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Possibilities of use of Gamete Breeding for Selecting of Plants Resistant to Water Deficiency

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Abstract. Creating a selective pressure during growth of the male gametophyte and selection microspores under selective conditions with obligatory receipt of haploid structures of constant form , that is, doubled haploid lines with given properties is one of the tasks of biotechnology and the goal of our research. Using ABA when pollen haploid breeding the most defensible way since this hormone plays a key role in the response to water stress . Pollen haploid breeding using ABA efficient way also because ABA is a fertility control.

Key word: haploid, doubled haploids, anther culture, stress, microspores, sporophyte, gametophyte, osmotic tolerance, selection.

I. INTRODUCTION

Resistance to abiotic stresses such as drought tolerance and salt tolerance takes a dominant position among the priorities of the world's wheat breeding. The combination of traditional and biotechnological methods of breeding is currently effective in creating new varieties while increasing productivity and resistance of plants to the above stressors. Accelerated doubled haploid breeding lines and their use in a variety of cross-breeding schemes gives tangible practical results.

At the IX Symposium in Canada for research and achievements of breeding and wheat genetic resources, it was noted that during the next 20 years to satisfy the growing needs of the world's population wheat production should grow by 1.6-2.6% per year and achieve 840-1000 million tons by 2020. (Rajaram, Brawn 2006)

The yield of spring wheat in Kazakhstan is less than 1 ton per hectare, well below the world average for 1990 - 2007, as shown in Table 1. This is due to the peculiarities of sharp continental climate of Kazakhstan, predominantly, the lack of water availability, as well as sharp fluctuations in temperature which deterrents wheat increase productivity. For example, wheat yield for 2007 -2012 years (1) in Kazakhstan environments 1,7 t/ha.

Wheat crops are suffering from drought for two years out of three and productivity in almost all regions of Kazakhstan is subject to many other abiotic stresses. In addition, wheat can not withstand the sharp fluctuations of temperature background in early spring, also it does not tolerate the cold, salinity.

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Table 1. Yields of wheat in Kazakhstan (data from Ministry of Agriculture)

Years	Area, million hectars	Yield, t / ha
Up to 1990	14.1	1.00
1991-95	13.1	0.80
1996-2000	10.7	0.85
2001	10.7	1.18
2002	11.6	1.08
2003	11.3	1.02
2004	11.8	0.84
2005	12.6	0.87
2006	12.4	1.09
2007	12.7	1.07

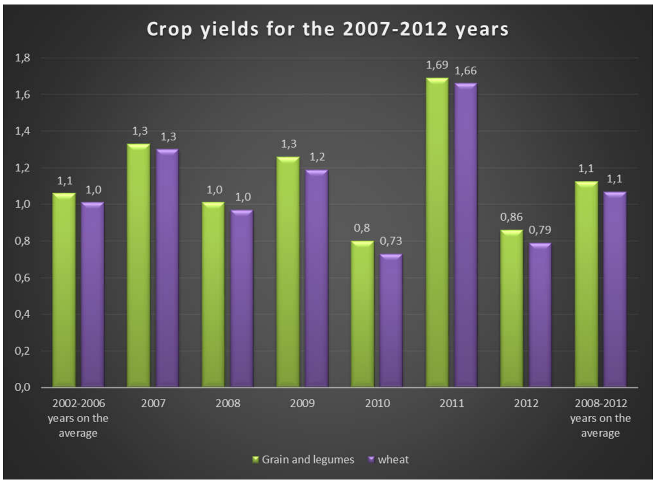

Fig. 1 The yield of grain and leguminous crops, including wheat for 6 years.

Biotechnology methods are alternative methods for studying stress resistance and selection osmo-tolerant cell mechanisms. Recently, studies of the drought problems and salt tolerance prefere genetic engineering studies and the study of the molecular mechanisms of stability control.

Plants exposed to a lack of moisture undergo certain changes. There are studies on gene loci in Trititicum aestivum, Hordeum vulgare, Secale cereale, responsible for resistance to dehydration and the important role belongs to the auxin signaling pathways (Dong-Woog et al., 2009, Dong-Wei Di et al., 2015).

