Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2018, 8(4), 213-221

ORIGINAL ARTICLE

Osmotic adjustment in spring durum wheat pollen grains under induced drought stress

L.P. Khlebova[1], O.V. Bychkova[1], A.M. Titova[1], M.A. Rozova[2], A.I. Ziborov[2]

[1]Altai State University, pr. Lenina 61, Barnaul, 656049, Russia
[2]Altai Research Institute of Agriculture, Barnaul, Russia
E-mail: hlebova61@mail.ru, olga4ka_asu@mail.ru, mrosova@mail.ru, aniish@mail.ru

Received: 18.10.2018. Accepted: 25.11.2018

Osmotic adjustment (OA) is recognized as one of the main mechanisms of drought tolerance in crops. OA is realized by reducing the osmotic potential due to accumulation of organic and inorganic osmolytes in response to a water deficit. This mechanism is manifested in all plant cells, including pollen grains. We carried out pollen analysis to differentiate the genotypes of spring durum wheat in tolerance to drought. Eight wheat assessments of different ecological and geographical origin from the collection of the Altai Research Institute of Agriculture, Russia were studied. They were Omskaya stepnaya, Zhemchuzhina Sibiri, Bezenchukskaya 210, Solnechnaya 573, Oazis, Pamyati Yanchenko, 12S1-14, and 12S2-24. Plants were grown at the field plots in 2016. Drought was simulated by adding 55% PEG 6000 to the culture medium. Pollen samples were incubated in the dark at 24 ± 1°C during 48 hours at 70-80% relative humidity. To assess the efficiency of cation accumulation due to transmembrane migration from the external environment, 10 mM KCL as the osmolyte was added to the pollen culture medium containing 55% PEG 6000. Plasmolytic phenomena were assessed under a microscope by changing the cytoplasm area by scanning the surface of each pollen grain. Quantitative measurements of the projected area of pollen cytoplasm were carried out. We have established significant differences among varieties of spring durum wheat relative to OA mechanisms responsible for dehydration avoidance. The response of pollen grains to *in vitro* induced osmotic stress reflected various strategies for the behavior of genotypes involving or not transporting K^+ cations from the cultivation medium. Most of the accessions tested are capable of supporting the cellular turgor, using only intrinsic adjustment mechanism, or combining it with osmolyte induced OA. One line has been shown to exhibit the induced osmotic adaptation, which caused some restoration of the projected cytoplasm area. One line did not resist osmotic stress, reducing the pollen turgor both under stress and in the presence of osmolyte. We succeeded in revealing a good correspondence between the pollen reactions under induced *in vitro* osmotic stress and the field drought tolerance index of genotypes. This made it possible to test the diversity of the accessions for tolerance to drought by scanning the pollen surface. Assessment of the behavior of the male gametophyte population of wheat under conditions of induced stress allows screening and determining drought-tolerant genotypes.

Keywords: drought, osmotic adjustment, pollen grain, durum wheat, field drought tolerance index

Many environmental stresses such as drought, extreme temperatures, salinity, heavy metals, mineral deficiency, pollutants, pathogen infection when they present either alone or in combination are responsible for low plant productivity under field conditions (Ramakrishna & Ravishankar, 2011; Gursoy et al., 2012; Suzuki et al., 2014). Among the various abiotic stresses, drought is the most common and fatal to plants, particularly in critical growth stages. In the worst-case scenario it may result in the complete damage to crops (Zivcak et al., 2016). It is currently estimated that one third of the land potential suitable for cultivation is not used because of water shortages. According to forecasts, the situation will worsen due to climate change processes (IPCC, 2007; Reynolds et al., 2016). Drought of varying degrees can be observed in almost all climatic zones (Passioura, 2007).

