UDC 620.92

MISCANTHUS: GENETIC DIVERSITY AND A METHOD OF PLOIDY VARIABILITY IDENTIFICATION USING FLUORESCENT CYTOPHOTOMETRY

N. S. Kovalchuk, M. V. Roik

Institute of Bioenergy Crops and Sugar Beets NAAS
25, Clinichna Str., Kyiv, Ukraine, 03110

e-mail: natalakovalcuk461@gmail.com, sugarbeet@ukr.net

Received on November 01, 2017

Aim. Due to the introduction of the *Miscanthus* species, attributed to the European gene pool, in Ukraine, it is necessary to develop methods for the determination of genome ploidy and adjust them to the foreign methods in order to ensure high purity of the planting material, to study genetic diversity, to produce new polyploid lines and select alternative *Miscanthus* × *giganteus* clones (*3x*). **Methods.** Cytological, biotechnological, fluorescence cytophotometry, field, laboratory. **Results**. Domestic diploid millet (*Panicum*) variety of Veselopodilska Research Breeding Station and grain sorghum (*Sorghum*) variety Dniprovsky, whose number of chromosomes was previously investigated, served as standard genotypes for the ploidy identification with Partec ploidy analyser (Germany). Using the technique, various species of miscanthus, namely *Miscanthus* × *giganteus* (*3x*), *Miscanthus sinensis* (*2x*), and *Miscanthus sacchariflorus* (*2x*) were selected and multiplied by clones. The heterogeneity of the *Miscanthus* × *giganteus* (*3x*) population of the two ecotypes was determined based on the level of genome ploidy in the vegetative reproduction of rhizomes which originated from Poland and Austria. **Conclusions.** Due to the complexity of cytological research, the need to involve the representatives of the *Miscanthus* genus in the development of bioenergy in Ukraine, and to differentiate them both *in vivo* and *in vitro* conditions to assimilate the European gene pool, a new methodology for identification of plant material of different miscanthus species using the method of fluorescence cytophotometry is presented. The ploidy of commercial foreign samples of miscanthus, introduced in the network of research and breeding stations of the Institute of Bioenergy Crops and Sugar Beets of NAAS, was identified.

Keywords. Partec ploidy analyzer, bioenergy, nuclear DNA histograms, genome ploidy level, *in vitro* culture, fluorescence cytophotometry, *Miscanthus* × *giganteus, Miscanthus sinensis, Miscanthus sacchariflorus*.

DOI: 10.15407/agrisp4.03.019

INTRODUCTION

A distinguished feature of potential varieties of bioenergetic crops is efficient transformation of free solar energy into the industrial biomass with minimal negative impact on environment [1]. It is due to high yield and absence of unfavorable factors for ecology, that such energy cereal grasses as *Miscanthus* genus representatives are a relevant energy crop for production [2]. Due to C4 type photosynthesis, carbon fixation occurs much faster in these plants. The use of nutrients, water, solar radiation is more efficient in these plants, compared to others [3]. According to the researchers of bioenergy crops, all these physiological features affect the adaptation to different soil and climatic conditions of Ukraine [4].

Until recently, the main criteria of taxonomy for *Miscanthus* Anderss. genus have changed a lot. Most scientists refer it to *Poaceae* genus [5], including about 12 varieties, the most valuable ones for biomass production being Miscanthus *sacchariflorus*, *Miscanthus sinensis*, *Miscanthus* × *giganteus* and *Miscanthus floridulus* [6]. In Europe, the cultivation of the varieties of *Miscanthus* genus is mainly concentrated on growing *M.* × *giganteus* of tropic and subtropic origin [6, 7]. *Miscanthus* × *giganteus* (2n = *3x* = 57) is an interspecies hybrid, obtained from natural hybridization of the diploid species *Miscanthus sinensis* (2n = *2x* = 38)

and tetraploid Miscanthus *sacchariflorus* (2n = *4x* = = 76) [8]. High biomass performance of the obtained allotriploid is determined by heterosis effect and the combination of three genomes, which occurs in hybrid combinations. As a result, the sterile *Miscanthus* × *giganteus* (*3x* = 57) is reproduced only in the vegetative way – via rhizomes, rootlets or *in vitro* culture [9, 10]. The specificity of its reproduction affects the risk of its leaving the ecosystem and leads to extremely limited genetic diversity and the need for breeding of clones, adapted to new natural and climatic conditions [11]. It should be noted that *Miscanthus* × *giganteus* (*3x*) was isolated from natural populations of Japan; it has a considerable potential as an alternative source of energy. The first clone of *Miscanthus* × *giganteus* was imported from Japan to Denmark in 1935 as a decorative plant, and later – to North America for clonal reproduction and use for commercial purposes [6]. The natural populations of tetraploid *Miscanthus sacchariflorus* (*4x*) and diploid *Miscanthus sinensis* (*2x*) were studied in southern Japan and the ploidy of the obtained samples of seeds was identified [12]. The triploid shoots, collected on the plants of *Miscanthus sacchariflorus* (*4x*), were isolated by the method of flow fluorescence cytophotometry. In the opinion of scientists, studying wild species of *Miscanthus* genus, it is quite possible that triploid plants, collected in Kushima, may have resulted from the hybridization between (*4x*) *Miscanthus sacchariflorus* and (*2x*) *Miscanthus sinensis,* or due to the self-compatibility of (*4x*) *Miscanthus sacchariflorus*, via the fertilization of a *2x* egg with 1*x* pollen [12]. The cultivation of genetically homogeneous clones requires the study on the risk of resistance to diseases and to cold [7, 13]. A current task is to expand the genetic database of *Miscanthus* × *giganteus* (3*x*) via the development of hybrids of wild paternal forms of *Miscanthus sacchariflorus* and *Miscanthus sinensis.* New clones may serve as a source of genetic variability, resistance to new diseases, identified for clone *Miscanthus* × *giganteus* [14].

