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Multi-Step Cellular and Tissue Selection of Soybean for Resistance to Osmotic Stress Using Peg 6000 Invitro Conditions

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ABSTRACT

Cell and tissue selection in vitro allows targeted selection of the desired traits under severe selective conditions at the level of individual cells and tissues. On the basis of multistage cell and tissue selection with the use of a selective agent - neutral osmotic polyethylene glycol 6000in increasing concentrations -5%, 10%, 15%, 20% of the final volume of the nutrient medium, callus of the Zhansaya soybean variety that are stably resistant to osmotic stress have been obtained. Regeneration from callus was noted only in 8.3% of the planted callus 6 soybean regenerants plants resistant to osmotic stress were obtained from callus.

Keywords- soybean, callus, cell selection, osmotic stress, resistance

I. INTRODUCTION

Soybeans are the world's leading oilseed crop. Soybeans are a rich source of vegetable oil and protein feed. Among the natural factors that have the greatest negative impact on all physiological processes of growth and development of soybeans and, ultimately, leading to crop losses, is water stress caused by drought. Lack of moisture reduces the yield of soybeans by about 40% [1]. Depending on the genotype, soybean plants use about 450-700 mm of water during the growing season. However, the most critical period for water stress in soybean plants is the flowering stage and the post-flowering period, i.e. formation and filling of seeds [2].

In recent years, soybean production in Kazakhstan has been steadily increasing. This is important for the agro-industrial complex of the country and contributes to solving the problem of protein deficiency in human nutrition and animal feeding, as well as diversifying crop production. However, the main region of soybean cultivation is the south and southeast of Kazakhstan. In 2016, with a total sown area in the Republic of Kazakhstan of 106.5 thousand hectares in the Almaty region, 97.7 thousand hectares were occupied by soybeans, i.e. more than 90%. The promotion of soybeans in the northern and eastern regions of the republic is the target indicator of the program for the development of the agro-industrial complex in the Republic of Kazakhstan for 2013-2020. For a wide variety of soil and climatic conditions, Kazakhstan requires soybean varieties that are resistant to various stresses, and, above all, varieties that are resistant to drought.

Such a method of biotechnology as in vitro cell selection makes it possible to expand the range of starting material and activate the selection process aimed at creating highly productive drought-resistant varieties. Genetic variation in this case is characterized by a wider spectrum, and the selection of the desired traits in the harsh selective conditions of the cell occurs purposefully at the level of individual cells and tissues.

At the cellular level, resistance to drought is expressed in the tolerance of cells to the presence of osmotically active substances in the nutrient medium that lower the external water potential [3]. For in vitro selection, polyethylene glycol, mannitol, sorbitol, NaCl are usually used as selective agents. The most successful selection factor is PEG with a molecular weight of 6000. Polyethylene glycol (PEG) is a non-penetrating osmotic that causes collapse of cell walls and compression of the protoplast, that is, it well simulates the water balance of a cell under conditions of osmotic stress [4].

Currently, selective systems for the selection of forms resistant to drought have been developed for the main cereals: wheat [5], rice [6], corn, [7], barley [8]. Osmotolerant sorghum callus clones were obtained [9].

Research on obtaining drought-resistant leguminous crops by biotechnological methods is extremely limited. Published works have shown mainly only the possibility of in vitro screening of genotypes for resistance to osmotic stress.

The work was carried out within the framework of the budget program 255 "Creation of conditions for the development of production, processing, sale of crop products" under the project "Increasing the productivity of oilseeds based on traditional and modern methods of breeding, development of technology for their cultivation and organization of primary seed production"

Purpose of the work: Creation of new drought-resistant soybean lines using cell and tissue selection against a water-deficient background using PEG 6000.

II. METHODOLOGY

The material for research was the soybean variety (Glycine max L.) Zhansaya of domestic selection, approved for use in the Republic of Kazakhstan.

Research methodology:

Soybean plants were grown to the stage of 5-7 day old seedlings in the greenhouse complex of the Kazakh Research Institute of Agriculture and Crop Production (KazNIIZiR) at a 16 hour photoperiod, lighting 10-15 thousand lux, air temperature 26-28 °C;

The cotyledon leaves of soybean seedlings were used as an explant for obtaining primary callus. The explants were sterilized using a 20% NaOCl solution with a Tween-80 drop for 8-10 minutes on a shaker, followed by washing with sterile distilled water (three times).

Obtaining the primary callus mass was carried out according to standard methods [10-12]. Soy tissue culture, regeneration, root formation [13];

For direct cell selection in vitro, the culture medium Murashige and Skoog was used with the addition of phytohormones (1 mg / L IAA and 1 mg / L kinetin), 30 g / L sucrose and 6 g / L agar, 2.5 mg / L ascorbic acid, pH -5.6-5.8.