For agriculture, drought is defined as insufficient moisture supply which causes a reduction in plant production. It is a gap between crop demand for water and the supply of water (Blum, 2011). Currently, the development of plant genotypes with an increased capacity to survive during water shortage combined with a high yield potential is the primary goal of agrobiology (Jarzyniak & Jasinski, 2014). This way is the most effective means to improve and stabilize yield under stress conditions (Blum & Jordan, 1985; Tester & Langridge, 2010). Modern approaches to produce drought-tolerant cultivars combine (or try to combine) conventional breeding tools with marker-assisted selection and genetic engineering technology (Richards et al., 2010; Blum, 2011; Farooq et al., 2011; Boopathi, 2013; Nezhadahmadi et al., 2013; Obata et al., 2015; Fleta-Soriano & Munné-Bosch, 2016). However, the success of any breeding program is determined by the availability of reliable markers, which allow identifying drought-tolerant genotypes. Crops show various morphological, physiological, biochemical, and molecular responses to solve the problem of water shortage (De Micco & Aronne, 2012; Nezhadahmadi et al., 2013; Jarzyniak & Jasinski, 2014; Rybka & Nita, 2015; Fleta-Soriano & Munné-Bosch, 2016; Reynolds et al., 2016; Bi et al., 2017). Common strategies for drought adaptation include escape, avoidance, and tolerance. Drought-escaping mechanism allows plants to complete their life cycle before developing a severe dry period. This is realized by active metabolism, rapid growth, and accelerated maturation. Avoidance strategies are based on the maintaining water uptake through the modification of root architecture. Decrease in water loss is also due to reduced stomatal and cuticular conductance and reduced evaporation surfaces. Drought tolerance mechanisms are associated with the ability of cells to counter low water potential. This is achieved via osmotic adjustment and the synthesis of low-molecular weight proteins that protect plants from damage caused by water deficiency (Ashraf & Foolad, 2007; Bacelar et al., 2012). For crops grown in conditions of water shortage in arable soils, the latest two strategies are relevant. The terms “avoiding drought” and “adapting to drought” are fundamental, determining the physiological status of plants in conditions of water scarcity (Rybka & Nita, 2015). It is worth mentioning another term used in the scientific literature, “drought resistance” (Blum, 2011), which is according to Blum, is more appropriate and does a working one main for breeders who create varieties with increased productivity for regions prone to droughts.

Osmotic adjustment (OA) is wide recognized as one of the key mechanisms of drought tolerance (or “dehydration avoidance” according to Blum) in crop plants. OA is realized by decreasing the osmotic potential due to accumulation of organic and inorganic osmolytes in response to a water deficit (Zhang et al., 1999). It should be emphasized that this term is used when there is an accumulation of new substances, rather than the concentration of existing solutes as a result of water loss (Babu et al., 1999). Thus, OA is an active process where solutes increase in plant cells to prevent dehydration main by maintaining the turgor and protecting specific cellular functions (Blum, 2017). For the same leaf water potential, a higher ability for osmotic adjustment determines the greater relative water content in the leaves, providing an increase in turgor, in comparison with plants with a low OA (Blum, 2011). Compatible solutes that reduce the osmotic potential of the cytosol are sugars, amino acids such as proline or glycine betaine, sugar alcohols including mannitol, and other low molecular weight metabolites (Ashraf & Foolad, 2007; Javadi et al., 2008; Marcińska et al., 2013; Pawar et al., 2015; Karpets et al., 2016). Osmolyte pool leading to the increased osmotic adjustment can also include inorganic ions such as potassium (Morgan, 1992) and sodium cations (Slama et al., 2015).

A wide range of genetic diversity for OA was found in many crops. They are wheat (Morgan, 1992; Blum et al., 1999; Moinuddin et al., 2005; Bănică et al., 2008; David, 2009; Ahmed et al., 2014), barley (Gonzalez et al., 1999; 2008), maize (Chimenti et al., 2006; Anjum et al., 2017), sorghum (Santamaria et al., 1990; Patil & Ravikumar, 2011), sunflower (Chimenti et al., 2002), chickpeas (Moinuddin & Khanna-Chopra, 2004). The capacity for OA is shown to be an inherited trait that in wheat, for example, is controlled by alternative alleles at a single locus located on the short arm of chromosome 7A, about 13 cM from the marker (Morgan & Tan, 1996). The recessive *or* gene, associated apparently with potassium accumulation (Morgan, 1999), is common in Australian wheat breeding programs (Richards, 2006).

