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ВАВИЛОВСКИЙ ЖУРНАЛ ГЕНЕТИКИ И СЕЛЕКЦИИ

СОДЕРЖАНИЕ • 2021 • 25 • 7

VAVILOV JOURNAL OF GENETICS AND BREEDING

CONTENTS • 2021 • 25 • 7

Шестая международная научная конференция PlantGen2021

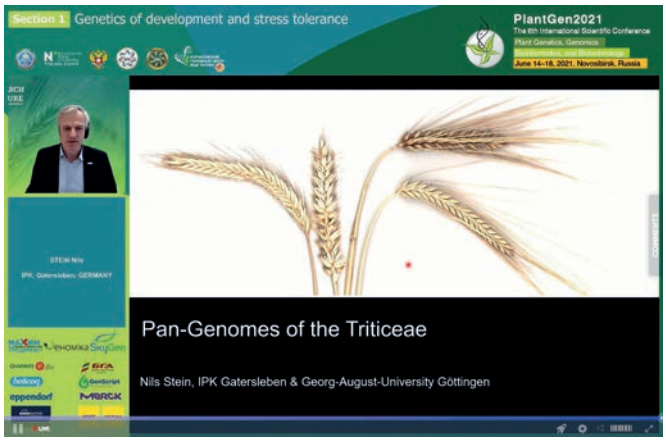


Дорогие читатели! Седьмой выпуск журнала тематический, он посвящен Шестой международной научной конференции «Генетика, геномика, биоинформатика и биотехнология растений» (PlantGen2021), которая состоялась 14–18 июня 2021 г. в новосибирском Академгородке. Главными организаторами конференции выступили Федеральный исследовательский центр «Институт цитологии и генетики Сибирского отделения Российской академии наук» (ИЦиГ СО РАН), Курчатовский геномный центр ИЦиГ СО РАН и Новосибирский государственный университет. Спонсоры конференции: ООО «Максим Медикал», ООО «Феномика», ООО «СкайДжин», ООО «Квадрос-Био», ООО «Компания Хеликон», GenScript Biotech (Netherlands) B.V., ООО «Био-Ген-Аналитика», ООО «Эпендорф Раша», ООО «Вайсс Климатехник», Компания «Мерк», ООО «Диаэм».

Конференция, проходившая как в очном, так и в онлайн-формате, включала в себя следующие секции: генетика развития и стрессоустойчивость; эволюционная и функциональная геномика; биотехнология растений в постгеномную эпоху; биоинформатика, системная биология растений и цифровое фенотипирование; промышленная биотехнология.

В работе PlantGen2021 приняли участие 317 ученых из 22 стран, в том числе 41 иностранный, что превысило число участников предыдущей конференции, состоявшейся в 2019 г., еще до начала ограничений, связанных с пандемией. Было представлено 240 докладов, из них 97 устных и 143 стендовых. Стоит отметить весомый показатель участников – молодых ученых: 41 % (130 человек). За время конференции зарегистрировано более 6000 заходов на ее официальный сайт, где транслировались заседания. Онлайн-трансляцию смотрели участники из 22 стран, наиболее активными среди них были представители из России, Казахстана, Германии, США и Белоруссии. Трансляцию смотрели также из Чехии, Молдовы, Украины, Узбекистана, Кыргызстана, Нидерландов, Армении, Азербайджана, Канады, Франции, Грузии, Индии, Саудовской Аравии, Южной Кореи, Таджикистана, Туркменистана и Англии.

На конференции были представлены результаты новейших исследований в области генетики, геномики,



биоинформатики и биотехнологии растений, обсуждены перспективные направления исследований (в том числе совместных) по фундаментальным и прикладным аспектам изучения генома растений. Многие доклады отражали историю развития тех или иных научных направлений в России. Вниманию слушателей были предложены новые результаты из Казани, Москвы, Новосибирска, Санкт-Петербурга, Уфы, где сегодня работают ведущие отечественные научные школы в области генетики растений.

Одновременно к участию в конференции были привлечены ведущие мировые эксперты по ряду наиболее «молодых» и популярных сегодня направлений. Например, с докладами о последних достижениях в геномном редактировании выступили Сергей Свиташев (США) и Иоганн Каумляйн (Германия).

Организаторы конференции планируют дальнейшее расширение программы, в частности, на PlantGen2023 запланировано выделить в отдельную секцию доклады по биохимической генетике и физиологии растений, увеличив число сообщений по этой тематике. Сегодня значительных успехов в этом направлении добились казанские ученые, и в связи с этим рассматривается вариант проведения следующей конференции в Казани.

С каждым разом расширяется число и география участников конференции PlantGen, что говорит о ее востребованности в мировом научном сообществе и служит объективным показателем ее успешности. Ученым есть из чего выбирать: число мероприятий биологической тематики год от года растет, но конференция PlantGen по-прежнему занимает среди них достойное место.

А.В. Кочетов,
член-корреспондент РАН, директор ИЦиГ СО РАН,
председатель конференции PlantGen2021

Е.А. Салина,
профессор, главный научный сотрудник ИЦиГ СО РАН,
сопредседатель конференции PlantGen2021

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Introgressions of *Vitis rotundifolia* Michx. to obtain grapevine genotypes with complex resistance to biotic and abiotic stresses

V.A. Volynkin[1], V.V. Likhovskoi[1], I.A. Vasylyk[1] ✉, N.A. Rybachenko[1], E.A. Lushchay[1], S.M. Gorislavets[1], V.A. Volodin[1], V.I. Risovannaya[1], E.K. Potokina[1, 2]

[1] All-Russian National Research Institute of Viticulture and Winemaking "Magarach" of the Russian Academy of Sciences, Yalta, Russia
[2] Saint Petersburg State Forest Technical University, St. Petersburg, Russia
✉ kalimera@inbox.ru

Abstract. *Vitis rotundifolia* Michx. is one of the species of the family Vitaceae, with resistance to both biotic and abiotic stresses. The present study reports new scientific knowledge about the inheritance of resistance to downy mildew, powdery mildew and frost by *V. vinifera* varieties from *V. rotundifolia*. Recombinant lines of three hybrid populations from the crossing of the maternal genotype ♀M. 31-77-10 with *V. rotundifolia* hybrids were used as the object of the study. As a result of laboratory screening, more than 40 % of recombinants of the ♀M. 31-77-10 × × [DRX-M5-734 + DRX-M5-753 + DRX-M5-790] population showed a high degree of frost resistance (–24 °C), while 6 % of transgressive recombinants were characterized by a very high degree of resistance (–27 °C). The maternal genotype ♀M. 31-77-10 does not carry alleles of resistance to powdery mildew at the *Run1* locus and in the field suffers from powdery mildew much more than the paternal genotypes. The prevalence of powdery mildew on vegetative organs in the three recombinant populations over the years varies on average between 3.2–17.1, 0.3–17.7 and 0.6–5.2 %, respectively. As a result, almost all recombinant genotypes that received a resistant allele from the paternal genome are highly resistant to powdery mildew.
Key words: grapes; *Vitis vinifera* L.; *Vitis rotundifolia* Michx.; backcrosses; biotic and abiotic stress; powdery mildew; frost; resistance; genes; introgression.

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Интрогрессии *Vitis rotundifolia* Michx. для получения генотипов винограда с комплексной устойчивостью к биотическим и абиотическим стрессам

В.А. Волынкин[1], В.В. Лиховской[1], И.А. Васылык[1] ✉, Н.А. Рыбаченко[1], Е.А. Лущай[1], С.М. Гориславец[1], В.А. Володин[1], В.И. Рисованная[1], Е.К. Потокина[1, 2]

[1] Всероссийский национальный научно-исследовательский институт виноградарства и виноделия «Магарач» Российской академии наук, Ялта, Россия
[2] Санкт-Петербургский государственный лесотехнический университет им. С.М. Кирова, Санкт-Петербург, Россия
✉ kalimera@inbox.ru

Аннотация. *Vitis rotundifolia* Michx. – один из видов в семействе Vitaceae, демонстрирующий устойчивость как к биотическим, так и к абиотическим стрессам. В процессе изучения получены новые научные знания о наследовании культурным виноградом от *V. rotundifolia* признаков устойчивости к патогенам, вызывающим милдью и оидиум, и к морозу. Объектом исследования служили рекомбинантные линии трех популяций от скрещивания материнской формы ♀M. 31-77-10 с гибридами потомства *V. rotundifolia*. Установлено, что признак морозостойкости, скорее всего, имеет полигенное наследование. По результатам лабораторного изучения, в популяции ♀M. 31-77-10 × [DRX-M5-734 + DRX-M5-753 + DRX-M5-790] более 40 % рекомбинантов характеризуются высокой степенью морозоустойчивости (–24 °C), в то время как 6 % трансгрессивных рекомбинантов – очень высокой степенью устойчивости (–27 °C). Материнский генотип ♀M. 31-77-10 не несет аллелей устойчивости к оидиуму в локусе *Run1* и сильнее, чем отцовские генотипы, поражается оидиумом в полевых условиях. Распространение оидиума на вегетативных органах в трех рекомбинантных популяциях в среднем за годы исследований колеблется в пределах 3.2–17.1, 0.3–17.7 и 0.6–5.2 % соответственно. Почти все рекомбинантные генотипы, получившие аллель устойчивости в локусе *Run1* от отцовского генома, обладают высокой устойчивостью к оидиуму.
Ключевые слова: виноград; *Vitis vinifera* L.; *Vitis rotundifolia* Michx.; беккроссы; биотический и абиотический стресс; мучнистая роса; мороз; устойчивость; гены; интрогрессия.

Introduction

Remote hybridization plays an important role in modern grape breeding. It allows combining in hybrid progeny traits of various *Vitis* species, which have significantly diverged in evolution; for example, high productivity and high berries quality of the *Vitis vinifera* L. varieties with resistance to biotic and abiotic stress of American *Vitis* species. Hybridization makes it possible, on the one hand, to obtain experimentally new forms and varieties, on the other hand, to study the relationship between genomes, structure and function of chromosomes, the patterns of inheritance of morphological and economically valuable traits. N.I. Vavilov (Vavilov, 1986) emphasized that employing remote hybridization is especially promising for the breeding of vegetative propagated plants, including grapes.

Significant success was achieved by grape breeders and growers in the development of interspecific hybrids and in the study of such important issues as the selection of parental pairs, dominance, coping with incapacity for hybridization, identifying the sources of inter-sterility and reduced fertility of hybrid plants. In contrast to the *V. vinifera* L. cultivars, many other *Vitis* species, native to North and Central America, especially *V. rotundifolia*, are distinguished by high resistance to pathogens, pests and frost. Therefore, breeders and grape growers have always found the creation of new cultivated varieties of grapes promising, combining productivity and quality of *V. vinifera* with resistance of American *Vitis* species, meaning to create a "perfect" grape variety. In the middle of the 20th century in Europe there even existed a "perfect variety" breeding program, which has been transformed in the 'Magarach' Institute into the breeding program "Analogue" (Volynkin et al., 2018). Currently, this breeding program has found its further development in the introgression of *V. rotundifolia* genes into *V. vinifera* genome (Volynkin et al., 2020a). It should be noted that the Institute of Viticulture and Winemaking 'Magarach' is one of the leading centers of grape breeding in the world (Volynkin et al., 2015), and its grapevine breeding program is based on the study of the world *Vitis* gene pool and international trends of viticulture (Volynkin et al., 2021a).

The significance of such a breeding program is explained by the fact that a considerable part of vineyards in the Russian Federation is located in the zone of risky viticulture and almost every year suffers from frost coupled with the intensive development of downy mildew (caused by *Plasmopara viticola* Berl. et De Toni) and powdery mildew (*Erysiphe necator* Schwein.). In these conditions, the period of growing season of grape plants is reduced. Besides, in winter, plants are exposed to temperatures lower than the biological adaptive capacity of this species allows.

The study of the inheritance of grape frost resistance in progeny made it possible to establish that the trait is determined, first of all, by biological specificity of a grape genotype. Some *Vitis* species die in mild frosts; others are able to survive in the most severe winters (Likhovskoi et al., 2019; Vasylyk et al., 2020). Frost resistance is also influenced by soil and climatic conditions as well as agrotechnical methods that provide plants with optimal conditions for nutrition, water supply and airing. Cultivated grapevine in natural field conditions usually do not achieve maximum frost resistance, since the conditions of their preparation for the winter period are often unfavorable (Pavloušek, Postbiegl, 2003; Xiaoyan et al., 2015; Polulyakh et al., 2017).

Diagnostics of the frost resistance of grape varieties plays an important role in breeding, because only if information about the degree of a trait assigned to a particular genotype is complete and accurate, it can be used as a source of a valuable trait in breeding (Kozma, 1998; Korbuly, 2000; Clark, Barchenger, 2015; Ivanisević et al., 2015; Gonçalves et al., 2016; Volynkin et al., 2020b, c). In modern research, scientists are searching for the ways of conducting express-diagnostics of the frost resistance degree based on correlations with morphological traits (Maltabar, Zhdamarova, 2012; Novikova, Naumova, 2018; Ilnitskaya et al., 2019; Volynkin et al., 2020d), or studying biochemical mechanisms of the resistance and adaptation of grape plants to environmental stress factors at the molecular level (Di Gaspero et al., 2007; Nenko et al., 2019; Ricciardi et al., 2021; Shen et al., 2021). The most complete and reliable information about the resistance of grape varieties to environmental stress factors can be obtained only as a result of combination of field and laboratory experiments (Korbuly et al., 2004; Read et al., 2004; Ulitin, Nudga, 2008; Zlenko et al., 2018).

The development of new grapevine varieties that ensure ecological purity of food based on genetically determined resistance to pathogens in combination with frost resistance is one of the priorities in modern grape breeding.

Materials and methods

Plant material. The studies were carried out in 2017–2020 in field and laboratory conditions. The object of the study was the recombinant lines of three populations obtained in the 'Magarach' Institute from the following crosses: ♀M. 31-77-10 × × [DRX-M5-734 + DRX-M5-753 + DRX-M5-790] (66 hybrids), ♀M. 31-77-10 × 2000-305-143 (43 hybrids) and ♀M. 31-77-10 × 2000-305-163 (30 hybrids). Hereinafter, they are referred to as populations 2-11, 3-11 and 4-11, respectively. The maternal genotype ♀M. 31-77-10 was obtained at the 'Magarach' Institute by crossing the cv. Nimrang (*V. vinifera*) with Seibel 13666 (a complex interspecific hybrid). In turn, the two paternal genotypes are progeny of the NC16-5 (*V. rotundifolia* × *V. vinifera*) backcrosses with various varieties of *V. vinifera*. To ensure the greatest reliability of the crosses performed, the maternal genotype taken for crossing possessed a functionally female type of flower, excluding the possibility of self-pollination.

Climatic conditions. The breeding plot was located in the South Coast of the Crimean Peninsula, on mild slopes of the South-West exposure, at an elevation of 123 m above the sea level. The breeding plot soils were rather heavy, clayey admixed with gravel.

The climate is mild warm Mediterranean sub-humid, characterized by a relatively small amplitude of daily and annual temperatures, with warm winters, mild hot summers and long warm autumns. The first frosts are usually registered in early December, and the last – in the middle of March. Thus, the growing season of grape begins from the first days of April finishing at the end of November. In very warm years, some late grapevine varieties retain their leaves until January.

Winter is mild, small frosts often alternate with frost-free periods. Frosts usually do not reach the level when damage of

buds on annual shoots is observed. In years of extremely cold winter the temperature drops to –12…–13 °C. Therefore, even non-frost-resistant varieties do not suffer from winter frosts in the Crimea. In the second half of March, with a noticeable increase in temperature, the buds begin to swell, and in the first or second decade of April – to burst. However, temperature rises relatively slowly in April and May due to proximity to the sea. The inhibitory effect of low temperatures also affects flowering, which is usually registered in the first half of June. The beneficial effect of the sea is observed in the second half of summer and in autumn when daily and monthly temperatures do not show any violent oscillations. Autumn is warm, mild dry, with a lot of sunny days. Summer and autumn months are characterized by a relatively low amount of precipitation and air humidity.

The conditions do not favor the distribution of such diseases as downy mildew, gray rot and anthracnose. Among fungal diseases powdery mildew causes the greatest harm to vineyards, while downy mildew spreads only sporadically. The most widespread grapevine pests on the South Coast of Crimea are phylloxera and European grape moth, which produces three generations per season here.

Laboratory testing of genotype resistance to low temperatures. The laboratory method of testing frost resistance was based on the recommendations of S. Pogosyan (1974) and M. Chernomorets (1985), with some methodology modifications (Zlenko et al., 2018). In short, the diagnostics of frost resistance of grape genotypes was carried out by stepwise hardening and freezing of two-eyed cuttings of mature shoots as follows: from +8 to +4 °C for 14 days (hardening stage I); from –3 to –5 °C for 11 days (hardening stage II); and –10 °C for 1 day (hardening stage III). Then cuttings were frozen stepwise in the temperature range: from –16 to –24 °C with a 2 °C temperature change interval; from –24 to –30 °C with an interval of 10 °C. After each of ten sequential freezing stages (–16 °C for 2 days; –18 °C for 3 days; –21 °C for 2 days; –24 °C for 2 days; –25 °C for 3 days; –26 °C for 2 days; –27 °C for 2 days) 5 cuttings of each genotype were placed to refrigerator with a temperature of +2 °C for 3 days for gradual defrosting. Then cuttings were water-soaked for 1 day and placed for sprouting in half-liter containers with water at a room temperature (+22 °C).

The assessment of frost resistance was carried out according to a 9 point scale of International Organization of Vine and Wine (OIV) descriptor, with the following points of resistance: 1 – very low (–15 °C), 3 – low (–18 °C), 5 – medium (–21 °C), 7 – high (–24 °C), 9 – very high (–27 °C and lower). The degree of genotype resistance to frost stress was determined after 4 weeks of sprouting in water by assessing the percentage of shoot development from buds after each stage of freezing. For a more objective assessment of the vine shoots vitality after freezing, the length of the developed shoots, the number and length of roots, as well as the development of inflorescences were additionally determined.

Determining the resistance to pathogens in the field. Phenotypic data were obtained by evaluating plants in the field against a natural infection background without the use of fungicides.

The nature and percentage of leaves damage were accounted according to generally accepted methods (Buga, 2007). Up to 30 leaves from different parts of a plant were examined on every accounting bush. Each season, we carried out two examinations: the first was performed 3 weeks after grape flowering, the second – at the beginning of grape ripening. The percentage of leaves affection and degree of disease development on leaves were determined using the following scale:

0 – no signs of affection;
1 – single and barely noticeable spots on leaves;
2 – up to 10 % of leaf surface is affected;
3 – 11–25 % of leaf surface is affected;
4 – 26–50 % of leaf surface is affected;
5 – more than 50 % of leaf surface is affected.

Disease development (*R*, %) for a specific genotype was calculated using the formula:

$$R = \frac{\Sigma(a \cdot b)}{N \cdot K} \cdot 100, \quad (1)$$

where, *a* is a score of the scale, according to which the lesion was evaluated in the experiment; *b* is the number of affected leaves within the range of this score; *N* is the total number of leaves evaluated (pcs); *K* is the highest score of the scale; and 100 is the conversion factor.

The data obtained were averaged, and then the results were interpreted according to the OIV international standards (O-452, O-454), where the degree of grape plant resistance to fungal pathogens was assessed by the degree of leaf affection using the following point scale: 1 – very low degree of resistance (extensive surface affection by the pathogen is more than 50 %); 3 – low degree of resistance (the area affected by the pathogen is 30–50 %); 5 – medium degree of resistance (the area affected by the pathogen is 20–30 %); 7 – high degree of resistance (weak pathogen affection – up to 10 %); 9 – very high degree of resistance (very small or no pathogen affection). For data analysis, the value of maximum degree of affection was used.

Laboratory testing of resistance to pathogens. In addition to the field evaluation, a phytopathological screening was carried out using the disk-test method. For the disk-test, the leaves of recombinant lines were collected in duplicate in June–July. The fourth and fifth young leaves starting from the shoot tip were taken from each hybrid plant. Grape plants, from which leaves were collected, were left unsprayed with fungicides. The disinfected leaves were placed in agar medium in Petri dishes. Visual assessment of lines resistance was carried out 6–12 days after inoculation using the OIV descriptors 452-1 ('Resistance degree of leaves to *Plasmopara viticola* in laboratory conditions (disk-test)'), 455-1 ('Resistance degree of leaves to *Erysiphe necator* in laboratory conditions (disk-test)') according to the above scale (Volynkin et al., 2021c).

Results

Resistance of grape genotypes of hybrid populations to low temperatures

Frost resistance was determined in laboratory conditions in 2019. The highest range of frost resistance variation (Fig. 1) among the genotypes of the studied cross combinations with the maternal form ♀M. 31-77-10 was observed in the population 2-11 (–15…–27 °C), which reflects diversity of

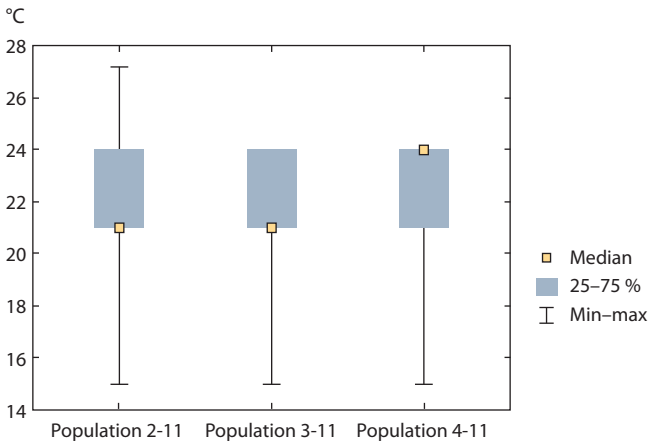

Fig. 1. Box-and-whisker plot reflecting variation of the trait "the lowest temperature in the experiment at which a plant survives" among the studied populations 2-11 (M. 31-77-10 × [DRX-M5-734 + DRX-M5-753 + + DRX-M5-790), 3-11 (M. 31-77-10 × 2000-305-143), and 4-11 (M. 31-77-10 × 2000-305-163).

the hybrids with varying degrees of frost resistance and, as a consequence, provides a broad spectrum of valuable genotypes as a source for breeding. This conclusion is confirmed by the calculated breeding value (45.5 %) of this cross combination (Table 1). The population 4-11 is distinguished by a higher average degree of resistance to low temperatures, and is characterized by the highest breeding value among the studied hybrid populations (56.7 % of genotypes inherited the high level of resistance of parental forms).

As a result of laboratory screening of the population M. 31-77-10 × [DRX-M5-734 + DRX-M5-753 + DRX-M5-790], about 40 % of recombinants were characterized by a high degree of frost resistance (–24 °C), and 6 % of transgressive recombinants showed a very high degree of resistance (–27 °C) (Fig. 2, see Table 1). In the populations M. 31-77-10 × × 2000-305-143 and M. 31-77-10 × 2000-305-163 (see Fig. 2), 44 and 56 % of recombinants, respectively, were characterized by a high degree of frost resistance (–24 °C).

In each studied population, there were several genotypes capable of sprouting 100 % of shoots from buds after freezing at –27 °C. In populations 2-11, 3-11 and 4-11, respectively 3, 7 and 17 % of such highly viable genotypes were discovered.

A specific combining ability was observed for each population. For example, in the combination M. 31-77-10 × 2000-305-163, almost half of progeny (56.7 %) has high frost resistance, whereas genotypes with true heterosis were not detected (Th = –16.2). Similar principle of seedling distribution was observed in the combination of M. 31-77-10 × 2000-305-143. Hybrids of the cross M. 31-77-10 × [DRX-M5-734 + DRX-M5-753 + DRX-M5-790] were distributed almost equally into groups of medium (42.4 %) and high (39.4 %) frost resistance. Genotypes with a true heterosis effect were identified in the population (Th = 14.5) (see Fig. 2).

The resistance of grape genotypes to *Erysiphe necator* and *Plasmopara viticola* in hybrid populations

The maternal genotype ♀M. 31-77-10 is not protected by the resistance alleles in the *Run1* locus and is much more affected

Table 1. Inheritance of resistance to low temperatures by grape genotypes in hybrid populations

Indicators	Populations		
	2-11	3-11	4-11
	M. 31-77-10 × × [DRX-M5-734 + DRX-M5-753 + + DRX-M5-790]	M. 31-77-10 × × 2000-305-143	M. 31-77-10 × × 2000-305-163
Number of seedlings, pcs	66	43	30
Points of resistance in parental genotypes:			
maternal ♀	7	7	7
paternal ♂	Nd	7	7
Distribution of seedlings in populations, according to points of resistance, %			
1 point (–15 °C)	3.0	4.7	3.3
3 points (–18 °C)	9.1	18.6	6.7
5 points (–21 °C)	42.4	32.6	33.3
7 points (–24 °C)	39.4	44.2	56.7
9 points (–27 °C)	6.1	0.0	0.0
Average point of resistance to low temperatures in the population	5.7	5.3	5.9
Breeding value of the population, %	45.5	44.2	56.7
Variation coefficient, %	11.6	12.5	10.4
Hypothetic heterosis, %	–4.5	–23.9	–16.2
True heterosis (Th), %	14.5	–23.9	–16.2

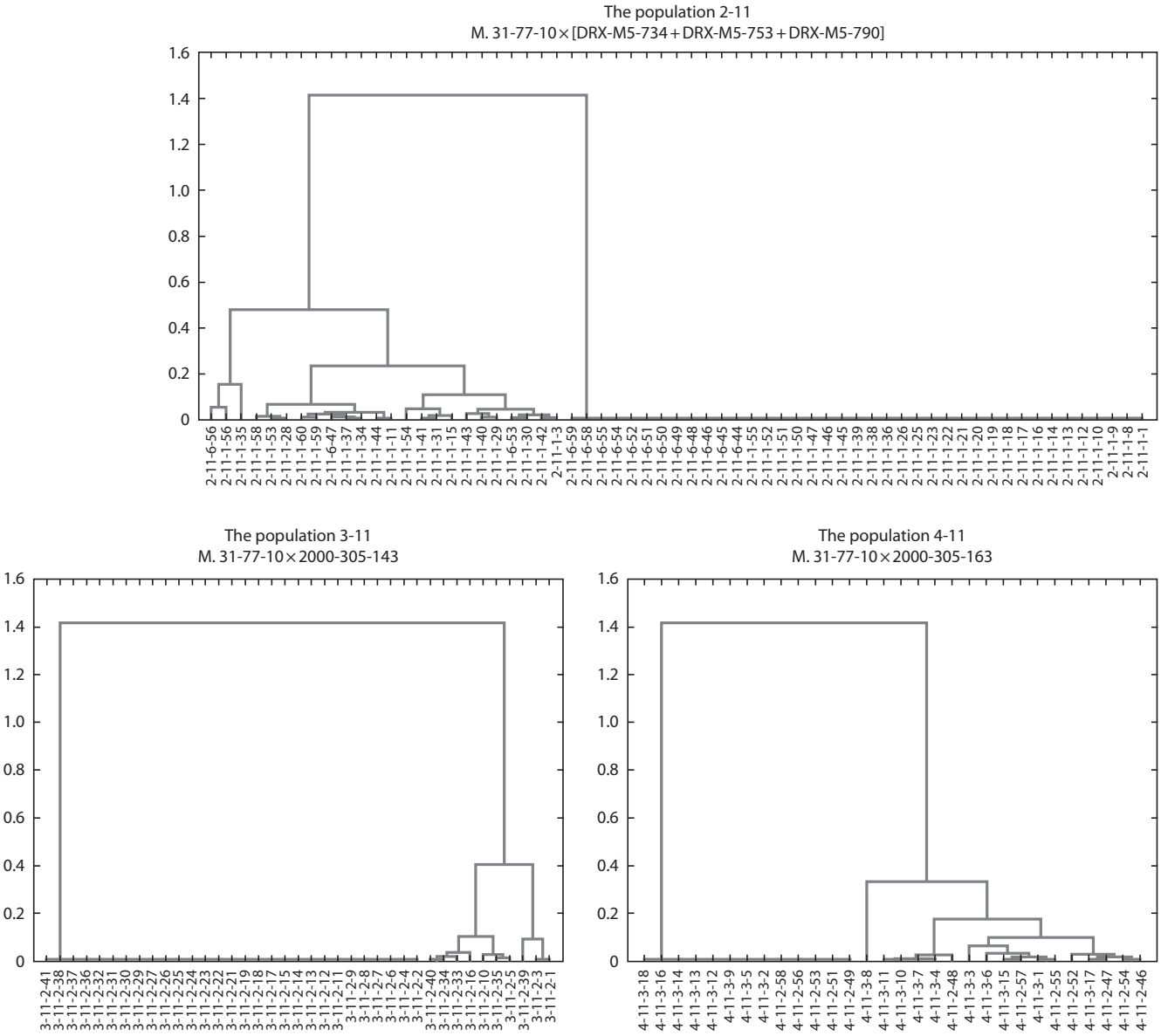

Fig. 2. Clustering of grape genotypes according to their degree of low temperature stress resistance, observed in the populations 2-11, 3-11, and 4-11.

by powdery mildew in the field compared to the paternal genotypes (e. g. 2000-305-143 and 2000-305-163) (Volynkin et al., 2021b). The percentage of oidium disease distribution on vegetative organs in the populations of recombinants varied over the years and for populations 2-11, 3-11 and 4-11 amounted to 3.2–17.1, 0.3–17.7, and 0.6–5.2 %, respectively. Due to the inheritance of resistant alleles from the paternal genome, some of the recombinant lines of hybrid populations showed a high level of resistance to *E. necator* (up to 26.7 %) (Table 2). Nevertheless, the average score for powdery mildew resistance among populations was lower than that observed for the paternal genotypes. The data obtained suggests that employing M. 31-77-10 as a parent in crosses with donors of resistance to *E. necator* allows to obtain a significant number of powdery mildew resistant genotypes in F_1.

The average scores of resistance to *P. viticola* established in the population 3-11 (M. 31-77-10 × 2000-305-143) and in the population 4-11 (M. 31-77-10 × 2000-305-163) were intermediate compared to parental genotypes (Table 3). The percentage of downy mildew distribution on vegetative organs in hybrid populations fluctuated in different years and amounted to 1.3–28.3, 0.2–14.8, and 0–18.6 % for populations 2-11, 3-11 and 4-11, respectively. Employing genotypes 2000-305-143 and 2000-305-163 in cross combinations as male parents allows producing 100 % sustained progeny. Remarkably, among the progeny of the cross M. 31-77-10 × [DRX-M5-734 + + DRX-M5-753 + DRX-M5-790] (population 2-11), 21.2 % of heterosis seedlings were observed to show the highest level of resistance (9 points).

The obtained results of the field evaluation of resistance to pathogens were confirmed by experiments on laboratory assessment of resistance using the disk test method (Volynkin et al., 2021c). The results indicate the great importance of remote hybridization of *V. vinifera* with *V. rotundifolia*, as well as derivatives of the cv. Seibel 13666 to obtain grapevine genotypes, resistant to fungi pathogens and frost.

Table 2. Inheritance of resistance to *Erysiphe necator* by grape genotypes in hybrid populations

Indicators	Populations		
	2-11	3-11	4-11
	M. 31-77-10 × × [DRX-M5-734 + DRX-M5-753 + + DRX-M5-790]	M. 31-77-10 × × 2000-305-143	M. 31-77-10 × × 2000-305-163
Number of seedlings, pcs	66	43	30
Rating of the trait in original forms:			
maternal ♀	7	7	7
paternal ♂	Nd	9	9
Distribution of seedlings in populations, %			
1 point	0	0	0
3 points	0	0	0
5 points	34.8	25.6	6.7
7 points	59.1	62.8	66.7
9 points	6.1	11.6	26.7
Average score of resistance in the population	6.4	6.7	7.4
Breeding value of the population, %	65.2	74.4	93.3
Variation coefficient, %	17.9	17.9	14.9
Hypothetic heterosis, %	–8.2	–16.0	–7.5
True heterosis (Th), %	–8.2	–25.3	–17.8

Table 3. Inheritance of resistance to *Plasmopara viticola* by grape genotypes in hybrid populations

Indicators	Populations		
	2-11	3-11	4-11
	M. 31-77-10 × × [DRX-M5-734 + DRX-M5-753 + + DRX-M5-790]	M. 31-77-10 × × 2000-305-143	M. 31-77-10 × × 2000-305-163
Number of seedlings, pcs	66	43	30
Rating of the trait in original forms:			
maternal ♀	7	7	7
paternal ♂	Nd	9	9
Distribution of seedlings in populations, %			
1 point	0	0	0
3 points	0	0	0
5 points	22.7	0	0
7 points	56.1	55.8	46.7
9 points	21.2	44.2	53.3
Average score of resistance in the population	7.0	7.9	8.1
Breeding value of the population, %	77.3	100.0	100.0
Variation coefficient, %	19.2	12.7	12.6
Hypothetic heterosis, %	–0.43	–1.45	0.83
True heterosis (Th), %	–0.43	–12.40	–10.37

Discussion

Among all grape species, the *V. rotundifolia* Michx. is the only one having a complex of biological properties, missing in *V. vinifera* L. grape varieties (Patel, Olmo, 1955).

Vitis rotundifolia is also the only native North American ancestor, the cultivated varieties of which were obtained without any genome introgression from other species of the *Vitis* genus, including *V. vinifera*. Difficulties in hybridization of genotypes of *V. vinifera* and *V. rotundifolia* are related to differences in the number of chromosomes (*V. vinifera* L., subgenus *Euvitis*, $2n = 2x = 38$ chromosomes; *V. rotundifolia* Michx., subgenus *Muskadinia*, $2n = 2x = 40$ chromosomes). For a long time, after such interspecific crossings attempts, breeders did not get fertile plants. The first fertile hybrid (F_1)

between *V. vinifera* and *V. rotundifolia*, the N.C. 6-15 hybrid ($2n = 2x = 39$) was obtained in the USA. Using a N.C. 6-15 hybrid, cross-pollinated with an unknown variety *V. vinifera*, R.T. Dunstan (1964) obtained the remote hybrid (F_2) – DRX-55 (Dunstan *Rotundifolia* crossing symbol) ($2n = 2x = = 39$). Of all remote grape hybrids, the DRX-55 was the only diploid-allotetraploid cytochimeric plant. Later on, other DRX hybrids were obtained from the same cross. By crossing remote hybrids F_4 DRX-M4-520, DRX-M4-510 ($n = 38$) with varieties GM-35-58, Cristal and Moldova, the fifth generation (F_5), combining hybrids with a somatic number of chromosomes $2n = 2x = 38$, was obtained (Alexandrov et al., 1998). Among the seedlings of hybrid population F_5, four synthetic genotypes were discovered, carrying a new grape genome with $n = 19$ ($2n = 38$), combining chromosomes of two species *V. vinifera* and *V. rotundifolia*.

In 2011, the pollen of three forms DRX-M5-790, -753, and -734 was kindly provided by Prof. Sh. Topale (Institute Vierul, Moldova) to the 'Magarach' Institute for hybridization experiments. At the same time, the hybrid genotypes 2000-305-143 and 2000-305-163 were received from Prof. R. Eibach (Federal Research Institute for Grape Breeding, Geilweilerhof, Germany). Those two genotypes were obtained by crossing French breeding line MTP3082-1-42, carrying resistance loci to powdery and downy mildew, with variety 'Regent'. The resistance loci were originally inherited from *V. rotundifolia* Michx.

In the USA, the Muscadine Grape Breeding Program is being developed in the University of Georgia. This is the oldest breeding program dedicated to the improvement of the muscadine grape. The UGA program began in 1909, and over the years has released over 30 cultivars. Current goals of the program include the development of new cultivars that combine large berry size with perfect flowers, earlier and later harvest dates, berries with dry stem scars and edible skins, and increased cold hardiness. The varieties can withstand frosts down to –25 °C (Morris, Brady, 2004).

Thus, *V. rotundifolia* can be considered as a potential donor of resistance genes to downy and powdery mildew pathogens in combination with frost resistance for breeding of new grape genotypes.

Conclusion

Remote hybridization involving *V. rotundifolia* can be considered as a modern and promising trend in grapevine breeding. It opens great prospects for obtaining new forms and breeding improvement of existing varieties, expands possibilities of creating new rootstocks and enriches the gene pool of cultivated grapes. It also provides a wide range of sources for breeding and conducting in-depth cytogenetic studies to reveal general patterns of diversity formation in F_1–F_5 interspecific hybrids, as well as for developing research sources for grape genetics.

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ORCID ID

V.A. Volynkin orcid.org/0000-0002-8799-1163
V.V. Likhovskoi orcid.org/0000-0003-3879-0485
I.A. Vasylyk orcid.org/0000-0002-8231-0613
N.A. Rybachenko orcid.org/0000-0001-5976-3756
E.A. Lushchay orcid.org/0000-0002-5695-5936
S.M. Gorislavets orcid.org/0000-0002-6749-8048
V.A. Volodin orcid.org/0000-0002-2842-6092
V.I. Risovannaya orcid.org/0000-0003-2208-798X
E.K. Potokina orcid.org/0000-0002-2578-6279

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Raise and characterization of a bread wheat hybrid line (Tulaykovskaya 10 × Saratovskaya 29) with chromosome 6Agi2 introgressed from *Thinopyrum intermedium*

Yu.N. Ivanova[1] ✉, K.K. Rosenfread[2], A.I. Stasyuk[3], E.S. Skolotneva[1], O.G. Silkova[1]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Novosibirsk State Agrarian University, Novosibirsk, Russia
[3] Kurchatov Genomic Center of ICG SB RAS, Novosibirsk, Russia
✉ kabanenko@bionet.nsc.ru

Abstract. Wheatgrass *Thinopyrum intermedium* is a source of agronomically valuable traits for common wheat. Partial wheat–wheatgrass amphidiploids and lines with wheatgrass chromosome substitutions are extensively used as intermediates in breeding programs. Line Agis 1 (6Agi2/6D) is present in the cultivar Tulaykovskaya 10 pedigree. Wheatgrass chromosome 6Agi2 carries multiple resistance to fungal diseases in various ecogeographical zones. In this work, we studied the transfer of chromosome 6Agi2 in hybrid populations Saratovskaya 29×Tulaykovskaya 10 (S29×T10) and Tulaykovskaya 10×Saratovskaya 29 (T10×S29). Chromosome 6Agi2 was identified by PCR with chromosome-specific primers and by genomic *in situ* hybridization (GISH). According to molecular data, 6Agi2 was transmitted to nearly half of the plants tested in the F_2 and F_3 generations. A new breeding line 49-14 ($2n = 42$) with chromosome pair 6Agi2 was isolated and characterized in T10×S29 F_5 by GISH. According to the results of our field experiment in 2020, the line had high productivity traits. The grain weights per plant (10.04 ± 0.93 g) and the number of grains per plant (259.36 ± 22.49) did not differ significantly from the parent varieties. The number of grains per spikelet in the main spike was significantly higher than in S29 ($p \le 0.001$) or T10 ($p \le 0.05$). Plants were characterized by the ability to set 3.77 ± 0.1 grains per spikelet, and this trait varied among individuals from 2.93 to 4.62. The grain protein content was 17.91 %, and the gluten content, 40.55 %. According to the screening for fungal disease resistance carried out in the field in 2018 and 2020, chromosome 6Agi2 makes plants retain immunity to the West Siberian population of brown rust and to dominant races of stem rust. It also provides medium resistant and medium susceptible types of response to yellow rust. The possibility of using lines/varieties of bread wheat with wheatgrass chromosomes 6Agi2 in breeding in order to increase protein content in the grain, to confer resistance to leaf diseases on plants and to create multiflowered forms is discussed.
Key words: alien introgression; chromosome substitution; GISH; molecular analysis; stem rust; brown rust; yellow rust; *Thinopyrum intermedium*; bread wheat.

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Получение и характеристика линии мягкой пшеницы (Тулайковская 10 × Саратовская 29) с интрогрессией хромосомы пырея *Thinopyrum intermedium* 6Agi2

Ю.Н. Иванова[1] ✉, К.К. Розенфрид[2], А.И. Стасюк[3], Е.С. Сколотнева[1], О.Г. Силкова[1]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Новосибирский государственный аграрный университет, Новосибирск, Россия
[3] Курчатовский геномный центр ИЦиГ СО РАН, Новосибирск, Россия
✉ kabanenko@bionet.nsc.ru

Аннотация. Пырей промежуточный *Thinopyrum intermedium* является источником агрономически ценных признаков для мягкой пшеницы, для передачи которых используют частичные пшенично-пырейные амфидиплоиды и линии с замещением хромосомами пырея. С использованием линии Агис 1 создан сорт яровой мягкой пшеницы Тулайковская 5, который входит в родословную сорта Тулайковская 10. В геноме сорта хромосома пшеницы 6D замещена хромосомой пырея 6Agi2, несущей комплексную устойчивость к грибным заболеваниям в различных эколого-географических зонах. В данной работе изучен характер передачи хромосомы пырея 6Agi2 в гибридных популяциях сортов Саратовская 29×Тулайковская 10 (С29×Т10) и Тулайковская 10×Саратовская 29 (Т10×С29). Хромосома пырея 6Agi2 идентифицирована с помощью хромо-

сомоспецифичных праймеров и методом геномной *in situ* гибридизации. Согласно молекулярному анализу, хромосома 6Agi2 передавалась почти половине изученных растений в F_2 и F_3 поколениях. В F_5 поколении Т10×С29 с помощью GISH выделена и охарактеризована новая селекционная линия 49-14 ($2n = 42$) с парой хромосом 6Agi2. По результатам эксперимента в полевых условиях 2020 г. линия имела высокие показатели продуктивности. Масса зерен с растения (10.04 ± 0.93 г) и число зерен с растения (259.36 ± 22.49) достоверно не отличались от родительских сортов. Число зерен на колосок в главном колосе у линии 49-14 было достоверно выше, чем у сортов С29 (при $p \le 0.001$) и Т10 (при $p \le 0.05$). Растения характеризовались способностью завязывать 3.77 ± 0.1 зерна на колосок, размах изменчивости признака варьировал от 2.93 до 4.62 у индивидуальных растений. Содержание белка в зерне составило 17.91 %, клейковины – 40.55 %. Согласно скринингу на устойчивость к грибным болезням, проведенному в полевых условиях 2018 и 2020 гг., хромосома 6Agi2 сохраняет у растений иммунность к западносибирской популяции бурой ржавчины и к доминантным расам стеблевой ржавчины, а также обеспечивает средний устойчивый и средний восприимчивый типы реакции к возбудителям желтой ржавчины. Обсуждается возможность использования линий/сортов мягкой пшеницы, несущих хромосомы пырея 6Agi2, в селекции на увеличение содержания белка в зерне, на устойчивость к листостебельным заболеваниям и на создание многоцветковых форм.
Ключевые слова: чужеродная интрогрессия; замещение хромосом; GISH; молекулярный анализ; стеблевая ржавчина; бурая ржавчина; желтая ржавчина; *Thinopyrum intermedium*; мягкая пшеница.

Introduction

Wild perennial common wheat relatives of the *Thinopyrum* genus are broadly polymorphic. They can be sources of commercially valuable traits: resistance to fungal and viral diseases (Friebe et al., 1996; Li H., Wang, 2009; Krupin et al., 2013, 2019; Davoyan et al., 2015; Leonova, 2018), tolerance of saline soils and drought, and high protein contents in the grain (Tsitsin, 1954; Upelniek et al., 2012). The *Thinopyrum* genus includes about 20 species of different ploidies: diploids, allotetraploids, allohexaploids, octoploids, and decaploids (Wang R., 2011). The genetic pools of two species are in the greatest use: elongate wheatgrass *Th. elongatum* (*Agropyron elongatum*) and intermediate wheatgrass *Th. intermedium* (*Ag. glaucum*). They became donors of genes for resistance to pests: *Lr19*, *Lr24*, *Lr29*, and *Lr38* to brown rust; *Sr24*, *Sr25*, *Sr26*, *Sr43*, and *Sr44* to stem rust; *Pm40* and *Pm43* to powdery mildew; *Bdv2* to barley yellow dwarf virus; and *Wsm1* to wheat streak mosaic virus (Li H., Wang, 2009).

Viable wheat–wheatgrass hybrids were first obtained by N.V. Tsitsin in 1930–1933. He crossed diploid, tetraploid, and hexaploid wheats to *Ag. elongatum* and *Ag. glaucum* (Tsitsin, 1954) and obtained octoploid forms of perennial and ratooning wheats known as intermediate wheat–wheatgrass hybrids, IWWHs (Tsitsin, 1954; Upelniek et al., 2012). Experiments on wheat hybridization to plants of the *Thinopyrum* genus were also carried out in the United States, Germany, Canada, and China. Various hybrid forms were obtained and annotated: partial amphiploids; high-protein addition, substitution, and translocation lines and forms resistant to barley yellow dwarf virus, wheat streak mosaic virus, powdery mildew, yellow rust, brown rust, and stem rust (Friebe et al., 1996; Fedak, Han, 2005; Li H., Wang, 2009; Chang et al., 2010; Hu L. et al., 2011; Fu et al., 2012; Zeng J. et al., 2013; Bao et al., 2014; Zheng et al., 2014; Danilova et al., 2017; Li D. et al., 2018).

Partial wheat–wheatgrass amphidiploids are used internationally for transferring valuable traits to common wheat (Jiang et al., 1993; Fedak, Han, 2005). In Russia, two groups of common wheat cultivars resistant to fungal pests have been raised via IWWHs at the Agricultural Research Institute of the South-East and the Samara Research Institute of Agriculture. In their genomes, wheat chromosome 6D is replaced by chromosome 6Agi from wheatgrass *Th. intermedium*. Chromosomes 6Agi1 and 6Agi2 are not identical, as they show different C banding patterns in Giemsa staining (Sibikeev et al., 2017). In the former case, 6Agi1 was inherited from substitution line S29-Agro139-M2-2, obtained by crossing spring common wheat Saratovskaya 29 to IWWH 139, and from cv. Mnogoletka 2. Then wheatgrass addition chromosomes recombined with each other (Sibikeev et al., 2017). The cultivars raised in Samara inherited wheatgrass chromosome 6Agi2 from substitution line Agis 1, obtained by crossing S29 to IWWH 644 (Sinigovets, 1976, 1988).

Since 1984, when Tulaykovskaya 5 was enlisted to the State Register of Selection Achievements, varieties with wheatgrass chromosome introgression bred in Samara retain their resistance to brown rust and powdery mildew in various ecogeographical regions of Russia (Salina et al., 2015; Leonova et al., 2017). It has been shown that the *Lr* genes on chromosome 6Agi2 are not allelic to the genes *Lr9*, *Lr19*, *Lr24*, *Lr29*, or *Lr47*, and the type of response to inoculation with *Puccinia triticina* Eriks. isolates confirms their not being allelic to *Lr19* or *Lr38* (Sibikeev et al., 2017). Testing of F_2 and F_3 hybrids of susceptible varieties with Tulaykovskaya 10 for brown rust resistance shows that chromosome 6Agi2 houses a locus for resistance to the West Siberian brown rust race (Salina et al., 2015). However, the copy number of resistance genes on 6Agi2 is still unknown. The loci have not been mapped on the chromosome either.

Molecular and cytogenetic markers are designed for detection of wheatgrass genetic material in the common wheat genome (Han F. et al., 2004; Li G. et al., 2016; Cseh et al., 2019; Kroupin et al., 2019). There are molecular markers specific to the *Th. intermedium* genome: simple sequence repeats (SSRs) (Ayala-Navarrete et al., 2010), mar-

kers designed on the base of expressed sequences (ESTs) (Wang M.J. et al., 2010; Danilova et al., 2017), and specific locus amplified fragments (SLAFs) (Li G. et al., 2016). There are several RFLP (Zhang Z.Y. et al., 2001), SCAR (Liu et al., 2007), and ISSR (Zeng Z.-X. et al., 2008) markers for *Pseudoroegneria spicata* (St genome), designed for identification of particular chromosomes of the St genome. The correspondence of wheatgrass chromosomes to homoelogical common wheat groups is tested with unique gene markers based on PCR (PLUG markers) (Ishikawa et al., 2009; Hu L. et al., 2014) and SNP markers (Cseh et al., 2019; Ma et al., 2019). Salina et al. (2016) designed markers specific to the long and short arms of *Th. intermedium* chromosome 6Agi2.

Varieties bred in Samara are used in Russian breeding programs (Martynov et al., 2016; Leonova, 2018). The goal of this work was to obtain breeding material with introgressed wheatgrass chromosome, test its commercially significant indices, and investigate the transfer of *Th. intermedium* chromosome 6Agi2 present in cv. Tulaykovskaya 10 by the example of a hybrid population with wheat cultivar Saratovskaya 29, which is a gold standard of grain quality. DNA markers specific to the long and short arms of 6Agi2 and genomic *in situ* hybridization (GISH) were used to identify the chromosome.

Materials and methods

Plants. Experiments were conducted with spring common wheat varieties Saratovskaya 29 (S29) and Tulaykovskaya 10 (T10) and with their hybrids S29 × T10 (generations F_2, F_3) and T10 × S29 (generations F_2–F_6). The hybrid generations were obtained by self-pollination of F_1 hybrids. Varieties S29 and T10 belong to the mid-season group. Saratovskaya 29 is highly susceptible to leaf diseases. Tulaykovskaya 10 is immune to brown leaf rust and medium-sensitive to powdery mildew (https://samniish.ru/pshenica-myagkaya-yarovaya-sort-tulajkovskaya-10.html).

Hybrids S29 × T10, generations F_2 and F_3, and T10 × S29, generations F_2, F_3 and F_5, were grown in a hydroponic greenhouse of the Laboratory of Artificial Plant Growth, Institute of Cytology and Genetics, Novosibirsk, in the autumn of 2017 and in the springs of 2019 and 2020, respectively. The temperature schedule was 22 °C in the daytime and 16 °C at night. The light/dark schedule was 16:8 h. Hybrid generations T10 × S29 F_4 and F_6 were grown in the field in the Moshkovo raion of the Novosibirsk oblast in the summers of 2018 and 2020, respectively; locality coordinates 55.14° N and 83.63° E.

Fluorescence *in situ* hybridization (FISH). Mitotic chromosome slides for FISH were prepared as in Ivanova et al. (2019). Use was made of the *Aegilops tauschii* pAet6-09 probe specific to chromosome centromeric repeats of rice, wheat, rye, and barley (Zhang P. et al., 2004) and wheatgrass genomic DNA isolated from *Th. intermedium* plants. A DNA sample of the pAet6-09 repeat was kindly provided by Dr. A. Lukaszewski (University of California, Riverside, United States). All slides were examined under an Axio Imager M1 microscope (Karl Zeiss, Germany). Images were captured with a ProgRes MF camera (Meta Systems, Jenoptic) in the Shared Access Center for Microscopy Analysis of Biologic Objects, Siberian Branch of the RAS, and processed with Adobe Photoshop CS2.

