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Development And Ecological Test Of The Early Maturing Spring Wheat Forms, Obtained By Cell Technology

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ABSTRACT

The 398 R0 plants were regenerated from long-term cultivated calluses of 28 commercially important wheat varieties. From these 110 plants of 18 varieties brought up R1 seed generation. 47 lines (37%) from those 110 lines of R1 we selected that had accelerated term of maturation on 3-6 days compared to initial varieties. Ecological trial of selected lines in R2 generation at North, Central and South-East Kazakhstan conditions allowed to prove the expression of the precocity trait (accelerated development on 1-8 days) and to select the precocious forms (25-47,7% from the number of tested lines) with high productivity and drought resistance traits which were most prospective for each region. Thus, we have shown the principal possibility of the creation of spring wheat precocious forms with the complex of valuable economic-biological traits for growing in North, Central and South-East Kazakhstan by the use of cell technology developed by us.

Keywords: callus tissues, early maturing, ecological test, regenerated plants, wheat.

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INTRODUCTION

Today, the problems of food security are considered as the main task in all countries of the world. The situation on the grain market of Kazakhstan has also a tendency to increase grain production and consumption, due to the global increase in population of the world and the dynamic development of animal husbandry and processing industry.

The main spring wheat acreage is concentrated in the area of arid climate of the Kazakhstan Republic. In the northern, western and eastern regions of the Republic on average of 10 years and 6 are dry and arid. The variety of climatic conditions on the territory of the Kazakhstan Republic determines the complexity and diversity of adaptive problems associated with global climate change.

The threat of man-made disasters, threatening serious environmental consequences that have also been reported by many scientists [4, 11,16].

The national concept for adaptation to climate change [14] presents the priority measures aimed at preventing and reducing the risks in agricultural production, including, development of drought-resistant and early maturing crops and varieties, introduction water saving technologies, improvement of specific methods of agricultural technologies, etc.

To speed up and improve the effectiveness of breeding process is now widely used methods of genetic and cell engineering. However, because of the important food and export values of wheat, cautious attitude of the public to the use of genetically modified genetic engineering methods for improving this crop in Kazakhstan is limited. In this regard, scientists [5, 13] consider the variability accumulated in the cells during in vitro culture, and transmits to regenerated plants - one of the major biotechnological tools to increase the genetic diversity of wheat.

There are a lot of researches about development of forms resistant to abiotic and biotic stresses by using biotechnological methods in the world [2, 10, 17]. However, the development of precocious forms of plants by using biotechnological methods are not well studied.

Cell technology of plant regeneration in vitro well developed by researches of the Institute of Plant Biology and Biotechnology in Almaty [1], and now obtained precocious form with complex agronomic traits (increased productivity, resistance to lodging and so on. d.) based on the two genotypes of spring wheat.
The aim of this work is to study the possibility of obtaining precocious forms of spring wheat using cellular technology and test this forms under the North, Central and South-East of Kazakhstan.

MATERIALS AND METHODS

The objects of study were 28 of commercially important varieties of spring wheat (Samgau, Almakom, Kazakhstan 75, Astana-2, Karabalyk 98, Baiterek, Novosibirsk 15, Omsk-36, Asar, Arai, Zhazira, Kazakhstan 17, Kazakhstanskaya rannespelaya, Kazakhstan 10 , Kazakhstan 15, Karaganda 22, Karaganda 30, Nadezhda, Irtysh 7, Pavlodar 93 Pavlodar 8, Seke, Bekzat, Conditerskaya yarovaya, Pavlodarskaya yarovaya, Lutescens 90, Otan, Tselinnaya 3C), grown in in northern and central, south-eastern regions of Kazakhstan.

Tissue culture done by conventional methods [6]. As explants to induce callus of wheat immature embryos were 1,0-1,4 mm in length, which are planted on an agar nutrient medium of Murashige and Skoog (MS) [9] supplemented with 1,0 mg /l 2,4-dichlorophenoxyacetic acid (2,4-D). Plants regenerated R0 generation was obtained from long-cultivated callus 4-5 passage and in the greenhouse of the Kazakh Research Institute of Agriculture and Plant growing for R1 seed. The plants-regenerant were studied on phenological and morphological traits in comparison to the initial variety.

The ecological test of the plant –regenerants carried out in the five breeding centers of Kazakhstan: there are Karabalyk Agricultural Experimental Station, North Kazakhstan Agricultural Experimental Station, Karaganda Institute of Plant and breeding, Kazakh Research Institute of Agriculture and Plant growing, Determine the periods of emergence, tillering, heading and maturation.

