненная в условиях Восточной Сибири оценка сортов овса по комплексу биохимических и физических показателей зерна позволила выделить потенциально ценный исходный материал. Для использования в селекции кормового направления можно рекомендовать сорта с минимальным содержанием β-глюканов в зерне – Тубинский, Вятский и Голец, в селекции сортов продовольственного направления – Местный Тунис 1, Медведь и Тайдон с максимальным содержанием β-глюканов в зерне.

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Transcriptomic analysis of *Medicago truncatula* calli with *MtWOX9-1* overexpression

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Somatic embryogenesis (SE) is the development of embryo-like structures from somatic plant tissues. This process rarely can be observed in nature, but for many plant species, *in vitro* protocols are developed, which allow to obtain somatic embryos formation directly from tissues of plant explant or from the embryogenic callus. SE is widely used for plant propagation and transformation; therefore, the search for SE stimulators and revealing of the mechanisms of their functioning are very important for biotechnology. Among the SE regulators, proteins of the WOX family play significant roles. WOX (WUSCHEL-RELATED HOMEOBOX) is a homeodomain-containing transcription factor family. Different *WOX* genes function in different plant organs and tissues, maintaining meristem activity and regulating cell proliferation and differentiation. Recently, we have shown that transcription factor MtWOX9-1, belonging to the WOX family, can stimulate SE in the *Medicago truncatula* callus culture. In this research, transcriptomic analysis of highly embryogenic calli with *MtWOX9-1* overexpression was performed in comparison to wildtype calli. It was shown that *MtWOX9-1* overexpression led to the activation of several groups of genes, including genes related to cell division, tissue differentiation, and seed development. Enriched GO pathways included several groups related to histone methyltransferase activity as well as DNA methylation and chromatin binding, suggesting major epigenetic changes that occur in call overexpressing *MtWOX9-1*. Using Medicago Truncatula Gene Expression Atlas, we also identified a group of genes coding for transcription factors that were both coexpressed with *MtWOX9-1* in different plant organs and differentially expressed in our samples. These genes are putative targets of MtWOX9-1, and they may act in the same pathway with this regulator during SE.
Key words: somatic embryogenesis; *Medicago truncatula*; plant regeneration; transcription factors; transcriptomic analysis.

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Транскриптомный анализ каллусов *Medicago truncatula* со сверхэкспрессией гена *MtWOX9-1*

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Соматический эмбриогенез (СЭ) – это развитие зародышеподобных структур из соматических тканей растений. Этот процесс редко можно наблюдать в природе, однако для многих видов растений разработаны протоколы культивирования в условиях *in vitro*, с помощью которых можно добиться формирования соматических эмбрионов напрямую из тканей растительного экспланта или из эмбриогенного каллуса. СЭ широко применяют в биотехнологии для размножения и трансформации растений, и в связи с этим поиск стимуляторов СЭ и изучение механизмов их работы представляют собой актуальную задачу. Белки WOX играют важную роль в регуляции СЭ. WOX (WUSCHEL-RELATED HOMEOBOX) – семейство гомеодомен-содержащих транскрипционных факторов. Различные гены *WOX* функционируют в разных органах и тканях растений, поддерживая активность меристем и регулируя пролиферацию и дифференцировку клеток. Ранее нами было обнаружено, что транскрипционный фактор MtWOX9-1, принадлежащий к семейству WOX, способен стимулировать соматический эмбриогенез в каллусной культуре у *Medicago truncatula*. В настоящем исследовании проведен сравнительный анализ транскриптома высокоэмбриогенных каллусов со сверхэкспрессией гена *MtWOX9-1* и транскриптома каллусов дикого типа. Показано, что сверхэкспрессия *MtWOX9-1* вызывает активацию нескольких групп генов, включая гены, связанные с делением клеток, дифференцировкой тканей, а также с развитием семян. Среди обогащенных наборов генов в терминах GO мы обнаружили несколько групп с активностью метилтрансфераз гистонов, метилированием ДНК и связыванием с хроматином, что предполагает существенные эпигенетические изменения, происходящие в каллусах со сверхэкспрессией *MtWOX9-1*. Используя базу данных Medicago Truncatula Gene Expression Atlas, мы идентифицировали также группу генов, кодирующих транскрипционные факторы, которые коэкспрессируются с *MtWOX9-1* в различ-

ных органах растений и характеризуются дифференциальной экспрессией в наших образцах. Эти гены представляют собой предполагаемые мишени MtWOX9-1, которые могут работать в одном пути с этим регулятором в ходе СЭ.
Ключевые слова: соматический эмбриогенез; *Medicago truncatula*; регенерация растений; транскрипционные факторы; транскриптомный анализ.

Introduction

Somatic embryogenesis (SE) is a process of regeneration by which plants use somatic cells to grow embryo-like structures, which eventually can give rise to the new plant. This process isn't observed often in nature, but when plant explants are cultivated *in vitro*, several factors can induce direct SE or SE from callus tissue. Such factors include specific hormones, the concentration of nitrogen compounds (Reinert at al., 1967), the stress impact (Nic-Can et al., 2016), etc. Most of existing methods of SE induction *in vitro* include treatment with hormones and mechanical injury, and it is supposed that SE acts as the mechanism of defense against stressful *in vitro* conditions.

Somatic embryo development occurs in general through the same stages as development of zygotic embryo, and therefore it is used as the model for studying embryogenesis. SE also has a lot of biotechnological applications in transformation of plants, artificial seeds production and micropropagation.

The way by which embryogenic cells are chosen among explant or callus cells is not fully investigated. In *Medicago truncatula*, one of SE model objects, somatic embryos are often derived from mesophyll cells near the damaged surface, which have to dedifferentiate, but some are derived from the stem-like vascular procambium cells (Wang et al., 2011; Rose, 2019). Auxin gradients are shown to play key role in determination of cells that will give rise to the embryo (Su et al., 2009).

Dedifferentiating cell, which will be capable to form somatic embryo later, undergoes a number of changes, including mitochondrial fusion and increase in peroxisomes (Tiew et al., 2015) and P-bodies (RNA processing bodies) numbers (Bhullar et al., 2017). The first one is used to provide a kind of quality control for mitochondrial populations in new generations (Rose, McCurdy, 2017). The second one is a kind of stress response, whereas the third one plays role in posttranscriptional gene regulation and cell reprogramming.

Genetic cascade that induces dedifferentiation of cell and development of somatic embryo is not fully uncovered. At the present time correlation with SE has been established for *LEAFY COTYLEDON1* (*LEC1*), *BABY BOOM* (*BBM*), *AGL15*, *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*SERK*) and other genes (Fehér, 2015). Among the regulators of SE, proteins of WOX family also play important roles. WOX (WUSCHEL-RELATED HOMEOBOX) is a homeodomain-containing transcription factors (TFs) family. Different *WOX* genes function in different plant organs and tissues, maintaining meristem activity and regulating cell proliferation and differentiation.

The most well-studied family members are *WUS* and *WOX5* genes, which are expressed in the organizing and quiescent center cells of the shoot and root apical meristem (SAM and RAM), respectively, and regulate their development (Laux et al., 1996; Sarkar et al., 2007). The mechanism of WUS action in stem cell niche is related to stimulation of cytokinins activity, by which it represses the differentiation of SAM cells (Leibfried et al., 2005). *WUS* is also expressed in the floral meristem, where it stimulates *AGAMOUS* expression, providing termination of floral meristem activity (Lenhard et al., 2001).

WOX5 is functional analog of *WUS* gene in root. It is expressed in the quiescent center in the root apical meristem (Sarkar et al., 2007) and in different irregular meristems, such as nodule meristems (Osipova et al., 2012) and meristem-like structures of agrobacterial and spontaneous tumors (Lebedeva et al., 2015; Vinogradova et al., 2015).

The participation in zygotic and somatic embryogenesis was demonstrated for many *WOX* family genes. For example, *WUS* is an important stimulator of SE in different species, such as *Arabidopsis thaliana* (Su et al., 2009), *Capsicum chinense* (Solís-Ramos et al., 2009) and *Gossypium hirsutum* (Bouchabké-Coussa et al., 2013; Xiao et al., 2018). *WOX5* is also involved in SE, participating in RAM development in somatic embryos (Su et al., 2015). Expression of *WOX1* and *WOX3* homologs was observed during SE process in different objects, such as *C. chinense* (Valle-Gough et al., 2015), *Vitis vinifera* (Gambino et al., 2011) and *Picea abies* (Alvarez et al., 2015). The *WOX11* and *WOX12* genes are expressed in the early stages of callus and adventitious roots development in *Arabidopsis*, stimulating the cambium cells proliferation (Liu et al., 2014). The expression of *WOX11* homolog during SE was shown in *V. vinifera* (Gambino et al., 2011).

The genes *WOX2*, *WOX8*, and *WOX9* play an important role in the zygotic embryogenesis, defining the differentiation of specific embryo domains: apical (*WOX2*), central (*WOX9*) and basal (*WOX8*) (Breuninger et al., 2008). In *A. thaliana*, *WOX2* and *WOX8* expression was detected in egg cell, though the *Nicotiana tabacum* homologs of these genes were shown to be *de novo* transcribed in zygote right after fertilization (Zhou et al., 2018). The *WOX2* homolog in *Larix decidua* is expressed during early embryogenesis, both somatic and zygotic (Rupps et al., 2016). The *WOX9* homologs are microspore embryogenesis markers in *Brassica napus* (Malik et al., 2007) and SE markers in *V. vinifera* (Gambino et al., 2011). Besides, expression of the *WOX9* homolog *MtWOX9-like* was demonstrated during SE in *M. truncatula* (Kurdyukov et al., 2014).

In our previous studies, three new *M. truncatula* genes of the *WOX* family, that are expressed during SE, were found: *MtWOX9-1*, *MtWOX11-like* and *STENOFOLIA* (*MtWOX1*) (Tvorogova et al., 2015). It was further shown that overexpression of *STF* or *MtWOX9-1* stimulates the emergence of somatic embryos (Tvorogova et al., 2016, 2019). In the present work, we concentrated on studying the functions of the *MtWOX9-1* gene, analyzing how its overexpression affects the expression profile of embryogenic callus.

Materials and methods

Plant growth, cultivation and sample collection. *M. truncatula* line R-108 (Hoffmann et al., 1997) and transgenic line with *MtWOX9-1* overexpression were used in the analysis.

Line with *MtWOX9-1* overexpression, obtained through argobacterial transformation of R-108 line plants with pMDC32 vector containing *MtWOX9-1* coding sequence under the control of 35S promoter (Wolabu, 2015), was kindly provided by the laboratory of Dr Million Tadege.

M. truncatula seeds were sterilized in sulfuric acid for 10 minutes, rinsed 10 times with sterile water, and then put onto 1 % agar and left to germinate at 4 °C for 7 days. After germination, seedlings were transferred in the soil (Terra Vita, Russia) mixed with vermiculite (2:1). Plants were grown at 21 °C at 16 h photoperiod. Before *in vitro* cultivation, leaves of 30 day old plants were sterilized in 70 % ethanol for 1 minute, then in 50 ml solution of 0.5 % hypochlorite with two drops of Tween-20 for 10 minutes, and then rinsed 5–7 times with sterile water. *In vitro* cultivation and obtaining of embryogenic calli was performed as described previously (Tvorogova et al., 2016).

Two biological replicates for R-108 wildtype calli (wt from this point onward) and two biological replicates for *MtWOX9-1* overexpressing calli (w9o from this point onward) were taken at 35th day of cultivation (5th day of cultivation of hormone-free medium). Plant material was divided into two parts, the first for transcriptomic analysis and the second for quantitative PCR (qPCR) analysis.

Transcriptome sequencing and bioinformatic processing. RNA extraction, library preparation and sequencing was performed by the Genoanalitica company (Moscow, Russia). Total RNA was extracted from calli with Trisol reagent and PureLink RNA Micro Kit (Invitrogen) according to manufacturer instruction. Quality was checked with BioAnalyser and RNA 6000 Nano Kit (Agilent). PolyA RNA was purified with Dynabeads® mRNA Purification Kit (Ambion). Illumina library was made from polyA RNA with NEBNext® Ultra™II RNA Library Prep Kit for Illumina® (NEB) according to manual. Sequencing was performed on HiSeq1500 with 50 bp read length.

Trimming of adapter sequences was performed with Trimmomatic (Bolger et al., 2014). Filtration of ribosomal RNA was performed with SortMeRNA (Kopylova et al., 2012) with rRNA sequences from *M. truncatula* Jemalong A17 genome assembly v5r1.6 used as reference database (Pecrix et al., 2018). Alignment on reference genome (assembly MedtrA17_4.0) was performed with HISAT2 (Kim et al., 2015), and reads were counted with Stringtie (Pertea et al., 2015) with the usage of reference genome mentioned before and without *de novo* assembled transcripts. DESeq2 (Love et al., 2014), GSEABase (Morgan et al., 2019), and WGCNA (Langfelder, Horvath, 2008) R packages were used for differential expression analysis, GO gene enrichment analysis, and coexpression analysis, respectively.

qPCR expression analysis. Total RNA was extracted from calli with Purezol reagent (Bio-Rad, USA) according to manufacturer instructions. RNA was treated with DNAse I (Thermo Scientific, USA) for DNA removal. cDNA synthesis was performed with RevertAid reverse transcriptase (Thermo Scientific, USA) with oligo-dT18 primer according to manufacturer instructions. cDNA samples were diluted with sterile water to the end volume of 100 µl. For qPCR, the reagent kit for qPCR with Eva Green (Syntol, Russia) was used. Quantitative estimation of analyzed gene expression was performed with 2-ΔΔCt method (Livak, Schmittgen, 2001). *Actin* gene (*MTR_3g095530*) and constitutive gene for histone-like protein *H3L* (*MTR_4g097170*) were used as reference genes, and their primer sequences (ActinF: TCAATGTGCCTGCCATG TATGT; ActinR: ACTCACACCGTCACCA; H3LF: CTTT GCTTGGTGCTGTTTAGATGG; H3LR: ATTCCAAAG GCGGCTGCATA) were taken from literature (Ariel et al., 2010; Zhang et al., 2014). Primers for BHLH TF-like protein gene (Medtr1g107185, F: GCAACCACCAAACCAACA CTG; R: GACCTTTCTGCCCTCCAACAC), bZIP TF gene (*Medtr7g104190*, F: CGGATGGAGGTGAGCAGAAAC; R: CCTTGGTGATGGAAGTGGAATG) and *MtWOX9-1* (*Medtr2g015000*, F: CCAGAACAAGAATCAGAACCAG AAC, R: TTAGGGAAACCAAGGGAAAATAC) gene were selected using Primer3 Select online software (Untergasser et al., 2012).

Results

To analyze the mechanisms of MtWOX9-1 functioning during SE, 35 day-old wt and w9o calli of *M. truncatula* were obtained. At this stage, somatic embryos, visible as green spots on callus surface, started to appear on w9o calli (Suppl. Fig. 1, *a*)[1], but not on wt calli which usually start form embryos later during cultivation. The RNA was extracted, reverse transcribed and sequenced from embryogenic wt and w9oe calli (two biological replicates for each variant). According to qPCR analysis, expression level of *MtWOX9-1* was several thousand times higher in w9o samples than in wt samples (see Suppl. Fig. 1, *b*). 4 complementary DNA libraries were sequenced with an average depth of approximately 15 millions of reads. After trimming and ribosomal RNA removing, about 12 millions of 35-bp reads were taken for analysis. After alignment with HISAT2, about 75 % of reads in each sample were uniquely mapped. Reads were counted by StringTie, and correlation analysis performed for DESeq normalized counts demonstrated high correlation between biological replicates (see Suppl. Fig. 1, *c*).

After the analysis of differential expression with DESeq package and imposition of 0.01 adjusted *p*-value and 1.0 log2 fold change cutoffs, 3133 genes out of 51628 analyzed were found to be differentially expressed (Suppl. Table S1), with 1608 and 1525 up- and downregulated, respectively, in w9o calli in comparison to wt calli. qPCR expression analysis of two differentially expressed genes (DEGs) supported transcriptome analysis data (Suppl. Fig. 2).

To find new potential stimulators and repressors of SE, we assessed expression levels of genes coding TFs among DEGs (selection of DEGs with GO annotation number 0006355, "regulation of transcription, DNA-templated"). We also added there five DEGs from WOX family, which happened not to be included in this GO group, but, according to numerous data, should have TF function. We found 173 DEGs coding TFs of which 94 gene was upregulated, including several TFs from NF-Y family, B3 domain TFs, MADS-box TFs etc (Suppl. Table S2).

[1] Supplementary Figures 1–4 are available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx13.pdf Supplementary Tables S1–S7 are available in .xls format on the link below https://drive.google.com/drive/folders/1ceP-r3pc_UFDWBy1o5DzVVhSb4u9bW25?usp=sharing.

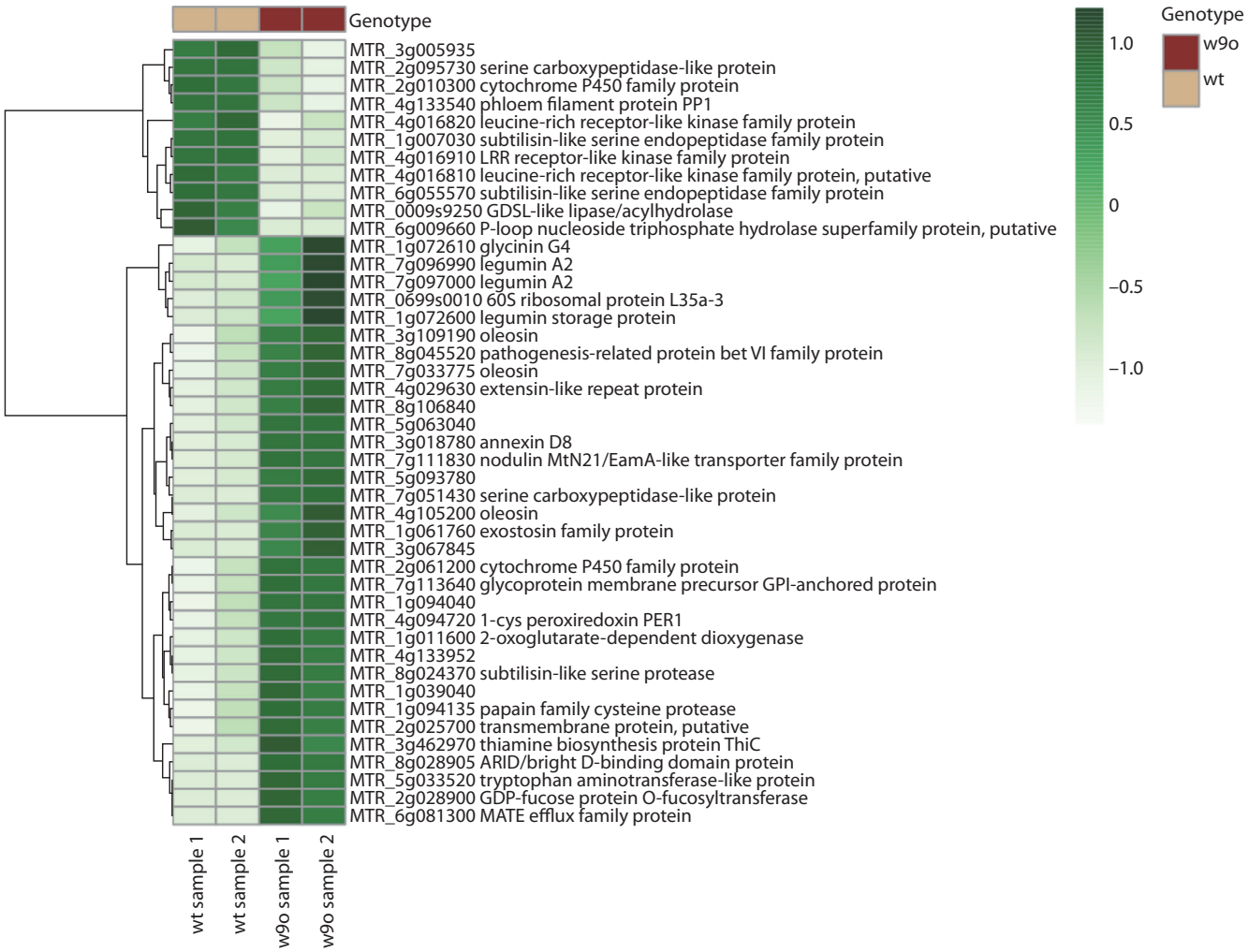

Fig. 1. Heatmap showing normalized expression levels of seed-specific genes differentially expressed between wt and w9o calli.

As well as w9o calli appear to be more embryogenic than wt ones according to our data (Tvorogova et al., 2019), we supposed that MtWOX9-1 should specifically stimulate expression of genes related with embryo development and development of pods. To check this hypothesis, we selected the genes with specific expression in seedpod (expression level in seedpod is at least 4 times higher than average expression level measured in seedpod, leafblade, nodule, root, flower and bud) from the *M. truncatula* genome database (Krishnakumar et al., 2015). Among these genes, only 3.7 % (44 genes) were differentially expressed in our experiment, but 75 % of this group of DEGs were upregulated (Fig. 1), which is consistent with our hypothesis.

We also performed gene enrichment analysis with GSEABase package using GO terms (Ashburner et al., 2000). Upregulated and downregulated genes were found to be enriched with genes from 17 and 21 GO "Molecular Function" groups, respectively (Fig. 2, Suppl. Fig. 3, Suppl. Table S3).

Interestingly, upregulated GO pathways included several groups related with histone methyltransferase activity as well as DNA and chromatin binding, suggesting major epigenetic changes occuring in w9o calli. We analysed *A. thaliana* homologs of upregulated histone methyltransferases and found that most of them are responsible for histone repressive marks associated with DNA methylation (Table).

49 and 40 "Biological Process" GO terms were overrepresented among upregulated and downregulated genes, respectively (Fig. 3, Suppl. Fig. 4, Suppl. Table S4). In accordance with the data above, genes from DNA methylation and gene silencing GO groups were found among upregulated genes.

To find the genes which may work together with *MtWOX9-1*, we performed coexpression analysis using Benedito et al. (2008) data from Medicago Truncatula Gene Expression Atlas and WGCNA R package (Langfelder, Horvath, 2008). We found 55 coexpression modules, containing from 35 to 4914 different genes and isoforms (Suppl. Table S5). The *MtWOX9-1* module contained 2337 genes, out of which 225 genes were differentially expressed in our calli samples according to transcriptome analysis (Suppl. Table S6). We searched for TF genes among this group using selection of genes with GO annotation number 0006355, "regulation of transcription, DNA-templated". We also added two DEGs from WOX family (including *MtWOX9-1* itself), which happened not to be included in this GO group, but, according to numerous data, should have TF function.

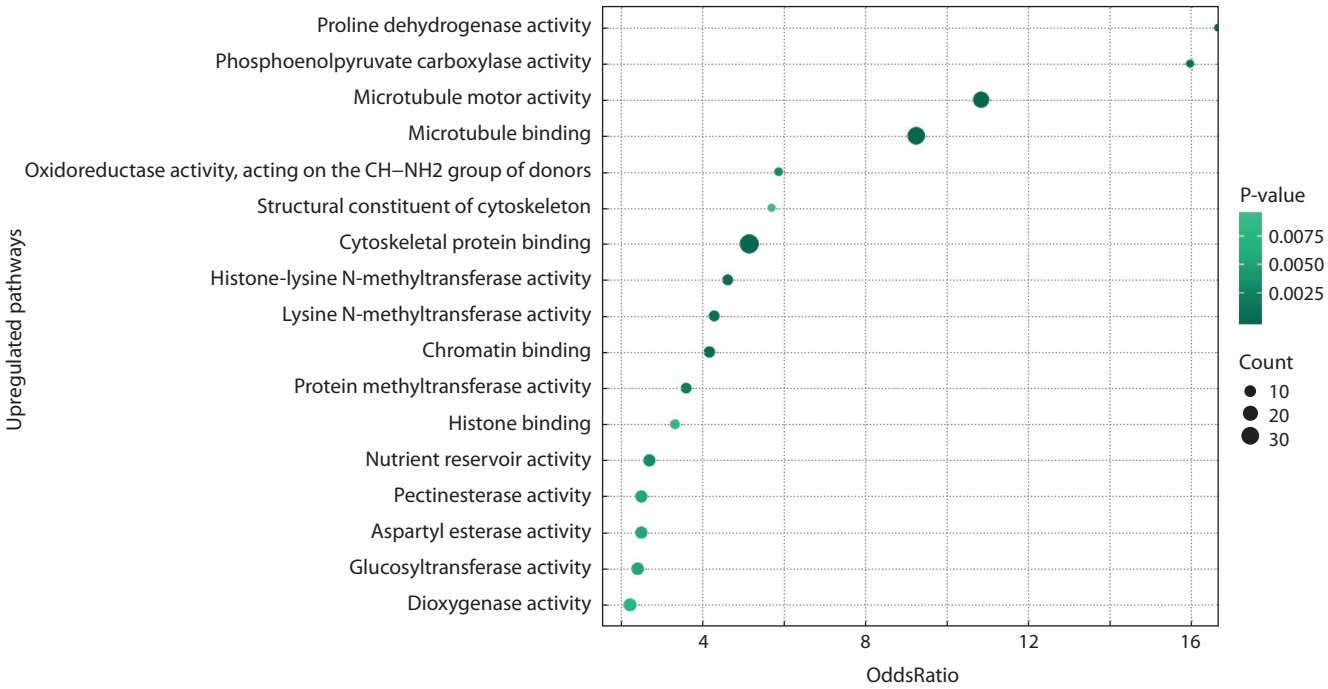

Fig. 2. Overrepresented "Molecular function" GO pathways in upregulated genes.

Upregulated histone methyltransferases in w9o calli, their *A. thaliana* homologs and possible functions

Gene	*A. thaliana* closest homolog	Histone mark	References
Medtr1g048950	*SUVH5*	H3K9me1/me2 (Repressive)	Ebbs, Bender, 2006; Rajakumara et al., 2011
Medtr5g016870	*CLF*	H3K27me3 (Repressive)	Schubert et al., 2006
Medtr5g018850	*SUVR5*	H3K9me2 (Repressive)	Caro et al., 2012
Medtr6g061200	*SUVH4/KRYPTONITE*	H3K9me1/me2 (Repressive)	Johnson et al., 2004
Medtr6g061270	*SUVH4/KRYPTONITE*	H3K9me1/me2 (Repressive)	
Medtr7g084090	*SUVH4/KRYPTONITE*	H3K9me1/me2 (Repressive)	
Medtr7g088370	*SUVH1*	H3K4me3 (Activating)	Li et al., 2016

The resulting TF gene list contained 18 genes, up- or down-regulated in w9o calli, including genes for three B3 domain proteins, several homeobox-containing factors, etc (Suppl. Table S7).

Discussion

In this study, we analyzed transcriptome of embryogenic calli, affected by overexpression of SE stimulator *MtWOX9-1*. It is important to note that in this study we used a single line with *MtWOX9-1* overexpression, therefore one cannot exclude the possibility that observed effects are related with specific location of transgenic insert in this line. However, according to our results (Tvorogova et al., 2019), the positive effect of *MtWOX9-1* overexpression on embryogenic capacity was observed after several independent transformation events, therefore we can assume that the majority of the effects demonstrated in this study are not unique for specific transgenic line.

Analysis of gene groups activated in w9o calli allowed us to suggest that *MtWOX9-1* overexpression and probably SE itself are associated with major epigenetic changes in embryogenic callus, such as DNA and histone methylation. Such changes are common for *in vitro* cultures (Kumar, Van Staden, 2017). Most of observed upregulated chromatin-related pathways were found to be repressive which is probably due to the start of differentiation of callus cells forming specific embryo tissues. Interestingly, such pathways as postembryonic development and seedling development were upregulated, suggesting important differences between zygotic embriogenesis and somatic embryogenesis, lacking dormancy stage.

As it was mentioned above, SE is the stress-induced process: temperature, wounding, starvation, heavy metal ions, and osmotic stress can lead to dedifferentiation of somatic cells and to other changes. In support of this, we also found some upregulated GO groups associated with stress response, including, for example, proline dehydrogenase and response to abscisic acid. Proline dehydrogenase enzyme is an important component of plants pathogen defense system which contributes to the hypersensitive response (HR) and disease resistance, and promotes the accumulation of reactive oxygen

Fig. 3. Overrepresented "Biological Process" GO pathways in upregulated genes.

species (ROS) and oxidative cell death (Cecchini et al., 2011; Monteoliva et al., 2014).

Several downregulated GO groups associated with stress response were also found, including phenylalanine ammonia-lyase activity (Wada et al., 2014) and chitin binding groups. Several other SE and cell division associated pathways were also found to be upregulated in w9o calli, including phosphoenolpyruvate carboxylase and pectin methylesterase (PME) activity, cytokinetic process etc. Phosphoenolpyruvate carboxylase modulates cell division and elongation of cotton fibers, the ovule epidermal cells formed during flowering process (Li et al., 2010). Changes in the methylesterification status of pectins have been associated with cell wall remodeling that occurs during diverse plant developmental processes (Levesque-Tremblay et al., 2015). It was shown that PME expression level increased in heart-torpedo embryos and mature cotyledonary embryos (Pérez-Pérez et al., 2018). This tendency is kept during somatic embryogenesis process among both woody and herbaceous plants (Solis et al., 2016). Besides their roles in plant development, PMEs are also involved in stress response and pathogen defense (Ma et al., 2013).

WOX genes are master regulators of development, and their direct targets often encode for other TFs. The results of our coexpression analysis allowed to identify several TF genes which may be the direct targets of MtWOX9-1. Therefore, the next step of research will be to check the relations between MtWOX9-1 and these genes using molecular biology methods.

Conclusion

The observed differences between w9o and wt calli and control can be considered as the specific effect of *MtWOX9-1* overexpression but, on the other hand, they may result from SE process itself, which tends to start earlier in w9o calli (Tvorogova et al., 2019). Thus, these data may be useful both for MtWOX9-1 target search and for the search for new SE-associated genes and SE stimulators.

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Mutants of inflorescence development in alfalfa (*Medicago sativa* L.)

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Alfalfa (*Medicago sativa* L., *Medicago varia* Mart., *Medicago falcata* L.) is a perennial leguminous plant well-known as the queen of forages cultivated all over the world. The general biology and morphology of the plant has been described in detail. The typical inflorescence of the plant is raceme. Due to the multistep inbreeding process in this cross-pollinated species, different mutant forms have been found in inbred progenies. They include long racemes, panicle-like racemes (with fertile and sterile flowers), complicated branched racemes, and fasciated inflorescences. The fasciation trait was discovered first in long racemes and then it was introduced into every mutant inflorescence type by hand pollination. By means of pair hybridization, transitional forms of some mutants were isolated and the new mutant forms combined two or three mutant genes. New gene names are proposed for new duplex and triplex mutant types: *lpfas*, *pi1lpfas*, *brilpfas*. *Medicago truncatula* is a conventional model species for legume genome research. *M. truncatula* and alfalfa share highly conserved nucleotide sequences and exhibit nearly perfect synteny between the two genomes. The knowledge about inflorescence development in model *M. truncatula* plants adds to understanding the genetic nature of mutant inflorescence development in alfalfa; therefore, we compiled the information on the genetic regulation of inflorescence development in *M. truncatula*. The *M. truncatula* mutant *mtpim* has a complicated inflorescence structure resembling panicle-like inflorescence in alfalfa. Presently, it is known that the inflorescence architecture in *M. truncatula* is controlled by spatiotemporal expression of *MtTFL1*, *MtFULc*, *MtAP1*, and *SGL1* through reciprocal repression. Some mutants isolated in *M. truncatula* resemble alfalfa mutants in phenotype. The mutant generated by retrotransposon insertion mutagenesis and named *sgl1-1* has a cauliflower-like phenotype looking just like the cauliflower mutant in alfalfa. New data concerning genes regulating inflorescence development in model legumes approach us to understanding the phenomenon of inflorescence mutations in alfalfa. The information of inflorescence mutants in nonmodel crops may augment our knowledge of plant development and help crop improvement.

Key words: *Medicago sativa* L.; alfalfa; mutants; inflorescences; plant development.

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Мутанты развития соцветий у люцерны (*Medicago sativa* L.)

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Люцерна (*Medicago sativa* L., *Medicago varia* Mart., *Medicago falcata* (L.)) – многолетнее бобовое растение, известное как «королева кормов» и культивируемое на всем земном шаре. Общая биология и морфология растения описаны в деталях, типичное соцветие люцерны – открытая брактеозная кисть. В процессе многоступенчатого самоопыления в инбредных поколениях люцерны определены мутантные по строению соцветий формы. Среди них удлиненные, метелковидные (фертильные и стерильные), ветвистые сложного строения, а также соцветия с фасциированными цветоносами. Признак фасциации соцветия выявлен у люцерны среди удлиненных соцветий и далее был введен в каждый мутантный тип соцветия путем скрещиваний вручную. Посредством парных скрещиваний созданы переходные гибридные мутантные формы, сочетающие два или три мутантных признака. Новые двойные и тройные мутанты получили названия: *lpfas*, *pi1lpfas*, *brilpfas*. *Medicago truncatula* – классический модельный объект проведения геномных исследований у бобовых. *M. truncatula* и люцерна посевная проявляют консервативную нуклеотидную последовательность и обладают высокой степенью синтении геномов. Знание о регуляции развития соцветий у модельного растения *M. truncatula* полезно для понимания генетической природы мутаций у люцерны, в связи с чем был проведен анализ информации о генетике развития *M. truncatula*. К настоящему времени известно, что архитектура соцветия у *M. truncatula* находится под контролем пространственно-временной экспрессии генов *MtTFL1*, *MtFULc*, *MtAP1* и *SGL1* посредством обратного подавления. Некоторые мутанты, выделенные у *M. truncatula*, имеют фенотип, схожий с фенотипом мутантов люцерны. Мутант *mtpim M. truncatula* обладает сложными соцветиями, напоминающими метелковидные соцветия у мутанта люцерны посевной. Мутант, полученный путем мутагенеза через инсерцию ретротранспозонами, под названием *sgl1-1*, имел фенотип типа «цветной капусты», присущий мутанту с аналогичным на-

званием у люцерны посевной. Новые данные о регуляции развития соцветий у модельных видов бобовых приближают нас к пониманию феномена мутаций соцветий у люцерны. Информация о мутантах соцветий у немодельных культур вносит свой вклад в науку о развитии растений и полезна для улучшения культур.
Ключевые слова: *Medicago sativa* L.; люцерна; мутанты; соцветия; развитие растений.

Introduction

In most traditional botanical terms inflorescence is a flowering shoot. Different theories of inflorescence classifications exist, especially in legume. In the present paper for characterization of the mutant inflorescences in alfalfa the terms of the axe of the first order, the axes of the second and higher orders will be used for convenience. Typical inflorescence of alfalfa is an open bracteous compound raceme. Flowering in the wild type inflorescence of alfalfa starts acropetally. In general flowering in angiosperms starts from transition of shoot apical meristem (SAM) to flower apical meristem (FAM). Mutants of inflorescence development in alfalfa demonstrate the wide range of variability of positions of FAM development.

Inflorescence type is one of the main traits in plant taxonomy. The shapes of flowers and their organization into branching systems, called inflorescences, dictate much of plant diversity. Development mutant deviations are good example of possible confusing in species taxonomic attribution using herbarium specimen. For example, panicle-look inflorescence, the most famous spontaneous mutation in alfalfa, transforms the habitus of the plant radically. Teratological events in plants attracted attention of botanists for a long time (Fedorov, 1958), but the genetic nature of some morphological deviations still remains not quite clear.

Alfalfa is a tetraploid cross-pollinated plant, self-pollination in few progenies allows to reveal the hidden polymorphism and sometimes leads to spontaneous mutations. Blossoming and pods setting is the top of individual plant development, the success or failure in ontogenesis is crucial. Inflorescence bearing flowers is the main construction for reproductive mission of the plant implementation. Deviations leading to seed reproduction failure should not maintain by natural selection, nevertheless some mutations are possible not to decrease but even to increase seed production. Other mutations in alfalfa are subjects of interest from the point of view of developmental genetics. Molecular and genetic studies show that the underlying mechanisms controlling flower development are largely conserved in distinctly related dicotyledons plants species. In the studies by M.F. Yanofsky (1995) early-acting genes were identified, that promote the formation of floral meristems, and later acting genes that determine the fate of floral organs primordia (Yanofsky, 1995). The events which determine transition of SAM to FAM are more early-acting than events coordinating differentiation of floral primordia, thus fate of inflorescences differentiation is resolved earlier than flowers whorls differentiation. The mutants of *M. sativa* with deviations in shoot meristems behavior and flower deviations are not under review in the present paper.

Materials and methods

All spontaneous mutants described below were got in VIR during large-scaled population screening in inbred progenies in the field conditions. Plants in individual standing were covered by isolators (one-half of the plant). Flowers under isolators were tripped artificially by hand and self- or cross-pollinated. Self-pollination and crossing were made without castration. Hybridization was made by pollination by pollen of desired parent under the isolators in field. Some material was grown and pollinated in 2017 in greenhouse without isolators in the lack of insects (Pushkin laboratories of VIR). No any chemical or radioactive mutagens were used. Field experiments were conducted in 1981–1992 at former VIR Aral Research Station (Kazakhstan) and in 2009–2011 at VIR Maykop Research Station (Adygeya Republic, Northern Caucasus).

Mutants description

Panicle-like mutants. Typical inflorescences of alfalfa – open bracteose raceme. Most famous spontaneous inflorescence mutation in alfalfa – panicle-like inflorescence, was discovered independently by several breeders (Dudle, Wilsie, 1956, 1957; Bayly, Craig, 1962; Murray, Craig, 1962; Pashenko, Rustamova, 1971; Mariani et al., 1976; Kinoshita, Suginobu, 1982; Dzyubenko N.I., Dzyubenko E.A., 1992). It is a compound inflorescence with fertile, semi-sterile and totally sterile flowers. On the axil of the first order instead of the FAM the second order axils are formed bearing flowers and pods (Fig. 1, *a*, Fig. 2, *b–c*).

This type of mutation was found in VIR in 1981 in self-pollinated progenies from crosses of the plants varieties Ellerslaier and Tibetskaya. The expression of the trait varied widely in the progenies. The mutant plants were divided into four groups depending upon the expression of the trait: a) plants with normal racemes and few panicle-like inflorescences; b) plants with few normal racemes, simple panicles and few large panicles with compound structure forming pods (fertile); c) plants with compound panicle inflorescences only, different violations of some flower structures may be observed, including actinomorphic petals, vestigial generative organs, pod setting decreased to some extent due to the presence of vestigial flowers (semi-fertile, semi-sterile); d) cauliflower-like inflorescences with rudimental flowers not forming pods (sterile).

Undifferentiated flower primordia stopped at their differentiation at the Va stadia of organogenesis (Kuperman, 1984). According to F.M. Kuperman (1984), inflorescence primordia and bracts are developing at the third stage of morphogenesis of higher plants, while differentiation of floral meristems occurs at the fourth stage of morphogenesis. Bracteas in cauliflower-like mutant are well-developed. The cauliflower phenotype appeares as a mutation in different plant genera and possible has common genetic regulation.

The panicle-like inflorescence trait is governed by a single recessive gene *pi-1* (for *M. sativa*) and *pi-2* (for *M. falcata*) (Dzyubenko N.I., Dzyubenko E.A., 1992). Z. Bodzon (2016) named this mutation as *br* and remarked that it enhanced 6–10 times floret number per inflorescence. Sterile panicle-like inflorescence, cauliflower type, was supposed to be controlled

Fig. 1. Picture and scheme of main mutant inflorescences in alfalfa.
a – panicle (*pi-1*); *b* – branched (*bri*); *c* – long petiole (*lp)*.

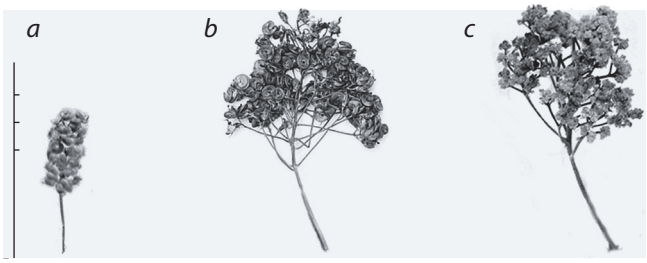

Fig. 2. Panicle mutants inflorescences.
a – normal inflorescence of alfalfa – a raceme; *b* – fertile panicle with pods (*pi-1*); *c* – sterile panicle (cauliflower type inflorescence).

Fig. 3. Wild type (left) and branched inflorescences.

by one recessive gene in nulliplex position (Kinoshita, Suginobu, 1982).

Mutation "Branched raceme". Most striking mutation, never found in alfalfa before, characterized by partial replacement of racemes by shoot-looking structures, bearing flowers (and setting pods) on the main axil at the bottom part and forming additional axes of the second order (sometimes even third or fourth orders) bearing flowers in its turn (Fig. 1, *b*). Flowers in the upper part may have some abnormalities such as flowers fused together or actinomorphic flowers with polymeric gynoecium. Progeny of self-pollinated mutants with branched racemes divided into groups of plants with different phenotypes.

Phenotype 1. Dwarf plants up to 30 cm, non-flowering and non-branching, with fragile shoots with short internodes, with dark green leaves.

Phenotype 2. Semi-dwarf plants 30–50 cm high with lonely almost sessile pale flowers, with fragile shoots and dark green leaves. Non-branching plants.

Phenotype 3. Plants of common size and dark green leaves and common inflorescences.

Phenotype 4. Plants with branched inflorescences. This inflorescence type does not fit any inflorescence type according any botanical classification, including last one suggested for legumes (Sinjushin, 2018). In the upper part of the raceme, the secondary axes are formed instead of flowers, some of them continue to produce axes of the third and higher orders, at the bottom part of the main axe normal flowers are set. Flowering starts acropetally by the bottom flowers at the axe of the first order and by the bottom flowers of the second order axes. Size and branching of axes of the second order demonstrated a large variability (Fig. 3). Mutation was named *bri* (Dzyubenko N.I., Dzyubenko E.A., 1992–1994, 1998, 2009, 2010). Branched inflorescences were characterized by high pollen and ovules fertility close to norm (Dzyubenko, 1990) and good seed production.

One can supposed that such kind of splitting in the self-pollinated progenies – presence of branched inflorescences type plants (abundance of SAM and FAM activity) together with the presence of dwarf plants with shortened internodes and dark green leaves (lack of SAM and FAM activity), may be connected with some gene system acting through hormones regulation, as it is described in Á. Dalmadi et al. (2008).

Long inflorescence. In the population of variety Vela k-42716 some plants of spontaneous mutants with long racemes were revealed. Plants with long racemes were self-pollinated. After self-pollination in progenies the length of the racemes in plants varied from 18 to 28 cm, amount of flowers per raceme – 10–32 cm. Fallen (un-pollinated) flowers and buds consisted up to 75 % from initial amount of flowers in some racemes, meanwhile in some racemes pod setting was good, promising for increasing the seed yield in alfalfa (Dzyubenko N.I., Dzyubenko E.A., 1991). Fertility of the pollen was high in most plants with long racemes, fertility of ovules was also close to norm (Dzyubenko, 1990).

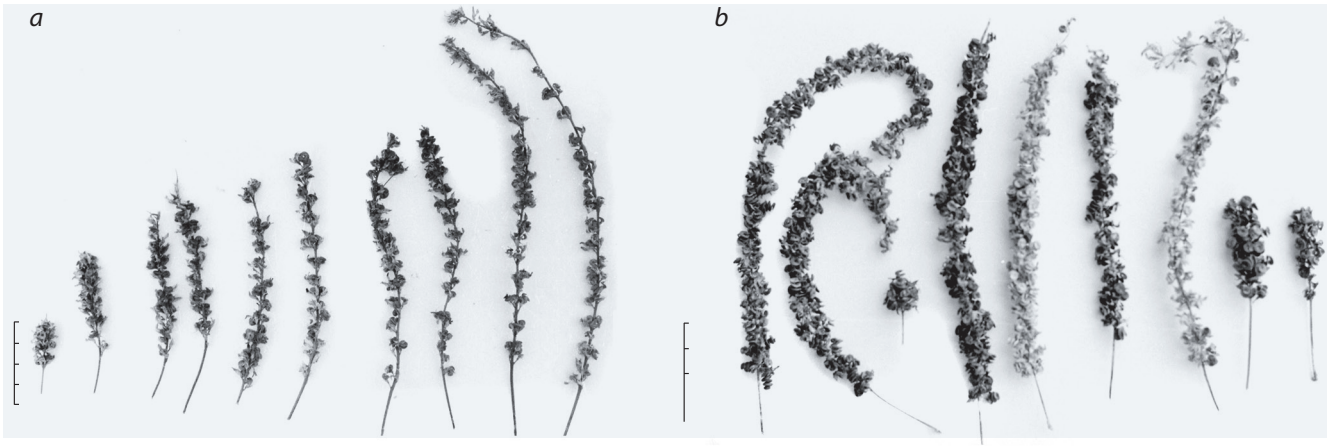

Fig. 4. Long inflorescences.

a – maloasian alfalfa S1 plants from VIR collection selections; *b* – spontaneous mutants.

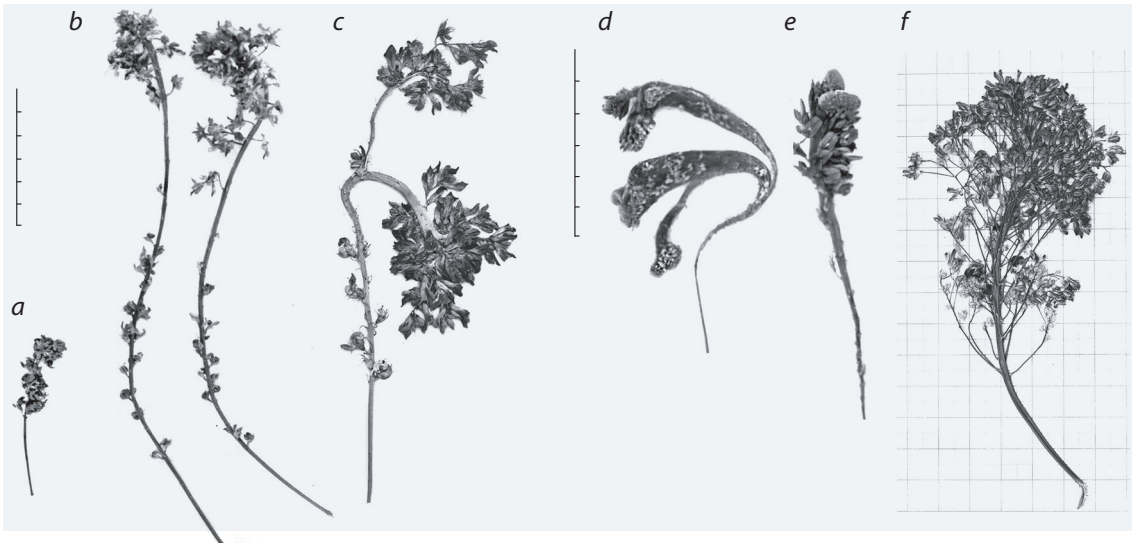

Fig. 5. Fasciated long petiole *lpfas* inflorescences and inflorescence of hybrid plants from hand crosses between different mutant types with fasciated long inflorescence.

a – wild-type raceme; *b* – branched (*bri*) × long fasciated (*lpfas*) = *brilpfas*; *c* – fasciated long petiole ((*lpfas*) with fertile flowers, splitting of peduncle; *d* – fasciated long petiole (*lpfas*) with sterile flowers; *e* – fasciated long petiole ((*lpfas*) with fertile flowers; *f* – panicle (*pi1*) × long fasciated (*lpfas*) = *pi1lpfas*.

Another source of long racemes in alfalfa – so called maloasian alfalfa from Turkey. VIR alfalfa collection was estimated by this trait and promising germplasm with high seed production was isolated (Dzyubenko N.I., Dzyubenko E.A., 1991) (Fig. 4). Other breeders also paid attention to long racemes in alfalfa as a potential for increasing seed yield (Staszewski, 1986; Bodzon, 1998). The *lp* trait is controlled propably by one recessive gene inherited in tetrasomic way (Dzyubenko N.I., Dzyubenko E.A., 1991; Bodzon, 1998).

Fasciation mutants' development in alfalfa. Broadening phenotypic diversity. Initially long inflorescences with fasciation were found in the self-pollinated progenies of the alfalfa accessions from Asia Minor from VIR collection. By means of deep inbreeding up to the fourth generations and subsequent selection the plants with wide fasciation at the top of inflorescence were obtained. Fasciation of the peduncle did not affect seriously the structure of the flowers. Nevertheless, up to one-half or the flowers dropped without pod setting. The dropped flowers were analyzed, they represented buds of different age. The amount of dropped buds increased with the extent of fasciation of peduncle. Expression of the trait "fasciated inflorescence" varied, the extreme manifestation was observed as 100 % fasciated inflorescences at the plant with up to 7 cm width of peduncles. All buds of the plant dropped at the stage 1–2 mm. Fasciated peduncle may split at the top into some sectors. The expression of the trait in self-pollinated progenies of the mutant plants varies from slight fasciation at the top of inflorescences to totally sterile inflorescences with flat peduncle. The trait "*fas*" was easily transferred to other inflorescence mutant forms (Fig. 5), using hand pollination.

In panicle-like mutants, especially in fertile forms, the flattening of the inflorescence peduncle does not lead to flowers fertility reduction. In branched inflorescences monstrous inflorescences with fasciated peduncles were obtained, semi-fertile,

Table 1. Plan of crosses between plants with different mutant inflorescences and generated double and triple mutant inflorescences in alfalfa

Crossing variant	Raceme with fasciated peduncle *fas*	Raceme with elongated peduncle *lp*	Raceme with elongated fasciated peduncle *lpfas*
Raceme with elongated peduncle *lp*	*lpfas*	–	–
Panicle-like inflorescence (fertile) *pi1*	*pi1fas*	*pi1lp*	*pi1lpfas*
Cauliflower panicle-like inflorescence (sterile) *pi1*	*pi1fas*	*pi1lp*	*pi1lpfas*
Branched inflorescence *bri*	*bri*	*brilp*	*brilpfas*

Fig. 6. Proliferation.
a – in cauliflower inflorescence; *b* – in long inflorescence.

some flowers had polymeric gynoecium, nevertheless most of the plants set the pods. In pea (Sinjushin, 2016) facsiation of the peduncle did not affected the viability of flowers in most cases, too. As a rule, fasciation affects most of SAM and FAM of the plant simultaneously, leading to the peduncles and stem flattening to the different extent. New combinations of mutant inflorescence types with fasciation and their designation are given in the Table 1.

Proliferation

Loss of FAM meristem identity in inflorescences leads to such phenomenon as proliferation with developing of vegetative shoot as prolongation of the inflorescence stalk or the axis of the second order, such cases were revealed in panicle inflorescences and long racemes (Fig. 6).

Mutants defective in their floral meristem identity (FAM) are possible to produce leaves after their transition to reproductive development, so some mechanisms cause "reprogramming" of FAM during this transition. Mutants defective in LEAFY/FLORICAULA (LFY/FLO) are available in various angiosperms, including tomato, pea, maize, snapdragon and *Arabidopsis*, and all show severe defects in flower development. For instance, in snapdragon *flo* mutant flowers are replaced by shoots (Coen et al., 1990).

Discussion

A main factor that shapes inflorescence architecture is the identity of the meristems produced in the inflorescence apex, what determines the relative position where flowers are formed. In *Arabidopsis*, upon floral transition, the vegetative meristem transforms into inflorescence meristem, which produces floral meristems in its turn. The development of the *Arabidopsis* inflorescence can be mostly explained by the function and mutual regulation of three genes: *TERMINAL FLOWER 1* (*TFL1*), *LEAFY* (*LFY*), and *APETALA 1* (*AP1*) (Shannon, Meeks-Wagner, 1993; Blazquez et al., 2006). These three genes act as opposing forces maintaining the balance between inflorescence and floral meristem identity at the inflorescence apex (Blazquez et al., 2006). By other definition, at least four genes are necessary for the specification of floral meristem identity in *Arabidopsis*: *LEAFY* (*LFY*), *CAULIFLOWER* (*CAL*), *APETALA1* (*AP1*), and *FRUITFULL (FUL*) (Weigel et al., 1992; Kempin et al., 1995).

