Resource potential of some species of the genus *Miscanthus* Anderss. under conditions of continental climate of West Siberian forest-steppe

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In the meantime, search for environmentally friendly renewable energy sources alternative to fossil fuel has been driven by energy security challenges including limited availability of fuel and energy price fluctuations. Therefore herbal perennial grasses with their rapid growth and prominent biomass yield increasingly make it a favorite choice as a valuable agricultural crop usable for cellulosic ethanol production. As an example, the genus *Miscanthus* Anderss. (silvergrass) comprises ca. 14–20 species including *M. sacchariflorus* (Maxim.) Hack., *M. sinensis* Anderss., *M. purpurascens* Anderss, and *M.* × *giganteus*, which appear to be an almost inexhaustible source of sustainable raw material, and several *Miscanthus* species were investigated as a potential biofuel energy crop with commercially viable way of its producing. Introduction and investigation of *Miscanthus* species were initiated in the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG SB RAS, Novosibirsk, Russia) based on the grass and ornamental plant collection in the late 1990s. The paper objective is studying the biological traits of three *Miscanthus* species introduced into the CSBG SB RAS, selection and genetic identification of cultivars and varieties as the most perspective agricultural crop. To evaluate the potential crop yield and selection prospects of *Miscanthus* species being competitive as a valuable biofuel energy crop, the authors have estimated seasonal rhythms of model species development in the continental climate conditions of West Siberia. The article characterizes different *Miscanthus* varieties obtained either by the *ex situ* or *in situ* methods; presents the biochemical analysis of plant material and molecular identification of three *Miscanthus* species introduced into the CSBG SB RAS. The seasonal development analysis of three selected varieties of *Miscanthus* (*M. sacchariflorus*, *M. sinensis*, and *M. purpurascens*) proved the hydrometeorological conditions to be advantageous for prominent biomass yield, e. g. contributory to use *Miscanthus* in West Siberia as an easy to grow cellulose-rich grass. Molecular markers applicable in DNA-identification and genetic passportization of *Miscanthus* varieties have been established, which are perspective as such an economically available plant material as alternative non-woody source of cellulose.

Key words: *Miscanthus*; bioenergy; phenology; biomorphology; reproductive biology; chemical composition; DNA sequencing; ITS locus; *trn*L-F intron.

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Ресурсный потенциал некоторых видов рода *Miscanthus* Anderss. в условиях континентального климата лесостепи Западной Сибири

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В настоящее время весьма актуальны задачи по поиску альтернативных древесине источников энергии, экологически безопасных и экономически доступных. В связи с этим особый интерес представляют виды травянистых растений с высокой скоростью роста и характеризующиеся высокими значениями нарастания надземной вегетативной массы, имеющие практическое применение в качестве источника биоэтанола. Примером может служить род *Miscanthus* Anderss. (веерник), включающий примерно 14–20 видов, в том числе *M. sacchariflorus* (Maxim.) Hack., *M. sinensis* Anderss. и *M. purpurascens* Anderss., а также *M.*×*giganteus*, которые являются практически неисчерпаемыми источниками возобновляемого сырья в области альтернативной энергетики. В Центральном сибирском ботаническом саду (ЦСБС) СО РАН (Новосибирск) на основе коллекции газонных и декоративных злаков в конце 1990-х гг. было начато формирование и изучение родового комплекса *Miscanthus* Anderss. Целью этого исследования стало изучение биологических особенностей видов *Miscanthus*: *M. sacchariflorus*, *M. sinensis* и *M. purpurascens*, интродуцированных в ЦСБС, отбор и генетическая идентификация перспективных форм в качестве технических сырьевых растений. Для оценки ресурсного потенциала и перспективы селекционной работы с родовым комплексом *Miscanthus* с целью хозяйственного использования в качестве технической (биоэнергетической) культуры в условиях лесостепи Западной Сибири были изучены сезонные ритмы развития модельных видов в условиях континентального климата в сравнении с муссонным и умеренно континентальным; охарактеризованы биоморфы, образующиеся *ex situ* и *in situ*; определен химический состав растительного сырья и проведена идентификация по молекулярно-генетическим мар-

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керам трех видов *Miscanthus*, интродуцированных в ЦСБС СО РАН. Анализ сезонного развития трех отборных форм веерников (*M. sacchariflorus*, *M. purpurascens* и *M. sinensis*) показал, что гидротермические условия благоприятствовали получению вегетативной массы растительного сырья, т. е. использованию в качестве технической культуры в условиях лесостепи Западной Сибири. Выявлены формы для дальнейшей селекции и молекулярные признаки для разных видов мискантуса, которые можно использовать для идентификации и паспортизации форм и линий, перспективных для получения экономически доступного растительного сырья – альтернативных источников целлюлозы недревесного происхождения.

