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Morphogenesis in Vitro in Maize Inbred Lines from the Lancaster Heterotic Group

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Abstract—The genotypic specificities of morphogenesis and regeneration have been studied in vitro in five maize inbred lines from the breeding-promising Lancaster heterotic group, compared with the representatives of other heterotic groups, that is, PLS61, A188, and Chi31. It has been shown that the ratio between the types of morphogenesis, such as organogenesis and embryoidogenesis, in a callus culture is predetermined by the explant genotype and sucrose concentrations in the medium for callusogenesis. The frequency of embryoidogenesis as the most efficient type of morphogenesis, considering further regeneration, was, on average, 40.0 ± 12.8% for maize inbreds from the Lancaster heterotic group and only 14.0 ± 4.0% from other heterotic groups. A sucrose concentration at a level of 30 g/L in the medium for callusogenesis further supported the process of regeneration through embryoidogenesis in inbreds of the Lancaster heterotic group at a level of 26.5 ± 15.4%, while the sucrose concentration of 60 g/L promoted regeneration up to a level of 57.7 ± 19.8%. Contents of sucrose in the medium for callusogenesis in the inbreds representing other heterotic groups did not affect the process of regeneration, and the level of embryoidogenesis with sucrose at the concentrations of 30 and 60 g/L was, respectively, 11.0 ± 7.0 and 15.0 ± 4.8%.

Keywords: maize, in vitro culture, morphogenesis, organogenesis, embryoidogenesis

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INTRODUCTION

An important end result in biotechnological studies, including cellular and genetic engineering in vitro, is associated with obtaining fertile regenerated plants able to grow and develop in vivo. The survivability of regenerated plantlets upon replanting them from the in vitro conditions into the soil depends on the type of morphogenesis due to which the samples are formed in a callus tissue culture. Morphogenesis with the subsequent in vitro regeneration of plantlets, according to Batygina et al. [1], can be realized, first, through organogenesis, i.e., due to the separate development of a shoot apical meristem (gemmogenesis), and a root system (rhizogenesis). Another pathway is somatic embryogenesis or embryoidogenesis, i.e., the development of an asexual bipolar structure, which structurally corresponds to a zygotic embryo and has the apexes of a stem and a root, as well as cotyledons.

A priority type of morphogenesis in obtaining somaclonal variations and in the genetic transformation of maize, as well as a valuable tool for the efficient formation of new genotypes, is somatic embryogenesis, since the latter can provide the development of regenerated plantlets with shoots and, at the same time, with their root system [2].

The dependence of regenerative ability on the genotype is described in many studies [3, 4]. However, Akoyi et al. [5] have shown for tropic maize inbreds that the formation of somatic embryos on a Murashige and Skoog (MS) medium with dicamba is independent of their genotype. To activate the process of embryoidogenesis, the culturing conditions should be optimized both for callusogenesis initiation and during the plant regeneration stage. One of the components for a nutrient medium for callusogenesis is sucrose, which can induce the callusogenic ability and somatic embryogenesis in vitro. The most frequently applied for inducing callusogenesis in maize are sucrose concentrations of 20 [6, 7], 30 [8, 9], and 60 g/L [10]. Sucrose at the concentration of 60 g/L is known to provide a higher level of callusogenesis than 30 g/L in a medium for callusogenesis [10]. There are other methods for culturing callus tissues, which include the induction of callusogenesis at the sucrose concentration of 30 g/L with a subsequent increase to 60 g/L of the sucrose content in the medium for the subcultivation of calli in order to provoke somatic embryogenesis [2, 5].

These data emphasize the importance of identifying the effects of certain concentrations of sucrose, as a component of the medium for callusogenesis, on the realization of certain types of morphogenesis for regenerated plants from particular groups of promising agriculturally valuable maize genotypes.