Plant DNA isolation. DNA was isolated from young leaves of hybrids and control plants with a Genomic DNA Purification Kit (Thermo Scientific, No. K0512) according to manufacturer's recommendations.

PCR analysis. DNA samples were analyzed with primers MF2/MR1r2 (amplicon size 347 bp) to the long arm of chromosome 6Agi2L of *Th. intermedium*, Te6HS476 (amplicon size 200 bp) to the short arm of chromosome 6Agi2S of *Th. intermedium*, and MF2/MR4 (amplicon size 328 bp) to the long arm of chromosome 6DL. The primers had been designed at the Laboratory of Plant Molecular Genetics and Cytogenetics, Institute of Cytology and Genetics (Salina et al., 2016). PCR was carried out in a Bio-Rad T-100 Thermal Cycler. The products were resolved in 1.5 % agarose gel with ethidium bromide and visualized with a Gel Doc XR+ gel documentation system (Bio-Rad, United States).

Assessment of commercially valuable traits. The T10 × S29 F_4 progeny selected with molecular markers was tested for resistance to brown rust *Puccinia triticina* Eriks. and stem rust *P. graminis* Pers. in field experiments in 2018. The F_6 progeny selected by molecular cytological analysis was tested for resistance to brown rust *P. triticina* Eriks., stem rust *P. graminis* Pers., and yellow rust *P. glumarum* Eriks. et Henn. in the field in 2020. The following parameters were recorded in generation F_6 selected by molecular and cytological methods in the field in 2000: the sprouting–flowering interval, plant height, productive tillering, main spike length, number of spikelets in the main spike, number of grains in the main spike, grain weight of the main spike, number of grains per spikelet in the main spike, grain number per plant, grain weight per plant, 1000 grain weight, and contents of protein and gluten in the grain. Grains were sown on May 9, 2020, in plots of 70 cm in width, 15 grains per row, and 25-cm intervals between rows.

The degree of injury by fungal pests was assessed according to the CIMMYT scale (Koyshybaev et al., 2014). The contents of protein and gluten were measured with an infrared OmegAnalyzer G (Bruins, Germany). The time from the mass-scale appearance of sprouts till the first appearance of yellow anthers in middle spikelets of spikes was taken to be the sprouting–flowering interval. Flowering dates were recorded in individual spikes. The significance of differences between two mean values of two samples was assessed by Student's *t* test.

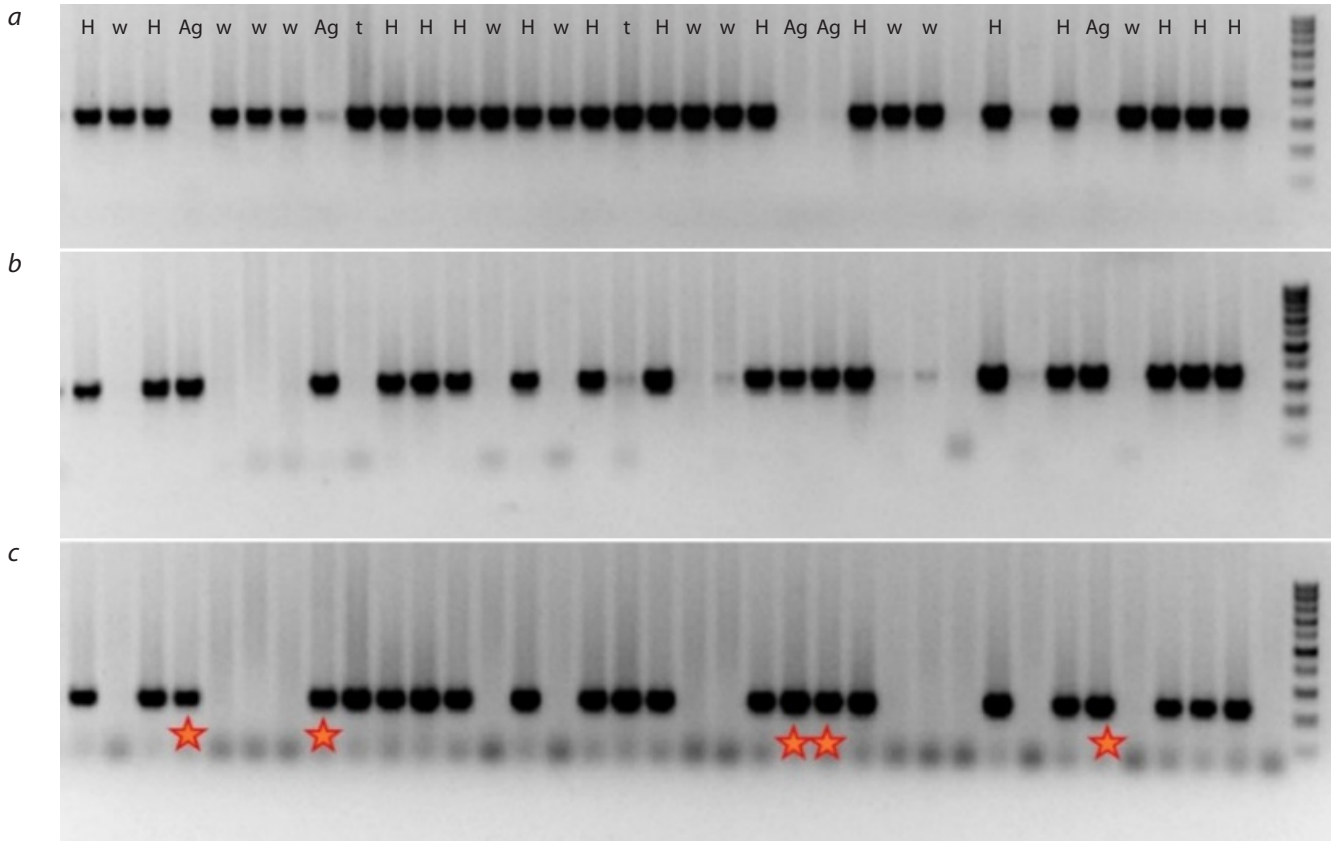

Fig. 1. Electrophoretic image of the amplification of markers in F_2 plants of the S29×T10 hybrid. Markers: *a*, to the long arm of chromosome 6DL; *b*, to the short arm of 6Agi; *c*, to the long arm of 6Agi.

Stars indicate plants with 6Agi2/6D substitution. Designations follow the text body and Table 1.

Table 1. The presence or absence of chromosomes or chromosome arms in generations $F_{2–3}$ of the S29×T10 and T10×S29 hybrids according to PCR data

Generation	Number of DNA samples tested	6AgiL only (t type)	6AgiS only (t type)	6Agi is present (Ag type or H type)	6Agi is absent (w type)
		Number/%			
F_2 S29 × T10	116	9/7.56	7/5.88	50/41.8	48/40.34
F_3 S29 × T10	20	2/10	4/20	3/15	11/55
F_2 T10 × S29	45	0	14/31.1	12/26.7	19/42.2
F_3 T10 × S29	35	1/2.86	4/11.43	14/40	16/45.71

Results

Identification of wheatgrass chromosome 6Agi2 in generations $F_{2–3}$ of the S29×T10 and T10×S29 hybrids with chromosome-specific primers

Chromosomes 6Agi2 of wheatgrass and 6D of wheat were present in the F_1 of S29×T10 and T10×S29 in the univalent state. Therefore, their presence or absence in DNA samples from generation F_2 was tested by PCR with primers specific to the wheatgrass chromosome. We tested 116 and 45 DNA samples from F_2 S29×T10 and T10×S29, respectively, and found samples with the absence of amplification with two primer pairs for the short and long arms of chromosome 6Agi2 and with amplification of the marker to chromosome 6D. Thus, there were no 6Agi2/6D substitution in these samples, designated as wheat (w) type (Fig. 1, Table 1).

The presence of chromosome 6D was also proven in samples with amplification of markers to either long or short arm, being indicative of the presence of telocentrics (t type; see Fig. 1, Table 1). Altogether, 12 telocentrics for the long arm and 29 telocentrics for the short arm were

detected in samples of generations F_2 and F_3, and the ratio of telocentrics for the short and long arm depended significantly on the cross direction. Telocentrics for the long arm were very rare in the T10 × S29 cross.

The presence of amplification fragments with two markers to the short and long arms pointed to the presence of the whole chromosome 6Agi2. With regard to the presence or absence of chromosome 6D, we suggest either full 6Agi2/6D substitution (Ag type) or the heterozygous state of the chromosome in the samples (H type).

For further analysis, plants with amplification of markers to the short and long arm of the wheatgrass chromosome were selected.

Karyotyping of generation F_5 of T10 × S29 hybrids

To verify the presence of one or two wheatgrass chromosomes in chromosome sets and to confirm stable inheritance of the substitution, we performed GISH of mitotic chromosomes at various self-pollination stages. The analysis of plants bearing substitutions according to PCR revealed 42 chromosomes, of which two were whole wheatgrass chromosomes (Fig. 2). Their long arms housed a large subtelomeric heterochromatin block, which is consistent with the locations of Giemsa C bands on chromosome 6Agi2 in Tulaykovskaya 10 (Sibikeev et al., 2017). The centromere-specific pAet6-09 repeat located on wheatgrass chromosomes showed weak signals, to demonstrate the poor hybridization of the repeat to centromeric DNA of wheatgrass chromosomes.

In situ hybridization confirmed the stable inheritance of the 6Agi2/6D substitution through generations.

Commercially valuable traits in T10 × S29 generations F_5 and F_6

Tulaykovskaya 10 is present in the pedigrees of many modern common wheat varieties. Its use in the breeding of new forms is based on its locus for brown leaf rust resistance, mapped on wheatgrass chromosome 6Agi2. In spite of the replacement of chromosome 6D by alien chromosome 6Agi2, the variety shows high grain yield, drought tolerance, and good baking quality (https://samniish.ru/pshenica-myagkaya-yarovaya-sort-tulajkovskaya-10.html).

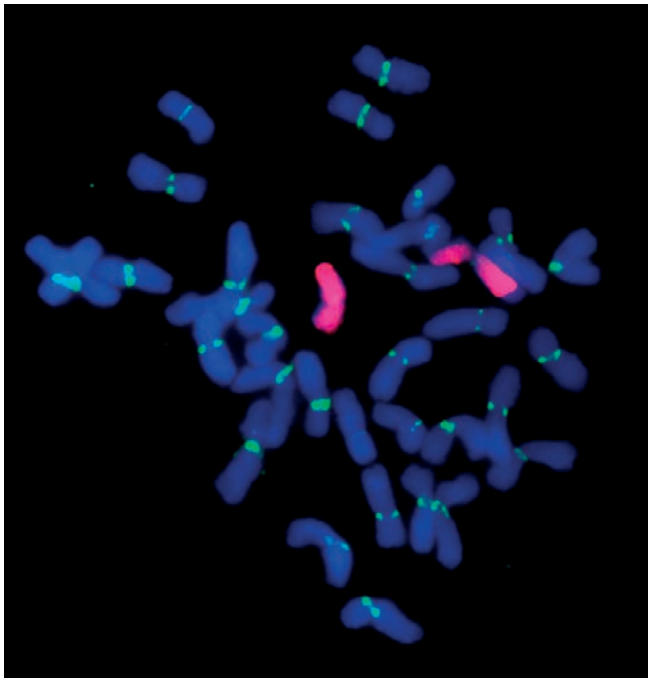

Fig. 2. Chromosomes of generation F_5 of T10 × S29 stained by GISH.
The two wheatgrass chromosomes are stained red, and centromeric regions, green.

Three lines were raised from F_4 plants of T10 × S29 with identified wheatgrass chromosomes: 33-2, 34-1, and 35-45. Analysis of the performance of T10, S29, and T10 × S29 F_5 lines grown in a hydroponic greenhouse showed that all the lines significantly outperformed T10 in all indices (Table 2). As compared to S29, the lines did not differ in productive tillering; lines 34-1 an 35-45 did not differ in grain number per plant or grain weight per plant; and in line 33-2, these indices were significantly lower. None of the lines outperformed S29 in 1000 grain weight; this index was significantly lower.

We selected the most productive plants of generation F_5 of line 35-45 to analyze performance indices and the duration of the sprouting–flowering interval in plants grown in the field in 2020. Thus, daughter line 49-14 was selected from the chosen segregating line 35-45.

Table 2. Performance indices in lines compared with varieties S29 and T10 (spring, 2019)

Index	T10	S29	33-2	34-1	35-45
Productive tillering, number of tillers	3.3 ± 0.03	4.9 ± 0.3	$4.6 \pm 0.2^{\#\#\#}$	$5.1 \pm 0.2^{\#\#\#}$	$5.5 \pm 0.3^{\#\#\#}$
Grains per plant	60.5 ± 2.8	154.0 ± 8.9	$109.7 \pm 5.8^{***\,\#\#\#}$	$165.3 \pm 6.5^{\#\#\#}$	$186.5 \pm 8.5^{*\,\#\#\#}$
Grain weight per plant, g	2.3 ± 0.1	6.8 ± 0.4	$3.9 \pm 0.2^{***\,\#\#\#}$	$6.4 \pm 0.3^{\#\#\#}$	$7.6 \pm 0.4^{\#\#\#}$
1000 grain weight, g	39.1 ± 0.4	43.9 ± 0.6	$35.1 \pm 0.9^{***\,\#\#\#}$	$38.5 \pm 0.3^{***\,\#\#\#}$	$41.05 \pm 0.5^{***\,\#\#\#}$
Total no. of plants	43	28	47	57	57

Note. Difference from S29 significant at $^{*}p \le 0.05$; $^{***}p \le 0.001$. Difference from T10 significant at $^{\#\#\#}p \le 0.001$.

Table 3. Performance and grain quality indices in the offspring of line 49-14 and cvs. T10 and S29, summer of 2020

Index	S29	Line 49-14	T10
Plant height, cm	108.33 ± 1.63	106.25 ± 1.44	98.54 ± 1.32$^{###}$
Productive tillering, number of tillers	6.63 ± 0.43*	5.27 ± 0.42	5.04 ± 0.4$^{#}$
Main spike length, cm	11.08 ± 0.31	10.75 ± 0.26	10.35 ± 0.19
Spikelet number in the main spike	17.25 ± 0.27	16.86 ± 0.25	18.02 ± 0.29$^{##}$
Grain number in the main spike	51.75 ± 1.62***	63.36 ± 2.19	56.66 ± 1.77$^{#}$
Grain number per spikelet in the main spike	3.01 ± 0.09***	3.77 ± 0.1	3.40 ± 0.08$^{#}$
Main spike density	1.57 ± 0.03	1.58 ± 0.03	1.68 ± 0.03$^{#}$
Grain weight in the main spike, g	2.51 ± 0.09	2.66 ± 0.13	2.42 ± 0.09
Grain number per plant	249.38 ± 19.14	259.36 ± 22.49	216.68 ± 19.18
Grain weight per plant, g	10.62 ± 0.89	10.04 ± 0.93	8.49 ± 0.83
1000 grain weight, g	42.53 ± 0.73***	38.44 ± 0.59	37.45 ± 0.91
Protein, %	15.88 ± 1.02	17.91 ± 1.23	18.81 ± 0.73
Gluten, %	35.56 ± 1.63	40.55 ± 2.47	40.00 ± 0.88

Note. Differences between S29 and 49-14 significant at $^{*}p \le 0.05$; $^{***}p \le 0.001$. Differences between T10 and 49-14 significant at $^{#}p \le 0.05$; $^{##}p \le 0.01$; $^{###}p \le 0.001$.

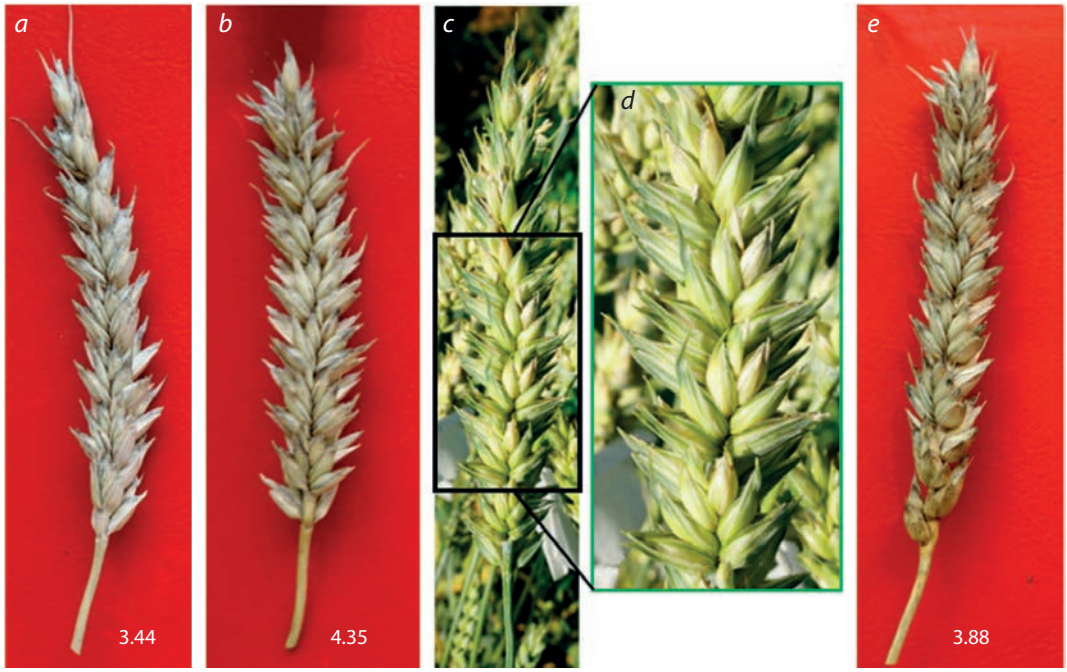

Fig. 3. The main spikes of plants with the best numbers of grains per spikelet in the main spike: *a*, S29 (3.44); *b*, line 49-14 (4.35); *c*, spike of line 49-14 at the waxy maturity stage; *d*, the zoomed central spike portion in (*c*); *e*, T10 (3.88).

Phenological observations revealed the shortest sprouting–flowering interval in line 49-14 (50.6 days), and in S29 and T10 it was one day longer. The flowering durations in the main spikes of individual plants were 11 days in 49-14, 10 days in T10, and 9 days in S29.

Comparison of performance indices in 49-14, S29, and T10 revealed no difference in main spike length, grain weight in the main spike, grain weight per plant, or grain number per plant (Table 3). Plants of line 49-14 were significantly taller than T10 but did not differ in height

from S29. Productive tillering and main spike density in 49-14 showed significant ($p \leq 0.05$) differences from the cultivars. The number of grains in the main spike in 49-14 was significantly greater than in S29 ($p \leq 0.001$) or T10 ($p \leq 0.05$).

The number of grains per spikelet in the main spike of line 49-14 was significantly higher than in S29 ($p \leq 0.001$) or T10 ($p \leq 0.05$). Line 49-14 set 3.77 ± 0.1 grains per spikelet on the average, and this trait varied among individual plants from 2.93 to 4.62 (Fig. 3, see Table 3). The 1000 grain weights in line 49-14 and T10 were significantly ($p \leq 0.001$) lower than in S29.

Grain quality analysis showed that S29, T10, and 49-14 had high contents of protein and gluten (see Table 3), characteristic of strong wheats. Grain quality in 49-14 was comparable with S29 and T10.

Screening of generations F_4 and F_6 of the T10 × S29 cross for resistance to fungal pathogens

The resistance of plants to brown rust and stem rust agents was tested in the field in 2018 and 2020. Field resistance to powdery mildew was not tested in those years, because weather conditions were unfavorable for the agent, as seen from the fact that the susceptible variety S29 was not injured.

In tests of the resistance to the Siberian population of the brown rust agent *P. triticina* conducted in 2018, S29 demonstrated the S (susceptibility) type of response, scoring 4 with about 100 % damage of leaf surface (Fig. 4, *c*). Tulaykovskaya 10 and F_4 plants of T10 × S29 showed the immune type without *P. triticina* pustules (see Fig. 4, *a*, *b*).

The hybrids tested and parental varieties produced a specific response to stem rust. Plants of S29, T10, and F_4 T10 × S29 showed generally the immune response except for a single case. One of the F_4 plants showed a specific type of interaction with the pathogen: occasional uredial pustules without chlorosis (5S) (see Fig. 4, *a*, ai). In practice, the detected local but pronounced syndrome is interpreted as a sign of a rare virulent fungus race in the local population (Roelfs et al., 1992). As reported by Skolotneva et al. (2020), the stem rust population in the Novosibirsk oblast is highly heterogeneous, as it is formed by southern and western migrants.

No signs of fungal diseases were detected in plants of the cultivars and line 49-14 at the stages of tillering and flowering in the field in 2020. Tests for plant resistance to the brown rust population at the milky ripeness stage showed type S (susceptibility) response in S29 plants (Fig. 5), score 4 with about 100 % leaf damage, whereas T10 and 49-14 demonstrated the immune response with no *P. triticina* pustules (Fig. 6).

At the milky ripeness stage, on August 2–5, the start of damage of S29, T10, and 49-14 by the yellow rust agent *P. striiformis* was noted. The percentage of leaf area injury

Fig. 4. Absence of damage by brown rust from F_4 T10 × S29 hybrids (*a*, *b*); brown rust damage of S29 (*c*); ai – culm damage by stem rust (zoomed).
Photographed August 18, 2018.

Fig. 5. Leaves of S29 injured by yellow and brown rusts.
Photographed August 2, 2020.

Fig. 6. Resistance of 49-14 plants to brown rust and different degrees of damage by yellow rust.
Photographed August 5, 2020.

in S29 was 50 to 75 (see Fig. 5), corresponding to medium susceptibility (MS).

Plants of T10 and 49-14 showed medium resistance (MR) and medium susceptibility (MS) to the yellow rust agent. The percentage of leaf area injury was 5 to 40, with chlorotic zones (see Fig. 6).

No damage by stem rust was seen in plants of S29, T10, or 49-14 in the summer of 2020.

Thus, the results of screening for resistance to a variety of plant pathogens conducted in the field in different years indicate that chromosome 6Agi2 retains the immunity of plants to the West Siberian brown rust population and immunity to dominant stem rust races. It also supports the medium resistant and medium susceptible types of response to yellow rust agents.

Discussion

Breeding line 49-14 ($2n = 42$) was isolated from generation F_5 of intervarietal hybrids T10 × S29, with introgression of a pair of wheatgrass chromosomes 6Agi2. It shows high performance indices and immunity to West Siberian populations of brown rust agents. The response of 49-14 plants to the yellow rust agent varies from medium resistance to medium susceptibility, probably because of the difference in aggressiveness among the agent races. Stem rust injury was noted in only one plant and was interpreted as immunity to dominant stem rust races.

Previously, it was demonstrated that the genetic material of chromosome 6Agi2 in common wheat varieties Tulaykovskaya 5, Tulaykovskaya 10, Tulaykovskaya zolotistaya, Tulaykovskaya 100, and Volgouralskaya retains the resistance to brown rust populations typical of the Lower and Middle Volga regions, Central and Ural regions, and West Siberia (Plakhotnik et al., 2014; Salina et al., 2015; Leonova et al., 2017; Askhadullin et al., 2019). The damage of Tulaykovskaya 10 by brown rust in infection nurseries of the Central Chernozem region reached 22 %, and the variety was assigned to group II of epidemic resistance (moderately resistant ER II) (Zeleneva, 2019). In Tatarstan, the damage of Tulaykovskaya 10 by stem rust was assessed as 5–10 % on the average, and the damage by powdery mildew scored 6; the type of response to brown rust remained immune (Askhadullin et al., 2019). The susceptibility of T10 to the powdery mildew population of the West Siberian region was assessed as resistance. A genome-wide association search (GWAS) mapped the *Pm6Agi2* gene on the long arm of wheatgrass chromosome 6Agi2, and this gene imparts resistance to the powdery mildew agent (Leonova, 2019). In experiments in the Middle Volga region, T10 showed immunity to brown rust and medium resistance (20 % injury) to stem rust, yellow rust, and powdery mildew (Syukov et al., 2016). Thus, T10 retains its immunity to brown rust populations in various ecogeographical regions. It is medium susceptible to stem and yellow rusts but shows diverse responses to the powdery mildew agent.

The substitution of wheatgrass chromosome 6Agi2 for 6D does not impair grain yield, grain quality, or drought tolerance (Filatova et al., 2010; Volkova et al., 2010), although in some cases of using T10 as a resistance gene donor, plants with lower productive tillering and 1000 grain weight appeared among the offspring with chromosome 6Agi2 (Stasyuk et al., 2017). The contents of protein and gluten in line 49-14 were about the same as in S29 or T10, corresponding to the grain quality of strong wheats (State Standard…, 2018, 2019). Line 49-14 lagged behind the parental varieties in productive tillering (S29), number of spikelets in the main spike (T10), and 1000 grain weight (S29). In spite of lower productive tillering, fewer spikelets in the main spike, and lower 1000 grain weight, the indices grain weight per plant and grain number per plant in line 49-14 did not differ significantly from the parental varieties owing to the significantly higher grain number per spikelet in the main spike of 49-14 than in S29 ($p \leq 0.001$) or T10 ($p \leq 0.05$). Plants of 49-14 set 3.77 ± 0.1 grains per spikelet, the range of variation in individual plants being 2.93–4.62, and up to 6 grains were set in spikelets of the middle spike part. Spikelets were fan-shaped (see Fig. 3). This shape is a specific sign of multiflowered spikelets in wheat (Martinek et al., 2005; Arbuzova et al., 2016).

Although common wheat has multiflowered spikelets, most of them set two or three grains. As the potential of forming more grains in wheat exceeds the actual yield by far, many studies are dedicated to seeking tools to control this process. The genetic and physiological grounds of breeding for more grains in spikes and spikelets and, ultimately, more grains per unit area are extensively investigated (Cui et al., 2012; Sreenivasulu, Schnurbusch, 2012; Arbuzova et al., 2016; Guo et al., 2016–2018; Bhusal et al., 2017; Philipp et al., 2018; Sukumaran et al., 2018; Wolde et al., 2019; Hu J. et al., 2020). Analysis of the reproductive developmental stages of spikes, spikelets, florets, and grains, as well as of their genetic regulation, is the best way to understand the formation of the trait 'grain number and spike fertility'. The 'grain number per spikelet' trait depends on the initiation of floret primordia, then on floret survival at the next stage, and then on their efficient pollination. Normally, up to 12 floret primordia form at the white anther stage, but later up to 60 % of the florets may remain underdeveloped (Guo et al., 2016, 2017). This applies especially to apical (uppermost) florets of a spikelet. As reported by Kuperman (1969), the growth rates of the two lowest and upper floret apices are nonuniform at organogenesis stage V; a spikelet may have up to five, less often, to seven florets. Lower florets very quickly form primordia of generative organs, stamens, and the pistil. A delay in organ formation is observed in the third and, particularly, fourth, fifth, and subsequent florets. Pistils most often remain underdeveloped in the uppermost florets. Chromosomes 4A, 5A, 6A, 7A, 2B, 5B, 7B, and 7D bear

QTLs responsible for the trait 'number of floret primordia per spikelet' (Guo et al., 2017). Also, the correlation and cluster analyses performed in the same study infer that the number of grains per spikelet does not depend on the maximum number of floret primordia per spikelet (Guo et al., 2017). Hence, the number of grains in a spikelet is determined by the fertility of each floret (Kuperman, 1969; Sreenivasulu, Schnurbusch, 2012).

A QTL responsible for greater numbers of grains per spikelet was detected on the long arm of chromosome 2A in GWAS of European common wheat varieties (Guo et al., 2017). Further studies of this locus mapped the *Grain Number Increase 1* (*GNI1*) gene, encoding a transcription factor with the HDZip1 homeodomain. Its mutation contributes much to greater numbers of fertile florets due to upper florets of the spikelet (Sakuma et al., 2019). Supposedly, *GNI1* was formed by gene duplication in wheat evolution, and its mutations were selected in domestication, as they increased the number of fertile florets, and, consequently, grains. Transcription factor *ARGONAUTE1d* (*AGO1d*) also affects the grain number in the spikes of common and durum wheats (Feng et al., 2017). *AGO1d* is important for the development of anthers and pollen at early developmental stages of wheat. Its malfunction shortens the spike, reduces anther size, decreases pollen fertility, and thereby decreases the number of grains in the spike (Feng et al., 2017).

The manifestation of traits in a plant is cumulatively affected by the genotype, ambient conditions, and farming techniques. All these factors greatly influence quantitative traits, including yield components (Piskarev et al., 2016; Stasyuk et al., 2017). The day/night regime and solar spectrum are particularly important ambient factors at organogenesis stages V and VI (Kuperman, 1969). Lower intensities of the red and infrared radiation reduce the number of fertile florets, number of grains per plant, and 1000 grain weight (Ugarte et al., 2010). The combinations of environmental factors required for each developmental stage stem from the conditions under which the species, varieties, and cultivars formed. With regard to their physiological developmental features, cultivars S29 and T10 belong to the Volga steppe and forest-steppe agroecological groups, respectively, or morphophysiological type II (Kuperman, 1969) (https://samniish.ru/yarovaya_myagkaya_pshenica.html). Such varieties utilize mainly winter and early spring precipitation in regions with water shortage in the second half of summer; that is, they are tolerant of summer drought. Cultivars bred in West Siberia belong to morphophysiological type V. The ecotype of Siberian forest-steppe wheats is determined by the climate: cold and dry April, May, and the first half of June; relatively ample precipitation in the second half of summer (July), and cold temperatures in August. The delay in organogenesis stage V allows much better use of late summer precipitation for the formation of large spikes and multiflowered spikelets. Owing to developmental physiological features and high drought tolerance, varieties of morphophysiological type II can be grown in steppe and forest-steppe regions of West Siberia (Kuperman, 1969). Thus, the genotypes of S29 and T10 are environmentally flexible. In the climate of West Siberian forest-steppe, they synchronize the metameric growth of spikelets to develop four, five, or more normal florets in a spikelet.

The genetic material of crested wheatgrass *Agropyron cristatum* is also beneficial for yield components. Addition lines with chromosome 6P of *Ag. cristatum* and, particularly, substitution lines 6P/6D show high productive tillering and significantly greater grain numbers in spikes and spikelets: up to 4.5 grains per spikelet (Wu et al., 2006; Han H. et al., 2014). It has been inferred that chromosome 6P houses genes controlling the numbers of florets and grains in a spike and spikelet (Wu et al., 2006). Conceivably, chromosome 6Agi2 of *Th. intermedium* bears gene(s) controlling the synchronous metameric growth of spikelets in T10, whereas the additive manifestation of the trait 'grain number per spikelet' is observed in line 49-14 (T10 × S29), where up to six normal florets develop in a spikelet.

Conclusion

Thus, the integrated analysis of the grounds of the multiflowered habit (Han H. et al., 2014; Arbuzova et al., 2016) and the raise and use of multiflowered forms in breeding (Guo et al., 2016; Sakuma et al., 2019) are means for improving wheat grain yield.

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ORCID ID

Yu.N. Ivanova orcid.org/0000-0002-9655-4539
E.S. Skolotneva orcid.org/0000-0001-8047-5695
O.G. Silkova orcid.org/0000-0003-3299-2975

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A prebreeding study of introgression spring bread wheat lines carrying combinations of stem rust resistance genes, *Sr22+Sr25* and *Sr35+Sr25*

S.N. Sibikeev[1] ✉, O.A. Baranova[2], A.E. Druzhin[1]

[1] Federal Center of Agriculture Research of the South-East Region, Saratov, Russia
[2] All-Russian Institute of Plant Protection, Pushkin, St. Petersburg, Russia
✉ sibikeev_sergey@mail.ru

Abstract. The *Sr22*, *Sr35*, and *Sr25* genes attract the attention of bread wheat breeders with their effectiveness against *Puccinia graminis* f. sp. *tritici* race Ug99 and its biotypes. The effectiveness and impact of *Sr22+Sr25* and *Sr35+Sr25* gene combinations on agronomic traits have not yet been studied. In the present article, these traits were studied using the spring bread wheat lines L503/W3534//L503, L503/*Sr35*//L503/3/L503 carrying the *Sr22+Sr25* and *Sr35+Sr25* genes during 2016–2020. These lines were assessed for resistance to *P. graminis* f. sp. *tritici* under natural epiphytotics and to the Saratov, Lysogorsk and Omsk populations of the pathogen and to the PgtZ1 (TKSTF) and PgtF18.6 fungus isolates in laboratory conditions (TKSTF + *Sr33*). The presence of the studied *Sr*-genes was confirmed by using molecular markers. Prebreeding studies were conducted during 2018–2020 vegetation periods. Under the natural epiphytotics of the pathogen and in the laboratory conditions, the *Sr22+Sr25* combination was highly effective, while *Sr35+Sr25* was ineffective. For grain yield, the lines with the *Sr22+Sr25* and *Sr35+Sr25* genes were superior to the recipient cultivar L503 in one year (*Sr22+Sr25* in 2019; *Sr35+Sr25* in 2018), with a decrease in 2020, but in general there were no differences. For the period 2018–2020, both combinations showed a decrease in 1000 grains weight and an increase in the germination-earing period. The line with *Sr22+Sr25* genes showed insignificant effects on gluten and dough tenacity, but the ratio of dough tenacity to extensibility was higher, and flour strength, porosity and bread volume were lower; in the line with *Sr35+Sr25* genes, the gluten content was lower, but the strength, tenacity of the dough and the ratio of dough tenacity to extensibility were higher, flour strength and the porosity of the bread were at the recipient level, but the volume of bread was lower. Key words: bread wheat; introgressive lines; *Sr22+Sr25* and *Sr35+Sr25* gene combinations; prebreeding studies.

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Пребридинговое изучение интрогрессивных линий яровой мягкой пшеницы, несущих комбинации *Sr22+Sr25* и *Sr35+Sr25* генов устойчивости к стеблевой ржавчине

С.Н. Сибикеев[1] ✉, О.А. Баранова[2], А.Е. Дружин[1]

[1] Федеральный аграрный научный центр Юго-Востока, Саратов, Россия
[2] Всероссийский научно-исследовательский институт защиты растений, Пушкин, Санкт-Петербург, Россия
✉ sibikeev_sergey@mail.ru

Аннотация. Гены *Sr22*, *Sr35* и *Sr25* привлекают внимание селекционеров мягкой пшеницы эффективностью против расы *Puccinia graminis* f. sp. *tritici* Ug99 и ее биотипов. К настоящему времени защитный эффект комбинаций генов *Sr22+Sr25* и *Sr35+Sr25* не исследован, неизвестно их влияние на агрономические показатели. В представленной работе эти показатели изучены с использованием линий яровой мягкой пшеницы Л503/W3534//Л503 (*Sr22+Sr25*) и Л503/*Sr35*//Л503/3/Л503 (*Sr35+Sr25*). Линии оценивали на устойчивость: к *P. graminis* f. sp. *tritici* в условиях естественных эпифитотий 2016–2020 гг., а также к саратовской, лысогорской и омской популяциям патогена и к изолятам гриба, PgtZ1 (TKSTF) и PgtF18.6, – в лабораторных условиях (TKSTF + *Sr33*). С помощью молекулярных маркеров подтверждено наличие изучаемых *Sr*-генов. Выявлена высокая эффективность комбинации генов *Sr22+Sr25* как при естественных эпифитотиях патогена, так и в лабораторных исследованиях. Комбинация *Sr35+Sr25* оказалась неэффективной. В среднем за 2018–2020 гг. у линий с обеими комбинациями генов отмечено понижение массы 1000 зерен и увеличение периода «всходы–колошение». У линии с комбинацией генов *Sr22+Sr25* обнаружены незначительные эффекты на показате-

ли клейковины и упругость теста, но отношение упругости теста к растяжимости было выше, а сила муки, пористость и объем хлеба – ниже; у линии с комбинацией *Sr35+Sr25* количество клейковины ниже, но крепость, упругость теста и отношение упругости теста к растяжимости выше, сила муки и пористость хлеба на уровне реципиента, но объем хлеба ниже.
Ключевые слова: мягкая пшеница; интрогрессивные линии; комбинации генов *Sr22+Sr25* и *Sr35+Sr25*; пребридинговые исследования.

Introduction

An increase in the harmfulness of the bread wheat diseases spectrum has been noted in the last decade for the zone of the Lower and Middle Volga regions of Russia. There was a strong epiphytoty of stem rust in 2016, a strong epiphytoty of leaf rust and Septoria tritici blotch (STB) in 2017, a strong epiphytoty of wheat tan spot in 2019 (Baranova et al., 2019; Sibikeev et al., 2020) and local but strong stem rust epiphytoty in 2020 (S.N. Sibikeev, unpublished data). Thus, there is a constant pressure of fungal diseases pathogens on bread wheat plants, in connection with which the increase in the yield of cultivated plants due to their resistance to biotic factors rises sharply.

Among the above-mentioned wheat diseases in the Saratov region, the role of the stem rust pathogen has sharply increased; the disease has become constantly present in the crops of spring and even winter bread wheat. So the development of *Puccinia graminis* f. sp. *tritici* on susceptible cultivars and lines of spring wheat was observed even in the dry growing seasons of 2018–2019. In terms of harmfulness, stem rust of wheat in the Saratov region began to occupy one of the first places. This fact is mainly explained by global climate changes in combination with agrobiological factors. Among the latter, two main factors are important. Firstly, most cultivars of bread wheat in the Saratov region are susceptible to this pathogen – namely, the following cultivars: Saratovskaya 55, Saratovskaya 68, Saratovskaya 70, Saratovskaya 73, Albidum 32, Favorit, Voevoda and Lebedushka. The cultivars Prokhorovka, Yugo-Vostochnaya 2 and Dobrynya were heterogeneous in terms of resistance (Baranova et al., 2019), the second factor is the presence of highly virulent population of *P. graminis* f. sp. *tritici*. So in the Saratov populations for 2016–2020 the proportion of highly virulent pathotypes (from 14 to 20 virulence genes) ranged from 35 to 60 % (O.A. Baranova, unpublished data).

To avoid economically significant losses from bread wheat diseases, including stem rust, constant scientifically based breeding for resistance to pathogens is required. This work should be based on knowledge of the pathogen biology, its virulence, resistance genetics of cultivated varieties and on a sufficient number and diversity of genes for resistance to pathogens; that is, it should be anticipatory (McIntosh, 1992; McIntosh, Brown, 1997).

As breeding practice shows, the most difficult task to solve is the expansion of the genetic diversity of effective resistance genes. Thus, studies by O.A. Baranova (2020) on a set of 32 new bread wheat cultivars, included in the "State Register of Breeding Achievements" of the Russian Federation for 2017–2018, showed that only 11 cultivars are highly effective against the causative agent of stem rust. Seven of them are protected by one *Sr31* gene, three cultivars – by the combination of *Sr31+ Sr57* genes, and one cultivar – by the combination of *Sr31+ Sr28* genes. Thus, protection can be determined by only one *Sr31* gene, since the interaction of genes in combinations has not been proven. In addition, the situation is complicated by the fact that the *Sr31* gene has been overcome by the Ug99 race, which currently consists of 13 biotypes (http;/globalrust.org/pathogens/pathogen-homepage). The Ug99 race is widespread in the countries of Africa and the Middle East, it migrates in the direction of Central and Southeast Asia, and it is possible to introduce it into the territory of the Russian Federation. In this regard, it is necessary to take this fact into account and include in the breeding process of bread wheat cultivars resistant to *P. graminis* f. sp. *tritici* the *Sr*-genes and their combinations effective against the biotypes of the Ug99 race.

At present, of the total number of identified genes for resistance to stem rust of wheat, 29 out of 61 have been transferred from the "alien" species (McIntosh et al., 2013, 2016, 2018, 2020). The genes *Sr25*, *Sr22*, and *Sr35* occupy a special place among them. They are all effective against the Ug99 race and its biotypes (http://rusttracker.cimmyt.org/?page_id=22). The *Sr25* gene was transferred from the tall wheatgrass *Agropyron elongatum* (2*n* = 70) into bread wheat as part of the 7DS-7DL-7Ae#1L translocation, the last two from the A genome of the cultivated einkorn into chromosomes 7AL and 3AL, respectively (McIntosh et al., 1995).

While the *Sr25/Lr19* gene complex (a gene for resistance to leaf rust) is widely used in cultivars and breeding material of spring bread wheat in the Middle Volga and Lower Volga regions (Gultyaeva et al., 2019, 2020), the *Sr22* gene is used only in the cultivars Schomburgk and BT-Schomburgk in Australia and in a set of near isogenic lines, and the *Sr35* gene has not been introduced into commercial cultivars (McIntosh et al., 1995, 2013). The limited use of the *Sr22* and *Sr35* genes in practical breeding is mainly due to the fact that they either do not compensate for the absence of wheat chromatin or contain undesirable genetic factors with negative effects (Paul et al., 1994). The *Sr25/Lr19* genes, or rather the 7DS-7DL-7Ae#1L translocation, has a positive effect on agronomic traits (Singh et al., 1998; Sibikeev et al., 2016, 2018). It was noted that an increase in grain productivity in the presence of this translocation is determined by a better utilization of assimilates by the reproductive organs (Miralles et al., 2007). However, to date, studies of the *Sr22+Sr25* and *Sr25+Sr35* gene combinations both in terms of effectiveness against the stem rust pathogen and the effect of introgressed genetic material on agronomically important traits (prebreeding studies) have not been carried out.

The aim of our research was to reveal the promising nature of the *Sr22+Sr25* and *Sr35+Sr25* gene combinations for practical breeding both in terms of effectiveness against *P. graminis* and in terms of their effect on productivity and grain quality.

Materials and methods

The material used included the following genotypes of spring bread wheat: cultivar – recipient L503, contains the 7DS-7DL-7Ae#1L translocation with the *Sr25/Lr19* genes (Badaeva et al., 2018); standard for the Saratov region cultivar Favorit, contains the substitution 6D(6Ag^{i}) (Sibikeev et al., 2017).

Introgression lines: L503/W3534//L503, where W3534 is a near isogenic line of the Marquis cultivar with the *Sr22* gene, namely W3534 = Marquis*5//Stewart*3/*T. monococcum*; L503/*Sr35*//L503/3/L503, where *Sr35* is a near isogenic line of the Marquis cultivar with the *Sr35* gene, namely *Sr35* = Marquis*5//G2919, G2919 Canadian source of *T. monococcum*. The lines W3534 and *Sr35* were kindly provided by Dr. R.A. McIntosh (Plant Breeding Institute, Gobbitty, Australia) and were used as paternal forms for crossing with spring bread wheat cultivar L503.

The studies included three stages: the first stage was to confirm the presence of the *Sr22+Sr25* and *Sr35+Sr25* combinations in the studied introgression lines. *Sr*-genes were identified using molecular markers for *Sr25* (Gb) (Prins et al., 2001), *Sr22* (*Xbarc121*, *Xcfa2123*, *Xcfa2019*, *Xwmc633*) (Khan et al., 2005; Yu et al., 2010), *Sr35* (*Xcfa2170*) (Zhang et al., 2010). Amplification was performed on C1000 Thermal Cycler (BioRad) amplifiers; amplification products were separated in 2 % agarose and 8 % polyacrylamide gels stained with ethidium bromide. The SWSR22TB line containing the *Sr22* gene and the W3435 (*Sr22*) parental line, as well as the Marquis*5//G2919 (*Sr35*), LC-SR25-ARS (*Sr25*) line was used as a positive control. The susceptible cultivar Khakasskaya served as a negative control, and a PCR mixture without the addition of DNA served as a control for contamination. A GeneRuler™ 50 bp DNA Ladder ("Fermentas") was used as a molecular weight marker. The amplification products were visualized using the ChemiDoc XRS+ (BioRad) gel documenting system. PCR was performed in two replicates.

The second stage was an evaluation of the lines resistance to the causative agent of stem rust in the field conditions in 2016–2020 – the phase of milky-wax ripeness (breeding sowing by the Federal Center of Agricultural Research of the South-East Region) against the natural background of the pathogen development. The stem rust infection type was determined using the A.P. Roelfs et al. (1992) scale, where R is resistant, MR is moderate resistant, MS is moderate susceptible, and S is susceptible, respectively. The degree of rust damage (%) was assessed according to the scale of R.F. Peterson et al. (1948). In the phase of seedlings (first leaf) in All-Russian Research Institute of Plant Protection, juvenile resistance of wheat samples to disease was studied according to the method of Y. Jin et al. (2007). Ten-day-old seedlings with a fully unfolded first leaf were inoculated with a urediniospore suspension of pathogen populations collected in the Omsk region, as well as in the Lysogorsky district of the Saratov region from the Favorit cultivar, which carries the 6Ag^{i}(6D) substitution, as well as two isolates of the fungus – PgtZ1 (TKSTF) and PgtF18.6 (TKSTF + *Sr33*). The virulence characteristic of the PgtZ1 and PgtF18.6 isolates is shown in Table 1.

The inoculums concentration was 1 mg of urediniospores in 1 ml water (Singh et al., 2008). The Khakasskaya spring bread wheat cultivar was used as a susceptible control. The results were taken into account on the 10th day according to E.C. Stakman et al. scale (1962), where 0 is the absence of symptoms; 0; – necrosis without pustules; 1 – very small pustules surrounded by necrosis; 2 – pustules of medium size, surrounded by necrosis or chlorosis; 3 – pustules of medium size without necrosis, 4 – large pustules without necrosis, X – pustules on the same leaf of different types, chlorosis and necrosis are present. Plants with reaction types 0, 0; 1, 2 were considered resistant, and 3, 4 and X were considered susceptible.

The third stage is the evaluation of grain productivity traits, physical properties of the dough and baking indicators in the introgression lines L503/W3534//L503 (*Sr22+Sr25*) and L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*) in comparison with the recipient cultivar L503 and the standard cultivar Favorit. The

Table 1. Virulence characteristic of *P. graminis* f. sp. *tritici* isolates used to inoculate introgression wheat lines in the seedling stage

Isolate	Race	Population	Virulence	Avirulence
Virulence/avirulence to *Sr*-lines from the "North American differential set"				
PgtZ1	TKSTF	Zernogradskaya	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 38, McN	11, 17, 24, 31
PgtF18.6	TKSTF	Lysogorskaya	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 38, McN	11, 17, 24, 31
Virulence/avirulence to additional *Sr*-near isogenic lines				
PgtZ1	TKSTF	Zernogradskaya	12, 15, 20, 25, 27, 28, 29, 32, 39, 7a+12, 7b+18, 17+13	2compl, 13, 22, 26, 26+9g, 33, 33+5, 35, 37, 40, 44
PgtF18.6	TKSTF+*Sr33*	Lysogorskaya	12, 15, 20, 5, 7, 28, 29, 32, 33, 39, 7a+12, 7b+18, 17+13	2compl, 13, 22, 26, 26+9g, 35, 37, 40, 44

studies were carried out in 2018–2020, of which 2020 was the most favorable; however, during this growing season, there was a deficit of precipitation from the flowering phase to full ripeness, and 2018 and 2019 were distinguished as severely droughty throughout the entire field season.

The experimental material was randomly sown in 7 m^2 plots in three replicates. The seeding rate was 400 grains per 1 m^2. The bread making quality was evaluated by the content of crude gluten, gluten strength and the indicators of the IDG-1 device (deformation index of gluten) and the Chopin alveograph with the baking of experimental bread samples. The protein content of grain, harvested in 2020, was determined on the Infratec™ 1241 Grain Analyzer. The data obtained were subjected to the appropriate statistical analysis using the Agros-2.10 software.

Results

Identification of resistance genes

To confirm the presence of *Sr22+Sr25* and *Sr35+Sr25* gene combinations in the introgression lines L503/W3534//L503 and L503/*Sr35*//L503/3/L503, *Sr*-genes were identified using molecular markers of the *Sr*-genes under study.

The *Sr22* gene is introgressed into tetraploid wheat from *Triticum monococcum* L. ssp. *aegilopoides* (synonym *T. boeoticum* Boiss.). For its identification, three molecular markers closely linked to it are usually used – *Xcfa2019*, *Xcfa2123* and *Xbarc121* (Yu et al., 2010). In the work (Olson et al., 2010), a set of lines with *Sr22* gene was obtained and the nearest flanking markers of this gene, *Xwmc633* and *Xcfa2123*, were proposed. In our work, we used all four *Sr22* markers: *Xbarc121*, *Xcfa2123*, *Xcfa2019* and *Xwmc633* (Fig. 1).

The size of the obtained PCR products with markers *Xbarc121*, *Xcfa2123*, *Xcfa2019* and *Xwmc633* is shown in Table 2. It was shown that when PCR was performed with the primers barc121F/R, cfa2123F/R, cfa2019F/R and wmc633F/R, fragments of different sizes were amplified and not only those that were declared as diagnostic. Thus, during amplification with primers wmc633F/R in the lines SWSR22TB and W3534 a diagnostic fragment of 117 bp size was obtained. In the introgression line L503/W3534// L503, the obtained fragment was about 211 bp. In the work of E.L. Olson et al. (2010), in the line U5616-20-154 with the small fragment of *T. monococcum* during amplification with primers wmc633F/R, a fragment of 229 bp was obtained, which was explained by recombination between the resistance gene and all markers mapped in this area.