Ключевые слова: род *Miscanthus*; биоэнергетика; фенология; биоморфология; репродуктивная биология; химический состав; секвенирование; локус ITS, *trn*L-F.

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According to domestic and foreign scholars, only 5 % of the vascular plants species of the world flora are studied regarding to their utility for humanity (Revin, 2000). Introduction in the culture of food, technical and ornamental plants of local and non-local flora allows reducing the load on resource species in their natural habitats. An integrated approach to solve these problems includes studying the biological and ecological characteristics of species *in situ* and *ex situ*, which has been discussed at the International Congresses of Botanical Gardens (Spain, 2004, Poland, 2007, Ireland, 2010, China, 2012) on repeated occasions.

A special group of objects is represented by species showing high polymorphism in nature and at the same time having a wide range of practical applications in various economic fields. The generic complex of *Miscanthus* Anderss. including *M. sacchariflorus* (Maxim.) Hack., *M. sinensis* Anderss., and *M. purpurascens* Anderss., originally known as ornamental grasses, is unique in this respect. However, over the past two decades, those species, as well as *M. × giganteus*, have been referred to the plant objects valued as practically inexhaustible sources of renewable raw materials for pulp production that in turn is a base product for developments in the field of alternative energy (Slynko et al., 2013).

The collection of lawn and ornamental grasses is a part of the bioresource scientific "Collection of Living Plants in the Open and Closed Ground" USU 440534 of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG SB RAS). Forming the collection of living plants and studying the *Miscanthus* family complex started in the late 1990s mainly due to receiving the cloned material from other botanical gardens.

Since 2003, the collection was widened by *Miscanthus* samples from their natural habitats at the Russian Far East. The most significant replenishments occurred in 2013 (samples from the Gamov Peninsula, Primorsky Krai) and 2017 (Chuguev and Khasan districts of Primorsky Krai, the Kuril Islands – Shikotan, Kunashir, Iturup, and Kamchatsky Krai). Selection of promising plant raw materials as a valuable alternative biofuel material being the ultimate research objective, there is a need to study genetic polymorphism and DNA marking in these *Miscanthus* species.

Miscanthus is one of the most efficient solar energy accumulators on the planet (Dohleman, Long, 2009). According to physiologists' research, *Miscanthus* species have the highest potential productivity. Obtaining up to 40 tons of dry biomass per hectare is associated with C_4-type photosynthesis typical for all representatives of this genus. Unlike most of the traditionally cultivated C_4 plants, such as sugar cane and corn, *Miscanthus* are able to maintain a high intensity of photosynthesis even under rather low temperatures (Naidu et al., 2003; Anisimov et al., 2016), which explains the high productivity of these plants grown under more severe climatic conditions than in natural ones.

The prospects of *Miscanthus* growing in the Ob forest-steppe region is conditioned by the fact that C_4 photosynthesis is ineffective in low-light terms due to high energy requirements according to some scholars. In *Miskanthus* natural habitats (Vladivostok), the average annual number of sunshine hours is 2140, while in Novosibirsk – 2077, that is much higher than in Moscow (1731) and approximates to the parameters of Krasnodar (2110) and Yalta (2185).

The study objective is to investigate the biological features of *Miscanthus* species (*M. sacchariflorus*, *M. sinensis* and *M. purpurascens*) introduced into the CSBG SB RAS, along with selection and genetic identification of promising forms as technical raw plants.

Materials and methods

Three species were chosen as model ones, their ecological and biological characteristics being studied since 2012 in the CSBG SB RAS. The introduced samples were collected at the Russian Far East. Taxonomic signs are presented by Voroshilov (1982) as follows:

M. sacchariflorus. A lemma has a short straight awn not protruding from a tylosis floccus. The floccus hairs are white, rarely reddish; a rhizome has thin creeping shoots. It grows at the territory of the Russian Federation in Primorye, the western and southern regions of the Amur River basin.

