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GETTING SEEDLINGS *MISCANTHUS x GIGANTEUS* BY METHOD OF CLONAL MICROPROPAGATION

The results of researches are shown on the getting of seedlings of plants Miscanthus x giganteus by biotechnological method. The presented comparative characteristics between plants are growing Miscanthus x giganteus from rhizomes and obtained by method clonal micropropagation.

***Keywords**: bioenergy, miscanthus, in vitro, adaptation.*

Introduction. Miscanthus - a perennial herb of the family *Poaceae*, which consists of about 40 species. Representatives of the genus distributed in tropical, subtropical and warm temperate areas of Asia, Africa and Australia. In Europe it been introduced in 1930 as an ornamental crops [5, 6, 7].

Miscanthus is unpretending to the soil. But worst grows on sandy and clay soils, the optimum pH – 5.5-7.5. Requires sufficient moisture, but can grow in relatively dry locations.

Plant of C_4 photosynthesis has erect stem height between 80-350 cm, leaves linear wide between 1-3 cm. Fan-shaped panicles long10-30 cm, spikelets length 0.3-0.7 cm. Plant form a rhizomes that store the apical bud and carry reduced leaves in the nodes. In the axils which are located of buds, from which form the ground shoots. Miscanthus propagates by seeds and rhizomes. Viability of seeds depends on the variety. Seed size 5x1 mm, weight of 1000 seeds 250 g [8].

Miscanthus is used as ornamental plants to control soil erosion, paper industry, production of building materials and as a bioenergy fuels [3].

For the growth of the commercial use of biomass are two types of *M. sinensis* (2n=2x=38) and diploid *M. sacchariflorus* (2n=2x=38) and tetraploid *M. sacchariflorus* (2n=4x=76). But sterile triploid hybrid *Miscanthus x giganteus* (2n=3x=57) is the most productive, it was obtained by crossing diploid *M. sinensis* and tetraploid *M. sacchariflorus. Miscanthus x giganteus* is sterile pollen and propagated vegetatively, by rhizomes, which are formed by 2-3 year of vegetation [8].

In recent years, great importance is the use of biotechnological methods in breeding high-breeding materials, and to generate new output forms of plants. The method of clonal micropropagation provides to a cured material for commercial value, it can propagate the breeding patterns throughout the year in a small laboratory space and increase the multiplication coefficient [1, 2].

Miscanthus x giganteus effectively propagated in culture *in vitro* [9], but impact level of ploidy on the multiplication coefficient miscanthus stills not studied and methods of approximation seedlings miscanthus in soil conditions is not designed.

Materials and methods of research. Research carried out in the sector of culture cells and tissues *in vitro* and in experimental field "Batyeva Ghora" of Institute of bioenergy crops and sugar beet NAAS Ukraine. The subject of the research were rhizomes, dormant buds *Miscanthus x giganteus*, inducing shoots by clonal micropropagation [4] and seedlings of *Miscanthus x giganteus* obtained by *in vitro*.

The basic material used dormant buds which were removed from rhizomes *Miscanthus x giganteus*.

The plant material was treated with 10 min. 0.005 % solution of potassium permanganate. Then buds dipped in a solution of mercuric chloride by weight 0.2% with exposure 1 h. The samples were washed with autoclaved distilled water for 1 h. Explants were cultured on modified medium Murashige and Skoog with the addition of 6-Benzylaminopurin (BAP) - 0.5 mg/l, kinetin - 1 mg/l, sucrose - 30 mg/l, pH 5.6-5.7.

Rooting of shoots was performed on a modified medium Murashige and Skoog with the addition of α-naphthylacetic acid (NAA) - 0.8 mg/l, kinetin - 1 mg/l, sucrose - 30 mg/l, pH 5.6-5.7. Cultivation was carried out in a thermal room with temperature 22-24 °C, relative humidity 70 %, light 3000 lux and 16 h photoperiod length.

