

INFLUENCE OF STRESS OF SALT IN VITRO ON THE DEVELOPMENT OF SHOOTS MISCANTHUS

The article presents the results of research of influence of stress of salt on the development of different species miscanthus in vitro on selective medium with different concentrations of NaCl. There are showed changes in the growth process, the amount of chlorophyll, the ability to rooting and dry matter accumulation miscanthus in this article.

Keywords: *miscanthus, in vitro, NaCl, chlorophyll, rooting.*

Introduction. Miscanthus is a perennial industrial crop, which is used mainly to produce thermal energy. For his growing is need to use the land on which crops can not grow and unprofitable. [1] These include saline soils that in Ukraine there are locally along the coast of the Black Sea, the Dnieper, on the terraces of the Southern Bug, Dniester, Danube [2]. The most harmful are chloride salinity soil, which is in violation of plants ultrastructure of cells, particularly in the structure of chloroplasts [3].

To speed up the selection process using biotechnology methods, including the method of culture in vitro, which allow a short time to get enough material for research [4]. In the application of biotechnology techniques have been received agricultural plants with signs of resistance to abiotic environmental factors, such as corn, sugar beets, tomatoes and so on. [5].

The aim of the study was to determine the effect of salt stress on the development of miscanthus shoots in culture and in vitro selection of tolerant forms.

Materials and methods. The study was carried out in the sector of culture cells and tissues in vitro and in control of seed specialized analytical and technological laboratory for sugar beet Institute of bioenergy crops and sugar beets in 2013.

Determination of resistance (tolerance) of different types of miscanthus to abiotic factors was performed by the method of selective media [6].

Shoots obtained by clonal micropropagation, selection of samples *Miscanthus giganteus*, *M. sinensis*, *M. sinensis Late*, *M. sinensis Early*, *M. sinensis New*, *M. sinensis Silberspinne*, *M. sacchariflorus*, cultured on modified nutrient Murasihe and Skoog, at temperature of 20-22 °C in the light room, light 2-3 thousand lux, relative humidity - 70 to 75%.

The study was conducted as follows: Control - modified culture medium MS (MS), I variant - MC + 0,25% NaCl; II variant - MS + 0,5% NaCl; III variant - MS + 1% NaCl; IV variant - MS + 1,5% NaCl; V variant - MS + 2,0% NaCl.

Rooting of shoots miscanthus in culture in vitro was performed as follows: Control - MS + NAA 0,8 mg/g + IAA 0,8 mg/g, I variant - MS + NAA 0,8 mg/g + IAA 0,8 mg/g + NaCl 1%, II variant - MS + NAA 0,8 mg/g + IAA 0,8 mg/g + NaCl 1,5%.

In each embodiment, the culture medium were planted on 20 shoots three species and four hybrid miscanthus.

Phenological observations and counts of growth processes were carried out every 2 weeks. Studies on the quantitative determination of chlorophyll content by spectrophotometric method were performed by the standard method [7]. After culturing the shoots on selective media with auxin and NaCl was performed counting the number of roots formed, their length and weight of the root system and accumulated dry matter content [8].

The results are statistically processed using the software Statistica 6.0 [9].

Results and discussion. Methods for biotechnology (microclonal method and selective medium) allow for a short period of time (1-2 months) conducting selective selection of genotypes tolerant to abiotic factors. For a small amount of salt in the culture medium (0,25%, 0,5%) observed

an increase in indicators of growth processes in all investigated samples within four weeks of cultivation. With increasing salt concentration (1%, 1,5%) reported a slight yellowing of leaves and shoots miscanthus tissue necrosis (Fig. 1). The concentration of salt (2,0%) in the culture medium can withstand only species *M. sinensis* and *M. sacchariflorus*: viability of shoots was 40 %, the average height of shoots ranged from 3-3,5 cm



Fig.1. The cultivation of miscanthus shoots to salt stress at different concentrations