According to the scientific literature, two or three identical clones are grown in the global bioenergy industry, but the researchers believe that there is an enormous probability of the fact that wide-scale production of miscanthus for biomass in Europe is based on the use of one clone only [6]. A similar situation is observed in North America, where the cultivated genotypes of *Miscanthus* × *giganteus* were obtained using vegetative reproduction from one clone of European origin [14]. Using DNA technologies, *Greef et al*. applied the AFLP method to select 31 sample of *Miscanthus* × *giganteus*, 11 clones of *Miscanthus sinensis* and 2 clones of Miscanthus *sacchariflorus,* suitable for cultivation in botanic gardens and plant beds of Central Europe [15]. The researchers in the fields of botany and taxonomy believe that the genotype pool of *Miscanthus* × *giganteus* is remarkable for low diversity, they managed to identify only three samples using molecular and genetic markers [6]. At the same time, the genotype pool of *M. sinensis* is noted for rather a wide diversity. Genetic diversity of a species may be used to create new polyploid lines and highly productive clones.

Hodkinson T. R., Chase M. W. established using ISSR molecular markers that the population of *M.* × *giganteus* (11 taxons) did not have any variations, and insignificant variations, found using AFLP markers, could have been an error (Great Britain) [5]. On the contrary, diploid samples of *M. sinensis* (50 taxons) have a high level of deviations both by molecular-genetic markers and by their ploidy. In another study, De Cesare *et al.* (2010) confirmed that 14 out of 15 samples of *M.* × *giganteus*, collected in the botanic gardens of the Trinity College (Dublin, Ireland) and Hohenheim University (Germany), which were analyzed by six cpSSR marker loci, belonged to one haplotype, whereas *M. sinensis* and *M. sacchariflorus* demonstrated a high level of polymorphism for some alleles [16]. As stated by Ma *et al*., *M. sinensis* is a highly heterozygous species due to its hybridization, and the capability of forming viable seeds ensures the blossoming of components and compatibility by the homology of chromosomes in natural populations [17].

In the 1970s, the variability of miscanthus by the ploidy was observed by foreign researchers using the cytological analysis of metaphase chromosomes in natural populations: from diploids – 38 chromosomes to hexaploids – 114 chromosomes [18]. The level of ploidy for the species of *Miscanthus* genus also changed from 2 to 6 according to Polish researchers [6]. As per the data of Clifton-Brown, J., the basic ploidy of *M. sinensis* is *2x*, but there are common natural and artificial polyploids (for instance, triploid *M. sinensis* Goliath) [19]. It was established that in natural populations of China, *Miscanthus sacchariflorus* usually has a diploid form, contrary to Japan, but this variety has a whole number of ploidy variants, including the hexaploid one. Tetra- and pentaploids have already been obtained based on the components of crossing *M.* × *giganteus* [19]. They are the source of improving the variety of *M.* × *giganteus* for biomass in new natural and climatic conditions.

Due to unavailability of information about the origin and ways of differentiating the representatives of *Miscanthus* genus of the European genepool in Ukraine, except that by morphological features, and due to the use of allotriploid *M.* × *giganteus* (*3x*) in bioenergy industry, the aim of scientific studies is to investigate genetic diversity of varieties and their origin, to optimize the method of differentiating the genome ploidy level, and to harmonize it with the European ones to ensure the purity of the planting material. The species of *Miscanthus* genus, identified by the ploidy level, will be used to create polyploids and select new clones, alternative to *M.* × *giganteus* (*3x*). This work is generally concentrated on three species of Miscanthus, used in Europe for biomass production, namely *M.* × *giganteus, Miscanthus sacchariflorus and Miscanthus sinensis*. The following tasks arise from this aim:

• to optimize and introduce the method of fluorescent cytophotometry and to adjust it to the European methods to identify the ploidy level for the genome of initial materials;

• to identify in terms of ploidy and to reproduce in *in vitro* conditions *Miscanthus giganteus, Miscanthus sacchariflorus* and *Miscanthus sinensis* for the selection of new polyploid lines and development of miscanthus selection in Ukraine;

• to investigate the heterogeneity by the genome ploidy level for the populations of *M.* × *giganteus* (*3x*), which originated from Poland and Austria.