M. sinensis. Inflorescence rami are branched; both spikes are at some distance from the axes of spigs. Glumes are asperous with long hairs. Tylosis flocci of a lemma are rather sparse, squarrose after flowering, dirty white, but quite often reddish as well. The lemma awn is considerably protruding from of a floccus, well visible, genuflexuous. A rhizome is shortened, thick. It occurs on Sakhalin and the Kuril Islands.

M. purpurascens. Inflorescence rami are unbranched (sometimes, except for a base itself); a sessile spikelet is located directly on the sprig axis. Glumes are only asperous. Flocci after flowering are often not so squarrose, denser and reddish. A rhizome is long, horizontal. The species inhabits Primorye. Records for Sakhalin and Kuril Islands are erroneous apparently.

Miscanthus growing rhythms and development were studied by techniques of phenological observations in the USSR botanical gardens (Methodology…, 1975) modified and supplemented in the course of the research by G.A. Zueva in compliance with objects' peculiarities. Hydrothermal characteristics of vegetation and rest periods were calculated from the data of Novosibirsk State Agrarian University and the weather station Ogurtsovo (Novosibirsk Region), and the climate monitoring data (http://www.pogodaiklimat.ru/) as well.

Biomorphological investigations were based on the data on *Miscanthus* life forms presented by A.B. and T.A. Bezdelevs (Bezdelev, Bezdeleva, 2006) for *M. sacchariflorus* and *M. sinensis*. Characteristics of *M. purpurascensis* life form are not given in this biomorphological report. For morphometric parameters, mean values (X), error (s_x), and a coefficient of variation (V, %) are presented.

The chemical composition of plant samples (leaves) collected in the CSBG SB RAS in the first decade of October was defined at the Laboratory of Bioconversion of the Institute for Problems of Chemical and Energetic Technologies SB RAS (Altaisky Krai).

Miscanthus plant specimens were grounded with scissors. Cellulose mass fraction determined by Kurschner method (in terms of absolutely dry raw materials – adrm), acid-insoluble lignin mass fraction (adrm), pentosan mass fraction (adrm), ash content (adrm), mass fraction of extractive substances – fat-waxy fraction (FF) (extractant – dichloromethane, adrm) were carried out according to standardized analysis of plant raw materials (Obolenskaya et al., 1991).

To extract the genomic DNA, CTAB method developed by Doyle and Doyle (1990) with dry plant material (leaves) was applied. The DNA concentration was determined by spectrophotometry using a BioSpectrometer Kinetic and µCuvette G1.0 microcuvettes (Eppendorf, Germany).

Primers ITS4 and ITS5 and the PCR protocol recommended by White et al. (1990) were used to amplify the internal transcribed spacer 1 and 2 regions (ITS1-2) of the nuclear ribosomal DNA. To amplify the *trn*L-F intergenic spacer of chloroplast DNA, universal primers "c" and "f" (Taberlet et al., 2007) were applied, as well as PCR protocol proposed by Amirahmadi et al. (2010). DNA loci amplification was performed on Thermal Cycler C1000 (Bio-Rad, USA). The length of the amplified fragments was analyzed by electrophoretic separation in an agarose gel (1.5 %) in Sub Cell Model 96 chambers under the current (4 V/cm). The amplified fragments were stained with SYBR-Green (Thermo Fischer Scientific, USA); visualizing and video recording of the separated PCR fragments were carried out with Gel-Doc XR + gel documentation and the Bio-Rad ImageLab Software systems.

The obtained individual DNA fragments of ITS1-2 and *trn*L-F intergenic spacers were purified from PCR components by AMPureXP sorption (Agencourt, USA) and sequenced directly using the BigDye Ready Reaction DNA Sequencing Kit v.3.1 kit (Thermo Scientific, USA). The Sanger reaction products were purified from unincorporated fluorescent dyes by centrifugation (900 g, 2 min) with a Sephadex G-50 Fine column (750 µl suspension) (GE Healthcare, USA), and scanned with 3130XL automatic genetic analyzers (Applied Biosystems, USA) at the Collaboration Centre "Genomics" (Novosibirsk). The obtained sequenograms were applied to mark species of the genus *Miscanthus*: *M. sacchariflorus*, *M. sinensis* and *M. purpurascens*. The comparison was made in nucleotide bases pairs (nbp).