The viability of shoots varied to control the environment in all samples and miscanthus was 85-100% (Table 1). In medium with 1% NaCl decreased viability relative to control shoots differs by 6-47% across the tested material, the lowest sensitivity to salinity 1% NaCl in culture in vitro samples showed *M. sinensis* - 6%, *M. giganteus* - 9%, *M. sinensis Early* - 10% of *M. sinensis Silberspinne* - 12%. If you salinity of 1,5% in the culture medium from control difference is 14-68% (*M. sinensis Early* - 14%, *M. sinensis Silberspinne* - 20%, *M. giganteus*, *M. sinensis* - 21%, *M. sinensis Late* - 22%, *M. sacchariflorus* - 42%, *M. sinensis New* - 68%) of live shoots. It should be noted that the presence of NaCl in selective medium inhibits the growth processes miscanthus shoots compared with control. In particular, the observed decrease in the height of shoots in all types of 0,1-1,7 cm also significantly reduced the formation of new shoots on a culture medium with 1% saline in all investigated samples at 0,2-1,3 pcs./shoot and on the selective medium with 1,5% NaCl in *M. sinensis*, *M. sinensis Late*, *M. sinensis Silberspinne* 0,8 pcs./shoot, and in *M. giganteus*, *M. sinensis Early*, *M. sacchariflorus*, *M. sinensis New*, an average of 1-1,4 pcs./shoot.

When cultured shoots miscanthus on selective media by a set of attributes (vitality, height of shoots and the formation of new clones) can distinguish *M. sinensis*, *M. sinensis Late* and *M. sinensis Silberspinne*.

In determining the chlorophyll content in shoots of miscanthus depending on the salinity of the culture medium NaCl was found that chlorophyll a is slightly increased salinity values at 1% compared with control in *M. sinensis Late* to 0,04 mg/g wet weight, respectively and the salinity 1,5% at 0,02 mg/g wet weight. It shows some tolerance to salinity substrate. In all other samples studied chlorophyll content and decreases with increasing amounts of salts and the difference compared to the control environment is 0,04-0,15 mg/g wet weight in medium with 1% NaCl and 0,04-0,16 mg/g wet weight at 1,5% NaCl.

Chlorophyll b in all samples miscanthus is reduced by 0,01-0,22 mg/g wet weight at 1% NaCl and at 0,01-0,24 mg/g wet weight in 1,5% NaCl compared with control. The trend to lower values of total chlorophyll a and b were observed in almost all genotypes, except for *M. sinensis Late*. Best of chlorophyll content showed *M. giganteus*, *M. sinensis*, *M. sinensis Silberspinne*, *M. sinensis Late*.

Table 1

Effect of salts on miscanthus *in vitro*

Selective number	Variant medium	Vitality of shoots, %.	Height of shoots, cm.	Formation of shoots, pcs.	Chlorophyll a, mg / g wet weight	Chlorophyll b, mg / g wet weight	Total content of chlorophyll a and b, mg / g wet weight
<i>M. giganteus</i>	Control	92	7,9	1,6	0,222	0,346	0,568
	1% NaCl	83	6,5	1,4	0,295	0,128	0,423
	1,5%NaCl	71	6,4	0,1	0,289	0,106	0,395
<i>M. sinensis</i>	Control	92	4,8	2,8	0,285	0,136	0,421
	1% NaCl	86	4,6	2,5	0,166	0,166	0,332
	1,5%NaCl	71	3,6	2,0	0,157	0,160	0,317
<i>M. sinensis Early</i>	Control	85	4,2	2,2	0,244	0,259	0,503
	1% NaCl	75	3,8	1,5	0,107	0,053	0,160
	1,5%NaCl	71	3,6	0,9	0,095	0,053	0,148
<i>M. sacchariflorus</i>	Control	100	4,5	2,5	0,268	0,087	0,354
	1% NaCl	61	4,0	1,2	0,155	0,041	0,195
	1,5%NaCl	58	3,6	0,2	0,143	0,043	0,186
<i>M. sinensis Late</i>	Control	100	3,5	1,5	0,127	0,076	0,202
	1% NaCl	80	3,5	1,3	0,160	0,066	0,225
	1,5%NaCl	78	3,4	0,7	0,146	0,063	0,209
<i>M. sinensis New</i>	Control	100	4,1	2,1	0,270	0,190	0,461
	1% NaCl	53	2,6	1,5	0,186	0,077	0,262
	1,5%NaCl	32	2,4	1,0	0,145	0,043	0,188
<i>M. sinensis Silberspinne</i>	Control	100	9,4	1,3	0,230	0,107	0,337
	1% NaCl	88	8,1	0,5	0,194	0,085	0,279
	1,5%NaCl	80	8,1	0,5	0,189	0,075	0,264
LSD ₀₅		-	0,4	0,5	0,22	0,14	0,36