As an ecological standards-regioalized differentiators were used widely cultivated by varieties in Kazakhstan Kazakhstan Early (middle-early), Karabalyk 90 (is middle).

The experiments laid in the optimal terms of sowing for each zone (25 April -25 May). Sowing of nurseries carried out by specially seed drills SSFK-7 of plots an area - 10 m2, the repetition of in experience 3-a multiple of. Phenological observations, visual assessments of the status and development of plants over the phases, the analysis of crop yield of structure was carried out by methods of Udolskoy N.L, Sosnin B.I. and Pastukhov A.I [15].

Selection of forms of wheat for precocity and photoperiodic sensitivity was performed according to the method lies in the growing hybrid population and the allocation of precocious forms on the timing of the appearance of the the spike stem tight fitting of the sheet. Thus plants have appeared early spike belongs to the precocious weakly susceptible to photoperiod forms, and late have appeared with a late-spike sensitive to photoperiod forms [12].

One indicator of drought-resistant varieties of spring wheat during the earing is the length of the upper internodes, which can be used when assessing source material on drought tolerance [7]. Statistical analysis was performed by methods of Lakin G.F. [8].

RESULTS AND DISCUSSION

Obtaining long-term embryogenic callus from varieties of spring wheat and regeneration of whole plants. In the MS medium supplemented with 1.0 mg /l 2,4-D, primary callus tissue derived from immature embryos, and 28 varieties of commercially important varieties of spring wheat. The primary callus tissue obtained from embry of 28 varieties of spring wheat were cultivated on a modified MS nutrient medium. To obtain the callus with regeneration ability embryogenic callus was transferred to medium of the same composition with a single salt content of the medium MS. As a result, we obtained embryogenic callus tissue with the beginnings of shoot (Figure 1), which maintain the ability for multiple plant regenerates were subcultured on MS medium supplemented with 1.0 mg /l 2,4-D, 1000 mg /l casein hydrolyzate and 500 mg /l of proline.

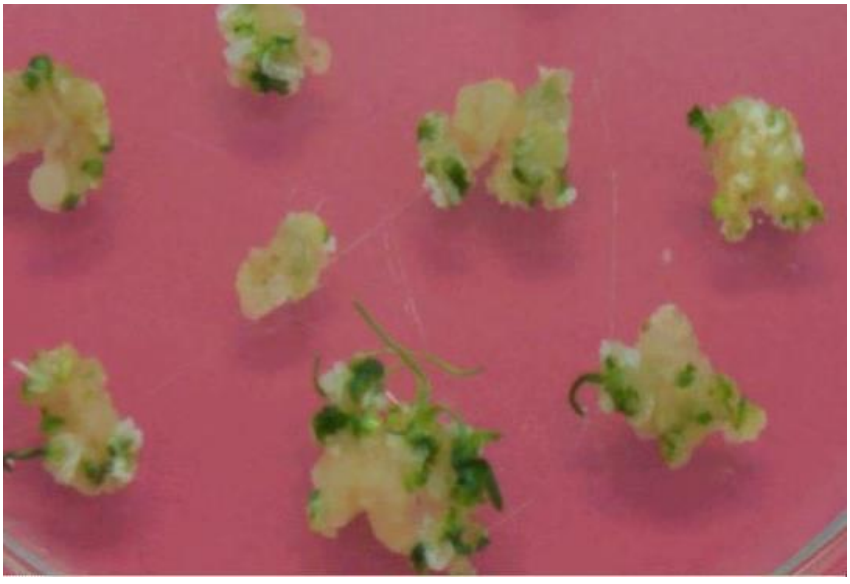

Figure 1. Embryogenic callus with the beginnings of shoot

The frequency of long-term cultured embryogenic tissue capable of repeated regeneration of plants (6-8 times), ranged from 6,3% to 77,4%, depending on the varieties (Table 1).