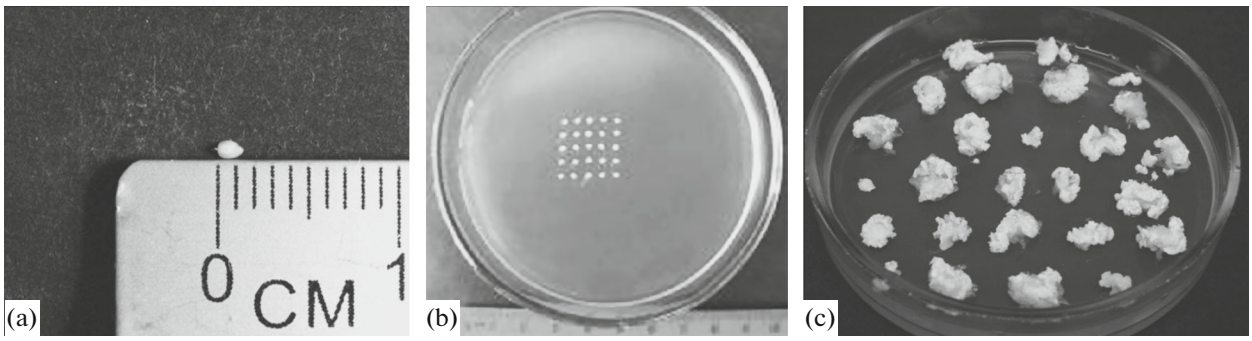

Fig. 1. Obtaining callus tissue from immature maize embryos in vitro: (a) an immature embryo before explantation; (b) immature embryos explanted on the nutrient medium for callusogenesis induction; (c) 30-day morphogenic callus tissue transplanted on the medium for regeneration.

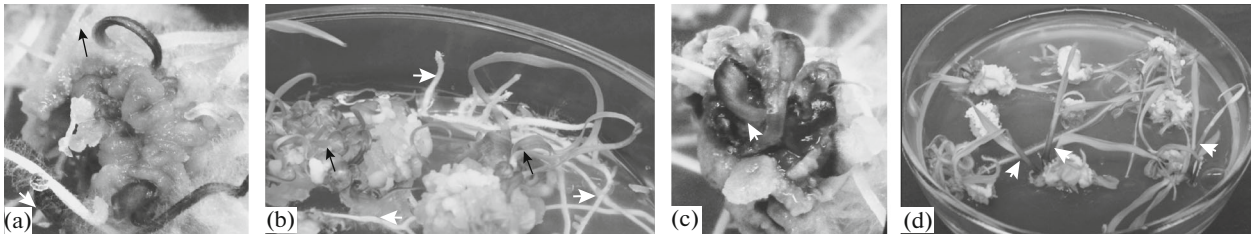

Fig. 2. Regeneration of plants via organogenesis in maize: (a, b) formation of leaflike structures (indicated by the black arrows) in callus tissue, the roots (indicated by the white arrows) develop separately; (c, d) formation of plantlets as shoots (indicated by arrows), roots are absent.

Widespread and commercially promising in the current breeding practice are heterotic groups, such as Lancaster, Iodent, Reid, BSSS, and Mindszenpuszta [11], for which the studies of their biotechnological properties are especially important. Among them is the Lancaster group, which is one of the most promising for cultivation in Ukraine, due to its early ripeness, drought resistance, and high yield potential [12].

The objective of our study was to compare the effect of genotypic specificities in maize inbred lines from the Lancaster heterotic group and the representatives of other heterotic groups on the relationship between the types of morphogenesis and plant regeneration in callus culture.

MATERIALS AMD METHODS

The Lancaster heterotic group of maize (*Zea mays* L.) was represented in our study by inbreds of the Ukrainian breeding—DK267, DK6080, DK420-1, DK298, and DK3070. The lines for comparison were represented by the model maize inbreds of foreign breeding—PLS61, A188, and Chi31—from the corresponding heterotic groups. Callusogenesis was induced in 10–12-day immature embryos of 1–1.5 mm in length (Figs. 1a, 1b), which were excised from field donor plants of the corresponding genotype. A modified N_6 medium [13] containing 30 or 60 g/L of sucrose was used to induce callusogenesis. The obtained 30-day callus tissue was planted on a regeneration MS medium [14] containing 20 g/L of sucrose. Embryos and calli were cultivated at 26°C in darkness, while regenerated plantlets were cultivated under a 16-h photoperiod.