MATERIALS AND METHODS

Miscanthus × *giganteus* (*3x*), *Miscanthus sinensis* (*2x*), *Miscanthus sacchariflorus* (*4x*), reproduced at Yaltushkivska Research Breeding Station of the Institute of Bioenergy Crops and Sugar Beets (RSS IBCSB), were used as initial materials to master the method of identifying the ploidy level of the genome as the main taxonomic index of *Miscanthus* genus. The selection station was used to investigate their morphological features, to determine the terms of blossoming, probabilities of seed formation, specificities of growth and development, and the formation of rhizomes in Ukrainian conditions. The descriptions of different species of Miscanthus, introduced at the Yaltushkivska RBS IBCSB are as follows:

*Miscanthus sacchariflorus ecotype I "*Poland*"* – is a tetraploid species and a component of crossing for the triploid clone *Miscanthus* × *giganteus* (*3x*). This is a species with a stem of 2.5 m which colonizes the soil space quickly, forming solid plantations. The tetraploid level of the genome in the material was not confirmed by the results of ploidy analysis, conducted using PA Partec.

Miscanthus sinensis (*2x* = 38) – in 2016, during the first vegetation years at the experimental field of IBCSB, Chinese miscanthus formed the stems of 1.5 m based on underground roots, obtained at Yaltushkivska RBS.

Miscanthus x giganteus ecotype 2 *"Austria"* – gigantic miscanthus; the plants of this species reach as high as 3 m on the second year of vegetation in Ukrainian conditions. This is natural allotriploid with 57 chromosomes.

First in Ukraine, *Miscanthus giganteus* as a new energy crop was obtained by the specialists of the laboratory of cultivation of bioenergy crops and sugar beet of IBCSB at the beginning of the XXI century using the collection samples of Poland. New initial material was reproduced using rhizomes and underground roots by the selection number of *Miscanthus* × *giganteus* (3*x*) *ecotype "*Poland*"* with the components of *Miscanthus sacchariflorus ecotype I "*Poland*"* and *Miscanthus sinensis ecotype I "*Poland*"*. A new ecotype of gigantic miscanthus *Miscanthus* × *giganteus ecotype 2 "Austria"* was obtained in 2012. Two different ecotypes of European origin were studied by us by the heterogeneity of populations of variability of planting material (rhizomes) on the experimental fields of IBCSB "Baranivka" and "Hradiv". In 2015, the specialists of Yaltushkivska RBS reproduced a new European clone – *ecotype 3 Miscanthus giganteus "Great Britain"*, which was characterized by cold resistance.

The following collection samples of Yaltushkivska RBS were used to master and introduce the method of fluorescent cytophotometry and to identify the ploidy:

• Miscanthus x giganteus (ecotype 1 "Poland", ecotype 2 "Austria", ecotype 3 "Great Britain");

• Miscanthus sinensis ecotype 1 "Poland";

• Miscanthus sacchariflorus ecotype 1 "Poland";

• Miscanthus mearly "Germany", Jelitto Company;

• Miscanthus latte "Germany", Jelitto Company;

• *Miscanthus sinensis new "Germany" ecotype 2, Jelitto Company*.

The selection of external standards and references by nuclear DNA histograms involved the study of the following species by the number of chromosomes:

• a domestic diploid millet variety *"Poliano"* (*2x* = 18);

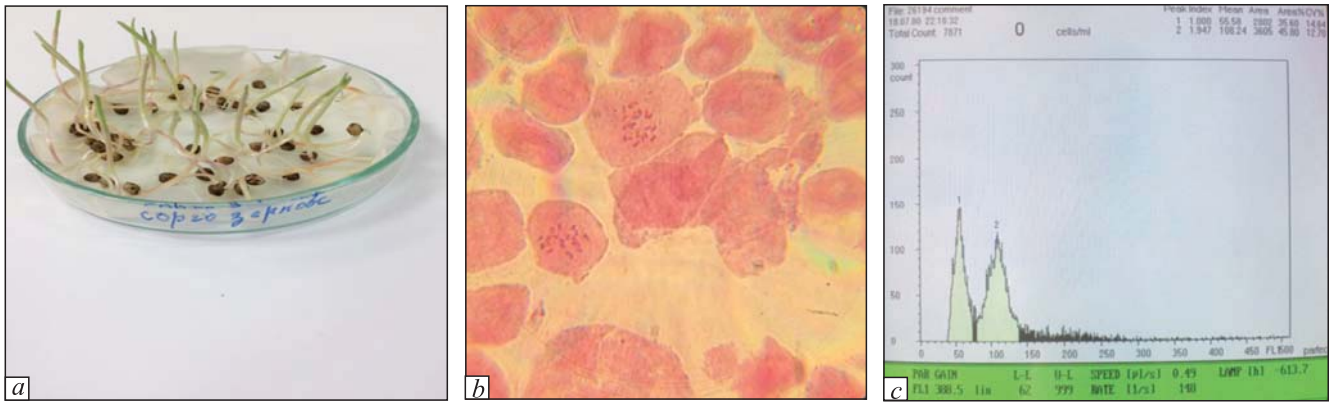

Fig. 1. The selection of the control genotype by the quantitative content of nuclear DNA at Partec PA: *a* – sorghum shoots as an object for ploidy determination; *b* – metaphase chromosomes of grain sorghum (2*x* = 20)*; c* – nuclear DNA histograms of grain sorghum, Dniprovsky variety

- grain sorghum (*2x* = 20), Dniprovsky variety;
- *Miscanthus sinensis* (*2x* = 38).