One of biochemical pathways, involving response to a moisture level of gene deficiency is metabolism-dependent abscisic acid (ABA). ABA hormone is essential for plant resistance to adverse environmental factors, and this may explain the increased interest of scientists to study the

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mechanisms of action of ABA..

The ABA study's authors prefer the isolation of mutant Arabidopsis plants with a target gene switching signal systems (Hetherington et al., 2001), the allocation of receptor protein ABA (Buchanan, 2000).

Abiotic stresses, especially such as drought and salinity, provoke similar cell disorders and activate similar signaling pathways. QTL-genes found in chromosome 5A, associated with the synthesis of ABA levels in wheat, are in a similar region in rice chromosome 9, and both are associated with osmoregulation (Pshenichnikova, 2006).

ABA-binding protein first isolated from the leaves of beans and analysis of changes in guard cells of stomata shows that "... ABA is not the signal circuit and the signaling network that allows alternative signal flow and the possibility of action of ABA signals the actions of other hormones, and it is very important for of economic genotypes "(Kefeli, 1989).

Expression of the gene encoding proline, increases at water stress, including signal ABA-dependent and ABA-independent reactions. Glycine betaine stabilizing the bifunctional function, a key enzyme of photosynthesis during water stress under in vitro and in vivo.

There exist a number of wideky known works on the use of ABA in research on drought resistance at the cellular level (Tuchin 2000; Lu et al, 1989.). A very important phenomenon under stress on a plant is the accumulation of low-molecular osmoprotectants performing a protective function. Plant cells cultured *in vitro*, are subjected to a number of genetic, epigenetic and morpho physiological changes. Features i*n vitro* methods for selection of drought-tolerant plants have been shown in earlier studies, using a selective agent mannitol, proline, etc. (Duncan et all, 1995; Mohamed et all, 2000).

The plant response to stress, as lack of water are known two ways, namely ABA-independent and ABA-dependent.

ABA-independent pathway activates cis-active element, which is called the "element of response to dehydration» (DRE; or C-repeat) and is currently isolated from many plants. Genes CBF1 (C-repeat binding factor1), (DREB1) found in plants of Arabidopsis - a small multigene family was discovered in rice and barley. In this study, researchers make an assumption that this regulatory factors which cause gene expression, which in turn may be orthologous genes of resistance of wheat. Arabidopsis Genes DREB respond to dehydration (dreb1, dreb2) induced by low temperature and water stress, and water and osmotic stress is not required for the expression of ABA. In the Nipponbare rice variety a sequence encoding a polypeptide having the amino acid composition of significant similarity to Arabidopsis CBF / DREB1 was detected (Dong-Woog et al., 2009).

ABA - dependent pathway is studied in Arabidopsis, which enhances the induction of A 382 BA genes. Open type of cis-element are dependent promoters of ABA genes. It was established that ABA is accompanied by significant changes in the genome. It is assumed that it is a signaling network that interacts with other hormones, and the reaction occurs in the cell stress response to reaching physiological effects and symptoms characteristic of stability (Kulaeva et al., 2009).

It is assumed that there are genes or TF - transcription factors containing three genetic factors - DRE / CRT and ABRE. These factors can trigger the function of salinity, drought and cold genes and ABA signaling pathways, and, thus, allow to provide stability to the complex **stress**. The review A. Roychoudhury et al examines in detail questions of ABA-dependent and ABA independent pathways of plant stress. Researchers also pay attention to the presence of transcription factors TFs, calling them trigger of salinity, drought and cold effects, which can provide a cross-tolerance. This factor mediated calcium binding and serves as a second messenger of the abiotic stress, endogenous ABA can induce synthesis the genes encoding enzymes of β-carotene (A. Roychoudhury et al.2013).

The genes of osmoregulation (or-genes) are closely related to plant yield under drought conditions. Or-gene is a recessive gene and is often used by Australian breeders in wheat breeding (Koshkin E., 2010).

Successful work on the study of plant signaling systems on abiotic and biotic stresses had been held in the Kazan Institute of Biochemistry and Biophysics plants. The role of ABA and other phytohormones synthesized in the regulation of a set of polypeptides in cells and their phosphorylation is revealed (Tarchevsky et al., 2001).

