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Gamete selection and field estimation of regenerant plant offspring (Triticum Aestivum L.)

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Abstract. While using abscisic acid (ABA) cell lines were obtained from embryoids in vitro at the level of reproductive cells, which formed more germinal roots compared with their original form (Erythrospermum 14). On the basis of the morphogenetic "cell-plant-cell" cycle drought-line AR2-45 is selected, which showed resistance to drought in field evaluation (7–9 points). Line differs in anatomy-morphological features and the general architectonics. Plants formed a large number of nodal roots and productive tillers under severe drought. The line of AR-45 significantly exceeded the standard by 0.29 t/ ha.

Key words. Haploid technologies, abiotic stress, anther.

1. Introduction

The yield of spring wheat in Kazakhstan over the past 10 years did not exceed 1 ton per hectare, which is far below the worldwide average value. This fact is caused by specifics of sharply continental climatic conditions of Kazakhstan, mainly by the lack of water supply, which constrain wheat productivity. After more than 30 year study of the environmental factors effecting wheat yield A. I. Barayev Research and Production Centre for Grain Farming (Barayev RPC GF) has identified high productivity-moisture supply correlation ($r = 0.645$).

Annual rate of precipitation 347 mm-min-220 mm, max–472 mm; summer precipitation (June-August) 143 mm-min 50 mm, max–257 mm; correlations between: yield—quantity of the year precipitation $r = 0.645$; yield–quantity of humidity in 1 m soil before planting $r = 0.735$; yield–precipitation during Tillering-Stem elon-

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gation stage (10–30 June) $r = 0.797^*$; yield–precipitation during Stem elongation-Heading stage (1–20 July) $r = 0.350$. (I. Vasko "Correlation of yield of spring wheat and meteorological factors", more than 30 years dat). All above mentioned show that wheat yield significantly depends on high productivity-moisture supply correlation. Worldwide average wheat yield amounts to 2.7 t/ha varying across countries and regions and the demand for grain will reach 840–1000 MT by 2020. Further expansion of planting acreage is practically impossible, and the only way to succeed is the increasing of wheat yield capacity [1]. Haploid technologies are known to be one of the efficient tools of biotechnology which increases effectiveness of practical plant selection [2–4]. It is currently known that the haploid technologies are the most promising way in breeding barley, corn, sorghum, oats and wheat, rye. For the moment the works showing the details of plant embryogenesis stages have been published. They write in particular about suspensor role, microspore embryogenesis and new view of androgenesis mechanisms. The present-day reviews discuss in details the success of the previous works on pollen embryogenesis induction as well as the usage of experimental research results in practical selection [5, 6].

ABA-independent path activates the cis-active element, which is called "element of the dehydration reaction" (DRE; or C-repeat) and is currently extracted from many plants. Genes CBF1 (C-repeat binding factor1), (DREB1) were found in Arabidopsis plants; a small multi-gene family was detected in rice and barley. ABA-dependent path is most studied for Arabidopsis where ABA increases induction of 382 genes. It is assumed that this refers to the network of signals which interacts with other hormones and the cell reacts to stress reaching a physiological effect and displaying the resistance characteristic. Interrelation between pollen and sporophyte resistance to environmental changes, pollen competitiveness and the sporophyte growth intensity, as well as between pollen viability and morphological indicators (excess and asymmetry) which characterize the adaptability of plants was determined [7].

2. Materials and methods

The purpose of this series of experiments is pollen embryogenesis induction in the anther culture in ABA presence. To do this ABA was added into nutrient medium during the anther cultivation in the following concentrations: 0.005 %, 0.01 %, 0.02 %, 0.05 %, 0.1 % and 0.5 %. Eritrospermum-14 recommended by Barayev RPC GF was used as the original breed. Unique single plants were propagated via microclonal propagation. Seeds of dihaploid plants were grown in Murashige and Skoog (MS) medium. Induction of tillering among young sprout was invoked by adding the gibberellic acid and kinetin. Henceforth formation of additional shoots was induced with using the alternation of hormonal and hormone free media.

3. Results

Seed offspring of the single regenerant plants was used in the second round selection (ABA in the concentration of 0.05 %, 0.1 % and 0.5 %). From regenerant plants selected under these circumstances the second seed offspring plants were received. They were re-injected into the anther culture and the 3^{rd} breeding cycle was conducted in ABA containing media. Regenerating capacity of embryoids induced by anthers culture media in the presence of ABA is shown in Table 1. The Table data demonstrate that regenerant plants have been received in all variants of selective media in the first and second cycle of breeding. However, in the process of further anthers cultivation (3-cycle breeding) in the selective media embryogenesis frequency sharply goes down. Growing media with ABA concentration of 0.05 % and 0.1 % were the exceptions. Regenerating capacity in these versions was equal to 100 % and 81.2 %, respectively. It can be assumed that the cells, which are resistant to water shortage, have been able to regenerate in selective media containing higher concentrations of ABA. The reproductive organ selection resulted in obtaining of regenerant plants and their seed offspring-L1 and L2.