MEGA 7.0.25 software (Kumar et al., 2016) was used to align the nucleotide sequences by the Clustal-W method, to calculate p-distances and to determine GC-nucleotides content; a scheme of genetic relationships of the analyzed samples was constructed by the Maximum Parsimony method.

Results

Miscanthus morphology and phenorhythm peculiarities under the conditions of West Siberian forest-steppe

To study the phenorhythmics in *Miscanthus* three species, the hydrothermal character of vegetation periods in various regions was analyzed (Table 1): (1) natural habitat zone (Vladivostok, mid-latitude monsoon climate); (2) region with a long-term successful experience of *Miscanthus* raw materials commercial production (Penza, mid-latitude moderate-continental climate); (3) experimental sites in the risk crop farming zone (Novosibirsk, mid-latitude continental climate).

Table 1 shows that for their spring regrowth and development *Miscanthus* gain from the thermal regime in conditions of Penza and even Novosibirsk (May), however, in late September–October this advantage over natural habitats is leveled. It is the heat accumulation in the autumn months that contributes to a full-fledged generative development and seed production in Primorsky Krai.

When comparing the temperature and solid precipitation in Novosibirsk, Penza and Vladivostok in winter, it was revealed that unlike the warmer growing conditions in Vladivostok in September–October, the winter temperature regime in natural habitats is more severe in December–January as opposed to Penza (Table 2). Compared to Novosibirsk, Vladivostok in winter has less snow precipitation, but this does not damage wintering reproduction buds *in situ*. Thus, 100 % winter hardiness of the genus *Miscanthus* representatives in Novosibirsk is explained by the historically developed and genetically fixed adaptive capabilities of the Far Eastern plants.

Studying the seasonal development of plants introduced into the culture makes it possible to draw more reliable conclusions on prospects, or, on the contrary, the inadequate stability of species and varieties, only if the long-term research period includes either dry vegetative season or the one with heavy winter precipitation, or extreme overwinter. For six-year long observations of *M. sacchariflorus*, *M. sinensis*, *M. purpurascens* in the forest-steppe conditions of West Siberia, it has been noted the following:

Table 1. Hydrothermal character of the vegetative season in geographical regions of *Miscanthus* cultivation, representing three types of climate

Indicator	April	May	June	July	August	September	October	November
Novosibirsk, type of climate: continental								
Mean maximum, °C	7.8	18.4	22.9	25.0	22.4	15.6	7.2	–3.7
Average temperature, °C	2.3	11.7	16.8	19.2	16.5	10.1	2.9	–7.1
Mean minimum, °C	–2.0	6.0	11.4	14.1	11.5	5.9	–0.3	–10.0
Precipitation norm, mm	28	34	50	72	49	42	46	38
Penza, type of climate: moderate continental								
Mean maximum, °C	12.1	20.7	24.5	26.4	24.6	18.1	9.7	0.6
Average temperature, °C	6.5	13.9	18.0	19.9	17.9	12.1	5.4	–2.3
Mean minimum, °C	1.5	7.5	12.0	13.8	12.0	7.2	1.9	–4.7
Precipitation norm, mm	33	41	63	59	50	52	46	45
Vladivostok, type of climate: monsoon climate								
Mean maximum, °C	9.4	14.7	18.1	21.9	24.1	20.2	13.4	3.3
Average temperature, °C	4.9	9.8	14.0	18.3	20.8	16.4	9.4	–0.3
Mean minimum, °C	1.9	6.7	11.4	16.2	18.5	13.5	6.4	–3.1
Precipitation norm, mm	62	95	89	164	145	92	59	41

Table 2. Hydrothermal character of the winter period in the geographical regions of *Miscanthus* cultivation in three climate types