The frequencies of organogenesis and embryoidogenesis were determined as a ratio between the number of regenerated plantlets obtained from a callus tissue through any type of morphogenesis and the total number of the regenerated plantlets obtained. The regeneration frequency was determined as the number of the obtained regenerated plantlets per 100 cultivated calli. The frequency of a certain type of morphogenesis and the frequency of regeneration were recorded every 30 days in dynamics, starting from the transplantation of callus tissue onto the regenerative medium to the 180th day of cultivation. The results were statistically processed according to the recommendations of Welham et al. [15]. The statistically treated data are represented in tables as $x \pm \Delta$, where x is the mean arithmetic value and Δ is the confidence interval, which is calculated as the mean arithmetic error and Student's t-test at the significance level of 0.05.

RESULTS AND DISCUSSION

The morphogenic callus tissue type I (according to the classification given in [16]), which was cultivated for 30 days (Fig. 1c) was obtained in the medium for the induction of callusogenesis on scutellums of immature embryos for all studied inbred lines with the

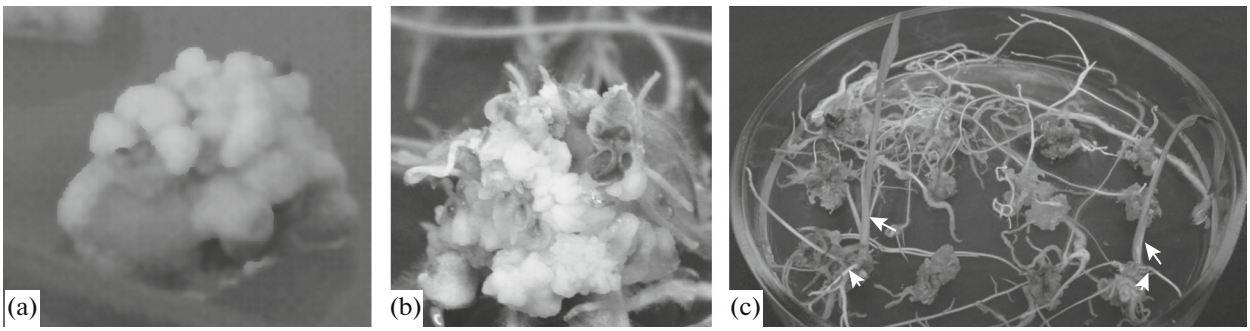

Fig. 3. Plant regeneration via embryoidogenesis in maize: (a) development of embryoids on a medium for callusogenesis; (b) further development of embryoids on a medium for regeneration; (c) plant regeneration via embryoidogenesis: the shoot (indicated by the long arrow) and the root (indicated by the short arrow) develop separately in a seedling.

frequency of 98–99%. Morphogenesis and plant regeneration in the obtained callus tissue took place both through organogenesis with the formation of leaflike structures or plantlets as shoots (gemmogenesis) and separate roots (rhizogenesis) (Figs. 2a–2b), and through the development of embryoids with the simultaneous formation of shoots and roots (Fig. 3).

Morphogenesis and plant regeneration from a callus tissue of the Lancaster group began already within the first 30 days of cultivation on a regeneration medium, while the maximum number of regenerants was formed predominantly from the 31st to the 60th days (Table 1).