The cytological analysis was conducted using the method, modified by us (Pausheva Z.P., 1980) involving the staining of meristem cells and shoots with acetoorsein [20]. The number of chromosomes was defined at the stage of mitosis metaphase. The apical meristems of newly formed side roots and shoots of underground branches (rhizomes) were analyzed with the chromosome decrease of 0.03 % using 8-orthooxyquinoline and cold pre-treatment for 6–12 hours at 4 ºC.

The selected samples of diploid sorghum (*2x* = 20), Dniprovsky variety, were let sprout till the formation of the first couple of actual leaves (Fig. 1, *a*, *b*, *c*). The isolation of the standard genotype for the optimization of the method and determination of the genome ploidy level using the Partec ploidy analyzer was coordinated and adjusted to previously published main indices of polyploid species of miscanthus, obtained by Japanese researchers Aya Nishiwaki, Aki Mizuguti *et al*. [12].

RESULTS AND DISCUSSION

The level of genome ploidy is one of the main taxonomic indices of miscanthus. The cytological methods for diploid species of miscanthus of 2n = *2x* = 38 chromosomes, triploid species of 2n = *3x* = 57 chromosomes and tetraploid forms of 2n = *4x* = 76 chromosomes are rather cost- and labor-consuming. New methods of identifying the genome status of *Miscan-*

Table 1. The determination of optimal objects for miscanthus ploidy analysis using PA Partec by the variability coefficient

No	Kinds of miscanthus	Ploidy	Objects for analysis	Coefficient of variability, % (CV*)
1	*Miscanthus species*	*2x*	vegetative shoots	5.06–6.00
			leaves	8.08–16.33
			generative shoots	2.55–3.07
			in vitro	5.02–9.05
2	*Miscanthus sacchariflorus*	*4x*	vegetative shoots	6.01–9.00
			leaves	9.10–14.58
			generative shoots	4.08–7.09
			in vitro	1.1–6.88
3	*Miscanthus* × *giganteus*	*3x*	rhizomes	8.60–13.73
			leaves	9.00–12.48
			generative shoots	5.99–7.08
			in vitro	4.98–7.09

Note: CV* – coefficient of variability within the cells of the main DNA fraction in the histograms of PA Partec

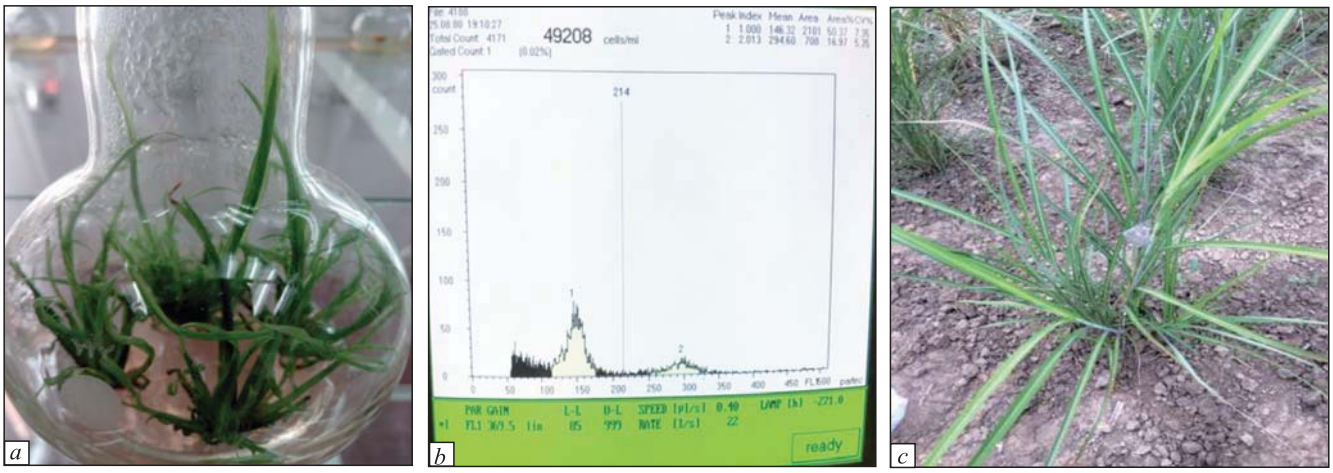

Fig. 2. The determination of ploidy for the representatives of *Miscanthus* genus*: a – Miscanthus sinensis* (2*x* = 38), in conditions; *b* – nuclear DNA histogram for *Miscanthus sacchariflorus* with max DNA on channels of 150 un. and 300 un.; *c – Miscanthus sacchariflorus* (2*x* = 38) of the first year of vegetation at the experimental field of IBCSB

thus genus species using the flow fluorescent cytophotometry and computer programs of PA Partec are introduced in different countries of the world and considered to be rather promising. To have a reference in terms of quantitative content of nuclear DNA for ploidy analysis on PA Partec, the researchers of *Miscanthus* genus use diploid plants of grain sorghum (*Sorghum bicolor*), green pea (*Pizum sativum*) and *Miscanthus sinensis*, previously defined by the number of chromosomes [7, 12, 13]. It was previously demonstrated using DNA-technologies that the genome of sorghum is closer to *Miscanthus* than to corn, rice and *Brachypodium distachyon* [6]. The species of *Sorghum bicolor* was first successfully used as a reference for the mass of nuclear DNA to study miscanthus in Japan [12]. The analysis of genome ploidy level is crucial for the classification of three main species of *Miscanthus* genus as well as the development of selection of new highly productive clones and purity of planting material.

