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VIABILITY OF MAIZE IN VITRO REGENERATED PLANTS HYBRIDS IN SOIL UNDER ARTIFICIAL CLIMATE

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Надано оцінку приживаності рослин-регенерантів гібридів кукурудзи української селекції у ґрунті в умовах штучного клімату. Виявлено генотипічний контроль над приживаністю у ґрунті. Встановлено гібридні комбінації з підвищеною приживаністю у ґрунті.

Ключові слова*: кукурудза, приживаність, in soli, in vitro, рослини-регенеранти, штучний клімат.*

Obtaining the fertility of in vitro regenerated plants is an important final result for the majority of plant biotechnological researches in vitro. The survival rate of in vitro regenerated plants in soli often depends on the genotype, the type of morphogenesis, conditions of artificial climate in which plants were grown, on the application of growth stimulators during planting in soil and further growth, on the size of regenerated plants, their stage of development ect.

Maize plants regenerated in vitro are usually planted in pots with soil, sand, vermiculite, moss etc. under certain combinations. For example, it is known that maize in vitro regenerated plants were planted in pots with peat moss, but some time later they were transplanted into pots which contained garden soil, humus and sand (2:2:1) [1, 2], into pots with Coco peat, vermiculite and sand (6:3:4) [3] or into pots with peat, vermiculite and sand (1:1:1) [4]. Also often a stage of adaptation in soil is necessary, while just planted plantlets are covered with transparent plastic bags with a few holes for a few days to maintain moisture for aboveground plant parts [1, 2]. It is desirable that artificial climate provides constant temperature and light conditions.

Most biotechnology researches with maize have genetical engineering and molecular and genetic aspects of application, in particular, through production of callus tissues and regenerated plants. Insufficient attention was paid to their rearing up to the end of the growing season. Survival rate of regenerated plants of promising maize Ukrainian inbreds and hybrids in soli remains unexplored. The aim of our work was to evaluate the survival rate of in vitro regenerated plants of maize hybrids of Ukrainian selection developed with the participation of Lancaster germplasm inbreds and highly sensitive to cultivation in vitro inbreds A188, Chi31and PLS61 in soil under artificial climate. Also we plan to identify of hybrid combinations with sufficient survival rate in soil to attract Lancaster inbreds to cellular and genetic engineering.

As the material for the study in vitro regenerated plantlets of hybrids of Ukrainian selection were used. Hybrid combinations were created with the participation of Lancaster national inbreds of different subgermplasms: DK267, DK212, DK6080 and DK420-1 of subgermplasm Oh43; DK633/266 and DK298 of subgermplasm Mo17/Oh43; DK633 of subgermplasm Mo17; DK3070 of subgermplasm Mo17/O92; DK236 of subgermplasm Mo17/F2; DK633/325 of subgermplasm Mo17/Mindzenpusta and lines A188, Chi31та PLS61 of the same name germplasms. The 30- and 60-day callus tissues of type I and type II according to the classification of [5] induced from immature embryos of 1–1,5 mm in length were used to obtain regenerated plants. For callus induction a modified N_6 medium with 30 or 60 g/l sucrose was used [6]. As regeneration medium MS medium [7] with 20 g/l sucrose modified with phytohormonal composition (6-benzylamino-purine or indolebutyric acid, or cefotaxime, or hormone free medium) was used. Rooting and adap-tation to in vivo conditions were performed in biological glasses on hormone free MS medium with half content of salts and 15 g/l sucrose. Landing in conditions of artificial climate was conducted when regenerated plants had reached about 10–20 cm in shoot length. Plants were transplanted into pots with a mixture of soil and sand (2:1). Conditions of artificial climate provided constant temperature 22–30 °C (day-night), 5000–10000 Lux light in the daytime, the darkness at night and the soil moistening if necessary.

Soil moisture was carried out with a solution of biohumat as a product of processing sunflower husks by the culture of worms *Eisenia foetida*.