Osmotic stress was simulated by the introduction into the nutrient medium of a selective agent, a neutral osmotic polyethylene glycol 6000 (PEG 6000), which simulates drought conditions in an in vitro culture. Callus were gradually introduced into in vitro culture on a nutrient medium containing PEG 6000 in increasing concentrations - 5, 10, 15, 20%. A medium without a selective agent was used as a control.

All obtained primary callus of the highly productive variety Zhansaya, 5-7 mm in size, were planted on nutrient media for callus formation - a control medium and a medium containing 5% PEG 6000 (200 callus for each variant). The duration of each passage was 20-25 days.

For the regeneration of plants in vitro, the nutrient medium Murashige and Skoog was used with the addition of phytohormones (BAP from 4 mg / L to 6 mg / L + 0.5 mg / L IAA), 30 g / L sucrose and 6 g / L agar, 1 ml / l antibiotic cefotaxime, pH -5.6-5.8.

III. PRIOR APPROACH

Observations of the dynamics of the growth of callus at the first stage of multistage cell selection of soybeans - 5% PEG 6000 were carried out every 10 days. Evaluation of the growth in the size of callus after 20 days of cultivation showed that the control recorded the growth of callus from 0.62 cm to 0.93 cm (Table 1). The increase in the size of callus was 0.31 cm. On a nutrient medium containing 5% PEG, the growth of callus from 0.57 cm to 0.97 cm was recorded. The increase in the size of callus was 0.4 cm, which is 129% of the control. All callus remained light after 20 days, darkening was observed on some callus of the control variant (Figure 1).

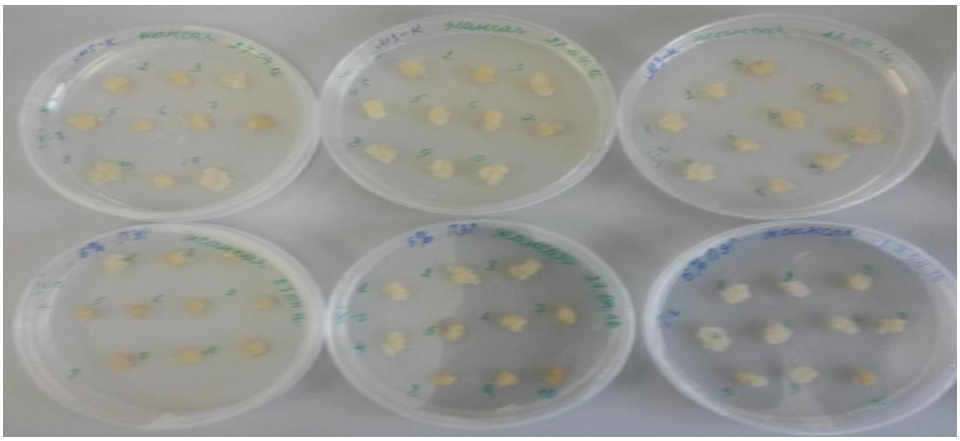

Figure 1: Cultivation of callus on control medium and medium containing 5% PEG600 (20 days)

Table 1: Results of multistage cell and tissue selection using increasing concentrations of polyethylene glycol 6000 in invitro

Variety name	Increase in the size of callus on the control medium, cm		Increase in the size of callus on a selective medium, cm		Callus outgrowth on selective medium relative to control (%)		The proportion of surviving callus, on a selective medium%
	after 10 days	after 20 days	after 10 days	after 20 days	after 10 days	after 20 days	
5 % PEG							
Zhansaya	0,25	0,31	0,21	0,4	84	129	98
10 % PEG							
Zhansaya	0,28	0,39	0,24	0,33	85,7	84,6	46

15% PEG							
Zhansaya	0,14	0,31	0,08	0,14	57,1	45,2	39
20% PEG							
Zhansaya	0,06	0,06	0,05	0,06	83,3	100	77,2

Thus, an intensive growth of callus was recorded, both in the control variant and in the medium with a selective agent. All callus were separated to 5-7 mm and transplanted to the second stage of cell selection - 10% PEG6000.

Evaluation after 20 days of cultivation of soybean callus with 10% PEG showed that the control variant recorded the growth of callus from 0.81 cm to 1.2 cm. The increase in the size of callus was 0.39 cm (Figure 2). On a nutrient medium containing 10% PEG, the growth of callus from 0.9 cm to 1.23 cm was recorded. The increase in the size of callus was 0.33 cm, which is 84.6% of the control.