Ploidy analyzer (PA) of Partec Company (Germany) is a cytometer, improved by computer programs which controls the analysis of nuclear DNA content in plant cells. In addition to the diploid sorghum, identified by the number of chromosomes and standard genotype, Polish researchers also use diploid forms of (*Miscanthus sinensis 2x* = 38), preserved and deposited in *in vitro* conditions [6].

The isolation of optimal objects and obtaining qualitative nuclear DNA histograms involved the studies of the following objects: the leaves of vegetative plants of different species of miscanthus; generative shoots; rhizomes of different species of miscanthus; leaves of miscanthus clones, reproduced in *in vitro* conditions (Table 1).

The experimental data in Table 1 include the information about the variability coefficient depending on the reproduction method *in vivo, in vitro* and the genome ploidy level. It was determined that a suitable object with a low coefficient of variability may be found in generative shoots and leaves of *Miscanthus* clones, reproduced *in vitro*. To prepare the control sample from the suspension of cells:

• the object to be analyzed is separated and cut in Petri dishes with the addition of 1.5 ml of the buffer solution;

• the buffer solution is used to extract and change the permeability of cellular membranes. It was found that a lysing buffer solution of Japanese researchers (Hamada and Fujcta, 1983) was suitable for application: 10 mM – aminomethane; 10 mM – Na2 – EDTA; 100 mM – NaCl; pH – 7.7; 100 ml – stock solution DAPI (Germany) [21];

• after the leaves are cut, 0.5 ml of fluorochrome DAPI solution (propidium iodide) is added to a Petri dish;

• the mixture is kept in Petri dishes for 5 min at ambient temperature and then filtrated through nylon filter to clean the nuclei from large cellular fragments and remains of leaves;

• the measurements of fluorescence and the number of nuclei in 1 cc of solution are performed at PA Partec with a multichannel analyzer. The tubes with cell suspension are switched on to electrodes.

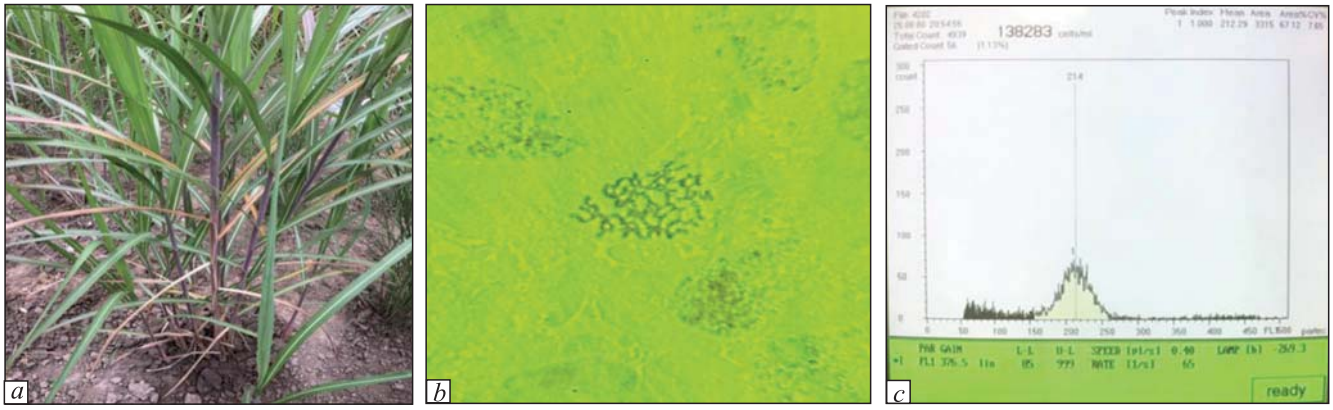

Fig. 3. The analysis of ploidy for *Miscanthus* (*3x*) by the number of chromosomes and mass of nuclear DNA: *a* – *Miscanthus x giganteus ecotype 2 "Austria" in vitro* conditions; *b* – metaphase chromosomes of *Miscanthus x giganteus* (*3x = 57*) at the increase of 12.5x100 magnification, determined by the analysis of rhizome shoots; *c* – nuclear DNA histograms for *Miscanthus x giganteus* with max DNA on the channels of 200 un. and 400 un.

The histograms describe the distribution of the investigated cellular substances, *i.e.* determine the number of cells with specific content of nuclear DNA: axis Ox (a channel) – quantitative classes of the investigated cellular substance (for instance, DNA); axis Oy (a count) – the number of cells in each channel; cells/ml – the number of cells in 1 ml; file – file number. The number of measurements of the device is from 2 to 150 thousand nuclei per sample.

To isolate the external standard by the quantitative content of nuclear DNA, the histograms of diploid millet variety of Veselopodilska RBS (*2x* = 18 and *4x* = = 36) and grain sorghum variety Dniprovsky (*2x* = 20) as well as diploid collection samples of *Miscanthus sinensis ecotype* 1 *"Poland"* were analyzed. The increase in the value of enhancing (FL1) was selected for G1 peak of the investigated nuclei, isolated from diploid grain sorghum, to be observed on channel 50 un. (G1) and 100 un. (G2).