Arabidopsis FAM forms simple racemes, not compound, so the reason for looking the appropriate models for responsible gene net in more relative model legumes plant is evident. Demonstrated macro- and microsynteny between the genomes of the model legume *M. truncatula* and other related species like diploid and tetraploid *M. sativa* (Choi et al., 2004) and pea (*Pisum sativum*) (Kalo et al., 2004) makes these plants easy targets to reveal gene functions.

In *P. sativum UNI*, *BROC*, and *PIM* genes all play roles in assigning floral meristem identity to the third-order branch. *Pim* mutants continue to produce inflorescence branches, resulting in a highly complex architecture and aberrant flowers, *uni* mutants initiate a whorl of sepals, but floral organogenesis is aberrant beyond that developmental point, and the double mutant *uni pim* lacks identifiable floral organs. A wild-type phenotype is observed in *broc* plants, but *broc* enhances *the pim* phenotype in the double mutant, producing inflorescences that resemble broccoli. Collectively these genes ensure that only the third-order meristem, not higher- or lower-order meristems, generates floral organs, thus precisely regulating the overall architecture of the plant (Singer, 1999).

Different reverse genetic and genomic tools providing to establish the function of candidate genes, responsible for architectural traits are available in several model and non-model legume species. *M. truncatula* is a classic model species for legumes. Through various international and national genomic initiatives sufficient amount of *M. truncatula* phenotypic

mutants were arisen using methods of forward and reverse genetics. Most used strategies for mutants generation were Tnt1 mutagenesis, TILLING and activation tagging. Both forward and reverse genetics screenings enabled to isolate interesting morphological mutants (extreme dwarf with dark green leaves, mutant with inflorescence and floral organ defects, unifoliate mutant with cauliflower-like inflorescences) (Tadege et al., 2008). Mutant populations generated by the retrotransposons *Tnt1* in *M. truncatula* are routinely used now for identification of mutants of genes of interest through reverse genetics (Cheng et al., 2011). Then, the virus induced gene silencing (VIGS) methods are available in several legume species including *M. truncatula* (Grønlund et al., 2008).

In *M. truncatula* the leaf development mutants with four alleles from a *M. truncatula* mutant collection generated by tobacco *Tnt1* retrotransposon insertion mutagenesis were isolated (Tadege et al., 2008). The mutants were named *sgl1-1* (single leaves) to *sgl1-4*, because all adult leaves were simple in these mutants, resembling the first leaf (juvenile leaf) developed in the wild-type plants. Flowers developed in *sgl1* mutants were abnormal and infertile, lacking petals and stamens and producing numerous flowers with cauliflower-like morphology. Because of their infertility, the *sgl1* mutants were maintained as heterozygotes. Progenies from self-pollination of heterozygous lines segregated wild-type-like and mutant plants in a 3:1 ratio, suggesting that the mutant phenotype was linked to a single recessive locus (Wang et al., 2008).

In pea development of inflorescences and flowers is under the control of few genes. *PIM* (*PROLIFERATING INFLORESCENCE MERISTEM*) was validated by A. Berbel et al. (2001), its homolog in *M. truncatula* was named *mtPIM* (Benloch et al., 2006). Corresponding *UNI* in pea (Hofer et al., 1997), *M. truncatula* homolog gene is *sgl1* (Wang et al., 2008). Homolog to *Arabidopsis* clue gene *LF* in pea is *lf* (Foucher et al., 2003), homolog in *M. truncatula* is unknown. Function of the gene *VEGETATIVE1* in pea (Berbel et al., 2012) is required for compound inflorescence development. Mutant *veg1* forms vegetative shoots instead of inflorescences. A. Berbel et al. (2012) found that genetic network controlling the legume compound inflorescence is distinct from that in grasses and Solanaceae.

Results of expression patterns analyses of *TFL1*, *FUL1*, *AP1* and *SG1* in *M. truncatula* indicated that they play specific role in identity determination of primary inflorescence meristem, secondary inflorescence meristems, floral meristems and common primordia, respectively (Cheng et al., 2018). In *M. truncatula* mutants *ap1* and *ap1 sgl1* manifestated proliferating inflorescences, double mutant *mtap1sgl1* completely lost floral identity, resembling cauliflower phenotype (Cheng et al., 2018). The conclusion was made that inflorescence architecture in *M. truncatula* is controlled by spatiotemporal expression of *MtTFL1*, *MtFULc*, *MtAP1*, and *SGL1* through reciprocal repression (Cheng et al., 2018). The data about homolog genes and mutants inflorescences in model plants, resembling mutants of *M. sativa* described above, are given in Tables 2–4.

The most unclear situation in alfalfa mutants arises in case of *lp* and *bri* mutants in the lack of resembling mutations within the model plants. Because of the complex segregation pattern of the tetraploid inheritance in *M. sativa*, geneticists often study the diploid alfalfa species and subspecies belonging to the *M. sativa* complex, such as diploid *M. sativa* ancestor *Medicago coerulea* (Kalo et al., 2000). P. Kalo et al. (2000) presented the improved genetic map of alfalfa, suitable for comparative mapping studies. Since the diploid and the cultivated tetraploid alfalfa are crossable and belong to the *M. sativa* complex the detailed genetic map of diploid *M. sativa* can facilitate mapping and tagging agronomically important traits in different alfalfa populations. The map can be used in map-based cloning approaches for isolating genes conditioning important agronomic traits in cultivated alfalfa, such as traits connected with seed productivity improvement (for example *lp – long peduncle*).

Table 2. Genes influencing compound inflorescence formation in *Arabidopsis* (flower-inflorescence transition), and their homologs in *P. sativum* and *M. truncatula*

Arabidopsis thaliana	*Pisum sativum*	*Medicago truncatula*
LEAFY (*LFY*) (Weigel et al., 1992)	*lf* (Foucher et al., 2003)	
	UNIFOLIATA (*UNI*) (Hofer et al., 1997)	*Sgl* (Wang et al., 2008)
APETALA (*AP1*) (Yanofsky, 1995)	*PROLIFERATING INFLORESCENCE MERISTEM* (*PIM/PEAM4*) (Hofer et al., 1997; Berbel et al., 2001)	*MtPIM* (Benlloch et al., 2006)
	VEGETATIVE (*VEG1*) (Berbel et al., 2012)	

Table 3. "Cauliflower" phenotype mutants in *Arabidopsis*, *Pea* and *Medicago*

Arabidopsis thaliana	*Pisum sativum*	*Medicago truncatula*	*Medicago sativa*
ap1cal (Kempin et al., 1995)	*unipim* (Singer et al., 1999)	*mtap1 sgl1* (Cheng et al., 2018)	*aaaa* (Kinoshita, Suginobu, 1982)

Table 4. Genes families regulating fasciation in *Arabidopsis* and *Pea*

Arabidopsis thaliana	*Pisum sativum*
CLAVATA (*CLV*) and *FASCIATA* (*FAS*) gene families (Williams, Fletcher, 2005)	*FASCIATA* (*FA*) and *FA* gene families (Sinjushin, Gostimskii, 2007)

Conclusion

Summarizing achievements in developmental genetics of inflorescence development in model plants relative to *M. sativa*, we approach to understanding of possible genetic network, regulating the mutant inflorescence deviations in cultivated alfalfa described above. In the nearest future no doubt the researchers will be able to identify genes responsible for these mutations.

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Variability of the structure of correlations between the morphological and commercial traits of soybeans with different growth habit and branching characters

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High yields of seeds, green pods and green biomass is the main goal of soybean breeding in many countries. An assessment of relationships between the productivity traits and their effect on the yield may be useful in developing effective crop cultivation programs. In soybean, the stem growth habit and the branching character are interrelated with plant productivity and in most cases determine it. Therefore, the aim of the present work was to study the variability of the level (strength) and the structure of correlations between 92 morphological, phenological, biochemical, agronomic traits of soybean accessions with different growth habits, and branching characters in different weather conditions. 270 soybean accessions of different ecological and geographical origin from the VIR collection have been grown in the Krasnodar region within 3 years. Field studies of the traits and biochemical analysis were carried out according to VIR guidelines. The variability of correlation matrices as regards the strength and structure of relationships was analyzed using the correlation and factor analysis (the principal component method), as well as the method developed by N.S. Rostova. A comparison of the level (R^2, coefficient of determination) and structure of correlations in different years has shown that the deterioration of external conditions is followed by an increase in the strength of relationships (R^2) between the traits and in the difference between correlation matrices' structure. Soybean adaptation to the changing conditions occurs due to the rearrangements of relationship systems, whereas the degree and direction of these changes are determined by the growing conditions and specificity of the accessions response. Under favorable conditions, the structure of correlations in soybeans with different growth habits, and branching characters has more similarity than in the conditions critical for development. The highest level of relationships (R^2) between the traits was observed in the year that was unfavorable for the growth of the semi-cultivated accessions (with the indeterminate growth habit and a large number of branches of the 1st and 2nd order). The green biomass productivity of accessions with the determinate growth habit and more than two branches is most strongly associated with the branch weight, while in accessions with the indeterminate growth habit and with (or without) 1–2 branches it depends on the growing season duration, one leaf weight and the number of leaves per plant. In the semi-cultivated accessions (with the indeterminate growth habit and numerous branches of the 1st and 2nd order), it correlates, besides the listed traits, with the number of nodes, the internode length, the main stem diameter, the weight of leaves, seed morphometric parameters and their quality.

Key words: soybean; genetic resources; growth habit; variability; correlations; multidimensional analysis.

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Изменчивость структуры корреляций морфологических и хозяйственных признаков у сои с разным типом роста и характером ветвления

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Высокая урожайность семян, зеленой массы, зеленых бобов – основная цель селекции сои во многих странах. Оценка связей между признаками продуктивности и их влияние на урожайность полезны при разработке эффективных программ по возделыванию культуры. У сои тип роста стебля и характер ветвления взаимосвязаны с продуктивностью растения и в большинстве случаев определяют ее. Проведено изучение изменчивости уровня (силы) и структуры корреляций 92 морфологических, фенологических, биохимиче-

ских, хозяйственных признаков у образцов сои с разным типом роста и различным характером ветвления в контрастных погодных условиях. 270 образцов сои разного эколого-географического происхождения из коллекции ВИР выращивали три года в Краснодарском крае. Изменчивость корреляционных матриц по силе и структуре связей анализировали с помощью корреляционного и факторного анализа (метода главных компонент) и по методу, разработанному Н.С. Ростовой. Сравнение уровня и структуры корреляций показало, что при ухудшении внешних условий увеличиваются сила связей между признаками и различие в структуре корреляционных матриц. Адаптация сои к меняющимся условиям происходит за счет перестроек систем связей, причем степень и направление этих изменений определяются условиями произрастания и спецификой реакции образцов. В благоприятных условиях структуры корреляций у сортов сои с разными типом роста и характером ветвления более сходны, чем в критических для развития. Самый высокий уровень связей (R^2) между признаками наблюдался в неблагоприятный для роста год у полукультурных образцов (с индетерминантным типом роста и большим числом ветвей первого и второго порядка). Продуктивность зеленой массы образцов с детерминантным типом роста и числом ветвей более 2 наиболее сильно связана со средней массой ветви; у образцов с индетерминантным типом роста и с 1–2 ветвями (или без них) она зависит от длины вегетационного периода, средней массы одного листа и числа листьев на растении. У полукультурных образцов с индетерминантным типом роста и множеством ветвей первого и второго порядка она коррелирует, кроме перечисленных признаков, с числом узлов, длиной междоузлия и диаметром главного стебля, массой листьев, морфометрическими параметрами семян и их качеством.
Ключевые слова: соя; генетические ресурсы; тип роста; изменчивость; корреляции; многомерный анализ.

Introduction

Soybean is one of the most economically important leguminous crops, ranking first among them in the world in terms of cultivated areas (http://www.fao.org/faostat). The numerous varieties that have been created by now differ by a huge variety of forms and demonstrate adaptation to various climatic conditions. Along with the specialized varieties, the semi-cultural forms are also industrially cultivated. The latter are commonly used for producing green fodder and green manure, as well as for the development of modern varieties. Soybeans are characterized by several types of the main stem growth: the varieties with indeterminate and determinate growth habit are distinguished. Since the determinate growth habit very rarely occurs in the wild-growing soybeans, this type of stem is associated with the domestication of the species (Liu et al., 2007; Tian et al., 2010). Previous studies have shown that the type of stem growth in soybean is mainly controlled by the *Dt1* locus; the indeterminate growth habit is dominant or not fully dominant with respect to the determinate *dt1* (Woodworth, 1932). Also known is the second locus, which controls stem growth and is designated as *Dt2*. The *Dt2* allele is almost dominant with respect to *dt2*. The *Dt2*/*Dt2* genotypes determine semi-determinate phenotypes in the *Dt1*/*Dt1* genetic background, while the *dt2*/*dt2* genotypes determine the indeterminate ones. However, the phenotype is determinate in the *dt1*/*dt1* genetic backgrounds, because *dt1* is epistatic to *Dt2* and *dt2* (Bernard, 1972). It has been reported about the identification of the third allele in the *Dt1* (*dt1-t*) locus, which produces a phenotype with some characteristics of both *dt1* and *Dt2* (Thompson et al., 1997). The *Dt1* gene (=*GmTfl1*) is homologous to the terminal flower 1 (*TFL1*) gene of *Arabidopsis*, i. e., the regulatory gene encoding the apical meristem signaling protein. The transition from the indeterminate to determinant type occurred through four independent single-nucleotide substitutions, each of which resulted in the replacement of amino acids (Tian et al., 2010).

The stem growth habit in soybean is an agronomically important trait, which is interconnected with many economically important characters. However, it is often difficult to distinguish between the indeterminate and determinate growth habit in field conditions, since their manifestation is influenced by the day length and the unfavorable growth conditions (Bernard, 1972). The branching character has not less influence on the grain and feed productivity of plants. The semi-cultivated varieties are distinguished by a large number of branches; the modern ones have no 2nd order branches, or form only the main stem.

Considering the importance of soybean as a food and forage crop, high yield of seed, green biomass and green pods is the main goal of soybean breeding in many countries. An assessment of relationships between the productivity component traits and their effect on the yield may be useful in developing effective crop cultivation programs. In this regard, a lot of research is devoted to the study of correlations between plant characters, as well as to the search for indicator traits which can be used for selecting accessions with the necessary economically important traits. In the works reviewed by the authors, seed productivity was connected with phenological and morphological characters, such as the ‘days before ripening’ and ‘grain filling period’ (Ferrari et al., 2018), plant height and number of branches (Aditya et al., 2011; Hakim, Suyamto, 2017), number of pods per plant (Board et al., 2003; Nagarajan et al., 2015; Rodrigues et al., 2015; Machado et al., 2017), number of nodes and pods with 2–3 grains (Machado et al., 2017), number of seeds per plant (Rozhanskaya et al., 2016), number of pods per plant and nodes on the main stem (Silva et al., 2015), as well as the number of seeds per plant and the 1000 seed weight (Vu et al., 2019). A number of works have noted a close relationship between seed productivity and green biomass (Leshchenko et al., 1987) or the above-ground mass of the plant (Huang et al., 2009).

There may be several reasons for so different, sometimes even opposite results concerning the relations between the traits of the seed and biomass yield obtained in studies of different authors. On the one hand, it can be explained by the nature of quantitative traits, which are characterized by continuous variability determined by the influence of a large number of genes, or by the redistribution of the number and range of genes due to the change of the limiting environmental factors. On the other hand, many authors studied correlations

using a limited number of genotypes, evaluated a different number of traits employing different statistical methods, and therefore obtained different results. Besides, the studies did not take the diversity of the studied forms into account. It is known that a change of only the sample size can reveal the previously inconspicuous connections (Rostova, 2002). For instance, a simultaneous study of hay, silage, and green fodder soybean accessions may yield one set of results, whereas their separate analysis may yield a different set of data (Burlyaeva, Rostova, 2014).

Though the amount of correlation studies in soybeans performed by now is quite huge, there is no common point of view on the relationships between the quantitative traits that determine the seed and green biomass productivity. In spite of the obvious difference in morphological and economically important traits between the accessions with different growth habit and branching characters, we failed to find any studies differentiating varieties according to these characters and analyzing the links between productivity elements taking the morphotype into account. The information about correlations is fragmented and is limited to a statement of facts; meanwhile, the study of the correlations variability allows making a judgment on the interrelationships between traits, inherited variability, and helps to choose the right breeding strategy.

The purpose of the present work was to study the variability of the level (strength) and structure of correlations between morphological, phenological, biochemical, economic traits in soybean accessions with different growth habit and branching characters in different weather conditions.

Materials and methods

To study the variability of the structure of correlations between morphometric, biochemical and economically important soybean traits, 270 accessions representing domestic and foreign varieties of different ecological and geographical origin were selected from the global VIR collection (the N.I. Vavilov All-Russian Institute of Plant Genetic Resources). Ninety-two traits of soybean varieties have been investigated (Supplement)1. The soybean plants included in the study significantly differed from each other. They were divided into three groups according to the growth habit and branching characters. The first group included accessions with the determinate growth habit and a large number of branches (more than two), the second group united the semi-cultivated accessions with the indeterminate growth habit and numerous branches of the 1st and 2nd order, while the third group consisted of the accessions with the indeterminate growth habit with 1–2 branches or without them.

The data selected for the analysis resulted from the field experiments, which were carried out in 1989, 1992 and 1994 at the Kuban Experiment Station of VIR (KOS VIR) located in the steppe area of the Prikubanskaya Plain. The trial years were characterized by contrasting meteorological conditions. In 1989, the growing degree days above 10 °C amounted to 3590 °C, 3156 °C in 1992, and 3578 °C in 1994. The amount of rainfall during the growing season in 1989 was 394.7 mm, 334.3 mm in 1992, and 177.1 mm in 1994. In 1989 and 1992, the rainfall exceeded the average long-term norm, whereas in 1994 it was significantly below the norm. The high moisture availability in 1992 was observed only in the first half of the growing season, while the second half of the summer was characterized by insignificant rainfall.

The accessions were sown according to the collection nursery pattern. Each variety was sown in a four-meter single-row plot, with the inter-row spacing of 70 cm and 10 cm between plants in a row. Phenological observations, botanical and morphological descriptions of the accessions were carried out in accordance with the The International COMECON List of Descriptors for the Genus *Glycine* Willd (1990). The green biomass yield was estimated in the mowing ripeness phase (the onset of pods filling). The weight of branches, leaves and pods was determined at the same time for 10 plants of each accession. After ripening, the analysis was carried out on 10 soybean plants selected from the middle of a row.

The content of dry matter, fiber, protein in green biomass, oil and protein in the seeds was determined in the biochemistry laboratory of the Kuban Experiment Station of VIR, while the content of trypsin, chymotrypsin in the seeds was analyzed in the Biochemistry Department of VIR according to the Methods of biochemical research in plants (Ermakov et al., 1987). The green biomass biochemical composition was analyzed when assessing the green biomass yield in the mowing ripeness phase.

The revealing of regularities in variability and of the degree of correlation of 92 economic and biological traits in different environmental conditions with different types of soybean growth and branching characters, determination of their information value, adjustment of the initial set of traits by discarding redundant and secondary characters were performed using statistical data processing, which included correlation analysis and factor analysis of the correlations system using the principal component method. The identification of the groups of the most interconnected traits (pleiades) was carried out by analyzing the systems of correlations when constructing the correlation circles (matrix images in the form of the correlation cylinder sections) (Terentiev, 1959). The analysis was performed for nine correlation matrices calculated for each sample (for three groups of accessions, composed according to the growth habit and branching characters, for three years of research). The correlations were compared concerning the strength (level) of relationships (R^2 – coefficient of determination) and their structure (traits rearrangement in the correlation pleiades). The differences between the correlations matrices in the strength of relationships were determined by comparing the average values of the determination coefficients (R^2) (Wright, 1920). Similarity of characters relationship systems (correlation matrices) was calculated between matrices with z-transformation (Rostova, 2002). The R. Fisher's z-transformation was introduced for converting the distribution of the correlation coefficients (r) to the normal one according to the formula $z = 0.5 \ln((1+r)(1-r))$. After the z-transformation, each of the compared correlations matrices (diagonal elements excluded) was rearranged into a vector. The obtained 9 vectors were used to form a new data array; in it, each matrix was regarded as a trait, and the individual coefficients in this matrix as values of the trait. The compared matrices were ordinated using the principal component method. The first main component was regarded as the factor of matrices similarity,

[1] Supplementary material is available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx14.pdf

and the proportion of variance corresponding to this component (FD1%) was used as an indicator of the degree of all the compared matrices similarity. The second main component was interpreted as an indicator of differences in the structure of matrices (Rostova, 2002). After grouping 9 correlation matrices by the principal component method and combining them with the correlation circles (matrix images in the form of the correlation cylinder sections), the regularities in the matrices distribution and changes in the structure of relationships in them were determined, i. e., the variability of the system of correlations in soybean accessions with different growth habit and branching character in different weather conditions have been revealed. Data analysis was performed using Statistica.7 and Excel 7.0 for Windows.

Results

At the initial stage of the study, the entire set of traits was analyzed in order to assess their relative informativeness. The factor analysis of all traits was performed using the data combined for three years of research and for each year separately. It has shown that the studied traits variability is associated with ten main factors. The studied traits got distributed into the factors of growth habit, seed weight and size, growing season duration, plant height, seed biochemical composition, leaf size and shape, green biomass biochemical composition, plant color (anthocyanin content in organs), the content of anti-nutrients in seeds, inflorescence parameters, and of green biomass yield. A more detailed description of this analysis is given in a previously published paper (Burlyaeva, Malyshev, 2013). As a result, 20 traits were selected as the most important ones for studying the accessions concerning their growth habit and green biomass productivity indicators. Also, the characters most strongly associated with the coordinated variability of plants in changing environmental conditions were identified. These included the weight of plants, branches and leaves; the number of leaves, branches and nodes; the average weight of one leaf and branch; stem diameter; plant length; internode length; middle leaf length and width; percentage of leaves from the total plant weight; growing season duration; the content of protein, fiber and dry matter in the green biomass; the content of protein and oil in seed; 1000 seed weight; and the seed hilum width.

Further studies of variability of the correlations level and structure were carried out using the adjusted set of traits. The evaluation of matrices for the relationships structure variability (by the traits rearrangement in the correlation pleiades) was carried out using the principal component analysis according to the method by N.S. Rostova (2002) described above in the Materials and Methods section. The first main component was interpreted as the factor of matrices similarity, while the second component reflected differences in the structure of matrices connection. The proportion of the main component variance was taken as the indicator of the degree of the compared matrices similarity.

A comparison of nine z-transformed correlation matrices (three groups for three years of study) has shown that the similarity of the structure of correlations between accessions from different groups is lower than within each group (52.2 % for all groups, 70.9 % for group 1, 52.4 % for group 2, and 65.5 % for group 3). When studying the variability of correlation matrices for each year, the largest differences between them were observed in 1994 with correlations similarity of 67.0 %, while in 1989 and 1992 variability of the trait correlation structure was approximately the same (73.4 % and 73.6 %, respectively) (Fig. 1).

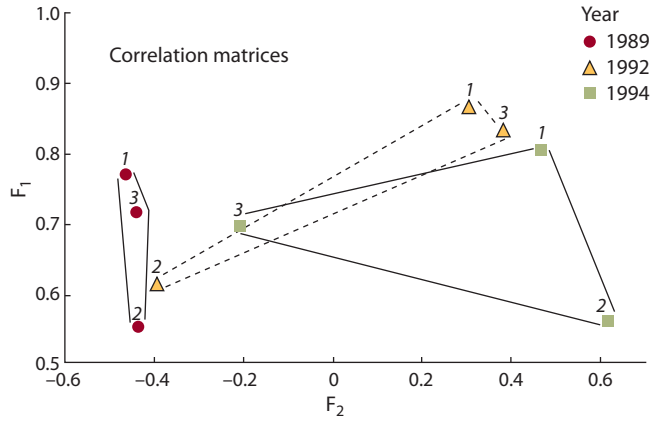

Fig. 1. Correlation structure variability in soybeans with different growth habit and branching characters in years of research.

F_1 – matrix similarity factor; F_2 – matrix specificity factor. *1* – group of accessions with the determinate growth habit and more than 2 branches; *2* – group of accessions with the indeterminate growth habit and numerous branches of the 1st and 2nd order; *3* – group of accessions with the indeterminate growth habit and 1–2 branches (or without them).

Strength of relationships in correlation matrices of the accessions with different growth habit and branching characters in different year of the study (1989, 1992, 1994)

Group	Year		
	1989	1992	1994
1 Accessions with the determinate growth habit and over 2 branches	0.060	0.103	0.086
2 Accessions with the indeterminate growth habit and numerous branches of the 1st and 2nd order	0.181	0.160	0.274
3 Accessions with the indeterminate growth habit and over 1–2 branches, or without them	0.063	0.134	0.077

The factorial variance of the correlation matrices of all accessions over the years of the study was 83.4 % and exceeded the factorial variance of the matrices calculated for groups (69.7 %). It follows, that the correlation structure variability for these accessions is largely affected by the genotypic properties of the variety. In 1994, the strongest differences were noted between the structure of correlations in matrices calculated for all groups; obviously, the conditions critical for growth caused serious and diverse changes in the structure of correlations in different accessions. The highest variability in the structure of correlations was noted for the semi-cultivated

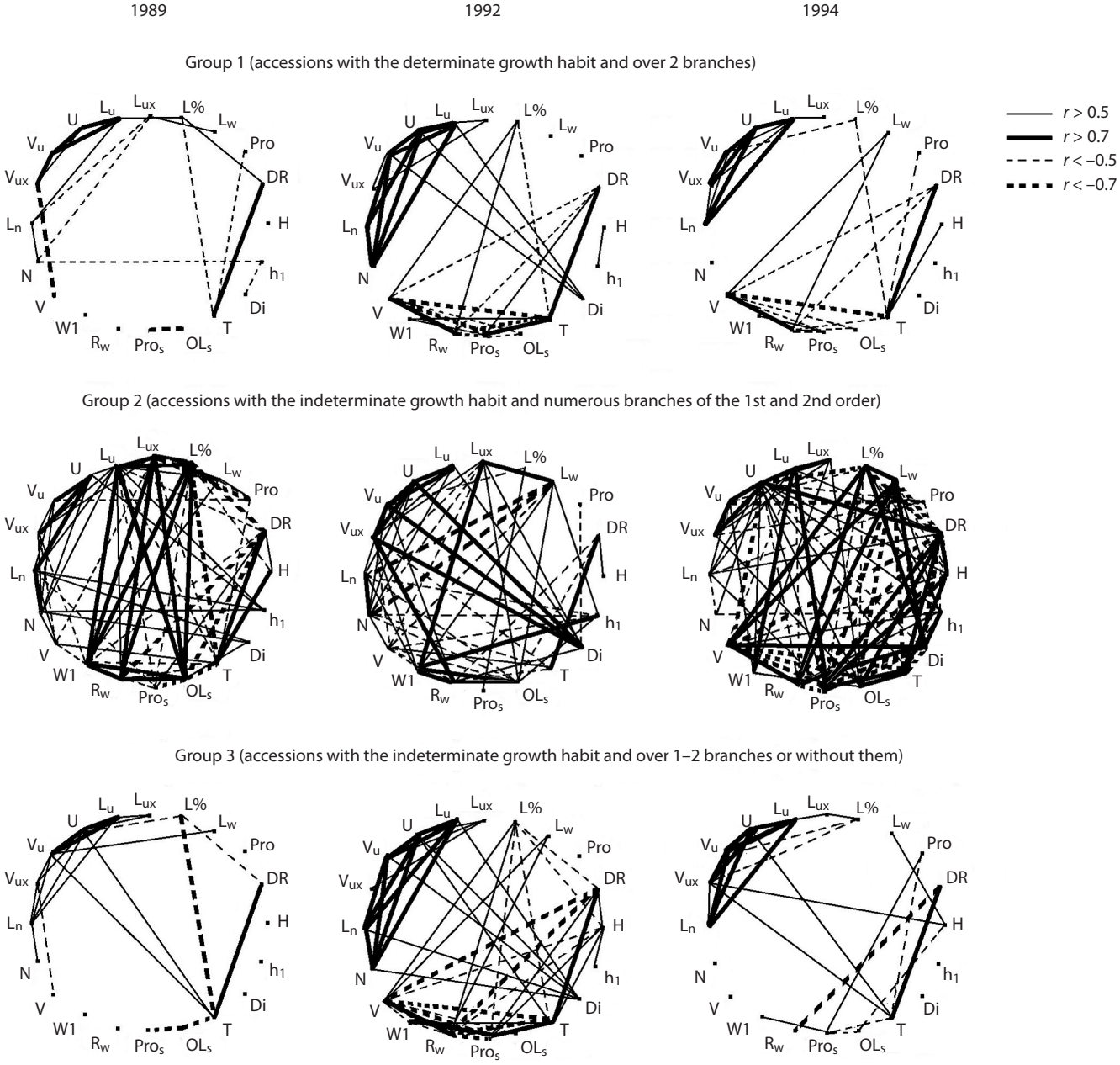

Fig. 2. Correlation structure of traits variability in accessions with different growth habit and branching characters in different years of the study.

U – plant weight in pod filling phase; L_u – leaves weight; L_{ux} – one leaf average weight; L% – leaves percentage of the total plant weight; L_w – middle leaf width; L_n – number of leaves per plant; V_u – branches weight; V_{ux} – one branch average weight; V – number of branches per plant; H – plant length; h_1 – average internode length; Di – stem diameter; N – number of nodes per plant; W1 – 1000 seed weight; R_w – seed hilum width; T – sprouting-to-maturity period duration; DR – dry matter content in green biomass; Pro – protein content in green biomass; Pro_s – protein content in seeds; OL_s – oil content in seeds.

accessions (with the indeterminate growth habit and a large number of branches of the 1st and 2nd order).

As the growing conditions changed, an instability of the structure of relationships between traits was also noted in this group, though the degree of traits determination (R^2, the relationships strength) was the strongest (Table).

When comparing the level and structure of correlations in different years, one can notice that the strength of relationships between the traits and the difference in the correlation matrices structure increase with the deterioration of external conditions. Under favorable conditions, the structure of correlations in soybean varieties with different growth habit and branching characters is more similar than in the conditions critical for vegetation. The adaptation of soybeans to the changing conditions in different groups occurs due to restructuring of the relationship system specific to a particular group.

To reveal more specific differences in the structure of trait relationships in accessions from different groups in different

years of the study, matrix images in the form of the correlation cylinder sections were used (Fig. 2). The correlation relationships in the green biomass productivity pleiad were the strongest and most stable in all accessions. In 1989, a year that was favorable for growth, the plant weight in accessions of the first group was associated with the weight of leaves, branches and the number of leaves. A strong and permanent correlation was also observed between the growing season duration and the dry matter content in the vegetative mass. In 1992, in the conditions of a cold and wet year, there was a strengthening of correlation relationships in the plant mass productivity pleiad, and the stem diameter character entry into the pleiad. A significant increase was also noted for the correlation between the growing season, the weight of 1000 seeds and protein content in them. Under severe drought conditions in 1994, the influence of the growing season on other traits increased. The plant length was determined by the growing season duration. The 1000 seed weight correlated with the high protein content in seeds both in 1992 and in 1994.

The second group composed of semi-cultivated accessions was distinguished by strong relationships between almost all traits, and the strongest ones were observed in 1994, a year that was dry and critical for soybean growth. In contrast to accessions from the first group, in 1989, a year that was favorable for vegetation, the green biomass productivity depended not only on the leaves weight and number, but also on the number of nodes and the internode length. Also, a very strong correlation was observed between the 1000 seed weight and the oil content in seeds. The growing season duration correlated with the plant length and with the content of dry matter in the green biomass. At low temperatures in 1992, the role of plant weight significantly increased in the total variability due to the increased strength of correlations with the weight of leaves and stem diameter. The leaf weight correlated with the 1000 seed weight, stem diameter and oil content in seeds. In contrast to 1989, the 1000 seed weight strongly correlated with the internode length. Similar to the accessions with the determinate growth habit, the relationship between the growing season duration and dry matter remained stable in all years of the study. Under the conditions of 1994, an increase in all correlations is accompanied by a weakened influence of the number of nodes on the variability of plant structures. The green biomass productivity, on the contrary, correlated with the majority of the studied parameters. There formed a relationship between the stem diameter and leaf characteristics, the percentage of leaves and the number of branches per plant. It is interesting to note that in contrast to 1992, there was a negative correlation between the leaf width and the protein and oil content in seeds.

In all the years of the study, the most stable and strong correlations in the third group of accessions (with the indeterminate growth habit and with 1–2 branches, or without them) were those between the green matter productivity, the weight of leaves, the number of leaves, and the growing season duration. In 1989 and 1994, there was a stronger negative relationship between the percentage of leaves per plant and the growing season duration than in the accessions from the first and second groups. The correlations between the traits of green biomass productivity were similar to the relationships revealed in the accessions with the determinate habit growth. The strongest relationships between the traits were observed in the conditions of 1992. That year, the stem diameter and the number of nodes per plant played a more significant role in the total variability of traits. The growing season duration correlated with the protein content in seeds. The trait of plant length had a greater significance than for the accessions from other groups. This trait was associated with the internode length, the growing season duration, dry matter content in green biomass and protein content in seeds. The drought of 1994 increased the strength of relationships between traits, though to a lesser extent than the growing conditions did in 1992. The structure of correlations in 1994 was close to the structure of relationships between traits in the accessions with the determinate growth habit.

Discussion

A comparison of the average level of traits determination (R^2) (Fig. 3) and stability of the structure of their relationships (correlations) (FD1%) performed for both different years and different groups has shown that the level of strongest relationships and similarity in the structure of correlations were observed for the vegetative mass productivity, growing season duration, and the number of branches per plant. A comparison of the R^2/FD1% ratios for nine correlation matrices (all years and groups) displays a noticeable decrease in the similarity in the structure of relationships between such traits as the growing season duration, seed hilum width and the number of branches per plant.

These traits are the main ones in the pleiades of the growing season duration (T), seed and pod parameters (R_w), stem characters, growth habit i. e., growth habit and branching characters (V); they have a high level of relationships with other plant traits (not included in their own pleiades) and are characterized by strong variability of these correlations, which depends on both conditions and genotype. It means that the same traits in different groups form relationships with different traits under the changing conditions. The lability of the trait correlations described above apparently plays a role in the plant adaptation to various growth conditions.

An analysis of the determination coefficients variability revealed the highest level (strength) of relationships between the traits in the pleiades of seed productivity, growing season, and plant characters (growth habit and branching characters) (Fig. 4). A lower level of determination and its relative stability were observed for such traits as stem diameter, leaf length, protein content in seeds, and dry matter content in green biomass. The traits in the pleiad of green biomass productivity, shoot, and leaf width were distinguished by a lower variability of the determination coefficients, i. e., displayed a stable level of relationships (correlations).

A detailed study of the correlation structure variability has shown that the relationships of green biomass productivity with the weight of branches and leaves, and with the number of leaves, are stable, display the highest level of relationships and are characteristic of all accessions. The green biomass increase in the varieties with the determinate growth habit and a large number of branches is influenced more by the average weight of a branch, while the growing season duration, one leaf weight, and the number of leaves per plant have a stronger impact on the accessions with the indeterminate growth

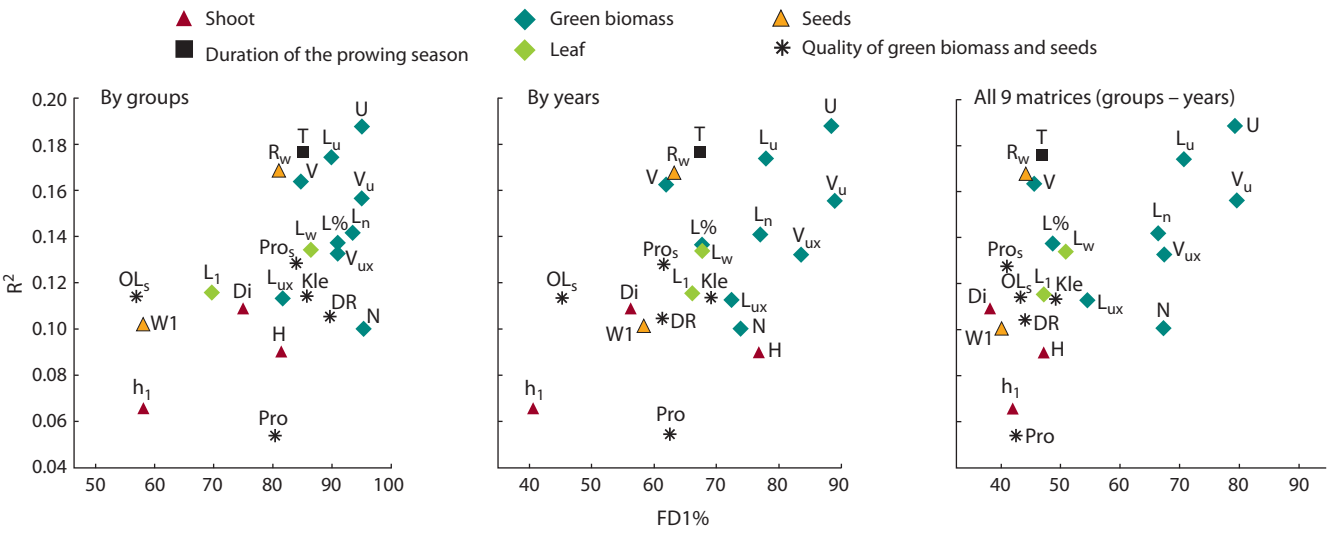

Fig. 3. Average level of trait determination (R^2) and stability of their relationships (correlations) structure (FD%).
For trait designations see Fig. 2.

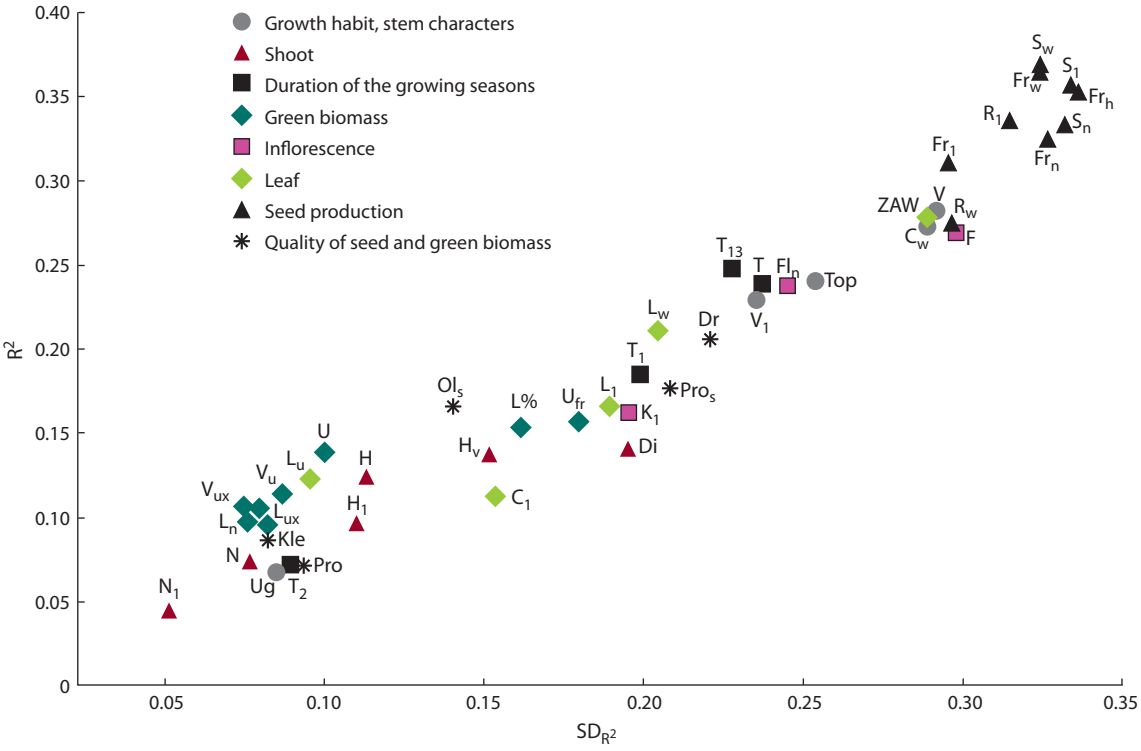

Рис. 4. Traits determination and variability of the strength of relationships between them.
X-axis – average determination (R^2); *Y*-axis – standard deviation of the average determination coefficient (SD_{R^2}). For trait designations see Supplement and Fig. 2.

habit, 1–2 branches or without them. In addition to the above mentioned traits, the green biomass productivity in the semi-cultivated accessions (with the indeterminate growth habit and numerous branches of the 1st and 2nd order) depends on the number of nodes and the main stem internode length. Changes in the environmental conditions cause both general rearrangements in the structure of correlation relationships, and individual ones, which are characteristic of plants with a certain growth habit and branching.

The studies of correlation relationships between the traits in soybean varieties with different growth habit and branching characters have resulted in revealing a regularity in these

characters variation under different environmental conditions. A minor deterioration in the growing conditions entails a slight decrease in the degree of correlation of all the traits. More tough conditions change the behavior of the traits of the generative and vegetative spheres. Fig. 4 shows the separation of these traits into two groups. R^2 values either diminish, or increase for the vegetative organ traits. Determination coefficients sharply increase for the traits associated with seed productivity. Separation of the green biomass and seed productivity traits in terms of the degree of correlation in varieties of the first and second groups (with numerous branches) was observed during their development during a drought. The accessions from the third group (with few branches) displayed such a separation of traits during vegetation in a year with excessive moisture in the initial phases of growth and insufficient moisture in the second half of summer. The difference between the varieties during critical periods was associated with the branching characters in these groups. In a dry year, the accessions with numerous branches experienced a greater moisture deficiency from the first phases of vegetation (from the branching period). The varieties characterized by a small number of branches did not experience such a severe influence of drought at that time. For them, the most unfavorable year was the one with a long period of vegetative organs growth (the phase before flowering) and insufficient moisture during the periods of mass flowering and pods formation.

Thus, the seed and green biomass productivity not always show a direct and strong correlation between them. The relationships between these traits is influenced by both the conditions of the year and the features of the variety. The variation of R^2 values for green biomass and seed productivity has its specificity in each group, which should be taken into account in breeding for the main economic traits.

Conclusion

A comparison of the correlation coefficient values calculated for all accessions, groups distinguished by the growth habit and branching characters, and for the years of study, yields results that are noticeably different. The data calculated for all accessions regardless of the growth habit and branching characters, characterize only the species specificity of the coordinated variability of soybean traits and do not reveal the features of varieties that are important for understanding their behavior in changing environmental conditions. This was not taken into account in most works that dealt with determining correlations between the traits and identifying indirect traits for selection for important economic characters. That is why the selection using the indicator traits identified by other researchers often did not yield a proper result.

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The *WF1* (*White Flower 1*) gene controlling the white color of petals and flowering time in lines from a mapping population of flax (*Linum usitatissimum* L.)

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Flax (*Linum usitatissimum* L.) is grown in different climatic zones as both a spring and winter crop. Adaptation to different growing conditions produced genotypes with different growth durations and degrees of photosensitivity. It was always of great importance for breeders to create varieties with rapid development, in particular, early-flowering ones. The evaluation of lines from the VIR flax genetic collection revealed a wide intraspecific diversity in the duration of growth phases, the number of leaves on the stem (physiological indicator of early flowering), and the degree of photosensitivity. Line gc-109, early flowering under the long-day conditions, but greatly photosensitive, was found to possess the *wf1* (*white flowers*) gene, associated with early flowering and a small number of leaves. This line was crossed to the late-flowering but low-photosensitive line gc-375, which had reddish purple flowers. The analysis of segregation in F_2 held under the long (19 hours) and short (12 hours, daylength at the equator) day conditions showed that the number of leaves on the plant stem was associated with the flowering time and controlled by close genetic systems only under the long-day conditions. In addition, no relationship between the flowering time and petal color was found under the short-day conditions. Thus, different groups of genes are active in different light schedules. More than 200 lines of the 6th generation of inbreeding were obtained from the plants of the hybrid population. Their field testing under the long-day conditions showed that although the majority of the lines with white petals flowered early and had a small number of leaves, some of them bloomed later and were leafier. On the contrary, the early flowering and less leafy lines appeared among the lines with colored flowers. Therefore, it is reasonable to assume that a crossover between the gene participating in the control of early flowering, which came from the gc-109 line, and its *wf1* gene occurred in meiosis of F_1. The linkage between the genes controlling early flowering and white petals suggests that flower color can serve as a marker of early flowering in the selection of early breeding material.
Key words: flax; photosensitivity; earliness; flower color; linkage.

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Ген *WF1* (*White flower 1*) белоцветковости и сроки цветения линий картирующей популяции льна (*Linum usitatissimum* L.)

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Лен (*Linum usitatissimum* L.) выращивают в различных климатических зонах как яровую и озимую культуру. Приспособление к различным условиям произрастания привело к появлению генотипов с разным вегетационным периодом и различной степенью фоточувствительности. В то же время для селекционеров быстро развивающиеся, и в частности раноцветущие, сорта всегда имели большое значение. Изучение линий генетической коллекции льна ВИР выявило широкое внутривидовое разнообразие по продолжительности фаз вегетационного периода, числу листьев на стебле (физиологическому показателю раннего цветения) и степени фоточувствительности. У раноцветущей в условиях длинного дня, но сильно фоточувствительной линии гк-109 был идентифицирован ген белоцветковости *wf1*, ассоциированный с ранним цветением и малым количеством листьев. Эту линию скрестили с поздноцветущей, но слабо фоточувствительной линией гк-375, имеющей красно-фиолетовые цветки. При изучении расщепления F_2 в условиях длинного (19 ч) и короткого (12 ч – длина дня на экваторе) дня установлено, что число листьев на стебле растения ассоциировано со временем цветения и контролируется близкими генетическими системами только на длинном дне. Кроме того, на коротком дне не проявлялась связь между временем цветения и цветом лепестков. Таким образом, при разных режимах освещения активны разные группы генов. Из растений гибридной популяции было получено более 200 линий 6-го поколения инбридинга. Их тестирование в полевых условиях длинного дня показало, что хотя большинство белоцветковых линий зацветало рано и имело малое количество листьев, часть из них цвела поздно и была более облиственна. Среди линий с окрашенными цветками наоборот появились раноцветущие и малооблиственные. Таким обра-

зом, можно сделать вывод о том, что в мейозе F_1 произошел кроссинговер между геном, участвующим в контроле раннего цветения, пришедшим от линии гк-109, и ее геном *wf1*. Сцепление генов раннего цветения и белоцветковости свидетельствует о возможности использования цвета лепестков как маркера раннего цветения при отборе скороспелого селекционного материала.
Ключевые слова: лен; фоточувствительность; скороспелость; окраска цветка; сцепление.

Introduction

Cultivated plant *Linum usitatissimum* L. is spread all over the world in different climatic zones, latitudes, and elevations above the sea level. It is grown as a spring and winter crop. Anyway, the earliness of a variety is one of the most important agronomic characters. This species is characterized by wide ranges of total growth duration and the duration of its main phases: from germination till flowering and from flowering till ripening (Brutch et al., 2011). Being a quantitative character, the duration of flax vegetative period is under polygenic control (Brutch, 2011). The two growth phases are controlled by different genetic systems (Brutch, 2011). It means that each phase makes its own contribution and is important for crop earliness. Also, the number of leaves on the stem below the inflorescence is considered to be an indicator of flowering time in different plants (Obraztsov, 1983). This fact was also confirmed for flax, and a certain similarity between the inheritance patterns of flowering time and leaf number was discovered (Brutch, 2011).

Though the rate of plant development is influenced by many genes, it is interesting for breeders to search for morphological characters associated with earliness, because they can simplify the selection of breeding material. Such work was done at VIR on the basis of its flax genetic collection. It was discovered that in line gc-109, selected from the Argentine variety Macovi (k-6099), the gene *wf1* (*white flower 1*), controlling white filaments and petal color, was associated with early flowering (Porokhovinova, 2000). A specific feature of this gene is that it is semi dominant for corolla color. As a consequence, heterozygotes for this gene have diluted petal colors. This fact helps genotyping flowering plants. Due to this phenomenon, the association of the *wf1* gene with early flowering was confirmed, because plant development accelerated proportionally to the number of mutant alleles in the genome (Porokhovinova, 2000).

As mentioned above, flax is cultivated at different latitudes as both a spring and winter crop. It means that plants develop under different photoperiodic conditions. It was believed for long that *L. usitatissimum* was entirely a long-day species. However, evaluation of accessions from the VIR genetic collection revealed genotypes not influenced by the short 12-hour day (daytime at the equator). Also, the conducted experiments demonstrated a wide range of photosensitivity among the evaluated lines (Brutch et al., 2008). Previously it was found in many crops that the absence of photosensitivity could be used as an indicator of flowering earliness (Koshkin et al., 2003). But in contrast to other crops, it was discovered that flax had genotypes with all possible combinations of flowering earliness and photosensitivity degree, i. e. early flowering and low photosensitivity, late flowering and low photosensitivity, early flowering and high photosensitivity, late flowering and high photosensitivity (Brutch et al., 2008; Domantovich et al., 2012). It was detected in those experiments that line gc-109, being early in flowering under long-day conditions, was highly photosensitive. On the contrary, line gc-375, selected from the Egyptian accession Giza purple (k-6263), being rather late in flowering under long-day conditions, demonstrated relatively low photosensitivity (Domantovich at al., 2012). This line, in contrast to gc-109, had red-violet flowers. The discovery of this unique plant material allowed further genetic analysis of earliness, particularly, time of flowering.

The present paper is devoted to the evaluation of the flax flowering time inheritance, the number of leaves on the stem, and association of these characters with the white flower gene under long- and short-day conditions. Finally, the structure of the mapping population, consisting of self-pollinated lines selected from the hybrid between contrasting parents, is described.

Materials and methods

The experiments were carried out in Saint Petersburg, Russia, at 60° N. Two inbred lines from the VIR genetic collection, differing by several characters, were chosen for hybridization. Early-flowering and highly photosensitive line gc-109 was used as the female parent. It had white flowers controlled by the genotype *wf1wf1 SFC3-2SFC3-2 CSB1CSB1* (Porokhovinova, 2011). The male parent was late-flowering and low photosensitive line gc-375 with reddish purple flowers, controlled by the genotype *WF1WF1 sfc3-2sfc3-2 csb1csb1* (Porokhovinova, 2011). Both parents and F_1 and F_2 hybrids were tested for flowering time and degree of photosensitivity in a special facility with transparent glass and non-transparent pavilions. In the end of May, 10 seeds of each genotype were sown in 5-liter pots placed on mobile trolleys in two variants. During the germination–flowering time, the tested plants were exposed to the short-day (12 hours) conditions by moving the trolleys into the lightproof pavilion every day. For this time control plants were placed in the transparent pavilion. Thus, they remained under natural illumination (17.5–19 hours), and the influence of other environmental factors was equal for both plant groups. The date of the first flower opening was recorded for each plant, and the germination–flowering time was estimated. The average duration of this phase under long (T1) and short (T2) day conditions was calculated for each genotype. The coefficient of photoperiodic sensitivity (CPhPS) was calculated as CPhPS = T2/T1. When plants matured, leaves on stems below the inflorescence were counted.

Field experiments were conducted at the same geographic location. The F_3–F_6 families were grown in the field with individual isolation of the plants. Those showing segregation in petal color were rejected. For the next generation, seeds from only one plant from each family were chosen. Finally, about 200 lines that had no segregation within six years were selected and propagated. The created lines were evaluated in the field on 1 m long rows spaced by 0.1 m.