The drought resistance mechanisms is very complex and not fully understood. Water deficit in plants is avoided by the following mechanisms: the escape from drought - period of rest during the drought, water conservation - closing of stomata, low cuticular conductance, weak absorption of PAR, maximum water consumption - a good root system.

The most important mechanism is stomatal apparatus which is associated with the synthesis of ABA bound with transpiration processes controlling wilting. Stomatal closing induced by the ABA, which included complex intracellular signaling where nitric oxide plays an important role. The hormone ABA is considered as regulator of stomatal apparatus, however, it is a complex process that is associated with the modulation of the concentration of this hormone in the guard cells of stomata and distant transport (Koshkin, 2010).

Drought tolerance of plants is closely associated with the root system in particular, with the number of germ roots and root anatomical structure. For example, Australian scientists studying the anatomical structure of the root, in particular, a sign of reducing the diameter of the xylem, increased of wheat productivity by 7% (Koshkin E., 2010). The decisive moment of plant adaptation to water scarcity are the processes of osmotic regulation of root and, of course, the genetic constitution of the type, which determines the maximum stomatal conductance, stomatal size, and this figures differ well from C3 and C4 plants.

When osmotic stress caused by the high concentration of NaCl in salt-tolerant varieties, increase of endogenous ABA content in plants is 30 times of the sensitivity of varieties. The use of exogenous ABA and the impact of salt stress (150 mM NaCl) increases the content of m-RNA in salt-tolerant varieties of Pokkali and Nonabokra rice varietes in the roots and leaves, than salt sensitive varietes TN-1 (S.Basu and A.Roychoudhury, 2014).

Particular attention on the study of drought tolerance should be paid during the growing season. For example, two wheat development period are very sensitive to soil moisture: the beginning of the period of formation of gametes and laid of grain. Functional disorders of the reproductive organs can cause irreversible reactions. In particular, the male gametophyte development disorders cause

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disturbances of metabolic processes. Water stress change distribution of starch in anthers and inhibits pollen outer shell intin. The development and fertility of male gametophyte is more sensitive to drought than the female reproductive organs (Koshkin, 2010).

Thus, the grain productivity depends on the normal passage of meiosis stages as the earliest disturbances of water scarcity and stress stability studies during the development of microspore and pollen show. Selective pressure during growth of the male gametophyte is one of the possible ways of creating a stress-resistant forms.

Currently, there is a growing interest in haploid technology. DH - lines are widely used in conventional breeding programs. Implementation of haploid technology in conjunction with the methods of classical genetics and breeding now allowed to select shapes, varieties and hybrid individuals with valuable economic traits (J. Murovec et.al, 2012, Sangam et.al, 2015). Androgenesis described for more than 240 species, 80 genera and 38 families of angiosperms. Cultures that have received DH-lines are presented on the website COST Action 851. The most studied - or rather, a model object in this direction is the culture of anther and microspore genus Brassica (Grishchenko, Blum 2001; Maluszyncki et al, 2003.). Third, doubled haploids are used for mapping genomic genetics and molecular markers in the preparation, as well as to serve as a target for microspore transgenic plants, etc.

In the Forster et al. review (Forster et al., 2007) the advantages of using haploid technology is discussed in detail, study focuses on the question of relevance of haploid issue. Previously, the use of haploid methodological difficulties has been limited, which is now resolved, and in the early works all kinds of benefits of the accelerated creation of DH-lines biotechnology techniques have been discussed in detail (Szarejko, Forster, 2006; Xu et al, 2007, Chiancone et al, 2015). The appearance of new methods of cell biology now allows detailing the stage of embryogenesis and a new look at microspore embryogenesis develops, in particular, the role of suspensors is described (Supena, 2008)

One possibility of the use of haploid technology is induction of gametic variability in the selection at the gamete level.

Adaptive selection closely linked with the gametophyte, it is possible that using a selection at the gamete level can alter the activity of genes responsible for stress resistance, those that induce epigenetic variability. Moreover, it can be assumed that the selective pressure of the male haploid phase leads to a change in nuclear-cytoplasmic ratio, which in turn implements a program of plant genetic adaptation to soil drought.