When researching opportunities miscanthus shoots form roots on selective media with NaCl was determined that the presence of NaCl in the medium retards the process of rooting 4-8 days in all investigated samples selection compared with the control (roots were formed in 10-12 days) (Fig. 2).

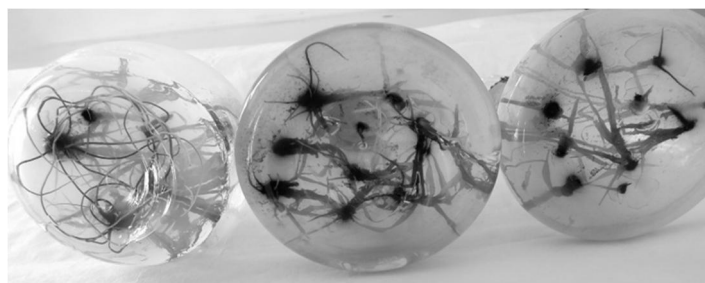


Fig. 2. Roots miscanthus *in vitro* under the influence of salt stress

Despite the fact that the average length of roots decreased in all investigated plants in both cases, the culture medium by an average of 0,5 cm (Table 2), it was determined that breeding specimens that showed the high potential of rooting plants have large number of indicators formed on the roots of salinity. The highest values of plant rooting and number of roots was obtained in samples of *M. sinensis*, *M. sinensis Early*, *M. sinensis Silberspinne*.

Table 2

Rooting miscanthus on the salt stress

Selective number	Variant medium	Rooting of plants, %	Weight of roots per plant, g	The average length of roots, cm	Number of roots, pcs/shoot	Dry matter content rooted in vitro of plants, %
<i>M. giganteus</i>	Control	100	0,075	5,8	5,2	16,08
	1% NaCl	67	0,012	1,1	3,8	14,79
	1,5% NaCl	29	0,001	1,4	1,3	12,85
<i>M. sinensis</i>	Control	100	0,043	2,0	2,4	16,48
	1% NaCl	70	0,040	1,3	4,3	13,30
	1,5% NaCl	59	0,010	1,2	2,3	12,47
<i>M. sinensis Early</i>	Control	60	0,031	1,5	4,0	13,68
	1% NaCl	54	0,017	0,8	2,2	12,38
	1,5% NaCl	46	0,010	0,4	1,4	12,03
<i>M. sacchariflorus</i>	Control	57	0,215	4,9	3,8	12,54
	1% NaCl	40	0,023	1,9	2,6	11,71
	1,5% NaCl	20	0,010	1,0	1,1	9,83
<i>M. sinensis Late</i>	Control	63	0,019	1,5	2,5	14,08
	1% NaCl	50	0,016	1,1	1,9	13,98
	1,5% NaCl	14	0,010	1,6	2,0	13,75
<i>M. sinensis New</i>	Control	56	0,044	1,1	3,6	15,17
	1% NaCl	19	0,013	0,4	1,7	12,02
	1,5% NaCl	-	-	-	-	-
<i>M. sinensis Silberspinne</i>	Control	93	0,020	2,7	2,6	20,21
	1% NaCl	57	0,009	2,1	2,5	16,39
	1,5% NaCl	50	0,004	0,8	1,4	16,30
LSD ₀₅		-	0,11	0,28	1,14	-