Evaluation of quantitative character inheritance is a very complicated task. Phenotypes are formed under the control of many genes whose expression is influenced by many en-

vironmental factors. In our experiment, the number of genes controlling the duration of the phase from germination to the opening of the first flower was analyzed by a computer program created by A.F. Merezhko (2005) on the Excel platform. The analyses were carried out individually for each year, characterized by specific weather conditions.

Statistical analysis of petal color association with quantitative characters was carried out using the bi-serial correlation coefficient:

$$r_{bs} = \frac{(x_{av1} - x_{av2})}{S_x} \sqrt{\frac{n_1 n_2}{N(N-1)}},$$

where x_{av1}, x_{av2} are the average character values for the groups with alternative colors; n_1, n_2 are the volumes of these groups; $N = (n_1 + n_2)$ is the sampling volume; S_x is the standard deviation for the whole sampling. The significance of r_{bs} was estimated by Student's *t*-test. The difference was considered significant when:

$$t_{\text{real}} = r_{bs} \sqrt{\frac{(N-2)}{(1-r_{bs}^2)}} \geq t_{\text{st}} \text{ for } k = N-2 \text{ and 5 \% significance level.}$$

Results

Several years of previous research showed that the early flowering parent gc-109 had high photosensitivity (CPhPS = = 1.26–1.45), and the late flowering, gc-375, low (CPhPS = = 0.95–1.07). High photosensitivity was dominant in F_1 (CPhPS = 1.28). The genetic analysis of flowering time performed with F_2 under the long-day conditions in 2008 revealed significant differences between the parental lines in two genes without dominance: one main gene and the other, 2.5 times weaker (Table 1). Exact determination of the number of genes controlling the number of leaves on the stem (physiological indicator of earliness) appeared to be impossible, but the most probable model of inheritance included three genes with the dominance degrees ranging from 0.0 to 0.5. One of the genes was 2.5 times stronger than the others. In addition, the correlation between the flowering time and the number of leaves on the stem in F_2 population under the long-day conditions was very close ($r = 0.91$). In 2009, the most reliable model of the flowering time genetic control included two genes with some epistasis, although a three-gene system was also probable (see Table 1). The inheritance of the leaf number character was very similar to that of the first variant of flowering time inheritance (see Table 1). The correlation between the flowering time and the number of leaves on the stem was 0.50. Thus, it can be supposed that the flowering time and the number of leaves are substantially determined by the same genes.

In 2008, the parental lines grown under the short-day conditions differed significantly from each other in five genes determining flowering time (Table 2). The first one was three times stronger than the others and had a partial dominance (–0.5) of early flowering. The inheritance of the leaf number on the stem was close to a three-gene model. One of these genes was four times stronger than the others. The correlation between flowering time and the number of leaves on the stem in the F_2 population was 0.57. In 2009, flowering time inheritance was close to a four-gene model in which one gene influenced the character three times stronger than the others, and the second, two times stronger than the remaining two genes. Leaf number inheritance also corresponded to a four-gene model where only one gene was 1.5 times as strong as the others. The correlation between flowering time and the number of leaves on the stem was 0.38. Thus, it is reasonable to

Table 1. Character inheritance under the long-day conditions in F_2 of the gc-109 × gc-375 hybrid in 2008 and 2009

Year	Number of genes	Dominance	Influence of genes	Epistasis	χ^2 observed	χ^2 theoretical
Developmental phase: from germination to the first flower opening						
2008	2	A = B = 0	A = 2.5; B = 1.0	No	5.48*	5.99
2009	2	A = 1.0; B = 0.4	Equal	B > A = 0.3	0.83*	3.84
	3	A = B = 1.0; C = 0.4		No	1.91*	3.84
Number of leaves on the stem						
2008	3	A = 0.5; B = 0.3; C = 0.0	A = 2.5; B = C = 1.0	No	1.00	0.00
2009	2	A = B = 1.0	A = 1.8; B = 1.0	No	4.43	3.84

A, B, C – gene symbols.
* Inheritance model is statistically significant.

Table 2. Character inheritance under the short-day conditions in the gc-109 × gc-375 hybrid in 2008 and 2009

Year	Number of genes	Dominance	Influence of genes	Epistasis	χ^2 observed	χ^2 theoretical
Developmental phase: from germination to the first flower opening						
2008	5	A = 0.5; B = C = D = E = 0.0	A = 3.0; B = C = D = E = 1.0	No	0.00*	0.00
2009	4	A = B = C = D = 1.0	A = 3.0; B = 2.0; C = D = 1.0	No	12.7	5.99
Number of leaves on the stem						
2008	3	A = 0.2; B = C = 0.0	A = 4.0; B = C = 1.0	No	4.37	0.00
2009	4	A = B = C = 1.0; D = 0.0	A = 1.5; B = C = D = 1.0	No	5.45*	5.99

A, B, C – gene symbols.
* Inheritance model is statistically significant.

Table 3. Comparison of genotypic classes in the segregation for quantitative characters in F_2 of the gc109 × gc375 hybrid under the long-day conditions in 2008

Characters of the 1st genotype			Characters of the 2nd genotype			t_{real}	t_{st}	r_{bs}	t_{real}	t_{st}
Genotype	$x_{av} \pm mx$	*n*	Genotype	$x_{av} \pm mx$	*n*					
Phase from germination to the first flower opening										
WF1WF1	47.1 ± 0.50	30	*WF1wf1*	44.7 ± 0.40	57	3.72*	2.00	0.37	3.62*	1.96
WF1wf1	44.7 ± 0.40	57	*wf1wf1*	43.1 ± 0.57	26	2.30*	2.00	0.24	2.26*	1.96
WF1WF1	47.1 ± 0.50	30	*wf1wf1*	43.1 ± 0.57	26	5.26*	2.00	0.58	5.28*	1.96
Number of leaves on the stem										
WF1WF1	102.7 ± 2.32	30	*WF1wf1*	88.2 ± 2.16	57	4.59*	2.00	0.23	2.14*	1.96
WF1wf1	88.2 ± 2.16	57	*wf1wf1*	79.9 ± 3.18	26	2.15*	2.00	0.62	5.89*	1.96
WF1WF1	102.7 ± 2.32	30	*wf1wf1*	79.9 ± 3.18	26	5.79*	2.00	0.42	4.25*	1.96

* Difference between groups is statistically significant.

Table 4. Comparison of genotypic classes in the segregation for quantitative characters in F_2 of the gc109 × gc375 hybrid under the short-day conditions in 2008

Characters of the 1st genotype			Characters of the 2nd genotype			t_{real}	t_{st}
Genotype	$x_{av} \pm mx$	*n*	Genotype	$x_{av} \pm mx$	*n*		
Phase from germination to the first flower opening							
WF1WF1	59.4 ± 0.86	52	*WF1wf1*	58.3 ± 0.55	89	1.06	2.00
WF1wf1	58.3 ± 0.55	89	*wf1wf1*	57.4 ± 0.75	48	0.95	2.00
WF1WF1	59.4 ± 0.86	52	*wf1wf1*	57.4 ± 0.75	48	1.72	2.00
Number of leaves on the stem							
WF1WF1	146.7 ± 2.45	52	*WF1wf1*	145.4 ± 2.43	89	0.38	2.00
WF1wf1	145.4 ± 2.43	89	*wf1wf1*	139.7 ± 3.97	48	1.22	2.00
WF1WF1	146.7 ± 2.45	52	*wf1wf1*	139.7 ± 3.97	48	1.50	2.00

conclude that the differences between the inheritance models of flowering time and the leaf number were more substantial under the short-day conditions than under long-day ones.

Further analysis of the previously identified association between the *wf1* gene controlling white petals (from line gc-109, Argentina) with early flowering in the F_1 generation showed that the expression of this gene correlated with early flowering and small number of leaves only under the long-day conditions. Thus, the results indicate that at different light-dark schedules, different genes controlling flowering time and the number of leaves on the stem are expressed. The gene(s) controlling early flowering and associated with the *wf1* gene does (do) not function under the short-day conditions.

Analyses of the F_2 segregation of *wf1* gene alleles according to petal color showed that the recessive allele was associated with early flowering and fewer leaves only under the long-day conditions (Tables 3–6). In 2008, the differences in flowering time between genotypes *WF1WF1*, *WF1wf1 and wf1wf1* under the long-day conditions were statistically significant, although *WF1wf1* plants bloomed only one day later than *wf1wf1* ones (see Table 3). In 2009, the first flower opening was synchronous with the last two genotypes (see Table 5). The same results were obtained for the number of leaves on the stems under the long-day conditions in 2008 and 2009 (see Tables 3, 5). Under the short-day conditions, significant differences between genotypes were found in neither flowering time nor the number of leaves (see Tables 4, 6). These results confirm that the gene involved in the control of flowering time and associated with the *wf1* gene functions only under long-day conditions.

Evaluation of F_3 families under the long-day field conditions showed that the segregation in the hybrid population resulted in the appearance of families with white flowers characterized by both early and relatively late flowering time. Also, early-flowering families appeared among families with colored flowers. Families heterozygous for this gene were divided into two groups: in the first the segregation in further generations showed significant association of the flowering time with petal color; whereas no such relationship was observed in the other. This suggests that one of the genes involved in the control of early flowering under the long-day conditions was linked to the *wf1* gene. As a result of chromosomal crossover, recombination occurred in several descendant families.

A set of about 200 I_6 lines was evaluated in the field under the natural long-day conditions in 2018. The groups of lines with white and colored flowers were not equal in size, so the results are presented as percentages of genotypes with approximately equivalent quantitative characters. The majority of the lines with white flowers were early flowering ones, and those that had colored flowers generally flowered later (Figure, *a*).

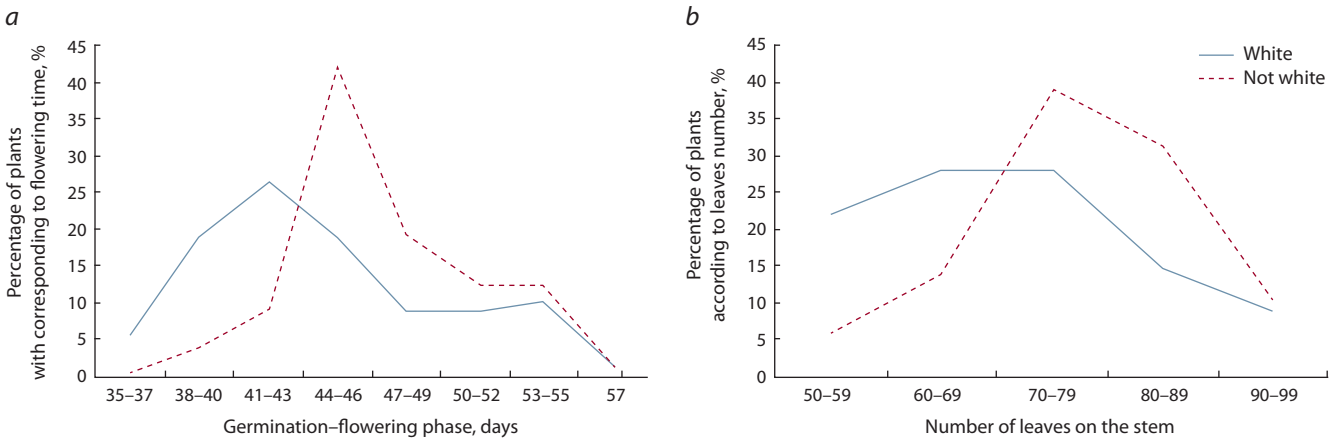
Table 5. Comparison of genotypic classes in the segregation for quantitative characters in F_2 of the gc109×gc375 hybrid under the long-day conditions in 2009

Characters of the 1st genotype			Characters of the 2nd genotype			t_{real}	t_{st}	r_{bs}	t_{real}	t_{st}
Genotype	$x_{av} \pm mx$	*n*	Genotype	$x_{av} \pm mx$	*n*					
Phase from germination to the first flower opening										
WF1WF1	55.9±0.42	64	*WF1wf1*	51.9±0.42	106	6.26*	2.00	0.44	6.26*	1.96
WF1wf1	51.9±0.42	106	*wf1wf1*	52.2±0.47	64	0.43	2.00	–	–	1.96
WF1WF1	55.9±0.42	64	*wf1wf1*	52.2±0.47	64	5.86*	2.00	0.44	6.06*	1.96
Number of leaves on the stem										
WF1WF1	88.7±2.45	58	*WF1wf1*	80.2±1.73	103	2.85*	2.00	0.22	2.85*	1.96
WF1wf1	80.2±1.73	103	*wf1wf1*	81.2±1.79	60	0.37	2.00	–	–	1.96
WF1WF1	88.7±2.45	58	*wf1wf1*	81.2±1.79	60	2.47*	2.00	0.93	26.52*	1.96

* Difference between groups is statistically significant.

Table 6. Comparison of genotypic classes in the segregation for quantitative characters in F_2 of the gc109×gc375 hybrid under the short-day conditions in 2009

Characters of the 1st genotype			Characters of the 2nd genotype			t_{real}	t_{st}
Genotype	$x_{av} \pm mx$	*n*	Genotype	$x_{av} \pm mx$	*n*		
Phase from germination to the first flower opening							
WF1WF1	66.1±0.94	52	*WF1wf1*	65.7±0.70	117	0.33	2.00
WF1wf1	65.7±0.70	117	*wf1wf1*	63.8±0.86	64	1.69	2.00
WF1WF1	66.1±0.94	52	*wf1wf1*	63.8±0.86	64	1.83	2.00
Number of leaves on the stem							
WF1WF1	113.5±3.01	49	*WF1wf1*	115.2±1.78	103	1.32	2.00
WF1wf1	115.2±1.78	103	*wf1wf1*	110.7±2.96	64	1.40	2.00
WF1WF1	113.5±3.01	49	*wf1wf1*	110.7±2.96	64	0.65	2.00



Distribution of lines with white and colored flowers according to their germination–flowering phase duration (*a*) and the number of leaves on the stem (*b*) in field conditions. Leningrad Province, 2018.

This was also true of the number of leaves (see Figure, *b*). In general, the correlation between the time of flowering and the number of leaves was retained ($r = 0.74$) within this sampling, because the experiment was conducted under the long-day conditions. The association of petal color with the time of flowering and the number of leaves on the stem was also still significant (Table 7). However, as already found for F_3 generation, some genotypes expressed other phenotypes. Some white-flowered lines were very late in blooming, and several lines with colored petals were very early.

Table 7. Comparison of genotypic classes in the segregation for quantitative characters in lines selected from the gc109 × gc375 hybrid under long-day field conditions in 2018

Characters of the 1st genotype			Characters of the 2nd genotype			t_{real}	t_{st}	r_{bs}	t_{real}	t_{st}
Genotype	$x_{av} \pm mx$	*n*	Genotype	$x_{av} \pm mx$	*n*					
Phase from germination to the first flower opening										
WF1-	47.3 ± 0.56	134	*wf1wf1*	44.5 ± 0.65	68	3.76*	2.00	0.28	4.14*	1.96
Number of leaves on the stem										
WF1-	76.6 ± 3.05	134	*wf1wf1*	70.2 ± 2.13	68	3.84*	2.00	0.27	4.03*	1.96

* Difference between groups is statistically significant.

Conclusion

Evaluation of the lines selected from the hybrid between the early flowering line gc-109 with white petals and the late-flowering gc-375 with reddish purple petals showed that one of the genes involved in the control of flowering time under the long-day conditions was linked to the white flower gene *wf1*. This marker can be used for the detection of early flowering genotypes in hybrid populations. It can be especially useful in cases when the progeny is grown under abnormal conditions (green house, etc.) when the real flowering earliness is not evident. In addition, the white color of petals indicates the high probability of earliness gene homozygosity.

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Diversity of photoperiodic responses in oats

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The article presents the results of an evaluation of the earliness and photoperiodic response (PPR) in the long-day oat accessions of various geographic origin. The material for this study were 139 oat accessions from the global collection of plant genetic resources maintained by the Vavilov Institute (VIR), which included landraces, breeding cultivars, and lines. In addition, the donors of low sensitivity to photoperiod developed at VIR were tested. A preliminary field study of the oat collection for early maturity and growing plants in the vegetation experiment was carried out according to the VIR Guidelines. The early accessions from VIR's oat collection identified in the field showed a great diversity of their photoperiodic responses during the vegetation experiment in a photoperiod facility. By origin, most of the accessions described in the vegetation experiment as earliness and weakly responsive to photoperiod were from Brazil (66 %); others from the USA, Portugal, Turkey, Colombia and Australia. Most of the Russian cultivars studied (77 %) were sensitive to a short photoperiod. Among donors with different photoperiodic responses, Skorospely 1 and Skorospely 2 were weakly responsive to photoperiod, while Srednespely 1 and Srednespely 2 showed medium responses. Many years of field studies and vegetation experiments with the oat genetic diversity from the VIR global collection have resulted in identifying genotypes characterized by earliness and weak photoperiodic responses. These accessions are of special value for breeders and currently being used to develop new early and productive oat cultivars.
Key words: oats; earliness; donors; photoperiodic sensitivity; photoperiod.

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Разнообразие фотопериодической чувствительности у овса

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В статье изложены результаты изучения скороспелости и фотопериодической чувствительности длиннодневной культуры овса различного географического происхождения. Материалом для исследования послужили 139 образцов овса из мировой коллекции генетических ресурсов растений Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР), в том числе местные селекционные сорта и линии. Кроме того, в анализ были взяты доноры слабой чувствительности к фотопериоду, созданные в ВИР. Предварительное полевое исследование коллекции овса на скороспелость и выращивание растений в вегетационном опыте проводили по методикам ВИР. Изученные в полевых условиях скороспелые образцы коллекции овса ВИР показали большое разнообразие по чувствительности к фотопериоду в вегетационном опыте в фотопериодическом павильоне. Большинство образцов, охарактеризованных в вегетационном опыте как скороспелые и слабочувствительные к фотопериоду, происходят из Бразилии (66 %), также были образцы из США, Португалии, Турции, Колумбии и Австралии. Большая часть изученных российских сортов (77 %) оказалась чувствительной к короткому фотопериоду. Среди доноров с различной фотопериодической чувствительностью слабочувствительными к фотопериоду были Скороспелый 1 и Скороспелый 2, а среднечувствительными – Среднеспелый 1 и Среднеспелый 2. В результате многолетнего полевого и вегетационного изучения генетического разнообразия образцов из коллекции ВИР выделены генотипы со скороспелостью и слабой фотопериодической чувствительностью, представляющие особую селекционную ценность, которые в настоящее время вовлекаются в процесс создания новых скороспелых продуктивных сортов овса.
Ключевые слова: овес; скороспелость; доноры; фотопериодическая чувствительность; фотопериод.

Introduction

The most important factors affecting the duration of a plant's growing season, especially its first half, are the daylight length and the temperature regime. The research on photoperiodism, started in the early 20th century, made it possible to understand and study in more detail mechanisms of the development of many crops (Garner, Allard, 1920, 1923; Gilbert, 1926).

Since the beginning of the 1920s, the effect of environmental factors on the growth and development of various plant species has been actively investigated. On N.I. Vavilov's initia-

tive, such research was conducted under the leadership of N.A. Maksimov, a well-known plant physiologist. Having analyzed literary sources and the intraspecific diversity of a number of crops, N.A. Maksimov (1929) made a conclusion about the effect of the daylight duration and temperature regime on the time between germination and flowering and the length of the whole growing season. N.I. Vavilov's geographic experiments (1928) confirmed the role of photoperiod in plant development. Afterwards, V.I. Razumov, Maksimov's disciple and follower, examining the intraspecific diversity of crops maintained in VIR's collection, identified plant species with strong and weak responses to daylength. He also noticed that their response to daylength was not always associated with their geographic origin and could be affected by definite growth conditions (Razumov, 1961).

By that time, it had been ascertained that a majority of cereal crops were long-day plants that needed a definite temperature regime, prolonged day and short night to reach the flowering phase (Wiggans, Frey, 1955). It was also shown that daylength and vernalization were two most important factors affecting the flowering process and, thereby, the yield formation (Sorrells, Simmons, 1992; Summerfield et al., 1997).

Some researchers have noted the effect of daylength on the passing of individual phases in oat development (Rodionova et al., 1994). Studying photoperiodic responses on a broad sample of common oar cultivars has helped to identify accessions highly responsive to the daylight duration and temperature regime as well as weakly responsive ones, the latter being recommended to breeders as sources of earliness (Merezhko, 1980; Rodionova et al., 1985; Merezhko, Ivanova, 1989; Ivanova et al., 1990; Koshkin et al., 2003, 2010).

For most of wild oat species, initial phases of their development have peculiarities in terms of their responses to environmental factors, including photoperiod and temperature (Paterson et al., 1976). A study of the ontogenesis of wild *Avena* L. species under long-day and short-day conditions showed that after 40 days of vernalization weak photoperiodic responses were typical for a number of accessions of diploid and tetraploid species, while various accessions of diploid, tetraploid and hexaploid oat species demonstrated a strong photoperiodic response (Loskutov, 2001). The most interesting among those accessions were early spring genotypes weakly responsive to daylength, originated from the Canary Islands (Spain), Corsica (France), Crete (Greece), Turkey, Tunisia, Lebanon and Ethiopia. All of them may potentially serve as source material for breeding early oat cultivars (Loskutov, 2007; Loskutov, Rines, 2011).

While studying cultivated oat species, a unique accession with a weak response to photoperiod was identified: a local cultivar from Turkey (CAV 2700) (Sampson, Burrows, 1972). On its basis a number of oat cultivars weakly responsive to photoperiod have been developed in Canada (Burrows, 1984, 1990, 1992). Low photoperiodic sensitivity was also described for accessions representing the endemic cultivated tetraploid species from Ethiopia *A. abyssinica* (Razumov, 1961; Arias, Frey, 1973).

For the *Di1* gene responsible for the effect of photoperiod in oats, two markers were identified using the RAPD analysis (Wight et al., 1994). Those molecular markers have been used to identify the dominant allele of *Di1* in oat breeding lines.

In Brazil, oat lines were studied using molecular markers (AFLP) associated with the flowering time; besides, their location on the genetic map was compared with other loci that may also affect the time of flowering. Those results were used for the development of oat breeding lines with an optimal set of alleles, providing earliness in the short-day environments of the southern hemisphere (Locatelli et al., 2006). The flowering time is an important factor in oat's adaptation to growth conditions. Genotypes differing in their responses to photoperiod and vernalization may prove useful when selecting parent pairs for the development of new cultivars, which would make use of the duration of the growing season with more efficiency (Locatelli et al., 2008). Some oat cultivars require low temperatures (vernalization) for flowering initiation. To analyze this factor, genes associated with oat vernalization were cloned in oat cultivars, and markers (QTL) connected with the response to vernalization were identified (Nava et al., 2012). In addition, the quantitative trait loci (QTL) controlling flowering periods in oats under varying durations of photoperiod and vernalization were mapped (Holland et al., 1997, 2002).

Analyzing responses to photoperiod and vernalization is of high practical importance, because these traits are closely linked with the growing season duration. At the same time, these factors may produce an effect on individual stages of plant development, especially on the durations of the periods between germination and booting and between germination and heading. It should be taken into account when definite accessions are used as source material for breeding (Koshkin et al., 2003, 2009, 2013).

The objective of this work has been to characterize a large number of accessions from the oat collection according to their photoperiodic responses and identify truly earliness source material with a weak response to photoperiod for further use in breeding programs in various regions of the Russian Federation.

Materials and methods

The experiments to assess photoperiodic responses were performed at the vegetation and photoperiod facilities of VIR's Department of Physiology on the grounds of Pushkin Laboratories of VIR in 2011–2018.

The material for the present research were 139 accessions of oats from the global collection of plant genetic resources held by the Vavilov Institute (VIR), which included landraces, breeding cultivars and lines from Russia, Ukraine, Norway, Slovakia, Germany, Turkey, Algeria, Portugal, Italy, China, Japan, Ethiopia, Canada, the USA, Mexico, Ecuador, Colombia, Peru, Brazil and Australia. The cultivar Privet (k-14787, Moscow Province) was used as the reference.

Also studied were the donors of low sensitivity to photoperiod developed on the basis of crosses between two references in the vegetation experiment: the Mexican cv. Chihuahua (k-12230, Mexico) with a weak response to photoperiod and the local cv. Anatolischer (k-14668, Turkey) with a strong response. The vegetation experiment under short-day conditions resulted in selecting early oat lines weakly responsive to photoperiod (k-15547, Skorospely 1, and k-15548, Skorospely 2), lines with medium photoperiodic response (k-15549, Srednespely 1, k-15550, Srednespely 2), and a late line with a strong

response to photoperiod (k-15551, Pozdnespely). The early cultivar Chihuahua (k-12230, Mexico) and the late cultivar Anatolischer (k-14668, Turkey) were used as the references for this vegetation experiment.

Preliminary field screening of the oat collection for earliness was performed using the VIR technique (Loskutov et al., 2012). In the vegetation experiment, the plants were grown according to the method described by V.A. Koshkin et al. (2013). Phenotypic description was made as follows: the date of heading was recorded for each plant after the emergence of a half of the panicle from the flag leaf sheath, the stems were labeled with paper tags, and the duration of the germination–heading period was calculated.

Photoperiodic response (PPR) was assessed according to the extent of heading delay under short day (SD) (T_2) compared with long day (LD) (T_1), and the PPR coefficient (C_{PPR}) was calculated using the formula $C_{PPR} = T_2/T_1$, where T_1 and T_2 are the durations of the germination–heading period (days) observed in oat plants, respectively grown under natural long-day (17–18 hrs) and short-day (12 hrs) conditions. Oat accessions delaying their heading under SD by 1–20 days versus LD and having C_{PPR} = 1.00–1.30 were classified as weakly responsive to photoperiod (Koshkin et al., 1994). Accessions with $C_{PPR} > 1.75$ were attributed to the category of strongly responsive cultivars. Mean errors were measured according to B.A. Dospekhov (1979).

Results

According to the data of the preliminary field screening (2010–2017) in the environments of Pushkin Laboratories of VIR (Town of Pushkin), all accessions demonstrated shortened germination–heading and germination–ripening periods by 8–15 days in all years of study, compared with cv. Privet that served as the reference in the field experiment, but under short-day conditions in the photoperiod facility they showed considerable differences in their heading time.

During the analysis of oat accessions for their photoperiodic responses in the vegetation experiment, the references showed the following results: the early Mexican cultivar Chihuahua in all years of study demonstrated a delay in heading under SD versus LD by 8.1 (5.8–9.3) days, and C_{PPR} = 1.21 (1.17–1.26), while the late Turkish local cultivar Anatolischer had a delay in heading by 35.8 (28.3–49.5) days and C_{PPR} = 1.79 (1.61–2.23).

All accessions of various origin involved in our research demonstrated under LD earlier heading than under SD. The photoperiodic response coefficient (C_{PPR}) varied in the studied sample of oat accessions from 1.02 to 2.23, while the heading delay under SD versus LD averaged from 0.7 to 49.5 days (Table 1, Figs. 1, 2).

The results for many years of study showed that the following accessions were highly responsive to photoperiod: k-15357, GN 08207, k-15361, GN 09146, k-15361, GN 09146 (Norway), k-15369, St. Aleixo (Portugal), k-15226, MF 9521-462, k-15258, PA 7836-2701 (USA), k-12235, Desnuda (Peru), and k-15031, Portuguesa (Brazil).

Early accessions with weak responses to photoperiod, selected in the field experiment and identified during many years of the vegetation experiment, are presented in Table 2. Most of them originated from Brazil: these are cultivars (URS Corona, URS Guara, URS Penca, URS Guana, URS Charrua, URS Guria, URS Brava, etc.) and breeding lines (UPF 77S090, UPF 798369-1-2, UFRGS1061503, UFRGS 20, UFRGS 077026-2, UFRGS 086004-1, etc.). Four accessions were from the United States: Common, breeding line C.I. 4627 from Mis-

Table 1. Distribution of the studied oat accessions by country of origin (Pushkin, 2011–2018)

Origin	Number of accessions studied	Number of unresponsive accessions	Mean C_{PPR}	Dispersion of C_{PPR}
Russia	13	3	1.39	1.20–1.85
Norway	5	0	1.85	1.76–2.07
Portugal	2	1	1.56	1.23–1.88
Italy	1	0	1.38	1.38
Germany	1	0	1.46	1.46
Slovakia	1	0	1.73	1.73
Ukraine	1	0	1.31	1.31
Turkey	3	1	1.58	1.16–2.23
India	1	0	1.68	1.68
China	7	1	1.46	1.29–1.63
Japan	1	0	1.58	1.58
Algeria	1	0	1.57	1.57
Ethiopia	1	0	1.49	1.49
Canada	2	0	1.38	1.32–1.43
USA	25	6	1.48	1.07–1.94
Mexico	3	1	1.37	1.19–1.60
Ecuador	1	0	1.57	1.57
Peru	6	0	1.65	1.30–1.90
Colombia	1	1	1.24	1.21–1.27
Brazil	57	31	1.33	1.02–1.91
Australia	6	2	1.39	1.2–1.69

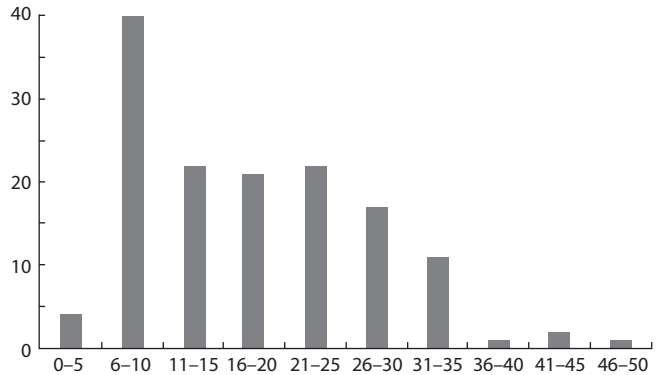

Fig. 1. Distribution of paniculation delay with short daylight vs. long daylight in spring oat varieties studied (Pushkin, 2011–2018).

Table 2. Characterization of oat accessions with weak response to photoperiod according to their PPR level (Pushkin, 2011–2018)

VIR catalog No.	Name	Origin	Germination to heading, days		T_2–T_1	C_{PPR}
			T_1	T_2		
12230	Chihuahua, st.	Mexico	37.3 ± 0.55	44.5 ± 1.00	8.1	1.21
14668	Anatolischer, st.	Turkey	46.6 ± 0.43	82.3 ± 2.07	35.8	1.79
15316	Dast	Russia	33.6 ± 0.16	43.5 ± 0.82	9.9	1.29
15547	Skorospely 1	Russia	36.0 ± 0.33	40.7 ± 0.41	4.7	1.21
15548	Skorospely 2	Russia	33.9 ± 0.10	40.8 ± 0.47	6.9	1.27
15106	St. Romao	Portugal	39.1 ± 0.38	48.1 ± 0.11	9.0	1.23
7751	Local	Turkey	37.4 ± 0.82	43.4 ± 0.67	6.0	1.16
4403	Common	USA	32.1 ± 0.23	34.5 ± 0.73	2.4	1.07
12836	C.I. 4627	USA	34.8 ± 0.55	41.5 ± 0.68	6.7	1.19
15153	B 525-336	USA	38.8 ± 0.61	48.5 ± 0.68	9.7	1.25
15216	P.I. 629063	USA	38.7 ± 0.26	48.6 ± 0.52	9.9	1.26
15111	L-15	Columbia	37.8 ± 0.40	45.8 ± 0.52	8.7	1.24
14009	UPF 77S090	Brazil	37.1 ± 0.23	43.2 ± 0.32	6.1	1.16
14010	UPF 798369-1-2	Brazil	39.5 ± 0.52	45.9 ± 0.91	6.4	1.16
14011	UPF 477S030	Brazil	37.0 ± 0.15	46.4 ± 0.92	9.4	1.26
15481	URS Corona	Brazil	37.6 ± 0.43	46.0 ± 1.07	8.4	1.22
15482	URS Guara	Brazil	34.7 ± 0.21	40.4 ± 0.54	5.7	1.16
15483	URS Penca	Brazil	34.1 ± 0.38	42.6 ± 0.94	8.5	1.25
15484	URS Guana	Brazil	39.2 ± 0.92	46.2 ± 0.46	7.0	1.18
15485	URS Tarimba	Brazil	32.9 ± 0.35	42.3 ± 0.30	9.4	1.29
15486	URS Charrua	Brazil	34.8 ± 0.33	39.9 ± 0.62	5.1	1.15
15487	URS Guria	Brazil	38.4 ± 0.52	46.1 ± 0.69	7.7	1.20
15488	URS Toprena	Brazil	41.3 ± 1.24	50.1 ± 1.39	8.8	1.21
15490	URS Brava	Brazil	38.9 ± 0.39	47.4 ± 0.78	8.5	1.22
15491	URS Estampa	Brazil	38.7 ± 0.30	48.6 ± 0.60	9.9	1.26
15492	UFRGS 017129-1	Brazil	35.1 ± 0.67	43.4 ± 0.82	8.3	1.24
15493	UFRGS1061503	Brazil	38.3 ± 0.62	43.9 ± 0.88	5.6	1.15
15533	UFRGS 8	Brazil	43.0 ± 0.26	52.7 ± 0.91	9.7	1.23
15534	UFRGS 9	Brazil	37.5 ± 0.34	46.8 ± 1.06	9.3	1.25
15541	UFRGS 17	Brazil	44.5 ± 0.58	52.9 ± 0.59	8.4	1.19
15543	UFRGS 19	Brazil	47.3 ± 1.71	51.4 ± 1.16	4.1	1.09
15544	UFRGS 20	Brazil	36.1 ± 0.28	42.9 ± 0.35	6.8	1.19
15546	UFRGS 22	Brazil	36.6 ± 0.60	46.0 ± 1.42	9.4	1.26
15598	UFRGS 077026-2	Brazil	39.2 ± 1.36	44.5 ± 0.37	5.3	1.14
15600	UFRGS 086208-3	Brazil	33.6 ± 0.45	43.1 ± 1.21	9.5	1.28
15609	UFRGS 953195	Brazil	36.2 ± 1.23	44.1 ± 0.59	7.9	1.22

Table 2. (End)

VIR catalog No.	Name	Origin	Germination to heading, days		$T_2–T_1$	C_{PPR}
			T_1	T_2		
15678	UFRGS 086004-1	Brazil	35.6 ± 1.12	42.1 ± 0.87	6.5	1.18
15681	UFRGS 086092-2	Brazil	35.4 ± 1.30	42.6 ± 1.41	7.2	1.20
15682	UFRGS 086136-5	Brazil	40.3 ± 2.05	41.0 ± 0.62	0.7	1.02
15683	UFRGS 086183-2	Brazil	33.4 ± 0.38	41.6 ± 0.58	8.2	1.25
8271	Gidgee	Australia	31.3 ± 0.30	37.6 ± 0.43	6.3	1.20
15173	Mitika	Australia	38.4 ± 0.68	46.4 ± 0.40	8.0	1.21

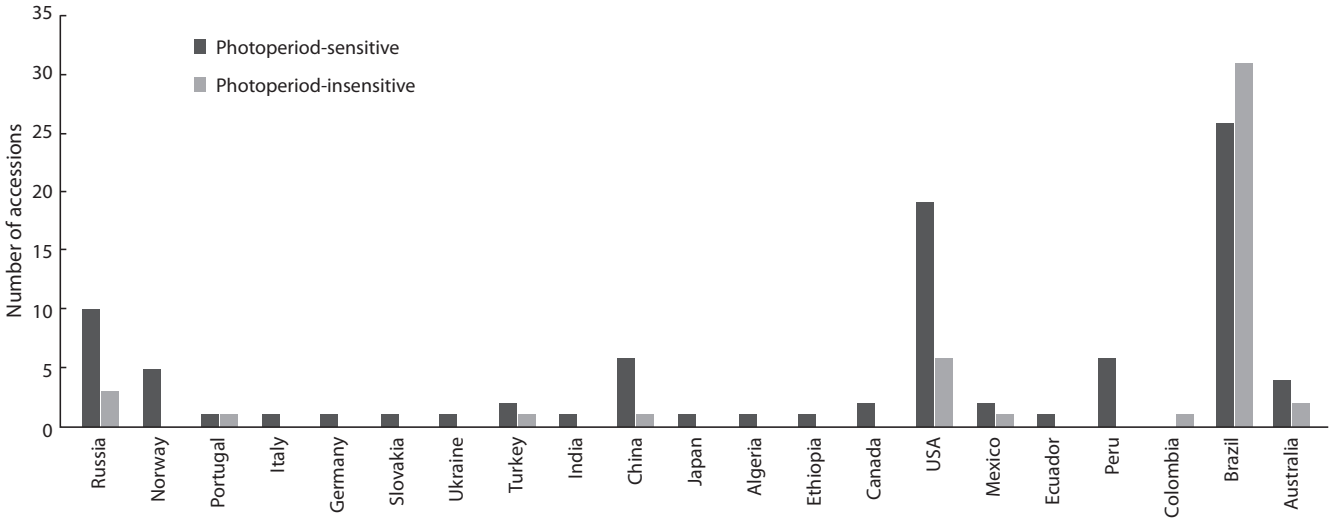

Fig. 2. Sensitive and insensitive oat accessions with regard to their geographic origin (Pushkin, 2011–2018).

Table 3. Characterization of oat donors with various photoperiodic responses according to their C_{PPR} (Pushkin, 2016–2018)

VIR catalog No.	Name	Origin	Germination to heading, days		$T_2–T_1$	C_{PPR}
			T_1	T_2		
12230	Chihuahua, st.	Mexico	37.3 ± 0.55	44.5 ± 1.00	8.1	1.21
14668	Anatolischer, st.	Turkey	46.6 ± 0.43	82.3 ± 2.07	35.8	1.79
15547	Skorospely 1	Russia	37.8 ± 0.58	46.5 ± 0.87	8.4	1.22
15548	Skorospely 2	Russia	34.9 ± 0.45	48.3 ± 0.85	11.3	1.30
15549	Srednespely 1	Russia	39.4 ± 0.37	57.6 ± 0.68	14.7	1.46
15550	Srednespely 2	Russia	36.2 ± 0.39	65.3 ± 1.77	22.9	1.56
15551	Pozdnespely	Russia	40.1 ± 0.53	64.9 ± 0.72	26.9	1.65

souri, etc., and the other accessions originated from Portugal, Turkey, Colombia and Australia.

Most of the accessions characterized in the vegetation experiment as early and weakly responsive to photoperiod came from Brazil (66 %). A majority of the tested Russian cultivars (77 %) appeared responsive to short-day photoperiod.

In addition to the accessions from the oat collection, in 2016–2018 a set of donors with various photoperiodic responses were examined (Table 3): two lines weakly responsive to photoperiod, Skorospely 1 and Skorospely 2, which had on average a minor delay under SD (8.4 and 11.3) versus LD and low C_{PPR} = 1.22, 1.30, whereas lines with medium response to

photoperiod, Srednespely 1 and Srednespely 2, considerably delayed their development under short-day conditions and had higher C_{PPR} = 1.46 and 1.56. The highest C_{PPR} = 1.65 was observed in the late line Pozdnespely.

Conclusion

Early oat accessions from the VIR global collection, screened in the field, showed greater diversity in their responses to photoperiod when tested in the vegetation experiment in the photoperiod facility. The early cultivars with weak responses to photoperiod, identified in the course of the present study, and the developed donors are of special importance for breeders, and are presently being involved in breeding programs in order to develop new earliness and high-yielding oat cultivars.

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Variability of the photoperiod response in guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes of different geographic origin

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The introduction of the new legume crop guar is of great practical importance for Russia, since it serves as a source of valuable vegetable raw material, guar gum, used for the food, gas and oil industry. The main problem with guar cultivation in the southern regions of the Russian Federation is that this plant should be grown under a short photoperiod. Prolonged daylight exposure is an obstacle to the timely transition of guar to flowering, which dramatically affects its productivity. In the study, 192 guar genotypes from the VIR collection were tested for the speed of transition to flowering on an extremely long photoperiod (18.2–18.9 h) in the greenhouse of the Pushkin experimental station of VIR (St. Petersburg). At the same time, the earliness of maturation of the same genotypes was estimated under the field conditions in the Kuban experimental station of VIR (Krasnodar area). Among the samples tested, genotypes with weak photoperiodic sensitivity (which were also early maturated under the conditions of Krasnodar), as well as the highly photoperiod-sensitive genotypes were identified. It has been established that for the same guar plant the critical photoperiod initiating the formation of buds may not coincide with the critical photoperiod required for their flushing (i. e. flowering *per se*). The observed fact confirms the hypothesis reported earlier about a two-stage launch of the flowering program in guar, according to which budding and flowering itself are controlled by independent gene systems. According to our results, the successful breeding of early mature guar varieties ultimately depends on the first gene system that controls the initiation of budding in response to a critical photoperiod. We suggest that another hypothetical gene system can influence the dates of guar flowering, which determines the speed of vegetative development of a specific genotype, measured as the number of days from germination to the appearance of the first true leaf. Thus, sensitivity to photoperiod in guar is only one of several factors that determine the speed of a plant's transition to flowering, and it should not be assessed on the basis of the length of the period from germination to flowering, which is common in breeding practice. The results of the study show that, although the photoperiod sensitivity of guar limits the range of geographic latitudes in which the legume crop can be successfully grown, there is a real opportunity to overcome this limitation by selecting and propagating photoperiod-insensitive genotypes from the enormous genetic diversity of this species.
Key words: *Cyamopsis tetragonoloba* (L.) Taub.; guar; photoperiod; bud formation; initiation of flowering.

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Амплитуда изменчивости фотопериодической реакции генотипов гуара (*Cyamopsis tetragonoloba* (L.) Taub.) разного географического происхождения

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Интродукция новой бобовой культуры гуар на территорию Российской Федерации имеет большое практическое значение, так как обеспечивает импортозамещение гуаровой камеди – растительного сырья, используемого для целей пищевой, газо- и нефтедобывающей промышленности. Основная проблема при возделывании гуара в южных регионах РФ – потребность культуры в коротком фотопериоде. Увеличенная продолжительность светового дня препятствует своевременному переходу гуара к цветению, что резко сказывается на его продуктивности. В проведенном исследовании 192 генотипа гуара из коллекции ВИР испытывались на скорость перехода к цветению на экстремально длинном для культуры гуара фотопериоде (18.2–18.9 ч) в условиях теплицы Пушкинского филиала ВИР (Санкт-Петербург). Параллельно оценивалась

скороспелость этих же генотипов в условиях поля Кубанского филиала ВИР (Краснодарский край). Среди испытуемой выборки были выявлены как генотипы со слабой фотопериодической чувствительностью (они же и более скороспелые в условиях Краснодарского края), так и сильно чувствительные к фотопериоду. Установлено, что у одного и того же растения гуара пороговый фотопериод, инициирующий образование бутонов, может не совпадать с критическим фотопериодом, необходимым для их распускания (собственно цветения). Наблюдаемый факт подтверждает выдвинутую ранее гипотезу о двуступенчатом запуске программы цветения у гуара, согласно которой бутонизация и собственно цветение контролируются независимыми генными системами. По результатам экспериментов, в успешной селекции гуара на скороспелость решающую роль играет именно первая генная система, контролирующая запуск бутонизации в ответ на критический фотопериод. На отсрочку даты начала цветения у гуара может влиять еще одна гипотетическая генная система, которая определяет скорость вегетативного развития конкретного генотипа, измеряемую как количество дней от всходов до появления первого настоящего листа, и не зависит от фотопериода. Таким образом, фотопериодическая чувствительность у гуара является лишь одним из факторов, определяющих скорость перехода растения к цветению, и ее не следует оценивать по признаку «всходы–цветение», распространенному в растениеводческой практике. Результаты проведенного исследования показывают, что, хотя фотопериодическая чувствительность гуара ограничивает диапазон географических широт, в котором эта культура может успешно выращиваться, есть реальная возможность преодолеть указанное ограничение, отбирая и размножая соответствующие генотипы из имеющегося огромного генетического разнообразия культуры.

Ключевые слова: *Cyamopsis tetragonoloba* (L.) Taub.; гуар; фотопериодическая чувствительность; сроки бутонизации; сроки цветения.

Introduction

Guar (*Cyamopsis tetragonolóba* (L.) Taub.) is an annual legume plant, traditionally cultivated in India and Pakistan as a fodder and vegetable crop, and also used as a green fertilizer (Kuravadi et al., 2012). The increased attention to guar all over the world in recent years is due to the high content of galactomannan polysaccharide reserved in guar seeds. Galactomannans quickly hydrate in cold water at low concentrations, forming a viscous colloidal solution – guar gum, which is used as a thickener and stabilizer in the food, textile, gas and oil industries. It was reported that various sectors of the Russian economy demand each year at least 15 thousand tons of guar gum, which is currently being covered exclusively by imports. Thus, the introduction of guar as the new legume crop to the southern regions of Russian Federation and breeding of new guar varieties adopted to new ecological conditions is a relevant and popular topic (Startsev et al., 2017).

Although the ecological optimum of guar perfectly matches to the conditions of the semi-arid climatic zone of the northwest of India (~27° N), several attempts have been made to introduce this crop into higher latitudes: in the southern states of the USA – Texas (~29° N), Arizona (~34° N), Oklahoma (~35° N) (Lubbers, 1987), in southern Italy (~39° N) (Gresta et al., 2018). The main complication for successful guar cultivation in the countries of the higher latitudes is the prolonged vegetation period of plants causing harvesting delay before the onset of autumn rains, which negatively affects the yield. For example, the comparative study of the length of the vegetation of 68 guar genotypes of different geographical origin (India, Pakistan, USA, Australia) in conditions of southern Italy showed that this trait ranged from 155–163 days for the earliest maturing varieties, up to 175–184 days for the late-maturing genotypes. Under these conditions, the guar plants completed their vegetation from mid-October to early November. Therefore, it was concluded that early maturity is a key breeding trait for this crop when cultivated in the Mediterranean region (Gresta et al., 2018).

The length of vegetation period and, consequently, maturation rate, is predominantly determined by the photoperiodic sensitivity (PPS) of a plant. Guar is a short-day plant (Lubbers, 1987). The length of the daylight during the growing season in Jodhpur province (India), where this crop is widely cultivated, varies from 12.7 to 13.8 h. In the Krasnodar region of Russia, where many attempts are made to introduce guar, the length of daylight in May–June is 14.3–15.6 h. Meanwhile, the critical photoperiod for different guar varieties was reported as 12–13 h for earliest genotypes or 13–15 h for the late maturing ones. Under conditions of the prolonged photoperiod plants start flowering with a strong delay, although it has been found that some genotypes are almost insensitive to the photoperiod (Lubbers, 1987). Selection of the genotypes with weak PPS from the large intraspecific genetic diversity of guar may solve the problem of successful introduction of guar in temperate latitudes, as it was done for soybean (*Glycine max* (L.) Merrill). The selected soya genotypes with the weak PPS allowed to expand the area of the short-day legume crop cultivation from the tropics to the 50th parallel of the northern latitude (Watanabe et al., 2012).

To date, 115 viable seed accessions of different geographic origin are maintained at the VIR guar germplasm collection. The representativeness of the genetic diversity is comparable with the collections of the USA and India. The most accessions came from India, there are 4 accessions from Pakistan, 6 from Australia, 4 from the USA, 1 accession was obtained from the UK. The collection contains both breeding varieties and local landraces cultivated by farmers in India. The collection includes guar varieties of vegetable, fodder and grain use. 15 accessions of the guar collection represent the new breeding varieties and lines developed in recent years in Russia to solve the problem of import substitution of guar gum used in oil and gas industry.

Having available the collection of guar genetic resources, we investigated the variability of the photoperiodic reaction

of genotypes of different geographical origin, growing them on a provokingly long photoperiod. The aim was to get an idea about the range of variability of their speed of development and timing of the transition to flowering on the long day conditions.

Material and methods

We examined 192 guar genotypes that have been selected during our preliminary study of the guar genetic resources collection in 2017 at the Kuban branch of VIR (Krasnodar region, South of Russia, 45°02'55" N). The genotypes were selected on the basis of the most contrasting manifestation of morphological characters (days to flowering, days to first mature pod, plant height, number of basal shoots, resistance to diseases), as well as their geographical origin. In this representation mostly the local varieties from India as well as known cultivars from USA (Kinman, Lewis, Santa Cruz) were presented. In 2017 the selected 192 plants were marked with labels, and seeds from each of the plants were collected individually. Half of the seeds of the offspring of each plant was used for a field experiment of the following year (2018) at the Kuban branch of VIR, other half of the offspring of the same plants was used for the vegetation experiment in the conditions of the greenhouse of Pushkin branch of VIR (St. Petersburg region, 59°53'39" N).

As previously reported, the provocative long photoperiod causes the wider amplitude of variability in the photoperiod response among the guar genotypes studied (Lubbers, 1987). Thus, it becomes more likely to divide the studied population into groups that differ in their photoperiod sensitivity. In our experiments, the speed of transition to flowering of 192 guar lines was evaluated under greenhouse conditions at the Pushkin branch of VIR in natural daylight, corresponding to the St. Petersburg latitude. During the experiment the photoperiod decreased gradually from 18.2 to 18.9 h (May–June) with an average daytime temperature of +27 °C and a night temperature of +18.0 °C. Each of the 192 guar lines was sown in replications: two blocks with 4 plants in each (a total of 8 plants per line). For all the plants the date of appearance of seedlings (germination), the date of appearance of the first true leaf, the date of appearance of the first floral bud and the date of the first floral bud opening (the date of the flowering *per se*) were recorded individually.

With the replications available, for the date of appearance of the first true leaf the broad sense heritability coefficient (h^2) was calculated with Statistica 12 package using ANOVA as suggested by See et al. (2002):

$$h^2 = \frac{(\text{entry MS} - \text{error MS})/r}{\text{error MS} + (\text{entry MS} - \text{error MS})/r},$$

where r – number of replications, entry MS – Mean of Squares explained by a genotype.

At the Kuban branch of VIR the seeds of the same 192 lines were sown in rows of 2 m and 50 seeds per each line. At the end of the growing season (147 days after the sowing date) 10 plants were selected per each line, for them the total number of pods per plant and the percentage of mature pods among them were calculated. The percentage of mature pods was considered as a "maturity index" estimated for each line.

Results

192 guar lines were sown under greenhouse conditions at the Pushkin branch of VIR in May 2018 at the daylight length of 18.2 h, and simultaneously sown in the field at the Kuban branch of VIR at the daylight length of 15.4 h. Only a few plants in the experiment at the Pushkin branch developed inferior pods that did not contain a single mature seed. A good-quality seed reproduction was obtained from all the plants grown under the conditions of the Kuban branch of VIR in Krasnodar region.

14 out of 192 lines at the Pushkin branch of VIR were excluded from the experiment because of a strong infection by *Fusarium* pathogen at the seedling stage. 12 of the remaining 178 lines of guar under the conditions of a long photoperiod did not go over to the generative stage, without even forming floral buds. The remaining 166 lines were represented in the experiment by 664 plants, the number of plants per line varied from 2 to 8 (due to the partial death of seedlings), an average of 4 plants per line.

In earlier studies, it was noted that the photoperiodic response of guar should not be assessed by the length of the period from germination to the appearance of the first flower. Photoperiodic sensitivity is only one of the factors that determine the speed of a plant's transition to flowering. For example, it was shown that the date of appearance of the first true leaf in guar significantly varies between genotypes. This trait affects the time of the appearance of the floral buds in different genotypes, and is not related to their PPS (Lubbers, 1987).

It was also established that genetic control of the transition to the generative phase in guar is carried out by two independent gene systems. The first gene system initiates the floral bud formation in response to a critical photoperiod, the second one determines the floral bud-opening when the corresponding critical photoperiod is achieved. These two phases of guar development determine how fast a plant goes through the stage of flowering, setting up the pods, maturing of seeds and the end of the vegetation (Lubbers,1987).