It was established that as for the species of *Miscanthus sinensis ecotype* 2 *"Germany"* (*2x* = 38) in terms of the mass of nuclear DNA of external standards, the diploid level of genome corresponds to the quantitative class on the channel of 150 un. and the class of cellular substance (G2) of the synthetic and post-synthetic periods of the cellular cycle on the channel of 300 un. (Fig. 2 *a*, *b*, *c*). The collection samples of *Miscanthus latte "Germany"* and *Miscanthus mearly "Germany"* (*2x* = 38), used by Jelitto Company, as decorative species, corresponded to the diploid level of the genome according to the nuclear DNA histograms as well. *Miscanthus x giganteus* (*3x*) *ecotype* 1*"Poland"* and *ecotype* 2 *"Austria"* were characterized by the distribution in terms of the quantitative content of nuclear DNA on the channels of 200 un. (G1) and 400 un. (G2) in accordance to the external standard for grain sorghum, Dniprovsky variety, determined by us (Fig. 3 *a*, *b*, *c*).

Three most relevant species, reproduced by clones *in vitro* for polyploidation, are *Miscanthus sacchariflorus ecotype I "*Poland*", Miscanthus sinensis ecotype I "*Poland*", Miscanthus sinensis new ecotype* 2 "*Germany", Miscanthus* × *giganteus ecotype* 1 *"*Poland*"* and *ecotype* 2 *"Austria"*.

The analysis of the structure in terms of ploidy level of the genome of vegetating plants of the second year was conducted using *Miscanthus* × *giganteus ecotype* 1 "Poland*"* and *ecotype* 2 "*Austria"* from the experimental fields of Hrady, Baranivka, the experimental field of IBCSB. The objects of studies were leaves, generative shoots, rhizomes. The data are presented in Table 2.

According to the results of ploidy analysis for triploid plants of *Miscanthus* × *giganteus* (*3x*) of the second year of vegetation, which originated from Europe and were vegetatively reproduced using rhizomes, in Ukrainian conditions, we identify merely an insignificant heterogeneity of populations and the presence of rhizomes with both hyperaneuploidy and hypoaneuploidy status of the genome. To determine the reason of genome instability, it is necessary to conduct the studies on the mitotic division of cells of triploid clones depending on the reproduction term. There is an intention to reproduce the selected initial material by the vegetative mass and ploidy of the planting material (rhizomes) and to replicate it for the restoration of the purity and productive features of *Miscanthus* × *gigan-*

Table 2. The variability in terms of genome ploidy level for the populations of *Miscanthus x giganteus* of the second year of vegetation

Kind of miscanthus and external standard	Experimental numbers	Study object	Number of analyses	Max DNA (Mean)** on Partec PA channels			Coefficient of variability, % (CV*)	Ploidy
				50, 100	150, 300	200, 400		
External standard* brown durra $2x = 20$		acrospires	6	50.20–52.65			3.05–14.72	*2x*
Miscanthus species	2–1; 2–10	generative shoots	7		150.10		5.62–15.81	*2x*
Miscanthus × × *giganteus "Austria"*	7–1;7–19	leaves	19		145.43–160.0		4.98–9.01	*3x*
Miscanthus × × *giganteus "Austria"*	8–25	leaves	51		133.63–188.68	221.69	3.43–7.27	*3x* *3x*–n*** *3x*+n**** 4*x*
Miscanthus × × *giganteus "Poland"*	3–1; 3–3; 3–7	rhizomes	30		151.07–158.09		2.52–5.96	*3x*
Miscanthus × × *giganteus "Poland"*	3–9; 3–14	rhizomes	20		141.02–178.99		5.83–7.88	*3x* *3x*–n *3x*+n
Miscanthus × × *giganteus "Poland"*	4–2; 4–6; 4–9; 4–11; 4–12	rhizomes	50		143.15–180.13		3.09–11.16	*3x* *3x*+n *3x*–n

Note: * 20-chromosome line of grain sorghum, Dniprovsky variety $2x = 20$, as an external standard, was determined by the quantitative amount of nuclear DNA; ** average value of the fluorescence intensity of the main DNA fraction which corresponds to the quantitative classes for this variability coefficient; *** hypoaneuploidy status of the genome in the population of *Miscanthus* × *giganteus* during the vegetative reproduction using rhizomes; **** hyperaneuploidy status of the genome in the population of *Miscanthus* × *giganteus*.

teus ecotype 2 "Austria" and *ecotype 1 "Poland"* in the field conditions.

CONCLUSIONS

The method of determining the ploidy of miscanthus using the computer programs of Partec PA according to the quantitative content of nuclear DNA in the cell was mastered and adjusted to the foreign methods.

The method was coordinated with Japanese, Korean, and Polish researchers of the species of *Miscanthus* genus using grain sorghum, Dniprovsky variety ($2x = 20$); *Miscanthus sinensis* $2n = 3x = 57$, previously defined by the number of chromosomes, as standards and references of nuclear DNA.

The ploidy of collection samples of the species of *Miscanthus* genus was determined for the creation of new polyploid lines and selection of highly productive clones.