For the *Xbarc121* marker, the 215 bp size amplicon described as a diagnostic fragment by L.X. Yu et al. (2010) was observed in the control lines SWSR22TB and W3534, as well as in the line L503/W3534//L503. When analyzing the PCR products for the *Xcfa2123* marker, our results coincided with the data of J.K. Haile et al. (2013). In the control lines SWSR22TB and W3534, the fragment of 234 bp size was amplified. A similar fragment was observed in the L503/W3534// L503 line, but it was also observed in the Khakasskaya cultivar. The amplicons size of the Inna cultivar was somewhat larger – 240 and 250 bp. Amplification with cfa2019F/R pri-

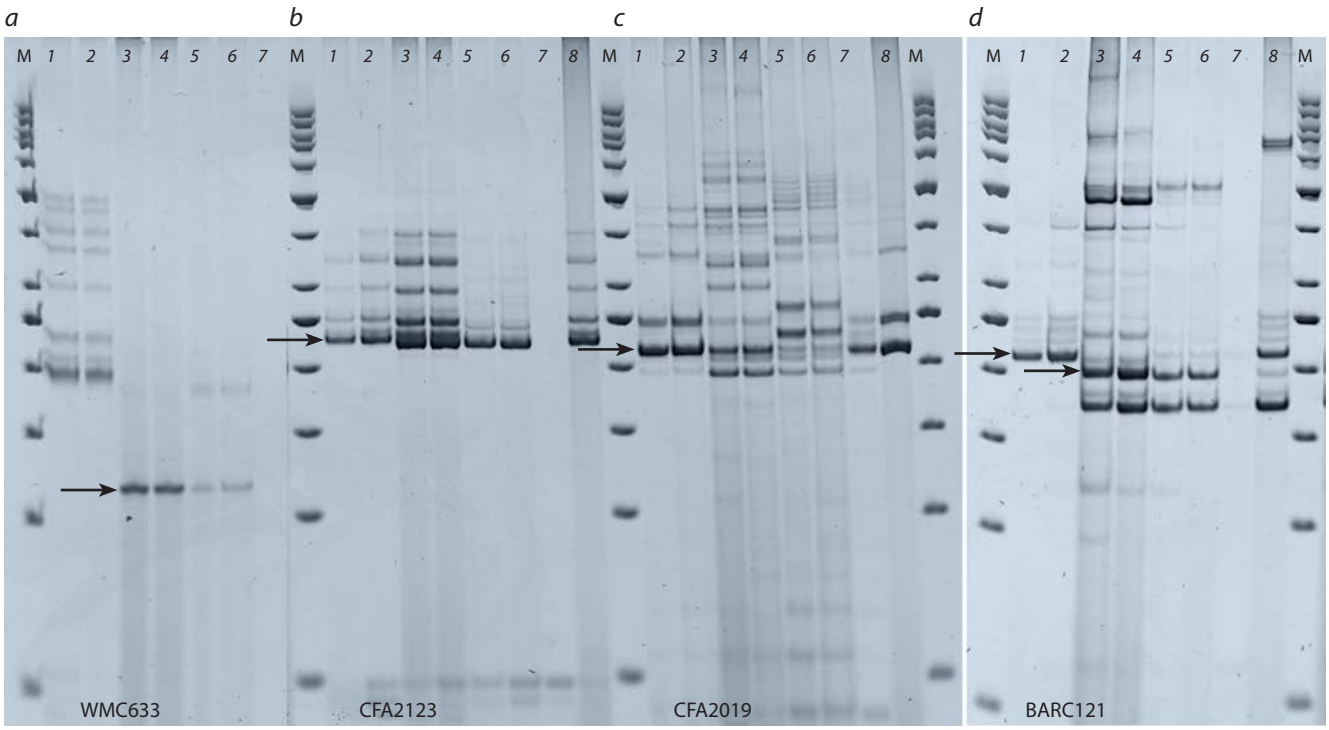

Fig. 1. Identification of the *Sr22* gene using molecular markers *Xwmc633* (*a*), *Xcfa2123* (*b*), *Xcfa2019* (*c*) and *Xbarc121*(*d*).

M – marker of molecular weight 50 bp "Fermentas"; No. *1*, *2* – line L503/W3534//L503; positive control of *Sr22*: *3*, *4* – line W3534, *5*, *6* – line SWSR22TB; negative control of *Sr22*: *7* – cultivar Khakasskaya, *8* – cultivar Inna.

Table 2. Polymorphism by the size of amplification fragments of molecular markers of the stem rust resistance gene *Sr22*

Wheat samples	Markers, amplicon size, bp											
	Xbarc121				*Xcfa2123*			*Xcfa2019*			*Xwmc633*	
	170	197	215	234	234	240	250	200	238	250	117	211
SWSR22TB (control +)	170	197	215	–	234	–	–	–	238	250	117	–
W3534 (control +)	170	197	215	–	234	–	250	200	238	–	117	–
L503/W3534//L503	–	–	215	–	234	–	–	–	238	250	–	211
Khakasskaya (control –)	–	–	–	234	234	–	–	–	238		–	–
Inna (control –)	–	–	–	–	–	240	250	–	238	250	–	–

Note. "–" – no amplicon.

mers in the SWSR22TB line revealed two fragments – 238 and 250 bp in our work, as in the line L503/W3534//L503. The line W3534 had fragments of 200 and 238 bp. However, it should be noted that the 238 bp fragment was also amplified in negative controls – the cultivars Khakasskaya and Inna. Amplification of the 238 bp diagnostic fragment for *Xcfa2019* was also shown in the work of E.L. Olson et al. (2010), which does not coincide with the data of J.K. Haile et al. (2013).

Thus, amplicons were identified by three markers to *Sr22* (*Xbarc121*, *Xcfa2123*, *Xcfa2019*) in the line L503/W3534//L503. In addition, this line was resistant to *P. garminis* isolates PgtZ1 and PgtF18.6, avirulent to the line with *Sr22* and virulent to the line with *Sr25*, and based on the pedigree data and identification of resistance genes, there are no other *Sr*-genes in this line. On this basis, we concluded that the L503/W3534//L503 line contains *Sr22* gene.

The *Sr35* gene was identified using the *Xcfa2170* marker (Fig. 2) in the L503/*Sr35*//L503/3/L503 line. The parental line Marquis*5/G2919 (*Sr35*) was used as a control.

When the *Sr35* gene was identified using the *Xcfa2170* marker, a 160 bp diagnostic fragment was obtained in positive controls, which coincides with the data of J.K. Haile et al. (2013). In addition to the *Sr22* and *Sr35* genes, *Sr25/Lr19* was also identified in the L503/W3534//L503 and L503/*Sr35*//L503/3/L503 lines using the Gb marker. It should also be noted that the L503/*Sr35*//L503/3/L503 line was resistant to the Ug99 race in Kenya (Baranova et al., 2021).

Thus, it was proved that the introgression lines L503/W3534//L503 and L503/*Sr35*//L503/3/L503 carry the combinations of *Sr22+Sr25* and *Sr35+Sr25* genes, therefore, the results of phytopathological and prebreeding studies presented below are correct.

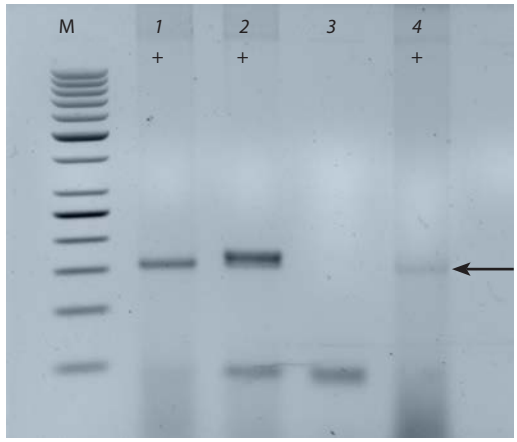

Fig. 2. Identification of the *Sr35* gene using the molecular marker *Xcfa2170*.

M – marker of molecular weight 50 bp "Fermentas"; No. *1* – line L503/*Sr35*//L503/3/L503; positive control of *Sr35*: *2* and *4* – line Marquis*5/G2919 (*Sr35*); negative control of *Sr35*: *3* – cultivar Khakasskaya. The arrow indicates diagnostic fragment with molecular weight of 160 bp.

Phytopathological analysis of resistance to the stem rust causative agent

Analysis of the reaction type to the stem rust causative agent was carried out both in the field with natural epiphytotics of the disease, and in laboratory conditions with artificial infection of seedlings. Evaluation of resistance to *P. graminis* f. sp. *tritici* under the conditions of epiphytotics 2016–2020 showed that the line L503/W3534//L503 (*Sr22+Sr25*) showed the type of reaction to the pathogen R, while in the line L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*) the type of reaction R was at epiphytotics of 2016–2019, and in 2020 – 20MS. At the same time, the reaction type of the recipient cultivar L503 (*Sr25*) in 2016, 2017 and 2020 was 25MS, 20MS, 30MS and in 2018, 2019 – R, and the reaction type to the pathogen of the cultivar Favorit (6D(6Ag^{i}) *LrAg*^{i}/*SrAg*^{i}) in epiphytotics of *P. graminis* f. sp. *tritici* in 2016–2020 was 75S, 50S, 10S, with the exception of 2019, when the 5S and R reaction types were observed on plants. This was caused by a severe drought and poor development of *P. graminis* f. sp. *tritici* in 2019 (Table 3).

The results of the evaluation of resistance to the stem rust in laboratory conditions at the seedlings stage are shown in Table 4. Laboratory evaluation at the seedlings stages of the studied lines showed resistant responses to the pathogen in the line with the *Sr22+Sr25* gene combination (ITs ranging from 0 to 2) and susceptible in the line with the *Sr35+Sr25* gene combination. It should be noted that these reaction types

Table 3. Field evaluation of spring bread wheat introgression lines for resistance to *P. graminis* f. sp. *tritici* in 2016–2020 (stage "milky-waxy ripeness")

Cultivars, lines	Field evaluation of disease resistance				
	2016	2017	2018	2019	2020
L503/W3534//L503 (*Sr22+Sr25*)	R	R	R	R	R
L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*)	R	R	R	R	20MS
L503 (*Sr25*)	25MS	20MS	R	R	30MS
Favorit (6D(6Ag^{i}) *LrAg^{i}/SrAg^{i}*)	75S	50S	10S	5S, R	50S

Table 4. Seedling resistance of spring bread wheat introgression lines to *P. graminis* f. sp. *tritici*

Cultivar, line	Infection types to *P. graminis*			
	Populations of *P. graminis* f. sp. *tritici*		Isolates of *P. graminis* f. sp. *tritici*	
	lysogorskaya	omskaya	PgtZ1	PgtF18.6
L503/W3534//L503 (*Sr22+Sr25*)	1^{+}	0;	0; 1	2
L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*)	3^{-}	2^{+}	3^{+}	3^{-}
Khakasskaya (susceptible control)	4	4	4	4^{+}

were related to both *P. graminis* f. sp. *tritici* populations and PgtZ1 and PgtF18.6 isolates.

Thus, the phytopathological analysis of resistance to the stem rust causative agent of introgression lines L503/W3534//L503 (*Sr22+Sr25*) and L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*), both in natural epiphytotics of *P. graminis* and with artificial infection, showed high and effective resistance of the *Sr22+Sr25* combination and the susceptibility of the *Sr35+Sr25* combination during epiphytotics in 2020 and laboratory evaluation.

Prebreeding studies of introgressive lines

The results of studying grain productivity in the introgression lines L503/W3534//L503 (*Sr22+Sr25*) and L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*) showed that, on average, for the period from 2018 to 2020, there were no significant differences in lines for grain yield compared to the recipient cultivar L503 and the standard cultivar Favorit (Table 5), which was expected, since the productivity indicators in 2020 were twice as high as the grain yield in 2018 and 2019. Nevertheless, the analysis of grain productivity by years revealed that, in 2018, under the background of severe drought throughout the growing season, the yield was the highest in the line with the *Sr35+Sr25* combination (significant excess of the recipient cultivar L503 and at the level of the standard cultivar Favorit). In the same line, there was an insignificant excess in grain yield of the cultivar L503 in the growing season of 2019 with a similar drought as in 2018. However, the line with the *Sr35+Sr25* combination was significantly lower for grain productivity under the conditions of the 2020 growing season, which was characterized by excess moisture and moderate air temperature from germination to the beginning of flowering, then a drought with high temperatures was noted until full maturation.

In general, for three years of testing in terms of the absolute indicator of grain yield, L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*) is equal to the recipient cultivar L503. L503/W3534//L503 (*Sr22+Sr25*) for grain yield was at the level of L503 in 2018, exceeded L503 in 2019 and was significantly lower than the cultivars L503 and Favorit in 2020. In general, over three years, in terms of absolute numbers for grain productivity, the line L503/W3534//L503 (*Sr22+Sr25*) is inferior to both L503 and Favorit. The line L503/*Sr35*//L503/3/L503 (*Sr35+Sr25*) is a more productive line (in absolute numbers), which was revealed when comparing the studied lines with each other.

On average, for 2018–2020, the analysis of 1000 grains weight, as one of the important elements of grain productivity, showed a significant decrease in lines with the combination of *Sr22+Sr25* (28.2 g) and *Sr35+Sr25* (30.1 g) genes compared with the recipient cultivar L503 (31.3 g). Moreover, this decrease was larger for the line with *Sr22+Sr25*, which was significantly inferior to *Sr35+Sr25*, while for the standard cultivar Favorit – 28.0 g, with LSD_{05} = 0.95 g and F* = 9.67. On average, for 2018–2020, in terms of the germination to earing period, significant differences were observed between the recipient cultivar L503 (44.3 days) and lines with *Sr22+Sr25* (47.7 days) and *Sr35+Sr25* (46.7 days) gene combinations, the differences between the lines were not significant, with LSD_{05} = 1.0 days and F* = 27.60. There were no differences in plant height between the studied lines and the cultivars L503 and Favorit.

An important stage in the production of bread wheat cultivars is the quality of the final product – flour and bread.

Table 5. The grain productivity of spring bread wheat introgression lines with the *Sr22+Sr25* and *Sr35+Sr25* genes combination and cultivars L503 and Favorit for the period of 2018–2020

Cultivar, line	Grain yield, kg/ha				Grain protein content, %
	2018	2019	2020	Average	2020
L503 (*Lr19/Sr25*)	616 a	991 a	2660 c	1422	16.4 b
L503/W3534//L503 (*Lr19/Sr25+Sr22*)	633 a	1170 b	1660 a	1154	16.9 b
L503/*Sr35*//L503/3/L503 (*Lr19/Sr25+Sr35*)	938 c	1036 ab	2388 b	1454	16.9 b
Favorit St (*Lr/Sr6Agi*)	852 bc	1067 ab	2743 c	1554	15.5 a
LSD_{05}	172	170	263	NS	0.5

Table 6. Bread making quality traits of spring bread wheat introgressive lines with the *Sr22+Sr25* and *Sr35+Sr25* genes combination and the cultivars L503 and Favorit (average for 2018–2020)

Cultivar, line	Gluten		Alveograph*			Bread**		
	%	Strength	P, mm	P/L	W, units	V, cm^3	Porosity, score	Crumb color
L503	38.0 b	84 bc	68 a	1.2 a	164 b	770 bc	4.9 b	Yellow
L503/W3534//L503	38.0 b	90 c	68 a	2.2 b	98 a	680 a	4.3 a	
L503/*Sr35*//L503/3/L503	31.6 a	73 a	103 b	3.0 c	187 b	690 a	4.9 b	
Favorit St	32.1 a	75 a	69 a	1.0 a	173 b	800 c	4.8 b	Cream

* Indicators of the alveograph: P – dough tenacity, P/L – tenacity to extensibility ratio, W – flour strength;
** Indicators of bread evaluation: V – bread volume, porosity.

Unfortunately, it is not uncommon for the involvement of alien genetic variability in the bread wheat gene pool to worsen some indicators of flour and bread quality. Over the period of research, it was revealed that the lines with the combinations *Sr22+Sr25* and *Sr35+Sr25* and the recipient cultivar L503 did not have significant differences in protein content, but exceeded the standard cultivar Favorit (see Table 5) on average. According to the indicators of gluten – the content and strength of gluten according to the IDG-1 device indicators, the following results were obtained: the line with the *Sr35+Sr25* combination significantly reduced the gluten content, but strength was significantly higher in relation to the cultivar L503, and the line with *Sr22+Sr25* combination did not differ from the recipient cultivar L503 according to this indicators. The values of dough tenacity indicators and the ratio of dough tenacity to extensibility (P/L) were distributed as follows: higher for the combination *Sr35+Sr25*, and the combination *Sr22+Sr25* did not differ in tenacity from the cultivar L503, but had a higher tenacity to extensibility ratio (P/L). The line with the *Sr22+Sr25* combination significantly reduced the flour strength, the crumb porosity and bread volume in relation to the recipient cultivar L503. At the same time, the line with the *Sr35+Sr25* combination had an insignificant increase in the flour strength, reduced bread volume, but had a high equal score of bread porosity in relation to L503.

In general, we can conclude that the *Sr35+Sr25* combination had a lesser effect on the flour and bread indicators (except for the gluten content) (Table 6).

Discussion

As noted above, all three studied genes *Sr22*, *Sr25*, *Sr35* are effective against the biotypes of the *P. graminis* f. sp. *tritici* Ug99 race (http://rusttracker.cimmyt.org/?page_id=22). However, our studies have shown that only the combination of *Sr22+Sr25* resistance genes is highly effective against the disease. There is reason to assume that in this case an additive effect or the so-called "forbidden combination" is manifested.

At the same time, in laboratory evaluation, the *Sr35* gene is separately effective against the Saratov population of 2016, 2017 and 2020 and the Lysogorsk population of the Saratov region in 2018 and 2019 (O.A. Baranova, unpublished data), but the *Sr35+Sr25* combination in the line L503/*Sr35*//L503/3/L503 showed susceptibility to the stem rust causative agent. There can be several explanations for this phenomenon. Firstly, in laboratory studies, seedlings were infected, but under field conditions, during epiphytotics of the pathogen, adult plants were evaluated at the stages of the beginning of grain filling or milky-wax ripeness. It is possible that the *Sr35* gene is resistant to this set of *P. graminis* f. sp. *tritici* populations only at the seedling stage. Secondly, it is possible that the *Sr35*

gene expression is suppressed by suppressor genes of cultivar L503. Similar cases were observed during the expression of the *Sr21* gene also transferred from *T. monococcum* L. (The, Baker, 1975; on: Leonova, 2018).

Analyzing the influence of the *Sr22+Sr25* and *Sr35+Sr25* gene combinations on agronomic traits, primarily grain productivity and flour and bread quality, it is necessary to take into account well-known individual effects of the studied genes of resistance to the stem rust pathogen. The *Sr25* gene was transferred to bread wheat as part of the 7DS-7DL-7Ae#1L translocation from chromosome 7Ae#1 of the tall wheatgrass, in which the following gene order was determined (from the centromere to the telomere end) – *Sd1-Xpsr165-Xpsr105-αAmy-D2-Xpsr129-Lr19-Wsp-D1-Sr25-Y-Ep-D1* (Prins et al., 1996). Without a doubt, the entire translocation has an impact on agronomic performance.

Our early studies showed that this translocation is neutral in relation to grain yield, significantly increases the gluten content without changing its quality and does not affect dough tenacity, the tenacity to extensibility ratio of dough and flour strength. However, it significantly reduces the volume of bread with the same porosity. 7DS-7DL-7Ae#1L translocation did not affect the germination-earing period and plant height (Sibikeev et al., 2018). Thus, this translocation does not worsen agronomic performance.

It is known that the *Sr22* gene was transferred into bread wheat from two diploid species carrying the A-genome – *T. boeoticum* Boiss. the source of G-21 (Gerechter-Amati et al., 1971) and *T. monococcum* the source of RL5244 (Kerber, Dyck, 1973). Transfers from these two sources include varying amounts of introgressed chromatin. The transfer from *T. boeoticum* contains almost entirely the long arm and part of the short arm of $7A^m$ (cv. Steinwedel), and from *T. monococcum*, the distal part of $7A^mL$ (cv. Marquis) (Kerber, Dyck, 1973; Paull et al., 1994).

Due to the fact that the recombination between the A-genome of bread wheat and the A^m-genomes of *T. boeoticum* and *T. monococcum* is limited due to the action of the *Ph* (pairing homeologous) gene system (Luo et al., 2000), introgressive material with the *Sr22* gene is inherited as a single block in most cases. As shown by the studies of J.G. Paull et al. (1994), introgression with the *Sr22* gene decreased grain yield and increased the germination-earing period. At the same time, the studies of T.T. The et al. (1988) revealed a slight decrease in grain productivity, depending on the recipient genotype (a decrease within 10 %). Successful attempts to reduce introgressive material with the *Sr22* gene from *T. boeoticum* for the possible improvement of agronomic performance have been undertaken (Olson et al., 2010). From the available sources, it is not known about the study of the *Sr22+Sr25* combination effect on agronomic performance and flour and bread quality. In addition, in our studies, we took a near isogenic line of the cultivar Marquis with the *Sr22* gene from *T. monococcum* (W3534), which carries a smaller block of introgression material from $7A^m$.

In terms of grain yield, from three years of study, one year (2019) was a significant excess of the recipient cultivar L503, but one year (2020), there was a significant decrease, in general there were no differences, but a decrease in productivity was noted in absolute numbers (1154 kg/ha in introgression line with *Sr22+Sr25* and 1422 kg/ha in cultivar L503). In terms of the germination-earing period, as in previous studies, there was an increase in the line with *Sr22+Sr25* for four days, at the same time there was a decrease in the weight of 1000 grains, there were no differences in plant height.

In our studies, it was found that the line with *Sr22+Sr25* has lower flour and bread making quality compared to the recipient cultivar L503, mainly due to the lower flour strength, coarser porosity and smaller volume of bread, at the same time, there was a high content of protein in grain – 16.9, against 16.4 % in L503.

Analyzing the *Sr35+Sr25* combination, it should be noted that the *Sr35* gene is localized on chromosome 3AL at 41.5 cm from the centromere (McIntosh et al., 1995) and has been studied in detail in terms of structure and regulation (Zhang et al., 2010; Saintenac et al., 2013), but, unfortunately, we could not find information about its effect on agronomic performance in the sources available to us. However, the absence of bread wheat commercial cultivars with this gene indicates a negative impact on the agronomic value (McIntosh et al., 2013).

In our studies, it was found that the line with the combination of *Sr35+Sr25* genes in three years of study conceded in grain productivity only in 2020 and exceeded in 2018. In general, there were no significant differences; in absolute numbers, the grain productivity for the period 2018–2020 for the line with the combination *Sr35+Sr25* was 1454 kg/ha, and for the cultivar L503 – 1422 kg/ha. In terms of the germination–earing period, the line with *Sr35+Sr25* is earing two days later than the cultivar L503, a significant decrease in the weight of 1000 grains was noted, and there were no differences in plant height with the recipient cultivar L503. In terms of flour and bread making quality, the line with *Sr35+Sr25* did not differ from the cultivar L503 except for a significant decrease in bread volume. In terms of protein content in grain, the line with *Sr35+Sr25* did not significantly exceed L503 – 16.9 and 16.4 %, respectively.

Conclusion

Thus, in general, for the entire studied complex of agronomic valuable traits, the combination of *Sr35+Sr25* genes looks more effective than the line with the combination of *Sr22+Sr25* genes. The study showed that the combination of *Sr35+Sr25* genes does not worsen the agronomic performance of wheat; however, it is possible that the expression of the *Sr35* gene in the L503/*Sr35*//L503/3/L503 line is suppressed by suppressor genes of the cultivar L503. It is necessary to further study the expression of the *Sr35* gene in combination with other resistance genes, such as *Sr31*, and its use in Russian breeding programs.

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ORCID ID

S.N. Sibikeev orcid.org/0000-0001-8324-9765
O.A. Baranova orcid.org/0000-0001-9439-2102
A.E. Druzhin orcid.org/0000-0002-3968-2470

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Complex resistance of spring and winter bread wheat lines to biotic and abiotic stresses

I.F. Lapochkina[1] ✉, N.R. Gainullin[1], O.A. Baranova[2], N.M. Kovalenko[2], L.A. Marchenkova[1], O.V. Pavlova[1], O.V. Mitroshina[1]

[1] Federal Research Center "Nemchinovka", Novoivanovskoe, Moscow region, Russia
[2] All-Russian Institute of Plant Protection, Pushkin, St. Petersburg, Russia
✉ inna-lapochkina@yandex.ru

Abstract. An original initial material of spring and winter bread wheat with group resistance to stem and leaf rust was developed using new donors of resistance to stem rust: winter soft wheat GT 96/90 (Bulgaria) and accession 119/4-06rw with genetic material of the species *Triticum migushovae* and (*Aegilops speltoides* and *Secale cereale*), respectively, a line of spring wheat 113/00i-4 obtained using the species *Ae. triuncialis* and *T. kiharae*, as well as spring accession 145/00i with genetic material of the species *Ae. speltoides* resistant to leaf rust. The transfer of effective *Sr*-genes to progeny was monitored using molecular markers. New lines underwent a field assessment of resistance to leaf and stem rust in the epiphytotic development of diseases in the Central Region of the Russian Federation, as well as in the North Caucasus and Western Siberia, and showed high resistance to these pathogens. Fourteen genotypes of spring wheat with group resistance to these diseases and parental forms that participated in the origin of the lines were evaluated for resistance to spot blotch (*Cochliobolus sativus*) and tan spot (*Pyrenophora tritici-repentis*) using isolates from Kazakhstan and Omsk in laboratory conditions. A highly resistant parental form of winter soft wheat from "Arsenal" collection 119/4-06rw (wheat-*Ae. speltoides*-rye hybrid $2n = 42$) with group resistance to two spots, four medium-resistant genotypes to both isolates of tan spot from Kazakhstan and Omsk populations of the pathogen, as well as genotypes resistant to the Omsk isolate of *P. tritici-repentis* (parental form 113/00i-4 and lines 1-16i, 6-16i, 9-16i) were isolated. Among the lines of winter wheat, four were identified with group resistance to spot blotch and tan spot. Additionally, the stress resistance of the lines to NaCl salinization and prolonged flooding of seeds with water was evaluated at the early stages of ontogenesis in laboratory conditions. Lines 33-16i, 37-16i, 32-16i and 9-16i showed a high ability to withstand excess moisture. Lines 33-16i, 37-16i, 32-16i and 3-16i were characterized by high salt tolerance, exceeding the average of 49.7 %. Among the winter genotypes, lines were identified with increased resistance to hypoxia (37-19w, 32-19w, 16-19w, 90-19w) and with increased salt tolerance (20-19w, 9-19w, 37-19w, 90-19w), significantly exceeding the standard cv. Moskovskaya 39. The listed lines are of interest as sources of resistance to anaerobic and salt stress, as well as donors of resistance to a group of fungal diseases: leaf and stem rust and tan spot. We attribute the increased level of resistance of the new initial material to the presence of alien translocations in the original parental forms involved in the origin of the lines.

Key words: common wheat; stem and leaf rust; spot blotch and tan spot; salt resistance; resistance to hypoxia.

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Комплексная устойчивость линий яровой и озимой мягкой пшеницы к биотическим и абиотическим стрессам

И.Ф. Лапочкина[1] ✉, Н.Р. Гайнуллин[1], О.А. Баранова[2], Н.М. Коваленко[2], Л.А. Марченкова[1], О.В. Павлова[1], О.В. Митрошина[1]

[1] Федеральный исследовательский центр «Немчиновка», пос. Новоивановское, Московская область, Россия
[2] Всероссийский научно-исследовательский институт защиты растений, Пушкин, Санкт-Петербург, Россия
✉ inna-lapochkina@yandex.ru

Аннотация. Оригинальный исходный материал яровой и озимой мягкой пшеницы с групповой устойчивостью к стеблевой и бурой ржавчинам создан с использованием новых доноров устойчивости к стеблевой ржавчине – озимой мягкой пшеницы GT 96/90 (Болгария) и линии 119/4-06rw с генетическим материалом соответственно видов *Triticum migushovae* и (*Aegilops speltoides* и *Secale cereale*), линии яровой пшеницы 113/00i-4, полученной с использованием видов *Ae. triuncialis* и *T. kiharae*, а также ярового образца 145/00i с генетическим материалом вида *Ae. speltoides*, устойчивого к бурой ржавчине. Передачу эффективных

Sr-генов потомству отслеживали с помощью молекулярных маркеров. Новые линии прошли полевую оценку устойчивости к бурой и стеблевой ржавчине при эпифитотийном развитии болезней в Центральном регионе Российской Федерации, а также на Северном Кавказе и в Западной Сибири и показали высокую устойчивость к этим патогенам. Четырнадцать генотипов с групповой устойчивостью к этим болезням и родительские формы, принимавшие участие в происхождении линий, в лабораторных условиях оценили на устойчивость к темно-бурой (*Cochliobolus sativus*) и желтой (*Pyrenophora tritici-repentis*) пятнистостям с применением изолятов из Казахстана и Омска. Выделена высокоустойчивая родительская форма озимой мягкой пшеницы из коллекции «Арсенал», 119/4-06rw (пшенично-эгилопсно-ржаной гибрид 2*n* = 42) с групповой устойчивостью к двум пятнистостям и четыре среднеустойчивых генотипа к обоим изолятам желтой пятнистости (из казахстанской и омской популяций патогена), а также генотипы, устойчивые к омскому изоляту *P. tritici-repentis* (родительская форма 113/00i-4 и линии 1-16i, 6-16i, 9-16i). Среди образцов озимой пшеницы выделено четыре с групповой устойчивостью к темно-бурой и желтой пятнистостям. Дополнительно оценена стрессоустойчивость линий в лабораторных условиях на ранних этапах онтогенеза к засолению NaCl и длительному затоплению семян водой. Линии 33-16i, 37-16i, 32-16i и 9-16i проявили высокую способность противостоять избытку влаги. Высокой солеустойчивостью, превышающей средний показатель, 49.7 %, характеризовались линии 33-16i, 37-16i, 32-16i и 3-16i. Среди озимых генотипов выделены образцы с повышенной устойчивостью к гипоксии, 37-19w, 32-19w, 16-19w, 90-19w, и солеустойчивостью – 20-19w, 9-19w, 37-19w, 90-19w, достоверно превышающие стандартный сорт Московская 39. Эти образцы представляют интерес как источники к анаэробному и солевому стрессу, а также как доноры устойчивости к группе грибных заболеваний: бурой и стеблевой ржавчинам и желтой пятнистости листьев. Повышенный уровень устойчивости нового исходного материала мы связываем с наличием чужеродных транслокаций у исходных родительских форм, участвовавших в происхождении линий.
Ключевые слова: мягкая пшеница; стеблевая и бурая ржавчины; темно-бурая и желтая пятнистости; солеустойчивость; устойчивость к гипоксии.

Introduction

The Non-Black Earth Region belongs to the zone of insecure agriculture, which has always been full of abiotic and biotic stress factors. Predominant fungal diseases are powdery mildew, leaf rust, and in 2010 stem rust, which had not been present for 27 years, returned to the fields. In recent years, due to global warming, wheat crops are periodically affected by leaf spots (spot blotch and tan spot) and septoria. The harmfulness of these diseases is high and yield losses can reach 40–50 % (Afanasenko et al., 2011; Mikhailova et al., 2012; Kim, Volkova, 2020).

On the one hand, among abiotic stresses, frequent May droughts that lead to crops getting thinned are observed, and, on the other hand, excess moisture, flooding of crops during snowmelt, snow mold damage are possible. Frequent heavy rainfall during growing season leads to lodged crop. Despite the significant success of breeders in creating highly productive varieties of spring and winter wheat for this zone, the development of the varieties resistant to biotic and abiotic environmental factors remains relevant, especially in recent decades, when we've been facing real facts of climate change leading to a change in the species spectrum of phytopathogenic fungi and their racial composition (Lekomtseva et al., 2007, 2008; Zeleneva et al., 2021).

The main goal of our research was the development of productive competitive spring and winter wheat lines resistant to stem rust *Puccinia graminis* f. sp. *tritici* (*Pgt*) and other dangerous pathogens (*P. triticina*, *Blumeria graminis*, *Pyrenophora tritici-repentis*, *Cochliobolus sativus*) and the identification of other economically valuable qualities and traits of the obtained material. The strategy and tactics of developing such an initial material were based on the previously created "Arsenal" bread wheat collection (Lapochkina, 2005), represented by genotypes with supplemented *Aegilops speltoides* chromosomes and alien translocations *Ae. speltoides*, *Ae. triuncialis*, *Triticum kiharae* and *Secale cereale*, as well as the search for new sources of resistance to the Ug99 stem rust race.

Materials and methods

History of the development of wheat lines with increased resistance to rust fungi began in 2010, when part of the "Arsenal" collection (90 accessions), as well as accessions from All-Russian Research Institute of Plant Genetic Resources (VIR) (129 accessions) were evaluated at the University of Minnesota for resistance to stem rust of the Ug99 race at the seedling stage. Seven genotypes of bread wheat with 2*n* = 42 and 2*n* = 44 from the "Arsenal" collection, as well as several genotypes from the VIR collection that showed resistance to this dangerous pathogen (type of reaction to the penetration of the fungus 0;, 1, 2) were selected. For further study and hybridization hexaploid accessions of known origin and accessions with alien material were left: winter wheat-*Ae. speltoides*-rye line 119/4-06rw (*Ae. speltoides*, *S. cereale*), a line from Bulgaria GT 96/90 with genetic material of the species *Triticum migushchovae*, winter wheat variety Donskaya polukarlikovaya (*Ae. squrrossa*) and a spring wheat accession 113/00i-4 with the genetic material of *Ae. triuncialis* and *T. kiharae* species.

The assessment of economically valuable characteristics in the field conditions of the Moscow region against the leaf rust infectious background highlighted a high resistance to the leaf rust population (0–5 % of severity) in all accessions. Accessions 113/00i-4 (further in Tables – 113) and 119/4-06rw (further – 119) were highly resistant to powdery mildew, and accessions from VIR (cv. Donskaya polukarlikovaya) and the GT 96/90 line) (further – D/p and 96) were susceptible to this disease. However, they had other economically valuable traits: precocity (early heading) and short stem. All accessions were

productive enough not to cause concerns about a decrease in productivity during hybridization.

The *Sr* genes were identified in the accessions using molecular markers recommended for marker-assisted selection (MAS). Molecular markers to 11 *Sr* genes were used: Xgwm533 – *Sr2* (Hayden et al., 2004); STS638 – *Sr15* (Neu et al., 2002); Wpt5343 – *Sr17* (Crossa et al., 2007); Xbarc121, Xcfa2123, Xcfa2019 – *Sr22* (Khan et al., 2005; Yu et al., 2010); Sr24#12, Sr24#50 – *Sr24/Lr24* (Mago et al., 2005); Scm9 – *Sr31* (Weng et al., 2007); Xbarc55, Xstm773 – *Sr32* (Somers et al., 2004; Dundas et al., 2007; Yu et al., 2009); Xwmc477, Xstm773-2 – *Sr36* (Tsilo et al., 2008); Sr39#22 – *Sr39* (Mago et al., 2009); Xgwm344 – *Sr40* (Wu et al., 2009); Xgwm501 – *Sr47* (Faris et al., 2008).

The PCR terms are given in the original works, but optimal conditions for each marker were selected. Both effective and non-effective genes for the Ug99 stem rust race, but showing resistance in the Non-Black Earth Region and the North Caucasus were identified (Baranova et al., 2015). Since we had three winter genotypes and only one spring genotype at our disposal, we considered the strategy for developing hybrid populations with a spring and winter pattern of life using different plant growing backgrounds.

Initially, the parental forms were crossed taking into account their alternative characteristics. Namely: a tall source was crossed with a short-stemmed one; a late-maturing source – with an early-earing one; a genotype resistant to powdery mildew was crossed with a susceptible one. In the first year, direct and reverse crosses of three samples were performed (GT 96/90, 119/4-06rw and 113/00i-4). The Donskaya polukarlikovaya variety's early start of heading made it impossible to carry out hybridization with it. F_2 seeds were divided in half and grown on different backgrounds.

To obtain spring genotypes, sowing was carried out in spring in the field, and the plants that completed the heading process were pollinated either with a recurrent parental form – line 113/00i-4 or with accession 145/05i, which was resistant to powdery mildew and leaf rust, but susceptible to stem rust.

The second half of the seeds was sown in heated ground in February. After seedling emergence, heating was turned off. The plants were vernalized and went through heading in natural conditions. Then, depending on their habitus, they were pollinated either with the Donskaya polukarlikovaya variety, with the GT96/90 line or the 119/4-06rw winter line. The use of heated background, conventional sowing and sowing in greenhouse vessels allowed to speed up the process of obtaining back-cross progeny of various saturation levels.

After self-pollination, individual plants with traits of resistance to leaf rust and powdery mildew, as well as with other valuable traits, were selected from this progeny against the infectious background of leaf rust. Identification of stem rust resistance genes of these plants was conducted using the molecular markers listed above, and plants with several resistance genes in a homozygous state and a complex of economically valuable characteristics were selected for field tests of resistance in the Moscow and Krasnodar Krai regions – as well as in Western Siberia (Omsk).

Immunological assessment of the lines resistance to stem rust in the Central and West Siberian regions was carried out to the natural population of the fungus in field conditions, and in the Krasnodar Krai region – against an artificial infectious background of stem and leaf rust development. North Caucasian populations of *Puccinia* spp were used as infectious material in the latter case. Plant damage level was recorded during the period of maximum development of diseases. The evaluation criteria were the type of reaction and the plant damage level according to the scale recommended by CIMMYT (Roelfs, Singh, 1992).

The resistance to spot blotch (*Cochliobolus sativus*) and tan spot (*P. tritici-repentis*) of the parental forms of crossing and lines with a complex of economically valuable traits was determined. For the latter, two isolates selected from *P. tritici-repentis* populations common in Western Siberia were used: (Omsk isolate) from the temperate climatic zone with a continental climate of forest-steppe and (Kazakhstan isolate) from the sharply continental zone of Northern Kazakhstan. Isolates differ in virulence. The assessment was carried out in laboratory conditions on leaf sections placed in the benzimidazole solution (0.004 %) according to the method of L.A. Mikhailova and co-authors (Mikhailova et al., 2012).

The stress resistance of spring and winter wheat lines to abiotic stresses, namely, water flooding (hypoxia) and NaCl salinization, was evaluated in laboratory conditions at the early stages of ontogenesis according to generally accepted methods (Beletskaya, 1976; Semushkina et al., 1976). The experiments were carried out in two replicates.

Statistical indicators and the reliability of their differences were determined in comparison with standard varieties using statistical analysis (Martynov, 1999).

Results and discussion

As a result of *Sr* genes identification using molecular markers recommended for MAS, both effective and non-effective with regard to Ug99 but demonstrating resistance in the Non-Black Earth Region, genes were identified (Baranova et al., 2015) (Table 1).

From 2 to 4 effective resistance genes were recorded in backcross progeny obtained after self-pollination. The genetic diversity of *Sr* genes among spring and winter wheat plants differed. Nine main gene combinations in spring plants

Table 1. *Sr* genes identified in the sources of resistance to stem rust race Ug99

Source of resistance	Genes of resistance to stem rust	
	Effective	Non-effective
119/4-06rw	*Sr22*, *Sr32*	*Sr9a*, *Sr17*, *Sr19*
GT 96/90	*Sr24*, *Sr36*, *Sr40*, *Sr47*	*Sr15*, *Sr17*, *Sr31*
Donskaya polukarlikovaya	*Sr32*	*Sr9a*, *Sr17*, *Sr19*
113/00i-4	*Sr2*, *Sr36*, *Sr39*, *Sr40*, *Sr47*	*Sr15*

Table 2. The results of the assessment of lines of spring and winter wheat to rust fungi and powdery mildew in various regions of the Russian Federation

Pathogen	Frequency of disease-resistant genotypes, %		
	Krasnodar	Omsk	Moscow
Spring wheat (198 lines), 2015			
P. graminis f. sp. *tritici*	81.0	66.5	–
P. triticina	82.0	98.0	–
B. graminis	–	–	36.0
Winter wheat (367 lines), 2016			
	Krasnodar	Moscow	
P. graminis f. sp. *tritici*	46.0	96.0	
P. triticina	97.0	98.7	
B. graminis	–	40.0	

were identified: *Sr2+Sr36*; *Sr2+Sr39*; *Sr2+Sr32*; *Sr2+Sr22*; *Sr2+Sr36+Sr40*; *Sr2+Sr32+Sr40*; *Sr2+Sr22+Sr40*; *Sr2+Sr32+Sr39*; *Sr2+Sr22+Sr32+Sr40*. It is twice as high in winter crops, but the frequency of occurrence of the *Sr2* resistance gene of adult plants was noted only in 35 % of individual plants selected for gene identification. And in half of the cases, the *Sr2* gene was in a heterozygous state. Winter wheat plants were characterized by a unique combination of resistance genes that are rarely used in the breeding process: *Sr22+Sr32*; *Sr22+Sr47*; *Sr32+Sr47*; *Sr2+Sr22+Sr32*; *Sr22+Sr32+Sr40*; *Sr36+Sr39+Sr47*. A plant with four resistance genes was revealed: *Sr2+Sr22+Sr32+Sr40*.

The evaluation of the progeny of 198 bread wheat spring lines and 367 lines of winter wheat with two-three *Sr* resistance genes was conducted in various geographical points of the Russian Federation to leaf and stem rust, differing in the spectrum of virulence genes (Lapochkina et al., 2016, 2018). This resulted in the selection of lines with group resistance to both pathogens (Table 2). Among the spring wheat lines, a high frequency of resistant genotypes to the North Caucasian population of stem and leaf rust was noted (81–82 %).

The frequency of occurrence of resistant genotypes to the West Siberian population of stem rust was lower (66.5 %). These data were facilitated by late sowing of spring crops, which was intentionally used as a factor stimulating affection by the pathogen. Due to the drought in 2015, 167 of the 198 lines survived, 111 of them were resistant to stem rust, and almost all of the material was resistant to leaf rust. It should also be noted that Western Siberia is characterized by the presence of aggressive population of the stem rust pathogen. This was demonstrated by the assessment results of the collection of isogenic lines and cultivars with known genes of resistance to stem rust, which showed differentiation only at the first assessment of the damage, and later the results were negated due to a strong disease development (Lapochkina et al., 2016), as well as by the results of the study on the racial composition of Western Siberian populations of this pathogen (Skolotneva et al., 2020).

In the Moscow region in 2015, the development of rust fungi was not observed, and an attempt to create an artificial leaf rust background failed due to high temperatures and low humidity of air and soil. However, 71 lines (36 %) with resistance to powdery mildew have been selected this year.

The resistance of 367 winter wheat lines was evaluated under the conditions of epiphytoty development of stem rust in Krasnodar and Moscow in 2016. In Krasnodar, 168 lines resistant to *P. graminis* were selected; almost all lines were resistant to leaf rust. Under the conditions of stem rust epiphytotic, a high yield of resistant genotypes 96–98 % to both pathogens) was also noted in Moscow. The frequency of occurrence of genotypes resistant to powdery mildew was about 40 %.

According to the assessment results, about 70 lines of spring wheat and more than 100 winter lines combining group resistance to rust fungi with a complex of other economically valuable characteristics (early heading period, optimal height, ear productivity of 1.7–2.5 g, large grain and high protein and gluten content in the grain) were selected.

Among the lines with a complex of economically valuable characteristics, an additional assessment of resistance to pathogens that cause spot blotch and tan spots development on the leaves was carried out. In total, 14 lines of spring wheat, 9 lines of winter wheat and the initial parental forms of crossing were evaluated (Table 3).

According to the evaluation results, the only medium-resistant accession 119/4-06rw from the "Arsenal" collection with reaction type 2 to spot blotch was identified. With regard to spot blotch, this is an excellent result. It is extremely rare for accessions resistant to spot blotch to be selected. Generally, reaction type 3–4 is traced in bread wheat accessions. The resistance to this pathogen is usually associated with the species *T. macha*, *T. vavilovii*, *T. timopheevii*, *T. monococcum* and *T. spelta* (Mikhailova et al., 2012).

Two isolates were used to infect the leaf segments with *P. tritici-repentis*: from Kazakhstan and Omsk. Accessions 119/4-06rw and GT 96/90 demonstrated high resistance to both isolates. High resistance to the Omsk tan spot isolate was found in the spring wheat line 113/00i-4 with the genetic material *Ae. triuncialis* and *T. kiharae*.

Among the 14 tested lines of spring wheat, no resistant genotypes to spot blotch were detected. Ten lines were resistant to the Omsk tan spot isolate, and 4 lines showed resistance to both the Kazakhstan and Omsk tan spot isolate (16i-16i, 17i-16i, 33-16i, 48-16i).

Among the 9 lines of winter wheat that got to check plant breeding nursery and competitive variety test, 4 were found to have resistance to spot blotch: 9-19w, 31-19w, 63-19w, 90-19w. Being infected with the most virulent tan spot isolate from Kazakhstan, the same four lines were selected with high resistance to *P. tritici-repentis*.

Table 3. The assessment results of the sources of resistance to stem rust and lines of spring and winter wheat obtained with the involvement of the sources to tan spot (*P. tritici-repentis*) and spot blotch (*C. sativus*)

Line, variety	Origin	Type of reaction to		
		tan spot		spot blotch
		Isolate from		
		Kazakhstan	Omsk	
GT96/90	Zhirovka/Mironovskaya poluintensivnaya	1/1 – R	1/1 – R	3
119/4-06rw	Rodina/*Ae. speltoides* (10 kR)/*S. cereale* (0.75 kR)	0/0 – R	0/0 – R	2
113/00i-4	Rodina/*Ae. triuncialis* (5 kR)/*T. kiharae*	2/2 – MR	2/1 – MR	4
Donskaya polukarlikovaya	Rusalka/Severodonskaya	2/2 – MR	2/2 – MR	3
1-16i	(96/113)/145/113	3/3 – S	2/1 – MR	3
6-16i	(96/113)/113	2/3 – MS	1/1 – R	4
9-16i	(96/119)113	2/3 – MS	1/1 – R	3
16-15i	(96/113)/113	1/2 – MR	2/1 – MR	3
17-16i	(96/113)	1/2 – MR	2/1 – MR	4
28-16i	(113/96)145/113	3/1 – MS	2/1 – MR	4
31-16i	(96/113)/113	2/1 – MR	3/2 – S	4
32-16i	(96/113)/113	3/3 – S	3/2 – S	3
33-16i	(96/113)/113/113	2/2 – MR	2/2 – MR	3
37-16i	(96/113)/145	2/2	2/2	4
44-16i	(113/119)/113	2/3 – MS	2/2 – MR	4
45-16i	(96/119)/113	1/3 – MS	1/2 – MR	4
48-16i	(96/119)/113	2/1 – MR	1/2 – MR	3
57-16i	(96/119)/113	2/3 – MS	1/3 – MS	3
Lada	(Obriy/Leningradka)/Moskovskaya 35	3/3	4/3	3
9-19w	(113/119)/D	1/1	–	2
16-19w	(113/119)/D/D/D	2/2	–	3
20-18w	(96/113)/96/96	1/1	–	3
31-19w	(96/113)/D	1/1, 2/1	–	2
36-19w	(96/113)/D/96	2/2	–	3
48-19w	(113/96)/D/D	2/2	–	3
63-19w	(113/96)/96	2/2	–	2
90-19w	(119/96)/119	2/2	–	2
92-19w	(119/96)/D	2/2	–	3
Moskovskaya 39	Obriy/Yantarnaya 50	2/2	–	3

Table 4. The reaction of spring wheat lines to water flooding of seeds

Line, variety	The number of normally sprouted seeds in		Resistance to hypoxia, %
	Control	Test	
Zlata (St)	100	47	47.0
Lada	96	82	85.4
33-16i	95	60	66.6
37-16i	88	51	58.0
32-16i	90	52	57.8
9-16i	92	52	56.5
3-16i	93	47	50.5
48-16i	87	40	46.0
57-16i	94	40	42.6
17-16i	88	7	8.0
Average	89.1	42.2	46.0
CV, %	13.2	48.2	43.2
LSD_{005}	–	–	7.7

Table 5. The reaction of winter wheat lines to water flooding of seeds

Line, variety	The number of normally sprouted seeds in		Resistance to hypoxia, %
	Control	Test	
Moskovskaya 39	98	75	76.5
37-19w	96	83	86.5
32-19w	94	78	83.0
16-19w	90	72	80.0
9-19w	98	75	76.5
4-19w	92	63	68.5
96-19w	96	65	67.7
20-19w	96	65	67.7
41-19w	100	62	62.0
36-19w	100	47	47.0
2-19w	96	31	32.3
Average as of the test	96.1	64.5	63.5
CV, %	3.0	30.5	29.6

The stress resistance of spring and winter wheat lines to water flooding (hypoxia) and NaCl salinization was evaluated under laboratory conditions at the early stages of ontogenesis. Progeny of 11 lines of spring wheat from the Nursery that had been tested for two years and two standard varieties (the modern variety Zlata and the previous variety Lada) were tested (Table 4). A high level of variation of the resistance to hypoxia basis (CV > 40 %) was recorded. Four lines (33-16i, 37-16i, 32-16i and 9-16i) reliably exceeded the indicators of the Zlata variety with regard to the mentioned stress factor; however, like this variety, they lagged behind the breeding masterpiece of E.D. Nettevich – the Lada variety (Nettevich et al., 1996).

As for the resistance to hypoxia, about two dozen lines of winter wheat were evaluated from the check plant nursery of 2019 (the conditions for seed formation were favourable) and from Competitive Plant Nursery of 2020 (the grain was formed in conditions of heavy rainfall and lodged crops). A high level of property variation was noted: the number of normally sprouted seeds (CV > 30 %). Four genotypes with resistance above the average as of the test and standard were identified: 37-19w, 32-19w, 16-19w and 9-19w (Table 5).

The results obtained with seeds of winter lines formed under unfavourable conditions in 2020 were markedly different. Only 50 % of seeds of the standard variety sprouted normally after flooding. Against this background, two lines, 90-19w and 16-19w, reliably exceeded the resistance to hypoxia level of the standard variety.

The harmful effect of NaCl salinization caused the depression of the length of seedlings in spring wheat. Both standard varieties and line 37-16i had high resistance to sodium chloride, exceeding the average results of the test (49.6 %). (Table 6). The comparison of the effect of anaerobic stress and salinization on spring wheat showed that the range of variability for salt tolerance fluctuated from 40 to 62 %, and for anaerobic stress – from 8 to 85 % that is a stronger differentiation of spring wheat genotypes with regard to water stress.

A high level of growth depression during salinization was also noted in winter wheat lines (CV = 28 %). A high ability to resist salt stress exceeding both the average of the test and the St variety level was revealed in the lines: 20-19w, 9-19w and 37-19w (Table 7).

The test with planted seeds formed in the unfavourable year of 2020 showed, on the one hand, a decrease in salt tolerance level in Moskovskaya 39 from 68 to 49 %, and, on the other hand, revealed another 90-19w line resistant to this stress, which has been in the nursery of competitive variety testing since 2021.