Inbred DK6080 has shown its regeneration ability only through embryoidogenesis. In other inbreds, morphogenesis took place both via organogenesis and embryoidogenesis. In inbreds DK267 and DK298, organogenesis dominated (65.0 and 72.7%, respectively), while embryoidogenesis prevailed in DK420-1 (66.7%). In DK3070 for the period of cultivation only two plants were formed on a medium for regeneration, including one formed through organogenesis and the other formed through embryoidogenesis. The frequencies of organogenesis and embryoidogenesis, in total, were 60.0 ± 12.8% and 40.0 ± 12.8% in the Lancaster group, i.e., they were 20% moved to organogenesis. But embryoidogenesis was the pathway of morphogenesis in 40% of cases and provided the development of well-developed plantlets. The most frequent regeneration within 180 days of cultivation among the inbreds of this group was in inbred line DK267 (51.3 plants per 100 calli), while the minimum frequency was shown by inbred line DK3070 (3.4 plantlets per 100 calli). Inbreds DK6080, DK298, and DK420-1 had intermediate values for this parameter (22.2, 15.9, and 20.4 plantlets per 100 calli, respectively). The mean regeneration frequency within 180 days among the Lancaster inbreds was 24.8 ± 5.6 samples per 100 calli.

The formation of regenerated plantlets in the inbreds representing other heterotic groups began 30 days after starting the cultivation of the callus tissue on the regeneration medium (Table 2), i.e., later, compared to Lancaster inbreds, and the mass plant regeneration took more time from the 31st to the 120th days and even from the 31st to the 180th days in the case of PLS61.

As for the type of morphogenesis, a significant prevalence of organogenesis over embryoidogenesis (86.0 and 14.0%, respectively) was observed in the representatives of groups PLS61, A188, and Chi31. The highest level of embryoidogenesis was recorded in inbred A188 (20.0%), while the lowest one was observed in PLS61 (12.0%). The level of regeneration frequency within the entire 180-day research period was the highest in PLS61 (234.1 samples per 100 calli); this value for inbreds A188 and Chi31 was 76.9 and 143.6 samples per 100 calli. The mean regeneration frequency within 180 days of cultivation for the representatives of non-Lancaster heterotic groups reached the level of 154.8 ± 13.1 samples per 100 calli, respectively.

The comparison of the data from Tables 1 and 2 indicate greater predisposition to embryoidogenesis in the Lancaster inbred lines than in other studied heterotic groups (40.0 ± 12.8% vs. 14.0 ± 4.0%). The total level of regeneration frequency for the entire period of cultivation on regeneration media in maize inbred lines from the Lancaster heterotic group was 24.8 ± 5.6 samples per 100 calli. The frequency of regeneration was almost six times higher, i.e., 154.8 ± 13.1 samples per 100 calli, in the inbreds from the analyzed non-Lancaster heterotic groups, which were recommended as model ones in the cell engineering of maize, due to their high regenerability [16, 17]. The decreased ability of the Lancaster group inbreds to form regenerated plantlets from callus tissue can be explained by the fact that the latter are those inbred lines from commercial heterotic lines, which had not passed through special selection for higher regenerability, compared with the model lines of other heterotic groups. Plant regeneration in the culture of callus tissues from Lancaster inbreds began earlier and lasted less than in model lines. We can suggest that this is a manifestation of predisposition of Lancaster genotypes in in vitro culture to an accelerated passage through the processes of growth and development, which provides their early ripeness in plant growth under field conditions. At the same time, the lower

Table 1. Relationship between the types of in vitro morphogenesis in maize inbreds from the Lancaster heterotic groups