Table 1. Plant regeneration in 3 cycles of breeding with use of ABA

ABA concentration (%)	Plant regeneration (%)		
	I-cycle	II-cycle	III-cycle
Control	75.0 (78.7–71.2)	100 (99.8–100)	71.0 (74.9–67.0)
0.005	68.4 (72.4–64.3)	61.5 (65.7–57.2)	-
0.01	100 (99.8–100)	75.0 (78.7–71.2)	-
0.02	52.9 (57.2–48.5)	50.0 (54.3–45.6)	-
0.05	50.0 (54.3–45.6)	40.0 (44.2–35.7)	100 (99.8–100)
0.1	50.0 (54.3–45.6)	33.3 (37.4–29.1)	81.2 (77.61–84.5)

I selection cycle – plant regeneration from Eritrospermum-14 cultivar embryoids; II cycle – 1^{st} seed offspring of L1 regenerant plants; III cycle – 2^{nd} seed offspring of regenerant plants obtained harvested from the embryoids induced on L2 ABA. However, the statistical analysis of the roots length of L1, L2 lines and Eritrospermum-14 original breed showed that the difference between two variants was insignificant (1–Eritrospermum 14 and L1: $t_{\text{pilot}} = 1$, $t_{\text{table}} = 2.31$, i.e., $t_{\text{pilot}} < t_{\text{table}}$ is statistically invalid; 2–Eritrospermum 14 and L2: $t_{\text{pilot}} = 0.19$, $t_{\text{table}} = 2.37$, i.e., $t_{\text{pilot}} < t_{\text{table}}$ is statistically invalid).

The embryonic roots of original breed and AR45 lines are depicted in Fig. 1.

Thus, osmo-tolerant cell lines were obtained *in vitro* at the level of the reproductive cells using abscisic acid. Based on “cell-plant-cell” morphogenetic cycle drought-resistant line AR45 was selected. It had demonstrated its resistance to drought in 9 points of field evaluation (Figure 2a). Further on the line was reproduced in the field (Fig. 2). In 2006 yield of Akmola-2 class was 2.6 t/ha and yield of AR-45 was 2.89 t/ha. AR-45 line reliably exceeded standard by 0.29 t/ha. For three years on

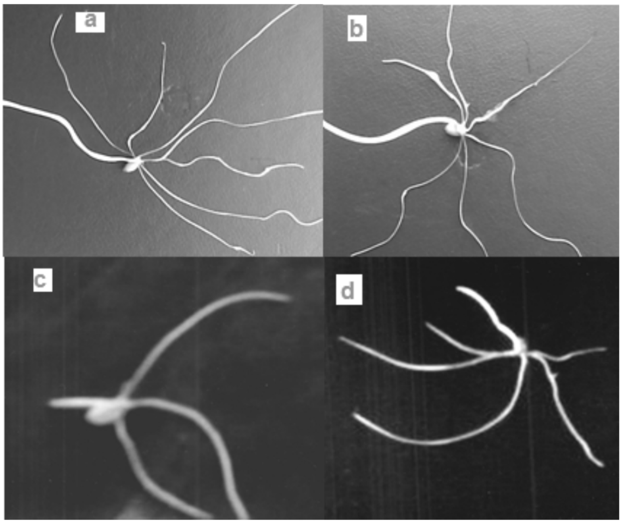

Fig. 1. Embryonic roots of original breed and AR45 lines: a–3d day (Eritrospermum 14); b–on the 5th day of seeds sprouting (Eritrospermum 14); c–sprouts which form more than 5 root in containing median ABA c (AR45); d–sprouts forming up to 7 roots in ABA containing medium

average AR-45 line has exceeded the standard by 0.45 t/ha. These lines can be attributed to the medium-slow maturing group per the length of the growing season (Fig. 2).

Lines which were obtained during gamete selection were distinct by anatomical and morphological characteristics and overall architecture - largest leaf surface, top sheet is located under much lower angle (45°–55°) with respect to stalk, which leads to the shifting phases of growth-earing for the better period of water availability. Analysis of the structural elements (Table 2) suggests that productivity comes from a higher productive tilling capacity, a number of grains in the spike, a weight of grain from the spike. Line AR45 showed drought resistance, and productive tilling capacity was 2.2 in Akmola region conditions. Kostanay region differs on climate and other agricultural factors and the line was inferior to standard in terms of Kostanay region, it is associated with the change of ecological-geographic zone.