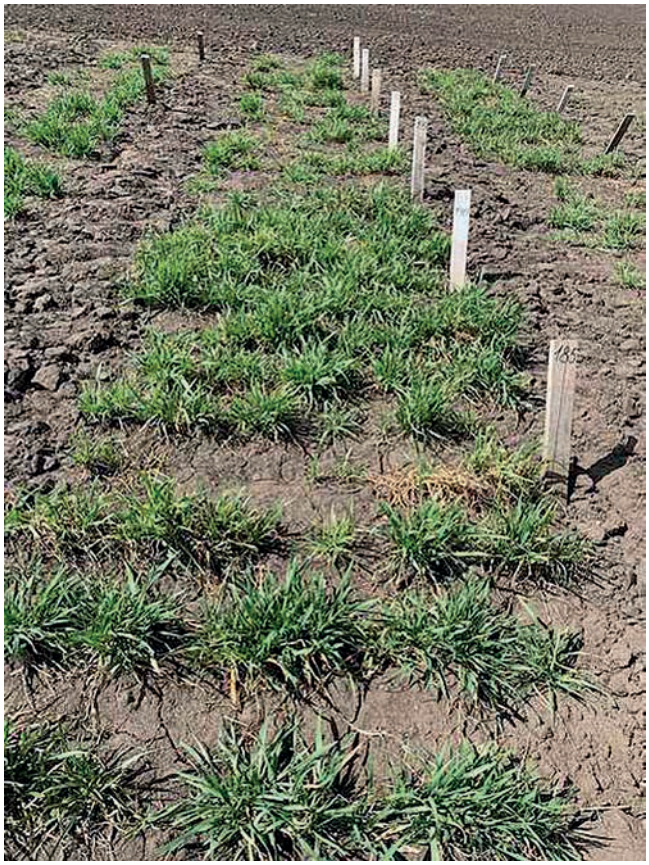
Some of the lines are already under additional environmental testing at the Federal Scientific Agroengineering Center VIM in the Ryazan region. Expansion of environmental testing of winter wheat lines to Western Siberia (the backcrossed progeny of individual plants was transferred to the Omsk State Agrarian University), makes it possible to develop a new winter wheat crop for this region. There is a high probability of selecting winter-hardy winter wheat lines with group resistance to fungal diseases that demonstrated a good overwintering level according to the results of assessments

Table 6. Depressive effect of NaCl on growth processes in spring wheat lines

Line, variety	Length of seedlings, mm		Salt tolerance, %
	Test	Control	
Zlata (St)	50.0	82.7	60.5
Lada	51.6	90.6	57.0
33-16i	55.5	107.3	51.3
37-16i	60.8	98.2	62.0
32-16i	54.5	101.2	54.0
9-16i	37.3	87.9	42.5
3-16i	53.5	103.3	51.4
48-16i	46.9	118.7	39.5
57-16i	55.0	111.9	49.2
16-15i	41.1	102.3	40.2
17-16i	45.1	96.1	47.0
Average	50.1	100.0	49.6
LSD_{005}			5.1

Table 7. Depressive effect of NaCl on growth processes in winter wheat lines

Line, variety	Length of seedlings, mm		Salt tolerance, %
	Test	Control	
Moskovskaya 39	62.5	92.3	67.7
20-19w	71.3	74.4	95.8
9-19w	95.0	98.2	96.7
37-19w	60.0	73.4	81.8
41-19w	62.8	83.6	75.1
32-18w	45.2	64.4	70.2
35-19w	53.2	76.6	69.5
74-19w	52.0	89.7	58.0
36-19w	37.6	60.5	54.1
16-19w	45.8	90.3	50.7
Average	55.1	79.4	69.0
CV, %	27.5	12.7	21.1
LSD_{005}			10.6



The winter wheat lines regrowth in Western Siberia in May 2021.

The photo was kindly provided for publication by Professor V.P. Shamanin, Omsk State Agrarian University.

performed in May 2021 (see the Figure). These lines are highly likely to be resistant to rust fungi, since this material has already been evaluated for resistance to these pathogens in the Krasnodar Krai region and in the Moscow region in 2016 during the epiphytotic stem rust. Since there are genotypes that are resistant to salinization among them, there is a high probability of selecting genotypes that are resistant to drought, which is important for Western Siberia, since both resistances are correlated.

Conclusion

After a comprehensive assessment of the obtained initial material and the identification of additional positive properties has facilitated the selection of breeding lines for reproduction and competitive variety testing. While in previous years we focused our attention on lines with a high protein and gluten content in grain and resistant to rust diseases, now we have lines with complex resistance to phytopathogens and abiotic stresses: spring wheat lines, 9-16i, 32-16i, 37-16i and 48-16i; winter wheat lines, 20-19w, 9-19w, 9-19w, as well as lines 31-19w and 48-19w with extended group resistance to fungal diseases (stem and leaf rust, powdery mildew, tan spot and spot blotch). This material can be used as a source of resistance to unfavourable environmental factors at the next stage of

improving bread wheat, as well as to identify its possibility to compete for productivity with modern wheat varieties.

The obtained initial material is of interest for molecular genetic mapping of resistance and QTL genes, as well as for MAS for *Sr* genes, especially for genes that are rarely used to increase immunity to stem rust: *Sr32*, *Sr39*, *Sr40*, and *Sr47*.

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ORCID ID

I.F. Lapochkina orcid.org/0000-0002-2328-2798
N.R. Gainullin orcid.org/0000-0002-0970-662X
O.A. Baranova orcid.org/0000-0001-9439-2102
N.M. Kovalenko orcid.org/0000-0001-9577-8816
L.A. Marchenkova orcid.org/0000-0001-6029-8089
O.V. Pavlova orcid.org/0000-0001-7705-075X
O.V. Mitroshina orcid.org/0000-0002-8321-0307

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Resistance of oat breeding lines to grain contamination with *Fusarium langsethiae* and T-2/HT-2 toxins

O.P. Gavrilova[1], T.Yu. Gagkaeva[1], A.S. Orina[1], A.S. Markova[2], A.D. Kabashov[2], I.G. Loskutov[3]

[1] All-Russian Institute of Plant Protection, Pushkin, St. Petersburg, Russia
[2] Federal Research Center "Nemchinovka", Novoivanovskoe, Moscow region, Russia
[3] Federal Research Center the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia
i.loskutov@vir.nw.ru

Abstract. Fusarium disease of oats reduces yield quality due to decreasing germination that is caused by then contamination of grain with mycotoxins produced by *Fusarium* fungi. The aim of this study was to characterize the resistance of naked breeding lines of oats to fungal grain infection and to contamination with T-2 and HT-2 toxins. Thirteen naked oat breeding lines and two naked varieties, Nemchinovsky 61 and Vyatskiy, as well as a husked variety Yakov, were grown under natural conditions in the Nemchinovka Federal Research Center in 2019–2020. The contamination of grain with fungi was determined by the mycological method and real-time PCR. The analysis of mycotoxins was carried out by ELISA. In oats, *Alternaria* (the grain infection was 15–90 %), *Cochliobolus* (1–33 %), *Cladosporium* (1–19 %), *Epicoccum* (0–11 %), and *Fusarium* (3–17 %) fungi prevailed in the grain mycobiota. The predominant *Fusarium* species were *F. poae* (its proportion among *Fusarium* fungi was 49–68 %) and *F. langsethiae* (29–28 %). The highest amounts of *F. langsethiae* DNA ((27.9–71.9) × 10^{-4} pg/ng) and T-2/HT-2 toxins (790–1230 μg/kg) were found in the grain of husked oat Yakov. Among the analysed naked oat lines, the amount of *F. langsethiae* DNA varied in the range of (1.2–42.7) × 10^{-4} pg/ng, and the content of T-2/HT-2 toxins was in the range of 5–229 μg/kg. Two oat breeding lines, 54h2476 and 66h2618, as well as a new variety, Azil (57h2396), can be characterized as highly resistant to infection with *Fusarium* fungi and contamination with mycotoxins compared to the control variety Vyatskiy.
Key words: *Avena sativa*; naked; breeding; resistance; *Fusarium*; DNA; mycotoxins.

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Характеристика устойчивости селекционных линий овса к заражению *Fusarium langsethiae* и накоплению T-2/HT-2 токсинов

О.П. Гаврилова[1], Т.Ю. Гагкаева[1], А.С. Орина[1], А.С. Маркова[2], А.Д. Кабашов[2], И.Г. Лоскутов[3]

[1] Всероссийский научно-исследовательский институт защиты растений, Пушкин, Санкт-Петербург, Россия
[2] Федеральный исследовательский центр «Немчиновка», пос. Новоивановское, Московская область, Россия
[3] Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова (ВИР), Санкт-Петербург, Россия
i.loskutov@vir.nw.ru

Аннотация. Фузариоз относится к важным заболеваниям овса, поскольку многие виды грибов *Fusarium* способны продуцировать микотоксины, негативно влияющие на качество зерна. Иммунных к заражению грибами *Fusarium* зерновых культур нет, однако наблюдаются различия генотипов по степени устойчивости. Целью исследования стала характеристика перспективных линий голозерного овса по устойчивости к зараженности зерна грибами и содержанию фузариотоксинов. Анализировали 13 селекционных линий и два сорта голозерного овса, Немчиновский 61 и Вятский, а также сорт пленчатого овса Яков, которые выращивали на естественном фоне в Федеральном исследовательском центре «Немчиновка» в 2019–2020 гг. Зараженность зерна грибами определяли микологическим методом, а также с помощью полимеразной цепной реакции (ПЦР) в реальном времени, анализ микотоксинов выполняли иммуноферментным методом. Проведенные анализы показали, что зерно всех образцов овса было заражено грибами, однако их численность и видовое разнообразие варьировали в зависимости от анализируемого генотипа и года исследований. Микобиоту генотипов овса преимущественно составляли виды родов *Alternaria* (15–90 % от всех выделенных грибов), *Cochliobolus* (1–33 %), *Cladosporium* (1–19 %), *Epicoccum* (0–11 %) и *Fusarium* (3–17 %). Основными

представителями фузариевых грибов были *F. poae*, продуцирующий ниваленол, и *F. langsethiae*, производящий Т-2/НТ-2 токсины. Наибольшее количество ДНК *F. langsethiae*, а также Т-2/НТ-2 токсинов содержалось в зерне пленчатого сорта Яков и составило (27.9–71.9)×10^{-4} пг/нг и 790–1230 мкг/кг соответственно. В зерне анализируемых линий овса содержание ДНК *F. langsethiae* варьировало в диапазоне (1.2–42.7)×10^{-4} пг/нг, Т-2/НТ-2 токсинов – от 5 до 229 мкг/кг. Две линии голозерного овса, 54h2476 и 66h2618, а также новый сорт Азиль (линия 57h2396) можно охарактеризовать в условиях эксперимента как высокоустойчивые к заражению грибами *Fusarium* и контаминации микотоксинами в сравнении с контролем – сортом Вятский.
Ключевые слова: *Avena sativa*; голозерный; селекция; устойчивость; фузариоз; ДНК; микотоксины.

Introduction

Over the past decade, the amount of information on Fusarium disease of oats (*Avena sativa* L.) has increased dramatically. The infection of oats caused by different *Fusarium* Link species is recognized as one of the most devastating diseases of this cereal crop. In addition to direct negative impacts on economically valuable traits, such as the loss of grain yield (Martinelli et al., 2014), the harmfulness of *Fusarium* fungi is determined by their ability to produce different mycotoxins that accumulate in infected grains. Mycotoxins produced by many *Fusarium* species remain in processed products and, when consumed by people or animals, can cause immunosuppression and various health issues (Foroud et al., 2019). Current studies of the *Fusarium* problem in oats concern the analysis of grain infection by different fungal species and the determination of mycotoxin contents in grain (Fredlund et al., 2013; Gavrilova et al., 2016; Hofgaard et al., 2016; Schöneberg et al., 2018), the study of host-pathogen interactions (Divon et al., 2012; Tekle et al., 2012; Martin et al., 2018; Wilforss et al., 2020) and the search for potential sources of resistance to the disease, including the use of molecular analysis methods (He et al., 2013; Bjørnstad et al., 2017; Isidro-Sánchez et al., 2020).

The composition and representation of *Fusarium* species causing the disease in oats vary significantly and depend on the place of cultivation and the prevailing weather conditions during the growing season (Schöneberg et al., 2018). As a rule, the main species of *Fusarium* fungi responsible for disease in oats are *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb. and *F. langsethiae* Torp & Nirenberg (Kurowski, Wysocka, 2009; Fredlund et al., 2013; Gavrilova et al., 2016; Hofgaard et al., 2016), while *F. graminearum* Schwabe (Schöneberg et al., 2018) and *F. avenaceum* (Fr.) Sacc. (Vargach et al., 2019) occur less often. All of the mentioned *Fusarium* fungi are capable of producing various mycotoxins. The results of numerous studies demonstrate a high contamination of grain with T-2 and HT-2 toxins produced by *F. sporotrichioides* and *F. langsethiae* (Opoku et al., 2013; Burkin et al., 2015; Hofgaard et al., 2016; Kononenko et al., 2020; De Colli et al., 2021).

In the breeding of oat varieties, the trait of resistance to Fusarium disease was not taken into account for a long time despite the problem with grain infection of this cereal crop. The main challenge of the evaluation of resistance of oat genotypes to the disease in the field is the absence or weak symptoms of *Fusarium* infection on oat panicles, in contrast to the noticeable specific symptoms on heads of other small-grain cereals (Tekauz et al., 2008; Imathiu et al., 2013; Martin et al., 2018; Zhuikova, Batalova, 2019). However, *Fusarium* fungi and mycotoxins in the grain of asymptomatic spikelets in panicles are often detected, and oat genotypes can be significantly different according to their amounts. In addition, it is already well known that the disease severity is determined by factors such as the weather and infection pressure.

There are no cereals that are immune to infection with *Fusarium* fungi; however, different degrees of resistance are observed among genotypes. Previously, it was mentioned that a wheat genotype resistant to infection with one *Fusarium* species also tends to be resistant to other species of this genus (Mesterhazy et al., 2005). Additionally, several types of resistance to Fusarium disease in cereals have been described and commonly divided into at least five separate types (Boutigny et al., 2008; Tekle et al., 2018): resistance against initial infection (type I), resistance against the spread of infection (II), resistance against grain infections (III), tolerance (IV), and resistance to mycotoxin accumulation or degradation (V). In the sowing oats (*A. sativa* L.), two subspecies, husked oats (*A. sativa* subsp. *sativa* L.) and naked oats (*A. sativa* subsp. *nudisativa* (Husn.) Rod. et Sold.), which differ from each other in their morphological characteristics, biochemical properties and resistance to abiotic and biotic factors, were described (Kobylyansky, Soldatov, 1994; Loskutov et al., 2020). The relatively high resistance of naked oats to *Fusarium* infection of grain, in comparison with husked oats, has been repeatedly noted (Tekauz et al., 2008; Yan et al., 2010; Gagkaeva et al., 2013; Martin et al., 2018; Chropová et al., 2020).

Earlier, information on the resistance of oat genotypes from the VIR collection to Fusarium disease, which was analysed under conditions of artificial inoculation with *F. sporotrichioides*, was systematized in the Catalogue (Gagkaeva et al., 2012). A successful example of combining the efforts of different research groups was the breeding of a new variety of naked oats, Vsadnik, which is the first officially registered variety in Russia characterized as relatively resistant to Fusarium disease. This variety accumulated significantly lower amounts of mycotoxins in the grain than the standard husked variety Konkur, which is cultivated over a wide area in Russia (Mishenkina, Zakharov, 2017).

At present, the attention of many Russian oat breeders is focused on the creation of naked oat varieties characterized by improved grain quality and resistance to fungal diseases (Kabashov et al., 2018; Batalova et al., 2019; Isachkova et al., 2019; Zhuikova et al., 2020). The progress achieved in the breeding process is evidenced by the increase in the number of naked oat varieties included in the “State Register of Selection Achievements…”, which in 2020 consisted of

Table 1. The breeding lines and varieties of oats included in the study

Breeding line/variety	Pedigree	Year of analysis
57h2396/Azil	Krestyansky local × Zalp	2019, 2020
2h2348	Krestyansky local × Rysak	
16h2476	32h1962 × AC Lotta (k-14619)	
54h2476	32h1962 × AC Lotta (k-14619)	
2h2532	AC Baton (k-14803) × 53h2035	
52h2467	28h1827 × Abel (k-14638)	
50h2613	Zalp × Bullion (k-14683)	
70h2613	15h1946 × Bullion (k-14683)	
55h2618	55h2106 × Pennline 2005 (k-14344)	
66h2618	55h2106 × Pennline 2005 (k-14344)	
4h2708	Vyatsky × Rysak	2020
16h2771	Krestyansky local × 14h2255	
15h2657	119h2093 × 37h2273	
Nemchinovsky 61	Krestyansky local × 15h1880	2019, 2020
Vyatsky (control)	Individual selection from the variety of naked oat Adam from the VIR collection (k-14253, Czech Republic), followed by multiple selection based on hulliness trait	
Yakov (standard)	Soroca (к-13243) × 36h1127	

121 varieties of husked oats and 15 varieties of naked oats[1]. Since 2010, 11 new varieties of naked oats have been included in the State Register.

The aim of this study was to characterize the resistance of naked oat lines to contamination of grain with *Fusarium* fungi and T-2/HT-2 toxins. These oat genotypes are the breeding material of the Federal Research Center "Nemchinovka" and were cultivated in field experiments under natural conditions.

Materials and methods

Oats breeding material. 10 and 13 naked oat breeding lines (*A. sativa* subsp. *nudisativa* (Husn.) Rod. et Sold.) were analysed in 2019 and 2020, respectively. In addition, the naked oat varieties Nemchinovsky 61 (NFRC) and Vyatsky as a control (Zonal North-East Agricultural Research Institute, Kirov region) and the standard husked variety Yakov (NFRC) were included in the study (Table 1).

Cultivation of breeding material. In 2019–2020, the analysed varieties and breeding lines of oats were grown after spring barley as the previous crop in the experimental 10 m^2 plots in the nursery of the NFRC according to the state variety testing methodology[2]. The harvesting of oats was carried out at the full-mature stage: August 8, 2019, and August 16, 2020. The weather conditions in the growing seasons of 2019 and 2020 were different (Table 2). The summer period of 2020 was characterized by an increased temperature in June-August compared to the long-term average values, as well as a 1.7–2.6 times excess of the total precipitation in May-July compared to this period in 2019.

Mycological analysis of grain infection. To evaluate the fungal infection and species composition of oat grain mycobiota, 100 seeds of each genotype were surface sterilized in 5 % sodium hypochlorite and washed with sterilized water. Then, grains were placed on potato sucrose agar medium (PSA) in Petri dishes (Orina et al., 2018), and incubated in the dark at 24 °C in an MIR-254 thermostat (Sanyo, UK). After seven days, the number and species diversity of fungi isolated from the grain were registered.

The taxonomic status of the isolated fungi was determined by the sum of macro- and micromorphological characters according to the manuals (Ellis, 1971; Gerlach, Nirenberg, 1982; Samson et al., 2002; Torp, Nirenberg, 2004).

The grain infection by definite fungi was quantified as the percentage ratio of the number of grains from which these fungi were isolated to the total number of analysed grains.

Analysis of *F. langsethiae* DNA content. Ten grams of grain of every oat genotype was homogenized separately using sterilized grinding chambers of a Tube Mill Control batch mill (IKA, Germany) at 25,000 rpm for 30–45 s. Total DNA from 200 mg of grain flour was isolated using the CTAB

[1] The State Register of Selection Achievements Approved for Use. Vol. 1. Plant Varieties (at February 26, 2020). https://gossortrf.ru/gosreestr//.
[2] Methodology for State Variety Testing of Agricultural Crops. Second edition. Grains, Cereals, Legumes, Corn and Fodder Crops. Moscow, 1989.

Table 2. The weather conditions of summer 2019 and 2020 in the Moscow region (meteorological station No. 27515)

Month	Temperature, °C			Average humidity, %	Total precipitation, mm	Number of days with precipitation
	average	min	max			
			2019			
May	+16.1	+1.3	+28.5	59	50	16
June	+19.6	+8.6	+31.3	57	60	11
July	+16.6	+8.3	+29.3	70	42	20
August	+16.2	+6.8	+27.9	71	36	14
			2020			
May	+11.5	+0.7	+24.1	68	124	24
June	+19.1	+8.0	+30.5	68	100	14
July	+18.3	+9.1	+30.5	78	110	20
August	+17.3	+8.6	+30.3	75	46	13

method (Gagkaeva et al., 2013). Genomic DNA was isolated from the mycelium of a typical *F. langsethiae* strain from the collection of the Laboratory of Mycology and Phytopathology of All-Russian Institute of Plant Protection using a Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's protocol.

The DNA concentrations from the grain samples and from fungal strains were determined using a Qubit 2.0 fluorometer with a Quant-iT dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). Before the start of quantitative PCR (qPCR), the concentrations of all DNA samples were aligned to 20–60 ng/μL.

The *F. langsethiae* DNA content in every DNA sample extracted from oat flour was estimated by qPCR with a TaqMan probe fluorescently labelled with Cy5 dye and a BHQ-2 quencher (Yli-Mattila et al., 2008).

Amplification reactions were run using the CFX 96 Real-Time System (BioRad, USA) according to the following protocol: 1 × [95 °C, 3 min]; 40 × [95 °C, 10 s; 60 °C, 10 s; 72 °C, 20 s]. The DNA content was calculated as the ratio of fungal DNA to total DNA in each sample (pg/ng).

Analysis of mycotoxin content. The mycotoxins were extracted from 1 g of oat flour with 5 mL of an acetonitrile:water mixture (84:16, *v/v*) for 14–16 h. The total amounts of T-2 and HT-2 toxins in the extracts were determined using an indirect competitive enzyme-linked immunosorbent assay. The diagnostic certified test system "T-2 toxin–ELISA" (All-Russian Research Institute for Veterinary Sanitation, Hygiene and Ecology, Russia) was used. The limit of mycotoxin detection was 4 μg/kg.

Statistical analysis. The contents of fungal DNA and mycotoxins in the grain of each genotype were analysed at least twice. The mean values, confidence intervals, Pearson coefficients of correlation (*r*) between quantitative parameters and variance analysis (ANOVA) were performed using Microsoft Excel 2010, Minitab 17 and Statistica 10.0 programs. Differences were considered significant at $p < 0.05$.

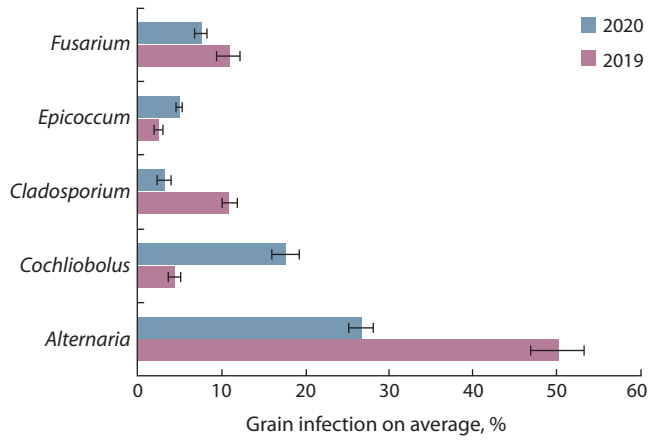

Fig. 1. Fungal infection of oat grain (Federal Research Center "Nemchinovka", Moscow region, 2019–2020).

Results

Fungal infection of oat grain

The predominance of fungi belonging to *Alternaria* Nees, *Cochliobolus Drechsler*, *Cladosporium* Link, *Epicoccum* Link, and *Fusarium* genera in the grain of the analysed oat genotypes was revealed by the mycological method. In addition, the fungi *Acremonium* Link, *Arthrinium* Kunze, *Gliocladium* Corda, *Microdochium* Syd. & P. Syd., *Mucor* Fresen., *Nigrospora* Zimm., *Penicillium* Link, *Phoma* Sacc., and *Trichothecium* Link genera were sporadically isolated from the grain.

Alternaria fungi were the most abundant in the oat grain mycobiota in both years of the study (Fig. 1). The majority of isolated *Alternaria* spp. was represented by fungi belonging to section *Alternaria* (86 % in 2019 and 84 % in 2020), and the remaining isolates were identified as *Alternaria* fungi belonging to section *Infectoriae*.

Grain infection with *Cochliobolus* fungi, including *Bipolaris sorokiniana* Shoemaker, *Drechslera avenae* (Eidam)

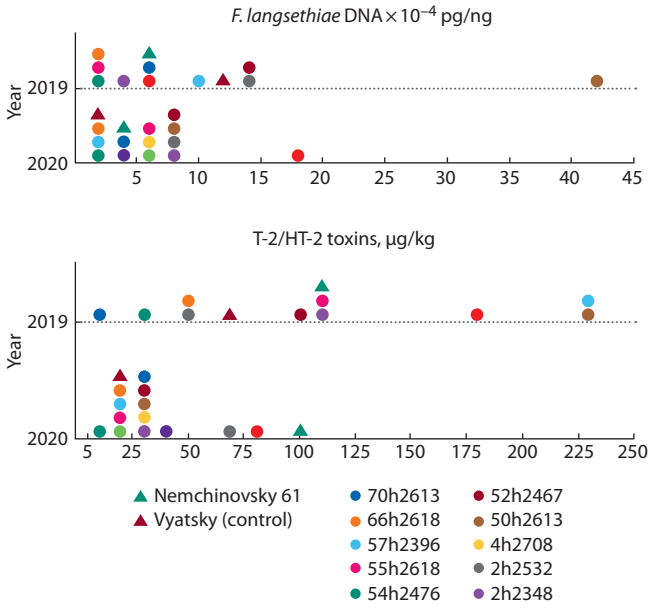

Fig. 2. Contents of *F. langsethiae* DNA and T-2/HT-2 toxins in the grain of the varieties and breeding lines of oats (Federal Research Center "Nemchinovka").

Sharif (*Pyrenophora avenae* Ito & Kurib) and others, differed significantly in the years of study ($p = 0.000012$). In 2019, oat grain infection with *Cochliobolus* spp. varied in the range of 1–13 %, whereas in 2020, its incidence was 6–33 %.

The proportion of grains colonized by *Fusarium* spp. did not differ significantly between the years of study. The incidences of *Fusarium* infection of grain of naked oat lines and the variety Nemchinovsky 61 varied from 5 to 17 % in 2019 and from 3 to 13 % in 2020. For the husked variety Yakov, the incidences of *Fusarium* grain infection were 26 and 17 % in 2019 and 2020, respectively. For the control variety Vyatsky, the incidences were 5 and 3 %, respectively. In both years of the study, only two lines, 54h2476 and 66h2618, were characterized by lower grain infection or coincidence with the control variety Vyatsky grain infection with *Fusarium* spp. At least nine *Fusarium* species were identified in the mycobiota of oat grains, but toxin-producing *F. poae* and *F. langsethiae* species prevailed in both years. The proportions of these fungi among *Fusarium* spp. isolates were 49–68 % for *F. poae* and 29–28 % for *F. langsethiae*.

Contents of *F. langsethiae* DNA and T-2/HT-2 toxins

The highest content of *F. langsethiae* DNA was revealed in the grain of the husked variety Yakov and amounted to 71.9×10^{-4} pg/ng in 2019 and 27.9×10^{-4} pg/ng in 2020. In the grain of the control naked variety Vyatsky, the *F. langsethiae* DNA content was significantly lower, reaching 11.0×10^{-4} pg/ng in 2019 and 1.2×10^{-4} pg/ng in 2020. In 2019, only three oat breeding lines, 2h2532, 52h2467, and 50h2613, contained more *F. langsethiae* DNA than the control variety Vyatsky. In 2020, the contents of fungal DNA in the grain of all analysed oat breeding lines were higher than that in the control variety (Fig. 2).

The content of T-2/HT-2 toxins was the highest in the grain of the husked variety Yakov – 1230 µg/kg in 2019 and 790 µg/kg in 2020. In the grain of the naked variety Vyatsky, these mycotoxins were detected in lower amounts of 71 and 23 µg/kg in 2019 and 2020, respectively. The content of T-2/HT-2 toxins in the grain of oat breeding lines varied in the ranges of 5–230 µg/kg in 2019 and 10–100 µg/kg in 2020 (see Fig. 2).

Discussion

The mycological analysis revealed the presence of fungal infection in the grain of all oat genotypes; however, the number and species composition of identified micromycetes varied depending on oat genotype and crop year.

The average temperatures during the vegetation seasons in both years were similar; however, the precipitation in May-August in 2020 was two times higher than that observed in the previous year. As a result, the average grain infection with *Cochliobolus* increased fourfold in 2020; at the same time, grain infections with *Alternaria*, *Cladosporium* and *Fusarium* fungi significantly decreased 1.4–3.5 fold.

With the high incidence of *Cochliobolus* infection of oat grain in 2020, a significant negative correlation between infection with *Cochliobolus* and *Alternaria* fungi was revealed ($r = –0.56$, $p = 0.024$). Previously, antagonistic relationships between these two groups of fungi associated with small-grain cereals were also established (Kazakova et al., 2016; Gannibal, 2018; Orina et al., 2020). Perhaps the *Cochliobolus* were more competitive in the wetter conditions, and these fungi had an advantage over *Alternaria* and *Fusarium* fungi.

Significant positive correlations between grain infection and *Alternaria* and *Fusarium* fungi ($r = 0.64$, $p = 0.019$) and *Epicoccum* and *Fusarium* fungi ($r = 0.57$, $p = 0.043$) were revealed in 2019. A symbiotic relationship between *Alternaria* and *Fusarium* fungi in cereal grain has been established repeatedly (Kosiak et al., 2004; Orina et al., 2017; Karakotov et al., 2019).

Among all *Fusarium* fungi isolated from oat grains, the *F. poae* and *F. langsethiae* strains were dominant. *F. poae* produce nivalenol and diacetoxyscirpenol and *F. langsethiae* is a strong producer of T-2/HT-2 toxins and DAS. In Russia, the amounts of T-2/HT-2 toxins are regulated in oat grains for food and feed, and the maximal permissible limit is 100 µg/kg[3, 4].

The relatively low infection of grain with *F. langsethiae* (with maxima of 14 and 5 % in 2019 and 2020, respectively) led to high amounts of detected mycotoxins. Therefore, we evaluated the breeding material by both the presence of *F. langsethiae* DNA and the accumulation of the sum of T-2 and HT-2 toxins in grain.

The highest incidence of infection with *F. langsethiae* and the maximal amounts of fungal DNA and T-2/HT-2 toxins were found in the grain of the husked variety Yakov. In comparison with this genotype, all naked breeding lines and varieties were

[3] Technical Regulation of Custom Union 015/2011 "About grain safety" with changes from 15 September 2017. Supplementary 2.
[4] Technical Regulation of Custom Union 021/2011 "About food safety" with changes from 8 August 2019. Supplementary 3.

less infected and contained significantly less fungal DNA and mycotoxins. Significant positive correlations between the amounts of *F. langsethiae* DNA and T-2/HT-2 toxins in the grain of naked oat genotypes were found ($r = 0.54$, $p = 0.069$ in 2019, and $r = 0.51$, $p = 0.054$ in 2020).

The results of our study demonstrated significant differences in oat breeding lines and varieties according to the content of *F. langsethiae* DNA in grain, although all genotypes were contaminated with T-2/HT-2 toxins. Thus, it is worth emphasizing again that the evaluation of oat resistance to *Fusarium* disease should be carried out according to several parameters.

It has been suggested that oat resistance type V to *Fusarium* disease depends on the mycotoxin type and that the QTLs associated with a low level of accumulation of deoxynivalenol in grain might not provide the resistance of the same genotype to other mycotoxins (He et al., 2013; Martin et al., 2018). However, comparison of the results obtained in our study (Gagkaeva et al., 2013) and later studies of the same oat genotypes under different conditions, genotype VIR-7766 (Hautsalo et al., 2021), varieties Argamak (Willforss et al., 2020) and Vyatsky (Chrpová et al., 2020), demonstrated a relatively high resistance of these oats to the accumulation of different mycotoxins, such as T-2/HT-2 toxins and deoxynivalenol.

The genetic basis of oat breeding lines plays a key role in their resistance to Fusarium disease. In the pedigree of two naked oat lines, characterized by high contents of fungal DNA and mycotoxins in 2019, the Zalp variety was recorded. Apparently, the crossing of breeding material with this variety can promote an increase in genotype susceptibility to Fusarium disease.

Conclusion

The breeding lines of naked oats created in the Federal Research Center “Nemchinovka” were evaluated by the sum of parameters characterized by different types of oat resistance to Fusarium disease. The amounts of *F. langsethiae* DNA and T-2/HT-2 toxins produced by this fungus were analysed, and based on the results obtained during a two-year study, under growth conditions, two lines of naked oats, 54h2476 and 66h2618, and the new variety, Azil (Line 57h2396 in 2019), demonstrated relatively high resistance to *F. langsethiae* infection and mycotoxin contamination compared with the control naked variety Vyatsky. These lines should be actively used to create new varieties that do not accumulate mycotoxins and are characterized by high-quality grain.

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ORCID ID

O.P. Gavrilova orcid.org/0000-0002-5350-3221
T.Yu. Gagkaeva orcid.org/0000-0002-3276-561X
A.S. Orina orcid.org/0000-0002-7657-6618
A.S. Markova orcid.org/0000-0002-2528-1422
A.D. Kabashov orcid.org/0000-0002-7450-3845
I.G. Loskutov orcid.org/0000-0002-9250-7225

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The gene *Sr38* for bread wheat breeding in Western Siberia

E.S. Skolotneva[1] ✉, V.N. Kelbin[1], V.P. Shamanin[2], N.I. Boyko[3], V.A. Aparina[3], E.A. Salina[1, 4]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia
[3] Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[4] Kurchatov Genomic Center of ICG SB RAS, Novosibirsk, Russia
✉ sk-ska@yandex.ru

Abstract. Present-day wheat breeding for immunity exploits extensively closely related species from the family Triticeae as gene donors. The 2NS/2AS translocation has been introduced into the genome of the cultivated cereal *Triticum aestivum* from the wild relative *T. ventricosum*. It contains the *Lr37*, *Yr17*, and *Sr38* genes, which support seedling resistance to the pathogens *Puccinia triticina* Eriks., *P. striiformis* West. f. sp. *tritici*, and *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn, which cause brown, yellow, and stem rust of wheat, respectively. This translocation is present in the varieties Trident, Madsen, and Rendezvous grown worldwide and in the Russian varieties Morozko, Svarog, Graf, Marquis, and Homer bred in southern regions. However, the *Sr38* gene has not yet been introduced into commercial varieties in West Siberia; thus, it remains of practical importance for breeding in areas where populations of *P. graminis* f. sp. *tritici* are represented by avirulent clones. The main goal of this work was to analyze the frequency of clones (a)virulent to the *Sr38* gene in an extended West Siberian collection of stem rust agent isolates. In 2019–2020, 139 single pustule isolates of *P. graminis* f. sp. *tritici* were obtained on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics SB RAS) from samples of urediniospores collected on commercial and experimental bread wheat fields in the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions. By inoculating test wheat genotypes carrying *Sr38* (VPM1 and Trident), variations in the purity of (a)virulent clones were detected in geographical samples of *P. graminis* f. sp. *tritici*. In general, clones avirulent to *Sr38* constitute 60 % of the West Siberian fungus population, whereas not a single virulent isolate was detected in the Krasnoyarsk collection. The Russian breeding material was screened for sources of the stem rust resistance gene by using molecular markers specific to the 2NS/2AS translocation. A collection of hybrid lines and varieties of bread spring wheat adapted to West Siberia (Omsk SAU) was analyzed to identify accessions promising for the region. The presence of the gene was postulated by genotyping with specific primers (VENTRIUP-LN2) and phytopathological tests with avirulent clones of the fungus. Dominant *Sr38* alleles were identified in Lutescens 12-18, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79/07, 9-31, and 8-26. On the grounds of the composition of the West Siberian *P. graminis* f. sp. *tritici* population, the *Sr38* gene can be considered a candidate for pyramiding genotypes promising for the Novosibirsk, Altai, and Krasnoyarsk regions.
Key words: *Puccinia graminis* f. sp. *tritici*; avirulent clones; resistance; *Triticum aestivum*; *Sr38*.

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Ген *Sr38*: значение для селекции мягкой пшеницы в условиях Западной Сибири

Е.С. Сколотнева[1] ✉, В.Н. Кельбин[1], В.П. Шаманин[2], Н.И. Бойко[3], В.А. Апарина[3], Е.А. Салина[1, 4]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Омский государственный аграрный университет им. П.А. Столыпина, Омск, Россия
[3] Сибирский научно-исследовательский институт растениеводства и селекции – филиал Федерального исследовательского центра Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[4] Курчатовский геномный центр ИЦиГ СО РАН, Новосибирск, Россия
✉ sk-ska@yandex.ru

Аннотация. Современная селекция пшеницы на иммунитет широко применяет генетический резерв близкородственных видов из семейства Triticeae. Транслокация 2NS/2AS привнесена в геном культурного злака *Triticum aestivum* от дикорастущего сородича *T. ventricosum* и содержит гены *Lr37*, *Yr17* и *Sr38*, которые отвечают за устойчивость пшеницы на уровне проростков к бурой, желтой и стеблевой ржавчине с соответствующими возбудителями: *Puccinia triticina* Eriks., *P. striiformis* West. f. sp. *tritici* и *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.

Данная транслокация известна в таких мировых сортах, как Trident, Madsen, Rendezvous, а также в отечественных сортах южной селекции Морозко, Сварог, Граф, Маркиз и Гомер. При этом ген *Sr38* до сих пор не введен в производственные сорта, высеваемые на территории Западной Сибири, поэтому сохраняет практическое значение для селекции на иммунитет в областях, где патогенная популяция *P. graminis* f. sp. *tritici* представлена авирулентными клонами. Основная цель работы состояла в анализе частоты а/вирулентных клонов к гену *Sr38* в расширенной западносибирской выборке возбудителя стеблевой ржавчины. В лаборатории с контролируемым климатом (Институт цитологии и генетики СО РАН) на проростках универсального восприимчивого сорта Хакасская выделено 139 монопустульных изолятов *P. graminis* f. sp. *tritici* из образцов урединиоспор Новосибирской, Омской областей, Алтайского и Красноярского края, собранных в 2019–2020 гг. на производственных и селекционных посевах мягкой пшеницы. Путем заражения тестерных генотипов пшеницы, несущих ген *Sr38* (VPM1 и Trident), выявлены вариации по частоте а/вирулентных клонов в географических образцах *P. graminis* f. sp. *tritici*. В целом текущая западносибирская популяция представлена на 60 % авирулентными клонами гриба к гену *Sr38*, при этом в образцах популяции из Красноярского края не выявлено ни одного вирулентного изолята. Поиск источников гена устойчивости к стеблевой ржавчине среди отечественного селекционного материала был выполнен с помощью специфических молекулярных маркеров на транслокацию 2NS/2AS. Исходя из перспективы использования в регионе, выбор проводили среди коллекции линий и сортов мягкой яровой пшеницы Омского ГАУ, адаптированных к условиям Западной Сибири. Присутствие гена постулировалось путем проведения процедуры генотипирования с помощью специфических праймеров (VENTRIUP-LN2) и фитопатологического тестирования авирулентными клонами гриба. Носителями доминантных аллелей гена *Sr38* оказались линии Лютесценс 12-18, Лютесценс 81-17, Лютесценс 66-16, Эритроспермум 79/07, 9-31 и 8-26. Полученные данные по составу образцов западносибирской популяции *P. graminis* f. sp. *tritici* позволяют рассматривать ген *Sr38* в качестве кандидата для включения в селекцию пшеницы в Красноярском крае, а также в составе генных пирамид в Новосибирской области и Алтайском крае.
Ключевые слова: *Puccinia graminis* f. sp. *tritici*; авирулентные клоны; устойчивость; *Triticum aestivum*; *Sr38*.

Introduction

Bread wheat *Triticum aestivum* has been cultivated in many countries for millennia. The exhaustion of the diversity of wheat genes potentially encoding commercially valuable traits, including pest resistance, is inevitable. Wild relatives in the Triticeae family are broadly used as genetic resources for modern wheat breeding for immunity. They include *Triticum monococcum* L., *T. speltoides* (Tausch) Gren., and *T. ventricosum* (McIntosh et al., 1995; Dubcovsky et al., 1996; Friebe et al., 1996). A long chromosome stretch (25–38 cM) hosting three genes for rust resistance was transferred to the genome of bread wheat variety VPM1 from *T. ventricosum* (Maia, 1967) and identified as a 2NS/2AS translocation (Bariana, McIntosh, 1993). The acquired genes *Lr37*, *Yr17*, and *Sr38* confer resistance against brown, yellow, and stem rusts, caused by *Puccinia triticina* Eriks., *P. striiformis* West. f. sp. *tritici*, and *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., respectively. The 2NS/2AS translocation was also introgressed to other commercial varieties: Trident, Madsen, and Rendezvous (McIntosh et al., 1995). Then it was extensively used in breeding in various regions of the world, where it provided efficient protection from rust agents and some nematode species attacking cereals (Dyck, Lukow, 1988; Robert et al., 1999; Seah et al., 2000). Cultivars with the identified *Lr37* gene for brown rust resistance and, correspondingly, with the *Sr38* and *Yr17* genes for resistance to stem and yellow rusts were raised at the Lukyanenko National Center of Grain, put on the Russian state register, and authorized for commercial use in the Central Chernozem, North Caucasian, Middle Volga, and Lower Volga regions. They include Morozko (2015), Svarog (2017), Graf (2018), Marquis (2019), and Homer (2020) (Bespalova et al., 2019a, b).

The *Sr38* gene became inefficient against stem rust in countries of Asia and Northern Africa when the aggressive southern race Ug99 started its expansion (Pretorius et al., 2000). However, this race has not yet been detected among wheat pathogens in Russia (Baranova et al., 2015; Skolotneva et al., 2020b). Moreover, it has been shown that low temperatures enhance *Sr38* expression (Helguera et al., 2003). Thus, it may be promising in wheat breeding in regions with temperate climate. As *Sr38* has not been widely introduced into commercial varieties grown in West Siberia (Sochalova, Lichenko, 2015), is remains of practical significance for breeding for resistance in regions where pathogenic *P. graminis* f. sp. *tritici* populations are represented by avirulent clones.

Several molecular markers of the 2NS/2AS translocation have been designed to facilitate the transfer of the *Lr37*, *Yr17*, and *Sr38* genes to commercial varieties. The first of the proposed markers was the dominant SCAR (Sequence Characterized Amplified Region) marker, located at 0.8 ± 0.7 cM apart from the *Yr17* gene (Robert et al., 1999). At present, two markers are widely used to identify the 2NS/2AS translocation in wheat genetic material (Helguera et al., 2003). The codominant CAPS (Cleavage Amplified Polymorphic Sequence) marker demands an additional step of digesting the diagnostic fragment with restriction endonucleases. The dominant PCR marker is targeted directly at a specific sequence of the typical allele inside the translocation. The amplification is done with the VENTRIUP-LN2 primer pair, and the products are resolved in agarose gel (https://maswheat.ucdavis.edu/protocols/Sr38), which is an obvious advantage of the marker.

Here we analyze the frequencies of clones (a)virulent against *Sr38* in a West Siberian collection of stem rust agent isolates extended by adding samples from the Krasnoyarsk region. Another objective of this work is the DNA marker-assisted search for *Sr38*-carrying accessions. The study involved a collection of spring bread wheat lines and cultivars adapted for growing in West Siberia.

Table 1. Percentages of avirulent *P. graminis* f. sp. *tritici* clones on *Sr38*-bearing tester wheat varieties

Sampling locality	Year	Number of single pustule isolates	Percentage of avirulent clones on testers with *Sr38*
Omsk region	2020	33	9
Novosibirsk region	2019, 2020	57	65
Altai region	2019	21	71
Krasnoyarsk region	2020	28	100
Total		139	60

Materials and methods

The extended West Siberian collection of the stem rust agent included samples from the Novosibirsk, Omsk, Altai, and Krasnoyarsk regions collected from commercial and experimental bread wheat fields in 2019–2020. A total of 139 *P. graminis* f. sp. *tritici* single pustule isolates were obtained from the collected urediniospores on seedlings of the standard susceptible cultivar Khakasskaya in an environmentally controlled laboratory (Institute of Cytology and Genetics, Novosibirsk) (Table 1).

The frequencies of clones avirulent to the *Sr38* gene were determined on tester wheat genotypes: an isogenic line and varieties from a set for differentiating stem rust races on wheats of the USA and Canada bearing the *Sr38* gene: VPM1 and Trident, respectively. Prior to the experiment, the seed material was verified with molecular markers to the gene, and plants *Sr38*-negative on the DNA array were rejected.

The protocols for seedling preparation and inoculation with fungus clones for the analysis of resistance are described in detail by Skolotneva et al. (2020a). The infection types on wheat tester lines were scored according to the Stackman four-point scale (Stackman et al., 1962).

The collection of 80 bread wheat lines and varieties adapted to the West Siberian conditions was kindly provided by Prof. V.P. Shamanin, Omsk SAU. DNA was isolated from seedling apices by the CTAB method (Rogers, Bendich, 1985). DNA was quantified with a Qubit 4 fluorometer (Invitrogen, United States).

The *Sr38* gene was identified in the material with the primers VENTRIUP (5′-AGGGCTACTGACCAAGGCT-3′) and LN2 (5′-TGCAGCTACAGCAGTATGTACACAAAA-3′) for the 2NS/2AS translocation. Amplification mixture: 1× SE-buffer AS (ammonium sulfate), 0.2 mM each dNTP, 0.2 μM each primer, 1.5 mM $MgCl_2$, 50 ng of genomic DNA, 1 U of Taq DNA polymerase (SibEnzyme, Russia), volume 25 μL. The reaction was carried out in a Bio-Rad T100 thermocycler (United States) according to the following program: predenaturation 7 min at 94 °C followed by 30 cycles: 94 °C, 30 s; 65 °C, 30 s; 72 °C, 30 s. Postextension was performed at 72 °C for 10 min. The products were resolved in 2 % agarose gel. Fragment sizes were assessed against the Step 50 plus DNA ladder (Biolabmix, Russia).

The final step of gene postulation was the phytopathological test of resistance with *P. graminis* f. sp. *tritici* isolates avirulent against *Sr38*. Plant resistance was assessed at the seedling stage as mentioned above. The Khakasskaya variety was chosen as the susceptible control. The experiment was carried out on ten plants of each genotype in two replications.

Results and discussion

While assessing stem rust agent isolates from various localities in West Siberia, we detected a variation in the frequencies of fungus clones not attacking tester genotypes with *Sr38*, that is, avirulent against them (see Table 1). The variation showed a longitudinal cline from the minimum frequency in the Omsk region to the nearly 100 % avirulence in the population of the Krasnoyarsk region. The polymorphism of the detected infection types in response to the inoculation with single pustule *P. graminis* f. sp. *tritici* isolates from different samples is illustrated in Figure 1. All types scoring 1, 2, 3, and 3+ were detected, but those corresponding to resistance and medium resistance were predominant in isolates from the Altai and Krasnoyarsk regions. Noteworthy is the occurrence of avirulent clones in the Novosibirsk and Altai samples, not observed in the analysis of the races of the West Siberian population in 2017 (Skolotneva et al., 2020b). This fact may be due to importation of *P. graminis* f. sp. *tritici* inoculum from southern regions. It is known that the *Sr38* gene is efficient in northern Kazakhstan and China (Koyshybaev, 2018; Li et al., 2018).

In general, clones avirulent against *Sr38* constitute 60 % of the West Siberian population. If we reject the collection from the Omsk region, where the gene has been considered inefficient against the local agent for several years (Shamanin et al., 2020), the frequency of fungus clones not injuring genotypes with *Sr38* increases to 78 %. Therefore, the gene can be useful in gene pyramiding for eastern West Siberia. The efficiency of the genotypes *Sr25+Sr38* and *Sr31+Sr38* has been demonstrated in the Urals, where *Sr38* alone cannot sufficiently protect plants from stem rust (Druzhin et al., 2018). An additional valuable feature of the 2NS/2AS translocation is that it bears the resistance genes *Lr37* and *Yr17*, which remain efficient against West Siberian isolates of brown and yellow rust agents (Skolotneva et al., 2018; Gultyaeva, Shaydayuk, 2020).

Donors of the *Sr38* gene were sought in the Russian breeding material with a specific molecular marker for the 2NS/2AS translocation. As the breeding programs should be targeted at West Siberia, the Omsk SAU collection of spring bread wheat lines and varieties adapted to the region was screened. The gene presence was postulated by genotyping with specific primers (VENTRIUP-LN2) and phytopathological tests with avirulent fungus clones.

Fig. 1. Infection types of *P. graminis* f. sp. *tritici* from various regions tested on genotypes with *Sr38*.

Reaction type scores with fungus isolates: from the Novosibirsk region: (*1*) 3+, (*2*) 3–, (*3*) 1; from the Altai region: (*4*) 1; from the Krasnoyarsk region: (*5*) 2; from the Omsk region: (*6*) 3, (*7*) 3+.

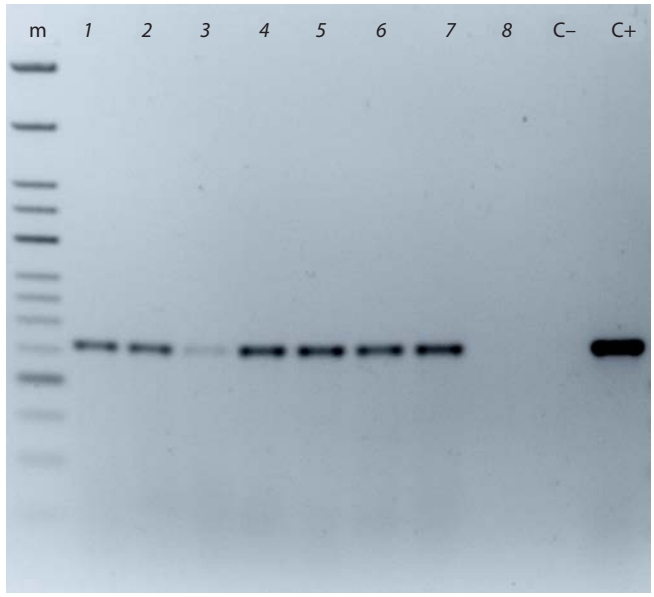

Fig. 2. Electrophoretic image of amplification with molecular markers to the *Sr38* gene on bread wheat DNA from the West Siberian collection of experimental lines, Omsk SAU.

Lanes: m, Step 50 plus DNA ladder (Biolabmix); *1*, Lutescens 12-18; *2*, Lutescens 34-16; *3*, Lutescens 81-17; *4*, Lutescens 66-16; *5*, Erythrospermum 79/07; *6*, line 9-31; *7*, line 8-26; *8*, genotype 2 from the Omsk SAU collection; "C–", negative control (cv. Khakasskaya); "C+", positive control (VPM1).