Considering the previously published reports, we separately analyzed the variability of three components of the composite trait "days from germination to flowering", which may potentially affect the early maturity of guar genotypes. In particular, the duration of three periods was investigated: (1) days from seedlings to first true leaf; (2) days from first true leaf to first floral bud; (3) days from first floral bud to first flower (floral bud-opening).

The variability of the trait "days from seedlings to first true leaf"

For the most of lines studied, the number of days from seed germination to the first true leaf varied from 4 to 14 (Fig. 1). A few plants were found that developed the first leaf 28–32 days after seed germination, and then they successfully switched to the generative phase of formation floral buds and flowers. For lines that were represented in the experiment by

at least three plants, the effect of genotype on the variability of the analyzed trait was estimated by ANOVA, and turned out to be highly significant ($p < 10^{-6}$) (Table 1).

An attempt was also made to calculate the proportion of variability of this trait explained by genetic factors (heritability in a broad sense), using ANOVA (See et al., 2002). The calculated coefficient of heritability in the broad sense (h^2) was 0.46, thus, the variability of the length of period from seedlings appearance to first true leaf in guar is almost half determined by the genotype.

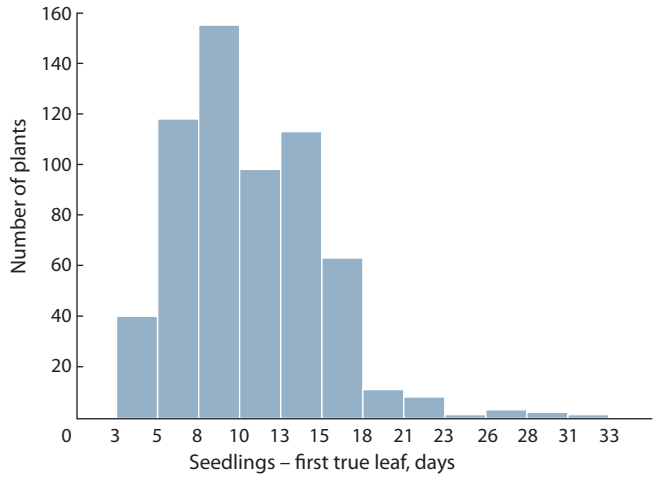

Fig. 1. The variability of the number of days from seedlings to first true leaf among the representation of guar genotypes of different geographical origin from the VIR collection.

Variability of "days from first true leaf to first floral bud" and "days from first floral bud to first flower"

The experiment with guar growing in the greenhouse of the Pushkin branch of VIR was conducted from May to October with a photoperiod natural to the latitude of St. Petersburg. The experiment allowed us to observe the reaction of different genotypes of the short-day legume crop to a gradually decreasing length of daylight: from the maximum (~19 h) on the day of the summer solstice, to a relatively short (11 h) in the first decade of October. Since each guar genotype in the experiment required a certain critical photoperiod that triggers the transition to the generative phase, as the length of the light day shrank, the lines one by one passed to flowering as soon as the photoperiod reached a certain threshold level. This allowed us to divide all the plants into groups with the same PPS. Thus, according to the dates of transition to the stage of floral bud formation, the guar plants were divided into "early" and "late", which formed the first floral bud with a day length of 17–18 and 12–13 h, respectively. At the same time, an intermediate group of plants was defined, in which the transition to floral bud formation was recorded at the 15-hour light day.

Since for all plants in the experiment, not only the date of the appearance of the first floral bud, but also the date of its opening (flowering) was recorded individually, some essential observations were made. Among the genetic diversity of the guar there were (i) genotypes that passed from floral bud formation to floral bud opening without delay (within 8 days); (ii) genotypes with delayed floral bud-opening. For those, from the moment a floral bud formation to first flower passed an average of 35 days, and in some cases, flowering did not occur even after 75 days.

Another important fact was recorded: on the long photoperiod, plants could have an equally short time interval between the first leaf and first floral bud (possible due to weak PPS), but at the same time, they differed greatly in the length of the period "seedlings – first true leaf" which significantly affected their flowering (first floral bud-opening) calendar date, as such. Thus, due to the delay of the vegetative development phase, plants can lately go over to floral bud formation, and, as a result, can be mistakenly classified as highly sensitive to photoperiod.

650 plants that have successfully switched to the generative phase under conditions of an extremely long photoperiod are arranged in Fig. 2 in order of increasing the time interval between the appearance of the first true leaf and the formation of the first floral bud. This time interval was considered in our experiment as the most accurate indicator of PPS. At the same time, for all plants in Fig. 2 days from seedlings to first true leaf and days from first floral bud to first flower are shown. Such a ranking made it possible to conditionally divide the entire sample into five groups.

The guar genotypes of the group 1 with the weakest PPS pass without delay to the formation of floral buds at a photoperiod of 18.6 h. They can be called "early" since: (i) only 34 days pass from the first leaf to first floral bud (Table 2); (ii) floral bud opening begins shortly after floral bud formation – on average, after 8 days. This means that an almost identical critical photoperiod is needed to trigger the floral bud formation and the floral bud opening.

The group 2 included "almost early" genotypes, which proceeded to the formation of floral buds when a daylight

Table 1. Results of analysis of variance (ANOVA) of the number of days from seedlings to first true leaf among the sample of guar genotypes

Effect	SS	df	MS	F	*p*-value
Intercept	64342.87	1	64342.87	4771.782	< 0.001
Genotype (a guar line)	6422.10	134	47.93	3.554	< 0.001
Error	6445.37	478	13.48		

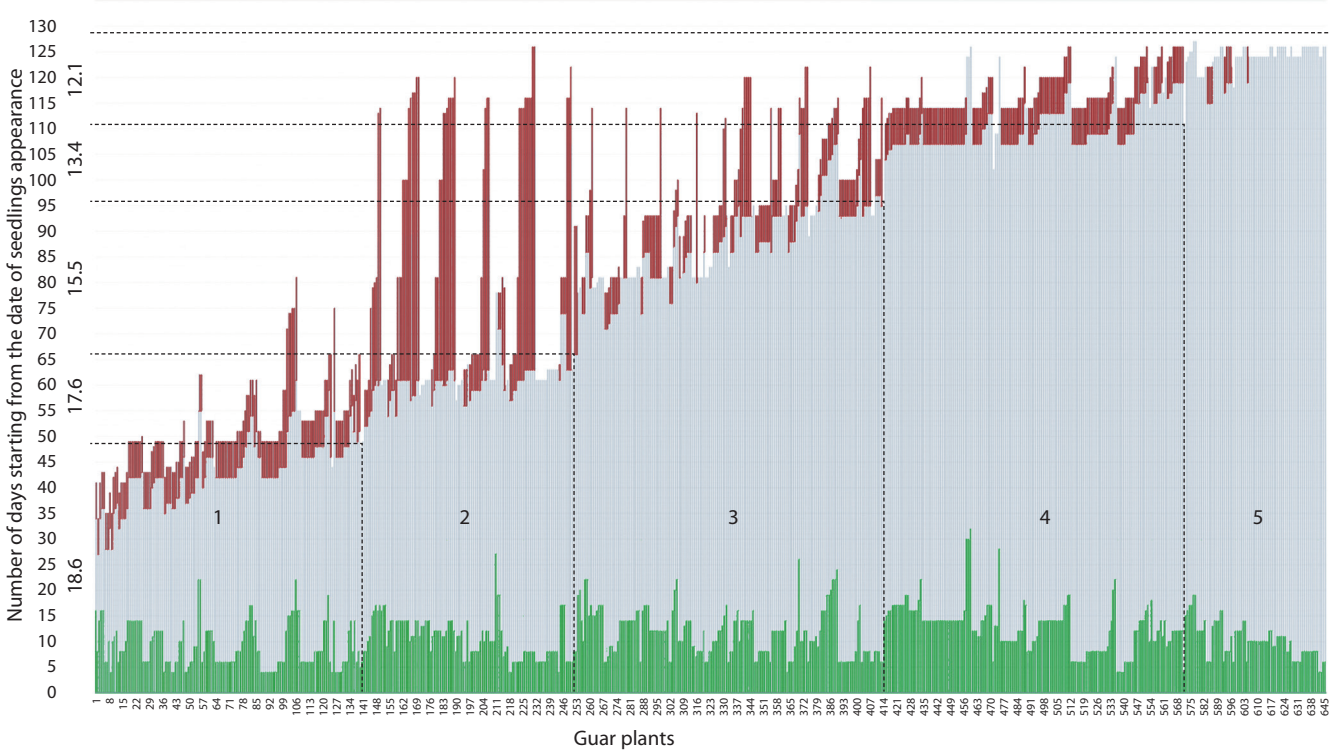

Fig. 2. The distribution of guar plants in groups No. 1–5, depending on the duration of the developmental stages and sensitivity to photoperiod.

The dotted line shows the boundaries of the groups (average photoperiod values (h) are given for each group). The time interval from seedling appearance to first true leaf is marked in green for each plant, the period from the first true leaf to bud formation is marked in gray, floral bud formation to floral bud opening – in red.

Table 2. Description of the speed of development and the critical photoperiod required to trigger floral bud formation and floral bud opening for guar lines with different photoperiod sensitivity

Groups of guar based on PPS	Floral bud formation				Floral bud opening			
	Critical photoperiod, h	Period "first true leaf –floral bud formation", days			Critical photoperiod, h	Period "first floral bud – floral bud opening", days		
		mean	minimum	maximum		mean*	minimum	maximum
1	18.6	34 ± 0.5	18	42	17.8	8 ± 0.9	5	20
2	17.6	50 ± 0.4	43	58	13.5	35 ± 2.3	7	75/∞**
3	15.5	76 ± 0.6	59	89	14.0	16 ± 0.7	7	35/∞**
4	13.4	97 ± 0.4	90	106	12.7	7 ± 0.0	7	7
5	12.1	113 ± 0.5	107	120	< 11	∞**	∞**	∞**

* The mean was calculated only for the plants, that passed to flowering.
** ∞ During the growing period no transition to flowering was recorded.

length did not exceed 17.6 h. A distinctive feature of this group is the delayed opening of the floral buds. Most of the plants formed their first floral buds in response to decreasing of the photoperiod to 17.6 h, while the floral buds opened only in 35–40 days, when the length of the day decreased to 12.6 h. Some plants of this group did not switch to flowering

at all, having stopped at the stage of floral bud formation. Nevertheless, the average time interval between the first true leaf and the floral bud formation for these genotypes was only 50 days.

Genotypes of the very heterogeneous group 3 formed their first floral buds with a photoperiod of 15.5 h (approximately 76 days after the first leaf was appeared). The heterogeneity of the group is explained by the fact that some of the plants began to flower soon after budding (after 7–10 days), while for another part of the plants a photoperiod of 15.5 h was sufficient to form floral buds, but not for their opening. Thus, the launch of the actual flowering occurred when the day length was further reduced to 12 h. There were also some genotypes, which formed floral buds at 15.5 h, but did not open them at all.

The group 4 of "late" genotypes was quite homogeneous, the plants started budding when the day length reached 13.4 h, while the time interval between the first true leaf and the floral bud formation for these "late" genotypes was, on average, 97 days. The same length of the photoperiod seems to be critical for triggering the floral bud opening. As a result, almost all plants of this group opened their floral buds during the week after the bud formation.

The group 5 of "very late" genotypes formed their floral buds approximately 113 days after the first true leaf was developed, and the critical photoperiod for the floral bud formation was 12.1 h. Actually, no floral bud opening was recorded for most plants of this group.

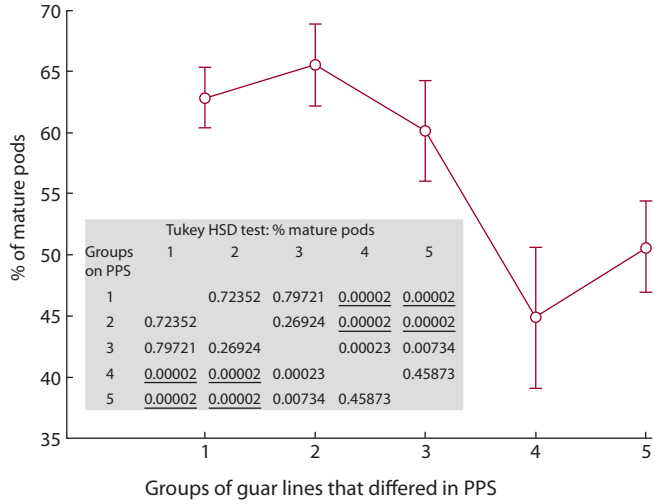

Fig. 3. Results of ANOVA showing significant difference in maturity index of the guar genotypes grown at the Kuban branch of VIR depending on their affiliation to the groups No. 1–5, registered at the Pushkin branch of VIR according to their photoperiod sensitivity.

Correlation of photoperiodic reaction of guar lines in greenhouse with their earliness in the field

Guar lines, which photoperiod sensitivity was tested in greenhouse conditions of the Pushkin branch of VIR with an extremely long day, were also studied in field conditions of Krasnodar region (at the Kuban branch of VIR). At the end of the field season, that is, 147 days after the planting date, for 10 plants of each line the percentage of mature pods ("maturity index") was estimated. Since each line was assigned to one of five groups that differed in the PPS (Table 2), we had the opportunity to assess the significance of the difference in the "maturity index" of guar lines with different photoperiodic reactions.

Fig. 3 shows that the "late" and highly photoperiod sensitive lines from 4th and 5th groups showed also the latest maturation in the field conditions of the Krasnodar region. Nearly half of the pods of such plants did not mature at the time of harvesting. The probable reason for this can be the length of daylight (12–13 h) that is critical for this group of genotypes to start flowering (see Table 2). That threshold is not reached during the guar vegetation period under conditions of the Krasnodar area, where the daylight length in May–July is 14.3–15.4 h, 15.4–15.6 h and 14.7–15.6 h correspondingly (https://voshod-solnca.ru). Other lines of the groups 1–3, which require a photoperiod shorter than 15.5 h to go over to the generative stage, started flowering in Krasnodar area at the end of June, as soon as this length of daylight period is reached.

Discussion

To date, the only monograph by E.L. Lubbers (1987) is devoted to a detailed description of characterization and inheritance of photoperiodism in guar. It describes the results of experiments with 330 guar genotypes, conducted in 1982–1983 in five geographical locations of the US (Arizona, Kansas and Texas), as well as the results of the evaluation of six guar varieties at different photoperiods under greenhouse conditions. It was found that, depending on the genotype, the critical length of daylight that triggers the transition to flowering in guar varies from 12 to 15 h. It should be noted that of the six varieties tested, one genotype was almost insensitive to the photoperiod, successfully proceeding to the flowering phase at 12, 13, 14 and 15 h of daylight.

In the author's experiments with crossings of guar genotypes of the contrast PPS, the pattern of offspring segregation indicated the presence of two genes controlling "days from first true leaf to first floral bud" and two or three genes determining "days from first floral bud to first flower" in response to a certain photoperiod (Lubbers, 1987). Our results support the hypothesis about two independent gene systems that control the two stages of flowering in guar.

It can be assumed that in our experiment, the "early" and "late" lines of guar (groups 1–2 and 4–5 respectively, see Table 2) had alternative alleles of the genes of the first gene system responsible for the formation of floral buds in response to establishing of a critical photoperiod. Due to alternative alleles of the genes of the second gene system the groups 1 and 4 possibly switched to floral bud opening without regard to the length of the daylight, while groups 2 and 5 did it only in response to a certain critical photoperiod. Thus, it seems likely that in guar the reception of the length of the daylight, regulating the transition to flowering, occurs twice.

The combination of the alleles of these two gene systems may explain the diversity of dates of onset and passage of the generative phase observed among guar lines, when grown under conditions that are extremal for the short-day plant. For example, the plants of groups 1 and 4 were equally fast opening their floral buds, but the difference in the dates of

these floral buds' formation in these two groups was almost 60 days. At the same time, as followed from the analysis of variance presented in Fig. 3, the alleles of the genes that are responsible for initiation of floral bud formation have a major effect on early maturity.

Following Lubbers (1987), our observations of the variability of the period "seedlings – the first true leaf" indicate that photoperiodic sensitivity is only one of the factors that determine the speed of a plant's transition to flowering. When the photoperiodic sensitivity of guar is recorded as "days from seedlings to first flower", it might be masked by other unrelated factors, e. g. by different rates of passage of the vegetative development phases preceding flowering. In this regard, it is necessary to record not only the calendar actual date of the flowers appearance, but to take into account the date of the first true leaf appearance.

The observations of Lubbers (1987), as well as our results, confirm the idea that, although the photoperiodic reaction of guar limits the range of geographic latitudes in which this crop can be successfully grown, there is a real possibility to overcome this limitation by selecting and reproducing of nearly day-neutral genotypes from the existing genetic diversity of this species. The good perspective of such an approach of breeding a short-day legume crop adapted to conditions of temperate latitudes is well illustrated by the example of another short-day legume plant – soybean. Most soybean varieties need a short day to initiate flowering, but the successful breeding of genotypes with low sensitivity to the photoperiod has made possible the large-scale promotion of this crop to temperate latitudes (Watanabe et al., 2012).

There are many reasons for comparing the experience of the introduction of soya and guar. Although the genus *Cyamopsis* belongs to the tribe *Indigofereae* (Schrire, 2013), by the polymorphism of the chloroplast and mitochondrial genomes guar and soybeans belong to the same monophyletic clade on the phylogenetic tree of the subfamily *Faboideae*, along with *Phaseolus*, *Vigna*, *Dolichos* and other short-day legume crops (Cronk et al., 2006). A recent transcriptome study of the structure of the coding part of guar genome showed that *Glycine max* is the closely related species for guar, demonstrating the maximum percentage 41.91 % of homologous genes in these two species (Tanwar et al., 2017).

At least ten genes/QTLs have been reported controlling the transition to flowering and maturing for soybeans (Bernard, 1971; Cober et al., 2010; Kong et al., 2014; Kim et al., 2018). However, the progress in the identification and cloning of these genes is not obvious, which may be explained by the large number of genetic factors required for the initiation of the generative phase in legumes. The results of our research indicate that the phenotyping of guar plants in order to identify genetic loci that determine the speed of transition to flowering should include an analysis of all components of the period between date of the seedlings appearance and date of first flower, since each of them can be controlled by an independent gene system.

Conclusion

The transition to flowering in guar occurs in response to a critical photoperiod. Furthermore, the floral bud formation may be triggered by the one certain length of daylight, but flowering per se (bud opening) – by another. In addition, the setting of floral buds also depends on genetic factors that determine the rate of seed germination and the formation of the first true leaf. In the VIR germplasm collection various guar genotypes are found that are insensitive to the photoperiod, both at the stage of formation of floral buds and at the bud opening. Genotypes with the lower photoperiod sensitivity are also early mature, showing the highest percentage of matured seeds by harvesting.

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Генетическое разнообразие и селекционная ценность синтетической гексаплоидной пшеницы, привлеченной в коллекцию ВИР

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Для успешного развития селекции пшеницы в России необходим генетически разнообразный и хорошо охарактеризованный исходный материал, в основном сохраняемый в коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР). С целью пополнения коллекции было изучено 36 образцов синтетической гексаплоидной пшеницы (СГП), созданных в CIMMYT путем скрещивания *Triticum durum* с *Aegilops tauschii*. Наше исследование было направлено на изучение линий (образцов) СГП по комплексу морфологических и хозяйственно ценных признаков в условиях Северо-Западного региона России (30° в. д., 59° с. ш.); оценку реакции СГП на фотопериод; определение генетической гетерогенности образцов СГП и сходства между ними с использованием глиадинов как биохимических маркеров. Результаты показали, что изменчивость различных признаков СГП укладывается в рамки рода *Triticum*, СГП можно классифицировать как слабо окультуренные формы. Их отличительная черта, ценная для селекции пшеницы, – высокая масса 1000 зерен (до 60.6 г). Этот признак характеризовался низкой степенью изменчивости и слабой корреляцией с другими признаками. Реакция растений пшеницы на продолжительность светового дня имеет решающее значение для их перехода от вегетативного развития к репродуктивному. Исследованные СГП отличались от мягкой пшеницы и друг от друга реакцией на короткий день и продолжительностью фазы всходы–колошение на длинном дне. Задержка развития растений в условиях короткого фотопериода составляла от 5.4 до 53.8 дня, на длинном дне продолжительность фазы всходы–колошение варьировала от 39.5 до 53.9 дня. Обсуждается возможная генетическая основа выявленных различий. Для оценки разнообразия СГП нами использованы также глиадины как информативные биохимические маркеры. Показано, что 21 образец был мономорфным, остальные – гетерогенными. У изученных СГП определено 44 различных биотипа, из которых 36 были уникальными. Взаимосвязи между биотипами продемонстрированы в кластерном анализе. Следует отметить, что 13 СГП были нестабильными. У каждого такого образца некоторые растения отличались от других комплексом морфологических признаков, реакцией на фотопериод и спектрами глиадина. Возможно, нестабильность образцов – результат перестройки генома у СГП. Образцы СГП и выщепившиеся из них формы рассмотрены в качестве источников новой генетической изменчивости для улучшения мягкой пшеницы.

Ключевые слова: пшенично-эгилопсные амфидиплоиды; полевое изучение; реакция на фотопериод; электрофоретический анализ глиадина; генетическое разнообразие; классификация; расширение генофонда пшеницы.

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Genetic diversity and breeding value of synthetic hexaploid wheat introduced into the VIR collection

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For the successful development of wheat breeding in Russia, a genetically diverse and well-characterized starting material, mainly stored at the VIR collection, is needed. To replenish the collection, 36 lines (accessions) of synthetic hexaploid wheat (SHWs) developed at CIMMYT by crossing *Triticum durum* with *Aegilops tauschii* were studied. Our research was aimed at studying the SHWs using a complex of morphological and economically valuable traits in the environments of European Russia's northwestern part (E30°, N59°), evaluating the reaction of the SHWs to a photoperiod and determining their genetic heterogeneity and similarities by gliadins as biochemical markers. The results showed that the variability of different traits for SHWs fits into the framework of the genus *Triticum*, and so SHWs can be classified as poorly domesticated forms. Their distinctive feature, valuable for wheat breeding, is a large weight of a thousand grains (up to 60.6 g). This trait was characterized by a low degree of variability and a low correlation with other traits. The reaction of wheat plants to the length of the day is crucial for their transition from vegetative to re-

productive development. The SHWs studied differed from common wheat and one another by responses to the short day and by the length of the 'emergence-heading' phase if they grew under the conditions of a long day. The delay in the development of plants with a short photoperiod ranged from 5.4 to 53.8 days. On a long day, the duration of the 'emergence-heading' phase varied from 39.5 to 53.9 days. A possible genetic basis for the differences identified is discussed. To assess the diversity of SHWs, we also used gliadin proteins as informative biochemical markers. It was revealed that 21 SHWs were homogeneous, and the rest, heterogeneous. Forty-four different biotypes were found for the SHWs studied, from which 36 were unique. Relationships between biotypes have been demonstrated using cluster analysis. It should be noted that 13 SHWs were unstable. In each of them, some plants differed from the others in terms of a complex of morphological characters, reaction to a photoperiod, and gliadin patterns. It is possible that the instability of accessions is the result of genome rearrangement in SHWs. SHW accessions and the forms isolated from them are considered as sources of new genetic variability to improve common wheat.
Key words: wheat-aegilops amphidiploids; field study; reaction to photoperiod; electrophoretic analysis of gliadin; genetic diversity; classification; enhancement of the wheat germplasm.

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Введение

Синтетическая гексаплоидная пшеница (СГП) – искусственно созданные амфидиплоиды ($2n = 6x = 42$, BBAADD) с тем же геномным составом, что и у мягкой пшеницы (*Triticum aestivum* L.), но отличающиеся от нее бóльшим числом аллелей генов благодаря использованию в скрещиваниях различных представителей тетраплоидных пшениц ($2n = 4x = 28$, BBAA) рода *Triticum* L. и вида *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD) (Ogbonnaya et al., 2013). Первые формы синтетической пшеницы были получены в 1940-е годы в процессе выяснения степени родства геномов мягкой пшеницы (Kihara, 1944; McFadden, Sears, 1946). Но лишь спустя несколько десятилетий появились статьи, указывающие на достоинства вида *Ae. tauschii* как полезного источника новых генов для улучшения возделываемой пшеницы (Gill et al., 1986). В настоящее время в мире известно свыше 1.5 тыс. образцов СГП, для которых материнскими формами были не только современные сорта твердой пшеницы *T. durum* Desf., но и образцы дикой двузернянки *T. dicoccoides* (Koern. ex Aschers. et Graebn.) Schweinf., культурной полбы (*T. dicoccum* Schuebl.), а также пшеницы карталинской (*T. persicum* Vav.), а донорами пыльцы – примерно 900 образцов *Ae. tauschii* различного географического происхождения (Ogbonnaya et al., 2013). Преобладающая часть всей этой работы выполнена A. Mujeeb-Kazi с коллегами (1995, 1996) в CIMMYT (The International Maize and Wheat Improvement Center, Мексика). По результатам изучения 521 образца СГП сформированы наборы Elite 1 из 95 образцов, отобранных в основном по агрономическим признакам (Mujeeb-Kazi et al., 2000), и Elite 2 – из 33 образцов, характеризующихся устойчивостью к различным болезням (Mujeeb-Kazi, Delgado, 2001). Доступность этого материала для широкого круга исследователей разных стран стимулировала как создание новых СГП, так и передачу их генетического материала в возделываемые пшеницы Австралии (van Ginkel, Ogbonnaya, 2007), Китая (Yang et al., 2009), Индии, Пакистана и других стран мира (Шаманин и др., 2018; Li et al., 2018).

Для расширения возможностей использования СГП в российской селекции нами были привлечены в коллекцию генетических ресурсов растений Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР) линии (образцы), созданные в CIMMYT. Включение в коллекцию любого нового материала предполагает его морфологическое описание и комплексную оценку по хозяйственно полезным признакам с целью ботанической идентификации образцов и раскрытия их потенциала для селекционного использования. Цель настоящего исследования – изучение образцов СГП по комплексу морфологических и хозяйственно ценных признаков в условиях Северо-Западного региона Российской Федерации, оценка их реакции на фотопериод, определение генетической гетерогенности образцов СГП и сходства между ними с использованием глиадинов в качестве биохимических маркеров.

Материалы и методы

Материалом для исследований послужили 36 образцов СГП из списка Elite 1, полученные в коллекцию пшеницы ВИР в 2006 г. из Wheat Genetic and Genomic Resources Center at Kansas State University (США). В создании этих СГП участвовали 19 различных гибридных форм и сортов *T. durum* и 31 образец *Ae. tauschii* (Приложение 1)[1].

Полевое изучение образцов СГП проводили в яровом посеве (карантинный питомник, г. Павловск, 2007 г. и опытный участок ВИР, г. Пушкин, 2008 г.; 30° в. д., 59° с. ш.). Сроки посева и агротехника – общепринятые для зоны. Посев, фенологические наблюдения и оценку элементов структуры продуктивности образцов осуществляли в соответствии с Методическими указаниями ВИР (Мережко и др., 1999). Использовали также шкалы Широкого унифицированного классификатора СЭВ рода *Triticum* L. (1989) для описания образцов в системе этого рода. Для оценки высоты растений и компонентов продуктивности отбирали по 10 растений каждого образца. Стандартом служил сорт Ленинградка (к-47882, Россия, Ленинградская область).

В вегетационном опыте изучали фотопериодическую чувствительность (ФПЧ) образцов СГП. В качестве стандартов использовали ультраскороспелый сорт Фотон (к-55696, Россия, Краснодарский край), слабо реагирующий на короткий день, и среднеспелый Ленинградка, задерживающий развитие на коротком дне на две-три

[1] Приложения 1–4 см. по адресу:
http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx15.pdf

недели. Растения выращивали на дерново-подзолистой почве в пластиковых пятилитровых сосудах. В одном варианте опыта все сосуды с растениями находились в условиях естественного длинного дня (от 17 ч 30 мин до 18 ч 52 мин), в другом – короткого (12 ч) светового дня (фотопериода). Короткий день создавали, закатывая вагонетки с вегетационными сосудами в светонепроницаемый павильон, в котором они находились с 20 ч вечера до 8 ч утра. Вагонетки с растениями в условиях естественного длинного дня на этот период помещали в стеклянный павильон. У растений пшеницы определяли продолжительность периода всходы–колошение (дни) в условиях длинного (T_1) и короткого (T_2) дней. ФПЧ вычисляли по величине задержки колошения на коротком дне по сравнению с естественным длинным днем (T_2–T_1) и по коэффициенту ФПЧ ($К_{фпч} = T_2/T_1$).

Для электрофоретического анализа запасного белка глиадина в 2009 г. на поле ВИР (Пушкин) были высеяны зерновки отдельных колосьев урожая 2008 г. от каждого из 36 образцов СГП. Полученные из них растения оценивали на однородность по морфологическим признакам, и у растений каждого морфологического типа изучали глиадин 2–11 зерновок.

Глиадин экстрагировали из отдельных зерновок раствором 2 М мочевины в течение 10–12 ч. Электрофоретический анализ глиадина в пластинах 6.5 % ПААГ (буфер 0.013 М уксусная кислота) проводили по методике, принятой в ВИР. Идентификацию компонентов и запись белковых формул выполняли в соответствии с эталонным спектром (Идентификация сортов…, 2000). Стандартом для оценки качества разделения белков и идентификации компонентов служил глиадин сорта Мироновская 808 (к-43920; СССР, Украинская ССР). Белковые формулы глиадина всей совокупности изученных зерновок 36 образцов СГП опубликованы ранее (Хакимова и др., 2018). Растения с разными типами спектра глиадина у образца рассматривали как биотипы, их обозначали тем же номером каталога, дополнительно указывая номер биотипа, например к-65490_1 и к-65490_2.

Статистическую обработку результатов проводили с использованием программного пакета Statistica 12. Для оценки взаимосвязей между биотипами образцов СГП по сходству электрофоретических спектров глиадина применяли кластерный анализ. Степень сходства между электрофоретическими спектрами всех возможных пар биотипов оценивали показателем подобия Жаккара. Расчет матрицы, в которой наличие компонента кодировали цифрой 1, отсутствие – 0, кластерный анализ (алгоритм UPGMA), построение фенограмм кластеризации осуществляли с помощью программного обеспечения DARwin 6.0.

Результаты

Образцы СГП представляют род ×*Aegilotriticum* P. Fourn., относящийся к трибе Triticeae Dum., семейству Poaceae Barnh. (UniProt, 2019). Отличительными морфологическими признаками образцов были наличие веретеновидного полуостистого или остистого колоса, имеющего светло-коричневую или серо-коричневую окраску, в разной степени ломкий стержень, жесткие колосковые чешуи и трудный обмолот. Характеристики каждого из 36 образцов СГП по морфологическим признакам растения и компонентам продуктивности колоса приведены в Каталоге мировой коллекции ВИР, вып. 870 (Хакимова и др., 2018). Поскольку система рода ×*Aegilotriticum* не разработана, положение каждого изученного образца СГП дано в системе рода *Triticum* L. (Приложение 2). По признаку «высота растения» образцы входили в три группы: полукарлики, среднерослые и высокорослые, в двух последних группах выделены подгруппы. Независимо от этого деления образцы имели преимущественно средний по размеру колос, лишь у пяти образцов колос был длинным. Число колосков у СГП не выходило за пределы классов «очень малое» и «малое», а число зерен в колосе довольно широко варьировало: от очень малого до большого. Такое варьирование этого показателя могло быть связано с различиями растений по фертильности. Масса зерна с главного колоса у 11.4 % линий была очень малой, у 65.7 % – в пределах 0.9–1.4 г, т. е. малой, у 22.9 % – средней (от 1.5 до 2.6 г). Напротив, масса 1000 зерен у 58.3 % образцов была большой и очень большой. В целом на основании полученных характеристик можно было заключить, что выявленное разнообразие образцов рода ×*Aegilotriticum* укладывается в рамки *Triticum* L.; образцы можно классифицировать как слабо окультуренные формы.

Наряду со степенью выраженности признаков у образцов СГП были изучены уровень их варьирования под влиянием условий внешней среды и взаимосвязи между признаками, для того чтобы прогнозировать результативность отбора по ним. Изучение изменчивости признаков показало, что наиболее варьирующими по результатам двухлетнего исследования в Павловске в 2007 г. и Пушкине в 2008 г. были признаки «масса зерна с главного колоса или одного растения», «число продуктивных стеблей» и «число зерен в колосе» (табл. 1). Напротив, наиболее однородной была выборка образцов СГП по признакам «продолжительность периода всходы–колошение», «масса 1000 зерен» и «длина колоса». Коэффициент вариации по признаку «высота растения» составил 16.0 % (Пушкин, 2008) и 18.6 % (Павловск, 2007).

Рассчитанные по средним значениям признаков парные корреляции продемонстрировали сходство структуры их сопряженной изменчивости (Приложение 3). Независимо от места и года выращивания образцов СГП, статистически значимые ($p < 0.05$) связи признаков сохранялись между высотой растения и длиной колоса ($r = 0.41_{Пав., 2007}$ и $r = 0.48_{Пуш., 2008}$), числом зерен в колосе ($r = 0.55_{Пав., 2007}$ и $r = 0.61_{Пуш., 2008}$), массой зерна с растения ($r = 0.55_{Пав., 2007}$) или колоса ($r = 0.59_{Пуш., 2008}$), числом продуктивных стеблей ($r = 0.53_{Пав., 2007}$ и $r = 0.44_{Пуш., 2008}$), а также массой зерна с растения или колоса и числом продуктивных стеблей ($r = 0.77_{Пав., 2007}$ и $r = 0.45_{Пуш., 2008}$ соответственно), числом зерен в колосе ($r = 0.49_{Пав., 2007}$ и $r = 0.92_{Пуш., 2008}$ соответственно). Такая сопряженная изменчивость признаков может быть обусловлена генетической гетерогенностью образцов и их однотипной реакцией на изменение условий внешней среды. Практически независимо от других признаков варьировали длина колоса и масса 1000 зерен.

Высокие значения признака «масса 1000 зерен», его низкая степень изменчивости и слабая взаимосвязь с другими признаками указывают на образцы СГП как

Table 1. Parameters of 35 SHW accessions grown in the Northwestern region of Russia*

Traits	Pavlovsk, 2007			Pushkin, 2008		
	Xcp ± s**	lim	CV, %	Xcp ± s	lim	CV, %
Height, cm	88.9 ± 2.8	56–126.3	18.6	96.3 ± 2.6	70.9–128.1	16.0
Spike length, cm	9.2 ± 0.2	5.6–11.5	13.8	9.5 ± 0.2	8.1–12.2	10.3
Number of grains per spike	20.7 ± 1.1	6.8–38.0	30.1	23.3 ± 1.1	9.7–38.8	28.2
Grain weight in the main spike or a single plant, g	(5.2 ± 0.7)	(0.6–15.5)	(81.9)	1.2 ± 0.1	0.4–2.0	28.7
1000 grain weight, g	50.4 ± 1.0	35.0–58.0	11.3	51.6 ± 0.8	41.3–60.6	9.7
Number of productive stems	5.2 ± 0.3	1.5–10.8	39.8	4.0 ± 0.3	1.4–8.7	37.5
Duration of the emergence–heading interval, days	58.4 ± 0.8	48–71	8.0	49.3 ± 0.4	44–55	4.3

* Accession k-65488 was not examined. ** Xmean, mean value; s, error of the mean; lim, limits of character variation; CV, coefficient of variation.

Table 2. The SHW accessions shown to be sources of high 1000 grain weight in a two-year field study in the Northwestern region of Russia

VIR accession number	Locality-year	Plant height, cm	Number of productive stems	Spike length, cm	Number of grains per spike	1000 grain weight, g
65501	Pushkin-2008	96.4	3.4	9.3	22.4	51.5
	Pavlovsk-2007	86.3	5.3	9.1	23	57
65503	Pushkin-2008	119.0	4.2	9.6	22.3	54
	Pavlovsk-2007	101.0	8.3	9.4	25	53.5
65505	Pushkin-2008	110.5	4.3	9.9	26.1	60.6
	Pavlovsk-2007	88.0	4.3	9.6	21.3	50
65506	Pushkin-2008	104.6	6.2	9.7	28.9	53
	Pavlovsk-2007	93.0	9.0	10.2	27.5	56.3
65507	Pushkin-2008	86.7	1.4	9.1	16.6	53.7
	Pavlovsk-2007	101.0	8.0	9.9	18.7	55
65513	Pushkin-2008	73.5	5.0	8.7	25	55
	Pavlovsk-2007	77.5	5.5	10	22.5	58
65514	Pushkin-2008	88.7	3.3	9.6	28.3	46.9
	Pavlovsk-2007	74.3	2.8	8.6	22.3	46

на ценные генетические источники. Некоторые из них по результатам двухлетнего изучения перечислены в табл. 2. В литературе имеются сведения о возможности увеличения массы 1000 зерен у сортов мягкой пшеницы путем скрещивания их с образцами СГП (del Blanco et al., 2001). О высокой массе 1000 зерен (более 60 г), в том числе образцов, получивших номера каталога к-65485, к-65488, к-65492, к-65507 и к-65509, сообщали и A. Gul Kazi с коллегами (2012). Коэффициенты сходства между образцами, рассчитанные ими при сравнении генотипов по RAPD- и SSR-маркерам, варьировали от 0.693 до 0.889, что свидетельствовало в пользу генетических различий образцов. В транскриптомном анализе продемонстрировано, что различия между СГП и мягкой пшеницей по массе и размеру зерновок со второго по пятнадцатый день после опыления сопряжены с разной скоростью их развития и уровнем экспрессии генов, контролирующих метаболизм сахаров (Yan et al., 2018).

Следует отметить, что среди растений 13 из 36 образцов, выращенных из зерновок отдельных колосьев, а именно у к-65487, к-65496, к-65498, к-65501, к-65506, к-65508, к-65509, к-65510, к-65511, к-65514 и к-65518 (посев 2009 г.), были выявлены растения, отличающиеся от других по морфологическим признакам и скорости развития. Эти растения были на 10–15 см выше остальных, более позднеспелыми, безостыми, имели более светлую окраску колоса и большее сходство с мягкой пшеницей. Группа растений этих образцов была условно названа «выщепившиеся СГП». После размножения этих растений на опытном поле ВИР (Пушкин, 2011 г.) с целью сравнения полученных линий с типичными растениями образцов изучали реакцию некоторых из них на фотопериод, а также глиадин.

Результаты изучения в вегетационном опыте реакции на фотопериод 20 образцов СГП, трех выщепившихся форм и двух сортов-стандартов показаны в табл. 3. В условиях

Table 3. The duration of the emergence–heading interval and the photoperiodic sensitivity coefficient of SHW accessions grown under long- and short-day conditions (Pushkin, 2011, greenhouse experiment)

VIR accession	Emergence–heading interval		T_2–T_1	K_{PHS} (T_2/T_1)
	T_1	T_2		
k-55696, Foton	32.3 ± 0.21	34.7 ± 0.21	2.4	1.07
k-47882, Leningradka	40.1 ± 0.10	58.5 ± 0.69	18.4	1.46
k-65484	47.1 ± 2.48	63.5 ± 1.13	16.4	1.35
k-65485	45.8 ± 0.88	58.8 ± 0.44	13	1.28
k-65487	46.0 ± 1.76	64.9 ± 1.04	18.9	1.41
k-65487a	41.6 ± 0.96	51.6 ± 2.12	10	1.24
k-65488	45.9 ± 0.56	63.8 ± 1.63	17.9	1.39
k-65489	47.9 ± 0.77	63.6 ± 0.44	15.7	1.33
k-65490	43.0 ± 0.00	60.0	17.0	1.40
k-65492	46.6 ± 0.16	57.8 ± 0.65	11.2	1.24
k-65496	48.1 ± 0.28	59.9 ± 0.65	11.8	1.25
k-65498	45.1 ± 0.46	69.9 ± 0.93	24.8	1.55
k-65500	43.6 ± 0.31	49.0 ± 0.33	5.4	1.12
k-65501	48.4 ± 1.17	68.5 ± 3.48	20.1	1.42
k-65502	45.1 ± 0.56	90.4 ± 2.15	45.3	2.0
k-65503	43.9 ± 0.35	97.7 ± 0.96	53.8	2.23
k-65506	47.0 ± 0.58	62.8 ± 2.46	15.8	1.34
k-65513	39.5 ± 0.27	50.9 ± 0.91	11.4	1.29
k-65513a	46.7 ± 2.06	66.0 ± 2.39	19.3	1.41
k-65514	47.2 ± 0.13	64.0 ± 0.90	16.8	1.36
k-65515	43.7 ± 1.65	61.5 ± 2.30	17.8	1.41
k-65516	42.1 ± 0.41	50.7 ± 1.12	8.6	1.20
k-65517	48.1 ± 0.69	61.9 ± 0.98	13.8	1.29
k-65518	43.3 ± 1.24	86.0 ± 2.29	42.7	1.97
k-65518a	53.9 ± 2.57	83.7 ± 4.51	29.8	1.55

Notes: T_1 and T_2 are durations of emergence–heading interval in plants grown at the natural long-daylight and at the short 12-h daylight, respectively. T_2–T_1 is the delay in plant heading at the short day as compared to the long day. $K_{PHS} = T_2/T_1$ is the photoperiodic sensitivity coefficient.

длинного дня продолжительность периода всходы–колошение у них варьировала от 39.5 до 53.9 дня, у сортов-стандартов Фотон и Ленинградка составила 32 и 40 дней соответственно. Продолжительность этого периода на коротком дне изменялась от 49.0 до 97.7 дня, у Фотона составила 34, у Ленинградки – 58.5 дня. Коэффициент ФПЧ у образцов и линий СГП изменялся от 1.12 до 2.23: Фотон – 1.07 и Ленинградка – 1.46.

На коротком дне развитие растений СГП задерживалось в разной степени: от 5.4 до 53.8 дня. Наиболее сильную реакцию на короткий день проявили образцы к-65502, к-65503, к-65518 и выщепившаяся форма к-65518а. Задержка колошения у них составила от 29.8 до 53.8 дня, при этом на длинном дне образцы выколашивались на 3–5 дней, а выщепившаяся форма на 22 дня позже сорта Ленинградка. Наименее чувствительными к короткому фотопериоду были сорт-стандарт Фотон, образцы к-65500, к-65513, к-65516 и выщепившаяся форма к-65487а (задержка колошения на 5–11 дней). На длинном дне все эти образцы выколашивались примерно в те же сроки, что и сорт Ленинградка, а на коротком – быстрее его на 7–8 дней. Остальные образцы и линии существенно не различались между собой. Следует отметить, что характер реакций выщепившихся форм на изменение продолжительности светового дня был разным. По сравнению с исходными образцами у к-65513а увеличилась продолжительность периода всходы–колошение и на длинном, и на коротком дне; напротив, у к-65487а она уменьшилась, а у к-65518а период всходы–колошение увеличился на длинном дне и практически не изменился на коротком.

Вегетационный опыт показал, что и образцы СГП, и выщепившиеся из них формы отличаются от сортов-стандартов и различаются между собой по продолжительности периода всходы–колошение на длинном дне и реакцией на короткий день. Возможно, это обусловлено генетическими различиями СГП и отличием их от мягкой пшеницы, прежде всего по аллелям гомеологичных генов *Ppd* (Response to Photoperiod).

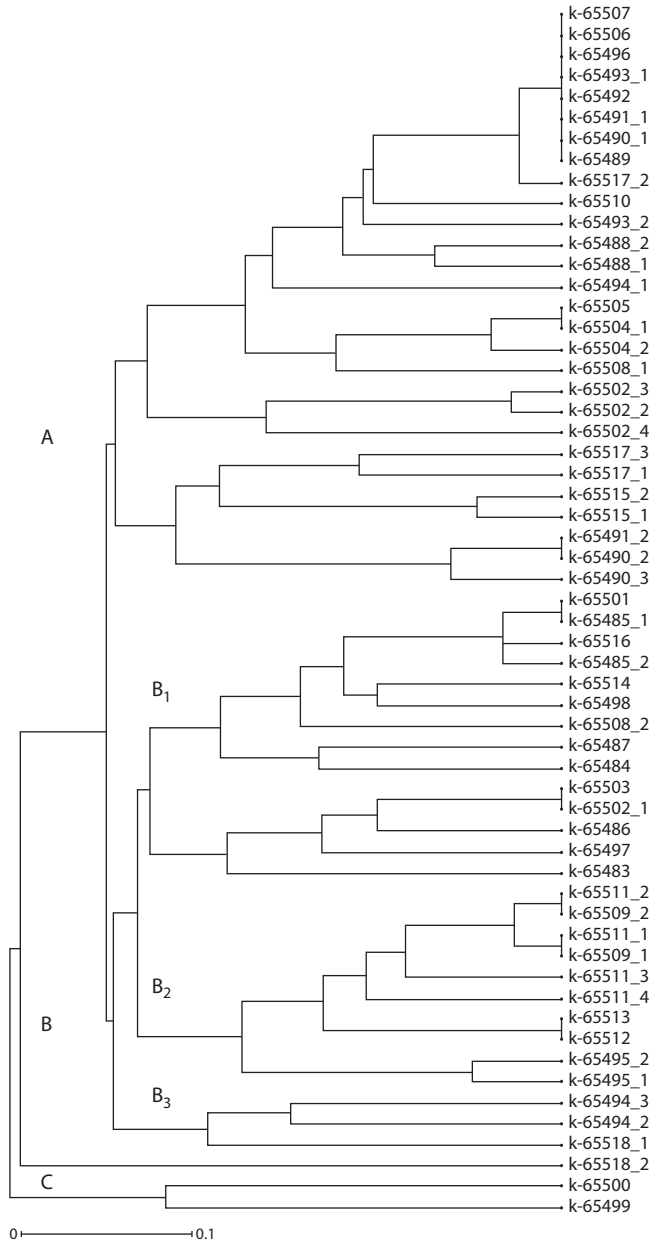
По данным L. Huang с коллегами (2012), образцы *Ae. tauschii* чувствительны к фотопериоду и характеризуются разной скоростью развития. Они имеют три гаплотипа (I–III), различающихся между собой по наличию–отсутствию делеций размером в 24 и 15 п. о., расположенных выше кодирующего участка гена *Ppd-D1*. Гаплотип I выявлен у образцов ssp. *tauschii*; а гаплотип III – у ssp. *strangulata*. В подтверждение тому, что представители ssp. *strangulata* были донорами генома D мягкой пшеницы, свидетельствует наличие у чувствительных к фотопериоду форм мягкой пшеницы гаплотипа III, но с дополнительной делецией 16 п. о. в экзоне 8. У нечувствительных к фотопериоду форм мягкой пшеницы выявлена делеция размером в 2089 п. о. выше кодирующего участка этого гена, какой нет у *Ae. tauschii* (Beales et al., 2007). Кроме того, в хромосомах 2А и 2В мягкой пшеницы показано присутствие гомеологичных генов *Ppd-A1a* с делецией в 1085 п. о. и *Ppd-B1a* с инсерцией в 308 п. о. или увеличенным числом копий (2–4) гена на хромосому, все эти изменения также определяют нечувствительность мягкой пшеницы к фотопериоду (Diaz et al., 2012; Nishida et al., 2013). В отличие от мягкой пшеницы формы *T. durum*, не чувствительные к фотопериоду, имеют ген *Ppd-A1* с делециями размером в 1027 или 1117 п. о., а мутаций гена *Ppd-B1*, связанных с нечувствительностью к фотопериоду, до сих пор не обнаружено (Wilhelm et al., 2009). Таким образом, образцы СГП, содержащие генетический материал твердой пшеницы и *Ae. tauschii*, являются источниками новых для мягкой пшеницы аллелей генов *Ppd*, которые можно использовать в селекции при создании сортов с разной продолжительностью фаз онтогенеза.

Для оценки степени гетерогенности и сходства образцов СГП, регистрации их разнообразия проведен электрофо-

ретический анализ запасного белка зерновки глиадина. В наиболее полном виде суммарный электрофоретический спектр этого белка содержал следующие компоненты: $\alpha 23456_1 6_2 7_1 7_2$, $\beta 12_2 3_1 3_2 3_3 4_1 4_2 5_1 5_2$, $\gamma 2_1 2_2 2_3 34_1 4_2 5$, $\omega 23_2 3_3 4_1 4_2 4_3 5_1 5_2 6_1 6_2 6_3 7_1 7_2 8_1 8_2 9_1 9_2 9_3 10_1 10_2$. Разнообразие по спектрам было обусловлено как наличием–отсутствием компонентов в α-, β-, γ- и ω-зонах, так и варьированием их интенсивности.

Сравнение электрофоретических спектров глиадина отдельных зерновок у каждого из 36 образцов СГП показало, что 21 образец был мономорфным, остальные – гетерогенными. Среди гетерогенных образцов десять имели по два варианта спектров глиадина, или биотипа; образцы к-65490, к-65494 и к-65517 – по три биотипа, а к-65502 и к-65511 – по четыре биотипа. У некоторых образцов и биотипов СГП были одинаковые спектры глиадина, при этом они имели разные родительские формы. Условно их можно объединить в группы: (1) 65489, 65492, 65496, 65506, 65507, 65490_1, 65491_1; (2) 65512, 65513; (3) 65504_1, 65505; (4) 65490_2, 65491_2; (5) 65485_1, 65501; (6) 65502_1, 65503; (7) 65509_1, 65511_1 и (8) 65509_2, 65511_2. Всего при анализе отдельных зерновок для 36 образцов СГП получено 44 типа спектра глиадина, которые различались по составу компонентов, из них 36 были уникальными: они встречались один раз в изученной выборке семян образцов. О генетическом разнообразии образцов свидетельствуют и данные изучения их по составу другого запасного белка зерновки – субъединиц высокомолекулярного глютенина (Rasheed et al., 2012). Широкое разнообразие образцов по двум запасным белкам зерновки (Хакимова и др., 2018) указывает на возможность регистрации и надежной идентификации их с помощью белковых маркеров, а также использования СГП для создания новых сортов мягкой пшеницы с желаемым составом запасных белков, влияющих на свойства клейковины, от которой зависит хлебопекарное качество муки.

Для классификации образцов и биотипов СГП по степени сходства электрофоретических спектров глиадина проведен кластерный анализ, основанный на UPGMA (рисунок). В составе построенной дендрограммы выделены кластеры А, Б и В. Суммарно кластер А включал 28 биотипов, принадлежащих 13 образцам. Кластер Б состоял из трех субкластеров, из них $Б_1$ содержал 14 биотипов, относящихся к 13 образцам, $Б_2$ – 10 биотипов пяти образцов, $Б_3$ – 3 биотипа двух образцов, т. е. биотипы, выявленные в составе отдельного образца, часто находились в одних и тех же кластерах. Исключение составили биотипы образцов к-65502 и к-65508, оказавшиеся в кластерах А и $Б_1$, а также к-65494, представленные в кластерах А и $Б_3$. Вне кластеров оказался биотип к-65518_2. Следует отметить, что наличие общей отцовской формы у пар образцов к-65488 и к-65489, к-65501 и к-65506, к-65516 и к-65517 не оказало влияния на их группировку, в то время как наличие общей материнской формы способствовало объединению биотипов в отдельных кластерах. Так, в кластер В вошли образцы к-65499 и к-65500, у которых материнской формой был сложный гибрид 68.111/RGB-U// Ward/3/FGO/4/Rabi/5/ (см. Приложение 1). В субкластере $Б_1$ и кластере Б объединились биотипы образцов, в родословных которых были сорта твердой пшеницы Croc 1



UPGMA dendrogram of the similarity of gliadin electrophoretic spectra for 58 SHW biotypes.

(к-65498, к-65501, к-65514) и Doy 1 (к-65483, к-65495, к-65511, к-65518) соответственно. Известно, что глиадиновые белки у пшеницы контролируются по меньшей мере шестью главными сложными локусами или блоками тесно сцепленных генов *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* и *Gli-D2*, локализованными в коротких плечах хромосом первой и шестой гомеологичных групп, а также несколькими минорными локусами *Gli-A3*, *-A5*, *-A6* (хромосома 1AS), *Gli-B3*, *-B5* (1BS), *Gli-D4*, *-D5* (1DS) (Wang et al., 2017). По-видимому, различия или сходство аллелей генов этих локусов у образцов СГП и определяют разнообразие или сходство их глиадиновых белков.