Inbred	Number of calli planted onto the medium for regeneration, samples	Cultivation period for calli on regeneration medium, days	Number of the obtained regenerated plantlets, samples	Type of morphogenesis, %	
				organogenesis	embryoidogenesis
DK267	78	1–30	6	50.0	50.0
		31–60	34	67.7	32.4
		61–90	0	0	0
		91–180	0	0	0
		In total, within 180 days	40	65.0	35.0
DK6080	18	1–30	1	0	100.0
		31–60	3	0	100.0
		61–90	0	0	0
		91–180	0	0	0
		In total, within 180 days	4	0	100.0
DK420-1	33	1–30	0	0	0
		31–60	2	50.0	50.0
		61–90	1	0	100.0
		91–180	0	0	0
		In total, within 180 days	3	33.3	66.7
DK298		1–30	0	0	0
		31–60	10	8	2
		61–90	1	0	100.0
		91–180	0	0	0
		In total, within 180 days	11	72.7	27.3
DK3070	54	1–30	1	0	100.0
		31–60	1	100.0	0
		61–90	0	0	0
		91–180	0	0	0
		In total, within 180 days	2	50.0	50.0
In total	59	1–30	8	37.5 ± 36.6	62.5 ± 36.6
		31–60	50	66.0 ±13.5	34.0 ± 13.5
		61–90	2	0	100.0
		91–180	0	0	0
		In total, within 180 days	60	60.0 ± 12.8	40.0 ± 12.8

regeneration intensity in inbreds from the Lancaster group provided higher frequency of well-developed plantlets obtained through embryoidogenesis (40.0 ± 12.8%), whereas high regenerability values in non-Lancaster inbreds were only 14.0 ± 4.0% associated with embryoidogenesis.

Thus, morphogenesis in maize inbred lines from the Lancaster group and other heterotic groups has certain differences. Plant regeneration through organogenesis and embryoidogenesis in Lancaster inbreds goes approximately with the same frequency and only with an insignificant shift towards organogenesis, whereas organogenesis significantly prevails in model inbreds of other heterotic groups. The frequency of embryogenesis as more efficient type of morphogenesis, considering further regeneration, reached, on average, 40.0 ± 12.8% for inbreds from the Lancaster heterotic group and only 14.0 ± 4.0% for those from other heterotic groups.

The research results concerning the effect of sucrose in the medium for callusogenesis on the morphogenic and regenerative potential of callus tissues from different heterotic maize groups are presented in Table 3.

The regeneration frequency increased with increasing the sucrose concentration in the medium for callusogenesis from 30 to 60 g/L for inbred lines of non-Lancaster groups (203.6 vs. 93.2 samples per 100 calli). On the contrary, the frequency of regeneration did not significantly change (27.2 ± 8.0 vs. 22.2 ± 7.7 samples per 100 calli) for the Lancaster group lines when the

Table 2. Relationship between the types of in vitro morphogenesis in maize inbreds of heterotic groups PLS61, A188, and Chi31

Inbred	Number of calli planted onto the medium for regeneration, samples	Cultivation period for calli on regeneration medium, days	Number of the obtained regenerated plantlets, samples	Type of morphogenesis, %	
				organogenesis	embryoidogenesis
PLS61	82	1–30	0	0	0
		31–60	66	80.3	19.7
		61–90	93	94.6	5.4
		91–120	21	100.0	0.0
		121–180	12	58.3	41.7
		In total, within 180 days	192	88.0	12.0
Al88	78	1–30	0	0	0
		31–60	28	71.4	28.6
		61–90	24	87.5	12.5
		91–120	7	100.0	0.0
		121–180	1	0.0	100.0
		In total, within 180 days	60	80.0	20.0
Chi31	39	1–30	0	0	0
		31–60	37	86.5	13.5
		61–90	0	0	0
		91–120	17	88.2	11.8
		121–180	2	50.0	50.0
		In total, within 180 days	56	85.7	14.3
In total	199	1–30	0	0	0
		31–60	131	80.2 ± 7.0	19.8 ± 7.0
		61–90	117	93.2 ± 4.7	6.8 ± 4.7
		91–120	45	95.6 ± 6.2	4.4 ± 6.2
		121–180	15	53.3 ± 26.7	46.7 ± 26.7
		In total, within 180 days	308	86.0 ± 4.0	14.0 ± 4.0

Table 3. Effects of different sucrose concentrations on the in vitro morphogenesis and regeneration in maize callus tissue