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Table 1. Morphogenesis and regeneration of plants in tissue culture of commercially important varieties of spring wheat

Varieties	long-cultivated embryogenic callus,%	The total number of regenerated shoots R0	Number of rooted shoots R0	Number of plants regenerants R0 rooted in the ground
Arai	29,0	9	1	-
Omskaya 36	38,2	22	19	19
Zhazira	68,8	12	4	4
Kazakhstanskaya 17	22,2	13	10	10
Kazakhstan rannespelaya	20,8	8	2	1
Karabalykskaya 98	75,6	19	9	9
Lutescens 90	21,4	8	2	2
Kazakhstanskaya 10	6,3	8	1	1
Baiterek	57,5	23	10	9
Bekzat	51,7	18	10	10
Karagandinskaya 22	**77,4**	**20**	**17**	**17**
Kazakhstanskaya 15	53,2	10	7	7
Kazakhstanskaya 75	65,8	17	14	14
Pavlodarskaya jubileinaya	25,8	10	5	5
Karagandinskaya 30	59,1	13	8	8
Asar	41,2	8	5	-
Astana 2	48,3	9	1	1
Pavlodarskaya 8	33,3	8	5	-
Samgau	10,0	10	7	-
Conditerskaya yarovaya	35,3	16	8	8
Irtysh 7	45,5	11	7	5
Pavlodarskaya 93	16,7	9	6	-
Novosibirskskaya 15	46,2	6	3	2
Almaken	7,2	13	8	4
Seke	50,0	8	3	2
Nadezhda	60,0	9	6	6
Tselinnaya 3C	43,3	33	28	26
Otan	46,7	48	45	44
Total:		397	221	214

The frequency of long-term cultured embryogenic callus from precocious varieties (22 Karaganda, Kazakhstan 75, 98 Karabalyk, Baiterek) more than 1.4-2.5 times higher compared to late-maturing genotypes (Lutescens 90, 17 Kazakhstan, Kazakhstan Early ripe, Pavlodar anniversary, Pavlodar 8 Almak, Confectionery spring), and is, accordingly, 57,5-77,4%. Probably, early maturing varieties of genetically fixed high speed allows the development of shoots often form separate homes regeneration in several outragerous centers. A small number of samples with long-cultured embryogenic callus are not allowed to reveal some regularities in the formation of this phenomenon, such as the dependence on the availability containing chlorophyll cells, callus on a medium-sized proliferation or culture settings on the stages of callus induction. For barley

experiment carried out on the material of Russian selection, showed a lower incidence of multiple plant regeneration in early ripening varieties in comparison with late-maturing [3].

Plant regenerants were subcultured on MS medium supplemented with 1.0 mg /l 2,4-D, 1000 mg / l casein hydrolyzate and 500 mg / L of proline. Shoots of plant-regenerants R0 was obtained from embryogenic callus and transplanted 4-5 times on nutrition medium for rooting - MS medium with 0.1 mg / l NAA and 0.5 mg / l IAA (Figure 2a).

a **b**

Picture 2. The callus with roots and planting in greenhouses of Institute

The total number of regenerated shoots was between 6 and 48 regenerated plants per cultivar (Table 1). It was noted that 83% of the samples with a long-cultured embryogenic calli were chlorophyll cells during passaging to their environment. Of these, only half formed a complete regenerated, which landed on the rearing. From planted regenerates 80% obtained from the callus that had a green color. This only shows that the presence of callus cells chlorophyll favored in most cases, the formation of additional points of growth, but it is not mandatory prerequisite.

397 plant-regenerants R0 generation was obtained and 221 (55.67%) of them are rooted on a rooting medium (table 1).

Rooted plants transferred from the tubes and planted into soil in the greenhouse conditions of the Kazakh Research Institute of Agriculture and Plant growing for obtaining the seeds. (Figure 2 b). As a result of the transfer into the ground had survived 214 plants regenerated R0 generation 28 varieties of spring wheat, which are were grown under greenhouse conditions (Table 1). It is noted that the samples with the highest number of regenerated of shoots (Otan, Tselinnaya 3C, Baiterek, Omsk 36, 22 Karaganda, Kazakhstan 75) more actively take root in the medium (14-45 of shoots), and take root in the ground under greenhouse conditions (14-44 regenerated plants) (Table 1).

Obtain the seeds of plant-regenerant R1 in the condition greenhouse. Phenological and morphological study of the plant- regenerants R1 generation in the field. 110 plant-regenerants belonging to 18 varieties of wheat: Almaken, Kazakhstan 75 Kazakhstan early maturing Bekzat, Baiterek, Novosibirsk 15, Omsk-36, Zhazira, Kazakhstan 15, Karaganda 22, Karaganda 30 Yertis 7, Seke, Confectionery spring, Otan, Tselinny 3C Karabalyk 98 and 17 give the seed. Other plants were sterile.