Indicator	December	January	February	March
Novosibirsk, continental climate				
Mean maximum, °C	–10.1	–12.2	–9.8	–2.1
Average temperature, °C	–13.9	–16.1	–14.3	–7.0
Mean minimum, °C	–17.5	–19.6	–17.9	–11.1
Precipitation norm, mm	31	26	15	17
Penza, moderate continental climate				
Mean maximum, °C	–4.4	–5,5	–5.1	1.0
Average temperature, °C	–7.4	–8.7	–9.1	–3.4
Mean minimum, °C	–10.3	–11.9	–12.5	–7.2
Precipitation norm, mm	41	38	31	35
Vladivostok, monsoon climate				
Mean maximum, °C	–6.0	–8.2	–4.1	2.1
Average temperature, °C	–9.9	–12.2	–8.5	–1.9
Mean minimum, °C	–12.6	–15.3	–11.3	–4.7
Precipitation norm, mm	26	10	25	35

– in 2012, the vegetation period was abnormally dry and hot, there was 38 % of the precipitation norm in June, and only 6 % in July; an average monthly air temperature exceeded the average annual one by 4.7 °C in June, and by 3.1 °C in July;
– in 2013, the vegetation period had excess moisture, the precipitation exceeded an average long-term data being as much as 115 % in July, 284 % in August (171 mm), and 135 % in September;
– the early wintering in 2013 (November) was relatively mild, especially considering the almost twofold excess of snow precipitation;
– in 2016 the early wintering was extreme, when in November during the entire second decade, the minimum air temperatures were below –20 °C, and even –25 °C for six days, with a slight excess of mean annual solid precipitation;
– the wintering in 2012–2013 was the harshest one (first of all, for phanerophytes, not for hemicryptophytes, which group the involved plants refers to), when 24 days in December were characterized by a minimum air temperature below –25 °C, of which 10 days were the coldest (from –30.0 to –41.5 °C).

However, the severe temperature conditions did not significantly affect *M. sacchariflorus*, *M. sinensis* and *M. purpurascens* winter hardiness.

It was found that during all the years of observation, the three plant species of *Miscanthus* had the same set of phenophases of vegetative and generative development (regrowth, tubing, earing and flowering). The exception was 2013, when the earing and flowering phases did not occur. It's explained not so much by the excessive precipitation leading to a turbulent vegetative development of plants similar to the Far Eastern monsoon climate, but lower air temperatures fixed in Novosibirsk at the same time. Probably, the heat shortage slowed the intrarenal differentiation of generative organs to such an extent that the plants couldn't reach even the earing phase before the first autumn frosts.

M. sacchariflorus stably differs from *M. sinensis* and *M. purpurascens* by earlier (5–10 days) regrowth; the advance persists even after entering the other phenophases. It should be noted that in its natural habitats of Primorsky Krai this species occupies the northernmost areas (e. g. Sikhote-Alin).

Table 3. Morphometric parameters of vegetative organs of *Miscanthus* in West Siberian forest-steppe (*ex situ*)

Length of vegetative shoot, cm		Number of leaves, pcs.		Leaf width*, cm		Leaf length*, cm		Straw diameter, cm	
$X \pm s_x$	V, %	$X \pm s_x$	V, %	$X \pm s_x$	V, %	$X \pm s_x$	V, %	$X \pm s_x$	V, %
M. purpurascens									
163.1 ± 4.3	13.1	10.5 ± 0.1	5.6	1.5 ± 0.1	13.4	57.2 ± 1.1	9.6	0.47 ± 0.02	21.80
M. sinensis									
213.0 ± 7.2	13.2	11.3 ± 0.2	6.4	2.1 ± 0.1	16.3	66.4 ± 1.8	10.3	0.51 ± 0.02	16.24
M. sacchariflorus									
220.7 ± 0.3	12.5	11.4 ± 0.2	7.1	2.1 ± 0.1	16.9	67.5 ± 2.2	10.9	0.51 ± 0.03	16.33

* The fourth top leaf.

Table 4. Chemical composition of *Miscanthus* samples ($X \pm s_x$, %) introduced in the CSBG SB RAS, Novosibirsk

Samples	Cellulose by Kurschner*	Lignin*	Pentosans*	Ash*	FF
M. purpurascens	48.4 ± 1.0	23.1 ± 0.5	21.7 ± 0.5	4.36 ± 0.05	2.2 ± 0.5
M. sinensis	49.1 ± 1.0	23.3 ± 0.5	20.7 ± 0.5	3.00 ± 0.05	2.6 ± 0.5
M. sacchariflorus	53.3 ± 1.0	28.1 ± 0.5	21.3 ± 0.5	5.66 ± 0.05	2.4 ± 0.5

Note: * In terms of absolutely dry raw material; FF – fat-waxy fraction.