Darkening was observed on almost all callus cultured on 10 PEG 6000 after 20 days.

82 callus resistant to osmotic stress were identified. All of them were separated (up to 150 callus) and transplanted with 15% PEG.

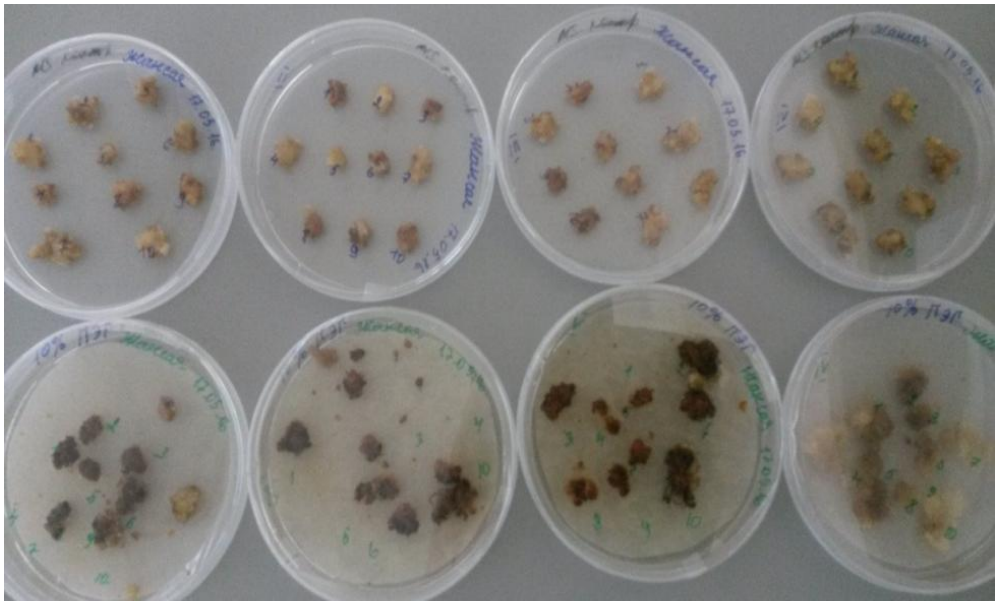

Figure 2: Cultivation of callus on control medium and medium containing 10% PEG600 (20 days)

The cultivation of surviving callus on a nutrient medium containing a selective agent of 15% showed that the increase in the control was 0.31 cm, and in the experiment 0.14 cm, which is 45.2% of the control. Death and darkening of callus were observed. The proportion of surviving callus was 39%. At the same time, the growth and division of individual groups of cells was recorded. All of them were selected and isolated as resistant callus.

The next passage and further cultivation of resistant callus on a selective medium with 20% PEG showed that all the isolated callus were light, but division was slow. The average growth on the selective medium after 20 days was 0.06 cm. The proportion of surviving callus from the transplanted 44 pieces was 77.2%. All surviving callus (34 pcs) were transplanted onto a nutrient medium without a selective agent. On a nutrient medium without a selective agent, they were subcultured for 2 passages. All resistant callus were propagated.

The selection of truly resistant callus cells was carried out upon repeated return to the selective medium with 15% PEG. The cultivation lasted 20 days. Callus were identified, in which adaptation was observed, and after the medium without the selective agent, resistance did not manifest itself. Of the 90 planted callus, resistance was shown to 75. After repeated return to selective conditions with 15% PEG, stable clones were selected, which were transplanted for regeneration.

The study of the regeneration of resistant callus obtained on the basis of cell selection showed that 94% of callus acquired a green color, points of formation of meristem foci were outlined (Figure 3). Regeneration from callus was noted only in 8.3% of the planted callus. The low percentage of regeneration, apparently, is a consequence of prolonged passaging of callus and a decrease in the morphogenetic potential. Six soybean regenerant plants resistant to osmotic stress were obtained from callus.

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Figure 3: Cultivation of Isolated resistant callus on a medium for plant regeneration

IV. CONCLUSION

On the basis of multistage cell and tissue selection using a selective agent - neutral osmotic polyethylene glycol 6000 at concentrations of 5%, 10%, 15%, 20% of the final volume of the nutrient medium, callus, which are stable to osmotic stress (75 pcs), of the Zhansaya soybean variety, were obtained.

Regeneration from callus was noted only in 8.3% of the planted callus. The low percentage of regeneration, apparently, is a consequence of prolonged passaging of callus and a decrease in the morphogenetic potential. Six soybean regenerant plants resistant to osmotic stress were obtained from callus.

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