Table 2. Structural elements of yield in Akmola region

Class, line	Plant height (cm)	Productive tilling capacity (PCs)	Number of grains in the spike (PCs)	Weight of grain from the spike	Weight of 1000 grains (g)	Protein
Akmola 2, st	85	1.9	28.4	1.4	42.7	13.6
AR-45	90	2.2	32.5	1.6	36.4	13.4

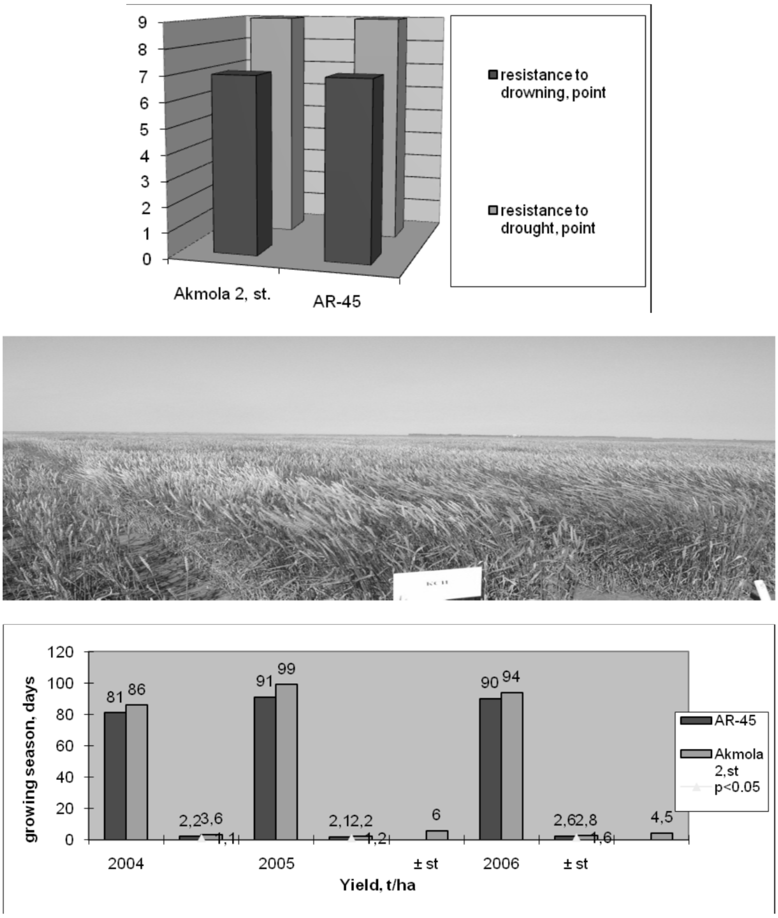

Fig. 2. Evaluation of AR-45 field and st. Akmola 2: top–resistance to drought and drowning; middle–reproduction of AR-45 line, bottom–yield and growing period

In 2008–2010, line AR-45 was inferior to standard class Karabalykskya 90 in productivity by 0.45 t/ha (Table 3). AR-45 ears and matures 3 days later than the standard, resistance to drowning is 5.0 points, whereas for standard it is 4.8 points.

4. Conclusion

Agronomic and physiological characteristics of plant productivity obtained in culture *in vitro* were discussed in our works [3, 7] under long-term research. This article provides information on the results of field tests of droughty 2006, total amount of precipitation for the plant growing period was 89.5 mm against long-time annual average rate of 134.7 mm, which is 45.2 mm less than normal. For the first time the information on embryo root of regenerant plants offspring and original breed in their amount has been provided, which is very important for creating the source forms to water deficiency, and as well as agronomic signs of AR-45 in Kostanay region. Weather conditions of Kostanay region are sharply different from weather conditions of Akmola region. The dependence of complex inheritance characteristics of polygenetic ecological-geographic zone is considered in detail in the work of V. A. Line AR-45 was used in process of hybridization and with the participation of this line 18 combinations of the breeders were conducted. As a result of selection 4400 elite ears have been selected in breeding nursery, and that is the positive outcome of the work. AR-45 line is distributed into two biotypes depending on plant height and maturation periods which are seeded separately in comparison with both the standard and original AR-45 form.

Table 3. Structural elements of yield and growing period of line AR-45 in small reproduction, average in years 2008–2010

Class, line	Plant height (cm)	Productive tilling capacity (PCs)	Number of grains in the spike (PCs)	Weight of 1000 grains (g)	Yield (t/ha)	Deviation from St, +; -	Growing period (day)	Sustainable to drowning (points)
Karabalykaya 90	89	1.56	28.6	35.6	2.38		82	4.8
AR-45	85	1.58	27.2	33.4	1.93	–0.45	85	5.0

Further on the potential lines will be delivered to the State System for Ensuring Uniform Measurement (SSM). Field testing of the line AR-45 obtained through anthers culture in the next years (2011–2014), on productivity and other valuable agronomic traits were stable and showed results which were discussed above. Thus, the *in vitro* selection scheme developed at the level of reproductive cells demonstrates its efficiency in drought-resistant plant selection where abscisic acid is used as a selective factor in adaptive cell water loss reactions. Long-continued field evaluation has confirmed morpho-physiological indicators of wheat resistance to drought and lodging.

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