The earliest entry into generative phases was marked for all the three species in 2014, which was characterized by early spring warming and thereafter by earlier soil thawing.

The most references (Greef et al., 1997; Nishiwaki et al., 2011; Gifford et al., 2015) devoted to various aspects of research and utilization of *Miscanthus* species, forms, hybrids and varieties point out that the concerned gene pool collection requires serious systematic verification.

To a large extent, the taxonomic accessory of vascular plants is traditionally determined by the quantitative and qualitative characteristics of generative organs and aboveground vegetative ones. However, in the *Miscanthus* species, the structure of the underground organs should also be taken into account. So, while studying *M. sacchariflorus* biomorphology in the CSBG SB RAS, it was revealed that the species life form in the Ob forest-steppe conditions matches its life form description made by A.B. and T.A. Bezdelevs (Bezdelev, Bezdeleva, 2006) for natural habitats in Primorye: a perennial summer-green herbaceous thin-long-rhizome sympodially growing polycarpic with an elongated erect shoot.

These authors described two biomorphs for *M. sinensis*. However, in our opinion, their description of the life form "a perennial summer-green herbaceous thick-long-rhizome loose-tussock sympodially growing polycarpic with a semi-rosellate erect shoot" is more relevant to *M. purpurascens*, while "a perennial summer-green herbaceous short-rhizome loose-tussock sympodially growing polycarpic with a semi-rosellate erect shoot" is referred to *M. sinensis*, for which particularization is character under local conditions.

The dynamics of shoot formation and morphometric parameters in culture were compared with shoots development of *M.* × *giganteus* under conditions of mid-latitude moderate continental climate (Penza). It is believed that one of the parental forms of *Miscanthus giganteus* is *M. sinensis* (Table 3).

Table 3 shows that the length of generative shoots in *Miscanthus* three species varies within 163–220 cm. In Penza the range of generative shoots of *Miscanthus giganteus* was 160–207 cm (Gushchina et al., 2016). The species studied in Novosibirsk (CSBG SB RAS) and Penza are similar in this parameter, as well as in the leaves width.

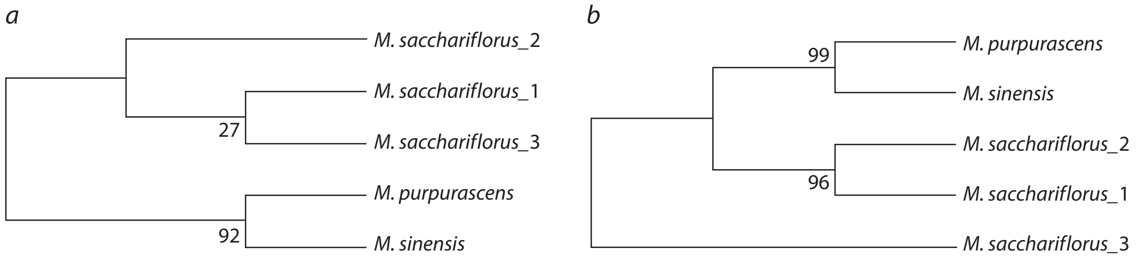
It should be noted that the stems and leaves characteristics are of particular importance when using *Miscanthus* as a technical crop. As these studies were carried on within the framework of SB RAS Integration Project "Fundamental basics of ethylene production from *Miscanthus*" (http://www.sbras.ru/files/files/pril_pso-230_15-08-17.pdf), a special attention was paid to obtaining raw materials and further analysis of its chemical composition for the content of cellulose, as well as lignin, pentosans, ash and fat-waxy fraction, which results are tabulated in Table 4.

As it turns out, *M. sinensis* and *M. purpurascens* are close not only by habitat regions, morphological features, but in chemical composition as well. *M. sacchariflorus* reveals higher values of both cellulose and lignin. Lignin high content can be associated with the greater ash content (6 %). In commercial production, it is caused by the higher concentration of soil acid-insoluble salts, which may result from the excessive mineral fertilizer application to obtain the maximum yield. However, to process such biomass into cellulose is much more difficult. It is noteworthy that under the CSBG SB RAS conditions, all the three *Miscanthus* species were grown on aligned agrarian background.

