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 Anders. 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 cytometry. 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 1x 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 (3x) 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, Greer 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 1 “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* (3x) ecotype “Poland” with the components of *Miscanthus sacchariflorus ecotype 1 “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);
• 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 °C.

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

Note: CV* – coefficient of variability within the cells of the main DNA fraction in the histograms of PA Partec
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thus genus species using the flow fluorescent cytophometry 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 (Pisum 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; 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 Fujita, 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.
The histograms describe the distribution of the investigated cellular substances, \textit{i.e.} determine the number of cells with specific content of nuclear DNA: axis (a channel) – quantitative classes of the investigated cellular substance (for instance, DNA); axis (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 (2n = 18 and 4n = 36) and grain sorghum variety Dniprovsky (2n = 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 nearly “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 \textit{in vitro} for polyploidation, are Miscanthus sacchariflorus ecotype 1 “Poland”, Miscanthus sinensis ecotype 1 “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-
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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.

Miscanthus: genetic diversity and a method of ploidy variability

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

<table>
<thead>
<tr>
<th>Kind of miscanthus and external standard</th>
<th>Experimental numbers</th>
<th>Study object</th>
<th>Number of analyses</th>
<th>Max DNA (Mean)** on Partec PA channels</th>
<th>Coefficient of variability, % (CV*)</th>
<th>Ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>External standard* brown durra 2x = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscanthus species</td>
<td>2–1; 2–10</td>
<td>acrospires</td>
<td>6</td>
<td>50.20–52.65</td>
<td>3.05–14.72</td>
<td>2x</td>
</tr>
<tr>
<td>Miscanthus × × giganteus “Austria”</td>
<td>7–1; 7–19</td>
<td>generative shoots</td>
<td>7</td>
<td>150.10</td>
<td>5.62–15.81</td>
<td>2x</td>
</tr>
<tr>
<td>Miscanthus × × giganteus “Austria”</td>
<td>8–25</td>
<td>leaves</td>
<td>51</td>
<td>133.63–188.68</td>
<td>3.43–7.27</td>
<td>3x</td>
</tr>
<tr>
<td>Miscanthus × × giganteus “Poland”</td>
<td>3–1; 3–3; 3–7</td>
<td>rhizomes</td>
<td>30</td>
<td>151.07–158.09</td>
<td>2.52–5.96</td>
<td>3x</td>
</tr>
<tr>
<td>Miscanthus × × giganteus “Poland”</td>
<td>3–9; 3–14</td>
<td>rhizomes</td>
<td>20</td>
<td>141.02–178.99</td>
<td>5.83–7.88</td>
<td>3x+n</td>
</tr>
<tr>
<td>Miscanthus × × giganteus “Poland”</td>
<td>4–2; 4–6; 4–9; 4–11; 4–12</td>
<td>rhizomes</td>
<td>50</td>
<td>143.15–180.13</td>
<td>3.09–11.16</td>
<td>3x+n</td>
</tr>
</tbody>
</table>

ACROSPIRIES GENERATIVE SHOOTS LEAVES RHIZOMES

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.

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 мілівості рівня плідності геному з використанням флуорисцентної цитофотометрії

Н.С. Ковалчук, М.В. Ройк

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 інтроductory видів роду Miscanthus європейського генофонду в Україні і насамперед поширенням єдиного стерильного алютропілюндного клону Miscanthus × giganteus (3х), як найбільш перспективної біоенергетичної культури, необхідно розробити і узгодити з зарубіжними методиками визначення рівня площинності геному для забезпечення сортової чистоти посадового матеріалу, одержання полілінійних рядів і селекції клонів альтернативних Miscanthus × giganteus (3х).

Методи. Цитологічні, біотехнологічні, флуорисцентні цитофотометрії, польові, лабораторні. Результати. Еталоном для визначення площі геному при вегетативному розмноженні ризома гібридного 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 розчинного матеріалу за рівнем площинності геному з використанням флуорисцентної цитофотометрії. Визначено площинність комерційних зразків клонів Miscanthus × giganteus

Ключові слова: аналізатор площинності (АП) «Par tec», біоенергетика, гістограми ядерної ДНК, рівень площинності геному, культура in vitro, флуоросцентна цитофотометрія Miscanthus × giganteus, Miscanthus sinensis, Miscanthus sacchariflorus.

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