Another indicator of sustainability is the dry matter content, which accumulates in plant stress conditions. In the study of this indicator is worth noting that *M. sinensis Early*, *M. sacchariflorus*, *M. sinensis Late* dry matter content in plants miscanthus on selective medium with 1-1,5% NaCl smaller compared with the control at 1-2%, and other samples – 2-4%. The highest dry matter content under control described *M. giganteus*, *M. sinensis*, *M. sinensis Silberspinne* - 16-20%. It should be noted that *M. sinensis Early* and *M. sinensis Late* had lost the least dry matter (1-2%) when cultured shoots to salinity compared to control.

Conclusions. The observed differentiation between the three species and four hybrids miscanthus shoots in vitro by the amount of chlorophyll accumulated dry matter content and processes of salinization in the culture medium (0,25-2% NaCl). Highlight breeding specimens (*M. giganteus*, *M. sinensis*, *M. sinensis Late*, *M. sinensis Early*, *M. sinensis Silberspinne*), are characterized by a high level of tolerance to salt stress.

References

1. Новая форма мискантуса китайского (веечника китайского *Miscanthus sinensis* Anders.) как перспективный источник целлюлозосодержащего сырья / [В.К. Шумный, С.Г. Вепрен, Н.Н. Нечипоренко и др.]. // Весник ВОГиС. – 2010. – Том 14, №1. – С. 122-126.
2. Назаренко І.І. Грунтознавство: підручник / І.І. Назаренко, С.М. Польчина, В.А. Нікорич. – Чернівці: Книги – ХХІ, 2004. – 400 с.
3. Строгонов Б.П. Физиологические основы солеустойчивости растений / Б.П. Строгонов. – М.: Изд. Академии Наук СССР, 1962. – 370 с.

4. Мельничук М.Д. Біотехнологія рослин / М.Д. Мельничук, Т.В. Новак, В.А. Кунах. – К.: ПоліграфКонсалтинг, 2003. – 520 с.
5. Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis / [R.W. Van den Bulk, H.J.M. Loffler, W.H. Lindhout, M. Koornneef] // Theor. Appl. Genet. – 1990. – № 80. – P. 817-825.
6. Отбор устойчивых к хлоридному засолению форм сахарной свеклы в условиях культуры тканей / [В.Ф. Зубенко, И.И. Ильенко, В.И. Редько, В.В. Редько] // Доклады ВАСХНИИЛ. – 1987. – №5. – С. 18-20.
7. Бессонова В.П. Практикум з фізіології рослин / В.П. Бессонова. – Дніпропетровськ: ПП Свідлера А.А., 2006. – 316 с.
8. Фізіологія рослин: практикум / [О.В. Войцехівська, А.В. Капустян, О.І. Косик та ін.]; за заг. ред. Т.В. Паршикової. – Луцьк: Терен, 2010. – 420 с.
9. Доспехов Б.А. Методика полевого опыта / Б.А. Доспехов. – М.: Агропромиздат, 1985. – 351 с.

Анотація

Коцар М.О.

Вплив сольового стресу in vitro на розвиток пагонів міскантусу

У статті наведено результати дослідження впливу сольового стресу на розвиток різних видів міскантусу, культивованих в умовах in vitro на селективному середовищі з різними концентраціями NaCl. Визначено зміни в ростових процесах, кількості хлорофілу, здатності до укорінення та накопичення сухої речовини міскантусу.

Ключові слова: міскантус, in vitro, NaCl, хлорофіл, укорінення

Аннотация

Коцар М.О.

Влияние солевого стресса in vitro на развитие побегов мискантуса

В статье приводятся результаты исследования влияния солевого стресса на развитие различных видов мискантуса культивируемых в условиях in vitro на селективной среде с различными концентрациями NaCl. Определены изменения в ростовых процессах, количества хлорофилла, способности к укоренению и накопление сухого вещества мискантуса.

Ключевые слова: мискантус, in vitro, NaCl, хлорофилл, укоренение