При сравнении компонентного состава глиадина у 13 выщепившихся форм было установлено 35 типов спектров глиадина, из них 28 уникальных (Приложение 4).

Изменения в спектрах, как и у исходных образцов, проявлялись по наличию–отсутствию компонентов разных зон и их интенсивности. Некоторые формы (они обозначены буквой «в») имели спектры, идентичные спектрам глиадина биотипов исходных образцов, а именно: к-65498_1в = к-65498_1; к-65501_2в = к-65501_2; к-65508_2в = к-65508_2; к-65511_1в = к-65511_2; 65511_4в = 65511_4; к-65518_1в = к-65518_1 и к-65518_2в = к-65518_2. Кроме того, у каждой выщепившейся формы также было обнаружено от одного до трех новых типов спектров этого белка. На дендрограмме (не приведена), построенной на основе сравнения всех полученных электрофоретических спектров, каких-либо отдельных кластеров биотипы выщепившихся форм не образовали. Они группировались вместе с биотипами исходных образцов.

Заключение

Включенные в коллекцию ВИР образцы СГП разнообразны по многим изученным признакам, примерно половина из них к тому же гетерогенная, что может быть следствием полиморфизма участвовавших в скрещиваниях родительских форм. Особо следует отметить нестабильность одной трети образцов и комплексный характер отличий выщепившихся форм от исходных образцов. Выявленная нестабильность, – возможно, результат продолжающейся реорганизации геномов СГП, изменений у них экспрессии генов (Щербань, 2013; Tonosaki et al., 2016). Данные по изучению характера конъюгации хромосом, подсчету мейотического индекса и определению числа микроядрышек в клетках пыльников у образцов СГП в сравнении с мягкой пшеницей подтверждают наличие такого процесса (Лапочкина и др., 2014; Frizon et al., 2017).

Вся совокупность образцов СГП, а также выщепившиеся из них формы – это оригинальная модельная популяция для оценки эволюционных изменений, происходящих в процессе адаптации межродовых гибридов, сходных по геномному составу с мягкой пшеницей, к различным эколого-географическим условиям. Они представляют собой ценный генетический ресурс для расширения генофонда мягкой пшеницы и ее селекционного улучшения не только по рассмотренным в этой статье признакам, но и по устойчивости к различным вредоносным болезням. В настоящее время нами завершено изучение образцов СГП по устойчивости к популяциям бурой ржавчины, собранным в различных регионах России, а также созданы линии F_5–F_7 поколений самоопыления от скрещивания отечественных сортов озимой мягкой пшеницы с отдельными образцами СГП с целью получения нового исходного материала озимого и ярового типов развития для селекции. Эти данные будут изложены в последующих публикациях.

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Biological and economic characteristics of the allotetraploid with genomic formula DDA^{u}A^{u} from the cereal family

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The synthesis of new allopolyploid cereal genotypes is an important task aimed at involving new genetic resources in breeding programs. Diploid species of the genera *Triticum* and *Aegilops* – bread wheat relatives – are an important source of agronomically valuable traits. A tetraploid synthetic with genomic formula DDA^{u}A^{u} was obtained by N.A. Navruzbekov through crossing *Aegilops tauschii* Coss. and *Triticum urartu* Thum. ex Gandil. The purpose of this work was to study the chromosomal composition and biological and commercially important traits of the tetraploid. Cytogenetic analysis using fluorescent *in situ* hybridization showed the presence of all chromosomes of the D genome in the chromosomal complement of the synthetic. By means of stepwise vernalization, the winter habit was established for the tetraploid synthetic with the optimum vernalization requirement of 45 days. Under greenhouse conditions, two groups of genotypes were found whose flowering dates differed by 6.5 days, which may indicate an allelism at the *Vrn-3* locus. The coloring of various organs of the tetraploid plant, such as coleoptile, stem, anthers, and glumes of the spike, was revealed. The coloration of the aleurone layer of the grain may indicate that the donor species *T. urartu* is a carrier of the *Ba* gene that controls its blue color. A new morphotype of leaf pubescence was found. In terms of productivity, the tetraploid is comparable to bread wheat. Grains are characterized by a supersoft structure and high wet gluten content, from 39–45 to 65 %, in the field and greenhouse conditions, respectively. Thus, the tetraploid can be used to create new wheat genotypes as a source of untapped genetic diversity, as well as a new genetic model for studying the patterns of evolution of polyploid plants.
Key words: *Triticum urartu*; *Aegilops tauschii*; synthetic allotetraploid; growth habit; flavonoid pigmentation; leaf and spike morphology; yield components; technological properties of grain and flour.

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Биологические и хозяйственные характеристики аллотетраплоида из семейства злаковых с геномной формулой DDA^{u}A^{u}

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Синтез аллополиплоидных генотипов злаков – важная задача, нацеленная на вовлечение в селекционные программы новых генетических ресурсов. Диплоидные виды родов *Triticum* и *Aegilops*, сородичей мягкой пшеницы, – существенный источник агрономически ценных признаков. Тетраплоидный синтетик (геномная формула DDA^{u}A^{u}) был получен Н.А. Наврузбековым путем скрещивания видов *Aegilops tauschii* Coss. и *Triticum urartu* Thum. ex Gandil. Целью настоящей работы было изучение хромосомного состава, биологических и хозяйственно важных признаков тетраплоида. Цитогенетический анализ с использованием флуоресцентной гибридизации *in situ* показал присутствие всех хромосом генома D в составе генома синтетика. С помощью ступенчатой яровизации установлен озимый образ жизни растений тетраплоидного синтетика с оптимальной потребностью в яровизации в 45 дней. В условиях теплицы обнаружено две группы генотипов с разницей по дате цветения в 6.5 дней, что может указывать на аллелизм по локусу *Vrn-3*. Наличие антоциановой окраски колеоптиле, стебля и пыльников предполагает регуляцию этого признака доминантными аллелями локусов *Rc-1*, *Pc-1* и *Pan-1*. Окраска алейронового слоя зерновки может свидетельствовать о том, что донорский вид *T. urartu* является носителем гена *Ba*, контролирующего голубую окраску. Был

также обнаружен новый морфотип опушения листа. По показателям продуктивности тетраплоид сопоставим с мягкой пшеницей. Зерно характеризуется супермягкой структурой, высоким содержанием сырой клейковины – от 39–45 до 65 % – в полевых и тепличных условиях соответственно. Таким образом, тетраплоид может быть использован при создании новых генотипов в селекции пшеницы как источник еще не освоенного генетического разнообразия, а также как новая генетическая модель для исследования закономерностей эволюции полиплоидных растений.
Ключевые слова: *Triticum urartu*; *Aegilops tauschii*; синтетический аллотетраплоид; образ жизни; флавоноидная пигментация; морфологические признаки листа и колоса; структура урожая; технологические свойства зерна и муки.

Introduction

The highland areas of the Caucasus are natural habitats of diploid species of the genera *Triticum* and *Aegilops*, which gave rise to cultural allopolyploid species of wheat. In the second half of the 20th century, many attempts were made to synthesize the new types of allopolyploids in order to engage in breeding and, subsequently, in economic activity, the new genetic resources from diploid species of the above mentioned genera. The tetraploid synthetic with a genomic formula DDA^uA^u was obtained by N.A. Navruzbekov (1982) by crossing *Aegilops tauschii* Coss and *T. urartu* Thum. ex Gandil. The character of meiosis was studied in amphidiploid and the karyotype was shown to do be stabile (Aminov, Navruzbekov, 1985). A similar hybrid with the participation of other initial samples of these species was named *T. erebuni* Gandul. (Gandilyan, 1984). These tetraploids, as well as the tetraploid species *T. palmovae* G. Ivanov (A^bA^bDD) carrying the genome A from the species *T. boeoticum* Boiss., are very limitedly involved in the researches of both applied and fundamental nature. Only the information about the use of two species as donors of fungal disease resistance genes in common wheat is available (Babayants et al., 2012; Davoyan et al., 2018). The biological characteristics of these tetraploid has not yet been conducted in more details. At the Dagestan experimental station of VIR allotetraploid of N.A. Navruzbekov with the genomic formula A^uA^uDD (AT Navruzbekov) is being maintained and successfully propagated. In this work, the chromosomal composition and biological and economically important traits were studied.

Material and methods

The tetraploid was obtained by a direct hybridization of diploid species *Ae. tauschii* and *T. urartu* Thum. ex Gandil. followed by colchicine treatment of the roots of F_1 hybrids (Navruzbekov, 1982).

Tetraploid plants were studied under natural conditions in the city of Derbent (Dagestan) at the experimental station of the VIR branch (St. Petersburg) and under the hydroponic greenhouse conditions of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk). The field study was conducted in a dry subtropical climate of Dagestan, in the southern plane zone. The average annual temperature of the area is +1…–1.4 °C with an annual amount of precipitation of 400–500 mm, 80–90 % of which falls in the autumn-winter period. Summer is hot and dry. Productivity indicators were studied in 2018 on a random sample of 20 plants. Plants were analyzed individually; spike morphology and productivity were assessed on the main spikes. In the hydroponic greenhouse the plants were grown on artificial soil, expanded clay, with daily double top dressing with Knop solution and under 14-hour lighting; day/night temperatures are 18/20 °C before and 20/22 °C after tillering. To determine the growth habit, the plants were grown without vernalization and under 30, 45, and 60 days of vernalizing at +4 °C. The seedlings were sown in bathtubs filled with expanded clay, 7–10 plants in a row with a length of 1 m. The observations were carried out for to the following traits: the day number from seedlings to flowering, the presence of pigmentation and pubescence on the plant organs, spike shape and awnedness, productivity indicators, technological properties of grain and flour.

Fluorescent *in situ* hybridization (FISH) was performed according to a previously published procedure (Salina et al., 2006). For the identification of chromosomes, pSc119.2 and (Bedbrook et al., 1980) and pAs1 (Rayburn, Gill, 1986) probes labeled with the nick-translation reaction were used. The pubescence of the leaf surface was studied in microphotographs of the folds of boot leaves according to the procedure described previously (Genaev et al., 2012). Grain was studied for the nine technological indicators using the methods recommended for variety testing of agricultural crops in Russia (Methods of State Tests of Crops, 1988), with modifications for small portions of grain. Raw gluten content was determined manually from the whole meal using a micro-method based on the state standard of the Russian Federation (State Standard R 54478-2011…, 2012). The diameter of the flour particles (μm) was determined on a PSH-4 device. Physical properties of dough: tenacity (mm), extensibility (mm), the balance of dough and flour strength (u. a.) were determined on a Chopin alveograph with 50-g mixer. Gluten content was determined in grain grown in greenhouse and under field conditions, the other parameters – in grain of field origin grown in Dagestan.

Results

Chromosomal composition. The analysis of the metaphase chromosomes of a tetraploid sample by the FISH method was carried out with the pSc119.2 and pAs1 probes, which

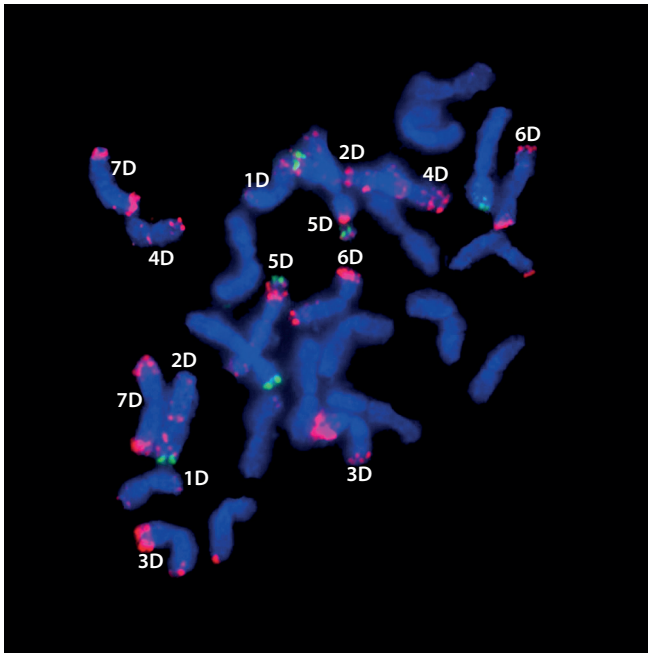

Fig. 1. Identification by fluorescent *in situ* hybridization (FISH) of the D-genome chromosomes of DDA^uA^u tetraploid.
Red signal – pAs1 probe, marking D-genome; green – pSc119.2 probe.

are most often used to identify chromosomes in wheat and a number of other cereals including the donors of the B and D genomes of polyploid wheat – *Ae. speltoides* Tausch. and *Ae. tauschii* Coss. (Badaeva et al., 1996; Schneider et al., 2003). The pSc119.2 probe hybridizes mainly with the chromosomes of the B genome of polyploid wheat, and the pAs1 sites are localized mainly on the chromosomes of the D genome. In the result of the study the sample was found to be a tetraploid synthetic wheat ($2n = 28$, DDAA), carrying 14 chromosomes of the D genome in the karyotype (Fig. 1). The sites of hybridization with pAs1 were also found at the ends of the long arms of one of the A-genome chromosome pair, which does not contradict the results of (Badaeva et al., 2015). The presence of the separate pAs1 blocks on different chromosomes of diploid species wheat were showed. The pSc119.2 probe is localized on the short arms of chromosomes 2D and 5D, which corresponds to the previously obtained data for *Ae. tauschii* (Badaeva et al., 1996). The Fig. 1 also contains a pair of submetacentric chromosomes of genome A with pSc119.2 signals in subtelomeric regions of short arms. According to previous studies, pSc119.2 sites on the A chromosomes are few. In tetraploid and hexaploid wheats this tandem repeats can be localized at 5AS and 4AL chromosomes (Schneider et al., 2003; Kubaláková et al., 2005). In diploid wheat, pSc119.2 almost does not occur. However, E.D. Badaeva et al. (2015) reported on the localization of this probe on a long arm, presumably on 2A chromosome, in one of the samples of *T. boeoticum*. Consequently, it is quite possible that the sample of *T. urartu* that participated in the obtaining of Navruzbekov's AT carries pSc119.2 sites on the short arms of one of the pairs of submetacentric chromosomes, of genome A possibly 5A.

Determination of growth habit. Growth habit and pace of development determine the adaptation of the genotype to environmental conditions through the timely formation of generative organs and successful seed reproduction. Under the conditions of Dagestan, tetraploid was sown in the fall. Due to the mildness of the climate it was not possible to determine the genetic status of vernalization genes. An experiment in the greenhouse showed that without vernalization and with 30-day vernalization, the plants do not pass to the generative phase. The most effective terms of vernalization were 45 and 60 days. This suggests that the sample carries the recessive alleles of the *Vrn-A1* and *Vrn-D1* genes. The winter growth habit was also noted in synthetic allotetraploid *T. palmovae* (DDA^bA^b) (Ivanov, 1984). The vernalized plants studied in the autumn and spring seasons under greenhouse conditions separated into two groups according to the flowering time. In the first group, the number of days was 40.5, while in the second group it was 46.8 days (the differences are significant at $p = 0.0014$). This may indicate that the sample carries different alleles of the *Vrn-3* locus in the chromosomes of the 7th homoeological group responsible for the "fine tuning" of the time of transition to flowering during the growing season, or other minor loci responsible for the timing of flowering *per se*.

Coloration of plant organs. Flavonoid pigments were synthesized at various stages of ontogenesis by various plant organs of the tetraploid synthetic. Their role in life processes is associated with adaptive responses to many abiotic and biotic stresses. The anthocyanin pigmentation was found on the coleoptile, stem and anthers (Fig. 2, *a*, *b*). These dominant traits in common wheat are controlled by *Ra*, *Pc-1* and *Pan-1* loci in the chromosomes of the 7th homoeological group (Khlestkina, 2012). Bright and stable color indicates a good expression of these genes in tetraploid. Among these genes, the genes of the 7D chromosome have the highest expression in bread wheat and are probably also determined by the D genome in the tetraploid. The ripened grain had a very rare blue color of the aleurone layer both in the field and in greenhouse conditions (see Fig. 2, *c*). Previously, the *Ba* gene that determines this trait was localized on chromosome 4A of the species *T. boeoticum* (Singh et al., 2007). No such genes were found in the D genome (Khlestkina, 2012). Thus, it can be assumed that the species *T. urartu*, which donated the genome to the synthetic tetraploid AT Navruzbekov, is the carrier of the dominant allele of this gene.

The tetraploid had colored spike glumes; the trait was well manifested in the field conditions but rather weakly in the greenhouse (see Fig. 2, *d*). This dominant trait in polyploid wheat is controlled by a series of homoeoallelic genes *Rg-1* in the chromosomes of the 1st homoeological group (Khlestkina et al., 2006). Among the diploid species,

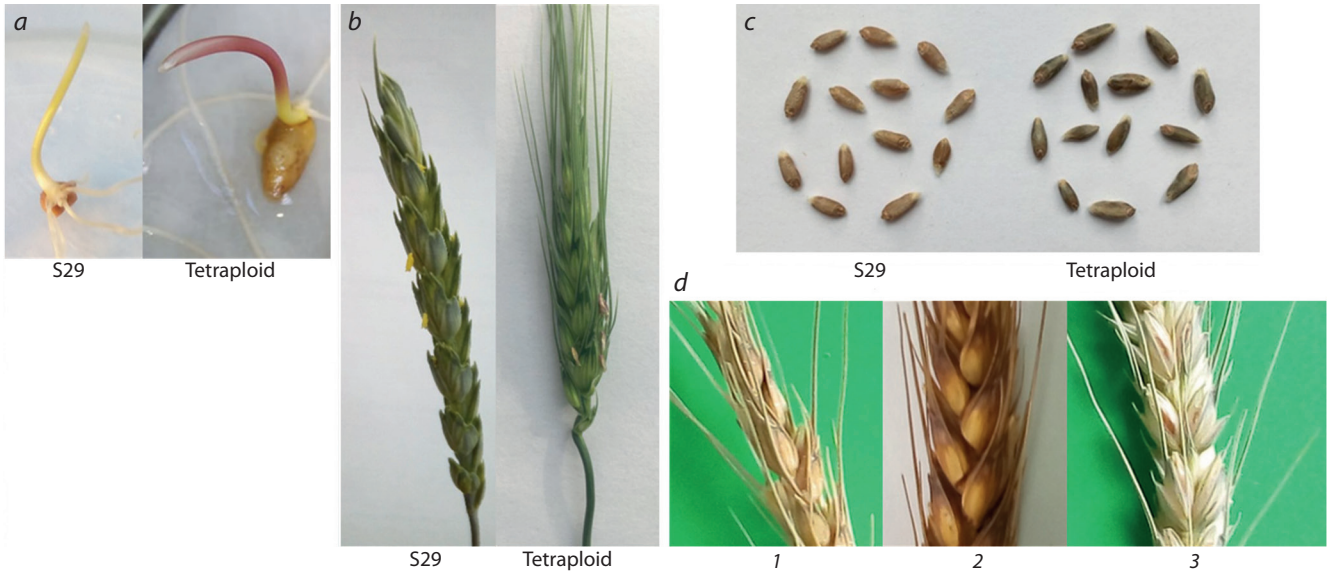

Fig. 2. Flavonoid coloration of different organs in DDA^{u}A^{u} tetraploid.

Spike coloration: *1* – in green-house; *2* – the same in field conditions, Derbent; *3* – uncolored spike of hexaploid wheat. S29 – hexaploid cultivar Saratovskaya 29. Explanations in the text.

Average values and variability of yield components of A^{u}A^{u}DD tetraploid un field growing on Dagestan experimental station (Derbent town)

Statistical parameter	Plant height, cm	Spike length, cm	Number of spikelets in spike	Spike density	Number of grains in main spike	Grain weight in main spike	1000 grain weight	Grain plumpness, score
(X ± Sx)	128.5 ± 5.62	11.6 ± 0.17	18.5 ± 0.50	19.4 ± 0.50	31.5 ± 1.81	1.2 ± 0.05	38.1	4.1 ± 0.08
Max	139.5	13.5	21.0	22.4	39.0	1.5	42.1	5.0
Min	115.4	8.5	17.0	17.0	19.0	0.8	38.5	3.0

the occurence of this trait has not been specifically studied. However, it is known that among the varieties of bread wheat the dominant allele is more common in elevated areas and in areas with a cold climate (Yakubtsiner, Savitsky, 1947). The allele of *Rg-D1* gene (chromosome 1D) which determines the gray-smoky (subsp. *cesium*) color of spikelet glumes is characterized of bread wheat. Earlier it was shown that *Ae. tauschii* species carries another than in bread wheat varieties allele of this gene, which gives a dark brown color (Pshenichnikova et al., 2005).

Morphobiological characteristics of the ear. Interestingly, that the studied genotype is close to a bread wheat in terms of the spike shape and density (see Table 1). It is well known that the A genome of the hexaploid precursor of bread wheat *T. spelta* carries the *Q* gene, which determines the speltoid form of the spike (Matsuoka, 2011). It can be assumed that the A^{u}A^{u} genome of the tetraploid under study carries an allele of this gene that does not affect the shape of the ear, and its relatively poor threshability is more closely linked to the *Tg* gene in chromosome 2D (McIntosh et al., 2013). The spike of Navruzbekov's AT is awned which is generally characteristic of wild and poorly cultivated relatives of common wheat.

Leaf blade pubescence and the presence of wax in plants. The presence of pubescence on the leaf blades was a characteristic feature of the AT Navruzbekov. Its morphology differed from the pubescence in bread wheat (Fig. 3). It was long, tough to the touch and rather rare. The adaptive significance of this trait is associated with a protection from damage by leaf-eating insects, as well as with the influence on the energy balance and gas exchange of the leaf by changing the thickness of the air boundary layer adjacent to its surface (Schuepp, 1993). The pubescence protects the photosynthetic apparatus of the leaf from excessive solar radiation and is especially common in plants whose growing areas are steppes, deserts, high mountains and tundra (Johnson, 1975). To date, the genes of bread wheat have been identified in chromosomes 4B and 7B (McIntosh et al., 2013), as well as in chromosome 5A of the diploid and tetraploid species *T. monococcum* and *T. timopheevii* (Jing et al., 2007; Pshenichnikova et al., 2019). Such genes have not yet been found for the D genome. We assume that

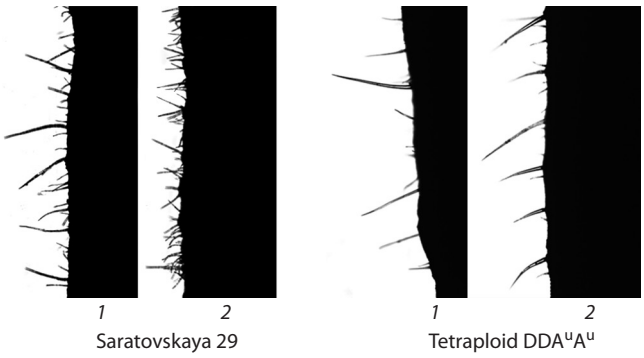

Fig. 3. Microphotograph of booting leaf folds of the hexaploid wheat Saratovskaya 29 (S29) and tetraploid DDA^uA^u.

Leaf surface: *1* – upper; *2* – bottom.

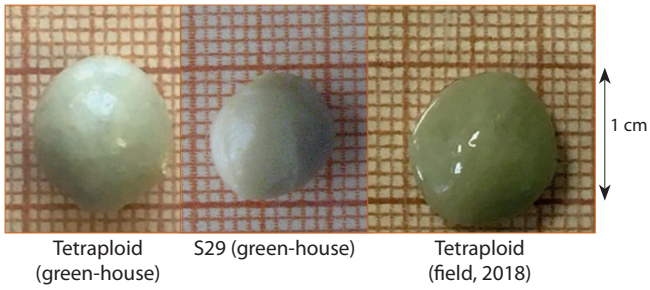

Fig. 4. Hand-washed gluten obtained from the whole meal of grain of DDA^uA^u tetraploid.

S29 – Saratovskaya 29.

the studied genotype is the carrier of a new gene from this genome. Earlier, we showed that among the samples of *Ae. tauschii* some carry the longest leaf pubescence; this expression level of the trait was inherited by the allopolyploid *T. palmovae*, which carries the D and A^b genomes (Pshenichnikova et al., 2017).

The plants of the sample studied were completely waxless. All einkorn wheats are the same (Tsunewaki, Ebona, 1999). No the inhibitor gene has been yet detected responsible for wax formation on einkorn wheat organs, however, the inhibitor gene is known on chromosome 2D (McIntosh et al., 2013).

Economically important traits. For plant height wheat the amphidiploid may be attributed to the intermediate forms, its average straw length is 128.5 cm (see Table). The straw is tough, but lodging resistance is low (3 points of the scale). The length of the ear varies from 8.5 to 13.5 cm. The spike and flower glumes are tough, with poor threshing. Ear density is average. The average weight of grains per spike is 1.2 g, the maximum number of grains per spike is 39. Visual evaluation of grain plumpness was 4,1 points out of 10 possible (see Table). This suggests a lack of uniformity of grain surface (see Fig. 2, *c*). However, the grain is quite large: thousand-grain weight of the main spike was 38.1 g under field conditions (see Table), and during the technological analysis of grain yielded in 2017 and 2018 it consisted 33.7 g on average.

Technological properties of dough. Technological properties of grain are the most important breeding characteristics that determine the production purpose of the harvested crop. For the first time in this work, the technological properties of synthetic tetraploid were studied. One of the main technological indicators in the classification of grain according to the production classes in Russia is gluten content in grain. This parameter was studied both in greenhouse and field grain. It turned out to be very high (Fig. 4). In the first case, the content was 65 %. In field conditions this figure was: in 2017 – 39 %, in 2018 – 45 %. Thus, A^uA^uDD tetraploid can be a source of improvement of this trait in breeding. The genotype may be characterized as soft-grained as the diameter of flour particles was 8.7 microns with a total grain vitreousness of 50 %. Such flour can be described as supersoft. Possibly, the tetraploid is the carrier of the dominant alleles of the *Ha* loci in both 5A and 5D chromosomes which synthesize the complete proteins PinA and PinB, providing the soft endosperm structure. This is characteristic of diploid ancestral species of wheat *T. monococcum* and *Ae. tauschii* (Gautier et al., 2000). It should be noted that the tetraploid species with the genomic composition BBAA completely lost the dominant alleles of the genes *Pina-A1* and *Pina-D1* and the DDA^uA^u tetraploid under study is unique in this respect. With its help it is possible to transfer into the bread wheat the dominant allele of *Pina-A1* gene from 5A chromosome, lost in the process of polyploidization. The physical properties of flour (on alveograph) were characterized by relatively high elasticity but low extensibility. The ratio of these rheological parameters was 6.1, which brings the tetraploid closer to durum wheat with the genomic composition of BBAA.

Conclusion

Estimating the breeding value of AT Navruzbekov it can be concluded that due to the noted shortcomings (lodging, poor threshing, low productivity, etc.) its direct use in production is not advisable. At this stage, it is necessary to determine the possible ways of transferring the identified valuable traits to a genotype that is more adapted to industrial cultivation or to obtain a new improved form on the basis of the amphidiploid. At present, there is only one paper concerning crossability of this tetraploid with certain samples of wheat, *Aegilops* and rye (Navruzbekov, 1984). It indicates that the best seed setting and germination is observed under hybridization with a cultivated emmer (over 80 %). With bread wheat this parameters are slightly lower. Seed setting under crossing with rye and various samples of *Aegilops* up to 10 %. These studies indicate the possibility of carrying out the breeding works with this tetraploid. Newly developed allopolyploids are of considerable interest for basic research. They simulate the processes of evolution occurring through polyploidy that is resulted in a simul-

taneous restructuring of the interactions between many genetic networks that existed in separate genomes. These processes largely determine both the adaptive capabilities and the future of the new genotype. The combination of the genomes A and B in cereals was very successful for the formation of a cytogenetically stable tetraploid, which adapted to environmental conditions and later gave rise to a large spectrum of species with the genomic formula BBAA. Later, the addition of the D genome was also evolutionarily favorable and gave the hexaploid *T. aestivum*, which spread widely across the globe.

A comprehensive study of artificial allopolyploids obtained in previous years, including by means of new molecular genetic technologies, will make it possible to master new genetic resources for practical purposes and create new genetic models for studying the patterns of plant evolution through polyploidy. The results obtained in this work indicate the prospects for a deeper genetic and breeding study of the Navruzbekovs' allotetraploid.

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Cytoplasmic genetic diversity of potato varieties bred in Russia and FSU countries

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Male sterility in potato is little studied since traditional breeding is based on the vegetative reproduction of highly heterozygous tetraploid varieties. The rapid development of hybrid diploid breeding contributes to growing interest in studying the male sterility of this important crop. In this work, a set of 6 cytoplasmic markers was employed to describe cytoplasmic genetic diversity of 185 potato cultivars bred in Russia and FSU countries. Three cytoplasm types were identified, T (40.0 %), D (50.8 %) and W/γ (8.7 %), which according to literature are associated with male sterility. With a single exception (0.5 %), cytoplasm types characteristic of male fertile forms (A, P) were not found in the subset of 185 cultivars. A comparison of these results with previously published data suggested expanding the subset to up to 277 cultivars, all developed in Russia or FSU countries; however, the resulting differentiation into three cytoplasm types (T, D and W/γ) was nearly the same. Fertility phenotyping helped identify both male-sterile and male-fertile genotypes within the three groups of varieties with T-, D- and W/γ-type cytoplasm. Fifteen genotypes differing in cytoplasm type and male sterility/fertility traits were selected for direct sequencing of 8 mtDNA loci. Fragments of the *nad2*, *nad7*, *cox2*, *atp6* and *CcmFc* genes were identical in all 15 selected genotypes. The polymorphism, detected in the *rps3*, *atp9* and *CcmFc* loci, was not associated with male sterility. Two SNPs in the *nad1*/*atp6* and *nad2* loci differentiated 7 genotypes with W/γ-type cytoplasm into five genotypes with tetrad sterility, and two with fertile pollen. The results of an NGS analysis confirmed the association of these SNPs with tetrad sterility in a larger set of 28 genotypes of different origin, all with W/γ-type cytoplasm. A heteroplasmy state was observed both in male-sterile and in male-fertile genotypes.
Key words: potato; *Solanum*; male sterility; cytoplasmic types; DNA markers.

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Генетическое разнообразие сортов картофеля российской селекции и стран ближнего зарубежья по типам цитоплазм

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Мужская стерильность у картофеля малоизучена, поскольку традиционное картофелеводство основывается на вегетативном размножении высокогетерозиготных тетраплоидных сортов. Быстрое развитие диплоидной гибридной селекции картофеля обусловливает возрастание интереса к изучению мужской стерильности у этой важной культуры. В настоящей работе охарактеризовано генетическое разнообразие по типам цитоплазм 185 сортов картофеля селекции России и стран ближнего зарубежья с использованием набора из шести цитоплазматических маркеров. Сорта выборки были дифференцированы на три группы с T (40.0 %), D (50.8 %) и W/γ (8.7 %) типами цитоплазм, которые, согласно литературным данным, ассоциированы с мужской стерильностью. За единичным исключением (0.5 %), типы цитоплазм, характерные для мужскофертильных форм (A, P), в изученной выборке не найдены. Сопоставление полученных результатов с ранее опубликованными данными позволило расширить выборку до 277 отечественных сортов, однако дифференциация на три типа цитоплазм (T, D, W/γ) сохранилась. На основании результатов фенотипизации среди сортов с T-, D- и W/γ-типами цитоплазм были выявлены не только мужскостерильные, но и фертильные генотипы.

У 15 отобранных генотипов, различающихся по типу цитоплазм и признаку мужской стерильности/фертильности, методом прямого секвенирования были проанализированы восемь локусов мт-генома. Фрагменты мт-генов *nad2*, *nad7*, *cox2*, *atp6*, *CcmFc* были идентичны независимо от типа цитоплазмы и мужской стерильности/фертильности сортов. Индели/замены нуклеотидов в локусах *rps3*, *atp9*, *CcmFc* не были ассоциированы с признаком мужской стерильности. На ограниченной выборке из семи образцов с W/γ-типом цитоплазмы были выявлены различия генотипов с тетрадной мужской стерильностью и с фертильной пыльцой по двум однонуклеотидным заменам в локусах *nad1*/*atp6* и *nad2*. Результаты NGS-анализа подтвердили ассоциацию этих SNP-вариантов с тетрадной мужской стерильностью на большей выборке из 28 образцов с W/γ-типом цитоплазмы различного происхождения. Показано, что гетероплазматическое состояние характерно как для генотипов с тетрадной мужской стерильностью, так и для генотипов с мужской фертильностью.
Ключевые слова: картофель; *Solanum*; мужская стерильность; типы цитоплазм; молекулярные маркеры.

Introduction

In more than 300 years, covering the breeding history of potato (*Solanum tuberosum*) outside South America, where it was domesticated, thousands of cultivars have been developed by breeders; they are presently cultivated in nearly 150 countries. This 300-year period may be divided into three stages: (1) the breeding process of the 18th–19th centuries, based on a restricted genetic diversity of relatively not numerous South America's indigenous cultivars introduced to Europe; (2) beginning from the early 20th century, targeted crossing of cultivated potato with wild species in order to introgress *R* genes, first of all, conferring resistance to fungal and viral pathogens; and (3) the third phase that we are now standing witness to is marked by the emergence of essentially novel trends in potato breeding: cysgenesis, genome editing, and diploid hybrid breeding. The latter trend, based on crossing inbred diploid lines in order to obtain in plenty hybrid seeds (true potato seeds, TPS) of heterotic F_1 hybrids, manifests a radical change of the paradigm in the breeding new potato varieties and in their reproduction (Lindhout et al., 2011; Jansky et al., 2016).

The rapid development of diploid hybrid breeding is coupled with the prospects to cardinally shorten the duration of the breeding process and reduce the costs of producing and multiplying healthy clones (Lindhout et al., 2011, 2018; De Vries et al., 2016; Jansky et al., 2016), because most of potato pathogens are not transmitted with pollen or TPS. The onset of diploid hybrid breeding called for a need to study genetic bases of self-incompatibility (Phumichai et al., 2005) and inbreeding depression in potato (Zhang et al., 2019) in order to obtain inbred diploid lines, as well as to promote research on male sterility and CMS-*Rf* genetic systems, aimed at the development of effective interline hybridization techniques (Anisimova, Gavrilenko, 2017). Previously, those challenges have had not been so relevant, so far as the conventional potato production is based on vegetative reproduction of highly heterozygous tetraploid cultivars.

Some few publications have addressed the problems of male sterility in potato, despite the fact that this trait is quite frequent in improved cultivars. There are several known types of mail sterility in potato:

(1) Genetic-cytoplasmic male sterility (Grun et al., 1962), expressed as various abnormalities in the development of reproductive organs and as the plant's inability to set berries, which is caused by interactions between dominant alleles of nuclear genes responsible for male sterility (for example, *Ms* gene) and genetic factors of the T (Tuberosum) type of cytoplasm (Grun et al., 1977; Iwanaga et al., 1991);

(2) Functional male sterility, when plants produce morphologically normal, well stainable, but non-functional pollen grains. This type of sterility has been described for hybrids, breeding clones and cultivars with the cytoplasm of wild Mexican species, *S. demissum* (Dionne, 1961; Hosaka, Sanetomo, 2012);

(3) Cytoplasmic male sterility (CMS), expressed in certain interspecies combinations. For example, virtually all studied cultivars and breeding clones with the cytoplasm of another wild Mexican species *S. stoloniferum* can participate in crosses only as female parents (Ross, 1986; Lössl et al., 2000; Song, Schwarzfischer, 2008), because they manifest tetrad sterility (tetrads do not disintegrate in the end of microsporogenesis, and over the entire process of subsequent microgametogenesis microspores remain united into 'permanent tetrads'). Using the method of metabolic profiling on anthers, it has been shown that tetrad sterility is associated with an abrupt disorder of the carbonic exchange in anthers, mainly as far as the polysaccharide spectrum is concerned, and the metabolism of amino and fatty acids (Shishova et al., 2019).

Among the carriers of the above-mentioned cytoplasm types, genotypes with male fertility occur with varied frequency. For example, H. Ross (1986) reported that a third of the varieties with the most widespread T-type cytoplasm were unable to develop berries. Studying the genetic control of fertility restoration resulted in identifying the nuclear *Rt* gene (a male fertility restorer gene) that can result in the recovery of male fertility in the T-type cytoplasm holders (Iwanaga et al., 1991; Ortiz et al., 1993). These authors reported a wide distribution of functional *Rt* gene alleles in the breeding gene pool. At the same time, the *Rt* gene, same as the nuclear *Ms* gene, has not been relevantly studied at the molecular level. Japanese researchers have screened hybrids and breeding clones carrying the *S. demissum* cytoplasm and selected genotypes that function in crosses as pollinators, which may be associated with effects of nuclear-cytoplasmic interactions (Sanetomo et al., 2011). At the same time, R. Ortiz et al. (1993, 2009) failed to detect the *Rt* or *Ms* genes among the samples with D-type cytoplasm. Recently, using the methods of comparative genomics, homologs of *RFL-PPR* genes were

identified in potato and their structural polymorphism was studied (Anisimova et al., 2019).

The data on structural rearrangements analysis of the mtDNA loci associated with the sterilizing cytoplasm of the wild Mexican species *S. stoloniferum* (Lössl et al., 1999) and the *S. demissum* (Sanetomo, Hosaka, 2011, 2013) are quite sporadic. At present, the potato cytoplasm types are identified with marker-assisted selection techniques, using organelle DNA markers (mitochondrial (mtDNA) and chloroplast (cpDNA)).

Plastid DNA markers. The first data concerning cpDNA polymorphism in potato species were obtained using restriction and RFLP analyses. As a result, the T-type of cpDNA was identified, with the 241 bp specific deletion in the *ndhC/trnV* locus, and 4 more types, differing in the restriction sites BamHI, HindIII and PvuII (Hosaka, 1986, 2003). The initial classification was later revised, so five main cpDNA types are now recognized in potato: A, C, S, T and W. Their identification requires employment of the entire package of SSR, STS and CAPS markers from the set of K. Hosaka and R. Sanetomo (2012) (Fig. 1).

Mitochondrial DNA markers. The mtDNA polymorphism was first reported for potato species using the data of RFLP analysis, which resulted in identifying there major (α, β, γ) and two minor (δ, ε) types of mtDNA (Lössl et al., 1999). Later, PCR primers were developed, with specificity to the regions of mt-genes *atp6*, *cob* and *rps10*, which detect α, β and γ types of mtDNA (Lössl et al., 2000). In time, it became clear that only one pair of primers, ALM_4/ALM_5 (specific to *rps10* mtDNA locus) was enough to identify major mtDNA types from the marker' set developed by A. Lössl et al. (2000) (Song, Schwarzfischer, 2008; Hosaka, Sanetomo, 2012; Antonova et al., 2018); these primers are also included in the set of Hosaka, Sanetomo (2012). Afterwards, markers amplifying different regions (Regions 1–3) of the chimeric fragment *Band1*, which incorporates a region of the *rps19* gene of mtDNA were developed (see Fig. 1). Chimeric fragment *Band1* is typical for the hybrids and cultivars having *S. demissum* in their maternal ancestry (Sanetomo, Hosaka, 2011, 2013).

Markers for cytoplasm types. The first set of PCR markers for potato cytoplasm identification, developed by A. Lössl et al. (2000), made it possible to identify three major combinations of cpDNA and mtDNA markers in improved cultivars: T/β for T-type cytoplasm, W/α for the cytoplasm of various wild species, including *S. demissum*, and W/γ for the cytoplasm introgressed from *S. stoloniferum*. Cultivars with cytoplasm of the W/γ-type were shown to display tetrad sterility in their pollen (Lössl et al., 2000; Song, Schwarzfischer, 2008).

Another set, offered by the Japanese researchers K. Hosaka and R. Sanetomo (2012), contains a larger number of markers for cpDNA, includes also a pair of ALM_4/ALM_5 primers from the set reported by A. Lössl et al. (2000) and, additionally, a D marker (Region 1) for identification of the D-type cytoplasm of *S. demissum*. With the help of this set, the modern classification of potato cytoplasm types has been developed; it includes 8 main types: A, M, P, W (W/α, W/β, W/γ), T (T/β) and D (see Fig. 1).

It should be kept in mind, however, that the cytoplasm types identified with the marker sets described by Lössl et al. (2000) and Hosaka, Sanetomo (2012) are not identical. A set of markers from the Japanese researchers' diagnoses 'the true W-type' (wild). When the set developed by Lössl et al. (2000) is used, the W-type, identified by the absence of the 241 bp deletion in the *ndhC/trnV* locus, combines the A, M, P and W types of cytoplasm (see Fig. 1) which can be found both in cultivated and in wild potato species. Thus, the W-type of cytoplasm is interpreted in publications twofold: as 'the true W-type' (Hosaka, Sanetomo, 2012; Sanetomo, Gebhardt, 2015; Gavrilenko et al., 2018) and as 'a contraposition to the T-type' (Lössl et al., 2000; Song, Schwarzfischer, 2008), which has been responsible for discrepancies in the interpretations of molecular screening results. Therefore, after 2012, the primers ALM_4/ALM_5 from the set described by Lössl et al. (2000) have been used to detect the mtDNA types, while the types of cytoplasm are commonly identified using the molecular marker set of Hosaka, Sanetomo (2012) (see Fig. 1).

None of the 8 cytoplasm types is species-specific, although the T-type is found in up to 90 % of Chilean indigenous varieties (Hosaka, 2003; Spooner et al., 2007; Gavrilenko et al., 2013) and in a majority of old European cultivars (Provan et al., 1999; Gavrilenko et al., 2007; Sanetomo, Gebhardt, 2015). In view of this, the T-type is often referred to as the 'cultivated' or Chilean. The A- and P-types of cytoplasm are characteristic of male-fertile diploid and tetraploid indigenous Andean cultivars, while the W/α and W/β types of many wild potato species (Hosaka, Sanetomo, 2012; Mihovilovich et al., 2015). The D-type of cytoplasm is typical for *S. demissum* and the cultivars developed with its involvement (as a maternal progenitor), and has been found among the accessions of wild allohexaploid and allotetraploid Mexican potato species (Hosaka, Sanetomo, 2012, 2014). W/γ cytoplasm type (and γ-type of mtDNA) has been detected in all hybrid clones and cultivars with the cytoplasm of *S. stoloniferum*, manifesting tetrad sterility (Song, Schwarzfischer, 2008; Antonova et al., 2018). At the same time, Hosaka and Sanetomo (2012) reported detection of W/γ-type cytoplasm in potato accessions of *S. chacoense*, *S. pampasense*, *S. pinnatisectum* and *S. vernei*.

Large subsets of foreign varieties, analyzed with the marker set of Hosaka, Sanetomo (2012), showed a low level of cytoplasm genome diversity: more than 90 % had three cytoplasm types, T, D and W/γ, associated with male sterility, while about 10 % possessed cytoplasm of rare types: W/β, A, M or W/α (without the D marker) (Sanetomo, Gebhardt, 2015). Varieties with P-type cytoplasm were not found in the European gene pool; however, the P-type was present in ~6 % of Japanese varieties (Hosaka, Sanetomo, 2012) (see Supplement 1)[1].

Studying agronomic traits in groups with different cytoplasm types revealed significantly higher starch content in hybrids and breeding clones with W/γ- and W/α-type cytoplasm than in those carrying the other cytoplasm types (Lössl et al., 2000). Higher starch content and later plant maturity were reported by R. Sanetomo and K. Gebhardt (2015) for the holders of W/γ-type cytoplasm, compared with those carrying the other types. The same authors also observed that the level of field resistance to late blight in potato accessions with D-type cytoplasm was higher than in those with the T-type.

The data on the distribution of different cytoplasm types in domestic varieties (bred in the USSR, Russia, or adjacent

[1] Supplementary Materials 1–10 are available in the online version of the paper: https://vavilov.elpub.ru/jour/manager/files/application/SupplGavrilenkoEngl.pdf

<table>
<tr><td colspan="4" rowspan="11">Identification of cytoplasm types in potato using marker set of Hosaka, Sanetomo (2012)</td><td colspan="5">Identification of cpDNA types using marker set of Hosaka, Sanetomo (2012):</td></tr>
<tr><td colspan="5">SAC-marker (locus cemA of cpDNA)</td></tr>
<tr><td colspan="2">Presence of the restriction in the BamHI site in the PCR-product of SAC primers</td><td colspan="3">Absence of the BamHI-restriction</td></tr>
<tr><td colspan="5">H1-marker (locus ndhC/trnV of cpDNA)</td></tr>
<tr><td>241 bp deletion in the PCR-product of H1 primers</td><td colspan="4">241 bp deletion is absent</td></tr>
<tr><td colspan="5">S-marker (locus rps16/trnQ of cpDNA)</td></tr>
<tr><td colspan="4">48 bp deletion is absent</td><td>48 bp deletion in the PCR-product of NTCP6 primers</td></tr>
<tr><td colspan="5">A-marker (locus rpl32/ccsA of cpDNA)</td></tr>
<tr><td colspan="3">Absence of the BamHI-restriction</td><td>Restriction of the BamHI site in the PCR-product of A primers</td><td>Absence of the BamHI-restriction</td></tr>
<tr><td colspan="2" rowspan="2">D-marker: amplification of chimeric fragment Band1 – PCR-product of primers D (Region 1)</td><td colspan="2">Identification of mtDNA types:</td><td colspan="5">Types of cpDNA:</td></tr>
<tr><td colspan="2">PCR with primers ALM_4/ALM_5 (Lössl et al., 2000), locus rps10 of mtDNA</td><td>T</td><td>W</td><td>C</td><td>A</td><td>S</td></tr>
<tr><td colspan="4"></td><td colspan="5">CYTOPLASM TYPES:</td></tr>
<tr><td>D</td><td>527 bp</td><td rowspan="2">α</td><td rowspan="2">2400 bp</td><td></td><td>D</td><td></td><td></td><td></td></tr>
<tr><td rowspan="3">Other types</td><td rowspan="3">Absence of PCR product</td><td></td><td>W/α</td><td></td><td></td><td></td></tr>
<tr><td>β</td><td>1600 bp</td><td>T</td><td>W/β</td><td>M</td><td>A</td><td>P</td></tr>
<tr><td>γ</td><td>Absence of PCR product</td><td></td><td>W/γ</td><td></td><td></td><td></td></tr>
</table>

Fig. 1. Identification of cytoplasm types using the marker set of Hosaka, Sanetomo (2012).

countries) are not numerious. The set of PCR markers developed by A. Lössl et al. (2000) was used to study 98 domestic cultivars (Gavrilenko et al., 2007). In the context of previous considerations, only 40 of them, with the T-type, match to the modern classification. The marker set described by Hosaka, Sanetomo (2012) was employed to determine cytoplasm types in 25 Russian cultivars preserved in foreign genebanks (Sanetomo, Gebhardt, 2015) and in 28 cultivars developed by Russian breeders (Gavrilenko et al., 2018) (see Supplement 1). The present research continues studying cytoplasm type genetic diversity of domestic potato varieties preserved in the VIR collection.

Materials and methods

The research material included 185 potato cultivars released in Russia and adjacent countries and preserved in the VIR collection (see the Table). For this cultivars the data on cytoplasm types were determined for the first time with the aid of the markers A, D, S, SAC and H1 from the marker set of Hosaka, Sanetomo (2012). For 158 cultivars of this set, mtDNA types were earlier identified using the pair of primers ALM_4/ALM_5, specific to the *rps10* locus of mtDNA (Gavrilenko et al., 2007; Antonova et al., 2018). Male sterility phenotyping and molecular screening with the *R1* and *R3a* markers involved additional varieties from an extended subset ($N = 277$), for which the types of cytoplasm had been identified earlier (Gavrilenko et al., 2007, 2018; Sanetomo, Gebhardt, 2015) (see Supplement 1).

DNA isolation was performed on plant leaves, using the CTAB extraction technique modified by Gavrilenko et al. (2013).

Molecular screening. Six STS/CAPS/SSR markers from the set of Hosaka, Sanetomo (2012) were used to identify cytoplasm types (see Supplement 2). Molecular screening for the presence of the *R1* and *R3a* gene markers controlling race-specific late blight resistance was conducted using the primers mentioned in Supplement 3.

The cytoplasm types of domestic potato varieties (*N* = 185) bred in Russia, USSR and adjacent countries which were identified in present research using marker set of Hosaka, Sanetomo (2012)

Cytoplasm types	*N* (%)	Variety name
T (T/β)	*N* = 74 (40.0 %)	Alisa, Ametist, Antonina, Avrora, Belorusskiy ranniy, Bezhitskiy, Brat-2, Bryanskiy delikates, Bryanskiy nadezhny, Bryanskiy priusadebny, Bryanskiy ranniy, Chayka, Druzhny, Falenskiy, Filatovskiy, Fioletovy, Gart, Gornouralskiy, Granat, Gulliver, Iskra, Kalinka, Katyusha (bred at the Polar station of VIR), Kemerovskiy, Khibinskiy ranniy, Kivi, Kolpashevskiy, Korenevskiy, Krasavchik, Krasnaya gorka, Krasnaya zarya, Krasnoufimskiy, Kristall, Kustarevskiy, Lakomka, Laymdota, Lekar, Lider, Lina, Lyuks, Musinskiy, Nadezhda, Nart 1, Narymka, Nauka, Ognivo, Pamyati Rogacheva, Pribrezhny, Prigozhiy 2, Primorskiy (=Pri-12), Rossiyanka, Rusalka, Ryabinushka, Safo, Sentyabr, Severyanin, Shaman, Shurminskiy 2, Sineva, Smena, Solnechny, Svetlyachok, Varsna, Vasilek, Virazh, Volzhanin, Volzhskiy, Vyatka, Yavar, Yubileyny Osetii, Yupiter, Zagadka, Zauralskiy, Zolskiy
D (W/α)	*N* = 94 (50.8 %)	Alena, Alpinist, Amur, Antoshka, Arkhideya, Babushka, Barin, Baron, Bashkirskiy, Belosnezhka, Belukha, Bolshevik, Bolvinskiy, Borodyanskiy rozovy, Bryanskiy yubileyny, Buket, Bylina Sibiri, Chaya, Delfin, Dina, Divo, Dontsovskiy, Effekt, Fermer, Garant, Gornyak, Goryanka, Gubernator, Irbitskiy, Kamenskiy, Kemerovchanin, Kormilets, Kortni, Krasavitsa, Krasnaya roza, Kuznechanka, Ladozhskiy, Lasunak, Lazar, Lazurit, Lyubava, Malinovka, Manifest, Mars, Matushka, Maugli, Nalchikskiy, Nesterovskiy, Nikulinskiy, Parus, Pransa, Prestizh, Prizer, Prolisok, Ramzay, Rapsodiya, Rassvet, Reggi, Rezerv, Romashka, Rosinka, Rumyanka, Rusich, Sambo, Saprykinskiy, Sarovskiy, Sintez, Skarb, Skoroplodny, Solnyshko, Start, Svenskiy, Tanay, Tango, Teshcha, Tomich, Tuleevskiy, Udacha, Ukrainskiy rozovy, Uspekh, Utenok, Veselovskiy 2-4, Veteran, Viza, Vympel, Vytok, Yugana, Yuna, Zhavoronok, Zhigulevskiy, Zhivitsa, Zlatka, Zov, Zvezdochka
W/γ	*N* = 16 (8.7 %)	Bryanskiy krasny, Barmaley, Fokinskiy, Ilinskiy, Kolobok, Korona, Meteor, Moskvoretskiy, Nakra, Olimp, Pogarskiy, Resurs, Sokolskiy, Vektar, Yubiley Zhukova, Zdabytak
A	*N* = 1 (0.5 %)	Katyusha (bred in Ukraine)

PCR was performed using a 20 μL reaction mixture, containing 10 ng of the total DNA of potato varieties, a 1× reaction buffer (Dialat Ltd, Moscow), 2.5 mM $MgCl_2$, 0.4 mM of each dNTP, 0.2 μM of the forward and reverse primer, and 1 unit of Taq polymerase (Dialat Ltd, Moscow).