Sucrose concentration in a medium for callusogenesis, g/L	Number of calli planted onto a medium for regeneration, samples	Type of morphogenesis, %		Frequency of regeneration, number of regenerated plantlets per 100 calli
		organogenesis	embryoidogenesis	
Inbred lines of Lancaster heterotic group (DK267, DK6080, DK420-1, DK298, and DK3070)				
30	125	73.5 ± 15.4	26.5 ± 15.4	27.2 ± 8.0
60	117	42.3 ± 19.8	57.7 ± 19.8	22.2 ± 7.7
Inbred lines of heterotic groups PLS61, A188, and Chi31(PLS61, A188, and Chi31)				
30	88	89.0 ± 7.0	11.0 ±7.0	93.2 ±5.4
60	111	85.0 ±4.8	15.0 ± 4.8	203.6 ± 27.7

sucrose concentration in the medium for callusogenesis was increased from 30 to 60 g/L.

According to Table 3, sucrose at the concentration of 30 g/L in the medium for callusogenesis contributed to the course of organogenesis in the Lancaster group inbreds (73.5 ± 15.4%), while the process of somatic embryogenesis was intensified at a sucrose concentration of 60 g/L, which made the frequency values of organogenesis and embryoidogenesis closer to each other (42.3 ± 19.8% and 57.7 ± 19.8%, respectively). No reliable differences have been found in maize inbreds of other heterotic groups in the ratios between the frequencies of organogenesis and embryoidogenesis at different sucrose concentrations in the medium for callusogenesis—a predominant number of regenerated plants, respectively, 89.0 ± 7.0 and 85.0 ± 4.8% was formed through organogenesis at the concentrations of 30 g/L and 60 g/L. Thus, the frequency of embryoidogenesis at a sucrose concentra-

tion of 60 g/L in the medium for callusogenesis, compared with the situation at a 30 g/L concentration, increased 31.2% in maize inbreds of the Lancaster heterotic group and only 4.0% in the inbred lines of other heterotic groups.

Thus, maize inbred lines from the Lancaster group are more sensitive to the effect of cultivation medium components than the inbreds representing other heterotic groups. For example, the level of embryoidogenesis increased up to 57.7% and reached, in fact, the level of organogenesis in the Lancaster group lines when 60 g/L of sucrose was used for the medium for callusogenesis. The level of sucrose concentrations in the medium for callusogenesis in the studied inbreds representing non-Lancaster heterotic groups (PLS61, A188, and Chi31) did not significantly affect the ratio between the types of morphogenesis in callus tissue.

CONCLUSIONS

Plant regeneration from callus tissue of maize inbred lines takes place through both organogenesis and embryoidogenesis, and the predominant type of morphogenesis is determined by the genotype of explants. We have succeeded in achieving a 2.1-fold increase in the frequency of embryoidogenesis, from 26.5 to 57.7%, in maize inbreds of the Lancaster heterotic group through modifying the composition of a nutrient medium for callusogenesis. The inbred lines of non-Lancaster heterotic groups that were insensitive to the effect of varying sucrose concentrations were characterized by a low frequency of embryoidogenesis (15.0%) and a predominant formation of regenerated plants through organogenesis. Thus, our recommendation is to use the sucrose concentration of 60 g/L for a medium for callusogenesis to improve the process of plant regeneration in the culture of callus tissue from maize inbred lines of the breeding-promising Lancaster heterotic group.