Using ABA in the pollen haploid selection the most defensible way since this hormone plays a key role in the response to water stress. Drought negatively affects the growth and development of wheat, but particularly affects plants during the formation of gametes and during flowering. Pollen haploid selection using ABA still efficient way because ABA controls fertility.

The efficiency of winter wheat pollen haploid selection system was described in 1992 (Hu Daofen, 1992). Pollen is widely used for selection to the chloride salinity and corn plants were selected at the pollen on the second checked sporophyte generation (Frascaroli, 2001).

Work on the haploid selection successfully carried out on wheat microspores. The relationship between the stability of pollen and sporophytes to changes in the environment, competitiveness of pollen and intensity growth of sporophyte and between pollen viability and morphological parameters (asymmetry and kurtosis) characterizing the plant adaptability. It is shown that the evaluation and selection of genotypes can be carried out on the stability of the pollen (Balashova et al, 1994.; Mulcahy, 1979) .

Successful work on the use of anther culture for the selection of salt-tolerant forms were carried out in the Picard's laboratory (Picard, 1989). For example, diploid barley lines was been selected on anther culture from a hybrid between the sensitive and resistant lines, using a medium with NaCl. Research is being conducted for the selection of salt-tolerant forms through microspore culture of rice (Chowdhury et al., 2001). Known works E. Heberle-Bors with staff on obtaining salt-tolerant cell colonies in microspores culture of wheat, which is used in the NaCl (op. At Touraev et al., 1995) as a selective agent. Also works in vitro selection on salt tolerance conducted in the Vijayan laboratory (Vijayan, 2003).

Unfortunately, due to the low frequency haploids output on media containing a selective agent experimental work on the use level selection for gametes are still not widespread. Perhaps the lack of modern detailing reviews associated with the above factors.

Gamete selection can serve as an ideal system for the study of specific selective pressure to produce plants resistant to abiotic environmental factors. Selective pressure plus gametic variability can lead to significant advances in breeding for resistance to stress, as they are inherited and stable.

Research on gamete selection are successful only when it is correctly selected endogenous and exogenous balance of plant hormones that depend from the genotype and physiological condition of the donor plants.

To determine the competence of microspores in the pollen embryogenesis when the pollen must be in sufficient detail to explore and identify the hormonal status of the donor plant. We decided to approach this problem from the other side - to determine the level of plant endogenous hormones and further to determine when, how and how processing plant phytohormones donor without changing the concentration of hormones in the induction medium. Consequently, we can assume that for pollen embryogenesis induction necessary high content of endogenous indole acetic acid (IAA) in anther time of inoculation, while differentially select exogenous IAA concentration for each varieties.

We have established an experimental system to study the effect of phytohormones on in vitro embryogenesis and finding ways to shift microspores with sporophytic to the gametophyte path of development through the identification of cells competent to induce embryogenesis. Exposure to hormones auxin type - IAA, 2,4-D in the processing of donor plants, taking into account the content of endogenous IAA may contribute to the achievement of success in the search for ways to improve androgenesis in vitro.

Increasing the concentration more than 0.5% reduces the pollen embryogenesis for all investigated genotypes.

Studies have shown that the studied varieties were characterized by a low content of endogenous IAA. The reason for this might be that this factor and the associated low yield haploids as intense mitotic processes

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cause the synthesis of the hormone necessary for initiation of DNA synthesis.

After determining the content of endogenous phytohormones it was found that in vitro induction androgenesis is directly dependent on the level of endogenous hormones.

To increase pollen embryogenesis it is necessary to take into account the level of endogenous IAA, ABA, GK (gibberellic acid) and CK (cytokinins) in the ultivation of the explants, and detailed works discussed the balance of endogenous and exogenous hormones and efficiency of their results for increasing the ability of embryogenic microspores (Iwona Z. et al, 2015;. Deepak P. et al., 2012). It was proved that capacity of haploid technology including gamete selection will be increased.