R1 generation seeds 110 lines regenerated plants obtained in the greenhouse, then grown in the experimental field and greenhouse IBBR KazNIIZiR and conducted phenological studies of regenerated plants R1 generation. Of the 110 plants regenerated R1 generation allotted 47- precocious lines (50.0% of the number of lines studied), outstripping by ripening for 3-6 days, belonging to 14 varieties (Almaken, Kazakhstan 75, Bekzat, Baiterek, Omskaya-36, Kazakhstan15, Karaganda 22, Karaganda 30, Novosibirsk 15, Ertis 7, Confectionery spring, Tselinnaya 3C, Kazakhstan early maturing, Otan) and one hybrid combination G4 (Kazakhstan Tselinnaya 3C x 15). Prepared seed R2 generation plants regenerated R1.

As a result of the structural analysis of 47 early maturing lines R1 generation selected 20 high-forms

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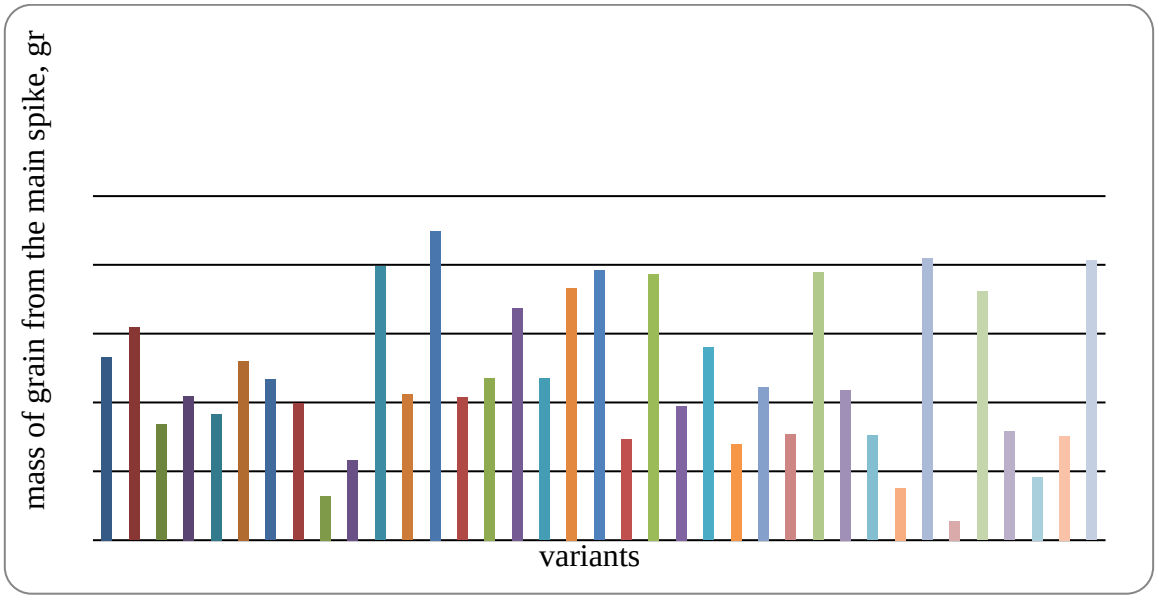
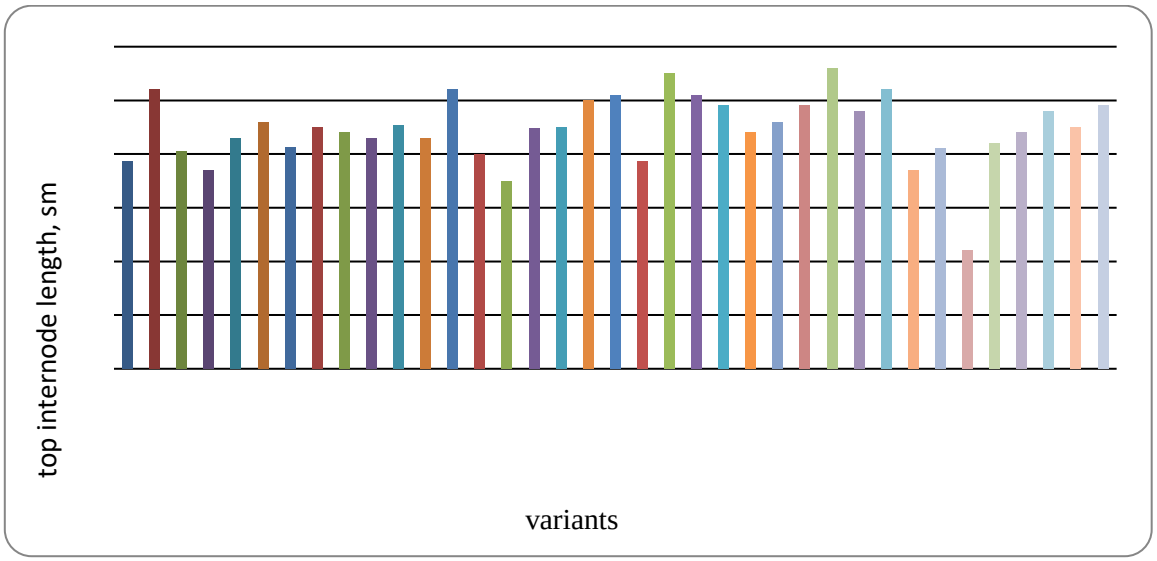
(42.6% of the number of precocious lines) related to 22 varieties of Karaganda, Karagandy 30 Bekzat, Almak, Confectionery spring, Baiterek, Kazakhstan, 75, Tselinnaya 3C, -7 Irtysh, Omsk 36, Otan and dihaploid hybrid line T4, which exceed the initial grade on the basis of "the mass of grains from the main ear" (Figure 3 a).