Amplification. The conditions of PCR complied with the recommendations of the primer developers (Supplements 2 and 3). In a number of cases, we optimized the programs using the TOUCHDOWN function. All reactions that involved the use of SCAR markers had no less than three replications.

Restriction. When CAPS markers were used, the treatment of PCR products with the BamHI restriction enzyme was performed in a 30 μL of reaction mixture according to the protocols of enzyme producers (SibEnzyme, http://russia.sibenzyme.com/).

Electrophoretic separation of fragments. PCR products were separated using horizontal 2 % agarose gel electrophoresis in a TBE buffer, followed by staining with ethidium bromide and UV visualization.

Sequencing. We developed primers for amplification of mtDNA loci (Supplement 4) on the basis of the complete sequence of the cultivated potato mt genome (GenBank number of the sequence: JF772172.1), using the Primer3Plus software. Amplified fragments were sequenced by the Sanger method on an ABI 3500x analyzer. High-throughput sequencing (NGS) was made according to the Illumina Inc. technology on an Illumina MiSeq (USA), using MiSeq® ReagentKit v3 (600 cycle) with twofold read coverage (2*300 nt).

Evaluation of male fertility. Pollen fertility (PF) was assessed using acetocarmine staining; no less than 300 pollen grains of each genotype were scanned in two replications. Preparations of stained pollen grains were analyzed under a light microscope Axio Scope.A1 (Zeiss, Germany), ×200 zoom. In addition, controlled crosses were made in 28 combinations, where 15 cultivars with D-type cytoplasm served as pollinators, and varieties with different cytoplasm types as female parents; immediately after the removal of yet unripened anthers, the emasculated flower buds were covered with isolators.

Statistic analysis. Data on the starch content and plant maturity score as well as foliage and tuber late blight resistance were taken from the State Register of Breeding Achievements (2010–2018) and the catalogues of potato cultivars for various years. Differences in these agronomic traits among the three groups of cultivars with the T-, D- and W/γ-types of cytoplasm were estimated by nonparametric statistic methods with the StatSoft Statistica 13.3 software tools (Khalafyan, 2010), using the Kruskal–Wallis test by ranks (one-way ANOVA on ranks) and the Mann–Whitney U test, at the significance level of 5 %. The same methods were used to assess correlation between type of cytoplasm and the presence/absence of molecular markers for the race-specific late blight resistance genes (*R1* and *R3a*).

Results

Identification of cytoplasm types in potato varieties

Types of cytoplasm were identified in 185 improved potato cultivars, and the resulting data are presented in the Table and Fig. 2. In this whole subset, only one cultivar, 'Katyusha' (bred

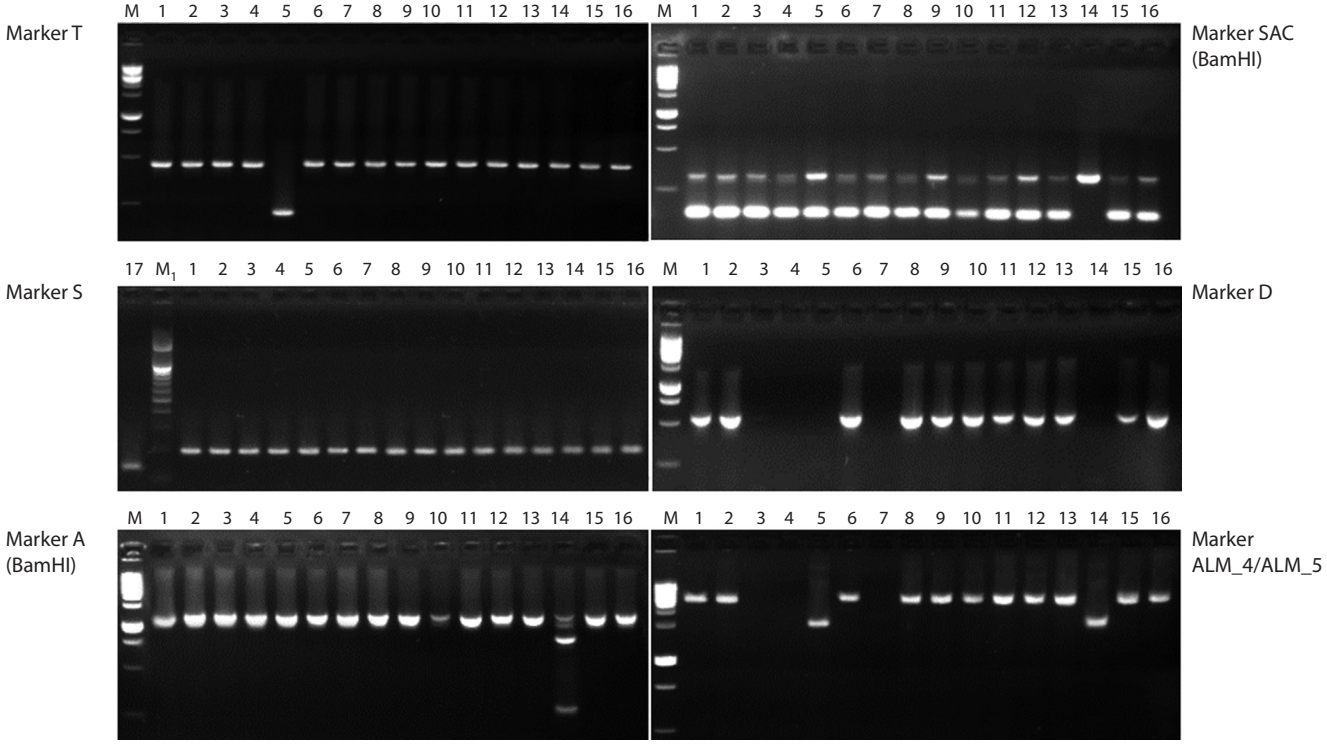

Fig. 2. Identification of cytoplasm types in potato varieties using markers set of Hosaka, Sanetomo (2012). Varieties: 1, Manifest (D); 2, Prestizh (D); 3, Kolobok (W/γ), 4, Nakra (W/γ), 5, Avrora (T); 6, Sintez (D); 7, Olimp (W/γ), 8, Tanay (D); 9, Zlatka (D); 10, Romashka (D); 11, Sambo (D); 12, Tomich (D); 13, Uspekh (D); 14, Katyusha (A); 15, Fermer (D); 16, Alpinist (D). Cytoplasm type is indicated in parentheses after the variety name. 17, control sample – *S. phureja* k-9386 with P-type cytoplasm having 48 bp deletion in the NTCP6 locus of cpDNA. M – DNA Ladder "Sibenzyme 1 kb"; M_1 – DNA Ladder "Sibenzyme 100 bp 1500".

in Ukraine), demonstrated the absence of the BamHI site in the PCR product of SAC primers (locus *cemA* of cpDNA); further restriction analysis resulted in detecting the fertile A-type cytoplasm in it (see Fig. 1, 2 and the Table). It should be kept in mind that among Russian cultivars there is one with the same name, 'Katyusha' bred at the Polar Experiment Station of VIR, which carries T-type cytoplasm. In the remaining 184 cultivars, the BamHI restriction site was detected in the PCR product of SAC primers (see Fig. 1), therefore they may be counted among the varieties with T-, D- and W/γ- types of cytoplasm, which are associated with the male sterility traits according to Hosaka and Sanetomo (2012). The Table shows the results of their subsequent differentiations. Seventy-four cultivars had a 241 bp specific deletion in the *ndhC/trnV* intergenic spacer of cpDNA (T-type cpDNA) and the β-type mtDNA (see the Table and Fig. 2).

Two pairs of primers developed for amplification of two different regions (Region 1 and Region 2) of the chimeric *Band1* fragment were used to identify the D-type cytoplasm in PCR analysis. The screening results appeared fully identical for both primer pairs. Approximately a half of the studied subset, 94 out of 185 cultivars, had cytoplasm of the D type (see the Table), which attested to the presence of *S. demissum* in their maternal ancestry. For all 94 cultivars with D-type cytoplasm, the ALM_4/ALM_5 primer pair detected the α-type mtDNA. All cultivars with D-type cytoplasm simultaneously possessed the 'true W type' of cpDNA – they had the BamHI restriction site in the PCR product of SAC primers, but did not have the BamHI restriction site in the PCR product of A primers and 241 bp deletion in the *ndhC/trnV* intergenic spacer of cpDNA (see Fig. 1, 2).

In the remaining 16 cultivars with 'the true W-type' of cpDNA, PCR products were not identified for the *Band1* fragment, hence they did not possess D-type cytoplasm (see Fig. 1, 2 and the Table). Since the same cultivars were earlier reported to have γ-type mtDNA due to the absence of amplification with the ALM_4/ALM_5 primers (Antonova et al., 2018), they may be recognized as having the W/γ-type of cytoplasm.

Thus, there were no cultivars with fertile cytoplasm types in the studied subset, except for the single (0.5 %) cv. 'Katyusha' (A-type) released in Ukraine, which confirmed the result earlier obtained by Hosaka, Sanetomo (2012) and Sanetomo, Gebhardt (2015), who had analyzed this cultivar. The remaining 99.5 % of the sampled cultivars possess three cytoplasm types: T, D and W/γ (see the Table), which are associated, according to Hosaka, Sanetomo (2012), with male sterility. With this in view, we carried out phenotyping of male sterility in the plants that took part in molecular screening.

Studying male sterility in plants

Pollen fertility (PF) was assessed in 121 cultivars from the extended subset (see Supplement 1): 45 with T-type, 60 with D-type, and 16 cultivars with W/γ-type cytoplasm. Of those, 26.7 % of cultivars with T-type cytoplasm developed colorless

and morphologically abnormal pollen grains; among them were 'Bryanskiy ranniy', 'Golubizna', 'Zolskiy', 'Lorkh', 'Pribrezhnyy', 'Rusalka' and 'Severyanin'. In 28.9 % of cultivars, the PF exceeded 60 %; in the rest it varied from 15 to 60 %. It should be mentioned that a considerable variability of pollen fertility was recorded in different years.

Out of the 16 cultivars with the sterilizing W/γ-type cytoplasm, tetrad sterility was observed in 14 (87.5 %). This trait was variable in different cultivars: for example, in cvs. 'Moskvoretskiy', 'Nakra', 'Olimp', 'Sokolskiy' and 'Yubiley Zhukova' up to 100 % of pollen grains remained clustered in 'permanent tetrads' at stage of opening flowers (Fig. 3, *a*).

Cvs. 'Bryanskiy krasnyy', 'Pogarskiy' and 'Korona' were observed to have about 50 % of 'permanent tetrads' against a background of sterile monads. Cvs. 'Kolobok' and 'Resurs' showed up to 20 % of 'permanent tetrads' with a background of sterile monads. Cvs. 'Barmaley' and 'Fokinskiy' with unknown pedigrees were two exceptions, as they developed only monads, 70 % of which were stainable with acetocarmine and had normal morphology (see Fig. 3, *b*).

In the cultivars with D-type cytoplasm, the pollen fertility varied from 20 to 80 %. Since Hosaka, Sanetomo (2012) reported functional pollen sterility for the holders of this cytoplasm type, we made 28 combinations of controlled crosses, where cultivars with D-type cytoplasm served as pollinators. Practically all crosses (except the combinations with cv. 'Skazka') resulted in berries with seeds, which is an evidence of functional pollen fertility in 14 out of 15 pollinators having the D-type cytoplasm of *S. demissum* (see Supplement 5).

Molecular screening with markers for the *R1* and *R3a* genes, introgressed into the breeding gene pool from *S. demissum*. Molecular screening of extended subset was performed to verify the assumption that, in the case of functional male sterility of D-type cytoplasm holders which took part in cultivar' pedigrees, markers of the *R1* and *R3a* genes can be detected only in the varieties with the cytoplasm of *S. demissum*; and when such forms possess male fertility – in varieties with various cytoplasm types. The results of molecular screening showed that markers of the *R1* and/or *R3a* genes had been identified in cultivars carrying various cytoplasm types in almost equal ratios (see Supplement 6).

Thus, the tree groups of cultivars with the T, D and W/γ cytoplasm types were shown to contain both male-sterile and male-fertile genotypes, which may be caused by the specificity of nuclear-cytoplasmic interactions and the polymorphism of mtDNA loci associated with the CMS trait.

Studying polymorphism of mtDNA loci. Based on the results of male sterility studies, 15 accessions were selected to form three groups with the T, D and W/γ cytoplasm types, so that each of them contained both male-sterile and male-fertile genotypes. They underwent direct sequencing to analyze 8 loci of the mt genome (fragments of the genes *atp6*, *atp9*, *cox2*, *CcmFc*, *nad2*, *nad7*, *rps3*, and the *nad1*/*atp6* intergenic spacer), whose polymorphism is often associated with CMS in various plant species (Ducos et al., 2001; Kim D.H., Kim B.D., 2006; Das et al., 2010; Liu et al., 2011). To amplify these 8 loci, we developed 8 pairs of primers, whose sequences are presented in Supplement 4. All the studied 15 genotypes, regardless of their cytoplasm type or male sterility/fertility traits, demonstrated identical sequences of the *nad2*, *nad7*, *cox2*, *atp6*, *CcmFc* loci, which matched with the corresponding regions on the complete mt genome sequence (JF772172.1) of cultivated potato. Nucleotide InDels/substitutions were identified in the *rps3*, *atp9* and *CcmFc* loci; however, they were not associated with the male sterility feature. For the *nad1*/*atp6* and *nad2* (the second intron) loci, single-nucleotide substitutions were identified, which differentiated 7 holders of the W/γ cytoplasm into two groups: five genotypes with tetrad male sterility, and two with fertile pollen. In the second intron of the *nad2* gene (position 216310 in the complementary strand of the sequence JF772172.1), an A→C transversion was found: SNP variant 'A' was present in male sterile genotypes, and 'C' – in male fertile ones (Fig. 4, *a*). As for the *nad1*/*atp6* intergenic spacer, SNP variant 'T' in position 48923 was identified for sterile genotypes, and 'G' for male fertile ones (see Fig. 4, *b*). In the accessions with cytoplasm of the T- and D-types, regardless of their male sterility/fertility traits, the sequences of these two loci were similar.

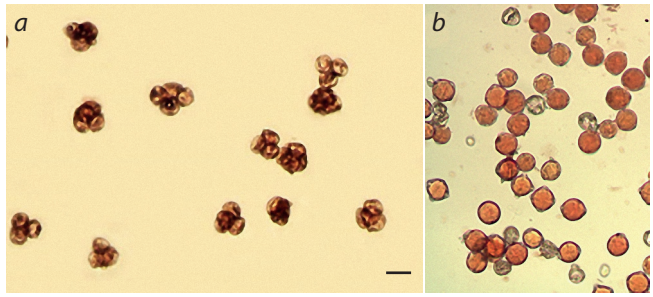

Fig. 3. Tetrad sterility in cv. 'Moskvoretskiy' (*a*) and pollen grains of cv. 'Fokinskiy' (*b*), both cvs. with W/γ-type cytoplasm.

These two SNP variants were identified while analyzing a limited number (7) of genotypes with W/γ-type cytoplasm. To confirm their association with tetrad sterility, we made an NGS analysis of the *nad2* and *nad1/atp6* loci on a larger subset of accessions of various origin, all having W/γ-type cytoplasm. The analysis covered 12 additional cultivars with W/γ-type cytoplasm, developed both in Russia and Europe having *S. stoloniferum* in their maternal ancestry, and 10 breeding clones with W/γ-type cytoplasm, developed on the basis of the Mexican species *S. guerreroense* (Zoteeva et al., 2017). These accessions were used to make three bulk samples, each uniting genotypes with W/γ-type cytoplasm of similar origin and with similar appearance of the male sterility or fertility trait (see Supplement 7). The results of the NGS analysis confirmed that SNP variants 'A' in the *nad2* locus (position 216310) and 'T' in the *nad1/atp6* spacer (position 48923) were associated with tetrad male sterility in all studied genotypes. In addition, minor mitotypes were identified in the polymorphic site for each bulk sample, which may be explained by either heterogeneity of a bulk sample or the heteroplasmic state of the analyzed genotypes – when substoichiometric quantities of different mtDNA variants are present in plant cells.

To find the cause of the observed heterogeneity, we developed a CAPS marker, Int2NAD2/Sse9I (F: GGGCTTCTT-GCTACGCTACA; R: TTAAGCCTGGGCGAAGATGG,

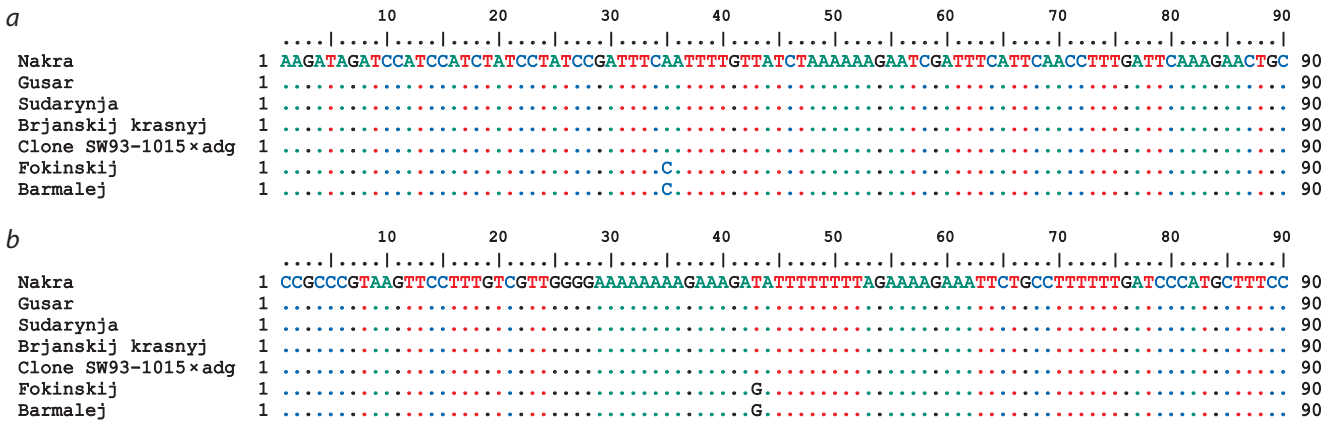

Fig. 4. Alignment of fragments (*a*) of the second intron of the *nad2* gene and (*b*) of the *nad1/atp6* intergenic spacer in 7 accessions having W/γ-type cytoplasm. From top to bottom – 5 genotypes with tetrad sterility: varieties Nakra, Gusar, Sudarynya, Bryanskiy krasny and breeding clone SW93-1015 × adg (Zoteyeva et al., 2017) and the last two: Fokinskiy, Barmaley – with fertile pollen grains forming only monads.

with subsequent restriction Sse9I), for the polymorphic SNP site A/C in the intron of the *nad2* gene. This marker makes it possible to distinguish the site AATT (genotypes with tetrad sterility) from the site CATT (genotypes with fertile pollen and W/γ cytoplasm). Each of the 28 selected accessions was analyzed individually with this marker, and all bulk samples appeared uniform – the accessions therein had a restriction site corresponding to the main nucleotide for the given group (see Supplement 7). Thus, the polymorphism of SNP variants in the second intron of the *nad2* gene may be explained by the presence of various mitotypes in substoichiometric amounts.

Differences between cultivars with different cytoplasm types in their agronomic traits. Pairwise comparison among the cultivar groups with different cytoplasm types, using the Kruskal–Wallis test by ranks and the Mann–Whitney U test, showed the absence of significant differences in starch content, field resistance of foliage to late blight, and presence of the markers of the *R1* and *R3a* genes, as well as the presence of significant differences in tuber late blight resistance between three cultivar groups ($p = 0.044$), with higher resistance in the varieties with W/γ-type cytoplasm. When the Mann–Whitney test was applied, the differences in earliness between the cultivars with D-type (mean = 6.4) and with W/γ-type (mean = 5.5) cytoplasm were significant ($p = 0.037$), but with the Kruskal–Wallis criterion the differences were not essential.

Discussion

According to the published data, more than 90 % of foreign potato cultivars (released in East Europe, North America and Japan) manifest three types of cytoplasm: T, D and W/γ, associated with variously expressed male sterility (see Supplement 1). The occurrence rate for cultivars with the A, M, W/β and W/α (without the D marker) cytoplasm types in total does not exceed 10 % (Hosaka, Sanetomo, 2012; Sanetomo, Gebhardt, 2015). Similar results were obtained in this research for potato cultivars developed in Russia and adjacent countries: 99.5 % of 185 cultivars were found to have three cytoplasm types: T (40.0 %), D (50.8 %), and W/γ (8.7 %). Except for cv. 'Katyusha" (A type, 0.5 %) released in Ukraine, no cultivars were observed to have fertile cytoplasm types. Comparing the obtained results with our previous results (Gavrilenko et al., 2007, 2018) and published data (Sanetomo, Gebhardt, 2015) suggested expansion of the sampled subset of domestic cultivars to 277, but their variability in cytoplasm types remained similar as it had been: T (48.4 %), D (44.4 %), W/γ (6.8 %), and A (0.4 %). Having screened the extended subset, we found neither any cultivars with A-type or P-type cytoplasm typical for male fertile tetraploid and diploid Andean landraces, nor any varieties with M, W/β or W/α (without the D marker) cytoplasm types characteristic of the related South American wild potato species: *S. acaule*, *S. spegazzinii* and *S. sparsipilum* (=*S. brevicaule*), frequently used in breeding programs. At the same time, many varieties from the studied extended subset had earlier proved to possess molecular markers of nuclear *R* genes conferring resistance to harmful organisms: *H1*, *Gpa2*, *Gro1-4*, *Rx1*, *Rx2*, and Ry_{adg}, introgressed into the breeding gene pool from the above-listed species (Gavrilenko et al., 2009, 2018; Biryukova et al., 2015; Antonova et al., 2016; Klimenko et al., 2017, 2019). It may be explained by male fertility of their hybrids, which participated in crosses, like the parental accessions of cultivated and wild South American species, as pollinators (Ochoa, 2004).

Wide distribution of the T-type cytoplasm, especially among old improved cultivars, may be due, on the one hand, to its positive effect on the yield (Plaisted, 1972) and, on the other, to the use of carriers of this cytoplasm type in breeding as female parents, because of the male sterility feature expressed in them (Grun et al., 1977). By the end of the 20th century, when interspecific hybridization had become the main tool of broadening the genetic diversity of breeding materials, the frequency of cultivars with T-type cytoplasm was abruptly decreased, whereas the occurrence rate of cultivars with the cytoplasm of wild Mexican species (D and W/γ) went up (see Supplement 8). It should be also mentioned that in the domestic gene pool the occurrence of cultivars with the D-type cytoplasm from *S. demissum* is two to three times higher than in West European of Japanese cultivars (see Supplement 1).

The spreading of the cytoplasm type inherent in wild Mexican species over the breeding gene pool is explained on the one hand by interspecific incompatibility (as a rule,

S. demissum and *S. stoloniferum* participate in interspecific crosses as maternal parents) and on the other hand – male sterility of the obtained hybrids (Dionne, 1961; Irikura, 1968; Song, Schwarzfischer, 2008; Hosaka, Sanetomo, 2012; Gavrilenko, Yermishin, 2017). Because of that, the introgression of nuclear genes responsible for race-specific late blight resistance (*R1–R3*, *R4*, *R8*, *R10*) from *S. demissum* and the Ry_{sto}/Ryf_{sto} genes of resistance to PVY from *S. stoloniferum* was simultaneously and unintentionally accompanied by a transfer of cytoplasmic determinants from wild Mexican species into the breeding gene pool.

It is reported in publications that the W/γ-, D- and T-types of cytoplasm have a specific effect on the male fertility/sterility traits, respectively inducing tetrad sterility, functional sterility of pollen, and abnormalities in the development of reproductive organs (see Hosaka, Sanetomo, 2012). According to the results of phenotyping performed in the framework of this research, each of the three groups of domestic cultivars with the T, D and W/γ cytoplasm types contained not only male-sterile, but also highly fertile genotypes. Male fertility in the T-type cytoplasm holders is explained in publications by the presence of a dominant allele of the *Rt* gene (a male fertility restorer gene) and, for a number of interspecies combinations, by the homozygosity (*ms*/*ms*) for the recessive allele of the *Ms* gene (male sterility gene) (Iwanaga et al., 1991; Ortiz et al., 1993; Mihovilovich et al., 2015). Among the varieties with T-type cytoplasm there are many efficient pollinators that have served as male parents in the development of many domestic cultivars, such as 'Avrora', 'Dina', 'Zarevo', 'Kameraz', 'Priyekulskiy ranniy', 'Smena' and 'Shurminskiy 2' (see the Table and Supplement 1).

The present results of successful crosses with pollinator varieties carrying D-type cytoplasm did not confirm the information about the functional pollen sterility in the *S. demissum* cytoplasm holders. An indirect evidence of male fertility in cultivars with D-type cytoplasm is the results of molecular screening with markers of the *R1* and *R3a* genes of race-specific late blight resistance, introgressed into the breeding gene pool from *S. demissum*. Markers of these genes were detected in cultivars with D-type, T-type and W/γ-type cytoplasm (see Supplement 9), which may be due to the involvement into the breeding process of donors of the *R1* and *R3a* genes (D-type cytoplasm holders) as pollinators.

Contrariwise, markers for the Ry_{sto} and Ryf_{sto} genes conferring extreme resistance to PVY, occur only in the cultivars having the W/γ cytoplasm transferred from *S. stoloniferum* (Flis et al., 2005; Song, Schwarzfischer, 2008; Antonova et al., 2018; Gavrilenko et al., 2018) (see Supplement 10), that confirms the sterilizing effect of this cytoplasm type. The analysis of the obtained results and published data (Song, Schwarzfischer, 2008) makes it possible to conclude that all genotypes with tetrad pollen sterility have W/γ cytoplasm type. On the other hand, the holders of this cytoplasm type may be sporadically interspersed with a few genotypes with fertile pollen, carrying cytoplasm of other wild species, for example, *S. vernei*, as shown by K. Hosaka and R. Sanetomo (2012). In our study, two (12.5 %) of the 16 cultivars with W/γ-type cytoplasm developed fertile pollen; it may well be that their maternal pedigree also included accessions of the South American species *S. vernei* or *S. chacoense*, whose W/γ cytoplasm type was ascertained by Hosaka, Sanetomo (2012).

The present research succeeded in differentiating sterile and fertile accessions both with W/γ-type cytoplasm according to single-nucleotide substitutions in two mtDNA loci (*nad1/atp6* and *nad2*). The CAPS marker Int2NAD2/Sse9I, developed by us, can be used in future for additional differentiation of the W/γ-type cytoplasm and for selection of male-sterile/fertile genotypes. The NGS analysis technique was for the first time applied to study the heteroplasmic state of mtDNA in potato genotypes with the sterilizing W/γ cytoplasm type. It has been shown that a heteroplasmic state is equally characteristic for the genotypes with tetrad sterility (where a minor mtDNA variant typical for genotypes with fertile pollen has been identified) and for male fertile cultivars (where a minor mitotype typical for cultivars with tetrad sterility has been found). The significance of stoichiometric quantities in the CMS phenotype expression has been demonstrated for various crop species: *Pennisetum glaucum* (Feng et al., 2009), *Beta vulgaris* (Bragin et al., 2011), *Brassica napus* (Chen et al., 2011), *Oryza sativa* (Bentolila, Stefanov, 2012), etc.

Conclusion

It may be summarized in the conclusion that Russian potato cultivars and those of the adjacent countries, along with the breeding gene pools in foreign countries, are represented mostly by plants with the T, D and W/γ cytoplasm types, while genotypes with fertile types of cytoplasm are practically absent. It seems obvious that breeders have not previously paid much attention to this trait, since the cultivated potato follows a vegetative reproduction pattern. In present research pairwise comparison of the cultivar groups with different cytoplasm types failed to find statistically significant differences in a number of economically useful traits, except for higher tuber late blight resistance in cultivars with W/γ-type cytoplasm.

The analysis of published data and results of the presented research makes it possible to conclude that the presently known cytoplasm markers for potato are not informative enough to be used in the selection of male sterile and male fertile genotypes. It is safe to assume that after the identification of the *Rt* and *Ms* genes in potato the efficiency of this research trend will be enhanced. Besides, further development of molecular markers is required to accomplish more detailed typing of mitochondrial DNA loci involved in male sterility control. Studying the CMS-*Rf* genetic systems and working out molecular marker techniques for sterilizing types of cytoplasm and fertility restorer genes are promising for the development of a new trend – heterosis-based potato hybrid breeding. Developing molecular markers for the CMS-*Rf* genetic systems are also promising for conventional breeding in the context of facilitating the selection of parental lines for crosses and, *inter alia*, pyramiding genes conferring resistance and other agronomical traits in one genotype.

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Культивирование зародышей *in vitro* гибридов раносозревающих сортов черешни (*Prunus avium* L.)

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Высокоадаптивных технологичных раннеспелых сортов черешни (*Prunus avium* L.) в России недостаточно, поэтому перед селекционерами стоит задача скорейшего их получения. Однако решение данной задачи осложнено очень низкой полевой всхожестью гибридных семян. Цель наших исследований состояла в подборе оптимальных сред и способов уменьшения инфицированности в условиях *in vitro* для получения наибольшего количества полноценных сеянцев сортов черешни от внутривидовой гибридизации и в ускорении селекционного процесса. Работа по культивированию *in vitro* зародышей внутривидовых гибридов от четырех комбинаций скрещивания перспективных сортов и доноров черешни: Валерий Чкалов × Свитхарт, Краснодарская ранняя × Крупноплодная, Ярославна × Свитхарт, Эйфория × Свитхарт по программе «раннее созревание и крупноплодность» была начата с предселекции, выбора материнских и отцовских форм черешни. В ходе исследований определены сроки «забора» плодов для высадки в культуру *in vitro*, соответствующие концу мая–началу июня. Оптимизирован процесс стерилизации от сапрофитной микрофлоры плодов, косточек и семян перед проведением процесса культивирования. Испытаны и оценены на пригодность для культивирования зародышей черешни три модифицированные среды с макро- и микроэлементами на основе сред Мурасиге и Скуга, Prunus и Смирнова. По результатам опытов наиболее оптимальной для прорастания и обеспечивающей превосходное питание зародышей признана искусственная питательная среда M_4 на основе среды Мурасиге и Скуга с добавлением в состав аскорбиновой кислоты и сахарозы. При применении разработанной схемы массовое прорастание из зародышей сеянцев черешни гибридных комбинаций скрещивания отмечено в наших опытах уже через полтора месяца после ввода их в культуру. Эффективность выращивания гибридных сеянцев черешни раннего срока созревания с применением культуры зародышей бесспорна, поскольку позволяет получать гибриды в первый год после проведения гибридизации.
Ключевые слова: черешня; селекция; внутривидовое скрещивание; зародыши; питательная среда; витамины; фитогормоны; *in vitro* культивирование.

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In vitro cultivation of the embryos of hybrid forms of early-ripening sweet cherry (*Prunus avium* L.) varieties

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Russia does not possess as many highly adaptable, technological, early-ripening varieties of sweet cherry (*Prunus avium* L.) as would suffice to feel comfortable. Therefore, breeders are faced with the task of obtaining hybrid seeds as soon as possible. This task is not easy because of low field germination rates of hybrid seeds. The goal of our research was to select the best environments and ways to reduce infection while obtaining the largest number of full-fledged sweet cherry seedlings from intraspecific hybridization *in vitro* and to accelerate the selection process. Work on the *in vitro* cultivation of germs of intraspecific hybrids from four combinations of crosses of the promising varieties and sweet cherry donors 'Valerij Chkalov' × 'Svithart', 'Krasnodarskaya Rannaya' × 'Krupnoplodnaya', 'Jaroslavna' × 'Sveethart', 'Eiforiya' × 'Sveethart' under the "early-ripening and large-fruited" program started with prebreeding and the selection of maternal and paternal forms of sweet cherries. In the course of research, the terms of taking fruits for planting *in vitro* culture have been determined, which correspond to the end of May and the beginning of June. The process of sterilization from saprophytic microflora of fruits, stone and seeds before cultivation has been optimized. Three modified media with macro- and microelements based on Murashige and Skoog, Prunus and Smirnova were tested and assessed for suitability for cultivation of cherry embryos. According to the results of the experiments, an agarized M_4 artificial nutrient medium based on the Murashige and Skoog formulations with

the addition of ascorbic acid and sucrose was proposed as the most optimal for germination and providing excellent nutrition. Mass germination from bud seedlings of these hybrid combinations of sweet cherry crosses, when applying the developed scheme, was noted in our experiments as early as in the first decade of July, i.e. about a month and a half after putting them into culture. The efficiency of growing hybrid seedlings of early-ripening sweet cherries with the use of embryo culture is indisputable, since it makes it possible to produce hybrids in the same year when the crosses are made.
Key words: sweet cherry; selection; intraspecific hibridization; embrio; nutrient medium; vitamins; phytohormones; *in vitro* cultivation.

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Введение

Плоды черешни (*Prunus avium* L.) открывают сезон свежих фруктов и ягод наравне с жимолостью и земляникой. Черешня – одна из ценных плодовых косточковых культур, которая пользуется большим спросом у населения. В настоящее время ее производят в Европе, Малой и Средней Азии, Индии, Японии, на Американском континенте, в Австралии. В России она культивируется в основном на юге и в Крыму (Витковский, 2003; Юшев, Еремина, 2007). Несмотря на широкое распространение культуры в мире, высокую ценность ее плодов и постоянно растущий на них спрос, потребности населения в России не удовлетворяются. Недостаточный объем производства товарной черешни обусловлен низкой продуктивностью существующих насаждений, которая зависит в первую очередь от оптимальных для определенного региона возделывания сорто-подвойных комбинаций.

Районированный сортимент для южных регионов РФ в настоящее время включает 24 сорта черешни (Юшев, Еремина, 2007). Однако он не соответствует запросам к культуре со стороны товаропроизводителей и потребителей. Согласно современным требованиям интенсивного садоводства, промышленный сорт черешни должен быть приспособлен к конкретным экологическим условиям, иметь компактную крону и умеренный рост, давать высокие регулярные урожаи, темноокрашенные плоды, желательно универсального назначения, с плотной мякотью, хрящеватой консистенцией и гармоничным вкусом (Алехина, Еремина, 2012). Наибольшую ценность для потребителя представляют сорта раннего срока созревания, но плоды у них более мелкие, чем у позднеспелых сортов. Своевременная всесторонняя оценка интродуцированных сортов и гибридов черешни с целью прямого вовлечения лучших из них в производство и выделение источников хозяйственно ценных признаков для создания новых сортов и гибридов являются актуальной задачей (Алехина, 2009, 2014, 2018; Михеев, Ревякина, 2012; Гуляева, 2015).

Работа по селекции сортов черешни на такие признаки, как раннее созревание и крупноплодность, начата на Крымской ОСС (филиал ВИР) еще в прошлом веке (Еремин, 1985) и продолжается по настоящее время. В ходе этой работы отмечено, что от скрещивания раносозревающих сортов черешни при посеве гибридных косточек в селекционный питомник получается мизерное количество сеянцев (0–1 %) из-за низкой всхожести. Анализ процесса развития зародыша от опыления до прорастания показал, что гибридные зародыши нежизнеспособны, начиная с ранних стадий их развития, или же происходит формирование недоразвитых зародышей (без корня, альбиносы и т. п.).

Метод культивирования зародышей *in vitro* уже несколько десятилетий занимает важное место при разработке различных селекционных программ по черешне в России (Здруйковская-Рихтер, 1964; Кухарчик, 1994; Ковальчук, Чуканова, 1997; Захарченко, Бунцевич, 2001; Коваленко, 2017) и за рубежом (Balla, Brozik, 1996; Stanys, 1998; Standardi, 2012; Asănică et al., 2016). Из анализа литературных данных следует, что на культуру эмбрионов влияют прежде всего генотип и искусственная питательная среда. Среды при культивировании зародышей составлялись преимущественно зарубежными авторами на основе солей питательных сред Мурасиге и Скуга (Asănică et al., 2016; Dulić et al., 2016). Использовались также варианты питательных сред, созданные на основе состава, предложенного De Fossard (1977). При этом период получения жизнеспособных зародышей составлял от двух до трех лет после ввода в культуру.

В настоящем исследовании приведены результаты разработки схем культивирования зародышей раннеспелых сортов черешни, способствующих быстрому росту сеянцев в тот же год, что и проведенная гибридизация.

Материалы и методы

Работа по выращиванию в культуре *in vitro* гибридов черешни, полученных на основе внутривидовой гибридизации, выполнялась в лаборатории биотехнологии и биохимии Крымской ОСС филиала ВИР (2017–2019 гг.) и базировалась на предыдущих разработках (Медведева, Старенко, 1998; Коваленко, Поливара, 2014, 2016). Исследования проводили по литературным и разработанным нами методикам (Здруйковская-Рихтер, 1962; Кухарчик и др., 2006; Коваленко, Поливара, 2016) с использованием искусственных питательных сред Мурасиге и Скуга (Murashige, Skoog, 1962), Prunus и Смирнова (Попов, 1979; Джигадло и др., 2005).

Материалом послужили коллекционные сортообразцы черешни Крымской ОСС филиала ВИР. Из них были выделены лучшие сорта раннего срока созревания (начало июня), пригодные для интенсивного выращивания в условиях юга России (Еремин и др., 2009, 2018). В качестве материнских форм в гибридизацию были взяты четыре сорта черешни раннего срока созревания плодов: Валерий Чкалов, Краснодарская ранняя, Эйфория (Восход), Яро-

славна, а в качестве отцовских – Крупноплодная и Свитхарт. Ниже приведено описание сортов в соответствии с каталогом паспортов доноров и источников селекционно значимых признаков вишни и черешни (Еремин и др., 2009, 2018).

Сорт черешни **Крупноплодная** (Наполеон белая × смесь пыльцы сортов Валерий Чкалов, Эльтон, Жабуле). Использование донора в селекции – создание крупноплодных высококачественных адаптивных сортов. Положительные признаки сорта – стабильная урожайность, высокая зимостойкость, частичная самоплодность. К отрицательным признакам относятся сильнорослость, склонность к растрескиванию плодов и поражение серой плодовой гнилью. С участием этого донора получены элитные формы 17-1-14, 17-3-209, 17-4-5, 17-4-99 (Северо-Кавказский федеральный научный центр садоводства, виноградарства, виноделия – СКФНЦСВВ), Метеотида, Наслаждение, Обещание, Орифлемма (НИИ орошаемого садоводства, Украина), Легенда млиевская (Млиевский НИИ садоводства, Украина).

Сорт **Краснодарская ранняя** (родословная неизвестна), создан в Северо-Кавказском зональном НИИ садоводства и виноградарства (СКЗНИИСиВ). Рекомендуется как донор признака «раннее созревание». Используется в селекции для создания адаптивных сортов. Положительные признаки сорта – зимостойкость, засухоустойчивость, устойчивость к болезням, продуктивность. Отрицательные признаки – плоды ниже среднего размера, склонность к перегрузке урожаем. С участием этого донора получены элитные формы 17-2-57, 17-2-136 (СКФНЦСВВ).

Сорт **Свитхарт** (Вен × Ньюстар) канадской селекции. Положительные признаки сорта – высокая урожайность, крупные плоды широкоокруглой формы и хороших столовых качеств. К отрицательным следует отнести слабую устойчивость к возвратным заморозкам, низкую засухоустойчивость и слабую устойчивость к грибным болезням.

Сорт **Валерий Чкалов** (сеянец от свободного опыления сорта Кавказская Розовая) выведен совместно ФНЦ им. И.В. Мичурина и Институтом орошаемого садоводства УААН. Обладает средней урожайностью, засухоустойчив. Плоды раннего срока созревания (начало июня), хороших столовых качеств. Отрицательными качествами являются средняя зимостойкость, слабая устойчивость к возвратным заморозкам и недостаточная устойчивость к грибным болезням.

Сорт **Эйфория** (клон сорта Восход) создан на Крымской ОСС ВИР. Плоды раннего срока созревания (начало июня), крупные, широкоокруглой формы, очень хороших столовых качеств. Зимостойкость, засухоустойчивость и урожайность высокие.

Сорт **Ярославна** (сеянец от свободного опыления сорта Дрогана Желтая) отобран на Артемовской ОСС (Украина). Плоды очень раннего срока созревания (конец мая), крупные, сердцевидно-округлой формы, хороших столовых качеств. Урожайность и засухоустойчивость высокие. Отрицательное качество сорта – слабая устойчивость к возвратным заморозкам.

В результате искусственного опыления получены плоды сортов черешни от гибридных комбинаций: Валерий Чкалов × Свитхарт, Краснодарская ранняя × Крупноплодная, Ярославна × Свитхарт, Эйфория × Свитхарт, которые использовались в дальнейших исследованиях.

Поверхностную стерилизацию семян от сапрофитной микрофлоры проводили последовательно, осуществляя промывку плодов с мылом, а затем их ополаскивание. Стерилизацию косточек и семян выполняли в водном растворе дезинфицирующего препарата HAZ TABS (на основе хлора) – 4.75 г на 500 мл H_2O. Зародыши высаживали в стерильных условиях в ламинарных боксах ВЛ-12, на ранее приготовленные питательные среды в стеклянные пробирки диаметром 30 мм и высотой 140 мм. Повторность опытов двукратная, по 20 пробирок в каждой повторности. Зародыши вводили в культуру на 33-й день после искусственного опыления цветков, без стадии покоя семян. Процесс культивирования проходил в специальной комнате – светозале, на стеллажах, при освещенности 1 м^2 стеллажа двумя лампами дневного света ЛД-40 с 16-часовым фотопериодом, при температуре воздуха +24 ± 1 °С.

При проведении скарификации косточки нами отмечено, что нередко они содержали «невыполненное» семя (пустые косточки), т. е. после опыления оплодотворение не произошло или зародыши не сформировались. Эмбриоспасение начинали с правильно подобранной питательной среды, компоненты которой должны соответствовать стадии развития зародыша. В ходе предыдущих наработок (Коваленко, Поливара, 2014, 2016; Коваленко, 2017) нами оптимизированы питательные агаризированные среды для культивирования зародышей черешни на основе состава среды Мурасиге и Скуга и Prunus. В результате выделены среды M_4 и Pr_1 (табл. 1). В 2017–2019 гг. при проведении испытаний была дополнительно включена питательная среда Смирнова (см. табл. 1).

Результаты

Ввод в культуру зародышей, полученных от четырех комбинаций скрещивания, на искусственные питательные среды был осуществлен в конце мая – начале июня. Выбраковку по степени инфицированности зародышей, введенных в культуру, проводили через каждые пять дней (табл. 2).

Процентный выход жизнеспособных сеянцев на трех изучаемых средах был примерно одинаков: от 40 до 75 % на Pr_1, от 40 до 80 % на M_4 и от 40 до 70 % на Смирнова, в зависимости от гибридной комбинации (см. табл. 2), что свидетельствует об идентичности растительного материала и способа его стерилизации. Наибольшая инфицированность семян наблюдалась у гибридной комбинации Ярославна × Свитхарт: 35 % на Pr_1, 40 % на M_4 и 50 % на среде Смирнова. Максимальное количество нежизнеспособных сеянцев, в том числе альбиносов, наблюдалось в комбинации Эйфория × Свитхарт: от 20 % на M_4 до 35 % на Pr_1 и питательной среде Смирнова. Очень хорошие показатели выхода жизнеспособных зародышей, судя по результатам проращивания сеянцев в культуре, получены нами в гибридной комбинации Краснодарская ранняя × Крупноплодная – 70–80 % (см. табл. 2).

В ходе наблюдений за скоростью прорастания и последующим морфогенезом зародышей в культуре отмечено существенное влияние генотипа на этот процесс.

Table 1. Optimal composition of nutrient medium for sweet cherry germ culturing

Component	Concentration, mg/L		
	M_4	Pr_1	Smirnova
KNO_3	1900	1800	80
NH_4NO_3	1650	4000	–
$MgSO_4 \cdot 7H_2O$	370	180	740
$CaCl_2 \cdot 2H_2O$	332	–	–
KH_2PO_4	270	270	–
$MnSO_4 \cdot 4H_2O$	24.1	76	–
$ZnSO_4 \cdot 7H_2O$	8.6	8.6	2.2
H_3BO_3	6.2	6.2	1.4
$CuSO_4 \cdot 5H_2O$	0.025	0.025	0.004
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	–
$CoCl_2 \cdot 6H_2O$	0.025	0.025	–
$FeSO_4 \cdot 7H_2O$	27.8	27.8	–
Na EDTA $\cdot 2H_2O$	37.3	37.3	–
Ascorbic acid	1.0	2.0	–
Thiamine-HCl	0.5	0.5	0.1
Pyridoxine-HCl	0.5	0.5	0.1
Nicotinic acid	0.5	0.5	0.5
Sucrose	20000	30000	20000
Agar	6.5	6.7	–
6-BAP	0.8	0.5	–
KJ	0.08	0.08	0.65
Na_2SO_4	–	–	200
$FeC_6H_5O_7 \cdot 3H_2O$	–	–	3.5
$MnCl_2 \cdot 4H_2O$	–	–	3.6
$Na_2MoO_4 \cdot 2H_2O$	–	–	0.025
KCl	–	–	65
$NaH_2PO_4 \cdot 2H_2O$	–	–	18.6
$Ca(NO_3)_2$	–	332	200
pH	5.6–5.8	5.7	4.8–5.2

Table 2. The results of the culturing of germs of sweet cherry intraspecific hybrids* on the modified nutrient medium

Hybrid combination	Number of embryos		
	infected	viable	unviable
Modified nutrient medium Pr_1			
Valeriy Chkalov × Sweetheart	10 (25)	26 (65)	4 (10)
Krasnodarskaya Rannyaya × Krupnoplodnaya	4 (10)	30 (75)	6 (15)
Yaroslavna × Sweetheart	14 (35)	16 (40)	10 (25)
Euphoria × Sweetheart	6 (15)	20 (50)	14 (35)
Modified nutrient medium M_4			
Valeriy Chkalov × Sweetheart	6 (15)	28 (70)	6 (15)
Krasnodarskaya Rannyaya × Krupnoplodnaya	4 (10)	32 (80)	4 (10)
Yaroslavna × Sweetheart	16 (40)	16 (40)	8 (20)
Euphoria × Sweetheart	12 (30)	20 (50)	8 (20)
Nutrient medium Smirnova			
Valeriy Chkalov × Sweetheart	14 (35)	20 (50)	6 (15)
Krasnodarskaya Rannyaya × Krupnoplodnaya	6 (15)	28 (70)	6 (15)
Yaroslavna × Sweetheart	20 (50)	16 (40)	4 (10)
Euphoria × Sweetheart	8 (20)	18 (45)	14 (35)

* Forty embryos for each hybrid combination.

Наблюдалась одинаковая реакция зародышей на баланс фитогормонов в питательной среде, независимо от их генотипического происхождения.

В отличие от питательной среды Смирнова, две другие среды (M_4 и Pr_1) помимо полного состава макро- и микроэлементов по прописи Мурасиге и Скуга имеют определенный лимит допустимых значений по цитокинину – 6-БАП от 0.5 мг/л в среде Pr_1 до 0.8 мг/л в среде M_4, а также аскорбиновой кислоты – 2.0 мг/л в питательной среде Pr_1 и 1.0 мг/л в среде M_4. Различаются эти две среды и по содержанию сахарозы (см. табл. 1). Пониженное содержание сахарозы получено также в питательной среде Смирнова. Обозначенные различия в составе питательных сред сказывались на дальнейшем развитии зародышей черешни. Наличие цитокининов (6-БАП) в средах M_4 и Pr_1 ускоряло морфогенез зародышей, стимулируя деление клеток, а культивирование зародышей на питательной среде Смирнова было успешным и без добавления 6-БАП.

Цитокинины индуцируют клеточное деление зародыша. Начало морфогенеза, по всей видимости, достигается воздействием фитогормона 6-БАП. В наших опытах наиболее активно это происходило при его концентрации 0.8 мг/л. В питательной среде M_4 отсутствовали ауксины, как и в среде Pr_1, где концентрация фитогормона 6-БАП составила 0.5 мг/г. Этим они отличались от более «бедной» среды Смирнова, без фитогормонов и с более низкой концентрацией витаминов.

Содержание в двух первых питательных средах нитратов, ионов аммония, калия, кальция, цинка, железа и магния в достаточном количестве, вероятно, способствовало более быстрому росту клеток в сравнении с третьей средой, о чем можно судить по количеству проросших зародышей (табл. 3). Как видно из результатов развития зародышей от всех гибридных комбинаций внутривидовых скрещиваний, более активный рост происходил на питательных средах M_4 и Pr_1, где только часть зародышей от скрещивания Эйфория × Свитхарт оставалась в покое даже через семь месяцев после посадки семян

Table 3. The results of germ culturing depending on the genetic origin of sweet cherry hybrids and on the nutrient medium

Hybrid combination	Viable, pc	Sprouts	
		pc	%
Modified nutrient medium Pr_1			
Valeriy Chkalov × Sweetheart	26	26	100
Krasnodarskaya Rannyaya × Krupnoplodnaya	30	30	100
Yaroslavna × Sweetheart	16	16	100
Euphoria × Sweetheart	20	12	60
Modified nutrient medium M_4			
Valeriy Chkalov × Sweetheart	28	28	100
Krasnodarskaya Rannyaya × Krupnoplodnaya	32	32	100
Yaroslavna × Sweetheart	16	16	100
Euphoria × Sweetheart	20	16	80
Nutrient medium Smirnova			
Valeriy Chkalov × Sweetheart	20	6	30
Krasnodarskaya Rannyaya × Krupnoplodnaya	28	18	64
Yaroslavna × Sweetheart	16	14	87.5
Euphoria × Sweetheart	18	6	33

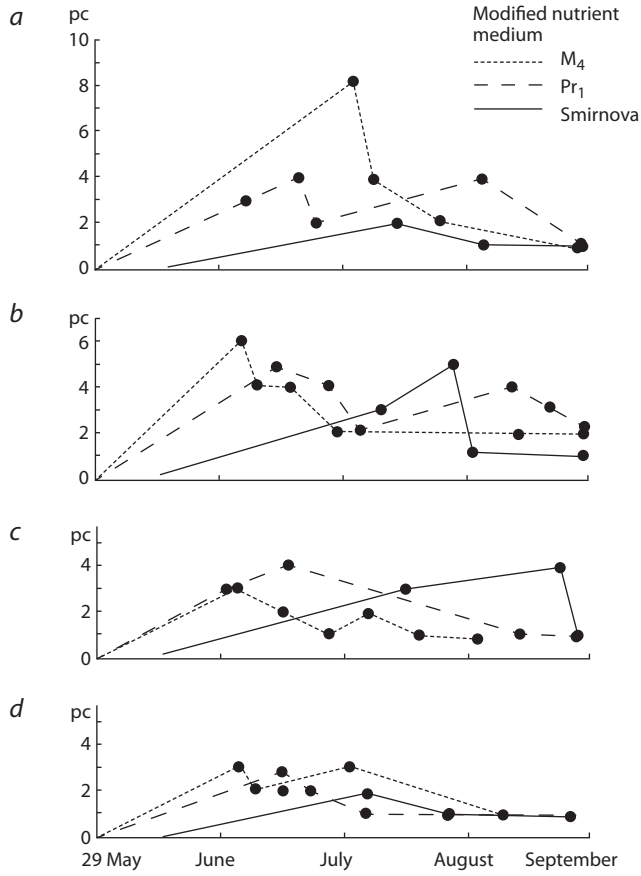

Fig. 1. The germination dynamics of germs from hybrid combinations: (*a*) Valeriy Chkalov × Sweetheart, (*b*) Krasnodarskaya Rannyaya × Krupnoplodnaya, (*c*) Yaroslavna × Sweetheart, (*d*) Euphoria × Sweetheart.