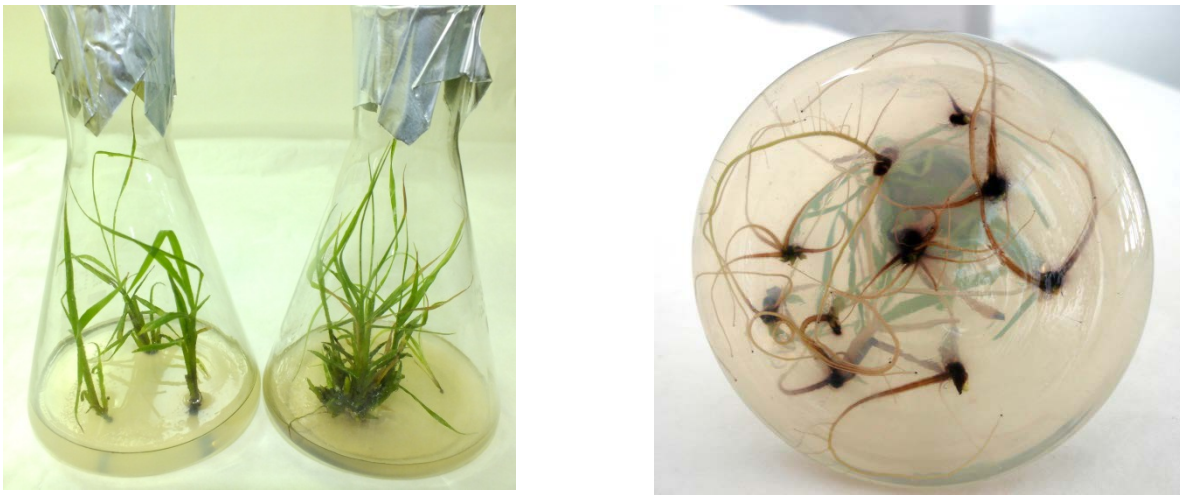
Adaptations of plants *in vitro* were performed in adaptive indoors in soil-perlitic mixture 3:1 - 2 months. Adaptation of micro-plants to environment was carried out at a temperature of 24-25 °C, humidity 70-80 %, light intensity fluorescent LD40 - 2000-3000 lux light period duration - 16 hours.

Rhizomes miscanthus are control. Adapted seedlings (I variant) and plants *in vitro* (II variant) *Miscanthus x giganteus* are landed simultaneously in late May in the soil of 100 pcs of 40 cm in length. During the growing season is accounting and observations conducted at the end of 1, 2, 3 months vegetation. There was noted plant height, number of shoots and leaves, length and width of the leaf blade.

Results of research. Research has shown that the difficulties sterilization associated with high infection initial explants. But a solution of mercuric chloride mass fraction of 0.2 % at 60-70 min, provides the highest percentage of sterile samples and allows the cultivation to get sterile germination dormant buds in a week. The formation of additional shoots in the number 2 pcs observed after 3-4 weeks of cultivation. Axillary branching is the primary method of conventional micropropagation. Of course, it is inhibited at one time or another by the presence of the main apical shoots, but the BAP in the culture medium removes apical dominance and causes branching shoots. The resulting lateral shoots were removed and transferred to fresh growth medium.

Researches have established the relationship between the concentration of cytokinins and formed numbers of shoots *Miscanthus x giganteus in vitro* culture: at a concentration of BAP - 0.5 mg/l in the culture medium was obtained with 1 shoot sprout up to 8 new. This multiplication coefficient allowed getting enough plant material for rooting after 5 passages (1 passage - 2 months). Shoots 5-6 cm in height were transferred to growth medium Murashige and Skoog of auxin. Use NAA

(0.8 mg/L) in the culture medium yielded 72 % of rooting plants. Formation of the root system is observed at 7-10 days of cultivation and is 2-10 roots of 1 shoot, with length of roots an average 1.3 cm (Fig. 1).



a) b)

Fig.1 Plants of miscanthus in vitro culture*:*

a) miscanthus by clonal micropropagation; б) formed of rooting by miscanthus.

Entrenched miscanthus plants that have high rates of shoots to 10 cm, well-developed root system, at least 4-5 roots with length an average of 1.1-1.5 cm, were planted for adaptation to soil-perlitic mixture into climatic boxes (Fig. 2). Coefficient of rooting was 99%.