Table 2 - Contents of the endogenous phytohormones in the aerial parts of the plants of spring wheat in the tillering phase (Bekkuzhina, 2014)

Treatment	IAA ng/ g dry weight	CK, mkg/ g dry weght	GK. ng/ g dry weight	ABA ng/ g dry weight
Yu 580R	18,50 ±1,2	2,26 ±0,40	44,98 ± 2,8	290,9 ± 2,8
LGV2-9 2-2	37 ± 3,1	1,17 ± 0,2	598,58 ± 1,3	145,5 ± 1,2
LGV3-6 92-3-5	18,5 ± 1,5	3,35 ± 1,1	364,68 ± 3,9	872,7 ± 1,9
LGV1-92-2	17,50 ± 2,3	1,94 ± 0,2	69,2 ± 2,3	436,4 ± 2,6

Benefits of the gamete selection and gametophyte screening for the solutions for breeding and genetic problems are described in detail in the review of Zhuchenko (Zhuchenko, 2003). Here are some of them:

- Improvement in reproductive structures themselves, and in particular pollen (change the aerodynamic properties and fertility; increase pollen productivity functions of plant as well as the ability of pollen to germinate at low or conversely high temperatures, humidity, light and other stressful situations);

- Change of the sporophyte signs by the evaluation and selection of genetically different quality of pollen grains, that is, through the use of correlations between the features of the gametophyte and sporophyte;

- Assessment of endogenous (including transgenic) and exogenous inducing frequency and spectrum of the recombination variability, manifestations of pollen traits in hybrids F1, the nature of their inheritance, as well as the differential growth of pollen tubes *in vitro* and *in vivo*;

- A study on characteristics of the haploid level, including taking into account that some signs of diploids are not shown;

- Improving the accuracy of hybrid analysis by taking into account possibilities of elimination of labeled recombinant microgametes, and changes in the relationships between plant phenotypic classes in fissile generations;

- The possibility of assessing the huge number of gametes for the direct selection of the desired genotypes in a specially designed and relatively easily adjustable backgrounds, and to increase the probability of identifying sporophytes based on correlation analysis (indirect gametophytic selection).

We have developed the *in vitro* wheat haploid selection technology in the microspores, that provide a stepwise selection for resistance to abiotic stress, followed by testing for resistance at the level of the whole plant.

In our opinion, the selection of the gamete level during the induction of pollen embryogenesis is fully justified way. Using microspores to select for resistance to stress is associated with resistance to changes in pollen at ambient temperature. Participation of gametophyte in the adaptive process and the effectiveness of gamete selection is discussed in Macovei (Macovei and al., 2001, 2010).

The prospect of using ABA hormone for selecting cells to a water shortage, at selection on gametes level related to fundamental theories of dehydration synthesis of proteins with the possibility of regulating the expression of genes coding for drought tolerance feature. For example, in maize studied clusters of genes that are responsive to ABA during embryogenesis (Wilen, 1990 et al .; Williams, 1994).

As a result of our experimental studies regenerated plants and their seed progeny were obtained during the selection at the level of the reproductive organs. Plants were selected *in vitro* culture are capable of creating more than 7 germinal roots.

According to the literature the number of germinal roots is the most accurate measure of wheat resistance to moisture deficit (Isabaev 2003; CIMMIT, 2004-2005).

Many years of experimental studies had increased the output frequency of haploid (Segui-Simaro, 2008, 2010), and because of this, such experimental studies as the selection on the reproductive cells level can be carried out.

II. CONCLUSION

In the field evaluation of the lines selected at the level of gametes compared with the standard, different anatomical and morphological features and architectonic occur, like that plant had to endure water scarcity by erecting and formation of narrow leaves with a waxy bloom.

There are additional attractive sides of the haploid technology. For example, the use of a marker breeding with double haploid technologies would increase the efficiency of conventional breeding.

Double haploid plants were collected on selective media are transferred to the breeding and testing centers for including in conventional breeding programs, which is discussed in detail in our publications.

It should be considered that wheat breeding is carried on a narrow genetic basis, and only a skilful combination of traditional and biotechnological methods is effective in expanding the genetic basis of wheat. Unequivocally, selection at the gamete level can adequately be one of the main directions in the search for ways of selecting plants to stress; however, it is not necessary to detract from the dignity of traditional breeding.

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