Table 2. Pedigrees of some wheat lines from the West Siberian collection (Omsk SAU) resistant to stem rust against the natural infectious background of the Omsk region, 2019

Breeding line	Pedigree	Field scores
Lutescens 12-18	MN6616M/3/NL456/VEE#5//DUCULA/4/KARAGANDINSKAYA 70	20MR
Lutescens 34-16	OMSKAYA 36/BAVIS//TERTSIYA	10MR
Lutescens 81-17	ERITROSPERMUM 55-94-01-20/5/PYN/BAU/3/MON/IMU//ALD/PVN/4/VEE#5/SARA//DUCULA/6/FITON 42	10MR
Lutescens 66-16	27.90.98.3/3/KA/NAC//TRCH/4/ALTAYSKAYA 530	25MR
9-31	UKR-OD 1530.94/AE.SQUARROSA(1027)/Pamyati Azieva	20MR
8-26	AISBERG/AE.SQUARROSA(369)/Omgau 90	20MR

Positive signals corresponding to the diagnostic 259 bp long amplicon were obtained from DNA templates of seven experimental wheat lines: Lutescens 12-18, Lutescens 34-16, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79/07, 9-31, and 8-26 (Fig. 2). The pedigrees of these varieties and hybrid lines are shown in Table 2. The dramatic variation in the origins of the supposed *Sr38* carriers deserves special attention, as it augments the value of the accessions as diverse resistance donors.

Puccinia graminis f. sp. *tritici* isolates eliciting stable responses on *Sr38*-bearing tester wheat genotypes were picked from infection samples of the Krasnoyarsk region for phytopathological tests of the West Siberian collection of bread wheat cultivars and hybrids. Infection types 0 and 1, indicative of resistance, were observed on inoculated plants of Lutescens 12-18, Lutescens 34-16, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79-07, 9-31, and 8-26 (Fig. 3). In addition to the susceptible control (cv. Khakasskaya), we added for reference genotype 2, which lacks *Sr38* according to genotyping with molecular markers. They showed the maximum development of stem rust signs, scored 3 and 4. Part of the tested Lutescens 34-16 plants were susceptible to fungal isolates avirulent against *Sr38* (45S and 45R in Fig. 3). They constituted 30 % of the tested sample. This observation indicates that the breeding material contained biotypes differing in stem rust resistance. The molecular marker is dominant; therefore, it cannot rule out heterozygosity for the character, as found in phytopathological tests. The presence of resistant *Sr38* alleles, expressing in response to the infection by avirulent clones of the fungus in accordance with Flor's gene-for-

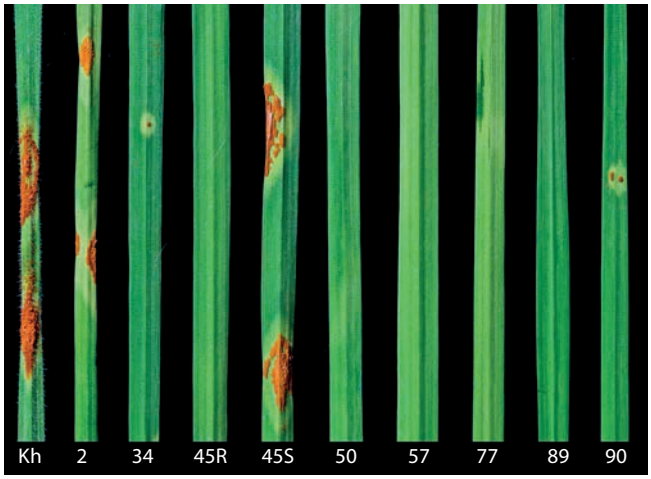

Fig. 3. Reaction type scores of bread wheat cultivars and hybrids from the Omsk SAU West Siberian collection inoculated with *P. graminis* f. sp. *tritici* isolates avirulent against *Sr38*.

Seedlings: Kh, cv. Khakasskaya (score 4); 2, genotype 2 from the Omsk SAU collection (score 3); 34, Lutescens 12-18 (score 1); 45R and 45S, Lutescens 34-16 (scores 0 and 4, respectively); 50, Lutescens 81-17 (score 0); 57, Lutescens 66-16 (score 0), 77, Erythrospermum 79/07 (score 0); 89, line 9-31 (score 0); 90, line 8-26 (score 1).

gene relationship, describing the interaction between a host and a pathogen, was proven in the remaining West Siberian bread wheat accessions: Lutescens 12-18, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79/07, 9-31, and 8-26. The results of immunological screening of these lines in field tests of breeding material against the natural infectious background point to medium stem rust resistance in *Sr38* carriers (see Table 2). This fact is consistent with phytopathological tests on seedlings with isolates from the Omsk *P. graminis* f. sp. *tritici* population.

Conclusion

The analysis of West Siberian *P. graminis* f. sp. *tritici* isolates shows that the *Sr38* gene is promising for wheat breeding in the Krasnoyarsk region and for gene pyramiding in the Novosibirsk and Altai regions. The following bread wheat cultivars and experimental lines from the Omsk SAU collection carry dominant *Sr38* alleles: Lutescens 12-18, Lutescens 81-17, Lutescens 66-16, Erythrospermum 79/07, 9-31, and 8-26. These accessions are adapted to the regional environment; therefore, they may be recommended as stem rust resistance donors for breeding programs in West Siberia.

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ORCID ID

E.S. Skolotneva orcid.org/0000-0001-8047-5695
V.N. Kelbin orcid.org/0000-0002-3455-5704
V.P. Shamanin orcid.org/0000-0003-4767-9957
N.I. Boyko orcid.org/0000-0002-5026-4907
V.A. Aparina orcid.org/0000-0003-2714-7216
E.A. Salina orcid.org/0000-0001-8590-847X

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Polymorphism of *CLE* gene sequences in potato

M.S. Gancheva[1] ✉, M.R. Losev[1], A.A. Gurina[2], L.O. Poliushkevich[1], I.E. Dodueva[1], L.A. Lutova[1]

[1] Saint Petersburg State University, St. Petersburg, Russia
[2] Federal Research Center the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia
✉ ganchovai@gmail.com

Abstract. CLE (CLV3/ESR) is one of the most important groups of peptide phytohormones: its members regulate the development of various plant organs and tissues, as well as interaction with some parasites and symbionts and response to environmental factors. In this regard, the identification and study of the *CLE* genes encoding the peptides of this group in cultivated plants are of great practical interest. Relatively little is known about the functions of CLE peptides in potato, since the *CLE* genes of the potato *Solanum phureja* Juz. et Buk. were characterized only in 2021. At the same time, potato includes plenty of tuberous species of the genus *Solanum* L., both wild and cultivated, and the diversity of its forms may depend on differences in the sequences of *CLE* genes. In this work, we performed a search for and analysis of the *CLE* gene sequences in three wild potato species (*S. bukasovii* Juz., *S. verrucosum* Schltdl., *S. commersonii* Dunal) and four cultivated species (*S. chaucha* Juz. et Buk., *S. curtilobum* Juz. et Buk., *S. juzepczukii* Juz. et Buk., *S. ajanhuiri* Juz. et Buk.). In total, we identified 332 *CLE* genes in the analyzed potato species: from 40 to 43 genes of this family for each potato species. All potato species taken for analysis had homologues of previously identified *S. phureja CLE* genes; at the same time, the *CLE42* gene, which is absent from the *S. phureja* genome, is present in all other analyzed potato species. Polymorphism of CLE proteins of *S. commersonii* is significantly higher than that of other analyzed potato species, due to the fact that *S. commersonii* grows in places outside the growing areas of other potato species and this potato is probably not one of the ancestors of cultivated potato. We also found examples of polymorphism of domains of CLE proteins that carried different functions. Further study of potato CLE proteins will reveal their role in development, including regulation of productivity in this important agricultural crop.
Key words: *CLE* genes; potato; *Solanum bukasovii*; *Solanum verrucosum*; *Solanum commersonii*; *Solanum chaucha*; *Solanum curtilobum*; *Solanum juzepczukii*; *Solanum ajanhuiri*.

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Полиморфизм последовательностей генов *CLE* картофеля

М.С. Ганчева[1] ✉, М.Р. Лосев[1], А.А. Гурина[2], Л.О. Полюшкевич[1], И.Е. Додуева[1], Л.А. Лутова[1]

[1] Санкт-Петербургский государственный университет, Санкт-Петербург, Россия
[2] Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова (ВИР), Санкт-Петербург, Россия
✉ ganchovai@gmail.com

Аннотация. CLE (CLV3/ESR) – одна из важнейших групп пептидных фитогормонов. Ее представители регулируют развитие различных органов и тканей растений, а также взаимодействие с некоторыми паразитами и симбионтами и ответ на факторы окружающей среды. В связи с этим идентификация и изучение генов *CLE*, кодирующих пептиды этой группы, у культурных растений представляют большой практический интерес. О функциях CLE пептидов у картофеля известно немного, поскольку гены *CLE* картофеля *Solanum phureja* Juz. et Buk. были охарактеризованы только в 2021 г. Вместе с тем картофель включает в себя много клубненосных видов рода *Solanum* L., как диких, так и культурных, и разнообразие его форм может зависеть в том числе от различий по последовательностям генов *CLE*. В этой работе мы впервые произвели поиск и анализ последовательностей генов *CLE* у трех диких видов картофеля (*S. bukasovii* Juz. et Rybin., *S. verrucosum* Schltdl., *S. commersonii* Dunal.) и четырех культурных (*S. chaucha* Juz. et Buk., *S. curtilobum* Juz. et Buk., *S. juzepczukii* Juz. et Buk., *S. ajanhuiri* Juz. et Buk.). У проанализированных видов картофеля выявлено 332 гена *CLE*: от 40 до 43 генов этого семейства для каждого вида картофеля. У всех видов картофеля, взятых в исследование, выявлены гомологи ранее идентифицированных генов *CLE S. phureja*; в то же время ген *CLE42*, отсутствующий в геноме *S. phureja*, найден у всех остальных проанализированных нами видов картофеля. Наибольшие отличия по аминокислотным последовательностям белков CLE оказались характерны для *S. commersonii* – вида, растущего вне ареалов культурных видов картофеля и, вероятно, не входящего в число их предков. Обнаружены

также примеры полиморфизма по аминокислотным последовательностям доменов белков CLE, несущих разную функциональную нагрузку. Дальнейшее изучение белков CLE картофеля позволит выявить их роль в развитии этой важнейшей сельскохозяйственной культуры, в том числе в формировании признаков продуктивности.
Ключевые слова: гены *CLE*; картофель; *Solanum bukasovii*; *Solanum verrucosum*; *Solanum commersonii*; *Solanum chaucha*; *Solanum curtilobum*; *Solanum juzepczukii*; *Solanum ajanhuiri*.

Introduction

The growth and development of higher plants, as well as their response to external stimuli, are regulated by intercellular communications mediated by phytohormones. In addition to the well-known and thoroughly studied "classical" plant hormones (IAA, cytokinins, ABA, etc.), numerous families of peptide hormones, which are mobile secreted oligopeptides or small proteins, play an important role in the coordination of plant development (Gancheva et al., 2019). One of the most famous families of peptide phytohormones with diverse functions is the family of CLE (CLV3/ESR) peptides. These peptides got their name from the first identified representatives: the CLAVATA3 (CLV3) Arabidopsis peptide (Clark et al., 1995) and the ENDOSPERM SURROUNDING REGION (ESR) maize peptide (Opsahl-Ferstad et al., 1997). Nowadays, genes encoding CLE peptides have been identified in all groups of terrestrial plants, as well as in the green alga *Chlamydomonas reinhardtii* (Oelker et al., 2008; Goad et al., 2017).

CLE genes encode proteins 100–150 amino acids (AA) long, which have a signaling domain (SD) at the N-terminus, a conserved CLE domain at the C-terminus, and a variable domain (VD) between them (Strabala et al., 2014). The CLE domain, consisting of 12 AAs, is a functional part of the CLE protein: immediately after synthesis, the precursor protein undergoes proteolytic processing and post-translational modifications (Kondo et al., 2006; Ni et al., 2011). As a result, what remains of it is the CLE domain with modifications (hydroxylation, arabinosylation) of conservative proline residues; this is the mature CLE peptide. CLE peptides that are secreted into the intercellular space become ligands for receptor kinases of Leucine Reach Repeats containing Receptor-Like Kinases (LRR-RLK) families and CRINKLY4 that are located on the plasma membranes of cells (Poliushkevich et al., 2020). By interacting with receptors, CLE peptides trigger a signaling cascade, the targets of which are homeodomain-containing transcription factors of the WOX family that regulate the maintenance of stem cell niches in plants (Tvorogova et al., 2021). The known functions of CLE peptides include control of shoot and root apical meristems and cambium activity, differentiation of vascular tissues, formation of lateral roots and nodules, early embryogenesis, stomatal development, and response to several environmental factors: water availability and changes in soil nitrogen composition (Yamaguchi et al., 2016; Fletcher, 2020) (Fig. 1).

In all angiosperm species studied, the CLE peptides are encoded by numerous genes. For instance, in the relatively small genome of *Arabidopsis thaliana*, there are 32 *CLE* genes (Sharma et al., 2003; Strabala et al., 2006), and each *A. thaliana CLE* gene is characterized by a unique spatial pattern of expression (Jun et al., 2010). However, some *CLE* genes encode the same CLE peptides. It is suggested that such an excess of CLE peptides is necessary for fine regulation of plant development (Kinoshita et al., 2007).

It is obvious that the family of *CLE* genes is not limited by the genes discovered to date. The accumulation of genomic data and computer software improvement make it possible to identify new members of this family. In our study, in the reference potato genome, which is the sequence of the doubled monoploid clone *Solanum phureja* DM-1-3 516R44 (Gancheva et al., 2021), 41 *CLE* genes that encode 37 unique CLE peptides were identified. Besides, many cultivated and wild-growing potato species are known. According to various authors, potatoes (*Solanum* L., section *Petota* Dumort.) include 112 to 235 species (Huamán, Ross, 1985; Spooner et al., 2014). The genomes of some potato species are currently sequenced and available in genomic databases.

In our work, we searched for and analyzed *CLE* genes in the genomes of seven potato species: three wild species (*S. bukasovii*, *S. verrucosum*, *S. commersonii*) and four primitive cultivated species (*S. chaucha*, *S. curtilobum*, *S. juzepczukii*, *S. ajanhuiri*). All genomes are provided in the NCBI database. In total, we found 332 *CLE* genes and identified unique peptides in individual potato species that can perform other unknown functions or be completely non-functional. In addition, we found similarities in the sequences of different CLEs, which may indicate their common genetic origin.

Materials and methods

We used the genome assemblies of various potato species presented in the NCBI database: wild species *S. commersonii*, *S. verrucosum*, and *S. bukasovii*, along with primitive cultivated species *S. chaucha*, *S. juzepczukii*, *S. curtilobum*, and *S. ajanhuiri*. In the present work the system of J. Hawkes was used.

Solanum commersonii is a widespread diploid potato species in South America, present at the coastal zone of the Atlantic Ocean, mainly located in Argentina and Uruguay. Its natural habitat extends from sea level to an altitude of 1300 m. *S. commersonii* is ruderal, and its primary habitats are rocky areas, dunes, and growing areas of cultivated plants (Hawkes, Hjerting, 1969).

Solanum verrucosum is a diploid potato, which, unlike other species studied in this work, is widespread in North America, more precisely in Mexico. Yet, it is believed that *S. verrucosum* is evolutionarily closer to the ancestors of cultivated potato species than *S. commersonii* (Hawkes, 1990). The habitats of this species are woodlands.

Solanum bukasovii is a diploid South American potato species that grows at an altitude of 3300–4000 m above sea level in Peru. It belongs to the group of wild potato species from which cultivated potato species are believed to have evolved (Li et al., 2018).

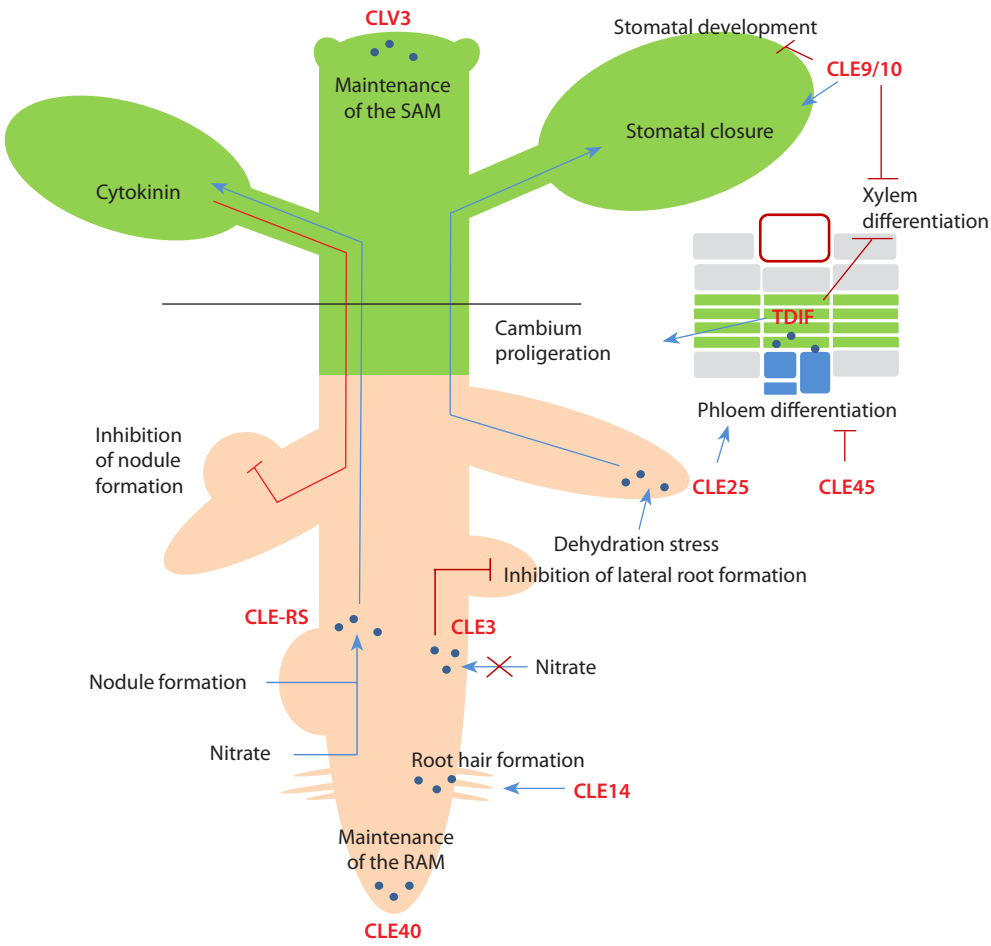

Fig. 1. Some functions of CLE peptides in plant development.
SAM – shoot apical meristem; RAM – root apical meristem.

Solanum juzepczukii and *S. curtilobum* are alpine triploid and pentaploid potato species, respectively, that grow in a very limited area. They belong to the group of "bitter" potatoes due to the high content of glycoalkaloids. Only some of their clones were found to be cultivated in the highlands of Peru and Bolivia, where other types of potatoes cannot be grown due to the conditions. They are more resistant to frost than any other domestic potato species (Lekhnovich, 1971).

Solanum ajanhuiri is a diploid alpine species. It is cultivated at an altitude of more than 3900 m in the area of Lake Titicaca. *S. ajanhuiri* is also hardy, but unlike *S. juzepczukii* and *S. curtilobum*, it has a significantly lower glycoalkaloids content in tubers (Hawkes, 1990).

Solanum chaucha is a triploid species found mainly in the northern mountainous regions of South America (Ecuador, Colombia) and northern Peru. It is cultivated at lower altitudes than the above-mentioned cultivated potato species (Hawkes, 1990).

The search for *CLE* genes in different potato species was carried out according to homology with genes from the CLE family in *S. phureja* (Gancheva et al., 2021), *A. thaliana* (Sharma et al., 2003; Strabala et al., 2006), and tomato *Solanum lycopersicum* (Zhang et al., 2014; Gancheva et al., 2021) using the Nucleotide Basic Local Alignment Search Tool (BLASTN) and the discontiguous megablast algorithm (Altschul et al., 1990) in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), where genome assemblies of all the studied potato species are available (see the Table). The alignment of amino acid and nucleotide sequences was carried out using the Muscle algorithm in the MEGA7 program (https://www.megasoftware.net/) (Kumar et al., 2016).

Phylogenetic analysis was performed in the MEGA7 program using the "nearest neighbour" method (Saitou, Nei, 1987) with default settings and a bootstrap of 1000 (Felsenstein, 1985). CLE protein signaling domains were predicted in the SignalP-5.0 program (http://www.cbs.dtu.dk/services/SignalP/). Consensus sequences of CLE proteins were visualized in the Geneious Prime software (https://www.geneious.com/features/).

Results

Using the NCBI database, we identified *CLE* genes in seven potato species by homology with the *CLE* genes of potato *S. phureja* (*SphCLE*), arabidopsis (*A. thaliana*), and tomato (*S. lycopersicum*). Species being studied were primitive domestic species *S. chaucha*, *S. curtilobum*, *S. juzepczukii*, *S. ajanhuiri*, and wild species *S. bukasovii*, *S. commersonii*, *S. verrucosum* (Supplementary Material)[1]. We also identified

[1] Supplementary Material is available in the online version of the paper: http://vavilov.elpub.ru/jour/manager/files/Suppl_Gancheva_Engl.pdf

The number of identified *CLE* genes in the potato species analyzed

No.	Species	Assembly	Abbreviation	*CLE* genes	Species
1	*S. chaucha* Juz. et Buk.	GCA_009849625.1	Sch	43	Primitive cultivated
2	*S. curtilobum* Juz. et Buk.	GCA_009849645.1	Scu	40	
3	*S. juzepczukii* Juz. et Buk.	GCA_009849685.1	Sj	42	
4	*S. phureja* Juz. et Buk.	GCA_009849755.1	Sph	41	
5	*S. ajanhuiri* Juz. et Buk.	GCA_009849805.1	Sa	40	
6	*S. bukasovii* Juz. et Rybin.	GCA_009849815.1	Sb	43	Wild
7	*S. commersonii* Dunal.	GCA_001239805.1	Sco	42	
8	*S. verrucosum* Schltdl.	GCA_900185145.1	Sv	41	

CLE genes in the primitive cultivated potato *S. stenotomum* and in the wild species *S. pinnatisectum*. However, due to the insufficient quality of the genome assemblies these genes were not selected for further analysis.

For each potato species, a different number of *CLE* genes was found, 40 to 43 (see the Table). This is primarily due to the different qualities of genome assemblies. For instance, the genome of *S. verrucosum* is published in the form of extended scaffolds, while the assembly of the *S. curtilobum* genome is presented at the contig level. As a result, some *S. curtilobum* CLEs were not included in the analysis due to contig breaks and the inability to analyze the entire gene sequence. Homologues of most previously identified *CLE* genes in *S. phureja* (*SphCLE*) (Gancheva et al., 2021) were found in all potato species analyzed. Furthermore, the gene that we named *CLE42* was not found in the *S. phureja* genome, although it is present in all other potato species studied.

Analyzing the amino acid sequences (AAS) of individual CLE proteins in different potato species, we found a high percentage of their similarity (78–98 % identical AA) (Fig. 2). Moreover, there are both completely identical AAS of proteins (for example, the CLE39 AASs are identical in six of the eight analyzed species) and variants with no identical AASs among the species (CLE3, CLE16, CLE40). However, the CLE proteins in the studied potato species are very similar to each other. Based on the complete protein sequence, individual CLEs form groups, each of which includes all homologues of one CLE protein from different potato species (Fig. 3). Among all analyzed potato species, the CLE proteins of *S. commersonii* contain a large number of unique AAs, which are absent in other species.

CLE proteins include three domains: the signaling domain (SD), the variable domain (VD), and the CLE domain. The CLE domain is the most conserved functional part of

Fig. 2. Consensus sequences of CLE proteins from 8 potato species.

IAA – percentage of identical AA.

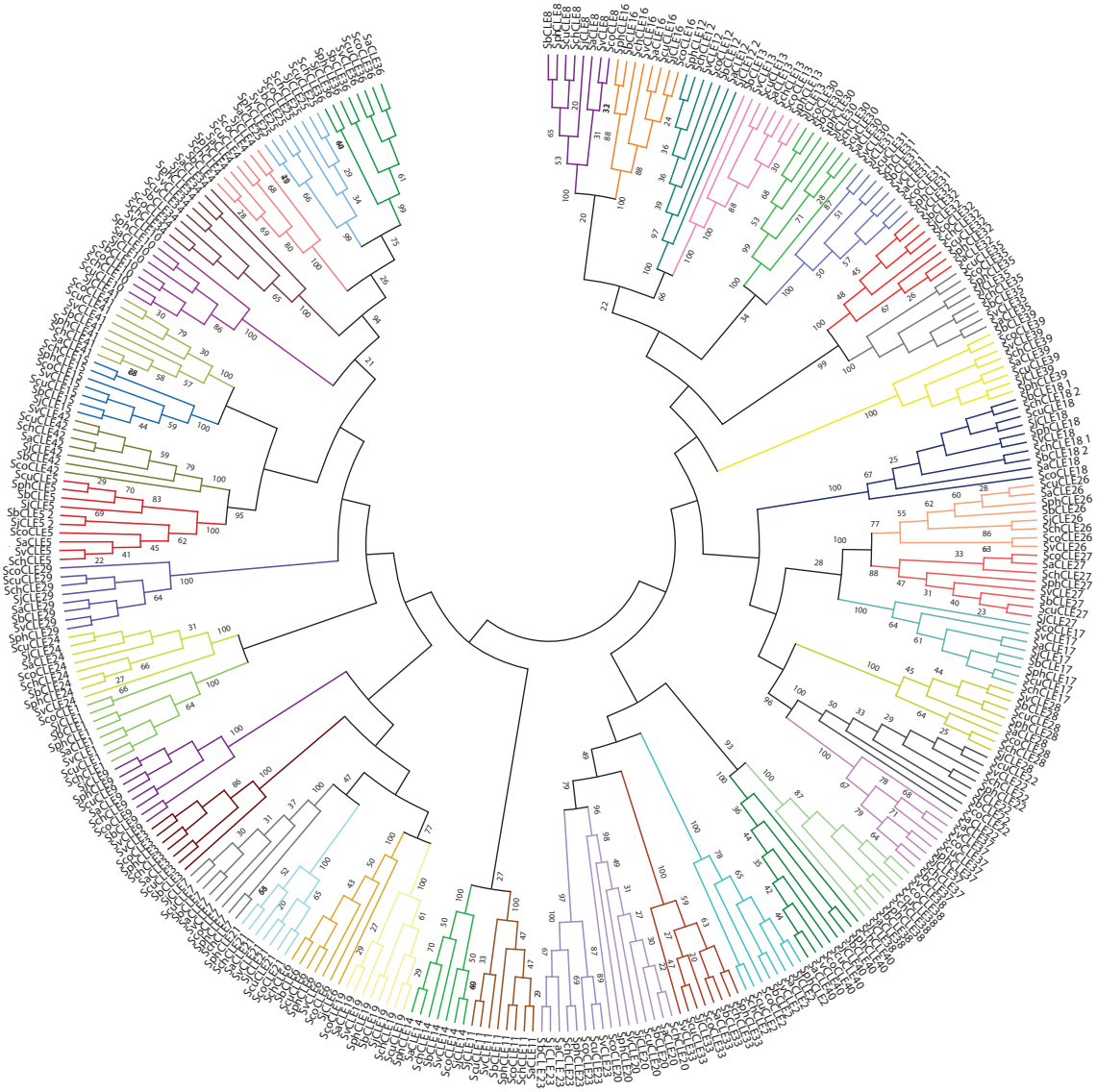

Fig. 3. Phylogenetic tree of potato CLE proteins.

the protein, and substitutions are rare here. In other protein domains, substitutions occur much more frequently. Although substitutions in the CLE domain will bring the greatest effect on the phenotype, SD and VD are also important and are involved in the implementation of CLE peptide function. SD and VD are active in the processing of CLE peptides and can thus influence the availability of mature peptides in certain cells and tissues (Meng et al., 2010). It turned out that, in different potato species, AASs of CLE domains are almost identical within each group. At the other extreme, the nucleotide sequences (NSs) of CLE domain in proteins of the same group vary between species. For example, proteins of the CLE12 group, although they have identical AASs of the CLE domains, differ in the NSs of the corresponding gene regions.

Although we identified 41 *CLE* genes in *S. phureja*, they encode 37 CLE peptides (Gancheva et al., 2021). This is because the AASs of CLE domains are identical in some proteins (for example, in CLE8 and CLE12). However, in other potato species, 20 additional CLE domains were found, which are absent in *S. phureja*. All these domains are similar to the CLE domains of *S. phureja* proteins, but differ from them by 1–4 AA (Fig. 4).

As mentioned above, in some CLE proteins of the same plant species, CLE domains are identical (for example, CLE41 and CLE44 domains of the arabidopsis). In potato, 10 pairs of such proteins with identical CLE domains were found. Mature peptides that have identical AASs of CLE domains are peptides from CLE10 and CLE38 groups, CLE17 and CLE18, CLE25 and CLE34, CLE6 and CLE19, CLE8 and CLE12. However, the remaining AASs of these proteins (outside the CLE domain) vary significantly within each pair.

At the same time, some potato CLE proteins have a similar AAS, but their CLE domains are not identical and differ by one or several AAs (for example, CLE32 and CLE35, CLE26 and CLE27). Interestingly, some proteins are grouped differently depending on whether the CLE domain or the rest of AAS of the protein is being compared. For instance, CLE37 is closer to CLE31 (they differ by 1 AA) when comparing their

CLE domains. However, the rest of the CLE37 protein forms a group with CLE22 protein. The same thing happens in the case of CLE25 and CLE34 proteins. These proteins have identical CLE domains, but their sequences outside the CLE domain are different and, comparing them, CLE34 is grouped with CLE36, not CLE25. Sometimes such a scenario occurs only within one potato species. For example, the CLE domain of ScoCLE4 differs by only 1 AA from CLE25 (while in other potato species CLE domains of CLE4 differ by 2 AA from CLE25); notably, protein sequence outside the CLE domain belongs to CLE4. Obviously, differences in the sequence of the CLE domain (and, consequently, of the mature peptide) should lead to the functional diversity of CLE proteins, despite the great similarity of their AASs. CLE proteins, similar in AASs, but distinct in the CLE domain, are also found in other plant species (for example, CLE41 and CLE42 or CLE25 and CLE26 of the arabidopsis). Their genes may have emerged as a result of duplications with subsequent mutations in the sequence of the CLE domain, which led to the emergence of new functions (Yaginuma et al., 2011; Takahashi et al., 2018). Indeed, some *CLE* genes in potatoes are duplicated, and their sequences weakly vary from each other. For instance, in all analyzed potato species, *CLE26* and *CLE2* genes are duplicated. Sometimes such duplicated genes have substitutions in CLE domain sequence, which leads to the emergence of a unique peptide (for example, *SvCLE2-2*). However, due to the high level of similarity, such genes are not counted in the Table.

Sometimes, CLE domains of some CLE proteins are identical in several potato species and differ from CLE domains of the same proteins in other species. For example, in the CLE26 proteins of *S. juzepczukii* and *S. chaucha*, I is in the second position of the CLE domain, while in other species there is L. The CLE11 proteins of *S. verrucosum*, *S. curtilobum*, and *S. juzepczukii*, in the fifth position of CLE domain have E, while in other species there is Q (see Fig. 4). The largest number of unique CLE domains, which differ from all other potato species studied, was found in *S. verrucosum*, *S. commersonii*, and *S. juzepczukii*.

Great interspecies differences in the AASs of CLE proteins relate to sequences outside the CLE domain. Thus, among the genes of the *CLE8* group, *SvCLE8* stands out, which has three additional nucleotides in the VD; it makes the protein 1 AA longer. CLE18 proteins of *S. verrucosum*, *S. commersonii*, *S. bukasovii*, *S. chaucha*, and *S. juzepczukii* have a region 5 AA in the VD, while CLE18 of other species does not have such a region. At the same time, *S. bukasovii* and *S. chaucha* each have two *CLE18* genes encoding proteins with and without this region (Fig. 5). A similar situation occurs for the CLE5 protein: a region 4 AA in the VD is present in *S. phureja*, *S. curtilobum*, *S. juzepczukii*, *S. bukasovii*, but absent in *S. chaucha*, *S. verrucosum*, *S. ajanhuiri*, *S. commersonii*, whereas *S. bukasovii* and *S. juzepczukii*

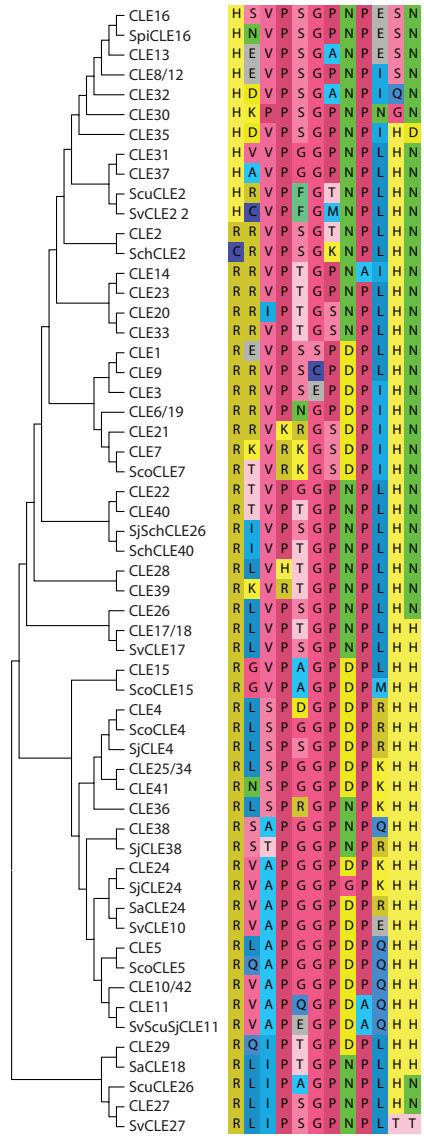

Fig. 4. Phylogenetic tree and alignment of unique potato CLE peptides.

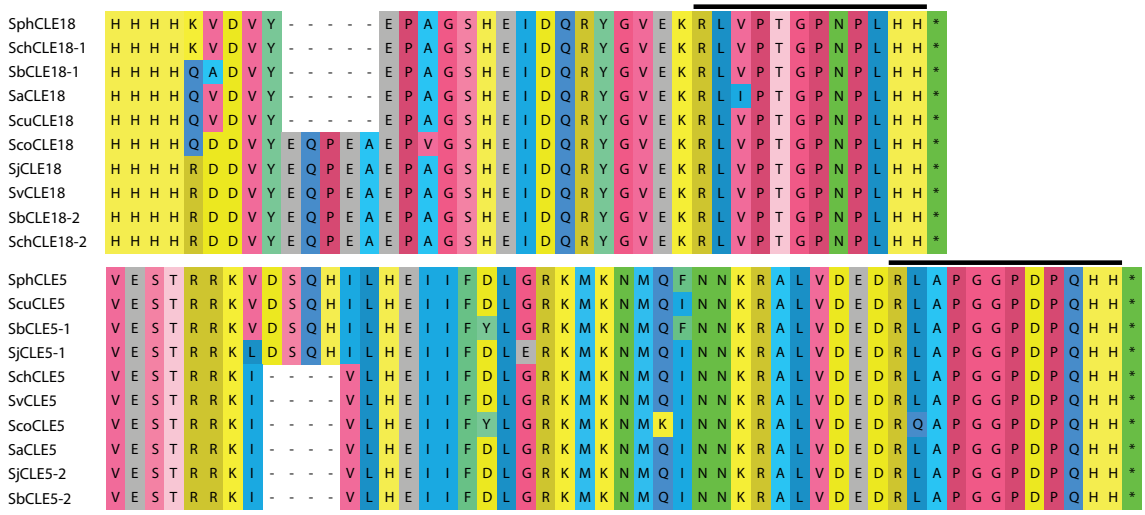

Fig. 5. Fragments of CLE18 and CLE5 protein alignments.
CLE domain is highlighted with a black line.

each have two CLE5 genes encoding proteins without this region and with it (see Fig. 5). Sometimes, great differences in the AASs of CLE proteins are observed only within one species. For example, protein SvCLE30, in contrast to CLE30 of other potato species, lacks 3 AAs in the VD. In the VD of ScoCLE20, there is no fragment of 8 AAs, and its SD is lengthened by 1 AA. *S. juzepczukii* has two *CLE12* genes, and one of them encodes a protein that differs from CLE12 of other species in that the onset of its SD is extended by 6 AAs, while 3 AAs are absent in the VD.

In sum, the analysis of CLEs nucleotide and amino acid sequences of the potato species studied revealed several examples of polymorphism in different regions of CLE carrying different functional loads. This polymorphism can affect the activity of CLE peptides. The differences relate to CLE domain itself, from which the actual CLE peptide is formed, or to sequences outside it, which can affect the processing of CLE protein. At the same time, accurate data on the functional difference between CLE proteins in potato species can be obtained only with a precise analysis of their functions. For example, in overexpression experiments of the corresponding genes or plant treatment with synthetic CLE peptides of different potato species. Our research can serve as the groundwork for further research in this area.

Discussion

In this research, we performed a search and analysis of the genes encoding CLE proteins in different species of potatoes: wild and primitive cultivated. Of the 332 identified *CLE* genes, we found 57 genes that encode unique CLE peptides. In total, we identified 42 genes that are present in almost all potato species analyzed. At the same time, there are genes that are very similar to each other in different species and those that have significant interspecific differences. Thus, in *CLE* genes of *S. commersonii*, presumably, the largest number of unique substitutions occurred, which led to serious differences in its CLE peptides compared with those in other potato species. *S. commersonii* is a wild potato that differs from the rest of the species analyzed by its habitat. It grows outside the growing areas of cultivated potatoes and, most likely, is not among the ancestors of cultivated potatoes (Juzepczuk, Bukasov, 1929).

Some of the revealed differences in the sequences of CLE proteins are unique for a certain species of potato, while other differences relate to several ones. Of special interest are the proteins CLE5 and CLE18, which vary in the presence or absence of a region of 4–5 AA. At the same time, there are potato species in which both protein variants are present (see Fig. 4), which may be associated with the natural hybridization of potatoes (Hawkes, 1990).

Additionally, in some potato species, substitutions occurred in CLE domain, which could affect the functions of the corresponding peptides. For example, divergence in 1 AA in CLE domain of *A. thaliana* CLE peptides results in the divergence of their functions: one peptide is involved in the response to water shortage (CLE25), while the other is not (CLE26) (Takahashi et al., 2018). Unique peptides that appear in individual potato species due to the differences in CLE domain can perform distinct functions or completely lose functionality. Furthermore, changes in the sequence outside the CLE domain can also affect the functioning of the CLE peptide and, in different potato species, changes in VD or SD may affect peptide activity. At the same time, the sequence similarity of different CLEs may point to their common origin. The presence of duplicated genes, such as *CLE2-2*, in which substitutions occur in CLE domain sequence and which may subsequently lead to the emergence of new genes confirms this hypothesis.

Conclusion

In summary, we found that CLE proteins in various potato species are similar; however, they also have differences that could affect their functioning. Further study of CLE proteins will reveal their role in potato development.

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ORCID ID

M.S. Gancheva orcid.org/0000-0002-9631-6143
M.R. Losev orcid.org/0000-0003-0968-8684
A.A. Gurina orcid.org/0000-0002-1791-3063
L.O. Poliushkevich orcid.org/0000-0003-2896-1893
I.E. Dodueva orcid.org/0000-0001-5282-718X
L.A. Lutova orcid.org/0000-0001-6125-0757

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Sugar transporters of the SWEET family and their role in arbuscular mycorrhiza

A.A. Kryukov[1] ✉, A.O. Gorbunova[1], T.R. Kudriashova[2], O.I. Yakhin[3, 4], A.A. Lubyanov[5], U.M. Malikov[6], M.F. Shishova[7], A.P. Kozhemyakov[1], A.P. Yurkov[1]

[1] All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia
[2] Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
[3] Institute of Biochemistry and Genetics – Subdivision of the Ufa Federal Research Center of the Russian Academy of Sciences, Ufa, Russia
[4] Tatar Research Institute of Agricultural Chemistry and Soil Science – Subdivision of the Federal Research Center "Kazan Scientific Center of the Russian Academy of Sciences", Kazan, Russia
[5] Research, Development and Production Enterprise "Eco Priroda", Ulkundy village, Duvansky district, Republic of Bashkortostan, Russia
[6] The Bonch-Bruevich Saint Petersburg State University of Telecommunications, St. Petersburg, Russia
[7] St. Petersburg State University, Biological Faculty, St. Petersburg, Russia
✉ rainniar@rambler.ru

Abstract. Plant sugar transporters play an essential role in the organism's productivity by carrying out carbohydrate transportation from source cells in the leaves to sink cells in the cortex. In addition, they aid in the regulation of a substantial part of the exchange of nutrients with microorganisms in the rhizosphere (bacteria and fungi), an activity essential to the formation of symbiotic relationships. This review pays special attention to carbohydrate nutrition during the development of arbuscular mycorrhiza (AM), a symbiosis of plants with fungi from the Glomeromycotina subdivision. This relationship results in the host plant receiving micronutrients from the mycosymbiont, mainly phosphorus, and the fungus receiving carbon assimilation products in return. While the efficient nutrient transport pathways in AM symbiosis are yet to be discovered, SWEET sugar transporters are one of the three key families of plant carbohydrate transporters. Specific AM symbiosis transporters can be identified among the SWEET proteins. The survey provides data on the study history, structure and localization, phylogeny and functions of the SWEET proteins. A high variability of both the SWEET proteins themselves and their functions is noted along with the fact that the same proteins may perform different functions in different plants. A special role is given to the SWEET transporters in AM development. SWEET transporters can also play a key role in abiotic stress tolerance, thus allowing plants to adapt to adverse environmental conditions. The development of knowledge about symbiotic systems will contribute to the creation of microbial preparations for use in agriculture in the Russian Federation.
Key words: arbuscular mycorrhiza; SWEET; sugar transport; sucrose; glucose; sugar transporter genes.

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Транспортеры сахаров семейства SWEET и их роль в арбускулярной микоризе

А.А. Крюков[1] ✉, А.О. Горбунова[1], Т.Р. Кудряшова[2], О.И. Яхин[3, 4], А.А. Лубянов[5], У.М. Маликов[6], М.Ф. Шишова[7], А.П. Кожемяков[1], А.П. Юрков[1]

[1] Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии, Пушкин, Санкт-Петербург, Россия
[2] Санкт-Петербургский политехнический университет Петра Великого, Санкт-Петербург, Россия
[3] Институт биохимии и генетики – обособленное структурное подразделение Уфимского федерального исследовательского центра Российской академии наук, Уфа, Россия
[4] Татарский научно-исследовательский институт агрохимии и почвоведения – обособленное структурное подразделение Федерального исследовательского центра «Казанский научный центр Российской академии наук», Казань, Россия
[5] Научно-производственное предприятие «Эко Природа», с. Улькунды, Дуванский район, Республика Башкортостан, Россия
[6] Санкт-Петербургский государственный университет телекоммуникаций им. проф. М.А. Бонч-Бруевича, Санкт-Петербург, Россия
[7] Санкт-Петербургский государственный университет, биологический факультет, Санкт-Петербург, Россия
✉ rainniar@rambler.ru

Аннотация. В продуктивности растений существенную роль играют транспортеры сахаров, поскольку с их помощью координируются и осуществляются потоки углеводов от листьев к органам потребления. Кроме того, с участием транспортеров сахаров регулируется значительная часть обмена питательными веществами с микроорганизмами ризосферы (бактериями и грибами), что является необходимым условием для формирования симбиотических отношений. В связи с этим в обзоре уделено особое внимание углеводному пита-

нию при развитии арбускулярной микоризы (АМ) – симбиоза растений с грибами подотдела Glomeromycotina, в результате которого растение-хозяин получает от микосимбионта микроэлементы, главным образом фосфор, а гриб взамен получает продукты ассимиляции углерода. Пути эффективного транспорта питательных веществ в АМ-симбиозе до сих пор не раскрыты. Одно из трех ключевых семейств углеводных транспортеров растений – SWEET, переносчики сахаров. Именно среди белков SWEET могут быть выявлены специфические для симбиоза с АМ-грибами транспортеры. В обзоре представлены данные по истории изучения, структуре, локализации, филогении и функциям белков SWEET. Отмечена высокая вариабельность как самих белков SWEET, так и их функций. При этом одни и те же белки у разных растений могут выполнять различные функции. Особая роль уделена участию транспортеров семейства SWEET в развитии АМ-симбиоза растений и грибов. Транспортеры SWEET могут также играть ключевую роль в устойчивости к абиотическим стрессам, позволяя растениям адаптироваться к неблагоприятным условиям окружающей среды. Развитие знаний о симбиотических системах будет способствовать созданию микробных препаратов для использования в сельском хозяйстве Российской Федерации.
Ключевые слова: арбускулярная микориза; SWEET; транспорт сахара; сахароза; глюкоза; гены транспортеров сахаров.

Introduction

Sugar transporters in plants are customarily divided into three major classes: SUT (SUC), MST (including STP, TMT, PMT, VGT, pGlct/SGB1, ESL, and INT subclasses), and SWEET (Sugars Will Eventually be Exported Transporters). The most well studied transporters are SUT and MST. SUT carry out long-distance transportation of sucrose from plant leaves to the targeted plant organs and tissues. They then disintegrate into monosaccharides and are subsequently transported by MST proteins. The major part of the transporters from SUT and MST classes are known to be non-specific to symbiotic plant-microbial systems such as arbuscular mycorrhiza (AM).

However, in 2010, Li-Qing Chen described a new transporter class SWEET. SWEET transporters carry out non-volatile bidirectional transportation of sugars in all plant organs and tissues. At present, the SWEET protein family is the least studied group of transporters. According to current knowledge, proteins specific to AM symbiosis may be detected inside the SWEET transporter group (Chen et al., 2010). Various sources in the literature provide conflicting information concerning the SWEET protein class. This survey is an attempt to tackle the issue and combine knowledge about the proteins of the group. Thus, the aim of the current research is to provide an overview of the data on gene phylogenesis inside the SWEET class and functions of the proteins encoded with the aforementioned genes as well as to assess their role in the sugar transportation process during the formation of AM symbiosis.

General information on the SWEET transporters

SWEET proteins, identified in the late 1990s, were first called MtN3 (involved in the development of *Medicago truncatula* Gaertn. nodules) and Saliva (first discovered in the salivary glands of *Drosophila* during embryonic development). That is why the transmembrane domains made of those proteins were named “MtN3/Saliva”, or “MtN3_slv domain” and are also known as “PQ loop” (Chen et al., 2010). In 2010, Li-Qing Chen was the first to isolate SWEET transporters into a separate family of proteins by providing a detailed description of those pertaining to *Arabidopsis thaliana* (L.) Heynh. Seventeen different transporters were discovered, described, and named according to the species belonging to a particular plant and the protein number (e. g. AtSWEET17). The same paper provides a detailed description of the SWEET proteins in *Oryza sativa* L. (Chen et al., 2010). Afterwards SWEET proteins were found in a number of other plant species and also in animals and prokaryotes (the latter were named SemiSWEET) (Chen et al., 2012; Feng et al., 2015; Patil et al., 2015; Manck-Götzenberger, Requena, 2016; Hu L.P. et al., 2017). Now all living organisms are generally considered to possess SWEET or SemiSWEET proteins (Feng et al., 2015). Newly found SWEETs are numbered according to orthology with Arabidopsis (*A. thaliana*) proteins. However, some discrepancies and variations in *A. thaliana* numbering have been noted (Supplementary Material 1)[1] (Doidy et al., 2019).

Structure and localization of the SWEET proteins on membranes

SWEET proteins are uniporters, transporting carbohydrates across membranes along a concentration gradient, typically localized on the plasma membrane (Chen et al., 2010). SWEET proteins in plants usually contain seven transmembrane (TM) helices (Xuan et al., 2013). Nevertheless, in 2015, G. Patil et al. discovered that *Vitis vinifera* L. SWEET is made of fourteen TMH (transmembrane helices), which proves that the SWEET protein helix structure may vary (Patil et al., 2015). Bacterial SemiSWEETs are the smallest among the known transporters. Consisting of about one hundred amino acids coiled in three spirals, i. e., three TMHs, they form a triple helix bundle (THB). The duplication of THB in prokaryotes leads to the emergence of eukaryotic SWEET transporters consisting of two THBs and an additional linker helix, numbered TMH4 (Feng et al., 2015). Moreover, it is noteworthy that the three TMHs in THB are not arranged in series on the membrane. The third TMH is squeezed between the first and the second one. There, the N-terminus of the protein is found on the outer side of the membrane, while the C-terminus is located on the inner side. In eukaryotes, the C-terminus is elongated and contains phosphorylation sites that can be used for post-translational modification (Jeena et al., 2019). The nucleotide sequence, encoding TMH4, is known to be the most variable of TMHs, and its origin is currently being debated (Jeena et al., 2019).

1 Supplementary Materials 1 and 2 are available in the online version of the paper: http://vavilov.elpub.ru/jour/manager/files/Suppl_Kryukov_Engl.pdf

Interestingly, some eukaryotes exhibit SWEET protein structures similar to the prokaryotic ones. For instance, wheat *Triticum aestivum* L. may also have a SemiSWEET consisting of three and four TMHs (Gautam et al., 2019). According to researchers, the presence of TaSWEET with 3, 4, 6, and 7 TMHs in wheat implies that both duplication and fusion of SWEET protein structures can occur in the genome (Gautam et al., 2019). SuperSWEET sugar transporters found in *Phytophthora* contain from 18 to 25 TMHs and are composed of 5–8 semiSWEET loops (Jia et al., 2017). Thus, the structures of SWEET proteins should be assumed to be highly variable.

Phylogenetics of the SWEETs, isoforms

In the construction of a phylogenetic tree, it was revealed that the SWEET genes of plants are still grouped into four clades, despite their low homology (Chen et al., 2015). This evolutionary division occurred long ago, and representatives of each of the clades are observed in almost all (possibly all) terrestrial plants. With all this, the second clade is the most ancient, and its representatives share certain homology with the SWEET proteins in algae (Li X. et al., 2018). In mammals and some microorganisms (e. g. *Chlamydomonas*), proteins have been found to fall into clade V, separate from the other SWEETs (Chen et al., 2012).