Miscanthus species genetic marking

To determine the genetic polymorphism and DNA marking in *Miscanthus* species, the variability of the length and nucleotide composition of ITS1-2 nDNA locus sequences and *trn*L-F chlDNA intergenic spacer were studied in three species: *M. sacchariflorus*, *M. sinensis* and *M. purpurascens*.

Based on the analysis of the ITS1-2 internal transcribed spacer of nuclear ribosomal DNA of *Miscanthus*, the variability of this region length was revealed, comprising



Genetic relatedness between *Miscanthus* representatives based on the ITS (*a*) and the *trn*L-F (*b*) data sets using Maximum Parsimony method.

The bootstrap values are given in the nodes.

440 bp in *M. purpurascens*, 486 bp – *M. sinensis* and 656–671 bp – *M. sacchariflorus*, correspondingly. The length of *trn*L-F xlDNA intergenic spacer chlDNA in *Miscanthus* varied in a smaller range: from 893 bp (*M. sinensis*) to 962 bp (*M. sacchariflorus*_2). The resulting *trn*L-F sequences are marked by a low content of G and C nucleotides (an average of 31.4 % for a locus) while for the ITS locus an average GC-nucleotide counts 61 %. ITS sequences are characterized by the presence of polyguanine fragments; *trn*L-F locus sequence – of a large number of poly-A and poly-T-blocks.

According to the sequencing data on nuclear (ITS) and chloroplast DNA (*trn*L-F) loci, the schemes were created (see the Figure), which reflect genetic polymorphism and related connections between representatives of the genus *Miscanthus*.

Both schemes reliably reveal the close relationship between *M. sinensis* and *M. purpurascens* (the bootstrap support value is over 90 %). Based on the analysis of different marker loci, two different models of clustering *M. sacchariflorus* samples were identified. According to sequencing ITS region, all three samples form a common clade (see the Figure, *a*). At the same time, a sample of *M. sacchariflorus*_3 and two samples of *M. sacchariflorus*_1 and *M. sacchariflorus*_2 form two separate clades according to the *trn*L-F locus analysis (see the Figure, *b*).

Discussion

The hydrothermal conditions of all vegetative periods (2012–2017), when the seasonal development of three selected forms of *Miscanthus* was studied (one of each species – *M. sacchariflorus*, *M. purpurascens* and *M. sinensis*), were favorable to the vegetative mass of plant raw materials production, i. e. their usage as a technical culture. Assessing the ornamental qualities of the objects revealed the only vegetative period of 2013, when not any sample entered the generative phase due to excess moistening. Neither earing nor flowering (when impressive thyrses develop being ornamental even over the winter snow) was observed.

Morphometric parameters of vegetative (technologically significant) Miscanthus organs in West Siberian forest-steppe were not inferior to the ones under the conditions of the moderate continental climate, varying at a low or medium level, thus making it possible to predict the yield (raw stock) rather precisely.

A general morphological peculiarity of *M. sacchariflorus* (which occupies northernmost habitats in monsoon and moderate continental climates) is the structure of underground organs, namely the thin-long-rhizome biomorph. Phenorhythm types and life forms of *M. sacchariflorus* and *M. sinensis* correspond to those under the natural conditions of Primorsky Krai. This testifies their rather high adaptive capabilities, and the prospects for selection of the most technologically (including biochemically) productive forms taking into account the intraspecific polymorphism.

Based on the analysis of the genetic relationships of the genus *Miscanthus* representatives and the sequencing data of two ITS1-2 and *trn*L-F loci, it should be concluded that obtained results are quite consistent in toto. The different nature of the phylogenetic trees branching can be conditioned by various types of inheritance (biparental and uniparental) character for nuclear and chloroplast markers, respectively, chosen to construct trees.

The revealed molecular features of *Miscanthus* different species can be used to identify and certify the *Miscanthus* forms and lines promising as available plant material, suitable for perspective environmentally safe alternative biofuel production.

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Conflict of interest

The authors declare no conflict of interest.

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