1. The survival rate in soil of regenerated plants of maize hybrids with the participation of Lancaster inbreds and PLS61

Hybrid	Number of regenerated plantlets transplanted into the soil	Number of regenerated plantlets that have finished a growing season	The survival rate in soil, %
Subgermplasm Oh43 of Lancaster germplasm			
DK267 x PLS61	34	23	67,7 ± 16,3
PLS61 x DK267	85	63	74,1 ± 9,6
DK212 x PLS61	9	5	55,6 ± 35,1
PLS61 x DK212	5	3	60,0 ± 49,0
DK6080 x PLS61	16	11	68,8 ± 23,9
PLS61 x DK6080	4	4	100,0 ± 0,0
DK420-1 x PLS61	23	14	60,9 ± 20,8
PLS61 x DK420-1	15	13	86,7 ± 18,2
In total for subgermplasm Oh43	191	136	71,2 ± 6,6
Subgermplasm Mo17/Oh43of Lancaster germplasm			
DK633/266 x PLS61	22	18	81,8 ± 16,8
PLS61 x DK633/266	22	11	50,0 ± 21,8
DK298 x PLS61	15	13	86,7 ± 18,2
PLS61 x DK298	4	1	25,0 ± 50,0
In total for subgermplasm Mo17/Oh43	63	43	68,3 ± 11,8
Subgermplasm Mo17 of Lancaster germplasm			
DK633/PLS61	6	5	83,3 ± 33,3
In total for subgermplasm Mo17	6	5	83,3 ± 33,3
Subgermplasm Mo17/O92 of Lancaster germplasm			
DK3070 x PLS61	33	18	54,6 ± 17,6
PLS61 x DK3070	1	1	100,0
In total for subgermplasm Mo17/O92	34	19	55,9 ± 17,3
Subgermplasm Mo17/F2 of Lancaster germplasm			
DK236 x PLS61	4	3	75,0 ± 50,0
PLS61 x DK236	0	0	0
In total for subgermplasm Mo17/F2	4	3	75,0 ± 50,0
Subgermplasm Mo17/Mindzenpusta of Lancaster germplasm			
DK633/325 x PLS61	18	13	72,2 ± 21,7
In total for subgermplasm Mo17/Mindzenpusta	18	13	72,2 ± 21,7
In total for germplasm Lancaster	316	219	69,3 ± 5,2

The survival rate of regenerated plants in soil was calculated as the percentage ratio of the number of regenerated plants that finished the growing season to the number of plants that had been transplanted into the ground. Statistical data processing was performed according to [8]. Data in tables are presented in the form $\bar{x} \pm mt_{0.05}$, where $\bar{x}$ is the arithmetic mean value of a parameter, m – an error of the arithmetic mean, $t_{0,05}$ – Student test at a significance level of 0,05.

The survival rate of regenerated plants of maize hybrids in soil are presented in tables 1–3. As it can be seen in table 1, the survival rate in soil of regenerated plants of maize hybrids created with the participation of various Lancaster inbreds and PLS61 ranged from 55,9 % (for subgermplasm Mo17/O92) to 83,3 % (for subgermplasm Mo17). The survival rate of hybrids created with the participation of PLS61 and other Lancaster subgermplasms had intermediate

values and were as follows: for subgermplasm Oh43 – 71,2 %, for subgermplasm Mo17/Oh43 – 68,3 %, for subgerm-plasm Mo17/F2 – 75,0 % and for subgermplasm Mo17/Mindzenpusta – 72,2 %. Among the hybrids the highest survival rate was observed for PLS61 x DK6080 – 100,0 %. The least survival rate in soil was recorded for PLS61 x DK298 – 25,0 %. The average survival rate for hybrids in this group was 69,3 ± 5,2 %.

As it can be seen from table 2, the survival rate in soil of regenerated plants of maize hybrids created with the participation of various Lancaster inbreds and A188 ranged from 45,7 (for subgermplasm Mo17/Oh43) to 82,4 % (for subgermplasm Mo17). The survival rate of hybrids created with the participation of PLS61 and subgermplasm Mo17/Oh43 made up 75,0 %. Among the hybrids the highest survival rate was observed for DK633 x A188 – 82,4 %. The least survival rate in soil was recorded for A188 x DK298 – 37,5 %. The average survival rate for hybrids of this group was 58,9 ± 13,3 %.