The increasing number of SWEET isoforms is a consequence of duplication or fusion of the THB genes. This contributes to the expansion of transporter functions and plant adaptation under new conditions (Li X. et al., 2018). The number of SWEET isoforms varies significantly among plant species. For example, unicellular and green algae have 1 to 3 SWEET isoforms, while monocots are observed to possess from 18 to 23, and dicots from 15 up to 68 (Li X. et al., 2018). According to other data, *T. aestivum* wheat (monocotyledonous) is known to have 108 isoforms of SWEET genes localized on 21 chromosomes, while some of them are orthologs of the SWEETs of Arabidopsis (of 14 genes of Arabidopsis), and some belong to three new types that do not have significant homology with Arabidopsis genes (Gautam et al., 2019). *M. truncatula* has up to 26 isoforms (Doidy et al., 2019). At the same time, it is likely that these are not all of the identified transporters, since, by 2015, only 24 of them were isolated (Chandran, 2015).

The representatives of the four clades are considered to be divided not only phylogenetically, but also functionally. Thus, most researchers argue that (1) the protein representatives of clades I and II transport hexoses, (2) proteins of clade III are mainly involved in the transport of sucrose, and (3) those of clade IV are principally involved in the transport of fructose (see Suppl. Material 1) (Chen et al., 2012; Feng et al., 2015). But this is not necessarily so. In 2019, B. Hu et al. showed that MtSWEET5b and MtSWEET7 (*M. truncatula*) are able to transport not only hexoses, but also sucrose. Other plants may also be exceptions, for instance LjSWEET3 (*Lotus japonicus* L.) also transports sucrose instead of hexoses. MtSWEET16 may be involved in the transportation process of sucrose and mannose. It is therefore impossible to speak strictly about the clade division of the SWEET genes for the types of the transferred substrate (see Suppl. Material 1) (Hu B. et al., 2019).

The nucleotide sequence analysis of the SWEET genes shows their significant variability. Between the four clades, it can reach up to 80 % (which is to say that, in some cases, there is homology of only 20 %) (unpublished data, Kryukov et al., 2021). With such variability, it is typically impossible to align sequences and then build phylogeny. In this regard, the existing phylogenetic trees of the SWEET genes should be treated with extreme caution. The intron-exon structure of the SWEET genes may also vary notably (Cao et al., 2019). Most of the *MtSWEET* genes (in *M. truncatula*) contain 5 introns, excluding the genes *MtSWEET4*, *MtSWEET6*, *MtSWEET7* and *MtSWEET13* which include 4 introns, and *MtSWEET2b* which contains 16 introns (Hu B. et al., 2019). The structure of the *M. truncatula* SWEET proteins is also heterogeneous: most contain 7 TMHs, but MtSWEET4 and MtSWEET11 have 6 TMHs, and MtSWEET2b contains 15 TMHs instead of 7 (Hu B. et al., 2019).

SWEET protein functions

As has already been mentioned, the representatives of the four clades can be divided in accordance with their functions. However, it should be noted that different authors provide varied data on functions of the certain SWEET proteins (see Suppl. Materials 1 and 2). This may be due to several possible reasons: (1) orthologs of the SWEETs can perform different functions in different species; (2) orthologs can perform different functions under different conditions and their genes are expressed in different ways; (3) possible paralogs within each clade may be similar and hence may be misidentified.

In all cases, SWEET proteins are non-volatile bidirectional uniporters. However, according to some researchers, the fact that all SWEET transporters are uniporters has not been completely proven (Chen et al., 2015). SWEET proteins are involved in a variety of processes, whether in plants (see Suppl. Material 1) or mammals. In addition to the transportation of carbohydrates, they are most likely to participate in the transport of other agents such as gibberellins, which is the case of Arabidopsis (Kanno et al., 2016). In peas (*Pisum sativum* L.), it was also discovered that the interaction between the SWEET transporters and CWINV (cell wall invertase) in the presence of cytokinins leads to the formation of multiple shoots and the loss of apical dominance during infection with the pathogen *Rhodococcus fastian* (Doidy et al., 2019).

SWEET transporters can also play a role in abiotic stress tolerance, allowing plants to adapt to adverse environmental conditions (see Suppl. Materials 1 and 2) (Chandran, 2015). Various authors have associated the accumulation of sugars in plants with abiotic stresses (Hu B. et al., 2019). Low temperatures, water, and other stressful environmental factors are able to induce the expression of the SWEET genes in plants, which leads to the assumption that these genes are associated with plant responses to these stresses (Kafle et al., 2019; Wei et al., 2020).

There is a great deal of literary data on the functions of the SWEET proteins in plants of various species. For example, LjSWEET3 mediates the transportation of sucrose (Sugiyama

et al., 2017) to nodules. The *AtSWEET1* and *AtSWEET5* genes are significantly expressed at different stages of pollen maturation. Almost all representatives of clade II are involved in the transportation of sugars to the reproductive organs, i. e., pollen, seeds, and some to fungal pathogens (Chen et al., 2010). Genes *AtSWEET11* and *AtSWEET12* have been established as important transporters of sucrose from parenchyma cells to phloem (Chen et al., 2012). At the same time, SWEET proteins of the clade III are associated with susceptibility and resistance to pathogens (Gautam et al., 2019). According to W.J. Guo et al., proteins of the clade IV – AtSWEET17, AtSWEET16 – are active in root cortical cells and are localized on the tonoplast (Guo et al., 2014).

Rhizosphere pathogens can cause an increased expression of clade III proteins, which leads to additional transport of sucrose to the roots and contributes to the nutrition of rhizosphere microorganisms (Doidy et al., 2019). In 2010, it was shown by L.-Q. Chen et al. that pathogenic bacteria, for example *Xanthomonas*, are able to enter tissues of the host plant and induce the expression of *SWEET* genes (primarily *SWEET11* and *SWEET14*, from clade III) to obtain sugars. Like symbiotic AM fungi, pathogenic fungi also have the ability to induce the expression of genes in order to get sugar for themselves (Chen et al., 2010).

The expression of a significant number of alterations in SWEET under the influence of stress factors such as water deficiency leads to a notably increased expression of the *MtSWEET3a*, *MtSWEET3b*, *MtSWEET9b* and *MtSWEET13* genes, while the expression of *MtSWEET1a*, *MtSWEET3c*, *MtSWEET15c* drops significantly (see Suppl. Material 2) (Hu B. et al., 2019). According to J. Doidy et al., *MtSWEET16* is unique in that its expression is mainly enhanced in leaves, whereas the pea ortholog *PsSWEET16* is expressed primarily in the roots and stem (Doidy et al., 2019). SWEET3 orthologs *PsSWEET3.1*, *MtSWEET3.3* and *LjSWEET3* (Sugiyama et al., 2017), SWEET11 orthologs *MtSWEET11* and *PsSWEET11* (Kryvoruchko et al., 2016) and SWEET15 orthologs *MtSWEET15.3* and *PsSWEET15.3* (Gamas et al., 1996) are specifically expressed in root nodules in leguminous plants.

J. Manck-Götzenberger and N. Requena note that numerous transporters show significant expression in AM symbiosis while being non-specific to it (Manck-Götzenberger, Requena, 2016). In turn, A. Kafle pointed out that SWEET1 orthologs (*MtSWEET1.2* and *PsSWEET1.2*) can be expressed in both mycorrhized roots and root nodules (Kafle et al., 2019).

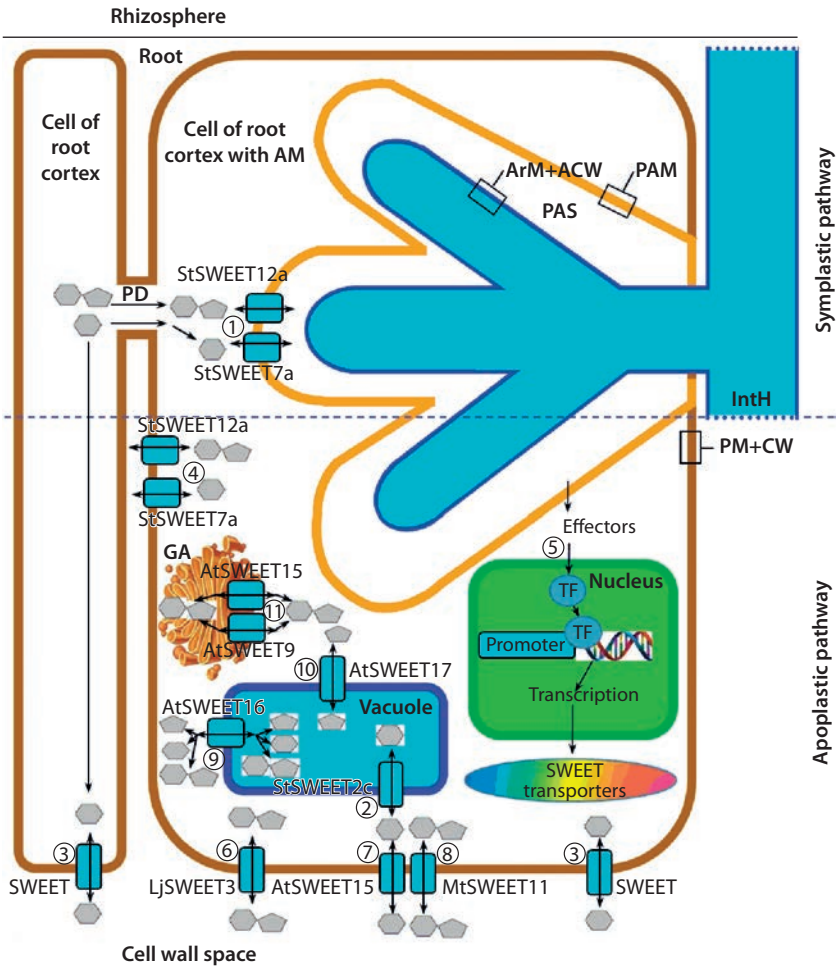
Localization and functions of the SWEET transporters in root cells of plants with arbuscular mycorrhiza fungus

As is known, according to the data of the transcription profiles, not all transporters of the SWEET family have yet been found, nor have all known transporters of this group been localized in a plant cell and their exact function established (Hennion et al., 2019). Only now is the localization of most SWEET sugar transporters receiving proper attention (see Suppl. Materials 1 and 2) as such study requires a separate examination of each individual transporter for each individual plant species. Their functions and localization also require confirmation. On the other hand, the question of the participation of SWEET proteins in the specific transport of sugars from the host plant to mycosymbiont AM fungi is quite urgent and requires detailed research, since knowledge of the mechanisms of active carbohydrate nutrition of a mycosymbiont will allow us to understand the mechanisms that lead to the formation and development of effective interaction between partners in AM symbiosis.

According to the literary data, it should be assumed that most of the SWEET transporters of the clades I, II, III are localized in the plasma membrane (see the Figure). The figure represents a root cell of a host plant with an arbuscule (arbuscule is the most common type of symbiotic structure formed during the development of AM; this is the invagination of plant plasmalemma into the plant cell at the site of penetration of the AM fungus hypha, followed by multiple branching of the trunk of the arbuscule with the formation of a new interface for the interaction of symbiotic partners – the periarbuscular space (PAS) – between the periarbuscular membrane (PAM) and the arbuscular membrane (ArM) with the arbuscular cell wall (ACW), formed in place of the cell wall of the host plant).

The peculiarity of transport processes under AM conditions is analyzed by comparing cells with and without arbuscules (Gaude et al., 2012). Thus, J. Manck-Götzenberger and N. Requena (Manck-Götzenberger, Requena, 2016) were the first to show that the main transport of sugars in *Solanum tuberosum* from the host plant to the AM fungus *Rhizophagus irregularis* can occur due to the facilitators of sucrose and glucose – StSWEET12 and StSWEET7a, respectively (①, see the Figure; Manck-Götzenberger, Requena, 2016; Hennion et al., 2019). StSWEET12 and StSWEET7a operate on PAM and transport sugars from the cytoplasm to the PAS and *vice versa*. From here, glucose is transported through the ArM from PAS in the arbuscule using the fungal monosugar transporter RiMST2 (*R. irregularis* (Błaszk., Wubet, Renker & Buscot)) (Hennion et al., 2019), or as a result of GpMST1 functioning (*Geosiphonomyces pyriformis* Cif. & Tomas) (Schüßler et al., 2006). The sucrose transportation through the ArM may occur via the fungal sucrose transporter RiSUC1 (Helber et al., 2011). Subsequently, sugar is transported along the intraradical mycelium as glycogen into the extraradical mycelium of the AM fungus (Hennion et al., 2019). On the other hand, the cytoplasm sugar in the cells of the root cortex can be regulated by their transfer from the vacuole by tonoplastic transporters, which include the glucose facilitator StSWEET2c (②; Hennion et al., 2019).

The sugar transportation apoplastic pathway is carried out to cells both with and without AM fungus via SWEET hexose transporters (③; Chardon et al., 2013; Ludewig, Flügge, 2013). It is assumed that there may be specific SWEET facilitators for AM symbiosis. For example, StSWEET12 and StSWEET7a proteins may carry out specific transportation of sucrose and glucose in *S. tuberosum* through the plasmalemma of root cortex cells containing arbuscules (④, Manck-Götzenberger, Requena, 2016; Hennion et al., 2019). Once there, the effectors secreted by AM fungi either directly activate the expression of the SWEET genes, or indirectly through the activation of transcription factors (⑤; Chandran, 2015; Jeena et al., 2018). The LjSWEET3 protein, which is responsible for the transportation of sucrose to the cells with arbuscules



Localization scheme of the SWEET transporters in a cell with an arbuscule (Guo et al., 2014; Lin et al., 2014; Chandran, 2015; Chen et al., 2015; Ait Lahmidi et al., 2016; Kryvoruchko et al., 2016; Manck-Götzenberger, Requena, 2016; Sugiyama et al., 2017; Hennion et al., 2019; Jeena et al., 2019; Yurkov et al., 2019).

PM+CW – plasmalemma and cell wall of the root cortex; PAM – periarbuscular membrane; PAS – periarbuscular space; ArM+ACW – arbuscular membrane and arbuscular cell wall; IntH – intercellular intra-root hypha of AM fungus; TF – transcription factor; GA – Golgi apparatus; PD – plasmodesmata. Description of the circuit is provided in the main article.

in *Lotus japonicus*, is claimed to be a specific facilitator as well (⑥, see the Figure; Sugiyama et al., 2017; Hennion et al., 2019). Proteins AtSWEET15 (previously called SAG29; Seo et al., 2011) and MtSWEET11 (Kryvoruchko et al., 2016) are known to be non-AM-specific SWEET transporters, localized on the root cell plasma membrane (⑦ and ⑧, respectively).

The discussion of the SWEET protein localization on the organelles of the root cell is controversial. Thus, according to some data, transporters of the clade IV (AtSWEET16 and AtSWEET17) can be localized in the tonoplast of the plant vacuole (⑨ and ⑩, respectively; Chardon et al., 2013; Guo et al., 2014; Jeena et al., 2019). On the other hand, clade III sucrose transporters AtSWEET9 and AtSWEET15 may be localized on the membrane of the trans-Golgi network (⑪, see the Figure).

Thus, summarizing the information on the localization of the SWEET transporters in AM, it can be concluded that none of the transporters has shown specific localization simultaneously in two or more plant species. Nor is there attested specific gene expression under the same conditions, as, for example, in the phosphate transporter (PT4) of *M. truncatula* and in a number of other plant species. The first to be verified are StSWEET12 and StSWEET7a.

The transporter functions in AM may be assumed on the basis of general information about the clades of proteins of the SWEET family, but it should be noted that there have been no detailed studies of both the localization and functions of these proteins in AM symbiosis yet. There are only assumptions about their role in AM. For instance, in a recent work by J. An et al. (2019), it has been noted that MtSWEET1b may supply AM glucose to fungi. According to the *M. truncatula* gene expression atlas (MtGEA; http://mtgea.noble.org/v3/), *MtSWEET1b* and *MtSWEET6* are highly expressed in arbuscular cells, and their putative orthologs *StSWEET1a*, *StSWEET1b*, and *StSWEET7a* (*S. tuberosum* L.) also demonstrate high transcription levels in mycorrhizal roots (Manck-Götzenberger, Requena, 2016). SWEET transporters of clade I are those most likely to participate in the supply of sugars to symbiotic systems, including AM (Doidy et al., 2019). Based on this information, it should be assumed that studies of the function of the SWEET proteins are still very fragmentary (see Suppl. Material 1). The confirmation in several plant species remains an urgent task.

Conclusion

SWEET proteins are essential for the transportation of carbohydrates in plants. Proteins specific to the various forms of symbiosis can be found amongst the SWEET class. Primarily, they can be located in clades I and III. SWEET transporters are quite variable, a change in external conditions may lead to the emergence of numerous isoforms with varying functions. Hence, SWEET protein identification and selection of primers for the gene amplification requires prudence. Close paralogs may be very similar; however, high variability between clades does not allow for the construction of a reliable phylogenetic tree with all the ensuing consequences. This high variability may account for the scatter of the data related to SWEET protein functions (see Suppl. Material 1). Still, a hypothesis about the universality of the range of SWEET genes may be put forward, mainly in case of similar gene structure. Furthermore, there are reasons to believe that not all of the genes from the SWEET class have yet been identified for *M. truncatula*. All this testifies in favor of the fact that the understanding of the functions of these transporters will be expanded in the coming years.

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ORCID ID

A.A. Kryukov orcid.org/0000-0002-8715-6723
T.R. Kudriashova orcid.org/0000-0001-5120-7229
O.I. Yakhin orcid.org/0000-0001-9039-7969
A.A. Lubyanov orcid.org/0000-0002-6134-0290
M.F. Shishova orcid.org/0000-0003-3657-2986
A.P. Kozhemyakov orcid.org/0000-0002-9657-2454
A.P. Yurkov orcid.org/0000-0002-2231-6466

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Development of a marker panel for genotyping of domestic soybean cultivars for genes controlling the duration of vegetation and response to photoperiod

R.N. Perfil'ev ✉, A.B. Shcherban, E.A. Salina

Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
✉ PerfilyevRN@bionet.nsc.ru

Abstract. Soybean, *Glycine max* L., is one of the most important agricultural crops grown in a wide range of latitude. In this regard, in soybean breeding, it is necessary to pay attention to the set of genes that control the transition to the flowering stage, which will make it possible to adapt genotypes to local growing conditions as accurately as possible. The possibilities of soybean breeding for this trait have now significantly expanded due to identification of the main genes (*E1–E4*, *GmFT2a*, *GmFT5a*) that control the processes of flowering and maturation in soybean, depending on the day length. The aim of this work was to develop a panel of markers for these genes, which could be used for a rapid and efficient genotyping of domestic soybean cultivars and selection of plant material based on sensitivity to photoperiod and the duration of vegetation. Combinations of 10 primers, both previously developed and our own, were tested to identify different alleles of the *E1–E4*, *GmFT2a*, and *GmFT5a* genes using 10 soybean cultivars from different maturity groups. As a result, 5 combinations of dominant and recessive alleles for the *E1–E4* genes were identified: (1) *e1-nl(e1-as)/e2-ns/e3-tr(e3-fs)/e4*; (2) *e1-as/e2-ns/e3-tr/E4*; (3) *e1-as/e2-ns/E3-Ha/e4*; (4) *E1/e2-ns/e3-tr/E4*; (5) *e1-nl/e2-ns/E3-Ha/E4*. The studied cultivars contained the most common alleles of the *GmFT2a* and *GmFT5a* genes, with the exception of the 'Cassidi' cultivar having a rare dominant allele *GmFT5a-H4*. The degree of earliness of cultivars positively correlated with the number of recessive genes *E1–E4*, which is consistent with the data of foreign authors on different sets of cultivars from Japan and North China. Thus, the developed panel of markers can be successfully used in the selection of soybean for earliness and sensitivity to photoperiod.

Key words: photoperiod; flowering period; gene marker; allele-specific primers; nonsynonymous substitution; indel; cultivar; soybean; maturity group.

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Разработка панели маркеров для генотипирования отечественных сортов сои по генам, контролирующим срок вегетации и реакцию на фотопериод

Р.Н. Перфильев ✉, А.Б. Щербань, Е.А. Салина

Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
✉ PerfilyevRN@bionet.nsc.ru

Аннотация. Соя (*Glycine max* L.) – одна из важнейших сельскохозяйственных культур, выращиваемая в большом диапазоне географической широты. В связи с этим в селекции сои необходимо обращать внимание на набор генов, контролирующих переход к фазе цветения, что позволит максимально точно адаптировать генотипы к локальным условиям произрастания. В настоящее время возможности селекции сои по данному признаку значительно расширились благодаря идентификации в ее геноме основных генов (*E1–E4*, *GmFT2a*, *GmFT5a*), контролирующих процессы цветения и созревания в зависимости от длины дня. Целью нашей работы являлось создание панели маркеров к этим генам, которая может быть использована для быстрого и эффективного генотипирования отечественных сортов сои и отбора растительного материала по признакам чувствительности к длине дня и продолжительности вегетационного периода. Проведено тестирование 10 комбинаций праймеров (как ранее разработанных, так и собственных) для выявления различных аллельных состояний генов *E1–E4*, *GmFT2a* и *GmFT5a* на выборке из 10 сортов сои из различных групп спелости. В итоге выявлено пять комбинаций доминантных и рецессивных аллелей по генам *E1–E4*: 1) *e1-nl(e1-as)/e2-ns/e3-tr(e3-fs)/e4*; 2) *e1-as/e2-ns/e3-tr/E4*; 3) *e1-as/e2-ns/E3-Ha/e4*; 4) *E1/e2-ns/e3-tr/E4*; 5) *e1-nl/e2-ns/E3-Ha/E4*. Проанализированные сорта содержали наи-

более распространенные аллели генов *GmFT2a* и *GmFT5a*, за исключением сорта Кассиди, у которого был обнаружен редкий доминантный аллель *GmFT5a-H4*. Степень скороспелости сортов положительно коррелировала с количеством рецессивных генов *E1–E4*, что согласуется с данными зарубежных авторов, полученными на выборках сортов из Японии и Северного Китая. Таким образом, разработанная панель маркеров может успешно использоваться в селекции сои на скороспелость и чувствительность к фотопериоду.
Ключевые слова: фотопериод; срок цветения; маркер гена; аллель-специфичные праймеры; несинонимичная замена; индель; сорт; соя; группа спелости.

Introduction

The genus *Glycine* consists of two subgenera, *Soja* and *Glycine*. The first subgenus includes the species *Glycine soja* (2*n* = 4*x* = 40), or the Ussuri soybean – a wild annual plant from Southeast Asia and the cultivated species of soybean – *Glycine max* L. (2*n* = 4*x* = 40) (Vavilov, 1926; Zhukovsky, 1964).

Soybean is cultivated in many countries of the world for food, animal feed and technical purposes due to its unique nutritional properties, including a high protein content (30–52 %). In terms of protein content, soybean surpasses all cultivated crops, in particular: wheat (9–26 %), rice (7 %), corn (10 %), etc., except for lupine. The value of soy protein is determined by the content of essential amino acids, the sum of which is 20 % of the total protein mass, and in wheat – 18 % (Gorissen et al., 2018). The degree of digestibility of soy protein has the highest index – 1, corresponding to proteins of milk, eggs, and casein and much higher than that of cereals (0.25–0.4) (Hoffman, Falvo, 2004).

Soybean was first cultivated in China 6000 BC. Then, as the main source for the production of vegetable protein and oil, soybean has spread to other countries of Southeast Asia: India, Korea, Japan, and Indonesia, where a variety of ways of eating it have been developed. Soybean appeared in Europe at the end of the 8th century. In Russia (the former USSR), soybean was brought to the Far East from China and this crop was introduced into production in the USSR in 1927.

In terms of the crop area in the world, soybean ranks first among leguminous crops. In 2019, it occupied 122 million hectares (https://www.kleffmann.com/). The world leaders in soybean production are Brazil and the United States. The cultivation area in these countries is 37 and 31 million hectares, respectively; average yield – 3.3 t/ha. According to the Federal State Statistics Service (Rosstat, https://rosstat.gov.ru/), in Russia in 2019, the total area under cultivated soybean was ~3 million hectares with yield – 1.0–2.0 t/ha. Five years later, the cultivation area of soybean in Russia has increased by 51 %. At the same time, the gross harvest increased by 1.6 times from 2.64 million tons in 2015 to 4.36 million tons in 2019.

The potential for increasing the yield of soybean in Russia is quite high and can be realized both by modernization of agrotechnical cultivation methods and through the development of new cultivars better adapted to the climatic conditions of specific regions (priority direction). The compatibility of the development phases with the optimum temperature for each phase plays an important role in plant adaptation. Soybean belongs to warm-season plants since the optimum temperature for the vegetative phase is +20…+25 °C and for seed germination – +12…+14 °C. Seedlings can withstand frosts down to –3 °C. During the period of flowering and pod maturity, the need for heat is greatest, with the optimum temperature during this period being +18…+20 °C.

Soybean is cultivated in a wide range of latitudes from 55° north to 35° south. However, the area of cultivation of each cultivar is limited to a very narrow range of latitudes and usually there is one cultivar per 1° of latitude (Agarkova et al., 2016). This is due to a strong reaction to the photoperiod. Soybean is a southern plant and it requires a short day to transition to flowering. In the long day environments in northern latitudes, the photoperiod-sensitive cultivars delay flowering and the pods do not have time to mature before the onset of frost in autumn. Reducing sensitivity to photoperiod allows the plant to start flowering earlier and reach maturity in the optimal period. On the other hand, in southern latitudes, in conditions of a short day and warm weather, soybean flowers too early and does not have time to form the vegetation mass necessary for the formation of a high yield.

Modulation of the maturity time, depending on the latitude of the area, is achieved by selecting an effective combination of gene alleles for this area, which are responsible for the photoperiodic reaction and the transition of the plant to flowering and maturation. At present, 11 major loci (*E1–E11*) affecting this trait have been identified in soybean (Jia et al., 2014; Tsubokura et al., 2014; Zhai et al., 2014, Samanfar et al., 2017; Wang et al., 2019). The function of genes *E1–E4*, which are directly involved in the regulation of flowering and maturity in various photoperiods, has been established in most detail (Xu et al., 2013). Combinations of the different alleles of these four genes account for 62–66 % variation in the length of the maturity time (Tsubokura et al., 2014). The *E1* gene is a flowering repressor and encodes a transcription factor that contains the putative nuclear localization signal and the B3 DNA-binding domain (Watanabe et al., 2012; Xu et al., 2015). The *E2* gene is an orthologue of the flowering regulator gene of the Arabidopsis *GIGANTEA* (Watanabe et al., 2011). The *E3* and *E4* genes encode phytochrome A: GmPHYA3 and GMPHYA2, respectively (Liu et al., 2008). Recessive alleles of genes *E1–E4* are the result of mutations (frame shifts, nonsynonymous substitutions, deletions), leading to dysfunction of proteins, which gives insensitivity to photoperiod (Xu et al., 2013).

The soybean genome contains 12 *GmFT* genes homologous to the flowering activator *FT* (*FLOWERING LOCUS T*) of Arabidopsis (Kong et al., 2010; Wu et al., 2017). Of them, genes *GmFT2a* and *GmFT4* were mapped as maturity genes *E9* and *E10*, respectively (Zhao et al., 2016; Samanfar et al., 2017). The *GmFT2a* and *GmFT5a* genes have the strongest influence on the flowering time (Guo et al., 2015; Takeshima et al., 2016). Several signaling pathways for the regulation of soybean flowering depending on the photoperiod have been proposed, including the *E1*-specific regulatory pathway. Ac-

cording to this pathway, photoreceptors *E3* and *E4* provide photosensitivity and induce the expression of the *E1* gene and its homologue *E1L*, which suppress the expression of *GmFT5a* and *GmFT2a* leading to a delay in flowering (Zhu et al., 2019).

Thus, the previous analysis of the main genes involved in the regulation of the maturity time in soybean made it possible to identify various dominant and recessive alleles of these genes, which cause different sensitivity to photoperiod, and to develop allele-specific markers for these genes. The aim of this work is to create a panel of molecular markers that can be used for fast and efficient genotyping of domestic soybean cultivars and selection of plant material in terms of sensitivity to day length and the duration of maturity.

Materials and methods

As a material, we used 10 cultivars of soybean with different maturity time. Seeds of 4 cultivars were provided by Siberian Research Institute of Forages SFSCA RAS (Novosibirsk); 6 cultivars – by EFKO company (Alekseevka, Belgorod region; www.efcoforms.com). The names, genotypes and maturity time of the studied cultivars are presented in Table 1.

Total DNA was isolated from 4-day-old individual seedlings grown on wet filter paper in Petri dishes. DNA isolation was performed according to the method described by Kiseleva et al. (2016). The amount of DNA was determined using a spectrophotometer NanoDrop 2000 (Thermo Scientific, USA).

To identify the various alleles of the studied genes, we used allele-specific primers synthesized by "Biosset" company (Novosibirsk) (Table 2). PCR was performed in a 25-µl volume using a HS-Taq PCR kit (Biolabmix, Novosibirsk). The reaction mixture contained 50–100 ng of DNA, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer and 1 U HS-Taq DNA polymerase. PCR protocol: 5 min at 95 °C; 35–40 cycles (95 °C, 10 sec; 55–60 °C, 20 sec; 72 °C, 30–40 sec); 5 min at 72 °C. PCR products were separated by electrophoresis in 1 % agarose gel.

To analyze the *E2* gene, we used the CAPS marker described by Watanabe et al. (2011). The PCR product obtained using *E2*-specific primers was digested by restriction enzyme *Dra* I (SibEnzyme, Novosibirsk). We added 1 U of the enzyme to the PCR mixture and incubated it at 37 °C overnight. The restriction products were separated in 2 % agarose gel. The results of electrophoresis were visualized and photographed in UV using Gel Doc™ XR+ (BioRad, USA).

For sequencing, PCR products were isolated from the gel and purified using a diaGene kit for DNA elution from agarose gel (DiaM, Russia) according to manufacturer's instruction. The sequencing of PCR products was carried out using a Bigdye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) and corresponding specific primers. Sequencing was performed at the SB RAS Genomics Core Facility using an automatic capillary analyzer ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA).

Table 1. Genotypes of the analyzed soybean cultivars by genes *E1–E4*, *GmFT*

Cultivar, region	Genotype						Maturity group (range of the growing season/ average value*)
	E1	*E2*	*E3*	*E4*	*E9* (*GmFT2a*)	*GmFT5a*	
Annushka, Belgorod region	*e1-as*	*e2-ns*	*e3-tr*	*e4*	*E9*	*GmFT5a-H1*	Ultra-early maturing/very early maturing (75–85/80)
Bara, Belgorod region	*e1-as*	*e2-ns*	*e3-tr*	*e4*	*E9*	*GmFT5a-H1*	Ultra-early maturing/very early maturing (85–95/90)
Gorinskaya, Western Siberia	*e1-nl*	*e2-ns*	*e3-fs* *e3-tr*	*e4*	*E9*	*GmFT5a-H1*	Early maturing (92)
SibNIIK-9, Western Siberia	*e1-nl*	*e2-ns*	*e3-fs*	*e4*	*E9*	*GmFT5a-H1*	Early maturing (90–98/94)
SibNIIK-315, Western Siberia	*e1-nl*	*e2-ns*	*e3-fs*	*e4*	*E9*	*GmFT5a-H1*	Early maturing (98–105/102)
Chera-1, Belgorod region	*e1-as*	*e2-ns*	*e3-fs*	*E4*	*E9*	*GmFT5a-H1*	Early maturing (94–116/105)
Persona, Western Siberia	*E1*	*e2-ns*	*e3-tr*	*E4*	*E9*	*GmFT5a-H1*	Early maturing (103–109/106)
Belgorodskaya 48, Belgorod region	*e1-as*	*e2-ns*	*E3-Ha*	*e4*	*E9*	*GmFT5a-H1*	Early maturing/medium early maturing (98–119/108)
Malaga, Belgorod region	*e1-as*	*e2-ns*	*e3-tr*	*E4*	*E9*	*GmFT5a-H1*	Medium early maturing (110–115/112)
Cassidi, Belgorod region	*e1-nl*	*e2-ns*	*E3-Ha*	*E4*	*E9*	*GmFT5a-H4*	Medium early maturing (110–120/115)

* The duration of growing season on a long day was taken from the website of the State Register of Breeding Achievements (https://reestr.gossortrf.ru/). Maturity groups are given according to the classification generally accepted in Russia (Korsakov, 1973).

Table 2. Primers used in the work

Gene/allele	Primer sequences	Length of PCR products, bp	*T*° annealing	Source
*E1/e1-fs/e1-nl**	E1F1:CACTCAAATTAAGCCCTTTCA E1R1:TCCGATCTCATCACCTTTCC	547	55	Xia et al., 2012
e1-as	e1asF:GGGAGCAGTGTCAAAAGAAGAC e1asR:GTGCTATCCCTTAGTTAATTAAATT	*e1-as*: 1403 *e1-nl*: – *E1*: –	60	Own developed
E1	E1F: GGGAGCAGTGTCAAAAGAAGAG E1R: GTGCTATCCCTTAGTTAATTAAATA	*E1*: 1403 *e1-as*: – *e1-nl*: –	60	
*E2***	E2F:GAAGCCCATCAGAGGCATGTCTTATT E2R: AAGCCTATGCCAGCTAGGTATTT	*E2*: 130 *e2*: 107+23	55	Watanabe et al., 2011
E3	E3F: TGGAGGGTATTGGATGATGC E3R1: CTAAGTCCGCCTCTGGTTTCAG E3R2: CGGTCAAGAGCCAACATGAG E3R3:GTCCTATACAATTCTTTACGACG	*E3-Mi*: 1339 *E3-Ha*: 558 *e3-tr*: 275	58	Watanabe et al., 2009
*E3/e3-fs**	E3fsF:GGGATAGTTCTGATGCTGTTCAA E3fsR:CCTTGTATCGATAGCATATGTGCT	*E3*: 758 *e3-fs*: 759	55	Xu et al., 2013
E4	E4F: AGACGTAGTGCTAGGGCTAT E4R1: GCATCTCGCATCACCAGATCA E4R2: GCTCATCCCTTCGAATTCAG	*E4*: 1229 *e4-SORE-1*: 837	58	Liu et al., 2008
E9 (*GmFT2a*)	E9F1:GCTCTCTCTCTTCCACTCTCTAGATGG E9F2: ACCCTCTCAAGTGGACATGT E9R: CTAGGTGCATCGGGATCAAC	*E9*: 440 *e9*: 307	60	Zhao et al., 2016
GmFT5a-H1/ *GmFT5a-H4**	FT5aF: GCATGGTTCATACATACTACAGGG FT5aR: AACTCAGTTGCGTACACATGCTG	*GmFT5a-H1*: 379 *GmFT5a-H4*: 330	60	Own developed

* Combinations used for sequencing.
** CAPS marker with *Dra* I restriction enzyme.

Comparison of the obtained sequences with those available from the NCBI database was performed using the BLASTN program (https://blast.ncbi.nlm.nih.gov/). Multiple alignment of DNA sequences was performed using the CLUSTAL Omega software (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Results

Previously, a number of studies carried out a detailed analysis of the structural organization of genes that determine the maturity time in soybean, including *E*-genes, as well as *GmFT* family genes (Liu et al., 2008; Xu et al., 2013; Jiang et al., 2014, 2019; Tsubokura et al., 2014). Molecular markers (PCR, CAPS markers) have been developed to identify different alleles of these genes, including the dominant alleles *E1–E4* for photoperiod sensitive plants and recessive alleles that cause insensitivity to the photoperiod and reduce the maturity time. In this work, we tested these markers on a set of soybean cultivars approved for use in Russia to create a panel of molecular markers. This panel will allow for accelerated screening of cultivars based on sensitivity to photoperiod and genotyping for all the indicated genes.

To analyze the *E1* gene, we initially used a combination of primers E1F1/E1R1 common for dominant and recessive alleles and flanking a region of the coding sequence (see Table 2). This region contains SNPs specific for two common *E1* recessive alleles: *e1-fs* and *e1-as* (Xia et al., 2012). As a result of PCR, a major 547 bp product was detected in 6 cultivars, while no PCR product was detected in the other 4 cultivars (result not shown). Then, we analyzed the nucleotide sequence of the obtained PCR product in 6 cultivars. Sequencing showed the presence of the *e1-as* allele in 5 cultivars and the *E1* allele in the 'Persona' cultivar. The recessive allele *e1-as* is characterized by a nucleotide substitution G→C in comparison with the dominant allele *E1* (Fig. 1). Based on the known sequences of the *E1* gene from the databases, we developed the allele-specific primers e1asF/e1asR, which allow us to identify the *e1-as* allele by the presence of a PCR product of 1403 bp (see Table 2). Figure 2, *a* shows the result of PCR with these primers. The next pair of primers (E1F/E1R) for the same region of the gene, specific for the dominant allele *E1*, gave an amplification only in the 'Persona' cultivar, which can be used as a control of *E1* (see Fig. 2, *b*). The absence of PCR products with all primers to different regions of the *E1* gene in cultivars 'Cassidi', 'SibNIIK-9', 'SibNIIK-315', 'Gorinskaya' can be explained by gene deletion, and this indicates the presence of the *e1-nl* allele, established by Xia et al. (2012).

We genotyped the *E2* gene in cultivars using CAPS marker (see Table 2). The 130 bp PCR product of the dominant allele is not digested by endonuclease *Dra* I. The recessive allele *e2*

```
e1-as (AB552966) GTCAAAAGAAGACGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
Belgorodskaya 48 GTCAAAAGAAGACGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
Annushka        GTCAAAAGAAGACGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
Chera-1         GTCAAAAGAAGACGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
Malaga          GTCAAAAGAAGACGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
E1 (AB552962)   GTCAAAAGAAGAGGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
Persona         GTCAAAAGAAGAGGAAATCCACCATATGCGAAGCCTCTAACTTTAGGACATCAAGGAGAA
                ************ **********************************************
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Fig. 1. Multiple alignment of the *E1* gene region containing an SNP characteristic of the recessive allele *e1-as.*

E1 and *e1-as* – *G. max E1* gene sequences of the 'Harosoy' cultivar (AB552962 and AB552966, respectively).

has a *Dra* I restriction site due to the A→T nucleotide substitution. The hydrolysis of the PCR product produces two DNA fragments 27 and 103 bp long. Figure 3 shows the presence of the recessive allele *e2* in all studied cultivars.

The *E3* gene has the most common recessive allele *e3-tr*, which is characterized by a deletion of 13 kb after the third exon (Watanabe et al., 2009). The dominant alleles *E3-Mi* and *E3-Ha* have the same effect on the phenotype, but the last allele is distinguished by the insertion of a retrotransposon into the third intron. A molecular marker for this gene allows the simultaneous identification of both the dominant and recessive allele of the *E3* gene (see Table 2). This marker revealed a 275 bp product characteristic of the recessive allele in the cultivars 'Annushka', 'Bara', 'Persona' and 'Malaga' and in one plant of the 'Gorinskaya' cultivar (Fig. 4). The rest of the samples had a PCR product corresponding to the dominant allele *E3-Ha* (see Fig. 4).

In addition to the 13 kb deletion for *E3*, other mutations lead to the formation of recessive alleles. Among them, the most common allele is *e3-fs* with the insertion of a T nucleotide in the first exon, leading to a frame-shift and the formation of a non-functional protein (Xu et al., 2013). We checked this mutation in all cultivars with *E3-Ha* alleles (see above) by sequencing a 759/758 bp PCR product obtained with primers E3fsF/E3fsR (see Table 2, PCR result not presented). It turned out that cultivars 'SibNIIK-9', 'SibNIIK-315', 'Gorinskaya', 'Chera-1' are carriers of the allele *e3-fs*, and cultivars 'Kassidi', 'Belgorodskaya 48' have a sequence corresponding to the dominant allele *E3-Ha* (Fig. 5).

There are several recessive alleles of the *E4* gene; the most common allele is *e4-SORE-1*, the result of the insertion of a 6,238 bp *Ty*1/*copia*-retrotransposon in the first exon (Liu et al., 2008). The molecular marker for this gene allows to identify simultaneously the dominant and recessive *E4* alleles by the presence of PCR products 1229 bp and 837 bp long, respectively (see Table 2). Using this marker, we identified the dominant allele in cultivars 'Cassidi', 'Chera-1', 'Malaga' and 'Persona', while the other cultivars have a recessive allele (Fig. 6).

Previously, molecular markers were developed for the flowering activator genes: *GmFT2a*, or the *E9* gene (Zhao et al.,

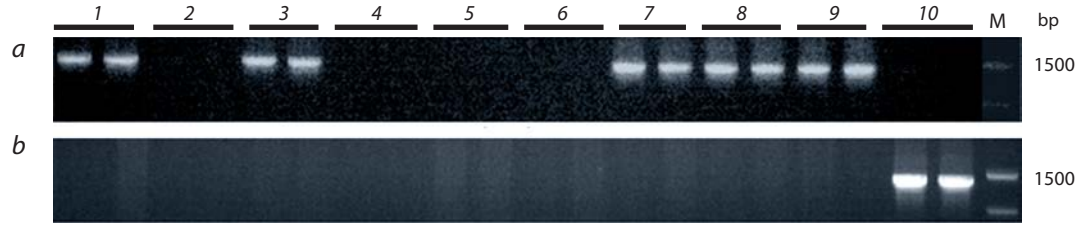

Fig. 2. Results of PCR obtained using primers e1-asF/e1-asR (*a*) and E1F/E1R (*b*).

Hereinafter: *1* – Annushka, *2* – Cassidi, *3* – Belgorodskaya 48, *4* – SibNIIK-9, *5* – SibNIIK-315, *6* – Gorinskaya, *7* – Chera-1, *8* – Bara, *9* – Malaga, *10* – Persona; M – "100+ bp DNA ladder". Two individual plants were analyzed for each cultivar.

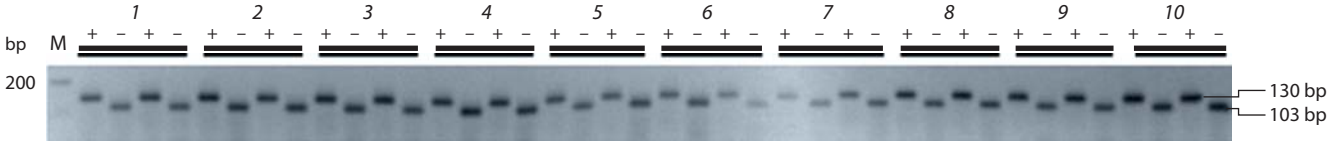

Fig. 3. Electrophoregram of CAPS marker of the *E2* gene.

Each plant sample is represented by a PCR product before (+) and after (–) restriction digestion.

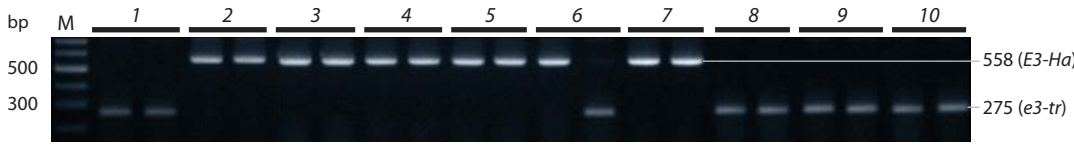

Fig. 4. Electrophoregram of PCR products of the *E3* gene.

The products of 558 and 275 bp long correspond to the dominant *E3-Ha* and recessive *e3-tr* alleles, respectively.

```
E3 (AB797201)      AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTC-TTGTGTGATATGCT
Belgorodskaya 48   AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTC-TTGTGTGATATGCT
Cassidi            AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTC-TTGTGTGATATGCT
e3-fs (AB766210)   AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTCTTTGTGTGATATGCT
Chera-1            AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTCTTTGTGTGATATGCT
Gorinskaya         AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTCTTTGTGTGATATGCT
SibNIIK-315        AGATTATTGAGAAGAACATCCTGCAAACTCAAACACTCTTTGTGTGATATGCT
                   ************************************ **************
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Fig. 5. Multiple alignment of the first exon region of the *E3* gene containing the insertion of T, leading to a frameshift mutation.

The reference sequences of *E3* and *e3-fs* alleles: AB797201 and AB766210, respectively.

2016) and *GmFT5a* (Takeshima et al., 2016). The recessive allele *e9* delays flowering due to lower gene expression caused by the insertion of the *SORE-1* retrotransposon into the first intron (Zhao et al., 2016). The marker (see Table 2) allows determining the dominant and recessive allele *GmFT2a*, by the presence of PCR products 440 and 307 bp long, respectively. Using this marker, we identified a 440 bp PCR product characteristic of the dominant allele *GmFT2a* in all analyzed samples (Fig. 7).

The *GmFT5a* gene has a dominant allele, *GmFT5a-H4*, which reduces the maturity time and differs from the recessive allele by a 49 bp deletion in 3′-UTR (Takeshima et al., 2016; Jiang et al., 2019). To identify both *GmFT5a* alleles, we used a combination of primers FT5aF/FT5aR flanking the deletion site (see Table 2). A 330 bp PCR product corresponding to the dominant allele was detected in only one cultivar – 'Cassidi'; the other cultivars had a 379 bp PCR product corresponding to the recessive allele (Fig. 8). We carried out sequencing of the PCR product in cultivars 'Cassidi' and 'Belgorodskaya 48' in order to search for the presence of different *GmFT5a* alleles. According to the sequencing result, the 'Cassidi' cultivar contained the *GmFT5a-H4* allele (result not shown).

Discussion

The high adaptation potential of soybean makes it possible to cultivate it outside the primary cultivation area – in a wide range of climatic conditions, including high-latitude regions with a temperate climate (Jia et al., 2014; Jiang et al., 2014). Soybean adaptation is achieved by the interaction of alleles of genes that control the date of flowering and maturity, depending on the length of the photoperiod (Saindon et al., 1989; Watanabe et al., 2012).

The maturity time of soybeans is 75 to 170 days. Depending on the maturity time, soybean cultivars are subdivided into: ultra-early maturing – less than 80 days; very early maturing – 81–90 days; early maturing – 91–110 days; medium early maturing – 111–120 days; medium maturing – 120–130 days; medium late maturing – 131–150 days; late maturing – 151–160 days; very late maturing – 161–170 days (Korsakov, 1973). In Russia, soybean is cultivated in the Far East, in the Central, Southern and Siberian regions. Each growing region is characterized by specific conditions of the climate; therefore,

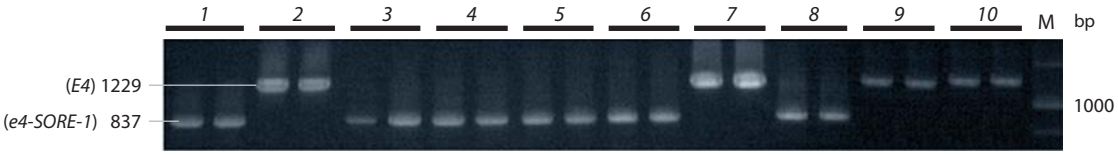

Fig. 6. Electrophoregram of PCR products of the *E4* gene.

The products 1229 and 837 bp long correspond to the dominant *E4* and recessive *e4-SORE-1* alleles, respectively.

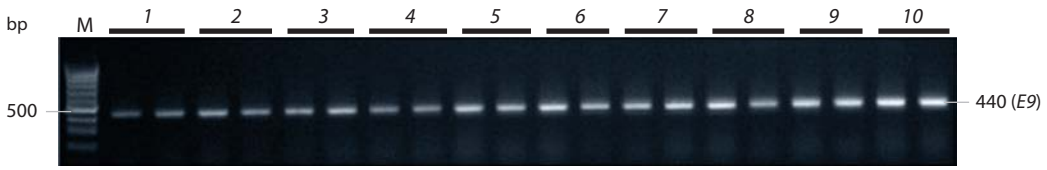

Fig. 7. Electrophoregram of PCR product of the *GmFT2a* gene.

The 440 bp product corresponds to the dominant allele of this gene.

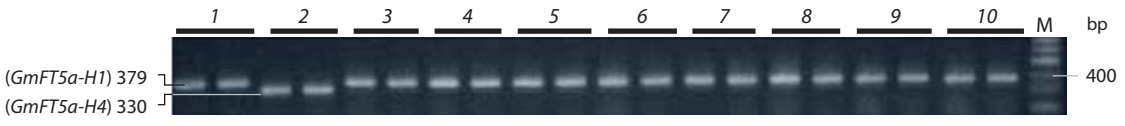

Fig. 8. Electrophoregram of PCR products of the *GmFT5a* gene obtained with primers GmFT5af2/r2.

The products of 379 and 330 bp long correspond to the recessive *GmFT5a-H1* and dominant *GmFT5a-H4* alleles, respectively.

it becomes necessary to select cultivars specifically adapted to a particular region using effective methods of marker-assisted selection. To demonstrate this possibility and create a working panel of DNA markers, we tested the previously developed combinations of primers for the main genes of photoperiod response: *E1–E4* and flowering activators *GmFT* (Takeshima et al., 2016; Wu et al., 2017). For this purpose, we used a set of 10 cultivars, differing in times of maturity: from the ultra-early maturing cultivar 'Annushka' to the medium early maturing cultivar 'Cassidi' (average maturity time – 80 and 115 days, respectively). The established genotypes of these cultivars for all studied genes are presented in Table 1. In total, 5 combinations of alleles for the *E1–E4* genes were identified: (1) *e1-nl(e1-as)/e2-ns/e3-tr(e3-fs)/e4*; (2) *e1-as/e2-ns/e3-tr/E4*; (3) *e1-as/e2-ns/E3-Ha/e4*; (4) *E1/e2-ns/e3-tr/E4*; (5) *e1-nl/e2-ns/E3-Ha/E4*.

All analyzed cultivars contained the most common, dominant and recessive alleles of the *GmFT2a* and *GmFT5a* genes, with the exception of the 'Cassidi' cultivar, which had a rare dominant allele *GmFT5a-H4*. The first combination *E1–E4* was found in two ultra-early-maturing cultivars and three early-maturing cultivars close to them in terms of maturity time. This genotype is characterized by the presence of recessive alleles for each of the *E1–E4* genes. The second combination with one dominant *E4* gene is present in cultivars 'Chera-1' and 'Malaga' (maturity time: 105 and 112 days, respectively). The third combination with one dominant *E3-Ha* gene was found in the 'Belgorodskaya 48' cultivar (108 days). The fourth combination includes the dominant genes *E1* and *E4*, found in the 'Persona' cultivar with a maturity time of 106 days. The medium early maturing cultivar 'Cassidi' contains the fifth combination with two dominant genes *E3-Ha* and *E4* and has the longest maturity time in this sample of cultivars. This cultivar has the *GmFT5a-H4* allele, which, according to Jiang et al. (2019), may influence the length of the maturity time. We have shown the predominant association of the genotype containing the recessive alleles of the *E1–E4* genes with a group of ultra-early maturing and very early maturing cultivars, while cultivars with a later maturity time have one or two dominant alleles for the *E1, E3,* or *E4* genes (see Table 1).