REFERENCES

1. Batygina, T.B., Kruglova, N.N., Gorbunova, V.Yu., Titova, G.Ye., and Seldimirova, O.A., *From Microspore to Variety*, Moscow: Nauka, 2010.
2. Gonzalez, G.A., Pacheco, M.G., Oneta, C.D., Ethchart, V.J., Kandus, M.V., Salerno, J.C., Eyherabide, G., Presello, D., and Lewi, D.M., Somatic embryogenesis and plant regeneration capacity in Argentinean maize (*Zea mays* L.) inbred lines, *Electron. J. Biotechnol.*, 2012, vol. 15, no. 1, pp. 1–7.
3. Bedada, L.T., Seth, M., Runo, S.M., Tefera, W., and Jesse, M., Regenerability of elite tropical maize (*Zea mays* L.) inbred lines using immature zygotic embryo explants, *Afr. J. Biotechnol*, 2012, vol. 11, no. 3, pp. 598–605.
4. Ali, F., Ahsan, M., Saeed, N.A., Ahmed, M., Ali, Q., Kanwal, N., Tehseen, M.M., Ijaz, U., Bibi, I., and Niazi, N.K., Establishment and optimization of callus-to-plant regeneration system using mature and immature embryos of maize (*Zea mays*), *Int. J. Agric. Biol.*, 2014, vol. 16, pp. 111–117.
5. Akoyi, J., Mgutu, J., Machuka, J., Lijsebettens, M.V., Taracha, C., and Anami, S.E., Dicamba growth regulator promotes genotype independent somatic embryogenesis from immature zygotic embryos of tropical maize inbred lines, *J. Life Sci.*, 2013, vol. 7, no. 7, pp. 677–689.
6. Morshed, S., Siddique, A.B., and Shahinul, IslamM.S., Efficient plant regeneration using mature and immature embryos of maize (*Zea mays* L.), *Int. J. Agric. Innovat. Res.*, 2014, vol. 3, no. 3, pp. 895–904.
7. Geetha, S., Suganthan, R.N., Prasanthi, P., Kumar, K.K., Kokiladeli, E., Arul, L., Balasubramanian, P., and Sudhakar, D., In vitro regeneration of Indian maize (*Zea maize* L.) inbred lines through immature embryo derived somatic embryogenesis, *J. Appl. Nat. Sci.*, 2015, vol. 4, no. 1, pp. 1–12.
8. Muoma, J., Muluvi, G., and Machuka, J., In vitro regeneration by indirect organogenesis of selected kenyan maize genotypes using shoot apices, *Biotechnol.*, 2008, vol. 7, no. 4, pp. 732–738.
9. Azad, M.A.K., Rahman, Md.W., Arifuzzaman, Md., Hasanuzzaman, M.D., Talukder, A.I., and Saha, R.K., Callus induction and plant regeneration from immature kernel of maize inbred line, *J. Chem. Biol. Physic. Sci.*, 2015, vol. 5, no. 4, pp. 4149–4161.
10. Thobunluepop, P., The somatic embryogenesis and plant regeneration from immature embryo of sweet corn inbred line, *J. Plant Breed. Crop Sci.*, 2009, vol. 1, no. 10, pp. 330–335.
11. Troyer, A.F., *Temperate Corn: Background, Behavior and Breeding*, Washington, DC: CBS Press, 2000.
12. Satarova, T.M., Derkach, K.V., and Abraimova, O.E., The estimation of reciprocal effect in in vitro culture for maize genotypes of Lancaster germplasm), *Bull. Inst. Graine Farming*, 2011, vol. 40, pp. 20–24.
13. Derkach, K.V., Abraimova, O.E., and Satarova, T.M., Callusogenic potential of maize lines of lancaster group in vitro, *Visnyk Dnipropetrovsk Univ. Biol. Ecol.*, 2011, vol. 19, no. 1, pp. 16–21.
14. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassay with tobacco tissue culture, *Physiol. Plant.*, 1962, vol. 15, pp. 473–497.
15. Welham, S.J., Gezan, S.A., Clark, S.J., and Mead, A., *Statistical Methods in Biology: Design and Analysis of Experiments and Regression*, Boca Raton, FL: CRC Press, 2014.
16. Green, C.E. and Phillips, H.L., Plant regeneration from tissues cultures of maize, *Crop Sci.*, 1975, vol. 15, no. 5, pp. 417–421.
17. Satarova, T.M., Piralov, G.L., Bodenko, N.A., and Abraimova, O.E., Callusogenesis of corn lines under auxin influence, *Bull. Inst. Grain Farming*, 2010, vol. 38, pp. 55–60.

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