2. The survival rate in soil of regenerated plants of maize hybrids with the participation of Lancaster inbreds and A188

Hybrid	Number of regenerated plantlets transplanted into the soil	Number of regenerated plantlets that have finished a growing season	The survival rate in soil, %
Subgermplasm Oh43 of Lancaster germplasm			
A188 x DK420-1	4	3	75,0 ± 50,0
In total for subgermplasm Oh43	4	3	75,0 ± 50,0
Subgermplasm Mo17/Oh43 of Lancaster germplasm			
DK633/266xA188	9	5	55,6 ± 35,1
DK298 x A188	18	8	44,4 ± 24,1
A188 x DK298	8	3	37,5 ± 36,6
In total for subgermplasm Mo17/Oh43	35	16	45,7 ± 17,1
Subgermplasm Mo17 of Lancaster germplasm			
DK633 x A188	17	14	82,4 ± 19,1
In total for subgermplasm Mo17	17	14	82,4 ± 19,1
In total for germplasm Lancaster	56	33	58,9 ± 13,3

As it can be seen from table 3, the survival rate in soil of regenerated plants of maize hybrids created with the participation of various Lancaster inbreds and Chi31, ranged from 33,3 (for subgermplasm Mo17) to 66,7 % (for subgermplasm Mo17/F2). The survival rate of hybrids created with the participation of Chi31 and subgermplasm Mo17/O92 was 55,6 %. Among the hybrids the highest survival rate was observed in DK236 x Chi31 – 66,7 %. The least survival rate in soil was recorded for DK633 x Chi31 – 33,3 %. The average survival rate for hybrids of this group was 53,3 ± 26,7 %.

Thus, it was observed a general trend of the increase of the viability in soil for hybrids between Lancaster inbreds in contrast with PLS61, A188 and Chi31.

3. The survival rate in soil of regenerated plants of maize hybrids with the participation of Lancaster inbreds and Chi31

Hybrid	Number of regenerated plantlets transplanted into the soil	Number of regenerated plantlets that have finished a growing season	The survival rate in soil, %
Subgermplasm Mo17 of Lancaster germplasm			
DK633 x Chi31	3	1	33,3 ± 66,7
In total for subgermplasm Mo17	3	1	33,3 ± 66,7
Subgermplasm Mo17/O92 of Lancaster germplasm			

DK3070 x Chi31	7	4	57,1 ± 40,4
Chi31 x DK3070	2	1	50,0 ± 100,0
In total for subgermplasm Mo17/O92	9	5	55,6 ± 35,1
Subgermplasm Mo17/F2 of Lancaster germplasm			
DK236 x Chi31	3	2	66,7 ± 66,7
In total for subgermplasm Mo17/F2	3	2	66,7 ± 66,7
In total for germplasm Lancaster	15	8	53,3 ± 26,7

Regenerated plantlets of hybrids between inbreds of subgermplasm Oh43 and PLS61 or A188 had the survival rate in soil at the level of 71,2–75,0 %, which was higher than that in hybrids between inbreds of subgermplasm Mo17/Oh43 and A188 or PLS61– 47,5–68,3 %. The viability in soil of hybrids involving inbred DK633 of subgermplasm Mo17 and A188 or PLS61 ranged in 82,4–83,3 %, but with the participation of Chi31 was much less, 33,3 %. The survival in soil of regenerated plants of hybrids involving inbreds of subgermplasm Mo17/O92 and Chi31or PLS61 made up of 55,6–55,9 %. The survival rate of hybrid plantlets created with the participation of subgermplasm Mo17/F2 and Chi31or PLS61 made up 66,7–75,0 %. Thus, a tendency to increasing survival was observed for such hybrid combinations as inbreds of subgermplasm Oh43 and PLS61 or A188, inbreds of subgermplasm Mo17/Oh43 and PLS61, subgermplasm Mo17 and PLS61 or A188, subgermplasm Mo17/O92 and PLS61 or Chi31, subgermplasm Mo17/F2 and PLS61, subgermplasm Mo17/Mindzenpusta and PLS61. Among three highly responsive maize inbreds namely PLS61 as a component of hybrids provides increased survival in soil. This fact can be used to improve regeneration capacity and viability in soil for perspective Lancaster inbreds though their hybrids with PLS61 and attraction them to the investigation in cellular and genetic engineering. That is, the relationship between survival rate in soli of a hybrid and genotype of regenerated plantlets components in hybrid combinations is observed. This dependence may be caused by genotypic peculiarities of in vitro ability of different maize hybrids, by features of morphogenesis type – organogenesis or embryoidogenesis, by features of response to alteration of in vitro conditions to soil ones, adaptability ect.

So, as a result of the study the survival rate of regenerated plants of maize Ukrainian hybrids in soil under conditions of artificial climate was evaluated. The effect of the genotype of regenerated plants on their survival in soil was determined. Specific forms of integration of maize inbreds in hybrid combinations to ensure appropriate survival in soil were recommended.

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