(см. табл. 3). Питательная среда Смирнова, которая не содержит ауксинов, цитокининов и витамина С и отличается довольно низким содержанием сахарозы (20 г/л), т. е. в целом «нейтральная» по составу, оказалась менее приемлемой для активного развития зародышей. Однако следует отметить, что на такой обедненной питательной среде хорошее развитие и рост зародышей наблюдались для комбинаций скрещивания Краснодарская ранняя × Крупноплодная и Ярославна × Свитхарт.

Продолжительность периода от даты посадки семян на питательные искусственные среды до их прорастания варьирует от 56 до 71 дня на среде M_4 и от 102 до 106 дней на среде Pr_1. При вводе в культуру зародышей черешни в конце мая их активное прорастание происходит в июле: в первой декаде – на питательной среде M_4, во второй – на Pr_1. На питательной среде Смирнова пик прорастания зародышей приурочен ко второй-третьей декаде августа, что в целом характерно для всех комбинаций скрещивания. Активность прорастания гибридных зародышей на трех средах в одной повторности представлена на рис. 1.

Питательная среда M_4 на основе солей среды Мурасиге и Скуга, имея в своем составе 0.8 мг/л 6-БАП, пониженное количество аскорбиновой кислоты (1.0 мг/л) и сахарозы (20 г/л), в сравнении с питательной средой Pr_1 обладает более приемлемым составом для ускоренного развития зародышей в семенах сортов черешни.

Использование культуры ткани имело положительный результат даже в тех случаях, когда проростки, полученные из ткани стебля, были лишены первичного корня. Особенно часто такие проростки наблюдались у гибридов Краснодарская ранняя × Крупноплодная (рис. 2).

Заключение

В ходе работы по программе «раннее созревание и крупноплодность» осуществлялось культивирование *in vitro* зародышей внутривидовых гибридов от четырех комбинаций скрещивания перспективных сортов и доноров черешни: Валерий Чкалов × Свитхарт, Краснодарская ранняя × Крупноплодная, Ярославна × Свитхарт, Эйфория × Свитхарт. Использованные сорта считаются перспективными для конкретной экологической зоны плодоводства и служат донорами признаков для получения селекционного материала сортов черешни раннего срока созревания. После искусственного опыления была применена культура зародышей *in vitro*, что дало более значимый эффект от гибридизации и высокий выход сеянцев в сравнении с таковым в полевых условиях селекционного питомника. Оптимизирован процесс обеззараживания плодов, косточек и семян черешни перед культивированием, который заключается в поверхностной стерилизации от сапрофитной микрофлоры: промывка плодов в мыльном растворе с последующим ополаскиванием водой; стерилизация выделенных косточек и семян водным раствором дезин-

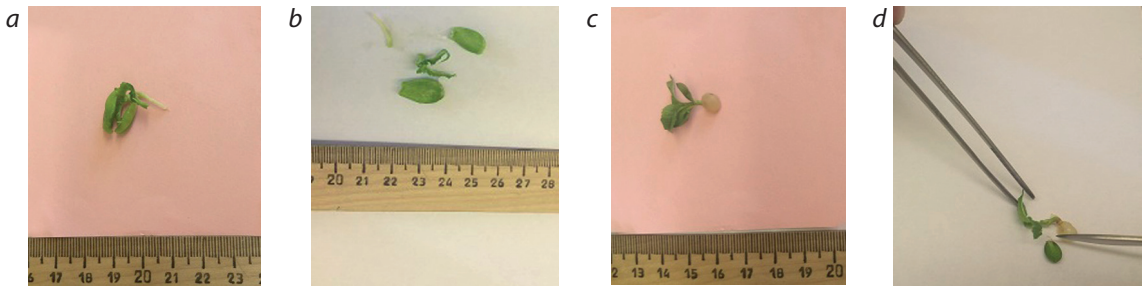

Fig. 2. Sweet cherry seedlings lacking the primary root: (*a*, *b*) Krasnodarskaya Rannyaya × Krupnoplodnaya, (*c*, *d*) Yaroslavna × Sweetheart.

фицирующего препарата HAZ TABS (на основе хлора) в концентрации 4.75 г на 500 мл воды.

По результатам выбраковки пробирочного материала с инфицированными и нежизнеспособными зародышами черешни, процент выхода жизнеспособных сеянцев на всех питательных средах (Мурасиге и Скуга, Prunus, Смирнова) был примерно одинаков – от 40 до 80 % в зависимости от гибридной комбинации, что свидетельствует об идентичности растительного материала и способа его стерилизации. Хорошие показатели выхода жизнеспособных зародышей наблюдались у гибридной комбинации Краснодарская ранняя × Крупноплодная.

Оптимальными параметрами питательных сред на этапе ввода в культуру зародышей гибридов черешни и дальнейшего их проращивания являются: полный состав макро- и микроэлементов по прописи Мурасиге и Скуга; биологически активные вещества (6-БАП 0.8 мг/л, без ауксинов) и витамины (аскорбиновая кислота 1.0 мг/л, тиамин-HCl 0.5 мг/г, пиридоксин-HCl 0.5 мг/л, никотиновая кислота 0.5 мг/л), при добавлении в среду 20 г/л сахарозы. При использовании трех питательных сред с различным составом активных веществ или даже их отсутствием (среда Смирнова) установлено влияние сортоспецифичности и концентрации веществ на начало роста зародышей, культивируемых *in vitro*. Активный рост сеянцев исследуемых гибридных комбинаций происходит в июле, т. е. примерно через 1.5 месяца после ввода в культуру.

Эффективность выращивания гибридных сеянцев черешни раннего срока созревания с применением культуры зародышей бесспорна, поскольку дает положительный результат в отношении не только выхода сеянцев, но и возможности получения гибридов в год проведения гибридизации, что сокращает селекционный процесс по меньшей мере на два года.

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Structuring ampelographic collections by phenotypic characteristics and comparing the reaction of grape varieties to climate change

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Modern climate changes task breeders to adapt viticulture to the new natural resource potential of the regions. A necessary condition is the assessment and analysis of current trends in changing the characteristics of contrasting groups of varieties. The aim of the study is to identify homogeneous groups of varieties of an ampelographic collection and to compare the rates of reaction of their agrobiological parameters to climate changes. Material for the study consists of observations of 21 agrobiological characteristics of 109 grape varieties from the Don ampelographic collection named after Ya.I. Potapenko (Novocherkassk) with an observation period from 10 to 36 years in 1981–2017. The sample included *Vitis vinifera* L. varieties and the *V. vinifera* L. × *V. labrusca* L. and *V. vinifera* L. × *V. amurensis* Rupr. interspecific hybrids, and hybrids from crosses between *V. vinifera* L. and several American species. Homogeneous groups of characteristics and varieties are identified by principal component analysis (PCA) and analysis of variance (ANOVA) methods. Trends in changing the agrobiological characteristics of the varieties and groups of varieties are calculated. PCA revealed that the main differentiating factor of the studied fragment of the ampelographic collection is the size of the bunch; the second, the yield; the third, the time of ripening. The values of the factors are contrasting in varieties of different directions of use and taxonomic origin, which was confirmed by ANOVA. The groups of the *V. vinifera* × *V. labrusca* and *V. vinifera* × *V. amurensis* hybrids do not differ significantly from each other in most indicators, exceeding *V. vinifera* varieties in the number of elements of productivity, winter hardiness and yield. Complex hybrids with American species have an intermediate position between these groups exceeding all groups in bunch weight. All groups of cultivars show trends towards a reduction in productive period, an increase in the mass of bunch and yield, sugar content and a decrease in acidity. The *V. vinifera* × *V. labrusca* hybrids are distinguished by the highest growth rate of the bunch mass caused by a reduction in the duration of active growth and a decrease in the percentage of fruit-bearing shoots. A higher reduction rate of the production period and a decrease in acidity were observed in later varieties. Regression analysis showed that the acceleration of the ripening of grapes is largely due to rising temperatures.

Key words: ampelographic collection; climate change; information system; time series; agrometeorology; adaptability.

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Структурирование ампелографической коллекции по фенотипическим характеристикам и сравнение реакции сортов винограда на изменение климата

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Современные изменения климата ставят задачу адаптации виноградарства к новому природно-ресурсному потенциалу регионов. Необходимым условием для этого являются оценка и анализ современных тенденций изменения агробиологических характеристик контрастных групп сортов. Целью исследования было выделение однородных групп сортов ампелографической коллекции и сравнение скоростей реакции их агробиологических показателей на климатические изменения. Материалом для исследования послужили наблюдения за 21 агробиологическим показателем 109 сортов винограда Донской ампелографической коллекции им. Я.И. Потапенко (г. Новочеркасск) с периодом наблюдения от 10 до 36 лет в 1981–2017 гг. В выборку вошли сорта *Vitis vinifera* L. и межвидовые гибриды *V. vinifera* L. × *V. labrusca* L., *V. vinifera* L. × *V. amurensis* Rupr., *V. vinifera* L. с несколькими американскими видами. Методами анализа главных компонент (PCA) и дисперсионного анализа (ANOVA) выделены однородные группы признаков и сортов. Рассчитаны тренды агробиологических показателей сортов и групп сортов. Метод PCA позволил выявить, что главным дифференцирующим фактором изученного фрагмента ампелографической коллекции является крупность грозди, вторым – урожайность, третьим – срок созревания. Значения факторов контрастны у сортов

разного направления использования и таксономического происхождения, что подтвердил метод ANOVA. Группы гибридов *V. vinifera*×*V. labrusca* и *V. vinifera*×*V. amurensis* достоверно не отличаются друг от друга по большинству показателей, превосходя сорта *V. vinifera* по количеству элементов продуктивности, зимостойкости и урожайности. Комплексные гибриды с американскими видами занимают по этим признакам промежуточное положение, однако превышают все группы по массе грозди. У всех групп сортов наблюдались тренды к сокращению продукционного периода, увеличению массы грозди и урожайности, росту сахаристости и уменьшению кислотности. Выделяются гибриды *V. vinifera*×*V. labrusca* наибольшей скоростью сокращения продолжительности активного роста, снижением доли плодоносных побегов и увеличением массы грозди. Бóльшая скорость сокращения продукционного периода и уменьшение кислотности отмечены у более поздних сортов. Регрессионный анализ показал, что ускорение созревания винограда происходит в значительной степени из-за роста температур.
Ключевые слова: ампелографическая коллекция; изменение климата; информационная система; временные ряды; агрометеорология; адаптивность.

Introduction

Modern climate change has become a significant factor for the viticulture in many countries (Jones, 2012; Vršič, Vodovnik, 2012; Quenol et al., 2014). A scientific basis for the program of adapting viticulture to current and expected climate changes are the creation of phenotypic databases of grape genetic resources (Delrot et al., 2019; Hausmann et al., 2019), an assessment of trends in the dynamics of economically important traits (Jones, 2012; Choudhury, Jones, 2014), as well as their modeling and forecasting (Molitor et al., 2014; Quenol et al., 2014). Plant phenology was found to be very sensitive to climate change (Cleland et al., 2007; Santibáñez et al., 2014), and this fact has revived interest in mathematical models of phenology, in particular, to the sum of growing degree days and the sum of effective daily temperatures during the growing season (Cleland et al., 2007; Molitor et al., 2014; Santibáñez et al., 2014). However, the accuracy of phenological models is still insufficient (Richardson et al., 2012).

Previously, the data accumulated from 1981 through 2011 for a sample of 20 grape varieties from the Don ampelographic collection was used in a study of the dynamics of the ripening time duration, which demonstrated uniformity of the varieties' response to changes in agro-climatic factors (Naumova, Novikova, 2013, 2018). To identify the agro-climatic factors determining the development of plants, a regression model (1) was constructed by a method modified for the time series, i. e. pre-differenced variables regression. The analysis in differences allows improving the quality of time series models by removing the agricultural engineering trend (Eliseeva et al., 2007; Sirotenko, 2012; Wenjiao et al., 2013; Iler et al., 2017). The method consists in the regression analysis of the relationship between the annual increments of the indicators, denoted by Δ. It was shown (Naumova, Novikova, 2013, 2018) that duration of the grape ripening time (N) depends on the total temperatures above 20 °C ($\sum T_{20}$) and on duration of the spring period with temperatures of 10–15 °C ($N_{10–15}$):

$$\Delta N = -0.154 - 0.013 \cdot \Delta\sum T_{20} + 0.353 \cdot \Delta N_{10–15},\ R^2 = 0.69, \quad (1)$$

where R^2 is the equation determination coefficient.

The adaptive potential of a grape plant is strongly dependent on the variety origin (Negrul, 1946; Troshin, 1999; Nosulchak, 2015; Zarmaev, Borisenko, 2018). In the 1960s-1980s, it became clear that the possibilities of intraspecific breeding of the European-Asian species *Vitis vinifera* L. had been exhausted, and breeding at the interspecific level was intensified, especially for obtaining varieties resistant to pests, diseases and low temperatures (Nosulchak, 2015). An American and East Asian species *V. labrusca* L. was widely used in breeding for increasing frost, disease and pest resistance, and *V. amurensis* Rupr. for creating early-maturing and frost-resistant varieties (Zarmaev, Borisenko, 2018). When working with large collections, including ampelographic ones, there appear the tasks of evaluating polymorphism, structuring the collections, and identifying homogeneous groups in them, which are solved by multidimensional statistics (Cunha et al., 2009; Leão et al., 2011; Lamine et al., 2014). Previously, the authors have studied dynamics of the main agrobiological indicators observed for at least 5 years and more for 106 varieties of *V. vinifera* L. and hybrids of *V. vinifera* L. × *V. amurensis* Rupr., and revealed differences in overwintering factors for the varieties of different origin (Novikova, Naumova, 2018). To date, the authors have expanded the database and created a program for the agrometeorological analysis of time series of observations over a large number of varieties, which allowed the inclusion of a group of other interspecific hybrids in the present study.

The purpose of the study was to identify homogeneous groups of varieties in the ampelographic collection and compare the rates of their agrobiological indicators response to climate change.

Materials and methods

The materials used in the study were the results of observations in 1981–2017 over 109 grape varieties from the Ya.I. Potapenko Don ampelographic collection. The study was carried out on the accessions with agrobiological data recorded within 10 to 36 years. Four groups of different taxonomic origin were studied (Table 1): varieties and intraspecific hybrids of *V. vinifera*, complex hybrids of *V. vinifera* with several American species (*V. rupestris* Scheele, *V. riparia* Michaux, *V. lincecumii* Buckley, etc.); as well as hybrids of *V. vinifera* × *V. labrusca*; and *V. vinifera* × *V. amurensis*. According to the type of use, two groups were distinguished, that is, of wine and table varieties. To enlarge these groups, 7 seedless varieties were attributed to the table, and 17 universal varieties to the wine-type ones. The presented varieties have an average ripening time from 99 to 152 days, which corresponds to 6 groups of the international Descriptor list (Code…, 1983).

Table 1. Distribution of accessions in the studied sample according to the taxonomic origin, type of use, and ripening time

Taxonomic origin	Type of use	Ripening time, days						
		Super-early, <105	Very early, 105–115	Early, 115–125	Mid-early, 125–135	Medium, 135–145	Mid-late, 145–155	Total
V. vinifera	Wine	0	1	0	7	19	6	33
	Table	4	7	4	5	2	0	22
Hybrids of *V. vinifera* with several American species	Wine	0	1	0	4	5	2	12
	Table	1	1	2	4	8	3	19
V. vinifera × *V. labrusca*	Wine	0	1	1	2	0	1	5
	Table	1	1	1	1	0	0	4
V. vinifera × *V. amurensis*	Wine	0	0	2	3	3	1	9
	Table	1	1	2	1	0	0	5
Total		7	13	12	27	37	13	109

Eighty-two varieties are cultivated as the covered and 27 as then the non-covered crop.

The study was carried out on varieties grafted onto the Kober 5 BB (*V. berlandieri* × *V. riparia*) rootstock. The plant spacing of 3.0 × 1.5 m was used, and no irrigation provided. Ground water occurred at a depth of 15–20 m and did not affect the development of vines. The used grape cultivation technology was common for the northern zone of industrial viticulture in the Russian Federation. The study of grape varieties (agrobiological characteristics measurement and calculation of indicators) was carried out using the methods commonly used in viticulture (Lazarevsky, 1963). The sugar content of berry juice and the titrated acidity were determined according to GOSTs. Twenty-one agrobiological indicators have been analyzed (Table 2), and the data from the weather station of the Ya.I. Potapenko Institute of Viticulture and Winemaking used.

The application of Statistica 13.0 (StatSoft Inc.) helped to reveal the main differentiating factors and contrasting groups of varieties by using the method of principal component analysis (PCA). The analysis of variance was applied to investigate the influence of the factors of taxonomic origin, type of use, and ripening time. The post hoc comparisons were made by the Tukey's test. Many agrometeorological studies of grapes employ the 'average varieties' notion (Davitaya, 1952; Lazarevsky, 1961), i. e., the annual data averaged for homogeneous varieties. The 'average varieties', i. e., the annual indicators averaged for a group, have been calculated for each of the identified homogeneous groups.

The VITIS TIME SERIES (VTS) program has been developed in Delphi 2006 for storing and analyzing the time series data from observations over agrobiological indicators. VTS was used for calculating agro-climatic characteristics for the years of observation (dates of temperature transition above 5, 10, 15, 20, 25 °C (Kelchevskaya, 1971), the sum of temperatures and precipitation for the periods between them), as well as the sums of temperatures for the varieties' interphase periods. The main tendencies in the dynamics (linear trends) in agrometeorological characteristics and agrobiological indicators of varieties and 'average varieties' have been determined. The trends were calculated as coefficients of linear dependence of the studied characters on the year (Eliseeva et al., 2007) and for convenience expressed as units per 10 years. Model (1) was validated by comparing the calculated and actual values for 2012–2017 which were not used in the model creation. By using model (1), the temperature-dependent trend in the ripening time duration was calculated. The significance level for a study was set at 5 %.

Results

Homologous groups identification. Principal component analysis. The PCA for 21 agrobiological indicators allowed identifying three factors which explain 28.9, 20.0, 18.1 %, and the total of 67 % of the variance, with eigenvalues of 6.1, 4.2, 3.8 (totaling 14.1). A scree plot of factor eigenvalues is given in Supplement 1[1]. The main factor differentiating varieties in the studied sample from the ampelographic collection are the bunch characteristics (Table 2), such as the bunch mass, the berry mass, the bunch length and width, and the berry length and width. There is a negative relation between sugar content and this factor. In terms of the first factor, there is a contrast between the groups of table and wine varieties (the level of significance between differences of the factor mean values is $p = 0.000$, Fig. 1). Supplement 2 presents the designations of the type of use and the origin of varieties in different figures.

The second factor is the yield and the positively associated with it percentage of bud breaks and the number of productivity elements, as well as the negatively associated date of the bud breaking onset. In terms of the second factor, there is a contrast between the varieties of *V. vinifera* and hybrids with *V. labrusca* and *V. amurensis* (Fig. 1), which are characterized by higher yields, high winter-hardiness, early bud break, a higher percentage of fruiting shoots and fruiting coefficient. The groups of *V. vinifera* × *V. amurensis* and *V. vinifera* × *V. labrusca* varieties do not differ between themselves by either the first factor ($p = 0.741$) or the second one ($p = 0.087$). At the same time, the average value of the second factor in all interspecific hybrids was significantly higher than that of *V. vinifera* ($p = 0.000$ for the hybrids with *V. amurensis* and *V. labrusca*; $p = 0.006$ for complex hybrids).

[1] Supplementary Materials 1–4 are available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx17.pdf

Table 2. Factor loads of 21 agrobiological indicators

Indicator	Factor 1	Factor 2	Factor 3
Onset of bud break	0.050	–0.654	0.096
Onset of bud break – onset of flowering	0.541	–0.112	–0.125
Onset of flowering – veraison	–0.120	–0.524	0.613
Veraison – full maturity of berries	–0.204	–0.433	0.702
Ripening time	–0.089	–0.584	0.741
Number of budbreaks, %	–0.354	0.622	0.276
Number of normally developed shoots	–0.478	0.420	0.206
Average bunches per developed shoot	–0.534	0.614	0.427
Average bunches per productive shoot	–0.413	0.488	0.349
Number of productive shoots, %	–0.549	0.592	0.410
Shoot productivity	0.480	0.522	0.497
Yield per vine	0.086	0.757	0.491
Bunch mass	0.907	0.017	0.137
Bunch length	0.707	0.174	0.101
Bunch width	0.730	0.270	0.148
Berry mass	0.855	0.119	0.297
Berry length	0.831	0.065	0.276
Berry width	0.782	0.198	0.163
Sugar content	–0.639	–0.249	0.003
Total acidity	–0.192	–0.466	0.718
Glucoacidometric index, GAI; sugar to acidity ratio	–0.118	0.372	–0.718

Note. The marked loads are above 0.70.

The third factor is the duration of the ripening time and interphase periods. There is a positive relation of acidity and negative of the glucoacidometric index (GAI) with this factor.

Analysis of variance. The influence of the type of use, taxonomic origin and the duration of the ripening time on the main indicators differentiating varieties within the sample was confirmed by the variance analysis. Fig. 2 shows the three main indicators identified by PCA for 8 groups composed according to the origin and type of use.

The two-factor analysis of variance showed that table varieties within the studied sample have, on the average, a significantly shorter ripening time (124 days) than the wine varieties (136 days, $p = 0.000$), and a higher bunch mass (300 and 196 g, respectively, $p = 0.000$). There are no significant differences in yield (4.7 and 4.1 kg, $p = 0.094$).

The taxonomic origin distinguished the group of *V. vinifera* × *V. labrusca* and *V. vinifera* × *V. amurensis* hybrids, which did not differ significantly from each other by the average values of the ripening time duration (123 and 127 days, respectively, $p = 0.834$), yield (6.8 and 5.8 kg, $p = 0.491$) and the bunch mass (190 and 210 g, $p = 0.943$). If compared with *V. vinifera* hybrids, those with *V. labrusca* and *V. amurensis* are characterized on the average by higher yields (3.7 kg for *V. vinifera*, $p = 0.000$ for both groups), a lower bunch mass (235 g for *V. vinifera*, $p = 0.450$ for hybrids with *V. labrusca* and $p = 0.758$ for *V. amurensis*). Also, there was no difference in the ripening time duration (131 days for *V. vinifera*, $p = 0.199$ and $p = 0.637$). Complex hybrids with American species are a very polymorphic group, which, on the average,

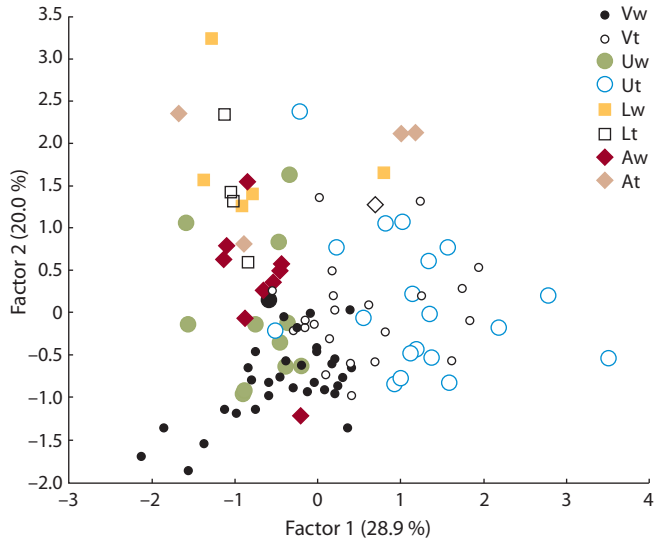

Fig. 1. 109 varieties in the space of the first two PCA factors.

Hereinafter: w – wine varieties, t – table varieties. Vw, Vt – *V. vinifera*, wine, table; Uw, Ut – hybrids with several American species, wine, table; Lw, Lt – *V. vinifera* × *V. labrusca*, wine, table; Aw, At – *V. vinifera* × *V. amurensis*, wine, table.

exceeds other groups by bunch mass (292 g, $p = 0.010$ when compared with *V. vinifera*, and $p = 0.018$ in comparison with hybrids with *V. labrusca* and *V. amurensis*). Concerning most indicators, these hybrids occupy an intermediate position between *V. vinifera* varieties and hybrids with *V. labrusca* and

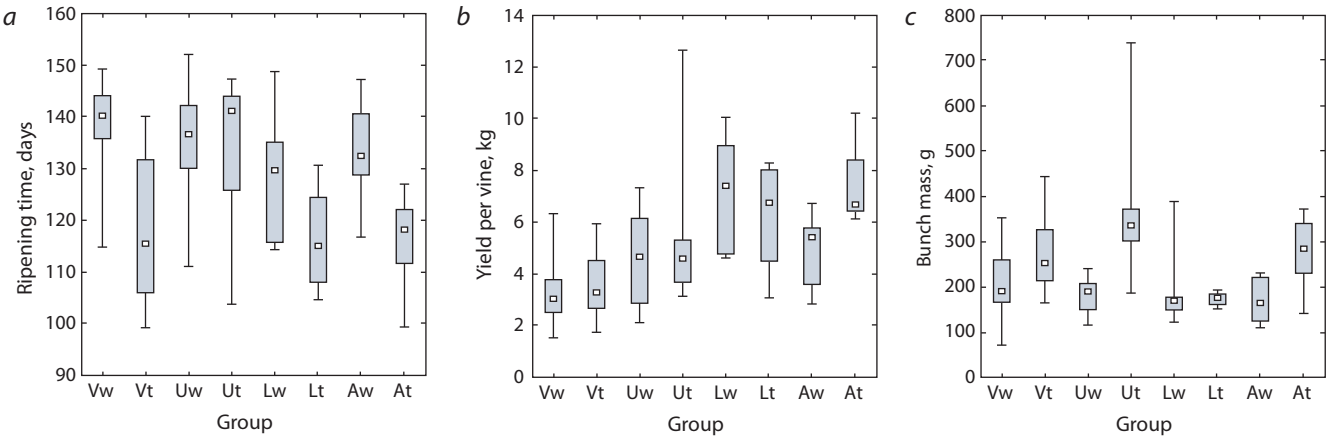

Fig. 2. Economically important characters of grape groups of different taxonomic origin and type of use: *a*, ripening time; *b*, yield per vine; *c*, bunch mass.

V. amurensis. If compared with *V. vinifera* varieties, complex hybrids are characterized on the average by higher yields (4.8 kg, $p = 0.001$), while they do not differ in the ripening time duration (135 days, $p = 0.411$).

The analysis of variance of six groups differing by the ripening time (see Table 1) confirmed the PCA-detected reliable association of the ripening time only with the interphase periods, acidity, and GAI. The average duration of the 'onset of flowering – veraison' period increases from 40 days in the group of super-early varieties up to 64 days for the mid-late ones, and that of the 'veraison – full maturity of berries' period from 25 days for super-early to 44 days for the mid-late varieties. The average acidity of the berries grows from 6.4 g/dm^3 in the group of super-early up to 10.2 g/dm^3 in the group of mid-late ones, and GAI decreases from 3.0 to 1.9 units accordingly.

Trends in agrobiological indicators. In 1981–2017, in Novocherkassk, the growing degree days for the period with temperatures above 10 °C has increased by 170 °C per 10 years, the amount of precipitation unreliably decreased over this period by 21 mm, the average temperature of the winter dormancy period (October 15 – April 15) has reliably increased by 0.5 °C/10 years, while the number of days during the winter with temperatures below –20 °C has not significantly changed. Trends were calculated for each trait of each variety, however, for most traits and most varieties trends were insignificant against the year-to-year fluctuations. The trends of the 'average varieties' in 8 groups composed according to the taxonomic origin and type of use demonstrated reliable values for a number of indicators (Fig. 3, Supplement 3). Berry quality indicators showed nonlinear dynamics: sugar content was decreasing till the mid-1990s and then started increasing, while acidity was at its maximum in the 1990s (Novikova, Naumova, 2013), therefore their trends have been calculated starting from 1995.

The groups of varieties composed according to the taxonomic origin and type of use are characterized by the same direction of change of the economically important traits, though with some exceptions. The onset of bud break does not change reliably in any group. The ripening time shortens in all groups and for the 'average variety' from 109 accessions for 2 days/10 years due to the shortening of all interphase periods. The yield increases in all groups of varieties by 1 kg/vine/10 years, on the average; the bunch mass increases by 10.2 g/10 years, on the average; the sugar content increases by 2 g/100 cm^3, on the average, and GAI does likewise by 0.6 units/10 years on the average, while acidity decreases by 1 g/dm^3, on the average. The sum of temperatures for the ripening time has increased by 71 °C/10 years, on the average, due to the growth of excess temperatures in mid-summer.

The group of wine hybrids with *V. labrusca* is distinguished by the highest rate of the ripening time reduction (18 days/10 years) due to shortening of all the interphase periods, including the 'onset of flowering – veraison' period (6 days/10 years), which, on the average, does not change in a sample of 109 varieties. The sum of temperatures for all the interphase periods and the ripening time for this group decreases on the whole by 140 °C/10 years. The percentage of fruiting shoots reliably decreases (by 11.1 %/10 years) only in wine hybrids with *V. labrusca*, which leads to the highest growth rate of the mass of the bunch (73 g/10 years) and berry (3 g/10 years) among the studied groups.

An analysis of trends in groups of different ripening time (Supplement 4) showed that even with such a division into groups, the direction of trends in different groups basically coincides. The early-medium, medium and mid-late varieties have a higher rate of the ripening time reduction (4–5 days/10 years) than the super-early, very early and early ones (2–3 days/10 years). Acidity decreases more rapidly (by 2 g/dm^3/10 years) in medium and mid-late varieties than in super-early and very early (0.3–0.4 g/dm^3/10 years) and in early and early-medium ones (1 g/dm^3/10 years), which is obviously related to the growing heat availability during the ripening of berries.

Model analysis of the ripening time duration. The observed trends may have contributions from such factors as climate change, agricultural engineering trends, and age-related changes of the bushes. To assess the contribution of climate change to the trend of the ripening time duration, a regression model (1) was used. At first, model (1) was checked using the data of 2012–2017, which was not used in its creation, the data calculated applying model (1) and the actual annual increments (ΔN) (Fig. 4).

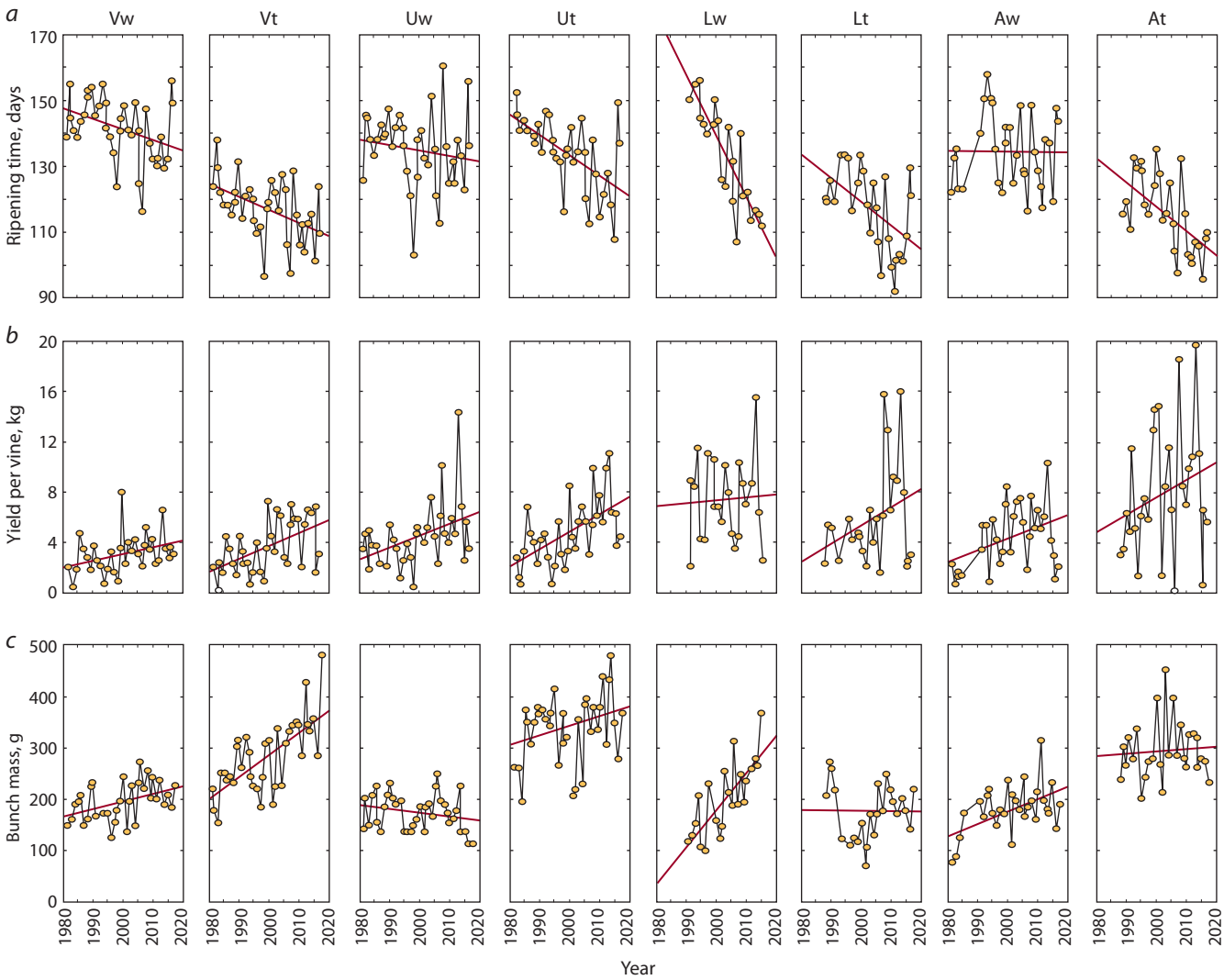

Fig. 3. Trends of economically important characters in grape groups of different taxonomic origin and type of use, 1981–2017, units per 10 years: *a*, ripening time; *b*, yield per vine; *c*, bunch mass.

The average absolute error of the model amounted to 8 days (with exception for 5 days in 2016), or 4 % of the ripening time duration, which confirmed its adequacy. The accuracy of the model is evidenced by the correlation ($r = 0.69$) of the actual and calculated data. The year 2016 was characterized by an anomalous precipitation of 338 mm during the ripening period against the average value of 214 mm, including the 'onset of bud break – onset of flowering' period with 182 mm against the perennial average of 65 mm. This might be one of the reasons for the 17-day increase in the ripening time duration compared to the average long-term value, which is not fully explained by the model.

The rate of independent variables change in 1981–2017 was $\Delta\Sigma T_{20} = 324.3$ °C/10 years, $\Delta N_{10–15} = –0.4$ days/10 years, therefore $\Delta N = –4.4$ days/10 years according to the formula (1). The model predicts a more significant ripening time reduction than the actually observed $\Delta N = –2.5$ days/10 years. The actual trend was significantly influenced by 2016 (see Fig. 3), while in 1981–2015 the actual trend showed $\Delta N = –4.3$ days/10 years, which corresponds to the calculated one. Thus, the decrease in the ripening time duration is largely due to the rising temperatures.

Discussion

The principal component analysis of the mean long term data on 21 indicators of 109 grape varieties has shown that the main differentiating phenotypic character of the ampelographic collection is the bunch and berry size, which is contrasting in the table and wine varieties. A large berry is characteristic of a group of table hybrids of *V. vinifera* with several American species. A significant part of this group is represented by varieties from the Republic of Moldova and by hybrids produced with the use of the American variety Save Villar and the Central Asian variety Katta-Kurgan. The Central Asian varieties known for large berries are absent in the used sample. The second factor, i. e. the yield and the number of fruiting shoots associated with it, winter hardiness and fruiting coefficient, is at its maximum in *V. vinifera* × *V. labrusca* and *V. vinifera* × *V. amurensis* hybrids, which retain winter resistance and a greater number of productivity elements from the Amur and

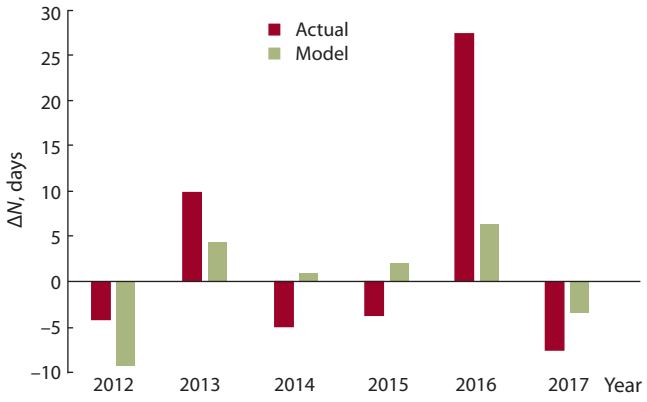

Fig. 4. Comparison of the actual and model-calculated annual increment to the grape ripening time duration (Δ*N*) in 2012–2017.

American species (Troshin, 1999; Nosulchak, 2015), while it is at its minimum in the European cultivated grapes. The third factor is the ripening time and interphase periods duration, and the related berry juice acidity and GAI.

The analysis of variance has confirmed a number of reliable differences of the average indicators between the groups of varieties composed according to the type of use, origin, and ripening time. The table varieties differ from the wine ones by a larger bunch and a shorter ripening time. On the average, interspecific hybrids exceed *V. vinifera* varieties in the number of productivity elements and yield. The groups of *V. vinifera* × *V. labrusca* and *V. vinifera* × *V. amurensis* hybrids do not differ significantly from each other by the average ripening time duration, yield and bunch mass values. The similarity of a number of characteristics of these species is noted in the literature (Negrul, 1946, p. 120). Complex hybrids with American species represent a polymorphic group and occupy an intermediate position concerning the majority of the studied parameters, with an exception for the characteristics of the bunch, between *V. vinifera* varieties and hybrids with *V. labrusca* and *V. amurensis*. The analysis of variance has confirmed the PCA-detected association of the ripening time only with the interphase periods duration, acidity, and GAI.

The analysis of trends in agrobiological indicators of homogeneous groups of varieties has confirmed that varieties do not differ in trends of the long-term dynamics of most indicators. All groups demonstrated a decrease in the ripening time duration (2 days/10 years on the average for 109 varieties), an increase in yield (1 kg/vine/10 years on the average) and sugar content (2 g/100 cm^3 on the average), a decrease in acidity (an average of 1 g/dm^3), which corresponds to the trends observed in other countries (Vršič, Vodovnik, 2012). The trends of the average indicators in the groups of varieties with different ripening time were also similarly directed. Thus, the thesis was confirmed that the impact of climate was greater than that of variety (Leeuwen et al., 2004).

The group of *V. vinifera* × *V. labrusca* hybrids of wine type is characterized by the most significant changes, e. g., by the highest rate of the ripening time decrease (18 days/10 years), a decrease in the fruiting shoots percentage (11 %/10 years) and an increase in the mass of the bunch (73 g/10 years) and berry (3 g/10 years).

The model of the ripening time duration dynamics (1) was validated using the data of 2012–2017, which were not used in model creation. The trend of the ripening time duration calculated using the model was Δ = –4 days/10 years, which corresponds to that observed until 2016. The increase in the ripening time duration in 2016 could not be explained by the model and was possibly due to the anomalous precipitation that year. Thus, it has been shown that the observed decrease in the varieties' ripening time duration was largely due to the growing temperatures.

Conclusion

Homogeneous groups of varieties in the ampelographic collection, composed according to the type of use, taxonomic origin and ripening time, are characterized by a similar response to the climate change. An increase in temperature in the northern zone of industrial viticulture in the Russian Federation leads to a reduction in the grapes ripening time, an increase in yield and sugar content, and a decrease in berry acidity.

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База данных генетических ресурсов коллекции озимой ржи ВИР как средство классификации генетического разнообразия, анализа истории коллекции и эффективного изучения и сохранения

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Озимая рожь – вторая хлебная и наиболее ценная кормовая культура, которую возделывают прежде всего в России, Германии, Польше, Беларуси, Украине, Скандинавии, Китае, Канаде и США. Посевные площади, отведенные под выращивание ржи в мире, сокращаются (1986 г. – 15.4 млн га, 2016 г. – 4.4 млн га). Во всех зонах возделывания рожь заслужила репутацию наиболее приспособленной к климатическим условиям страховой культуры низкого экономического риска. Для расширения посевов и увеличения валовых сборов зерна необходимо создание новых сортов ржи. В настоящее время в 94 генбанках мира хранится 22200 образцов озимой и яровой ржи. Крупнейший генный банк (3260 образцов) находится в России, это Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова. Коллекция мировых генетических ресурсов ржи, сосредоточенная в хранилищах и размножаемая на полях, содержит сорта, доноры, популяции и линии культурной, сорно-полевой, дикорастущей, озимой и яровой ржи. Идет постоянное обновление и пополнение коллекции новыми образцами, усовершенствуется система надежного хранения и поддержания высокой жизнеспособности семян, проводится их изучение и выявление среди них источников ценных для селекции признаков, создание доноров. В настоящей работе проанализирована и кратко охарактеризована коллекция ржи ВИР. Рассмотрена история развития предселекционного изучения, этапы создания и использования доноров для различных проблем селекции, создана паспортная база данных по озимой и яровой ржи.
Ключевые слова: озимая рожь; генетические ресурсы; коллекция ВИР; образец; сорт; донор.

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The database of genetic resources in the VIR winter rye collection as a means of classification of genetic diversity, analysis of the collection history and effective study and preservation

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Winter rye is the second bread and the most valuable forage crop. Rye is cultivated primarily in Russia, Germany, Poland, Belarus, Ukraine, Scandinavia, China, Canada and the United States. The acreage allocated for the cultivation of rye in the world is declining (from 15.4 million ha in 1986 to 4.4 million ha in 2016). In all areas of cultivation rye has earned a reputation as the most adapted to the climatic conditions of the insurance culture of low economic risk. For the expansion of crops of rye and an increase in the gross yield of grain, it is necessary to create new varieties of rye. Currently, 94 gene banks in the world store 22,200 samples of winter and spring rye. Gene banks are located around the world; the largest of them – the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (3260 samples) – is located in Russia. The collection of the world's genetic resources of rye, concentrated in storage and propagated in the fields, contains varieties, donors, populations and lines of cultural, weed-field, wild, winter and spring rye. The collection is being constantly updated and replenished with new samples, the system of reliable storage and maintenance of the high viability of seeds is being improved, the sources of traits with value for breeding are being identified and studied, and donors are being created. Scientific, breeding and educational institutions are being supplied with source material. An electronic passport documentation system of the collection is being developed and integrated into the international system of genetic banks. In this paper, a brief analysis and cha-

racterization of the VIR rye collection is given. The history of the pre-selection study and the stages of the creation and use of donors for various problems of selection are reviewed, a passport database on winter and spring rye has been created.

Key words: winter rye; genetic resources; collection of VIR; sample; variety; donor.

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Введение

Озимая рожь – важная продовольственная зерновая культура, вторая по значению после пшеницы. Ее хозяйственное значение обусловлено еще и тем, что рожь является одной из наиболее ценных кормовых культур. Зерно ржи содержит полноценные, богатые незаменимыми аминокислотами (особенно лизином) белки и витамины А, С, Е и группы В. Рожь превосходит другие зерновые культуры не только по холодостойкости, но и по устойчивости к почвенной засухе. Она способна успешно произрастать на песчаных и малоокультуренных почвах, где при соответствующей агротехнике опережает по урожайности пшеницу (Кобылянский, 1989; Гончаренко, 2014).

По мнению ряда ученых, род *Secale* возник в средне- и верхнетретичном периодах кайнозойской эры в Закавказье и прилегающих районах Передней Азии. В начале XX в. непосредственным родоначальником культурной ржи считали многолетний сборный вид горной дикой ржи *S. montanum* Guss. и сходные с ним многолетние дикие виды *S. anatolicum* Boiss. и *S. dalmaticum* Viss. Однако Н.И. Вавилов доказал, что дикие виды послужили лишь первоначальным материалом для образования сорно-полевой ржи, из которой и произошла впоследствии культурная рожь (*S. cereale* L.). В настоящее время это представление является общепризнанным (Вавилов, 1987).

В последние десятилетия посевные площади, отведенные под выращивание ржи в мире, сокращаются. Если в 1986 г., по данным ФАОСТАТ, они составляли 15.4 млн га, то к 1996 г. этот показатель снизился до 11.1 млн га, а к 2016 г. – до 4.4 млн га, т.е. с 1986 по 2016 г. посевные площади были сокращены на 71 %. Общий объем производства ржи за тот же период уменьшился с 30 до 13 млн т, или на 57 % (www.fao.org/faostat/en/#data/qc).

Основные ржаносеющие регионы в России расположены в Приволжском федеральном округе, где сосредоточено более 78 % всех посевных площадей ржи в стране. Доля остальных административных субъектов в структуре ржаного клина составляет: Южный федеральный округ – 7.7 %, Центральный – 7.2 %, Сибирский – 5.3 %, Уральский федеральный округ – 1.5 %. Незначительные площади заняты под посевы в Северо-Западном, Северо-Кавказском и Дальневосточном округах (Кедрова, 2000). Во всех зонах возделывания рожь заслужила репутацию наиболее приспособленной к климатическим условиям страховой культуры низкого экономического риска. В пользу экономической целесообразности расширения посевов ржи и увеличения валовых сборов зерна свидетельствуют: относительно низкая себестоимость зерна; пригодность к возделыванию в севооборотах, насыщенных зерновыми культурами, где рожь увеличивает эффективность применения азотных удобрений; появление новых высокоурожайных и устойчивых к полеганию сортов и гибридов; возможность эффективного использования зерна ржи на корм животным и для промышленной переработки (Шакирзянов и др., 2010).

Целью настоящей работы было выделить доноры хозяйственных признаков и предложить их для использования в селекции, основываясь на анализе паспортной базы данных изучения и сохранения генетического разнообразия коллекции ржи.

Мировое многообразие коллекции ржи

Обновление генетического материала за счет использования новых исходных форм лежит в основе селекции любой сельскохозяйственной культуры. Для эффективного создания конкурентоспособных сортов необходимо располагать генетически разнообразным и комплексно изученным исходным материалом (Пономарева и др., 2018).

Рожь, как аллогамное растение, является сложным объектом для поддержания образцов в генных банках (Шевелуха, 2000), поэтому в сравнении с ячменем или пшеницей коллекция ржи *ex situ* значительно меньше. В 94 генбанках мира хранится 22200 образцов рода *Secale*, тогда как генресурсы пшеницы насчитывают более 732 тыс. образцов, ячменя – 453 тыс. образцов (Шлегель, 2015). Среди разнообразия образцов ржи 73 % документированы в 66 коллекциях Европы, 16 % – в шести коллекциях Северной Америки, 6 % – в пяти коллекциях Азии; 2 % – в семи коллекциях Африки, 3 % – в двух центрах CIMMYT и ICARDA. Генные банки ржи рассредоточены по всему миру. Крупнейший из них (3260 образцов) находится в России, это Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова (ВИР). Следующими по величине являются Западная региональная станция интродукции растений Службы сельскохозяйственных исследований Департамента сельского хозяйства США, Университет штата Вашингтон (2802 образца); Генный банк Института генетики растений и растениеводства им. Лейбница, Германия (2515 образцов); Национальный центр исследований зародышевой плазмы малых зерновых Службы сельскохозяйственных исследований Департамента сельского хозяйства США (2107 образцов); Институт селекции и акклиматизации растений, Польша (1918 образцов) и хранилище "Plant Gene Resources of Canada" Саскатунского научно-исследовательского центра, Канада (1501 образец) (рис. 1).

Коллекция мировых генетических ресурсов ржи, сосредоточенная в хранилищах и размножаемая на полях ВИР, включает сортовые и другие популяции и линии возделываемой культурной, сорно-полевой и дикорастущей

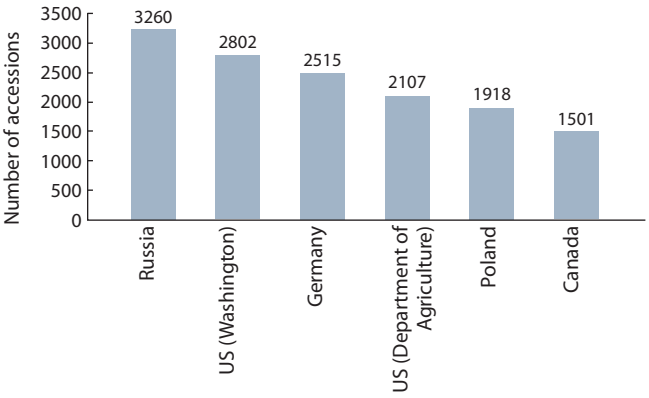

Fig. 1. The numbers of rye accessions in world's gene banks.

ржи. Этот генофонд служит источником безвозмездного обеспечения селекционных центров России исходным материалом при создании новых сортов, является стратегическим потенциалом и основой устойчивого производства кормового и продовольственного зерна ржи. Эффективное сохранение и использование коллекции имеют ключевое значение для обеспечения продовольственной и пищевой безопасности как в настоящем, так и в будущем.

Главный документ, свидетельствующий о составе коллекции и наличии в ней образцов, – паспортная база данных (БД). Она содержит основные сведения об образце, сопровождающие его при поступлении в коллекцию или, возможно, приобретенные и уточненные впоследствии: название, статус образца, происхождение, год поступления в коллекцию и т. д.

Пополнение коллекции осуществляют путем сбора образцов во время экспедиций ВИР на территории Российской Федерации и в другие страны, выписки материала из генбанков, селекцентров, семенных фирм, научно-исследовательских и учебных учреждений мира. В ходе этой работы необходимо уделять особое внимание первоочередному удовлетворению потребностей отечественной селекции и растениеводства; поиску новых ботанических видов, форм, местных и селекционных сортов; выделению источников и доноров носителей ценных генов хозяйственно и биологически полезных признаков, еще отсутствующих в коллекции; воспроизведению максимально возможного фено- и генотипического разнообразия собираемых видов.

Пополнение коллекций по выписке и обмену должно идти главным образом за счет привлечения новых сортов и особо ценных селекционных линий, а также линий с известными генами. Для этой цели используют следующие источники информации: 1) базы данных национальных и международных генбанков в Интернете; 2) отечественные и зарубежные публикации, включая каталоги, монографии, статьи в периодических изданиях; 3) отчеты сельскохозяйственных делегаций о зарубежных поездках; 4) личные сообщения селекционеров, сортоиспытателей и генетиков; 5) заявки на семена, в которых есть образцы, отсутствующие в коллекции.

Необходимо обратить особое внимание на обязательную передачу селекционерами страны их лучших селекционных достижений и константного селекционного материала в коллекцию ВИР. Утрата генетического разнообразия ржи снижает возможность устойчивого и гибкого управления сельским хозяйством в условиях неблагоприятной окружающей среды, а также резкого колебания климатических условий.

При поступлении нового семенного материала в отдел интродукции ВИР после карантинной проверки, которая проходит в карантинных питомниках института, новые образцы с номером интродукции (И-) поступают в отдел генетических ресурсов овса, ржи, ячменя ВИР, где их регистрируют в предварительном (временном) (ПР-) каталоге отдела или в основном (постоянном) (К-) каталоге ВИР и заносят в соответствующую паспортную базу данных. Паспортизация образцов, поступающих в коллекцию, многие годы осуществлялась заполнением записей в журналах, называемых каталогами.

Записи в каталогах имеют следующую структуру: № каталога, название образца, ботаническое название, откуда получен образец, год поступления, № интродукции, происхождение, в каком виде получен образец (семена, колос), примечание. Паспортная БД должна постоянно пополняться новыми созданными сортами озимой и яровой ржи, адаптированными к специфическим условиям конкретных агроэкосистем.