The established genotypes with a predominance of recessive alleles for the main genes of the photoperiod are typical for most cultivars from the northern regions of China (Jiang et al., 2014) and Japan (Xu et al., 2013). Thus, in the first work, it was found that the sensitivity to the photoperiod and the maturity time decrease with the accumulation of recessive alleles *E1–E4*. The cultivars with the genotype *e1*/*e2*/*e3*/*e4* have the least sensitivity to photoperiod and are common in the northern latitudes of China. These cultivars belong to the MG000 maturity group of very early cultivars according to the international classification and correspond to ultra-early maturing and very early maturing cultivars according to our domestic classification. The MG00 and MG0 maturity groups of early and medium early cultivars have genotypes with one or two dominant genes, mainly *E3* and *E4* on the background of recessive alleles *e1* and *e2*. These maturity groups have a maturity time of 91–110 and 111–120 days, respectively, which corresponds to our early and medium early maturing cultivars. Finally, MGI–MGIV maturity groups usually have genotypes with three or four dominant alleles: *E1*/*e2*/*E3*/*E4*, *e1*/*E2*/*E3*/*E4*, or *E1*/*E2*/*E3*/*E4*. These genotypes are common in the middle and southern regions of China, whose climatic conditions favor later maturation (Jiang et al., 2014). Thus, the analyzed cultivars have a maturity group MG000–MG0 and a genotype for genes *E1–E4* similar to varieties from the northern regions of Southeast Asia, which are closest to the territory of the Far East – the region of primary soybean cultivation in our country. Soybean germplasm from this region has spread to the Southwestern part of Russia, Siberia and other regions.

Alleles *E1–E4* have a different effect on sensitivity to photoperiod and maturity. Previous research shows that the *E1* and *E2* genes have a greater influence on the development prior to flowering. The loci *E3* and *E4* affect not only the previous, but also the subsequent phases of flowering and maturation (Xu et al., 2013; Jiang et al., 2014). Consequently, the last loci are more important in breeding for productivity. Of these genes, the *E4* gene has the greatest effect on light sensitivity, the recessive form of which is quite widespread in northern latitudes, which is also confirmed by our data. Of the first two genes, the *E1* gene presumably plays a key role in photoperiod-induced flowering (Xia et al., 2012). This is confirmed by the data of comparing the genotypes *E1*/*e2*/*E3*/*E4* and *e1*/*E2*/*E3*/*E4*, which showed a more significant decrease in photoperiod response in the genotype with *e1* (Jiang et al., 2014). Almost all cultivars studied by us, with the exception of the 'Persona' cultivar, contain non-functional alleles *e1-as* and *e1-nl*, which, apparently, make the main contribution to the shortening of the maturity time. The recessive allele *e2* was found in all studied cultivars. Our result is consistent with the data from the Amur region, which showed the presence of the dominant allele *E2* in only one cultivar out of 18 (Jia et al., 2014).

The genes of the *GmFT* family are flowering activators, and their transcription negatively correlates with the expression of the flowering repressor *E1* (Xia et al., 2012). The most important genes of this family are genes *GmFT2a* and *GmFT5a* (Takeshima et al., 2016). Despite the fact that the *GmFT2a* gene showed different transcriptional profiles under different environmental conditions and in individual cultivars differing in sensitivity to photoperiod, nevertheless, its polymorphism was not associated with the maturity time (Jiang et al., 2013). In some cultivars, the insertion of the *SORE-1* retrotransposon in the first intron of *GmFT2a* was identified, which suppressed the transcription of this gene and led to a delay in flowering (Zhao et al., 2016). Using the marker flanking the insertion (see Table 2), we established the intact form of the *GmFT2a* gene in all analyzed cultivars.

A 49 bp deletion in the 3′-UTR of the *GmFT5a* gene was found in a number of foreign cultivars of the MG000 and MG00 maturity groups (these groups also include the cultivars we analyzed), which reduces the flowering time relative to cultivars with a recessive allele of the gene (Takeshima et al., 2016; Jiang et al., 2019). We developed primers that amplify the site of the deletion, and using PCR and subsequent sequencing of the PCR product we showed the presence of this deletion in the 'Cassidi' cultivar (see Fig. 8). In addition

to the indicated dominant allele *GmFT5a*, potentially shortening the flowering time, this cultivar contains two dominant alleles *E3* and *E4*, which can have the opposite effect on the maturity time. However, the mechanism of interaction of these genes and their combined effect on the maturity time is yet to be clarified.

Conclusion

In this work, using the material of soybean cultivars cultivated in Russia in the regions of Western Siberia and Belgorod region, we for the first time tested molecular markers for various alleles of the *E1–E4*, *GmFT* genes, which are responsible for sensitivity to photoperiod and the maturity time. Cultivars from these regions have a shorter maturity time and low sensitivity to photoperiod. These features correlate with the number of recessive alleles of the *E1–E4* genes, so the cultivars with the shortest maturity time (ultra-early maturing) predominantly have the *e1-nl*(*e1-as*)/*e2-ns*/*e3-tr*(*e3-fs*)/*e4* genotype. The cultivars with a later maturity (early maturing and medium early maturing) have a genotype with one or two dominant alleles, mainly for the *E3* and *E4* genes. Our result of genotyping 10 soybean cultivars is consistent with the data of foreign authors obtained on a wide set of cultivars from the geographical regions of Japan and North China, close in climatic conditions to the Far East – the region of primary soybean cultivation in our country. Thus, the tested set of molecular markers can be used for breeding the domestic soybean cultivars based on sensitivity to photoperiod and maturity time, on which the productivity of soybean largely depends, especially in a temperate climate atypical for its cultivation.

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ORCID ID
A.B. Shcherban orcid.org/0000-0003-1000-8228
E.A. Salina orcid.org/0000-0001-8590-847X

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Using the synthetic form RS5 to obtain new introgressive lines of common wheat

R.O. Davoyan[1] ✉, I.V. Bebyakina[1], E.R. Davoyan[1], Y.S. Zubanova[1], D.M. Boldakov[1], D.S. Mikov[1], V.A. Bibishev[1], A.N. Zinchenko[1], E.D. Badaeva[2]

[1] National Center of Grain named after P.P. Lukyanenko, Krasnodar, Russia
[2] Vavilov Institute of General Genetics of the Russian Academy of Sciences, Moscow, Russia
✉ davoyanro@mail.ru

Abstract. The use of the gene pool of wild relatives, which have a significant reserve of genetic diversity, is of immediate interest for breeding common wheat. The creation and use of synthetic forms as "bridges" is an effective method of transferring valuable genetic material from wild relatives to cultivated wheat. For this purpose, genome addition, genome substitution and recombinant "secondary" synthetic forms have been created in the P.P. Lukyanenko National Center of Grain. The synthetic recombination form RS5 (BBAASD^t), in which the third genome consists of chromosomes of *Aegilops speltoides* (S) and *Aegilops tauschii* (D^t), was obtained from crossing the synthetic forms Avrodes (BBAASS) and M.it./*Ae. tauschii* (BBAAD^{t}D^t), in which the D genome from *Ae. tauschii* was added to the BBAA genomes of the durum wheat cultivar Mutico italicum. Introgression lines resistant to leaf rust, yellow rust and powdery mildew have been obtained from backcrosses with the susceptible common wheat cultivars Krasnodarskaya 99, Rostislav and Zhirovka. Twelve resistant lines that additionally have high technological characteristics of grain and flour have been selected. The cytological study (C-banding) has revealed chromosomal modifications in 6 of 8 lines under study. The rearrangements mainly affected the chromosomes of the D genome, 1D, 3D, 4D, 6D and 7D. It was found that in most cases the genetic material from the synthetic form RS5 in the studied lines was represented by substituted chromosomes from *Ae. tauschii*. In line 5791p17, the substitution of chromosomes 6D from *Ae. tauschii* and 7D from *Ae. speltoides* was revealed. Substitutions 4D(4D^t), 6D(6D^t) from *Ae. tauschii* and 7D(7S) from *Ae. speltoides* were obtained for the first time. Molecular analysis of 12 lines did not reveal effective leaf rust resistance genes, presumably present in synthetic forms of M.it./*Ae. tauschii* and Avrodes. It is assumed that the lines may carry previously unidentified genes for fungal disease resistance, in particular for resistance to leaf rust, from *Ae. tauschii* and *Ae. speltoides*.
Key words: common wheat; synthetic forms; disease resistance; protein; gluten; cytological analysis; C-banding; substituted chromosomes; translocations.

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Использование синтетической формы RS5 для получения новых интрогрессивных линий мягкой пшеницы

Р.О. Давоян[1] ✉, И.В. Бебякина[1], Э.Р. Давоян[1], Ю.С. Зубанова[1], Д.М. Болдаков[1], Д.С. Миков[1], В.А. Бибишев[1], А.Н. Зинченко[1], Е.Д. Бадаева[2]

[1] Национальный центр зерна им. П.П. Лукьяненко, Краснодар, Россия
[2] Институт общей генетики им. Н.И. Вавилова Российской академии наук, Москва, Россия
✉ davoyanro@mail.ru

Аннотация. Актуальной задачей селекции мягкой пшеницы является вовлечение генофонда диких сородичей, обладающих значительным запасом генетического разнообразия. Эффективный метод передачи ценного генетического материала от диких сородичей в культурную пшеницу – создание и использование в качестве «мостиков» синтетических форм. С этой целью в Национальном центре зерна им. П.П. Лукьяненко созданы геномно-замещенные, геномно-добавленные и рекомбинантные «вторичные» синтетические формы. Синтетическая форма RS5 (BBAASD^t), у которой третий геном состоит из хромосом *Aegilops speltoides* (S) и *Aegilops tauschii* (D^t), была получена от скрещивания синтетических форм Авродес (BBAASS) и M.it./*Ae. tauschii* (BBAAD^{t}D^t), у которой геном D от *Ae. tauschii* был добавлен к геномам BBAA твердой пшеницы Mutico italicum. От беккроссов с восприимчивыми к листовой ржавчине, желтой ржавчине и мучнистой росе сортами мягкой пшеницы Краснодарская 99, Ростислав и Жировка были получены устойчивые к этим болезням интрогрессивные линии. Отобраны 12 линий, которые наряду с устойчивостью к болезням имеют высокие технологические характеристики зерна и муки. Цитологический анализ (C-banding) выявил хромосомные перестройки у шести из восьми исследуемых линий. Перестройки в основном затронули хромосомы

генома D – 1D, 3D, 4D, 6D и 7D. Установлено, что генетический материал от синтетической формы RS5 в изученных линиях в большинстве случаев представлен в виде замещенных хромосом от *Ae. tauschii*. В линии 5791п17 обнаружено замещение хромосом 6D от *Ae. tauschii* и 7D от *Ae. speltoides*. Хромосомные замещения 4D(4D^t), 6D(6D^t) от *Ae. tauschii* и 7D(7S) от *Ae. speltoides* получены впервые. Молекулярный анализ 12 линий не выявил у них эффективных генов устойчивости к листовой ржавчине, предположительно присутствующих в синтетических формах M.it./*Ae. tauschii* и Авродес. Сделано предположение, что линии могут нести не идентифицированные ранее гены устойчивости к грибным болезням, в частности к листовой ржавчине, от видов *Ae. tauschii* и *Ae. speltoides*.
Ключевые слова: мягкая пшеница; синтетические формы; устойчивость к болезням; белок; клейковина; цитологический анализ; C-banding; замещенные хромосомы; транслокации.

Introduction

Common wheat (*Triticum aestivum* L.) is one of the main food crops. The constantly growing need to increase its productivity against the background of global climate changes requires further intensification of the breeding process. One of the main conditions for this is the presence of sufficient genetic diversity and, in particular, disease resistance genes. An actual and effective way to expand the genetic diversity of common wheat is to use its numerous related wild and cultivated species as sources of valuable breeding traits (Rasheed et al., 2018). It should be noted that almost all effective diseases resistance genes of common wheat originate from the gene pool of its wild relatives (McIntosh et al., 2015).

One of the most effective methods of transferring valuable genetic material from wild relatives to common wheat is the creation and use of synthetic forms as "bridges". An original approach was developed at the P.P. Lukyanenko National Center of Grain, which made it possible to create genome substituted, genome added and recombinant "secondary" synthetic forms (Zhirov, Ternovskaya, 1984; Davoyan R.O. et al., 2012). The genome substitution form of Avrodes (BBAASS) was used to create recombinant synthetic forms (RS-forms), in which, against the background of BA genomes, the third genome was recombinant and simultaneously consisted of two different wild species genomes (Davoyan E.R. et al., 2012). This form, due to the presence of the S genome from *Ae. speltoides*, has the ability to promote homoeologous pairing of chromosomes (Tsatsenco et al., 1993), which should have contributed to the production of new translocations and recombinations between chromosomes of different species.

The aim of the study was to use a synthetic form of RS5 (BBAASD^t), in which the third genome consists of *Aegilops speltoides* (S) and *Ae. tauschii* (D^t) chromosomes, to obtain new introgression lines of common wheat. This paper presents the results of cytological and molecular analysis, evaluation of resistance to fungal diseases, productivity components, technological qualities of grain and flour of common wheat introgression lines obtained using this synthetic form.

Materials and methods

Introgression lines of common wheat (BC_2F_6–BC_3F_5) obtained with the participation of a synthetic form of RS5 made up the material for this study. Common wheat varieties Krasnodarskaya 99 (lines 4942p17, 5038p17, 5658p19, 5714p18, 5766p19, 5791p17, 5845p18), Rostislav (lines 5001p17, 5656p19) and Zhirovka (lines 5725p18, 5733p19, 5785p18), susceptible to leaf rust, yellow rust and powdery mildew, were used as recipient varieties. The Zhirovka variety has a translocation of 5BS.5BL-5GL, obtained from the species *T. militinae* through the synthetic form *T. miguschovae*. Translocation 1RS.1BL from rye was detected in variety Rostislav.

The study of chromosome pairing in metaphase I of meiosis was carried out in maternal pollen cells on pressed preparations stained with acetic acid hematoxylin according to the generally accepted method (Pausheva, 1974). The number of cells studied in the lines ranged from 169 to 248.

The assessment of resistance to leaf and yellow rust was carried out at the stage of adult plants in the field, against the background of artificial infection. To assess the resistance to yellow rust, the Gassner and Straib scale was used (Gasner, Straib, 1934). Resistance to leaf rust was determined according to the Mains and Jakson scale (Mains, Jakson, 1926). Plants with reaction type 0 (immune), 1 (highly resistant) and 2 (moderately resistant) were classified as resistant. The resistance of plants with an intermediate type of reaction from 0 to 1 (single very small pustules with necrosis) was indicated by a score of 01. Plants with reaction type 3–4 were considered susceptible. Resistance to powdery mildew was evaluated on a natural infectious background according to the Geschele scale (Peresipkin, 1979). Plants with a degree of powdery mildew damage of 0–20 % were classified as resistant.

DNA extraction was carried out using the Plaschke et al. method (Plaschke et al., 1995). To identify the *Lr* genes, primers marking the *Lr28*, *Lr35*, *Lr39* and *Lr51* genes were used – CS421570-R, CS421570-L; BCD260F1, 35R2; GDM35-L, GDM35-R; S30-13L, AGA7-759R, respectively (Seyfarth et al., 1999; Singh et al., 2004; Cherukuri et al., 2005; Helguera et al., 2005). The PCR reaction was performed according to the conditions recommended by the authors. Electrophoresis of the PCR fragments was carried out similarly to those previously described (Davoyan E.R. et al., 2018).

Differential staining of chromosomes (C-banding) was performed at the Vavilov Institute of General Genetics according to the method developed by Badaeva and co-authors (Badaeva et al., 1994).

Technological quality of grain and flour was studied at the department of grain technology and biochemistry, P.P. Lukyanenko National Center of Grain, according to the Methods of State Crop Variety Trial (1988). Statistical processing of the obtained results was carried out using the AGROS-2.10 program.

Results

The synthetic form RS5 showed high resistance to leaf and yellow rust and moderate resistance to powdery mildew, while having very low fertility. To transfer resistance and restore fertility, this form was crossed with susceptible to these diseases common wheat varieties Krasnodarskaya 99,

Rostislav and Zhirovka. The first generation of hybrid plants was partially fertile and showed resistance to a complex of wheat diseases. Depending on the level of fertility of these plants, backcrossing with common wheat was performed from 1 to 3 times, but in most cases two backcrosses were sufficient to restore it. The plants obtained from backcrosses had from 40 to 42 chromosomes. The results of the cytological study of chromosomal associations in metaphase I of meiosis are shown in Table 1.

In general, the percentage of plants with multivalents did not differ by crossing combinations.

A large number of multivalents (75 %) was observed in F_1 plants obtained from crossing the recombinant RS5 form with common wheat, which is explained by the direct influence of the S genome chromosomes, which are a part of the recombinant sterile form, on the pairing of different genomes chromosomes. Further, along with the increasing number of backcrosses, which were also carried out in order to overcome the low fertility of F_1 hybrid plants, the number of plants with multivalents significantly decreases (up to 9 %). Examples of chromosome pairing in metaphase I of meiosis in hybrid plants are shown in Fig. 1.

The selection of plants for fertility and disease resistance, self-pollination contributed to the meiosis stabilization and necessary signs consolidation. As a result of the plants selection by the chromosomes number close to common wheat (42), 82 lines have now been obtained from the population of hybrid plants obtained on the basis of RS5 synthetics. This article presents the results of studying 12 lines that are closest to the recipient varieties according to the phenotype.

When using the RS5 form, the main purpose was the transmission of common wheat disease resistance. In this regard, an assessment of lines was fulfilled for the most common and harmful diseases – leaf rust (*Puccinia triticina* Eriks.), yellow rust (*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*). Characterization of introgression lines RS5 × *T. aestivum* for disease resistance for 2019–2021 is given in Table 2.

Eleven lines were resistant to leaf rust. Eight lines showed high resistance with reaction type 01 and 1: 4942p17, 5656p19, 5733p19, 5766p19, 5714p18, 5725p18, 5785p18 and 5845p18. The line 5001p17 was susceptible. The remaining lines had moderate resistance to this disease.

Resistance to yellow rust was carried by all 12 lines, 4 of which, 5656p19, 5725p18, 5791p17 and 5845p18 have the type of reaction to infection 01 and 1.

Resistance to powdery mildew was shown by 10 lines, with the exception of lines 5038p17 and 5785p18.

Of particular value for breeding are lines that are resistant to a complex of diseases. Three lines, 5001p17, 5038p17 and 5785p18, had group resistance to two and nine lines to all three diseases. The 5845p18 line had high resistance to all three diseases. The diversity of disease resistance lines may indicate different introgressions of foreign genetic material into the genome of common wheat.

In order to determine the form of the transferred material from the synthetic RS5 form, the studied lines were crossed with one of the most meiotically stable varieties of common wheat Krasnodarskaya 99 and meiosis was studied in hybrid F_1 plants (Table 3).

Table 1. Results of the study of chromosome pairing in metaphase I of meiosis of generation F_1 and BC_1F_1–BC_3F_1

Generation	Total plants studied	Number of plants with multivalents
F_1	12	9 (75 %)
BC_1F_1	31	16 (52 %)
BC_1F_2–BC_2F_1	45	12 (27 %)
BC_2F_2–BC_3F_1	80	7 (9 %)

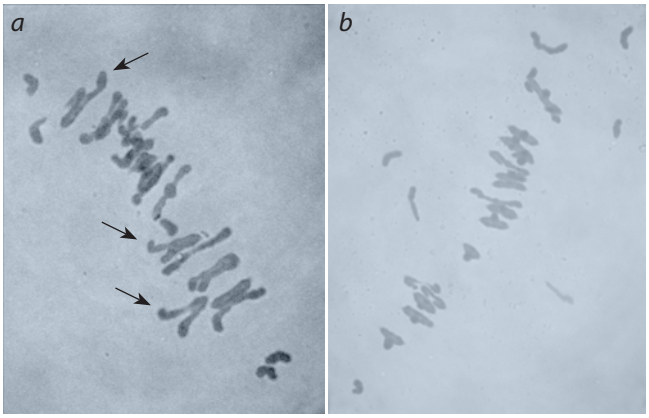

Fig. 1. Chromosome pairing in metaphase I of meiosis in RS5 × Krasnodarskaya 99 hybrid plants: *a*, BC_1 ($14^{II} + 4^{I} + 2^{III} + 1^{IV}$); *b*, BC_2 ($19^{II} + 4^{I}$). Multivalents are indicated by arrows.

Table 2. Disease resistance of introgression lines RS5 × *T. aestivum* for 2019–2021

Line, variety, synthetic	Resistance		
	to leaf rust (type of reaction)	to yellow rust (type of reaction)	to powdery mildew, %
4942p17	1	2	15
5001p17	3	2	20
5038p17	2	2	25
5656p19	01	01	20
5658p19	2	2	15
5733p19	1	2	20
5766p19	1	2	15
5714p18	01	2	20
5725p18	1	1	20
5785p18	1	2	25
5791p17	2	01	15
5845p18	01	1	10
Krasnodarskaya 99	4	3	25
Rostislav	4	4	30
Zhirovka	3	3	30
RS5	01	01	15

Table 3. Analysis of meiosis in metaphase I in maternal pollen cells F_1 hybrids obtained from crossing cytologically stable RS5 × *T. aestivum* lines with Krasnodarskaya 99

Plant material	Cells studied	21^{II}	$20^{II}+2^{I}$	$19^{II}+4^{I}$	Cells with multivalents
		%			
4942p17×K99*	214	77.4	16.6	4.7	1.3
5001p17×K99	185	80.7	10.3	6.4	2.6
5038p17×K99	190	67.4	32.6	–	–
5656p19×K99	237	80.5	15.4	3.3	1.8
5658p19×K99	248	65.4	30.4	4.2	–
5714p18×K99	185	48.7	38.4	10.7	2.2
5725p18×K99	210	56.2	29.5	12.4	1.9
5733p19×K99	317	68.6	19.2	9.8	2.4
5766p19×K99	262	67.2	23.7	8.4	1.7
5785p18×K99	247	77.4	12.8	7.5	2.3
5791p17×K99	169	44.7	43.1	12.2	–
5845p18×K99	223	58.3	36.8	4.9	–
Krasnodarskaya 99	112	91.0	6.3	2.7	–

* Hereinafter: K99 is a variety of wheat Krasnodarskaya 99.

The association of chromosomes of hybrid plants F_1 $20^{II}+2^{I}$ and $19^{II}+4^{I}$ may indicate the substitution of one or two pairs of wheat chromosomes with foreign ones. Such substitutions can occur in 4 lines out of 12 analyzed – 5038p17, 5658p19, 5791p17 and 5845p18. The hybrids of Krasnodarskaya 99 with the other lines have the presence of multivalents, which indicates that they can carry translocations from the RS5 synthetic, Rostislav and Zhirovka varieties. Hybrid plants of the lines 5714p18, 5725p18, 5733p19, 5766p19 along with multivalents form a significant number of cells (about 30 %) with the association of chromosomes $20^{II}+2^{I}$ and $19^{II}+4^{I}$. Probably, both translocations and substituted chromosomes may be present in these lines.

To identify the genetic material from the RS5 synthetic and changes in the genome of the obtained lines, the C-banding method was used. Of the eight analyzed lines, six revealed transfer from RS5 synthetics (Table 4).

Table 4. Results of the analysis RS5 × *T. aestivum* introgression lines by C-banding

Line	Identified translocations and substitutions
5656p19	T1BL.1RS
5658p19	2A(2A^t); 3D(3D^t)
5714p18	T1BL.1RS; T2AL?; del.3BS; 4D(4D^t)
5725p18	T1BL.1RS; T5BL.5GL; 4D(4D^t); 6D(6D^t)
5733p19	T5BS.5BL-5GL; 1D(1D^t); 6D(6D^t)
5785p18	T1BL.1RS; T5BL.5GL
5791p17	6D(6D^t); 7D(7S)
5845p18	1D(1D^t); 6D(6D^t)

The rearrangements mainly affected the chromosomes of the D genome. In most cases, the lines carry substituted chromosomes from *Ae. tauschii*. The most common rearrangements affect chromosomes 1D, 4D and 6D (Fig. 2).

Substitutions 2A(2A^t) and 3D(3D^t) were identified in line 5658p19. The line 5791p17 has a 6D chromosome substitution from *Ae. tauschii* and 7D from *Ae. speltoides*. It should be noted that introgression lines with chromosomal substitutions 4D(4D^t), 6D(6D^t) from *Ae. tauschii* and 7D(7S) from *Ae. speltoides* were obtained for the first time. Translocation T1BL.1RS from the recipient cultivar Rostislav was revealed in line 5656p19. Translocation T5BL.5GL obtained from the recipient cultivar Zhirovka is present in three lines – 5725p18, 5733p19 and 5785p18. The obtained introgressive lines are of particular interest as possible new disease resistance genes donors, in particular, to leaf rust, transferred from the species *Ae. tauschii* and *Ae. speltoides*. Currently, 5 resistance genes from *Ae. tauschii*: *Lr21*, *Lr22a*, *Lr32*, *Lr39*, *Lr42* and 6 resistance genes transmitted from *Ae. speltoides*: *Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51*, *Lr66* (McIntosh et al., 2015) are added to the catalog of wheat gene symbols. DNA markers were used to identify genes for resistance to leaf rust. Earlier (Davoyan E.R. et al., 2012, 2018), we analyzed the synthetic forms Avrodes and M.it./*Ae. tauschii* for the presence of effective leaf rust resistance genes *Lr28*, *Lr35*, *Lr47*, *Lr51* from *Ae. speltoides* and *Lr39* from *Ae. tauschii*. The resistance gene *Lr36* was not

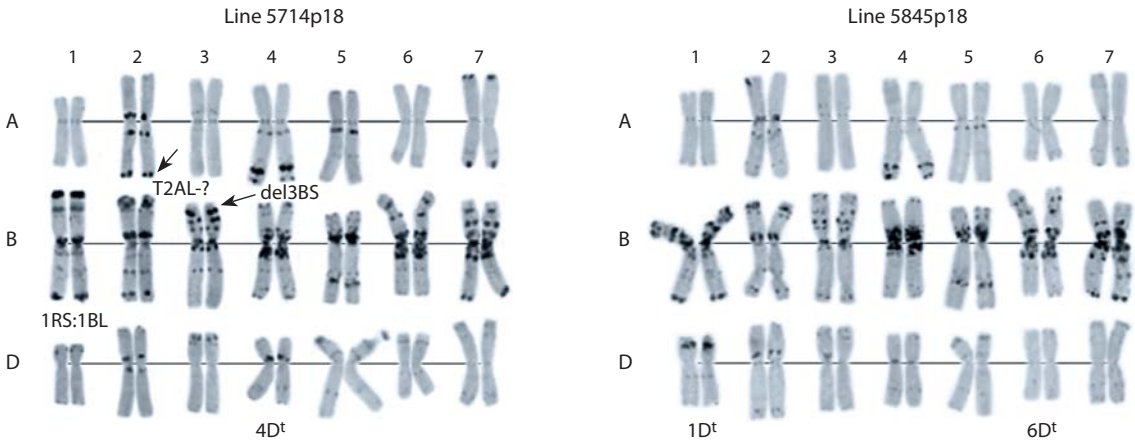

Fig. 2. Karyotypes of introgression lines 5714p18 and 5845p18 with genetic material of the recombinant synthetic form RS5.

Table 5. Technological characteristics of grain and flour RS5 × *T. aestivum* introgression lines of common wheat

Line	Protein content, %	Gluten content, %	Gluten deformation index, drop unit	Volume of bread, ml	Overall baking value, points
5656p19	18.6	36.8	88	950	4.7
5658p19	15.8	29.3	82	820	4.5
5684p18	17.1	33.6	75	850	4.5
5725p18	17.9	37.0	81	860	4.5
5733p19	16.4	31.6	68	850	4.8
5766p19	14.9	27.9	80	860	4.7
K99	14.4	26.0	65	800	4.6
LSD_{05}	0.3	1.4	2	19	–

included in the analysis due to the lack of an effective molecular marker for it. Identification of the *Lr66* gene was not performed at this stage. It was found that the synthetic form of Avrodes has only *Lr28*, *Lr35* and *Lr51* of the listed genes, and the synthetic form M.it/*Ae. tauschii* has the *Lr39* gene. Based on this, the obtained introgressive lines were analyzed only for the presence of effective leaf rust resistance genes *Lr28*, *Lr35*, *Lr39* and *Lr51*. The presence of the desired genes has not been established in any of the 12 lines.

To determine the prospects for involving the obtained lines in breeding practice, they were evaluated according to the technological qualities of grain and productivity components. This paper presents the results of six most phenotypically interesting lines evaluation of the 2019 harvest.

One of the most important agronomic traits, especially for lines carrying alien genetic material, is the technological characteristics of grain and flour. Alien introgression can significantly affect the technological qualities of grain and flour. The results of the analysis of lines for some technological parameters are presented in Table 5. The protein and gluten content of the lines largely depend on the conditions of the growing season. All studied lines exceeded the best recipient cultivar Krasnodarskaya 99 in terms of protein and gluten content. The lines 5656p19 and 5725p18 had the highest levels – 18.6 and 17.9 % protein, 36.8 and 37.0 % gluten, respectively. The protein and gluten content of the Krasnodarskaya 99 variety was 14.4 and 26 % (see Table 5).

Grain technological characteristics are determined by the protein and gluten content, as well as the qualitative indicators of gluten, which, in turn, determine such important characteristics as bread volume, crumb color, bread taste characteristics, etc. As a rule, the lines with alien genetic material have deterioration in the gluten quality. Thus, all the analyzed lines have high levels of gluten deformation index compared to the recipient variety Krasnodarskaya 99. However, the lines 5684p18 and 5733p19 had the gluten quality corresponding to group I according to State Standard, and the lines 5656p19, 5658p19, 5725p18 and 5766p19 had quality group II according to State Standard, which is a good indicator in general for introgressive lines. The volume output of bread in two lines 5658p19 and 5656p19 was 820 and 950 ml, respectively, exceeding the volume of bread of the recipient variety Krasnodarskaya 99 (800 ml). There were significant differences between the lines according to the indicator of the

Table 6. Yield components of RS5 × *T. aestivum* introgression lines

Line	Weight of 1000 grains, g	Number of spikes per 1 m², pieces	Grain weight per 1 m², g
5656p19	40.3	307.7	483.5
5658p19	43.2	238.7	532.7
5684p18	42.3	223.5	486.5
5725p18	40.7	329.2	466.0
5733p19	38.0	305.8	583.5
5766p19	43.9	284.3	600.3
K99	37.4	321.7	603.7
$LSD_{0.5}$	0.62	16.9	16.5

general baking assessment. Three lines: 5658p19, 5684p18 and 5725p18 (4.5 points) were inferior in this indicator to the recipient variety Krasnodarskaya 99 (4.6 points), and two lines, 5656p19 and 5766p19, having a score of 4.7 points, slightly exceeded the indicator of the Krasnodarskaya 99 variety. The line 5733p19 had the best baking rating out of all the lines – 4.8 points.

To study productivity, the following characteristics were used: the weight of 1000 grains, the weight of the grain, and the number of spikes per square meter (Table 6). The weight of 1000 grains in the lines varied from 38.0 (5733p19) to 43.9 g in line 5766p19, with an average value of the Krasnodarskaya 99 variety – 37.4 g. All lines, with the exception of 5733p19, significantly exceed the Krasnodarskaya 99 variety in this sign. Lines 5658p19, 5684p18 and 5766p19 form a smaller number of spikes per 1 m^2. In the other three lines, the differences from the Krasnodarskaya 99 variety were insignificant. The highest yield (600.3 g/m^2), comparable to the Krasnodarskaya 99 variety (603.7 g/m^2), had the line 5766p19. The other lines were significantly inferior to the Krasnodarskaya 99 variety.

Discussion

The creation and use of the synthetic form of RS5 was primarily associated with the possibility to transfer new introgressions from *Ae. tauschii* and *Ae. speltoides* to common wheat and, as a result, new disease resistance genes. Along with the selection of stable hybrid plants, their cytological study is important. The study of chromosome pairing in metaphase I of meiosis in RS5 × *T. aestivum* hybrid plants revealed a relatively large number of plants with multivalents in the early generations of F_1 and BC_1F_1 – 75 and 52 %, respectively. Such results are due to the ability of the synthetic form of Avrodes, obtained with the participation of *Ae. speltoides*, to cause homeologous pairing of chromosomes (Tsatsenco et al., 1993). A significant decrease in the number of plants with multivalents in subsequent generations of BC_2F_1–BC_3F_1 (9 %) may be associated with the stabilization of the number of chromosomes and their association in meiosis towards common wheat, as well as a decrease in the genetic material *Ae. speltoides* in them.

The 12 RS5 × *T. aestivum* lines of the BC_2F_6–BC_3F_5 generation selected for the study differed in resistance to leaf rust, yellow rust and powdery mildew. Lines with the types of reaction to leaf rust 01, 1 and 2, to yellow rust 01, 1 and 2, with a degree of powdery mildew damage of 10, 15 and 20 % were identified. The lines differ in their resistance to the complex of these diseases as well. The diversity of disease resistance lines may indicate different transfers of the RS5 genetic material in the genome of common wheat and the possible transfer of a new resistance gene(s).

Cytological analysis (C-banding) revealed chromosomal rearrangements in 6 out of 8 studied lines. The rearrangements mainly affected the chromosomes of the D genome – 1D, 3D, 4D, 6D, and 7D. In most cases, the genetic material from the synthetic RS5 form in the studied lines was found to be presented in the form of substituted chromosomes from *Ae. tauschii*. In one line – 5791p17 the substitution of chromosomes 6D from *Ae. tauschii* and 7D from *Ae. speltoides* was identified. It should be noted that chromosomal substitutions 4D(4D^t), 6D(6D^t) from *Ae. tauschii* and 7D(7S) from *Ae. speltoides* were obtained for the first time. Active participation in rearrangements of chromosomes of the D genome is explained by the fact that, firstly, *Ae. tauschii* is a donor of the D genome, secondly, in the synthetic form of Avrodes (BBAASS), the D genome of common wheat is replaced by the S genome from *Ae. speltoides*. In line 5656p19, translocation T1BL.1RS from the recipient cultivar Rostislav was revealed. At the same time, in contrast to the Rostislav variety, this line is resistant to leaf rust (01) and yellow rust (01) and has high levels of protein and gluten (18.6 and 35.8 %, respectively). Probably, the transfer of genetic material from RS5 of this line occurred through recombination, which is not detected by the C-banding method. The T5BL.5GL translocation obtained from the recipient cultivar Zhirovka was found in three lines – 5725p18, 5733p19, and 5785p18. Currently, this translocation does not provide resistance to leaf rust, yellow rust and powdery mildew.

The genes of resistance to leaf rust *Lr21*, *Lr22a*, *Lr32*, *Lr39*, *Lr42* from the species *Ae. tauschii* and *Lr28*, *Lr35*, *Lr36*, *Lr47*, *Lr51*, *Lr66*, *LrASP5* from *Ae. speltoides* were transferred to common wheat (Adonina et al., 2012; McIntosh et al., 2015). These genes were transferred from *Ae. tauschii* to the wheat chromosomes 1D, 2D, 3D, 2D and 1D, respectively; from *Ae. speltoides* – to 4A, 2B, 6B, 7A, 1B, 3A and 5B, respec-

tively (Friebe et al., 1996; Helguera al., 2000, 2005; Marais et al., 2010). Despite the rather large number of transferred genes, it is possible that other leaf rust resistance genes may be present in these species, which is also evidenced by the results obtained earlier (Davoyan R.O. et al., 2017).

Based on the marker analysis, it was previously assumed that the synthetic form of M.it/*Ae. tauschii* has *Lr39* of the listed genes, while Avrodes has only three: *Lr28*, *Lr35*, and *Lr51*. The desired genes were not detected in any of the 12 analyzed lines. Probably, these lines may have new leaf rust resistance genes derived from *Ae. tauschii* and *Ae. speltoides*.

Genetic material of wild relatives in introgression lines of common wheat, along with positive traits, can also carry undesirable ones, such as lengthening the growing period, deterioration of baking qualities, lodging tendency, decreased yield, etc. (Knott, 1989; Brevis et al., 2008; Timonova et al., 2012; Leonova, Budashkina, 2016).

The study of the 6 most interesting lines by phenotype revealed their diversity in productivity and technological characteristics of grain and flour. The studied lines exceeded the recipient cultivar Krasnodarskaya 99 in protein and gluten content. The lines 5656p19 and 5725p18 had the highest indices – 18.6 and 17.9 % protein, 36.8 and 37.0 % gluten, respectively. Despite the fact that all the analyzed lines have high levels of gluten deformation index compared to the Krasnodarskaya 99 variety, they form gluten corresponding to the first and second groups of state standard and have either an equal with Krasnodarskaya 99, or a higher overall baking rating. Thus, along with disease resistance, the studied lines can be used as donors to improve the technological qualities of grain and flour.

All lines, with the exception of 5733p19, significantly exceeded the weight of 1000 grains of the Krasnodarskaya 99 variety. According to the number of spikes per 1 m^2, the lines have either equal (5656p19, 5725p18, 5733p19) or lower indicators (5658p19, 5684p18 and 5766p19) compared to the Krasnodarskaya 99 variety. With the exception of the 5766p19 line, all the others were significantly inferior to the Krasnodarskaya 99 in terms of grain weight per 1 m^2. Based on the obtained data, the reduced productivity of the lines compared to Krasnodarskaya 99 can be tentatively attributed to the fact that against the background of a significantly high protein content (with the exception of the 5766p19 line), which, as a rule, negatively correlates with yield, they form either an equal or significantly smaller number of spikes per 1 m^2. It should also be noted that Krasnodarskaya 99 is one of the high-yielding varieties of winter common wheat.

Conclusion

Thus, the obtained results indicate a wide variety of created introgression lines and the effectiveness of using the synthetic RS5 form for transferring genetic material from *Ae. tauschii* and *Ae. speltoides* to common wheat.

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ORCID ID
R.O. Davoyan orcid.org/0000-0002-6771-5161

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The subcompartmented oxphosomic model of the phosphorylating system organization in mitochondria

I.V. Ukolova

Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia
irina@sifibr.irk.ru

Abstract. The *oxidative phosphorylation (OXPHOS) system* of mitochondria supports all the vitally important energy-consuming processes in eukaryotic cells, providing them with energy in the form of ATP. OXPHOS enzymes (complexes I–V) are located in the inner mitochondrial membrane, mainly in the cristae subcompartment. At present, there is a large body of data evidencing that the respiratory complexes I, III_2 and IV under *in vivo* conditions can physically interact with each other in diverse stoichiometry, thereby forming supercomplexes. Despite active accumulation of knowledge about the structure of the main supercomplexes of the OXPHOS system, its physical and functional organization *in vivo* remains unclear. Contemporary models of the OXPHOS system's organization in the inner membrane of mitochondria are contradictory and presume the existence of either highly organized respiratory strings, or, by contrast, a set of randomly dispersed respiratory supercomplexes and complexes. Furthermore, it is assumed that ATP-synthase (complex V) does not form associations with respiratory enzymes and operates autonomously. Our latest data obtained on mitochondria of etiolated shoots of pea evidence the possibility of physical association between the respiratory supercomplexes and dimeric ATP-synthase. These data have allowed us to reconsider the contemporary concept of the phosphorylation system organization and propose a new subcompartmented oxphosomic model. According to this model, a substantial number of the OXPHOS complexes form oxphosomes, which in a definite stoichiometry include complexes I–V and are located predominantly in the cristae subcompartment of mitochondria in the form of highly organized strings or patches. These suprastructures represent "mini-factories" for ATP production. It is assumed that such an organization (1) contributes to increasing the efficiency of the OXPHOS system operation, (2) involves new levels of activity regulation, and (3) may determine the inner membrane morphology to some extent. The review discusses the proposed model in detail. For a better understanding of the matter, the history of development of concepts concerning the OXPHOS organization with the emphasis on recent contemporary models is briefly considered. The principal experimental data accumulated over the past 40 years, which confirm the validity of the oxphosomic hypothesis, are also provided.
Key words: system of oxidative phosphorylation; mitochondria; oxphosome; models of the OXPHOS organization; supercomplexes.

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Субкомпартментационная оксфосомная модель организации фосфорилирующей системы митохондрий

И.В. Уколова

Сибирский институт физиологии и биохимии растений Сибирского отделения Российской академии наук, Иркутск, Россия
irina@sifibr.irk.ru

Аннотация. Система окислительного фосфорилирования (ОКСФОС) митохондрий поддерживает все жизненно важные энергозатратные процессы в клетках эукариот, обеспечивая их энергией в форме АТФ. Ферменты ОКСФОС (комплексы I–V) локализуются во внутренней мембране митохондрий, преимущественно в кристном субкомпартменте. К настоящему времени получен значительный объем данных, указывающих на то, что дыхательные комплексы I, III_2 и IV в условиях *in vivo* могут физически взаимодействовать друг с другом в различной стехиометрии, образуя суперкомплексы. Несмотря на активное накопление знаний о структуре основных суперкомплексов системы ОКСФОС, ее физическая и функциональная организация *in vivo* остается неясной. Современные модели организации ОКСФОС во внутренней мембране митохондрий противоречивы и предполагают существование либо высокоорганизованных дыхательных цепочек, либо, наоборот, набора случайно расположенных дыхательных суперкомплексов и комплексов. При этом предполагается, что АТФ-синтаза (комплекс V) не образует ассоциаций с дыхательными ферментами и работает автономно. Наши последние данные, полученные на митохондриях этиолированных побегов гороха, указывают на возможность физической ассоциации ды-

хательных суперкомплексов и димерной АТФ-синтазы. Эта информация позволила пересмотреть существующие представления об организации фосфорилирующей системы и предложить новую субкомпартментационную оксфосомную модель. Согласно новой модели, значительная часть комплексов ОКСФОС формирует оксфосомы, которые в определенной стехиометрии включают комплексы I–V и располагаются преимущественно в кристном субкомпартменте митохондрий в виде высокоорганизованных цепочек или «патчей», представляющих собой «мини-фабрики» по производству АТФ. Предполагается, что такая организация способствует увеличению эффективности работы системы ОКСФОС; открывает новые возможности для регуляции ее активности и в той или иной степени может определять морфологию внутренней мембраны митохондрий. В обзоре подробно обсуждается предлагаемая модель. Для лучшего понимания вопроса кратко рассмотрена история развития представлений об организации системы ОКСФОС с акцентом на современные модели, а также приведены накопленные за последние сорок лет основные экспериментальные данные, подтверждающие обоснованность оксфосомной гипотезы.
Ключевые слова: система окислительного фосфорилирования; митохондрии; оксфосома; модели организации ОКСФОС; суперкомплексы.

Introduction

The *system of oxidative phosphorylation* (*OXPHOS*) of mitochondria is the main source of energy generated in the form of ATP, which is necessary for maintaining all vitally important metabolic processes taking place in the cells of aerobic eukaryotic organisms. The OXPHOS enzymes are localized in the inner membrane of mitochondria and include five functional complexes I–V, each representing a complexly organized molecular machine: complex I (NADH-dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome-*bc1*-complex), complex IV (cytochrome *c* oxidase) and complex V (ATP-synthase). The four first enzymes form the respiratory chain and are sequentially involved in the process of transfer of electrons from the oxidizable substrate upon the molecular oxygen. This process in complexes I, III and IV is coupled with translocation of protons across the inner mitochondrial membrane, as a result of which an electrochemical proton gradient is formed, which is used by ATP-synthase for ATP synthesis. Furthermore, mobile electron carriers, ubiquinone and cytochrome *c* (Enríquez, 2016), as well as the translocators of adenine nucleotides and inorganic phosphate coupled with ATP-synthase are attributed to the OXPHOS system (Luzikov, 2009).

The components of the energy transformation system make up the bulk of proteins of the inner mitochondrial membrane and, according to various sources, occupy from half to two-thirds of its hydrophobic volume (Vonck, 2012; Schlame, 2021). To date, a lot of data evidencing the higher-ordered organization of the OXPHOS enzymes *in vivo* have been accumulated. Existence of respiratory supercomplexes, which include respiratory complexes I, dimer III_2, and IV in various stoichiometry, as well as the presence of oligomeric ATP-synthase in the inner mitochondrial membrane have been proven (Vonck, 2012; Chaban et al., 2014). It is believed that such a compact supramolecular organization gives the possibility of avoiding nonspecific aggregation of enzymes and deformation of the lipid bilayer (Guigas, Weiss, 2016), increases the efficiency of respiration, and protects the cell from oxidative stress (Lenaz, Genova, 2012).

However, despite the fact that the structure of the main supercomplexes has been sufficiently studied, the physical and functional organization of the OXPHOS system *in vivo* remains unknown and still is a matter of controversy. Active interest in this issue may be explained by the fact that correct understanding of native organization of the energy system in mitochondria not only opens new opportunities for further development of mitochondriology, but also determines new ways of solving such vitally important problems of mankind as therapy of diseases associated with mitochondrial dysfunctions.

To date, possible variants of the arrangement of supercomplexes in the inner mitochondrial membrane are considered in contemporary models of the phosphorylating system organization, which are sometimes contradictory and assume existence of either highly organized respiratory strings, or, *vice versa*, respiratory supercomplexes and complexes freely diffusing in the membrane plane. Furthermore, it is assumed that ATP-synthase does not form associations with the respiratory enzymes and functions autonomously.

Recently, on the basis of our data obtained with the use of mitochondria from pea shoots, a subcompartmented oxphosomic model of organization of the phosphorylating system has been proposed (Ukolova et al., 2020). This model, in contrast to existing ones, postulates that a substantial part of population of the respiratory supercomplexes interacts with dimeric ATP-synthase *in vivo*, thereby forming the oxphosomes, which are located mainly in the cristae subcompartment of mitochondria as highly organized strings or patches (Fig. 1, *f*). Such an organization is expected to substantially elevate efficiency and involve additional levels of control over the operation of the OXPHOS system. This new model is discussed below. For the purpose of better understanding the issue and assessing the validity of the model, the review provides a brief history of evolution of the views on the organization of the OXPHOS system, and also literary data maintaining the oxphosomic hypothesis.

A brief history of development of ideas on the mitochondrial OXPHOS system organization *in vivo*

The physical integrity of the respiratory chain (i. e. the unity of its components) was assumed long ago in publications by D. Keilin and his co-authors in the 1930s–1940s (Keilin, 1930; Keilin, Hartree, 1939, 1949). For a long time, it was believed that all the enzymes of the respiratory chain interact stably to form “respiratory assemblies” (Chance, Williams, 1956;

Lehninger, 1959). Such an aggregation state of the respiratory chain was called "solid" (Lehninger, 1959; Rich, 1981) (see Fig. 1, *a*). As new data became available, the "solid" model was replaced with the "fluid" one (Hackenbrock et al., 1986) (see Fig. 1, *b*). This model excluded the physical association of OXPHOS components and postulated that all the redox components involved in electron transfer and the proteins required for synthesis of ATP represent "independent lateral diffusants" that interact in course of multiple collisions.

Despite the existence of a large amount of data confirming the validity of the "fluid" model, facts indicating the existence *in vivo* of (i) associations of respiratory chain complexes and (ii) oligomeric ATP-synthase continued to accumulate. The year 2000 became a turning point, when H. Schägger and his colleagues (Schägger, Pfeiffer, 2000), using the method of blue native electrophoresis (BN-PAGE) (elaborated by them earlier), obtained convincing evidence of physical interaction between the respiratory complexes leading to formation of supercomplexes. Thus they actually updated and returned the "solid" model, proposing the respirasome model (see Fig. 1, *c*). According to this model, the found supercomplexes are the "building blocks" that "can interact to form a network of respiratory chain complexes that may be called a respirasome". Later, the authors (Schägger, 2002) began to call a separate supercomplex, comprising complexes I, III_2, and IV the respirasome, because this superstructure could independently "respire", i.e. provide for the entire cycle of electron transfer from the oxidized substrate to the molecular oxygen. As a result, this term has taken root and is currently used in the literature in this context.

Contemporary understanding of the energy system organization in mitochondria

Phylogenetic conservation of organization of OXPHOS components

With the advent of BN-PAGE and further successful combination of this method with cryoelectron microscopy, in-gel enzyme activity assays, and other methods, the study of supramolecular organization of the OXPHOS system in mitochondria of various organisms has reached a principally new level. Further investigations of this system in organelles from mammalian, plant, fungi, yeast, algae, and some protozoa revealed a similar composition of the supercomplexes (Krause et al., 2004; Chaban et al., 2014). All supramolecular associations of the OXPHOS components, obtained as a result of solubilization of mitochondria with the use of mild detergents, may be subdivided into four main groups: (1) supercomplex I_1III_2; (2) supercomplexes $III_2IV_{1–2}$; (3) respirasomes $I_1III_2IV_{1–4}$; and (4) dimeric ATP-synthase. In some species, other respiratory supercomplexes of distinct compositions and stoichiometry were found (see Ukolova et al., 2020). Dimers of ATP-synthases *in vivo* assemble into long oligomeric rows at the cristae rims (Kühlbrandt, 2019). There are convincing data proving that it is the dimerization of ATP-synthase followed by oligomerization that engenders high local membrane curvature and promotes the formation of cristae.

Presently, there are two alternative models of arrangement of respiratory supercomplexes and OXPHOS complexes in the inner mitochondrial membrane, which in fact are contemporary versions of the "solid" and "fluid" models, namely, a model of highly organized respiratory strings and patches (Nübel et al., 2009; Wittig, Schägger, 2009) and a "plasticity" model (Acín-Pérez et al., 2008; Enríquez, 2016), respectively. The first model describes the strings of respiratory supercomplexes associated with each other (see Fig. 1, *d*), while the second postulates a random distribution of supercomplexes and complexes in the membrane (see Fig. 1, *e*). Moreover, both models assume separate location and autonomous functioning of respiratory supercomplexes and oligomeric rows of ATP-synthases.

Respiratory strings and patches

The first model is a development of the previously proposed model of respirasome (Schägger, Pfeiffer, 2000). On the basis of new data obtained by using BN-methods, H. Schägger and his colleagues (Nübel et al., 2009; Wittig, Schägger, 2009) put forward an assumption that respiratory supercomplexes in the inner mitochondrial membrane can be "building blocks" for larger structures, i.e. for respiratory strings and even for patches. Respiratory strings are linear rows of supercomplexes associated with each other in a certain order (see Fig. 1, *d*). Depending on the organism and the species, either dimers or tetramers of complex IV may be the connecting links between the supercomplexes (Wittig, Schägger, 2009). The authors assumed that the respiratory strings can be spatially oriented parallel to each other in the membrane plane and interact via complex I monomers, while forming higher-order structures called "patches" (Nübel et al., 2009).