Miscanthus: генетична різноманітність видів та методика дослідження мінливості рівня плоїдності геному з використанням флуорисцентної цитофотометрії

Н.С. Ковальчук, М.В. Роїк

e-mail: natalakovalcuk461@gmail.com, sugarbeet@ukr.net

Інститут біоенергетичних культур і цукрових буряків НААН
Вул. Клінічна, 25, Київ, 03110, Україна

Мета. У зв'язку з інтродукцією видів роду *Miscanthus* європейського генофонду в Україні і насамперед поширенням єдиного стерильного алотриплоїдного клону *Miscanthus* × *giganteus* (*3x*), як найбільш перспективної біоенергетичної культури, необхідно розробити і узгодити з зарубіжними методики визначення рівня плоїдності геному для забезпечення сортової чистоти посадкового матеріалу, одержання поліплоїдних рядів і селекції клонів альтернативних *Miscanthus* × *giganteus* (*3x*). **Методи.** Цитологічні, біотехнологічні, флуоресцентної цитофотометрії, польові, лабораторні. **Результати.** Зовнішнім стандартом для визначення плоїдності з використанням аналізатора плоїдності (АП) «Partec» (Німеччина) виділений вітчизняний диплоїдний сорт проса «Поляно» Веселоподільської дослідно-селекційної станції (ДСС) та сорго зернове сорт «Дніпровський». З впровадженням методики флуоресцентної цитофотометрії відібрані за плоїдністю і розмноженні клонами різні види роду міскантус, *Miscanthus* × *giganteus* (*3x*), *Miscanthus sinensis* (*2x*), *Miscanthus sacchariflorus* (*2x*). Встановлена гетерогенність популяції *Miscanthus* × *giganteus* (*3x*) двох екотипів за рівнем плоїдності геному при вегетативному розмноженні ризомами, походження із Польщі і Австрії. **Висновки.** Через складність цитологічних досліджень, необхідність залучення представників роду *Miscanthus* для розвитку біоенергетики в Україні та диференціації представників роду *Miscanthus* в умовах *in vivo* та *in vitro* задля освоєння європейського генофонду, викладено нову методику ідентифікації рослинного матеріалу за рівнем плоїдності геному з використанням флуоресцентної цитофотометрії. Визначено плоїдність комерційних зразків міскантуса іноземного походження, що інтродуковані в мережі дослідно-селекційних станцій Інституту біоенергетичних культур і цукрових буряків.

Ключові слова: аналізатор плоїдності (АП) «*Partec*», біоенергетика, гістограми ядерної ДНК, рівень плоїдності геному, культура *in vitro*, флуоресцентна цитофотометрія *Miscanthus* × *giganteus, Miscanthus sinensis, Miscanthus sacchariflorus*.

Miscanthus: генетическое разнообразие видов и методика исследования изменчивости уровня плоидности генома с применением флуорисцентной цитофотометрии

Н. С. Ковальчук, Н. В. Роик

e-mail: natalakovalcuk461@gmail.com, sugarbeet@ukr.net

Институт биоэнергетических культур
и сахарной свеклы НААН
Ул. Клиническая, 25, Киев, 03110, Украина

Цель. В связи с интродукцией видов рода Miscanthus европейского генофонда в Украине и прежде всего распространением единого стерильного алотриплоидного клона *Miscanthus* × *giganteus* (*3x*), как наиболее перспективной биоэнергетической культуры, необходимо разработать и согласовать с зарубежными методики определения уровня плоидности генома для обеспечения сортовой чистоты посадочного материала, получения полиплоидных рядов и селекции клонов альтернативных *Miscanthus* × *giganteus* (*3x*). **Методы.** Цитологические, биотехнологические, флуорисцентной цитофотометрии, полевые, лабораторные. Результаты. Эталоном для определения плоидности по количеству ядерного ДНК с использованием анализатора плоидности (АП) *«Partec*» (Германия) выделены отечественный диплоидный сорт проса "Поляно*"* Веселоподольской опытно-селекционной станции (ОСС) и сорго зерновое сорт "Днепровский". С внедрением методики отобраны по плоидность и размноженные клонами различные виды рода мискантус, *Miscanthus* × *giganteus* (*3x*), *Miscanthus sinensis* (*2x*), *Miscanthus sacchariflorus* (*2x*). Установлена гетерогенность популяции *Miscanthus* × *giganteus* (*3x*) двух экотипов по уровню плоидности генома при вегетативном размножении ризом происхождения из Польши и Австрии. Выводы. Из-за сложности цитологических исследований, необходимости привлечения представителей рода *Miscanthus* для развития биоенергетики в Украине и дифференциации различных видов рода *Miscanthus* в условиях *in vivo* и *in vitro* с целью освоения европейского генофонда, изложена новая методика идентификации растительного материала с использованием флуоресцентной цитофотометрии. Определена плоидность коммерческих образцов *Miscanthus* × × *giganteus* (*3x*) иностранного происхождения, которые введены в систему опытно-селекционных станций Института биоенергетических культур и сахарной свеклы.