Fig. 2 Adaptation of miscanthus in soil-perlitic mixture

When planting the II variant in the soil, necessary conditions are covering of insulation caps to create a microclimate for 7-10 days (Fig. 3).

Fig. 3 Planting cultural plants of miscanthus in soil

Researches have shown that coefficient of rooting was quite high for all variants and was 79-95% (Table 1).

Table 1

Features of development of plants *Miscanthus x giganteus* different ways of breeding

№	Number of breeding	Coefficient of rooting, %	Month	Number of shoots, pcs.	Height of plants, cm	Number of leaves, pcs.	Length of leaf, cm	Width of leaf, cm
1	Control *Miscanthus x giganteus* з ризом	95	1 months vegetation	2	64	5	32	1.3
			2 months vegetation	4	81	6	43	1.3
			3 months vegetation	5	102	9	54	1.4
2	I variant *Miscanthus x giganteus* with adaptation	92	1 months vegetation	2	28	7	15	0.5
			2 months vegetation	6	60	9	29	0.7
			3 months vegetation	9	84	9	40	0.8
3	II variant *Miscanthus x giganteus* without adaptation	79	1 months vegetation	4	38	7	20	0.6
			2 months vegetation	5	69	9	36	0.8
			3 months vegetation	11	90	10	42	0.9
4	HIP_{05}		1 months vegetation	1.61	15.95	1.72	7.74	0.10
			2 months vegetation	2.40	17.90	1.33	8.67	0.10
			3 months vegetation	4.14	13.62	1.41	7.11	0.08

Development of plants *Miscanthus x giganteus* were obtained by clonal micropropagation (I-II variants) per 1 month growing season inferior of plants obtained from the rhizomes of all parameters by 1.5 times. At the end of 3 months growing season plants of I-II variants has growth rates that are consistent with those of control 2 month growing season.

Active formation of shoots in cultural plants observed during the second half of the growing season. Plants I and II variants above control to formed the number of shoots during the growing season in 2 times. This is due to the action of cytokines on growth medium that has been accumulated in the tissues of *Miscanthus x giganteus* during cultivation in vitro (Fig. 4).

a) b)

Fig. 4 Plants miscanthus 3 month growing season: a) *M. x giganteus* - from rhizomes b) *M. x giganteus in vitro* culture

Comparing the I and II variants can be noted that the acclimated plants have lower rates of growth biomass during the growing season. Damage to the root system of plants miscanthus when transfer out boxes, which carried adaptation to the soil causes a delay of growth processes of plants and reduces the growth rates of plant height of 4 cm, leaf length 2 cm, leaf shape width 0.1 cm and formed at least 3 shoots.

Conclusions. The developed method of growing *Miscanthus x giganteus* using biotechnology methods allows accelerating breeding in commercial quantities.

Dormant buds of rhizomes of *Miscanthus x giganteus* can be used for vegetative propagation *in vitro*, which will get genetically identical plants to 3.000 pcs from 1 source explant.

The established peculiarities of development of plants *Miscanthus x giganteus* I year growing season obtained *in vitro*, which exceed the number of shoots of plants *Miscanthus x giganteus* obtained from the rhizomes by 2 times.

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Анотація

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Отримання розсади miscanthus x giganteus методом клонального мікророзмноження

Наведено результати досліджень з отримання розсади рослин Miscanthus x giganteus біотехнологічним методом. Представлена порівняльна характеристика вегетації рослин Miscanthus x giganteus, вирощених з ризом та отриманих методом клонального мікророзмноження.

Ключові слова: біоенергетика, міскантус, in vitro, адаптація.

Аннотация

Роик Н.В., Бех Н.С., Коцар М.О.

Получение рассады Miscanthus x giganteus методом клонального микроразмножения.

Приведены результаты исследований с получения рассады растений Miscanthus x giganteus биотехнологическим методом. Представлена сравнительная вегетационная характеристика между растениями Miscanthus x giganteus выращенных из ризом и полученных методом клонального микроразмножения.

Ключевые слова: *биоэнергетика, мискантус, in vitro, адаптация*