Identification (with the use of modified native gels with large pores) of multimeric respiratory supercomplexes with visible masses from 4–8 to 35–45 MDa was a convincing argument in favor of this model (Strecker et al., 2010). The authors also relied on the earlier pioneering work of R.D. Allen and his colleagues (Allen et al., 1989), who managed (with the aid of cryoelectron microscopy) to reveal not only oligomeric rows of ATP-synthases along the outer curve of tubular cristae of *Paramecium multimicronucleatum* but also additional rows of large particles along their inner curve, which were regularly arranged and corresponded in size to the dimeric complex I. H. Schägger and I. Wittig assumed that this additional group of projections represents a respiratory string and proposed a variant of such a string for mammalian mitochondria (see Fig. 1, *d*) (Wittig, Schägger, 2009). Variants of respiratory strings for potato and *Polytomella* sp. were proposed by other investigators (Bultema et al., 2009; Miranda-Astudillo et al., 2018). At that, the respiratory strings were located parallel to the oligomeric rows of ATP-synthases (Miranda-Astudillo et al., 2018).

The "plasticity" model

The study of the composition of the OXPHOS system in various species with the aid of BN-PAGE revealed that – after solubilization of mitochondria with detergents – a part of the population of respiratory complexes remained in a free state,

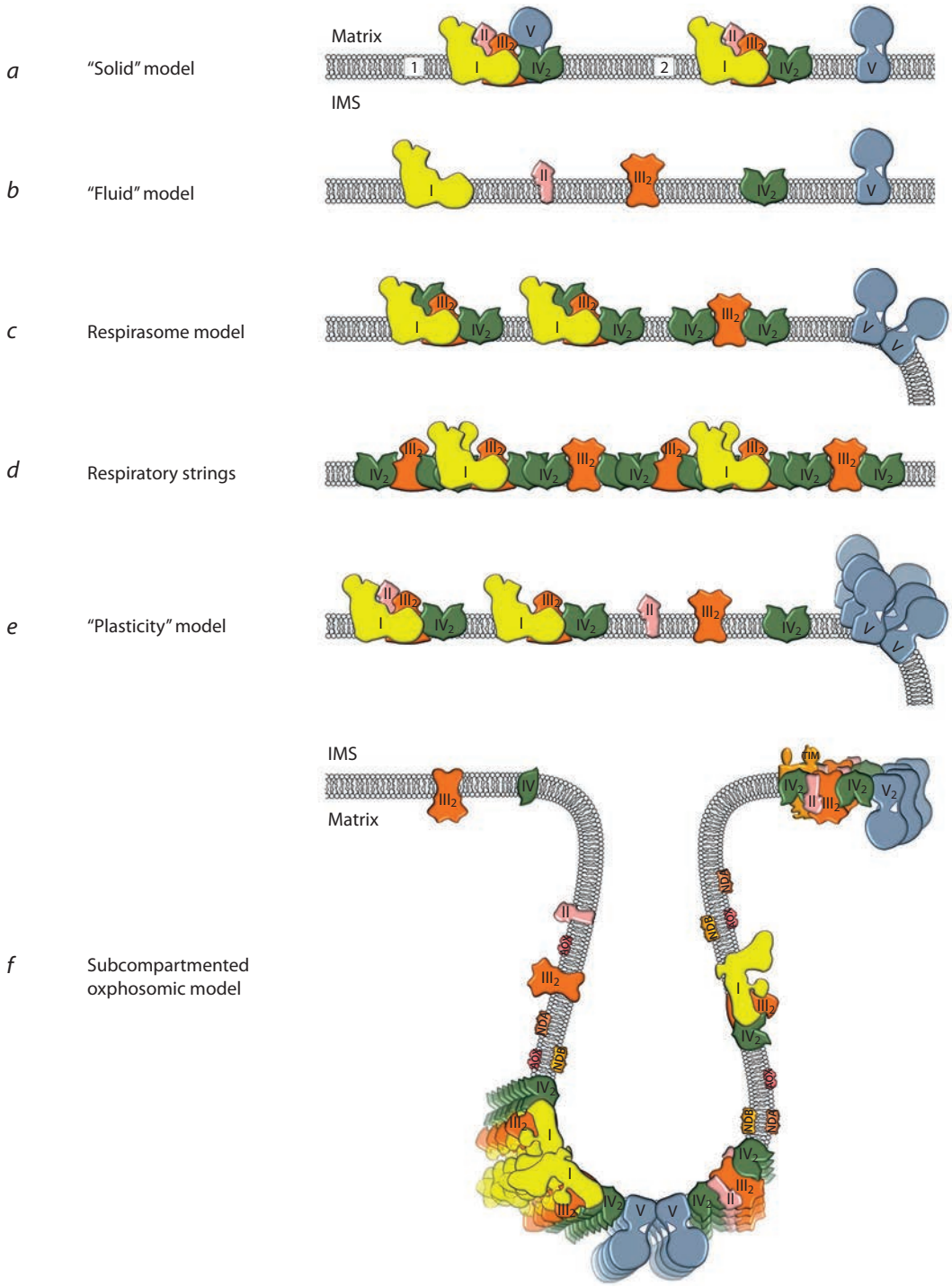

Fig. 1. Development of ideas related to the conception of the OXPHOS system organization in mitochondria, from the initial "solid" model to the proposed subcompartmented oxphosomic one.

The interpretation of the models is presented taking into account recent data on the structure of OXPHOS complexes of mammals (*a*–*e*) and plants (*f*). According to the literary data, all the principal models (with the exception of model *f*) are given for mammalian mitochondria. Denotations: (1) initial and (2) later "solid" models; matrix and IMS – matrix and, respectively, intermembrane space of mitochondria; complexes I, II, III_2, IV and IV_2 of the respiratory chain and ATP-synthase (complex V) are shown in yellow, pink, orange, green and blue, respectively. It is shown on more recent schemes that ATP-synthase dimers bend the membrane and, thereby, are involved in the formation of cristae (*c*, *e*, *f*). Description of the models can be found in the text. The respirasome model (*c*) is given in accordance with the schemes of H. Schägger (Schägger, Pfeiffer, 2000; Schägger, 2002); it reflects the principal postulate of the model, which determines the ratio of the large supercomplex $I_1III_2IV_4$ and the small supercomplex III_2IV_4 as 2:1. The model of respiratory strings (*d*) is shown according to the scheme of H. Schägger and I. Wittig (Wittig, Schägger, 2009), but, unlike the representation in the original, the figure shows a side view (in the plane of the membrane). There are other variants of the respiratory strings (Bultema et al., 2009; Miranda-Astudillo et al., 2018). In the oxphosomic model (*f*), developed for the mitochondrial OXPHOS system of etiolated pea shoots (Ukolova et al., 2020), in addition to the main OXPHOS complexes, there are also freely located alternative enzymes, which indicates a more complex organization of the phosphorylating system in plants.

while another part was present in the supercomplexes (Enríquez, 2016). It was noted in a number of investigations that the relative amount of free and superassembled respiratory enzymes as well as the ratio of supercomplexes of diverse stoichiometry varied depending on the type of cells and the physiological state of the organism (stage of development, stress exposure, disease). On the basis of these data, J.A. Enríquez and his colleagues (Acín-Pérez et al., 2008; Acín-Pérez, Enríquez, 2014) proposed the "plasticity" model that postulated balanced coexistence of free respiratory complexes and supercomplexes of diverse composition and stoichiometry, the ratio of which corresponded to the physiological status of the cell (see Fig. 1, *e*). The proposed model was considered by the author mainly as a "refined revision of the fluid model" (Enríquez, 2016), since individual respiratory complexes and supercomplexes freely diffused in the plane of the inner mitochondrial membrane.

The new subcompartmented oxphosomic model of organization of the phosphorylating system in mitochondria

According to the new model, a substantial part of the respiratory supercomplexes physically interacts with dimeric ATP-synthases, thereby forming oxphosomes, which are located mainly in the cristae subcompartment of mitochondria in the form of highly organized megastructures that are, in fact, "mini-factories" involved in ATP production (Ukolova et al., 2020) (see Fig. 1, *f*). At the same time, the rest of the respiratory complexes and supercomplexes obviously remain in free form. It is supposed that the ratio between the assembled oxphosomes and free respiratory supercomplexes and complexes depends on the type, physiological status and energetic needs of the cell. Actually, the model integrates the contemporary "solid" and "fluid" models (see Fig. 1, *d*, *e*), while adding a new layer of complexity related to the oxphosomic organization as well as to the structural and functional subdivision of the inner membrane into subcompartments.

Experimental data that contributed to the emergence of the model

As noted above, the model was developed on the basis of our data obtained recently by analysis of mitochondria from etiolated pea shoots (Ukolova et al., 2020). The usage of freshly isolated organelles for digitonin solubilization of OXPHOS supercomplexes and complexes, application of multimeric electrophoresis system based on BN-PAGE, and mild electrophoretic separation conditions made it possible to identify supercomplex IV_1Va_2 and demonstrate the possibility of physical interaction between ATP-synthase and respiratory complex IV. Furthermore, in addition to the canonical associations I_1III_2, $I_1III_2IV_n$ and III_2IV_{1-2}, dimer V_2 and free complexes I–V, we were able to reveal new structures that had not been not detected earlier, i. e. the second form of ATP-synthase Va having a higher molecular weight, respirasome $I_2III_4IV_n$ with two copies of complex I and double dimer III_2, as well as a megacomplex $(II_xIII_yIV_z)_n$ of high molecular weight. Simultaneous isolation of supercomplex IV_1Va_2, respirasomes $I_{1-2}III_{2-4}IV_n$ and megacomplex $(II_xIII_yIV_z)_n$, in which complex IV was bound up either with ATP-synthase or with respiratory complexes, allowed us to suppose that all the OXPHOS complexes *in vivo* can physically interact with a definite stoichiometry to form a larger structure that may be called an "oxphosome". Thus, putative oxphosome represents a structure, in which complexes I (and/or, possibly, II), III_2, IV and V are associated in strictly definite stoichiometry, and which can autonomously fulfill the whole cycle of reactions from the substrate oxidation to ADP phosphorylation, i. e. may "breathe" and produce ATP (Fig. 2).

It is possible to suppose that the connecting link between the respiratory and the phosphorylating parts of the oxphosome are dimers or tetramers of complex IV (see Fig. 2). However, the potential ability of complex IV to bind only respiratory supercomplexes to each other (via the formation of dimers and tetramers) has already been considered earlier in models of respiratory strings (Bultema et al., 2009; Wittig, Schägger, 2009; Miranda-Astudillo et al., 2018). Taking into account high abundance of free forms of complex IV (IVa/b and IV_2) after solubilization with a detergent, both in our work (Ukolova et al., 2020) and in other works (Eubel et al., 2003; Krause et al., 2004; Acín-Pérez et al., 2008), it may be assumed that this connecting link is detergent-sensitive and represents the break point of the oxphosome in course of solubilization. The dimers of complex V are also sensitive to detergent treatment and dissociate into monomers under these conditions, as it was shown for mitochondria of many species (Schägger, Pfeiffer, 2000; Eubel et al., 2003). Such sensitivity might explain the minor amount of the new supercomplex IV_1Va_2 in digitonin-solubilized mitochondria of pea shoots (Ukolova et al., 2020).

Some indirect literary data indicate that similar associations exist in other species and organisms. For example, Z.H. Qiu et al. (Qiu et al., 1992) demonstrated the possibility of reconstructing the association of complexes IV–V in proteoliposomes from highly purified complexes IV and V isolated from the bovine heart. It was demonstrated in the investigations conducted on the yeast that the absence of dimer-specific ATP-synthase subunits in mutant strains (i. e. absence of ATP-synthase dimers) reduced the activity of complex IV and the rate of ATP synthesis, altered the kinetic control of complex IV over oxidative phosphorylation (Boyle et al., 1999), as well as affected the stability of supercomplex III_2IV_2 (Saddar et al., 2008). These facts allow one to assume that the oxphosomic organization of the phosphorylating system may have conservative features and be typical of many species, although, apparently, it also has to possess taxon-specific traits.

The assumed organization of oxphosomes

All the presently known supramolecular structures of the OXPHOS system have strictly defined stoichiometry and spatial organization. In order to determine the stoichiometry of oxphosomes and develop a model of their organization *in vivo* for a specific species or an organism, it is necessary to take into account (i) the quantitative ratio of the OXPHOS complexes and (ii) the available data on the structure and spatial organization of respiratory complexes and supercomplexes, as well as ATP-synthase dimers.

Analysis of the literary data has given evidence that the ratio of complexes can vary depending on organism, type and physiological status of the cell (Schägger, 2002; Dubinin et

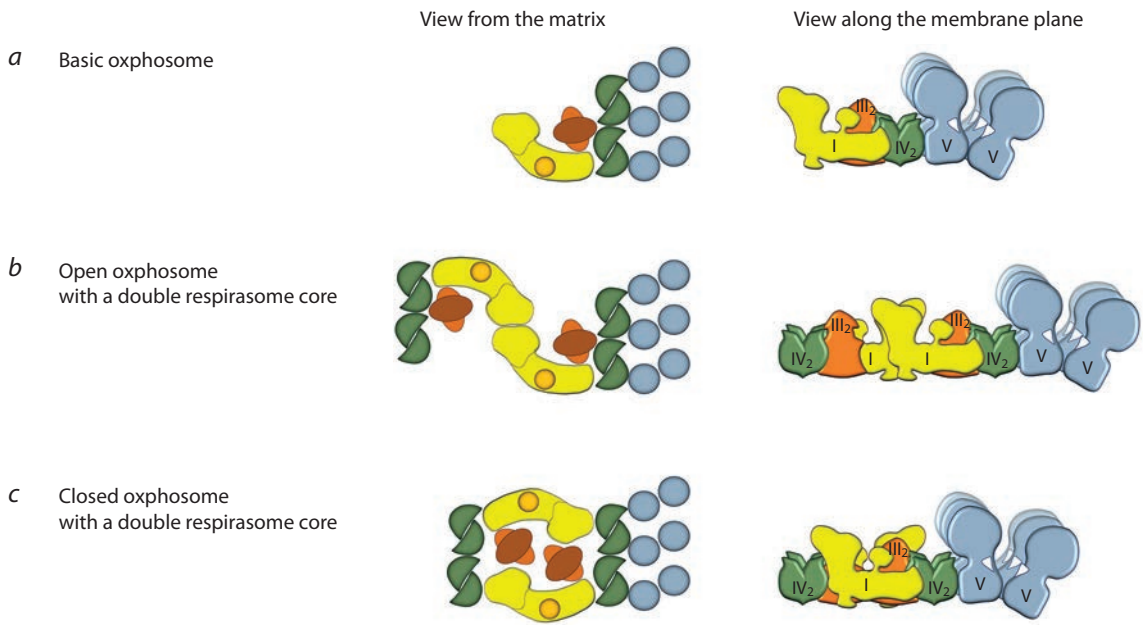

Fig. 2. Putative organization of the basic oxphosome and oxphosomes with double respirasome core of open and closed types.

The schematic view of putative oxphosomes from pee shoot mitochondria is shown. The view from the matrix side of the mitochondrial inner membrane and the side-view (along the membrane plane, profile projection) are shown. The color code is the same as in Fig. 1.

al., 2011; Peters et al., 2012). This means that the model of the OXPHOS system organization in each definite case may have specific features. Nevertheless, taking into account high conservation of the studied OXPHOS superstructures, it is possible to assume that the basic principles of the supramolecular arrangement of the system, i. e. the formation of oxphosomes, oxphosomic strings and free supercomplexes with definite stoichiometry and spatial architecture, shall be retained.

The exact ratio of complexes I:II:III:IV:V has been determined so far only for bovine heart mitochondria and is approximately 1:1.5:3:6:3 (Schägger, 2002). According to H. Schägger, at this overall stoichiometry of the OXPHOS complexes, for every 2 complexes I there are 3 complexes II, 3 dimers III_2, 6 dimers IV_2 (or 3 tetramers IV_4) and 3 dimers V_2. On the basis of these data, the respirasome model for bovine mitochondria, which considers associations only of respiratory complexes I, III, and IV, was developed. The model postulates that the "building blocks" for the network of mammalian respiratory chain complexes are the large supercomplex $I_1III_2IV_4$ (presently known as respirasome) and the small supercomplex III_2IV_4, which exist in a 2:1 ratio in the inner membrane (see Fig. 1, *c*). The exact overall stoichiometry of the OXPHOS complexes for mitochondria of pea shoots has not yet been determined. Meanwhile, our data showed that 2/3 of complex III population represent a part of respirasomes (Ukolova et al., 2020), which is consistent with the model of H. Schägger. This made it possible to develop the preliminary model of the OXPHOS organization for pea shoot mitochondria based on the ratio given for bovine OXPHOS complexes (see Fig. 1, *f*). Further investigation of abundance and the ratio of energy system's enzymes in pea shoot organelles will make it possible to clarify the proposed model.

Thus, based on the data discussed above, it is possible to assume that the main structural components or "building blocks" of the OXPHOS system in the mitochondria of pea shoots are represented by: the basic oxphosome $I_1III_2IV_4V_6$, respirasome $I_1III_2IV_4$ and supercomplex III_2IV_4. The basic oxphosome represents respirasome $I_1III_2IV_4$ bound up with three ATP-synthase dimers (see Fig. 2, *a*). The second respirasome $I_1III_2IV_4$ and supercomplex III_2IV_4 can also be bound up with the basic oxphosome, while forming higher order oligomeric structures (see Fig. 1, *f* and Fig. 2, *b*, *c*). Taking into account (i) the data of J.B. Bultema with co-authors (Bultema et al., 2009), who, using electron microscopy, have shown the presence of open and closed conformations of the supercomplex I_1III_2 from mitochondria of potato tubers, and (ii) our electrophoretic data indicating a difference in the structure of the two "heaviest" supercomplexes with the composition $I_2III_4IV_n$, it is possible to assume the formation of "open" and "closed" oxphosomes with a double respirasome core (see Fig. 2, *b*, *c*). It is likely that these two forms can transform one into the other and, so, participate in regulation of the activity of the OXPHOS system. The small supercomplex III_2IV_4 can *in vivo* associate with complex II or alternative NAD(P)H dehydrogenases directing electrons from oxidizable substrates to complex III_2 (see Fig. 1, *f*). The spatial organization of the putative oxphosomes has been developed given the available cryoelectron microscopy data on the structure of individual OXPHOS complexes and supercomplexes in plant mitochondria (see Fig. 2).

Presently, the "degree of the energy system assembly" in pea shoot organelles is not clear. It is not clear as well what part of the inner membrane is occupied by oxphosomic suprastructures in mitochondria of this and other species and organisms. It may be assumed that the ratio and the composition of free

and assembled (into oxphosomic patches) respiratory supercomplexes and complexes may depend on the physiological status and energy requirements of the cell. For example, in cells with higher needs for energy (cells of muscles, heart, brain of mammals), it is logical to expect a higher abundance of assembled oxphosomic patches, oriented to production of large amount of ATP.

Subcompartmental localization of OXPHOS components

The inner mitochondrial membrane is subdivided into two morphologically and presumably functionally distinct subcompartments: the cristae and the inner boundary membrane domain. It has been shown that OXPHOS complexes are predominantly localized in the cristae domain (Gilkerson et al., 2003; Vogel et al., 2006). So, according to the data of R. Gilkerson with co-authors (Gilkerson et al., 2003), about 94 % of complex III of the respiratory chain and ATP-synthase is localized in the cristae, and only 6 % – in the inner boundary membrane. On the basis of available literary data, it may be assumed that the oxphosomes are predominantly located in the cristae domain, where they can form highly organized oxphosomic strings or patches (see Fig. 1, *f*). At the same time, data of F. Vogel with co-authors (Vogel et al., 2006) showed that dimers of ATP-synthase were also present in the inner boundary membrane, which allowed one to assume the formation of oxphosomes in this subcompartment as well. Oxphosomes and individual supercomplexes located in the inner boundary membrane could efficiently support the potential-dependent and ATP-dependent processes that are most active in this domain, for example, protein translocation and assembly.

The facts confirming the functional validity of the oxphosomic model

The first hypothesis proposed the mechanism of proton coupling of oxidation and phosphorylation in a "rigidly" fixed assembly of OXPHOS enzymes, which later received experimental confirmation, was the hypothesis of local coupling proposed by R.J. Williams in 1961 (Williams, 1961). The hypothesis stated that such a spatially fixed organization of enzymes creates the conditions for the formation of protons in a high local concentration and for their direct (without crossing the hydrophobic membrane barrier) transfer to ATP-synthase (Williams, 1961; Skulachev, 1982). It is curious that the conception of R.J. Williams was proposed in the same year as P. Mitchell's chemiosmotic hypothesis (which later became a theory), and was an alternative to the latter. The hypothesis of P. Mitchell (Mitchell, 1961) postulated that protons H^+ are transported by proton pumps through the inner mitochondrial membrane into the water phase and do not bind to the membrane, while forming a delocalized electrochemical potential, which is used by ATP-synthase (Skulachev, 1982). Later, this mechanism was interpreted in favor of free (dissociated) distribution of enzymes in the membrane, that is, in favor of the "fluid" model.

Despite the huge amount of experimental data confirming the chemiosmotic hypothesis, the data supporting the concept of local coupling, and, consequently, the physical association of respiratory enzymes with ATP-synthase gradually accumulated, namely: (1) existence of mutual regulation between mitochondrial ATP-synthase and complexes of the respiratory chain was shown (Tu et al., 1981; Krasinskaya et al., 1984); (2) evidence was obtained for the existence of a non-equilibrium membrane-bound fraction of hydrogen ions forming locally as a result of functioning of proton pumps in the respiratory chain (Antonenko et al., 1993; Motovilov et al., 2009); (3) participation of the fraction of membrane-bound protons in ATP synthesis was revealed (Solodovnikova et al., 2004; Eremeev, Yaguzhinsky, 2015); (4) catalysts of release of membrane-bound protons from the outer surface of the inner membrane were revealed, with their help the possibility of switching the phosphorylating system from the local coupling mode to the transmembrane proton transfer mode was shown (Yaguzhinsky et al., 2006). These data support the oxphosomic hypothesis and suggest that the OXPHOS system can use both localized and delocalized electrochemical potentials of hydrogen ions.

Conclusion

New discoveries concerning organization, structure and functioning of the phosphorylating system of mitochondria periodically induce a revision of existing conceptions and initiate transition to a new level in understanding of the system's arrangement and functioning. Emerging models either completely disprove the previous ones, as was the case in the late 1970s – early 1980s when the "solid" model was replaced with the "fluid" one (see Fig. 1, *a*, *b*), or are based on the previous ones, expanding and developing them, as, for example, in case of the model of respiratory strings, which developed the respirasome model (see Fig. 1, *c*, *d*).

Presently, despite the active acquisition of data on spatial organization, structure and functional activity of respiratory supercomplexes and oligomeric ATP-synthase, the supramolecular organization of the OXPHOS system *in vivo* remains unclear. The latest data obtained in our investigations on mitochondria of etiolated pea shoots gave evidence of the possibility of physical association between respiratory supercomplexes and dimeric ATP-synthase (Ukolova et al., 2020). This information led to revision of the existing contemporary ideas of organization of the OXPHOS system (Wittig, Schägger, 2009; Acín-Pérez, Enríquez, 2014; Miranda-Astudillo et al., 2018) and to the proposal of the subcompartmented oxphosomic model (see Fig. 1, *f*).

This model presupposes the existence *in vivo* of highly organized associations between the oligomeric rows of ATP-synthase and respiratory supercomplexes, which represent oxphosomic strings or patches that are localized predominantly in the cristae subcompartment. It is assumed that the oxphosomic organization allows a significant increase in the functionality of the OXPHOS system and efficiency of its operation. Firstly, association of oligomeric ATP-synthase with respiratory supercomplexes could enable the system to use not only protons in the bulky water phase of the intermembrane space, but also membrane-bound protons forming in high local concentrations as a result of operation of the assembled respiratory enzymes. Secondly, such an organization could involve additional layers of the system's activity regulation (for example, transforming an open, possibly more active, form of oxphosome to a closed one, or changing the ratio of associated with ATP-synthases

and free supercomplexes). Thirdly, the "anchorage" of the respiratory supercomplexes on the oligomeric rows of ATP-synthase could determine the morphology of the membrane to some extent. Further investigations will make it possible to clarify and probably visualize the supramolecular structure of the OXPHOS system in mitochondria of various organisms *in vivo*.

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A bioinformatics approach for identifying the probable cause of the cross-interaction of antibodies to the antigenic protein HPV16 L1 with the HPV6 L1 protein

A.S. Stolbikov[1, 2] ✉, R.K. Salyaev[1], N.I. Rekoslavskaya[1]

[1] Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia
[2] Irkutsk State University, Irkutsk, Russia
✉ valkir5@yandex.ru

Abstract. This paper describes an attempt to analyze, with the aid of bioinformatics resources (programs and databases), the probable cause of the cross-interaction of antibodies against HPV16 L1 with antigenic protein HPV6 L1, which has been revealed in the investigation of the candidate vaccine obtained on the base of a plant expression system (tomato plants). In our opinion, the most likely reason for the cross-interaction of antibodies with antigens of different pathogenic HPV types is the similarity of their antigenic determinants. In this work, the amino acid sequences of HPV16 L1 and HPV6 L1 used for the development of a binary vaccine against cervical cancer and anogenital papillomatosis have been analyzed. For the analysis of antigenic determinants, the programs BepiPred-2.0: Sequential B-Cell Epitope Predictor, DiscoTope 2.0 Server and SYFPEITHI have been used. As a result of the analysis of probable B-cell linear determinants (epitopes), it has been found that in both types of HPV the proteins have approximately the same location and size of linear antigenic determinants; the difference is observed only in the form of small shifts in the size of several amino acid residues. However, there are some differences in the amino acid composition of epitopes; therefore, the possibility for cross-interaction of the antibodies with the antigens due to the similarity of linear antigenic determinants for B-cells is very small. The analysis of potential three-dimensional epitopes for B-cells has shown that due to little difference between them the HPV16 L1 and HPV6 L1 proteins have no prerequisites for cross-interaction of the antibodies with the antigens belonging to the two different pathogenic HPV types. The analysis of probable linear epitopes for T-cells has revealed a common antigenic determinant in the two protein sequences. According to the rank made with the SYFPEITHI program, the amino acid sequence AQL(I)FNKPYWL is the second most likely antigenic determinant for T-cells. Meanwhile, the amino acid sequences of this determinant in HPV16 L1 and HPV6 L1 are virtually identical. There is a difference in only one position, but it is not critical due to the similarity of the physicochemical properties of amino acids, for which there is a replacement in the amino acid sequence of antigenic determinants. Consequently, some moderate cross-interaction of the antibodies to HPV16 L1 with the antigens of HPV6 L1 may be expected.

Key words: human papillomavirus; HPV6 L1; HPV16 L1; bioinformatics analysis.

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Использование биоинформационного анализа для определения вероятной причины перекрестного взаимодействия антител к антигенному белку ВПЧ16 L1 с белком ВПЧ6 L1

А.С. Столбиков[1, 2] ✉, Р.К. Саляев[1], Н.И. Рекославская[1]

[1] Сибирский институт физиологии и биохимии растений Сибирского отделения Российской академии наук, Иркутск, Россия
[2] Иркутский государственный университет, Иркутск, Россия
✉ valkir5@yandex.ru

Аннотация. С помощью биоинформационных ресурсов (программ и баз данных) предпринята попытка проанализировать вероятную причину перекрестного взаимодействия антител против ВПЧ16 L1 с антигенными белками ВПЧ6 L1, которое было выявлено при изучении кандидатной вакцины, полученной на основе растительной экспрессионной системы (растений томата). По нашему мнению, наиболее вероятной причиной перекрестного взаимодействия антител с антигенами, принадлежащими к разным патогенным типам вируса папилломы человека (ВПЧ), является сходство антигенных детерминант. В ходе исследования были проанализированы аминокислотные последовательности ВПЧ16 L1 и ВПЧ6 L1, которые использовались при разработке бинарной вакцины против цервикального рака и аногенитальных папилломатозов. Для анализа антигенных детерминант использовались программы BepiPred-2.0: Sequential B-Cell Epitope Predictor, DiscoTope 2.0 Server, SYFPEITHI. В результате исследования вероятных

линейных детерминант для В-клеток установили, что у обоих типов ВПЧ белки имеют примерно одинаковое расположение и размер линейных антигенных детерминант, отличие наблюдается только в виде небольших сдвигов в несколько аминокислотных остатков. Однако выявлено некоторое различие в аминокислотном составе эпитопов, поэтому потенциал перекрестного взаимодействия антител с антигенами за счет сходства линейных антигенных детерминант для В-клеток незначителен. Анализ потенциальных трехмерных эпитопов для В-клеток показал, что по сумме различий белки ВПЧ16 L1 и ВПЧ6 L1 не имеют предпосылок для перекрестного взаимодействия антител с антигенами, принадлежащими к двум разным патогенным типам ВПЧ. Анализ вероятных линейных эпитопов для Т-клеток обнаружил у двух белковых последовательностей общую антигенную детерминанту. Согласно рейтингу, составленному программой SYFPEITHI, аминокислотная последовательность AQL(I)FNKPYWL представляет собой вторую, по вероятности, антигенную детерминанту для Т-клеток. При этом аминокислотная последовательность данной детерминанты у ВПЧ16 L1 и ВПЧ6 L1 практически идентична. Отличие имеется лишь по одной позиции, но оно не является критичным в силу сходства физико-химических свойств аминокислот, по которым наблюдается замена в аминокислотной последовательности антигенных детерминант. Исходя из этого можно ожидать умеренно выраженное перекрестное взаимодействие антител к ВПЧ16 L1 с антигенами ВПЧ6 L1.
Ключевые слова: вирус папилломы человека; ВПЧ6 L1; ВПЧ16 L1; биоинформационный анализ.

Introduction

Tens of millions of people are infected every year with various types of human papillomavirus (HPV), and this accounts only for regions of the world where appropriate medical observations and statistics are conducted (McLaughlin-Drubin, Münger, 2009). Therefore, the development of preventive vaccines against HPV is one of the current challenges to curb the increase in the number of diseases caused by this type of infectious agents.

The development of candidate vaccines based on plant expression systems is a relatively new field of biofarming. Plant expression systems have certain advantages over other systems. First of all, these advantages are related to safety due to the absence of prions, mammalian pathogens, transposons and dangerous viruses in a latent state, as well as the relative cheapness of obtaining vaccines, which generally contributes to wider commercialization and scaling. In our previous investigation, we attempted to develop candidate tetravalent oral vaccine based on transgenic plants against four types of HPV (16, 18, 31, 45) capable of causing cervical cancer. In this work, we planned to develop a vaccine that would provide maximum protection against cervical cancer by using the main antigenic protein L1 of the viral envelope of four highly oncogenic types of human papillomaviruses (HPV16, HPV18, HPV31 and HPV45), which are responsible for most cases of cervical cancer.

It has been revealed that the antibodies to the antigenic protein HPV16 L1 successfully interact with the HPV18 L1, HPV31 L1 and HPV45 L1 antigens (Salyaev et al., 2017). Based on the data obtained, it was assumed that the cross-interaction of the antibodies with the antigens belonging to different pathogenic types of HPV may be due to the similarity of antigenic determinants. This assumption was verified with a bioinformatic approach, where common linear determinants for T cells and B cells were found in all four types of L1 viral proteins. In addition, similar three-dimensional antigenic determinants were found for B cells in HPV16 L1 and HPV18 L1 (Stolbikov et al., 2020). When working on the binary vaccine containing HPV16 L1 and HPV6 L1 antigenic proteins, Western blot hybridization revealed a cross-interaction of serum antibodies against HPV16 L1 with antigenic protein HPV6 L1 (Salyaev et al., 2017; Rekoslavskaya et al., 2021). Human papillomavirus type 6 does not cause cancer, but can lead to the development of anogenital and respiratory papillomatoses. Despite the fact that these diseases rarely lead to death, they are widespread and highly contagious (WHO, January 11, 2020).

Such a wide range of cross-interaction between antigens and antibodies, which goes beyond the viruses that cause cervical cancer and belong to another family, seemed extremely interesting to us. In this regard, in this work, the antigenic determinants of HPV16 L1 and HPV6 L1 have been subjected to a comparative bioinformatic analysis. The data obtained during this work can be used to optimize the development of candidate vaccines using fewer HPV types due to the cross-interaction between antibodies and antigens of unrelated types, which, in turn, will reduce the labor intensity and cost of production of vaccines against dangerous types of human papillomaviruses.

Materials and methods

Alignment of the amino acid sequences of HPV16 L1 and HPV6 L1. As the first stage of the analysis of the antigenic determinants of HPV16 L1 and HPV6 L1, paired alignment of HPV isolates of each type was conducted. For this purpose, their full-size amino acid sequences encoded by nucleotide sequences previously used in genetic constructs in the development of the binary vaccine against cervical cancer and anogenital papillomatosis were found and processed in the NCBI database (GenBank) (Salyaev et al., 2017). This was necessary for the subsequent determination of the difference in the antigenic determinants of the two types of HPV. Whole set of full-size amino acid sequences of HPV16 L1 and HPV6 L1 was also extracted from the GenBank database. The alignment of amino acid sequences was carried out using the editor of multiple alignment of nucleotide and amino acid sequences BioEdit. The phylogenetic tree was constructed using the program "Simple Phylogeny" (EMBL-EBI) by Nearest Neighbor Algorithms (the neighbor-joining method) and unweighted pairwise mean (UPGMA).

Identification of potential antigenic determinants. For the second stage of the analysis of antigenic determinants, the program "BepiPred-2.0: Sequential B-Cell Epitope Predictor" was used (http://www.cbs.dtu.dk/services/BepiPred/) (Jespersen et al., 2017). This bioinformatic resource allowed us to identify potential linear antigenic determinants for B cells.

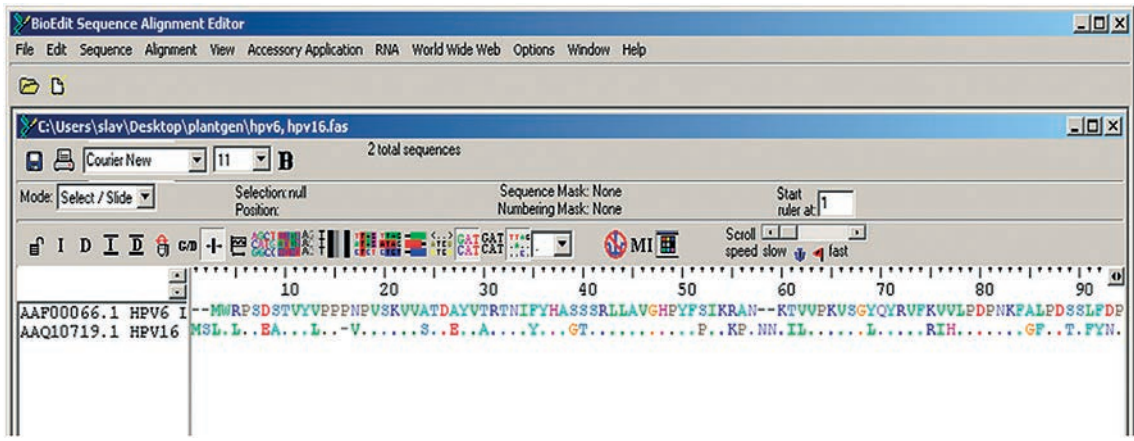

Fig. 1. The alignment of amino acid sequences of HPV6 L1 and HPV16 L1 in the BioEdit program.

To determine the three-dimensional antigenic determinants for B cells, the program "DiscoTope 2.0 Server" was used (http://www.cbs.dtu.dk/services/DiscoTope/) (Kringelum et al., 2012). When working with this program, the strictest conditions were set: sensitivity 47 %, specificity 75 %. Three-dimensional models of proteins that were analyzed using the program DiscoTope 2.0 Server were found in the Protein Data Bank (PDB) database. The programs BepiPred-2.0: Sequential B-Cell Epitope Predictor and DiscoTope 2.0 Server were publicly available on the server of the Danish Technical University (DTU). The search for potential antigenic determinants for T cells was performed using the SYFPEITHI program, which is in the public domain http://www.syfpeithi.com. This bionformatic resource ranks all possible variants of antigenic determinants according to the probability of their interaction with T cells (Rammensee et al., 1999).

Results

Amino acid alignment

The amino acid sequences of the L1 capsid proteins of HPV16 and HPV6 viruses were downloaded from the NCBI international database and aligned in the BioEdit program (Fig. 1).

According to the results of the alignment, phylograms were built. To emphasize the evolutionary differences between the 6 and 16 types of HPV, the comparative analysis used HPV31, which belongs to the same species *Alphapapillomavirus* 9 as HPV16 (Fig. 2). The phylogenetic comparison data showed significant differences between HPV6 L1 and HPV16 L1.

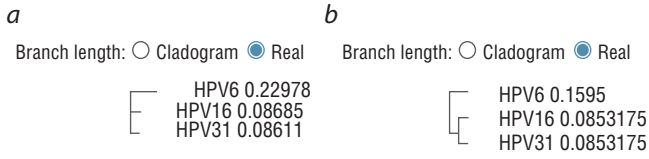

Fig. 2. The phylograms constructed using the program Simple Phylogeny (EMBL-EBI) by the neighbor-joining method (*a*) or by the UPGMA method (*b*).

Analysis of linear antigenic determinants for B cells

The amino acid sequences of two viral proteins HPV6 L1 and HPV16 L1 were analyzed for the presence of potential linear antigenic determinants for B cells using the program BepiPred-2.0: Sequential B-Cell Epitope Predictor. The study showed that the HPV6 L1 protein has the following antigenic determinants: 14–25, 79–83, 85–91, 120–141, 162–177, 208–216, 230–238, 260–283, 308–314, 345–358, 391–439, 447–457, 468–497 (Fig. 3).

Previously, the following antigenic determinants were identified in HPV16 L1: 8–28, 83–95, 123–143, 166–177, 213–220, 234–243, 264–286, 350–369, 396–421, 426–444, 452–462, 473–502 (Stolbikov et al., 2020).

As one can see, the analysis of linear determinants gave evidence that in the HPV types studied, the proteins have approximately the same location and size of linear antigenic determinants, the difference is observed only in the form of small shifts in several amino acid residues. A substantial difference consists only in the presence of linear determinants in the HPV6 L1 protein: 79–83, 308–314, which are not observed in HPV16 L1. In addition, the antigenic determinant 426–444 is present in the HPV type 16 protein, which has not been revealed in the virus type 6 protein.

In order to draw the conclusion that there are similar linear antigenic determinants in the two HPV types under consideration, it was necessary to compare the amino acid composition of the proposed epitopes. The difference in the amino acid composition can lead to a decrease in the degree of affinity with antibodies. The absence of substitutions in amino acid sequences or substitutions with amino acids similar in properties can preserve the level of antibody affinity. Therefore, it is important to determine the presence and evaluate the quality of amino acid substitutions in the proposed epitopes of the HPV6 L1 and HPV16 L1 proteins.

The paired alignment of protein sequences demonstrated substantial difference in the amino acid composition in most of the antigenic determinants. However, definite similarity was found between some amino acid sequences located within the boundaries of antigenic determinants close to both proteins. For example, in the determinant 166–177 for HPV16 L1, there was a difference in two amino acids: lysine was replaced

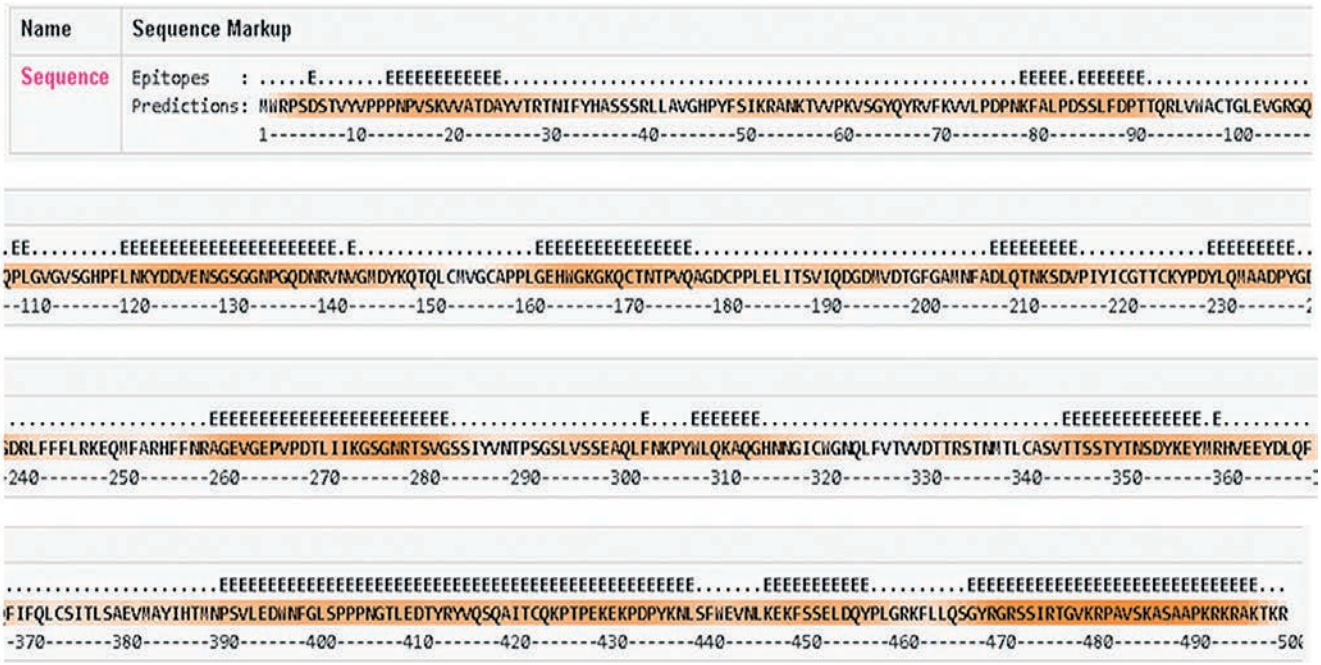

Fig. 3. Probable antigenic determinants of protein HPV6 L1 (BepiPred-2.0: Sequential B-Cell Epitope Predictor).
Epitope Threshold = 0.5; E – antigenic determinants.

with serine, glutamine with proline (Fig. 4). Serine is a polar uncharged oxycarboxylic amino acid, and lysine is a polar positively charged one. Proline is a heterocyclic nonpolar amino acid, and glutamine is a polar uncharged one. Taking into account the difference in the physico-chemical properties of amino acids, for which there are differences in the supposed antigenic determinants, as well as the difference in the length of linear epitopes, we may assume a weak cross-interaction with antibodies.

In the determinant 213–220 for HPV16 L1, some difference of two amino acids was found to be relative to the HPV6 L1 sequence: threonine was replaced with alanine, and aspartic acid was replaced with glutamic acid (Fig. 5). Since threonine substantially differs from alanine in physical and chemical properties, such a replacement can affect the antigenic properties of the L1 protein. In case of the second substitution, we deal with polar negatively charged amino acids with almost identical physical and chemical properties. Considering this fact, as well as the fact that the amino acid substitutions in the sequences are located at some distance from each other,

HPV6 L1 162-177	GEHWGKGKQCTNTPVQ
HPV16 L1 166-177	GEHWGKGSPCTN----

Fig. 4. The result of amino acid alignment of presumed antigenic determinant 166–177.

HPV6 L1 208-216	DLQTNKSDV
HPV16 L1 213-220	-LQANKSEV

Fig. 5. The result of amino acid alignment of the presumed antigenic determinant 213–220.

we can assume that there are similar antigenic properties of this region for the two viral proteins.

Search for antigenic determinants for T cells

In order to obtain additional evidence of influence of the similarity of the antigenic determinants of viral proteins HPV6 L1 and HPV16 L1 on the cross-interaction of the antibodies with the antigens, which belong to the two pathogenic types of human papillomavirus, a search for potential antigenic determinants for T cells was conducted with the aid of the bioinformatic resource SYFPEITHI. This program ranks possible variants of antigenic determinants according to the probability of their interaction with T cells.

Analysis of the protein sequences of the two scrutinized viral proteins allowed us to reveal a common probable antigenic determinant for T cells, which was located in HPV6 L1 at position 300–309, and in HPV16 L1 at position 304–313 of the amino acid sequence. According to the rating compiled by program SYFPEITHI, the amino acid sequence AQL(I) FNKPYWL was the second most likely antigenic determinant for T cells (Fig. 6).

When comparing the amino acid sequence of this determinant, a difference of only one amino acid was revealed in the two proteins, in HPV16 L1 leucine was replaced with isoleucine. Such a replacement should not lead to a change in the antigenic properties of the determinant, since leucine and isoleucine belong to the same class of amino acids and have similar physico-chemical properties. As a result, it should be noted that this antigenic determinant in the HPV6 L1 and HPV16 L1 proteins may have similar antigenic properties. Furthermore, multiple alignment of all the full-length amino acid sequences of HPV16 L1 and HPV6 L1 presented in NCBI has shown that this segment of the sequence is probably highly conserved and is almost identical in these two proteins.

a

Pos	1 2 3 4 5 6 7 8 9 0	Score
86	S L F D P T T Q R L	23
300	A Q L F N K P Y W L	22
101	G L E V G R G Q P L	21
455	D Q Y P L G R K F L	21
65	Y Q Y R V F K V V L	20
209	L Q T N K S D V P I	20

b

Pos	1 2 3 4 5 6 7 8 9 0	Score
12	Y L P P V P V S K V	22
304	A Q I F N K P Y W L	22
372	L Q F I F Q L C K I	22
460	D Q F P L G R K F L	21
68	L Q Y R V F R I H L	20
213	L Q A N K S E V P L	20

Fig. 6. Probable antigenic determinants for T cells (HLA-B13 decamers) in the protein sequences HPV6 L1 (*a*) and HPV16 L1 (*b*) according to program SYFPEITHI.

Investigation of potential three-dimensional epitopes for B cells

Only one three-dimensional model of the HPV6 L1 protein was found in the PDB database (6L31, DOI 10.2210/pdb6l31/pdb). Unfortunately, this model was not informative for the program DiscoTope 2.0 Server, so we conducted a comparative analysis of three-dimensional antigenic determinants using literary data.

According to some scientific publications, the following domains are isolated from the HPV6 L1 protein, forming three-dimensional epitopes that can interact with B cells: F49, R53, A54; K52, R53, A54, N55; Y123, N128; G130, S131, G132; K169, T172, N173, P175, V176, Q177, A178; E262, V263, E265, P266; V344, T345, T346; S353. Critical paratopes for recognition are domains F49, R53, A54 and K169, T172, N173, P175, V176, Q177, A178 (McClements et al., 2001).

It was shown in our previous publication that the HPV16 L1 protein has a spatial epitope in domain K53–L61, which partially coincides with the critical domain F49, R53, A54 of the HPV6 L1 protein. In addition, the location of the epitope S353 of the HPV6 L1 protein coincides with the three-dimensional antigenic determinant T350–Y355 of the HPV16 L1 protein (Stolbikov et al., 2020). In order to determine the level of similarity of the immunological properties of these two proteins, we analyzed the paired alignment of the amino acid sequences in the domains of their assumed three-dimensional antigenic determinants. Inconsistencies in amino acid residues were found in the supposed epitopes of antigenic proteins. In the position of 53 amino acid sequence in HPV16 L1, arginine is replaced with lysine, and in the position of 353 – serine with glutamic acid. These amino acid substitutions may be considered insignificant due to the similarity of the physico-chemical characteristics of the corresponding amino acids.

Discussion

According to the results obtained by us, it can be stated that there is a definite similarity between the antigenic determinants of HPV16 L1 and HPV6 L1 proteins. At the same time, with regard to B cells, the potential for cross-interaction of antibodies with antigens due to the similarity of linear antigenic determinants and three-dimensional epitopes is not substantial. However, for two types (16 and 6) of L1 viral proteins, there is a substantial similarity of linear antigenic determinants for T cells. According to the results obtained with the aid of program SYFPEITHI, these determinants are in the second position, but, despite this, these have a fairly high probability score. At the same time, the amino acid sequences of these epitopes are almost identical. There is some difference only in one position, but it is not critical due to the similarity of the physico-chemical properties of amino acids, according to which there is a replacement of antigenic determinants in the amino acid sequence. Based on the above results, when immunizing HPV16 L1, we can expect a fairly reasonable cross-interaction of antibodies with HPV6 L1 antigens.

The results obtained give a definite explanation of the cause of the effect of cross-interaction of antibodies with antigens belonging to different pathogenic types of HPV identified earlier. However, in order to obtain a more complete understanding of the mechanism of cross-interaction, it is desirable to study the phenomenon of polymorphic distribution of epitopes and the process of induction of *de novo* antibody synthesis (Brown et al., 2009; Kemp et al., 2011; Scherpenisse et al., 2013; Nakagawa et al., 2015).

Conclusion

The efficiency and expediency of the approaches and methods used in this work is confirmed by publications of other scientists. For example, it is known from the literary sources that a team of authors (Namvar et al., 2019) have used the same bioinformatic resources as our team (BepiPred-2, SYFPEITHI) to conduct an investigation of the cross-immune response to the surface proteins L1 and L2 of human papillomaviruses of highly oncogenic types 16 and 18. In this work, much attention was paid to the comparison of amino acid properties, such as hydrophobicity, the surface area accessible to the solvent, the charge and the secondary structure of the identified similar antigenic determinants of two different types of HPV. As a result of this investigation, a candidate multiepitope vaccine was created, the tests of which on laboratory mice showed rather good results. Application of this recombinant vaccine helped to induce a sufficiently strong immune response and protected mice from tumor cells with an efficiency of about 66.67 % (Namvar et al., 2019).

In conclusion, it should be noted that application of the research methods discussed above can substantially accelerate the development of efficient broad-spectrum vaccines against highly dangerous types of HPV. To date, there is a vaccine ‘Gardasil-9’ that provides protection against 9 types of oncogenic HPV, but it already contains the maximum permissible amount of antigenic proteins (270 μg of protein in one dose),

while it does not provide protection in about 10 % of cases (Li et al., 2018). Therefore, an extended study of cross-interaction of antibodies with antigens belonging to different pathogenic types of HPV conducted with the aid of bioinformatic analysis techniques can help in development of multi-epitope wide-action vaccines without increasing the number of antigenic proteins in the vaccine preparations.

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ORCID ID

A.S. Stolbikov orcid.org/0000-0002-6392-9365
R.K. Salyaev orcid.org/0000-0002-7602-7301
N.I. Rekoslavskaya orcid.org/0000-0003-3480-9855

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