On the traits of "the number of grains in main spike" in R1 generation 10 somaclonal lines allocated (21.3% of the number of precocious lines) varieties Karaganda 30 Almak, Confectionery spring, Baiterek, Kazakhstan, 75, Tselinnaya 3C Ertis -7, exceeding the original variety.

On the traits of "the length of the upper internode" is correlated with drought resistance of 47 precocious lines R1 generation highlighted 4 potentially drought-resistant lines (8.5% of the number of precocious lines) varieties of Karaganda, 30, 75, Kazakhstan, Tselinnaya 3C, Omsk 36, with a length upper internodes 59-70 cm, exceeding the initial variety Otan (54.0 cm) (Figure 3b).

As a result, breeding (on site IBBR and greenhouse KazRIAP) of 110 lines regenerated plants R1 generation derived seeds 284 R2-generation lines, which belong to 18 wheat varieties (Almak, Kazakhstan 75 Kazakhstan early maturing Bekzat, Baiterek, Novosibirsk 15 Omsk -36, Zhazira, 15 Kazakhstan, Karaganda 22 Karaganda 30 Ertis 7, Seke, Confectionery spring, Otan, Tselinny 3C Karabalyk 98 and Kazakhstan 17).





Legend: № 2 Karagandinskaya-30; № 2.1.1 Karagandinskaya-30 somaclonal line; № 5 Kazakhstanskaya - 15; № 5.1.1 Kazakhstanskaya - 15, somaclonal line; № 9 DG-4; № 9.2.1 T 4 dihaploid line; № 7, Omsk-36; № 7.2.1 Omskaya 36 somaclonal line; № 7.2.2 Omskaya 36 somaclonal line; № 7.3.1 Omskaya 36 somaclonal line; № 4Almaken, control; № 4.2.1 Almaken, somaclonal line; № 4.3.1 Almaken, somaclonal line; № 1 Konditerskaya yarovaya; № 1.1.1 Konditerskaya yarovaya, somaklonal line; № 2 Karaganda-30 variety; № 2.2.1 Karaganda-30 somaclonal line; № 5Baiterek ; № 5.1.1 Baiterek, somaclonal line; № 8, Kazakhstanskaya-75; № 8.2.1 Kazakhstanskaya-75 somaclonal line; № 8.2.3 Kazakhstanskaya-75 somaclonal line; №9 Tselinnaya; № 9.3.1 Tselinnaya, somaclonal line; № 9.3.2 Tselinnaya 3C somaclonal line; № 9.5.1 Tselinnaya 3C somaclonal line; № 9.7.1 Tselinnaya 3C somaclonal line; № 9.7.3 Tselinnaya 3C somaclonal line; №9.7.4 Tselinnaya 3C somaclonal line; № 9.8.1 Tselinnaya 3C, № 10 Otan; № 10.5.1 Otan, somaclonal line; №10.6.2 Otan, somaclonal line; № 11.1.1. Novosibirsk-15 somaclonal line; № 6 Irtysh 7; № 6.1.1 Irtis 7; № 6.2.1 Irtis 7.

Figure 3. (a) – Variation of the trait "the grains mass from main spike" of somaclonal lines; (b) - Variability of the trait "the length of the upper internodes"

Ecological test of selfpollinated offspring in leading breeding centers of Kazakhstan. Seeds 284 R2 generation lines derived from 110 lines R1-regenerated plants transferred to the selection of the country's leading centers for the study of the subject on the display trait earliness and presence of economic-biological traits.