Паспортная БД играет ключевую роль в сохранении, обеспечении доступности и использования широкого спектра генетического разнообразия растений для улучшения сельскохозяйственных культур. Создание БД по ржи было начато в 1980-х гг. в отделе серых хлебов ВИР, позднее данные из письменного каталога были перенесены в электронную версию. В настоящее время в паспортной БД по ржи есть 33 поля разной степени заполненности. Сформированная в программе Excel, она имеет текстовые и числовые поля, благодаря чему можно легко получить информацию об образце, ускорить выборку, поиск и обновление последней репродукции образца, отслеживать восстановление жизнеспособности каждого коллекционного образца ржи (Кобылянский и др., 2015).

Объем коллекции ВИР и ее видовое разнообразие

Коллекция ржи ВИР включает все многообразие диких, культурных видов и сорно-полевых форм, яровых и озимых, диплоидных и тетраплоидных, староместных и селекционных сортов. В коллекции представлены образцы (доноры и источники признаков озимой ржи), используемые в качестве исходного материала при селекции на такие признаки, как высокая урожайность, зимостойкость (морозостойкость, устойчивость к выпреванию), устойчивость к полеганию, устойчивость к прорастанию зерна в колосе, высокое содержание белка в зерне, устойчивость к болезням (мучнистая роса, бурая ржавчина, корневые гнили, снежная плесень и др.), низкопентозановые сорта. Образцы имеют разный селекционный статус: местные и сорно-полевые образцы – 43.7 %, селекционные сорта – 52.7 %.

Страны Европы: Россия (967 обр.), Беларусь (104 обр.), Украина (257 обр.), Болгария (114 обр.), Германия (86 обр.), Польша (169 обр.), Испания (26 обр.), Ита-

лия (23 обр.), Чехия (23 обр.), Югославия (107 обр.), Финляндия (191 обр.), Австрия (36 обр.), Норвегия (18 обр.), Швеция (73 обр.), Португалия (36 обр.), Дания (13 обр.), Франция (18 обр.). Основное поступление образцов в коллекцию из Европы приходится на селекционные сорта.

Страны СНГ: Азербайджан (176 обр.), Армения (118 обр.), Грузия (46 обр.), Казахстан (55 обр.), Кыргызстан (5 обр.), Таджикистан (60 обр.), Туркменистан (9 обр.), Узбекистан (12 обр.). Из стран СНГ, наоборот, преимущественно поступают местные и сорно-полевые образцы.

Остальные страны Европы и Америки, СНГ представлены в коллекции ВИР небольшим количеством образцов ржи (табл. 1). Самые крупные поступления в коллекцию за многолетнюю историю были из Европы – 2520 образцов, из СНГ – 495 образцов.

Table 1. The VIR rye collection size as of 2018

Region	Local and contaminating varieties	Elite varieties	Total
Europe	999	1521	2520
CIS	424	71	495
Asia	115	20	135
America	3	97	100
Australia	–	5	5
Africa	–	5	5
Total	1541	1719	3260

Table 2. Species composition of the collection of rye *Secale* L. (as of 2018) included in the main catalogue

Species	Number of accessions
Genus *Secale* L.	
S. cereale L.	3207
S. silvestre Host.	14
S. iranicum Kobyl.	1
S. montanum Juss.	38
Total	3260

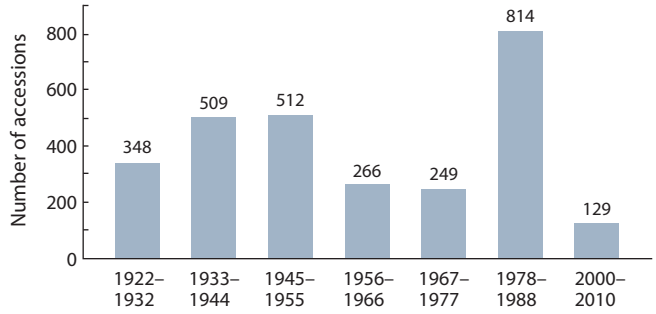

Fig. 2. The number of rye accessions coming into the VIR collection in different time intervals.

Статус образцов ржи

Более 90 % коллекции составляет рожь культурная *S. cereale* var. *vulgare* Körn. Это основной и практически единственный вид культурной ржи, возделываемый в РФ в качестве продовольственной культуры.

Остальные три вида являются дикими родичами культурной ржи. *S. silvestre* Host. Однолетняя рожь, по образу жизни растения яровые или озимые, слабозимостойкие, очень скороспелые, созревают на 15–20 дней раньше культурной ржи. Используется в селекции как донор короткостебельности и устойчивости к прорастанию зерна в колосе. *S. iranicum* Kobyl. Однолетнее растение, рожь иранская хозяйственного значения не имеет, сильно поражается всеми видами ржавчины, мучнистой росой и корневыми гнилями, но несет ценные для селекции ржи признаки самофертильности и короткостебельности. Используется в Германии для создания самофертильной короткостебельной культурной ржи путем гибридизации. *S. montanum* Juss. Многолетнее растение, объединяет все известные формы многолетней ржи, которые в разное время были описаны как самостоятельные виды. Широко вовлекается в скрещивание с культурной рожью при селекции многолетней кормовой ржи, а также для получения сортов, устойчивых к грибным болезням, ржавчине и мучнистой росе. Некоторые формы используют для скрещивания с пшеницей при селекции тритикале.

Яровую рожь высевают преимущественно в Забайкалье, где озимая рожь не зимует. Важное народно-хозяйственное значение имеет озимая рожь, которая возделывается в основном в европейских странах, несколько меньше – в Северной Америке, в странах Ближнего и Дальнего Востока (табл. 2).

Рожь – культура универсального применения. Свыше 60 % зерна используют для питания человека, 30 % – на кормовые цели, и 10 % имеют техническое назначение.

История создания коллекции ржи

В Российской Федерации создание коллекции ржи было начато в 1922 г. в Отделе прикладной ботаники при Ученом комитете Министерства земледелия и государственных имуществ под руководством Н.И. Вавилова. Благодаря многочисленным экспедициям Н.И. Вавилова и его соратников в разные страны мира, коллекция пополнялась в основном местными и сорно-полевыми образцами ржи. Первыми выведенными сортами были: рожь Лисицына (1929), Омка (1938), Онохойская (1943), Приекульская (1949), Вятка 2 (1950) и др. Подавляющее большинство возделываемых сортов ржи того времени не достигло максимального для этой культуры уровня урожайности. Сорта не удовлетворяли требованиям возрастающей интенсификации производства зерна: многие из них были высокорослыми, склонными к полеганию и прорастанию зерна в колосе, поражались болезнями или оказались недостаточно зимостойкими. Все сорта ржи склонны к поражению болезнями и вредителями.

«…Для обследования посевов хлебных злаков, в частности пшеницы и ржи», Н.И. Вавиловым в район Северного Кавказа и Предкавказья был командирован В.И. Антропов (Архив ВИР. Личное дело Антропова В.И. Фонд 5, оп. 2-1, д. 44, л. 8). Следует сказать, что уникальность кол-

лекции ржи ВИР состоит в том, что бóльшая ее часть собрана до 1940 г. Изучением морфологических признаков многих образцов, находящихся в коллекции, занималась В.Ф. Антропова (Антропова и др., 1970). Результаты описаний легли в основу внутривидовой классификации рода *S. cereale* L. – культурной ржи. Полученные новые морфологические данные вошли в монографию «Хлебные злаки. Рожь, ячмень, овес» из серии «Культурная флора СССР» (Антропов и др., 1936). Во время Великой Отечественной войны поступление новых образцов в коллекцию прекратилось. В первые послевоенные годы отдел возглавил А.П. Иванов. Следовало привести коллекции в порядок, восстановить всхожесть семян и оформить документацию, а также удовлетворить потребности государственных селекционных станций, научно-исследовательских и учебных учреждений и колхозников-опытников в исходном материале.

В 1948–1978 гг. во главе отдела серых хлебов (современное название – отдел генетических ресурсов, овса, ржи и ячменя) встала А.Я. Трофимовская. Под ее руководством были разработаны подходы к изучению всего разнообразия ячменя, овса и ржи. Группу ржи в 1964 г., после защиты кандидатской диссертации «Дикие виды ячменя», возглавил В.Д. Кобылянский, который провел исследования по филогении и систематике рода *Secale* L. с использованием морфолого-биологических, анатомических, генетических, цитоэмбриологических и кариологических методов. Он разработал современную ботаническую систему рода *Secale* L., включающую 4 вида и 9 подвидов. Им установлен один новый вид *S. iranicum* Kobyl. и 4 новых подвида: subsp. *vavilovii* (Grossh.) Kobyl. (рожь Вавилова); subsp. *tetraploidum* Kobyl. (рожь тетраплоидная); subsp. *derzhavini* (Tzvel.) Kobyl. (рожь Державина) и subsp. *tsitsinii* Kobyl. (рожь Цицина). В.Д. Кобылянский разработал внутривидовую классификацию культурной ржи *Secale cereale* L., включающую 40 разновидностей диплоидной и тетраплоидной ржи, в том числе 11 новых, а также создал определитель, облегчающий их распознавание. Данная ботаническая система рода *Secale* L. широко используется ведущими генбанками Европы.

Создание и использование доноров для селекции ржи

1. Гетерозисные гибридные сорта. Решение этой проблемы связано с открытием в 1962 г. у ржи явления цитоплазматической мужской стерильности (ЦМС). В ВИР были получены результаты, подтверждающие ЦМС, определен моногенный рецессивный контроль признака и созданы доноры ЦМС R-типа (русского типа). В генетической системе ЦМС получены стерильные линии (МС), закрепители стерильности (ЗС) и восстановители фертильности (ВФ). В коллекции ВИР такие линии созданы с высокой ОКС и СКС по комплексу хозяйственно ценных признаков, они могут служить донорами для улучшения сортовых популяций по лимитирующим селекционным признакам. Исследования по гетерозисной ржи проводили Н.С. Лапиков и А.Г. Катерова.
2. Короткостебельные неполегающие сорта. Заслуга В.Д. Кобылянского состоит в том, что он впервые осуществил генетическую дифференциацию признака короткостебельности у ржи и выделил неизвестный ранее доминантный ген *Hl*. Это открытие способствовало развитию нового направления селекции неполегающих сортов ржи. Первичные доноры признака – естественный мутант ЕМ-1, Малыш 72, и его производные, а также местная рожь (к-10028) из Болгарии.
3. Сорта, устойчивые к основным болезням. В.Д. Кобылянский совместно с О.В. Солодухиной, сотрудником отдела генетики ВИР, разработали стратегию селекции озимой ржи на устойчивость к грибным болезням: мучнистой росе, бурой и стеблевой ржавчине. Установлен генетический контроль признака иммунитета к этим болезням, идентифицированы гены и получены 63 эффективных донора групповой устойчивости ржи к московской и петербургской популяциям стеблевой ржавчины, бурой ржавчине, мучнистой росе, обладающих короткостебельностью и другими ценными селекционными признаками: Оргиб *Hl*, *Lr4*, *Sr1*, *Er*; Гетера 3 *Hl*, *Lr4*, *Sr1*, *Rm2*; Ловашпатонае 2 *Hl*, *Lr8*, *Er*; Ярославна 2 *Hl*, *Lr10*, *Sr1* и др. Созданы устойчивые к болезням сорта озимой ржи: Ника, Кировская 89, Снежана, Эстафета Татарстана, Эра и др.
4. Сорта с хорошими хлебопекарными качествами. Селекция ржи на качество зерна на протяжении всего исторического периода производства этой культуры была направлена на улучшение хлебопекарных свойств, среди которых главными являются низкая амилолитическая активность фермента альфа-амилазы, расщепляющего крахмал, и высокое содержание водорастворимых некрахмальных полисахаридов (пентозанов), обеспечивающих лучший подъем теста, структуру хлебного мякиша, определяющих свежесть и продолжительность хранения хлеба. Созданы и выделены сорта: Амилот, Альфа, Otello (Швеция).
5. Низкопентозановые сорта универсального использования. С 2004 г. в ВИР проводится работа по выведению популяционных сортов озимой ржи, пригодных для кормовой и комбикормовой промышленности. В 2016–2018 гг. в Государственный реестр селекционных достижений, допущенных к использованию, включены новые сорта низкопентозановой озимой ржи, созданные в ВИР: сорт Вавиловская (с участием Тульского НИИСХ), сорт Берегиня (с Котласской Россельхозакадемией), сорт Подарок (с Татарским НИИСХ), сорт Янтарная (с Уральским НИИСХ), сорт Красноярская универсальная (с Красноярским НИИСХ).

Хранение коллекции озимой ржи

Н.И. Вавилов уделял особое внимание сохранению собранного со всего мира генетического разнообразия культурных растений и их диких сородичей. Со временем любой образец коллекции может утратить свои качества и генетическую однородность в связи с частым пересевом образцов для восстановления всхожести. Для обеспечения жизнеспособности коллекционных образцов при минимальном числе пересевов их необходимо сохранять в контролируемых условиях в специализированных низкотемпературных хранилищах.

Современные низкотемпературные хранилища были установлены в 1976 г. на Кубанской опытной станции ВИР,

куда заложено 1806 образцов ржи, и в 2000 г. в зданиях ВИР в Санкт-Петербурге (Лоскутов, 2009). Коллекция ржи ежегодно закладывается на оперативное (+4 °С) и длительное хранение (–10 °С). На сегодняшний день на оперативное хранение заложено 2670 образцов ржи, а на длительное – 2380 образцов.

Изучение образцов коллекции ржи

Коллекционные образцы ВИР – это основной исходный материал для выведения новых сортов, которым широко пользуются селекционные учреждения РФ. От наличия хорошего, разносторонне изученного исходного материалы и правильного его подбора зависит успех селекционной работы. Наряду с комплексной полевой оценкой отделом генетических ресурсов овса, ржи и ячменя совместно с методическими отделами ВИР иследуется и выделяется ценный генофонд для решения проблем устойчивости к важнейшим заболеваниям, короткостебельности, качества зерна и зерновой продуктивности.

Изучение генетических ресурсов озимой и яровой ржи на всех этапах формирования коллекции ВИР было и остается важнейшим направлением работ, позволяющим систематизировать коллекцию, а также выявить источники и доноры ценных признаков. С целью дальнейшего расширения научных связей с селекцентрами, углубленного изучения и эффективного использования селекционного потенциала растительных ресурсов озимой и яровой ржи систематически издаются каталоги с характеристикой морфологических и хозяйственно ценных признаков, технологических свойств зерна, источников и доноров устойчивости к грибным болезням.

С 1970 по 2018 г. изучено свыше 2300 коллекционных образцов ржи, наибольшее количество составили сорта из России, Польши, Украины, Болгарии, Беларуси. По этим данным было издано 23 каталога мировой коллекции ВИР. Благодаря начатым еще в 1936 г. исследованиям разнообразия коллекции ржи, стало возможным целенаправленно снабжать селекцентры страны исходным материалом. Специалисты ВИР и селекционеры по ржи из 22 селекционно-семеноводческих учреждений тесно сотрудничают в рамках селекционных программ. Используя коллекцию ВИР по озимой ржи, селекционеры вывели 95 % всех допущенных к использованию сортов. В создании сортов участвовали отечественные селекционеры Ф.Т. Кондратенко, А.А. Гончаренко, У.С. Бамбышев, Т.Я. Ермолаева, А.А. Тороп, М.Л. Пономарева, С.Н. Пономарев, В.Д. Кобылянский, О.В. Солодухина, Л.И. Кедрова, Е.И. Уткина, Н.Г. Попов, К.П. Веселова, Н.Г. Пугач, Н.В. Трусов, К.Н. Курмангалин, В.Т. Васько, Л.С. Грачева, Г.С. Попова, М.А. Тимина, Н.С. Владимиров, Е.Г. Мухордов, А.Н. Ковтуненко, А.П. Романов, Г.А. Сюкова, Б.В. Попов, С.А. Кунакбаев, Н.И. Лещенко, В.А. Мызгаева, А.Х. Шакирзянов. На 2018 г. в Государственный реестр селекционных достижений России включено 80 сортов российской селекции, среди которых следует отметить новые сорта: Московская 12, Московская 15 (ФИЦ Немчиновка), Берегиня, Вавиловская, Красноярская универсальная (ВИР), Саратовская 6, Саратовская 7, Марусенька (НИИ сельского хозяйства Юго-Востока), Таловская 41, Таловская 44 (НИИ сельского хозяйства Центрально-Черноземной полосы им. В.В. Докучаева), Радонь, Тантана, Эстафета Татарстана, Подарок (ФИЦ Казанский научный центр РАН), Фаленская 4, Снежана, Рушник, Флора и Графиня (Федеральный аграрный научный центр Северо-Востока им. Н.В. Рудницкого), Эра, Славия, Памяти Попова (Ленинградский НИИ сельского хозяйства «Белогорка»), Синильга (ФИЦ Красноярский научный центр СО РАН), Влада, Сибирская 87 (ФИЦ Институт цитологии и генетики СО РАН, г. Новосибирск), Иртышская, Сибирь 4 (Омский аграрный центр), Паром, Алиса (Уральский НИИ сельского хозяйства), Чулпан 3, Чулпан 7, Памяти Кунакбаева (Башкирский НИИ сельского хозяйства) (Государственный реестр селекционных достижений РФ, 2018). Эти сорта отличаются высокой урожайностью, отличными хлебопекарными качествами, устойчивы к полеганию, меньше поражаются болезнями. Новые сорта Берегиня, Подарок, Красноярская универсальная, Янтарная допущены к использованию как низкопентозановые в 2018 г.

Заключение

В силу сложившийся традиции коллекцию генетических ресурсов ржи трактуют как базис исходного материала для селекции озимой ржи, выполнения основополагающих исследований и экспериментов. Систематическое предселекционное исследование сортов и образцов озимой ржи имеет свою предысторию и сложившую систему. В настоящее время оно осуществляется, прежде всего, для обнаружения среди образцов источников и доноров ценных признаков по устойчивости к заболеваниям, полеганию, по качеству зерна и др. Знание характера и генетических различий и взаимосвязей способствует не только целенаправленному привлечению в коллекцию ВИР нового исходного материала, но и повышению эффективности формирования выборок для селекционного использования.

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The Russian Brassicaceae collection – from N.I. Vavilov and E.N. Sinskaya till nowadays

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This article presents the history of the formation of the Russian state Brassicaceae collection maintained at the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). Nowadays this one of the world's richest collections encompasses more than 10,750 accessions of different status from 32 species and 11 genera: vegetable, fodder, oilseed, spicy, ornamental crops and continues to grow through collecting missions and exchange of material. The first intraspecific botanical and agrobiological ecologo-geographical divisions of many crops – cole, turnip, radish, small radish, Swede – were performed by E.N. Sinskaya and T.V. Lizgunova over years of research. These unique works have been continued by M.A. Shebalina and L.V. Sazonova: the cultivar types of Chinese cabbage and pakchoi have been determined; the development of the classifications of white cabbage, broccoli, small radish, turnip is being continued. The objective laws of variability of valuable biochemical traits are presented; a comparative analysis of nutritive and biologically active substances, primarily secondary metabolites, allowed us to determine specific biochemical compounds: those common for the related species *Brassica oleracea* and *B. rapa* but occurring in them at different frequencies and those unique for species, subspecies and separate cultivar types; this is the beginning of taxonomic studies. With phytopathological studies, the common diseases of Brassicas in the northwestern part of Russia were determined, and the level of their distribution and development depending on the crop was shown. Genetic studies of the Brassicaceae collections at VIR include DNA analysis to search for duplicates in the collections, to compare original seeds and the seeds after reproduction and to assess the authenticity of saved accessions, to assess biodiversity, including that of new material from collecting missions, to develop phylogenetic studies. Chromosome loci controlling flowering time, morphological and biochemical traits were determined by QTL analysis and association mapping, the molecular markers found are used for screening the collection and breeding material. The sources and donors of traits valuable for modern breeding directions have been found for use in various breeding programs.
Key words: Brassicaceae collection; formation; complex evaluation; biochemical and immunological studies; QTL analysis; association mapping.

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Российская коллекция Brassicaceae: от Н.И. Вавилова и Е.Н. Синской до наших дней

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Представлена история создания российской государственной коллекции семейства Капустные (Brassicaceae), сохраняемой в ФИЦ Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова (ВИР). В настоящее время это одна из самых обширных в мире коллекций, включающая более 10 750 образцов различного статуса, принадлежащих 11 родам и 32 видам овощных, кормовых, масличных, пряно-вкусовых и декоративных культур. Коллекция пополняется за счет регулярных экспедиционных сборов и обмена материалом. Первые внутривидовые ботанические и агробиологические эколого-географические классификации многих культур – капусты, репы, редьки, редиса, брюквы – были сделаны Е.Н. Синской и Т.В. Лизгуновой в процессе многолетнего изучения. Затем эти уникальные работы продолжили М.А. Шебалина и Л.В. Сазонова. Исследования проводятся в ВИР и сегодня: выделены сортотипы пекинской и китайской капусты, в стадии доработки находится агробиологическая классификация белокочанной капусты, брокколи, редиса и репы. В статье приведены результаты изучения закономерностей изменчивости ценных биохимических признаков. Так, сравнительный анализ накопления питательных и биологически активных соединений, прежде всего вторичных метаболитов, позволил установить для родственных видов капуста и репа общие компоненты биохимического состава, встречающиеся с различной частотой и уникальные у видов, подвидов и отдельных сортотипов, что является подходом к хемосистематике. Благодаря фитопатологическим исследованиям определены общие заболевания капустных культур на Северо-Западе России, показана степень распространения и развития болезней в зависимости от культуры. Генетические исследования коллекций Brassicaceae в ВИР включают ДНК-анализ для поиска дублетов,

сравнения оригинальных семян с репродукциями и установления аутентичности сохраняемых в генном банке образцов, оценки биоразнообразия, в том числе биоразнообразия нового экспедиционного материала, проведения филогенетических исследований. С помощью QTL-анализа и ассоциативного картирования капустных культур были установлены хромосомные локусы, контролирующие время перехода к цветению, морфологические и биохимические признаки, при этом найденные молекулярные маркеры используют для скрининга коллекционного и селекционного материала. Выделены источники и доноры ценных признаков по новым направлениям селекции для различных селекционных программ.
Ключевые слова: коллекция Brassicaceae; создание; комплексное изучение; биохимические и иммунологические исследования; QTL-анализ; ассоциативное картирование.

VIR Brassicaceae collections formation and modern condition

Family Brassicaceae Burnett is one of the largest, young, fast evolved family in plant kingdom with approximately 338 genera and 3709 species (Warwick, Hall, 2009), encompassing wild and many cultivated oilseed, vegetable, spicy, ornamental species that included in the basis of worldwide economy. N.I. Vavilov, famous botanist, phytopathologist and geographer, began to be Bureau of Applied Botany – future VIR – Director in 1920 and he had invited to VIR many scientists, among them E.N. Sinskaya, who worked with fodder, oilseed and rooted brassicas, T.V. Lizgunova, curator of vegetable cole collections. E.N. Sinskaya continued the Vavilov determination of centers of origin and biodiversity of cultural crops and their wild relatives (Vavilov, 1926), prepared a significant work "Historical Geography of Cultivated Flora" (1969).

The formation of VIR Brassicaceae collection had begun in 1923 after N.I. Vavilov visit West-European countries, USA and Canada (1921–1922) and from All-Russian agricultural exhibitions. In 1926 VIR scientists started to study 450 acc. of white cabbage and cauliflower, 150 turnip acc., 90 radish acc., 180 mustard acc.

1500 accessions of vegetable brassicas and 450 oilseed accessions were collected by N.I. Vavilov himself and his colleagues till 1940: for instance, in expeditions 1925–1929 231 acc. were collected by N.I. Vavilov in the countries of Mediterranean basin, Ethiopia, China, Korea, Japan, Taiwan, 360 acc. were collected by P.M. Zhukovskiy in Minor Asia and Syria, 90 acc. by V.V. Markovich in India, China, Japan, 241 acc. by E.N. Sinskaya in Japan. Collection had grown by exchange of valuable material with different Institutes and companies in Western Europe and North America. But only 20 % brassicas accessions were saved from this time during the 2nd World War. From 1941 till 1945 the collections were stored in Leningrad; a small part of valuable vegetable brassicas accessions was evacuated to Ural.

Resumption of VIR brassicas collection started from 1946, when loss material was collected from the same locations as in N.I. Vavilov time and bought from the same breeding companies like Vilmorin (France), Sluis en Groot, Nickerson-Zwaan B.V. (Netherlands), Dippe, Lembke (Germany), etc. The first round of new reproduction and new characterization and evaluation trials has begun from 1946. From 1950 VIR collections enriched very intensively. Local resources from ex former USSR, China, India, Japan, Syria, Turkey, and Australia were collected very full.

Nowadays the Russian state worldwide Brassicaceae collection keeping in N.I. Vavilov All-Russian Research Institute of Plant Genetic Resources (VIR) is one of the largest in the world; the largest Indian collection includes 11238 accessions, other large Brassicaceae collections are stored in United Kingdom (5329 acc.), Germany (4303 acc.), USA (4993 acc.), China (5500 acc.) and Japan (1831 acc.). In European data base 25901 brassicas are located. VIR Brassicaceae collection consists of 10759 accessions of 32 species belonging to 11 genera: vegetable, fodder, oilseed, spicy, ornamental crops. Among them it includes large collections of traditional crops: cole crops *Brassica oleracea* L. – 2420 accessions, Asian and European leafy and rooted crops *Brassica rapa* L. – more than 1500 acc., radish and small radish *Raphanus sativus* L. – 2390 acc., rapeseed *Brassica napus* L. *oleifera* Metzg. – 1300 acc., Indian mustard *Brassica juncea* (L.) Czern. – 1260 acc., and also old quite rare crops but becoming popular now in Russia again Swede *Brassica napus* L. *rapifera* Metzg., water cress *Lepidium* L. sp., salad rocket *Eruca sativa* L., turnip rape *Brassica rapa* L. *oleifera* Metzg., white mustard *Sinapis alba* L., false flax *Camelina* L. sp. and others.

According N.I. Vavilov worldwide collection should reflect natural biodiversity of cultural crops and their wild relatives and also general genepool of modern breeding achievements. The VIR collection includes the accessions of different status: landraces (30 %), old and advanced breeding cultivars (58 %), inbred and double haploid lines, hybrid populations, mapping populations (12 %). VIR Brassicaceae collections compose around 80 % unique accessions.

Nowadays big attention takes to collect local types and morphotypes with high level of resistance to biotic and abiotic stresses, valuable biochemical composition, decorative characters; introduction of the new for Russia crops and cultivar types; collection of the best world achievements on the newest breeding directions; addition of the new genetic material. Very important unique local accessions of white cabbage, turnip and radish have been collected during last five years in South part of Russia, Altay, Far East, and also in Armenia, Azerbaijan, Tajikistan, China. Earliest accessions of cauliflower, broccoli and kohlrabi have been received from the Dutch and Japanese breeding companies, turnip and leafy Asian crops accessions – from Chinese Institutions and a company from USA, vegetable types of Indian mustard – from Chinese Genbank.

In VIR the procedures of storage, regeneration, multiplication, distribution of accessions to users have been managed as in modern Genebank, including safety duplication of large part of collections in two locations: in St. Petersburg in the freezers under –10 °C and at Kuban station (Southern part of Russia) underground under +4 °C. Most accessions from the base collections stored in St. Petersburg under +4 °C for short-term storage. Most brassicas accessions have coefficient of safety duplication 2.5–2.8. 5 % brassicas accessions have been stored in Svalbard Global Storage. VIR Brassicaceae

crops and wild relatives Herbarium includes 1673 accessions of 105 species, 16 genera; weed Brassicaceae Herbarium includes representatives of 56 genera.

The main goals of PGR evaluation at VIR

Intraspecific division on the base of ecologo-geographical evaluation

The VIR evaluation strength is complete phenotyping of all accessions in the different ecologo-geographical zones of Russia according the same methodic during three years. The VIR evaluation data bases according original VIR descriptor include 47–55 morphological and phenological characters for vegetable crops, 18–23 characters for oilseeds, and separate immunological, physiological, and biochemical data bases too (Methodics, 1988, 1989).

As results of characterization and evaluation of brassicas collections for many years and phylogenetic studies VIR scientists created intraspecific ecologo-geographical classifications of cole, turnip, Swede, radish, Indian mustard and other mustard crops, prepared descriptors for brassicas and studied variability of many traits (Sinskaya, 1928, 1939, 1969; Lizgunova, 1959, 1965, 1984; Shebalina, 1974; Shebalina, Sazonova, 1985). They divided each cultivated species on subspecies, convarieties, varieties, botanical forms, agro-biological eco-geographical groups and cultivar types. This work is being continued in VIR nowadays. Need to note that the taxonomy and phylogeny of Brassicaceae is under discussion and determination in the world till now despite of great efforts to delimitate tribes and species. Many types of molecular markers have been carried out including different DNA regions as barcodes for taxonomic identification where ITS were the best (Warwick, Hall, 2009; Warwick et al., 2010).

All modern classifications of crops including molecular genetic works are finishing on botanical varieties and forms and modern researchers use agro-biological classifications developing by VIR scientists (Bradshaw, 2010; Wang, Kole, 2015). For example, *Brassica rapa* L. contains a number of crops with great economic importance of different use. After C. Linneus, F.I. Ruprecht, and L. Bailey E.N. Sinskaya described 10 separate species: *B. campestris* L., *B. chinensis* L., *B. chinoleifera* Viehoever, *B. dubiosa* Bailey, *B. glauca* Wittm., *B. narinosa* Bailey, *B. nipposinica* Bailey, *B. pekinensis* Rupr., *B. rapa* L., *B. trilocularis* Hook. et Thompson (Sinskaya, 1969). Modern classifications determined all these crops as subspecies and varieties into *Brassica rapa* species (Hanelt, 1986; Gladis, Hammer, 1992; Specht, Diederichsen, 2001), but final common intraspecific classification does not exist till now.

According to E.N. Sinskaya, turnip and turnip rape had a wild progenitor; it was form, closely related to cultivated winter turnip rape, with inclination for enlarged root formation. European forms were domesticated in Minor Asia, and Asian – in Afghanistan and North India. The oleiferous forms also could be domesticated separately: in Mediterranean center of origin and in Central Asia, Afghanistan and North-East India. In India three ecotypes of oleiferous *B. rapa* exist: yellow sarson, toria and the oldest brown sarson was evolved in North-East India from original initial plant of *B. rapa* (Sinskaya, 1928). Nowadays *B. rapa* is considered originated in two independent centers of origin – Mediterranean area as the primary center for oleiferous/rooted forms and Central Asia for turnip forms, then turnip distributed to different directions where different types were developed. In India the primitive types developed oilseed forms, in China – oilseed and leafy forms (Wang, Kole, 2015).

E.N. Sinskaya divided turnip *B. rapa* L. on two species *B. rapoasiatica* Sinsk. and *B. rapoeuropea* Sinsk., in the first she included oldest primitive Afghan form and originated from it Indian, Middle Asian, Japanese turnips, in the second – primitive Minor Asian turnips and originated from them European turnips and determined 9 eco-geographical groups: (1) Teltov; (2) Western European; (3) Russian yellow flash; (4) Intermediate type between Russian and Asian; (5) Minor Asian with distinct leaves; (6) Afghanis; (7) Japanese with entire leaves; (8) European with entire leaves; (9) Chinese with entire leaves without hairiness. Then M.A. Shebalina (1974) divided turnip on 5 varieties: European, Minor Asian, Iraqi, Central Asian, and Japanese; European variety divided on 25 cultivar types, but according her meaning, these cultivar types do not include all biodiversity of turnip cultivars. Now on need to divide the last M.A. Shebalina varieties on morpho-physiological cultivar groups.

E.N. Sinskaya, then M.A. Shebalina and L.V. Sazonova (1985) divided the radish *Raphanus sativus* L. on 3 subspecies: European, Chinese, Japanese, and each subspecies on varieties, correspondingly 6, 5 and 2. Small radish accessions have been divided on European and Chinese convarieties, including 4 and 2 varieties, generally corresponded of root colour, and 14 cultivar types. Follow by modern evaluation in VIR small radish collection was divided on 6 groups on the base of morphological characters of root, leaf rosette, phenological traits, resistance to bolting, etc., and DNA data (Artemyeva et al., 2017).

Accordind to E.N. Sinskaya opinion, rapeseed, turnip rape, black and charlock mustard are authentically unknown in a nature as wild forms. They seem to be originated from Mediterranean region, and probably had independent history of domestication at several spots. As results of investigation of the British oilseed rape samples, E.N. Sinskaya concluded that oilseed rape putatively amphidiploid, originated from the cross of ancient Atlantic form of wild kale with turnip rape (Sinskaya, 1960, 1969). According to De Candolle opinion, rapeseed has entered to Russia from Ukraine territory. This theory can be proved by a fact, that 20 % of the rapeseed landraces and old cultivars were collected at Ukraine (among accessions, collected before 1982).

T.V. Lizgunova believed the modern recognized cultural varieties of cole crops *B. oleracea* (Gómez-Campo, Prakash, 1999) by separate species in a composition of *B.* aggr. *oleracea* (L.) Lizg. (Lizgunova, 1965, 1984). She divided cole crops collections on cultivar types. Nowadays origin of some Russian and European white cabbage types – Savinskaya, Schweinfurter, Dobrovodske by hybridization of European and oriental types have been proposed; Italian sprouting broccoli have been divided on three types according the primer and second centers of origin Italy, USA and Japan (Artemyeva et al., 2017).

VIR Chinese cabbage collection intravariety classification has been done, 14 cultivar types have been determined:

into *B. rapa pekinensis dissoluta* Li – type Dunganskaya, var. *infarcta* Li – types Shantung, Siao, Nagasaki, Chirimen, var. *laxa* Tsen et Lee – Chosen, Kashin, var. *cephalata* Tsen et Lee – Chee-Foo (Wong-Bok) (subtypes Chee-Foo and Matsushima), Hotoren, Kaga, Aichi (subtypes Aichi and Nozaki), var. *cephalata* f. *cylindrica* Li – Granat (Michihli) (subtypes Granat and Khe-tou), Da-zin-kou, var. *cephalata* f. *depressa* Li Kensin. According large biodiversity into Granat cultivar type possibly to determine Khe-tou subtype as separate type. *B. rapa chinensis* collection has been divided on 5 cultivar types Taisai, Piorbai, Syusman, Yu Tsai, Lei choi (Artemyeva, 2001, 2004; Artemyeva, Abremskaya, 2017).

VIR brassicas collections have been structured. Genetic collection includes accessions with identified genes: for example, rapeseed genetic collection includes lines with identified genes controlled morphological traits, fatty acids composition, resistance to *Fusarium*, CMS polima system; pak-choi genetic collection includes double haploid lines with identified and localized chromosome loci determined many morphological, biochemical and immunological characters.

Trait collections present a system of intraspecific variability of studied traits, determination of the sources and creation of donors of these traits. Amplitude of natural variation of productivity, earliness, resistance to biotic and abiotic stresses, features of biochemical composition in brassicas is enormous. The limits of variation of quality traits in each crop have been determined, and accessions divided on statistically distinct groups. For instance pak-choi collection is divided for the quantity traits of plant size (plant diameter, height, lamina and petiole lenght and width) and productivity on 6–9 groups. The trait collections have been developed for all characters of breeding interest: vegetation period, weight, productivity, size, shape, colour of productive organ, habitus of leaf rosette, leaf morphological characters, resistance to diseases and insects, edaphic factors, cold, frost and heat, valuable nutritive and biologically active compounds, dwarfism. The agrobiological models of the future cultivars for different breeding directions and for different climatic conditions have been created in VIR for all brassicas crops.

Biochemical studies

Food nutrition is becoming one of the most important factors in the choice of products in modern conditions. *Brassica* crops are characterized by low caloric value; contain high quality protein, carbohydrates, fiber, minerals, and biologically active compounds: vitamins, enzymes, pigments, secondary metabolites. In humans, the last mentioned reduce risk of chronic diseases development having anticarcinogenic, antioxidant, antibacterial and antiviral effects, stimulate the immune system and reduce inflammation. Brassicas also prevent from development of cardiovascular diseases and illnesses associated with ageing. These arguments enable to recommend increased consumption of brassicas. In the number of researches analysis of bioactive compounds accumulation, the mechanisms of their action in Brassicaceae family and the biotechnological approaches enriching the content of antioxidant compounds in the plants have being considered (Raiola et al., 2018).

In VIR classical biochemical analysis of brassicas accessions, determination of resources of valuable compounds for breeding programs has been continued from 1936. 50–90 % accessions of collections from all cultivars types were evaluated for dry matter, sugars, vitamin C, organic acids, protein, carotene, chlorophylls, total glucosinolates and nitrates content. The cultivars types of all crops display a wide diversity of characters and among them the sources of nutritive and bioactive substances were revealed.

Nowadays objective laws of biochemical compounds accumulation by vegetable brassicas and their cultivar types have been found in VIR by metabolome analysis and chemosystematics studies using gas and liquid chromatography (GLC). Biochemical analysis of 180 accessions of cole crops, 320 accessions of *B. rapa* crops, including core collection, mostly leafy and rooted crops, 70 accessions of radish, 90 accessions of small radish and 10 accessions of vegetable Indian mustard have been studied, some of accessions both in the field and in greenhouse conditions. The large potential of variability of nutritive and bioactive substances between and within Brassicaceae species, subspecies, varieties, cultivar types was found.

Comparative biochemical analysis with chemotaxonomic issue for *B. oleracea* (white cabbage, tronchuda, cauliflower) and *B. rapa* leafy crops (Chinese cabbage, pak-choi, tatsoi, tsoisum, wutacai, mizuna, leafy turnip, broccoletto, oilseeds) has been done. For instance, in *B. rapa* crops 16 sugars (84 % monosugars) have been found, natural variation: 0.02–5.56 % (average 0.83 % per fresh matter), in *B. oleracea* crops – 15 sugars. The common sugars for two relative species were glucose, sorbose, mannose, galactose, fructose, sucrose; highest amount of glucose and sucrose have been observed. In some Chinese cabbage accessions tetra saccharide stachyose was found. Total 16 and 17 alcohols have been found in *B. rapa* and *B. oleracea* correspondingly, among them 8 biologically active. The common alcohol for two species was inositol; glycerol and sitosterol have been determined with high frequency. Variability of free amino acids content was significant for studied accessions: 8.5–716 mg/100 g (average 143 mg/100 g). Among 29 founded amino acids the common were glutamine, glutamine acid, asparagine, asparagine acid, serine, oxyproline – their content is highest, alanine and indispensable amino acids valine and threonine have been found common too. Other indispensable amino acids have been determined to be present with different frequency among crops, but mostly in headed Chinese cabbage and white cabbage.

In a study 35 and 32 organic acids have been found in two *Brassica* species, from that 23 and 18 acids were found in more than 15 % accessions; 9 organic acids were common, among them highest content of maleic, ascorbic, phosphoric acids have been noted. *B. rapa* accessions had a lot of fumaric acid. Some organic acids are biologically active, for instance pipecolic, abietic, ursolic acids. Pak-choi and leafy turnip were the richest by organic acids, although Chinese cabbage had the most content of phosphoric acid, broccoletto – caffeic acid. Among organic acids 13 phenol compounds have been determined; variability of them is from trace to 262 mg/100 g (average 44 mg/100 g). The common phenol compounds for *Brassica* is quinic acid, for *B. rapa* – sinapic and ferulic acids too. Abietic and caffeic acids and tocopherol were found in all *B. rapa* crops except Chinese cabbage, chlorogenic acid presented in pak-choi and tatsoi, hydroxycinnamic acid – in pak-choi only. Significant positive correlations between con-

tent of amino acids alanine and tyrosine and phenol compounds nicotinic, sinapic, benzoic acids and tocopherol have been found. Among founded free fatty acids – 17 in *B. rapa* and 12 in *B. oleracea* – common for *Brassica* was palmitic acid, with high frequency linoleic and linolenic acids have been determined, common for *B. rapa* crops – stearic and oleic acids.

Generally *B. rapa* crops composed significantly less nutritive compounds sugars than *B. oleracea* crops, but more or significantly more of all studied biologically active compounds. Valuable for human nutrition Brassicaceae accessions with high level of dry matter, sugars, ascorbic acid, biologically active alcohols, indispensable amino acids, phenol compounds, fatty acids have been found. Among *B. rapa* crops the most useful for human nutrition are tatsoi, pak-choi (cultivar types Leichoi and Yu Tsai), leafy turnip (hiroshimana type), late types of broccoletto; among cole crops – Russian and Dutch types of white cabbage, early types of cauliflower, tronchuda; among small radish – extra early red round and Chinese late types, among turnip – old Russian types Petrovskaya and Grobovskaya and French dry type, among Swede – Scandinavian types.

More than 700 accessions of rapeseed and turnip rape had been studied on oil content and fatty acids composition. Agricultural production is one of the main diesel fuel consumers. Emissions of internal combustion engines are one of the main causes of environmental pollution, so recently the requirements for the diesel fuel quality have become more stringent. In addition, the design of diesel engines is improving continuously which also requires the use of better quality diesel fuel. Biodiesel is synthesized from renewable raw materials; the sulfur content is lower than in diesel fuel and also diesel fuel has a higher cetane number and flash point. The range of raw materials for biodiesel production is constantly expanding. However, non-erucic rapeseed oil is a food product and its use for technical purposes is impractical. There are high-erucic technical varieties of rapeseed, but their crops are limited as spatial isolation from food rapeseed is required to avoid plant pollination. The cultivation of this crop requires certain costs due to the repeated use of insecticides against numerous insect pests during the growing season. In connection with the above, a comprehensive study of non-traditional Brassicaceae family oil crops less affected by pests was undertaken (Nagornov et al., 2017; Sainger et al., 2017). These crops include *Camelina sativa* (L.) Crantz, *Crambe abyssinica* Hochst. and *Eruca sativa* Mill. Studies conducted in VIR jointly with All-Russian Scientific Research Institute for the Use of Machinery and Oil Products in Agriculture have shown that the use of camelina, crambe and eruca raw material for biodiesel production is cheaper and environmentally friendlier compared to rapeseed. Biodiesel produced from these crops oils was obtained; their physical and chemical characteristics were established. It is established that biodiesel fuel created on the basis of these crops matches the requirements for this type of product (Romantsova et al., 2012).

Oilseed crops of family Brassicaceae as biodiesel resources were considered in a special study. 1038 accessions of rapeseed *(Brassica napus* ssp. *oleifera* Metzg.), 60 accessions of *C. sativa* L., 45 accessions of *C. abyssinica* Hochst. and 60 accessions of *E. sativa* Mill. from VIR collection were analyzed. Natural contents of erucic acid varied in rapeseed from 10 up to 61 %, but in food varieties there is less than 1 % of erucic acid. In camelina content of erucic acid varied from 2.6 up to 7.5 % (Ghamkhar et al., 2010). In crambe it varied from 54 up to 66 %, in eruca – from 42 up to 55 %. It is determined, that with the increase of erucic acid level the content of the saturated fatty acids (palmitic and stearic) decreases that is important for the increasing of biodiesel resistance to congelation. As a result the initial material for spring and winter rapeseed breeding with more than 40 % of erucic acid was described.

Immunological studies

Brassicaceae plants have been infected by bacterial, viral, many fungal diseases, more harmful in the central and Southern part of Russia on industrial growing. Resistance to diseases has been studied in VIR generally in the field natural conditions in St. Petersburg area, North-west Russia, to clubroot and black rot in artificial conditions. More than 1700 vegetable brassicas accessions have been tested, partly jointly with All-Russian Research Institute of Plant Protection, on level of distribution and degree of resistance to leaf spot (*Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schwein.) Wiltshire), clubroot (*Plasmodiophora brassicae* Woron.), black rot and leaf spot (*Xanthomonas* sp.), downy mildew (*Peronospora parasitica* (*brassicae*) (Pers.) Fr.), powdery mildew (*Erysiphe communis* f. *brassicae* Hammarl.), wilt (*Fusarium oxysporum* Schlecht. emend. Snyder & Hansen) (Gasich et al., 2013).

The most distributed pathogens in North-west area belong to *Alternaria* species; 80–100 % of white cabbage, cauliflower and Chinese cabbage accessions and 40–100 % of red cabbage accessions depending of year were susceptible to this pathogen, with low and middle degree of susceptibility 0.25–2.5. Several accessions of cauliflower from Russia, Australia, Japan are high tolerant to *Alternaria* leaf spot. The highest level of resistance showed the accessions with red colour of leaves and head: red cabbage, pak-choi, tatsoi, mizuna: 0.0–0.35.

Under artificial conditions in our studies tatsoi Bitaminna (k-213, Japan) was resistant to clubroot. High level of resistance to this pathogen has been shown by leafy turnip Kurona (k-264, Japan), local Chinese cabbage Shantai (k-68, China), turnip Mommersteegis Clubroot Resistant (k-1119, Sweden), Red Top Globe (k-1226, Denmark), Tartonda (Tetraploid) (k-1294, Germany). We can confirm that the sources of resistance have been originated from areas with strong development of clubroot.

Different species of genera *Xanthomonas* with several pathotypes cause diseases with different symptoms – black rot and leaf spot. Plant breeding on resistance is difficult because of less number of resistant sources and many races of the pathogen. Races 2 and 4 of *Xanthomonas campestris* Pam. (Dow.) are the most widely distributed, including Russia, Europe and Japan. High level of tolerance to these races was found in different varieties of *B. oleracea* L.

Susceptibility to downy mildew is common in *Brassica* crops: 10–50 % accessions of cole crops and Chinese cabbage were susceptible with degree 0.5–1.8, these meanings were less in red cabbage and pakchoi accessions. Several resistant to downy mildew accessions of cauliflower from Italy and Japan were revealed. Opposite powdery mildew is rare pathogen

in North-west part of Russia: distribution was 0–20 % with degree 0.2–1.0 on white cabbage, cauliflower and Chinese cabbage accessions, strong susceptibility has been noted on some kale accessions from Scandinavian countries and France. Wilt is also rare pathogen in this area, its development has been determined on several white cabbage accessions.

Resistance to the most harmful insect for brassicas cabbage root fly (*Delia radicum*) in Scandinavian resources of kale, turnip, Swede has been found, resistance to crucifer flea beetle (*Phyllotreta cruciferae*) – in Swede genotypes, mostly from Russia and central Europe, resistance to cabbage scoop (*Mamestra brassicae*) and cabbage butterfly (*Pieris brassicae*) – in kale and several local white cabbage accessions from Southern Russia and Balkan countries, that correlates with high level of glucosinolates content. Resistant sources in VIR collections are useful for the new breeding programs.

Genetic studies

The *B. rapa* whole genome sequence was a start of new era in Brassicaceae researches (Wang et al., 2011), then *B. oleracea* genome sequence (Liu et al., 2014; Parkin et al., 2014) and *B. napus* genome sequence (Chalhoub et al., 2014) were published. The reference genomes provided possibilities to explore the genetic variation by GWAS, for that large number of SNPs has to be generated.

DNA analysis has been used in VIR to search the duplicates in the collections, compare of original seeds and reproductions, evaluate biodiversity, variability within landraces, phylogenetic studies, screening collection and breeding material for marker assisted selection (MAS). QTL (Quantitative Trait Locus) analysis and association mapping have been developed.

Large variability within cole landraces has been determined in our investigations using SSR markers, when landraces compassed from 5–8 biotypes, and sometimes distance between biotypes into population is higher than between cultivars.

Morphological study of authenticity of VIR white cabbage collection after more than 10 rounds of reproduction and complicated regeneration procedure showed stability of morphological characters in core collection. Head weight is insignificantly increased during 50–70 years that can be explained by improved selection during reproduction process. Period of vegetation is decreased significantly in two locations; possibly it is connected with climate change. A set of 12 SSRs has been used for comparison of original seeds and the seeds after reproduction. 88 % coincidence between old/original and fresh seeds has been observed that permit to conclude good authenticity of VIR collection.

For QTL analysis a large number of studies – 40–50 articles per year last years – using different types of biparental mapping Brassicaceae populations have being developed to determine genetic control of flowering time, morphological traits from seed to seed, productivity, resistance to clubroot, mosaic virus, downy mildew, biologically active substances, mostly secondary metabolites. The bolting and flowering time is the most important characters in brassicas, but the genetic control of this process is complicated. Co-localized QTLs for different traits, generally for flowering time and plant size were found (Xiao et al., 2014).

For *B. oleracea* a major genomic region Ef2.1, harboring a robust flowering time QTL containing 29 genes, on chromosome 2 was identified using combined a next-generation sequencing-based whole-genome QTL-seq strategy and classical QTL analysis in mapping populations broccoli × cabbage. Major gene *BolGRF6* is a possible candidate gene for early flowering in broccoli line. The identified candidate genomic regions and genes may be useful for molecular breeding (Shu et al., 2018).

In VIR QTL analysis has been developed to identify chromosome loci related to a wide range of phenotypic, morphological, biochemical, immunological traits in *B. rapa* using two DH mapping populations resulted from crosses between leafy, rooted and oilseed lines created in Wageningen University, Netherlands, and *B. oleracea* using DH mapping population resulted from crosses between broccoli and kailan.

The *B. rapa* populations were evaluated for 47 morphological and phenological traits (bolting and flowering time, growth-related traits, leaf, seed, flower and fruit traits), for biochemical traits (content of protein, ascorbic acid, carotene, chlorophylls) and for resistance to four *Xanthomonas campestris* pv. *campestris* Pam. (Dow.) races. 140 QTLs controlling morphological and biochemical traits in DH30 и DH38 *B. rapa* L. and 10 and 11 co-adaptive blocks of genes correspondingly have been found (Artemyeva et al., 2016). The most important QTLs in both populations were in the bottom of A03, where *BrFLC5* that determines flowering time, productivity and biochemical traits is located. The inheritance of broad-spectrum quantitative resistance of *B. rapa* to *X. campestris* pv. *campestris* has been elucidated and 10 QTLs in DH30 and 19 QTLs in DH38 that control resistance to four races of the pathogen (1, 3, 4, 5) were found (Artemyeva et al., 2018).

In AGDH *B. oleracea* mapping population QTLs determined plant size traits have been found in the lower part of C01, C07, on the top of C04, C05, C09; QTLs for ascorbic acid content was found in the lower part of C01, C03, C08, protein content in the middle of C07, dry matter and carotenoids content in the lower part of C09.

Association mapping studies using collections of natural heterogeneous and heterozygous accessions and breeding material are widely developed in Brassicaceae. Pino Del Carpio et al. (2011) studied the secondary metabolite lutein, chlorophylls, carotene variation in *B. rapa* collection from 168 accessions. Wang, Kole (2015) proposed a strategy of combination both QTL and association mapping studies to identify candidate genes explaining trait variation.

Marker-phenotype significant associations in VIR *B. rapa* core collection of 96 accessions have been found for the same traits as in QTL analysis using 258 SSR and S-SAP molecular markers. Chromosome loci determined bolting time were found on the top of A02, in the bottom of A03, in the middle of A05 and A06, determined plant weight – in the bottom of A03, A09, in the middle of A05. 11 blocks of co-adapted genes for morphological traits were found; the most important blocks were located on the top of A01, A10, on the top and the bottom of A02, in the bottom of A03, in the middle of A05 and A06. The coincidence of positions of chromosome loci determined the same traits by QTL and association mapping on A02, A03, A05 and A06 have been found that judges a rightness of the results. Coincidence of positions of chromosome blocks controlling the biochemical traits on the

bottom of A03 and in the middle of A05 has been noted too. The most important loci for *B. rapa* on the top of A02, on the bottom of A03, in the higher part of A10 correspond to genes positions controlling flower time *BrFLC2*, *BrFLC5*, *BrFLC1*, that confirms importance of bolting time that determines many morphological and biochemical traits in *Brassica* plants.

Conclusion

Screening of brassicas accessions by SSR markers, associated with valuable traits, choosing in QTL analysis and association mapping, confirmed their perspectives for MAS. New VIR cultivars of pak-choi Meggy and VitaVIR, turnip Palitra, included in Russian State Register of Selection Achievements Admitted for Usage available to growing in a territory of Russia, and a lot of breeding material of all brassicas had been created in VIR using MAS.

VIR Brassicaceae collection is an extremely valuable tool for comprehensive fundamental and applied studies.

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