Ключевые слова: анализатор плоидности (АП) «Partec», биоэнергетика, гистограммы ядерной ДНК, уровень плоидности генома, культура in vitro, флюоресцентна цитофотометрия, *Miscanthus* × *giganteus, Miscanthus sinensis, Miscanthus sacchariflorus.*

REFERENCES

1. *Heaton EA, Clifton-Brown J, Voigt TB, Jones MB, Long SP.* Miscanthus for renewable energy generation: European Union experience and projections for Illinois. *Mitigation and Adaptation Strategies for Global Change*. 2004;**9**(4):433–51.
2. *Smeets EMW, Lewandowski IM, Faaij APC.* The economic and environmental performance of miscanthus and switchgrass production and supply chains in a European setting. *Renewable and Sustainable Energy Reviews*. 2008;**13**(2009):1230–45.
3. *Heaton EA, Voigt T, Long SP.* A quantitative review comparing the yields of two candidate C_4 perennial biomass crops in relation to nitrogen, temperature and water. *Biomass and Bioenergy*. 2004;**27**(1):21–30.
4. *Humentyk M.* Effect of planting method on productivity

of miscanthus rhizomes under the conditions of Forest-Steppe of Ukraine. *Bioenergy*. 2017;**9**(1):26–9.

5. *Hodkinson TR, Chase MW, Lledo MD, Salamin N, Renvoize SA.* Phylogenetics of Miscanthus, Saccharum and related genera (Saccharinae, Andropogoneae, Poaceae) based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL intron and trnL-F intergenic spacers. *J. Plant Res.* 2002;**115**(5):381–92.
6. *Maksimović J, Pivić R, Stanojkov-Sebiić A, Vučić-Kišgeci M, Kresović B, Dinić Z, Glamočlija D.* Plating density impact on weed infestation and the yield of *Miscanthus* grown on two soil types. *Plant, Soil and Environment*. 2016;**62**(8):384–8.
7. *Zub HW, Brancourt-Hulmel M.* Agronomic and physiological performances of different species of Miscanthus, a major energy crop: A review. *Agronomy for Sustainable Development*, 2010;**30**(2):201–14.
8. *Linde-Laursen I.* Cytogenetic analysis of Miscanthus 'Giganteus', an interspecific hybrid. *Hereditas*. 1993; **119**(3):297–300.
9. *Atkinson CJ.* Establishing perennial grass energy crops in the UK: A review of current propagation options for *Miscanthus*. *Biomass and Bioenergy*. 2009;**33**(5):752–9.
10. *Gubišová M, Gubiš J,* Žofajová *A, Mihálik D, Kraic J.* Enhanced *in vitro* propagation of *Miscanthus* × *giganteus*. *Industrial Crops and Products*. 2013;**41**:279–82.
11. *Rayburn AL, Crawford J, Rayburn CM, Juvik JA.* Genome size of three *Miscanthus* species. *Plant Molecular Biology Reporter*. 2009;**27**:184–8.
12. *Nishiwaki A, Mizuguti A, Kuwabara S, Toma Y, Ishigaki G, Miyashita T, Yamada T, Matuura H, Yamaguchi S, Rayburn AL, Akashi R, Stewart JR.* Discovery of natural Miscanthus (Poaceae) triploid plants in sympatric populations of *Miscanthus sacchariflorus* and *Miscanthus sinensis* in southern Japan. *Am J Bot*. 2011;**98**(1):154–9.
13. *Sacks EJ, Juvik JA, Lin Q, Stewart JR, Yamada T.* The gene pool of *Miscanthus* species and its improvement. *Genomics of the Saccharinae*. 2013:73–101.
14. *Glowacka K, Adhikari S, Stewart RJ, Nishiwaki A, Yamada T, Jørgensen U, Hodkinson TR, Gifford J, Juvik JA, Sacks EJ.* Genetic diversity in *Miscanthus* × *giganteus*: an abstract nr P0719. *Plant and Animal Genome XXI*, online materials. 2013, https://pag.confex.com/ pag/xxi/webprogram/Paper7634.html.
15. *Greef JM, Deuter M, Jung C, Schondelmaier J.* Genetic diversity of European Miscanthus species revealed by AFLP fingerprinting. *Genetic Resources and Crop Evolution*, 1997;**44**(2):185–95.
16. *De Cesare, Hodkinson TR, Barth S.* Chloroplast DNA markers (cpSSRs, SNPs) for *Miscanthus*, *Saccharum* and related glasses (Panicoideae, Poaceae). *Molecular Breeding*. 2010;**26**(3):539–44.
17. *Ma X-F, Jensen E, Alexandrov N, Troukhan M, Zhang L, Thomas-Jones S, Farrar K, Clifton-Brown J, Donnison I, Swaller T, Flavell R*. High resolution genetic mapping by genome sequencing reveals genome duplication and tetraploid genetic structure of the diploid *Miscanthus sinensis*. PLoS ONE7(3). 2012:e33821. https://doi.org/10.1371/journal.pone.0033821.
18. *Adati S, Shiotani I.* The cytotaxonomy of the genus Miscanthus and its phylogenic status. *Bulleting of the Faculty of Agriculture*. 1962;**25**:1-14.
19. *Clifton-Brown J, Chiang Y-C, Hodkinson TR.* Miscanthus: Genetic resource and breeding potential to enhance bioenergy production. In. W. Vermerris (Ed.), Genetic improvement of bioenergy crops. Springer, New York. 2008:273–94.
20. *Pausheva ZP.* Practical course on plant cytology. Moscow: Kolos. 1980: 300 p.
21. *Hamada S, Fujcta S.* DAPI staining improved for quantitative cytofluorometry. *Histochemistry*. 1983;**79**(2): 219–26.