Of these 38 lines which received from 11 varieties (there are: 6 lines of Almaken, 2 lines of Kazakhstanskaya 15, 5 lines of Kazakhstanskaya 75, 3 lines of Karabalykskaya 98, 4 lines of Karagandinskaya 22, 5 lines of Karagandinskaya 30, 4 lines of Omskaya 36, 5 lines of Zhazira, 2 lines of Yertis 7, 1 line of Kazakhstanskaya 17 and 1 line of Kazakhstanskaya-skorospelaya) studied in Karabalyk Agricultural Experiment Station (Kostanai region of Kazakhstan).

56 lines received from 9 varieties (8 lines from Karagandinskaya 30, 6 lines varieties Omskaya 36, 6 lines varieties Almaken, 6 lines from Baiterek, 9 lines from Bezostaya, 6 lines from Tselinnaya 3C, 6 lines from Karagandinskaya 22, 4 lines from Kazakhstanskaya 75, 3 lines from Zhazira, 8 lines from Otan) studied in Karaganda Institute of Plant and breeding (Karaganda region of Kazakhstan).

185 line received from 18 spring wheat varieties studied breeding centers in North and Central Kazakhstan in 2014. Seeds of 99 lines studied in Kazakh Research Institute of Agriculture and Plant growing (Almaty region) - 4 lines from Konditerskaya yarovaya, 5 lines from Karagandinskaya 30, 4 line from Karagandinskaya 22, 5 lines from Omskaya 36, 6 lines from Almaken, 3 lines from Zhazira, 7 lines from Baiterek, 1 line from Yertis, 3 lines from Kazakhstanskaya 75, 8 line from Tselinnaya 3C and 51 line from Otan.

The study of 284 lines regenerated plant R2 generations in different ecological zones of the South-East, North and Central Kazakhstan selected early-maturing forms with valuable traits. Thus, in the Almaty region allocated 28 lines (28.3% of the number of lines studied in this region, ripening on 2-8 days earlier than the initial variety); in Kostanai region - 17 lines (44.7% of the tested lines, ripening on 1-8 days before); Karaganda region - 14 lines (25.0% of the lines tested ripening for 3 days before). A total of 284 lines regenerated plant R2-generation 100-maturing lines were selected (35.2% of the number of lines tested).

A total of 284 lines regenerated plant R2-generation 100-maturing lines were selected (35.2% of the number of lines tested). Selected early maturing lines, exceeding the initial variety in productivity indicators: under the Karaganda region - 9 lines (64.3% of the tested lines), - 3 (8.7 % of the number of lines tested).

The appearance somaclonal variants in the form of mutant plants regenerated is explained, in our opinion, the appearance of genetic changes in the cells of callus tissue. As a result, dedicated lines from differ initial forms in early-matureg and other useful characteristics, as shown in our experiments.

Assessing the effectiveness of cell technology developed by us for obtaining early-maturing forms, we can conclude that:

- the presence long-term plant regeneration in callus culture of wheat produces a higher yield of seed progeny

R0, while maintaining this trait regenerant after planting in the ground. However this trait is seen in regenerated ripening varieties, but completely disappears in the process of rearing regenerated from the late-samples;
- In relation to the total number of lines studied somaclonal R1 generation (110) output ripening form (47) may be 42.7%;
- The number of varieties, which can produce a ripening form R1 generation through this technology can be 50.0% in relation to the amount of initial varieties;
- The number of maturing forms (47) R1 generation of 42.6% may have increased productivity, 17% - higher rates of symptoms correlated with drought resistance, 10.6% - while increasing productivity and drought indicators.
- Based on the total number of students of somaclonal lines generation R1 (110) maturing high-yield forms - 18.2%, maturing forms with enhanced drought tolerance - 7.3%, maturing forms with complex valuable traits - higher rates of productivity and drought tolerance traits - 4.5%.
Thus, the regenerated analysis from the callus culture of wheat, showed considerable variation timing of plant maturation and reproductive traits, namely the weight of grain from the main spike, number of grains per ear, which remained in vegetative R1 and R2 generations, which indicates the possibility of selection of somaclonal options. The fact that changes in wheat regenerated plants have a genetic or epigenetic nature and preserve the phenotypic expression in the environmental trial in various regions of Kazakhstan, points to the possibility of using them to accelerate the breeding process of spring wheat.

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