Osmotic adaptation is manifested in all plant cells, including pollen grains. To date, it has been established that up to 70% of structural sporophyte genes are expressed at the gametophyte level. So, comparison of the sporophyte and gametophyte transcriptomes of *Arabidopsis thaliana* revealed 61% overlap (Honys & Twell, 2004). Overlap in barley is estimated to be 60% (Pedersen et al., 1987) and in maize it amounts to 72% (Sari-Gorla et al., 1986). This allows selecting valuable genotypes at gamete level. The advantages of microgametophyte selection are appreciated by many researchers in breeding programs for different crops (Clarke et al., 2004; Patil et al., 2006; Rani & Ravikumar, 2006; Ravikumar et al., 2007; Totsky & Lyakh, 2015). They are the microscopic size of the pollen, many genotypes, and the haploid state of pollen genotype, which ensures the expression of recessive genes, many of which determine the economic value of cultivated plants (Beyaz et al., 2011). Also, gametophyte selection creates an opportunity to regulate the “rigidity” of selection in conditions that are strictly controlled.

The purpose of this study is to assess the diversity of spring durum wheat genotypes for tolerance to drought, using the response of pollen grains to induced *in vitro* osmotic stress.

Material and Methods

Eight genotypes of spring durum wheat of different ecological and geographical origin from the collection of the Altai Research Institute of Agriculture, Barnaul, Russia were studied. They were Omskaya stepnaya, Zhemchuzhina Sibiri, Bezenchukskaya 210, Solnechnaya 573, Oazis, Pamyati Yanchenko, 12S1-14, and 12S2-24. Accessions were grown at the field plots in 2016. Mechanisms of osmotic adaptation were evaluated *in vitro* using pollen grains exposed to different levels of osmotic stress. To do this, freshly cut ears were delivered to the laboratory just before to the start of the experiment. Drought was simulated by adding a high molecular weight osmotic agent, polyethylene glycol 6000 (PEG 6000), to the culture medium.

Pollen grains from ripened but not cracked anthers were immersed over microscope slides in a drop of 30% PEG 6000 (control) as well as 55% PEG 6000 and incubated in the dark at 24 ± 1°C during 48 hours at 70-80% relative humidity. PEG is a non-penetrating osmotic that causes the collapse of cell walls and protoplast compression imitating well the water balance of the cell under conditions of osmotic stress. Also, a variant of cultivating pollen grains in 55% PEG 6000 containing 10 mM KCl solution as an osmolyte was studied. The use of 30% PEG 6000 as a non-stressing control is due to the fact that at lower osmotic concentrations the pollen grains showed bursting (Banica et al., 2008; Patil, Ravikumar, 2011). Cytological analysis was carried out under the Olympus BX-S1 microscope at a magnification of 10×15×40. Plasmolytic phenomena were assessed by changing the cytoplasm area by scanning the surface of each pollen grain using the SellensStandard Program and expressed by the number of pixels per unit square. The following parameters were evaluated: A – projected area of pollen cytoplasm in the absence of osmotic stress (30% PEG 6000); B – projected area of pollen cytoplasm under conditions of osmotic stress (55% PEG 6000); C – projected area of pollen cytoplasm under conditions of osmotic stress (55% PEG 6000) with addition of 10 mM KCl solution. Two hundred pollen grains on a genotype in four replicates were estimated. The field drought tolerance index (TI) was calculated for the genotypes as a ratio between the mean yield obtained under drought stress and the corresponding trait value in favorable conditions (Rozova & Ziborov, 2016).

ANOVA as well as covariance analysis of the data received were carried out using the Microsoft Excel 2010 software package. The relationship between variables was analyzed by regression. The reliability of the differences among the means of a pair of genotypes was tested using the Least Significant Differences ($LSD_{0,05}$).

Results and Discussion

The revealed high-level of overlapping in the genes expressed during the gametophytic and the sporophytic generations (Mascarenhas, 1990) and the similarity in their behavior under the influence of various external factors give a basis for studying the response of plants to different stresses by screening the pollen produced by those plants (Hormaza & Herrero, 1996). Besides to reducing the cost of screening, it becomes possible to analyze the stress reaction of pollen produced by a single plant without destroying this plant. To differentiate homozygous and heterozygous lines of bread wheat for use them in backcross programs, Morgan (1999) designed an experiment to assess the possibility that the gene responsible for osmotic adjustment in leaves (*or*) is also expressed in pollen. He managed to find the corresponding allelic differences in the pollen grains subjected to osmotic stress induced by PEG solution. We applied Morgan̓s pollen test to identify durum wheat genotypes that are capable of OA, with a view to their further use in breeding programs for the creation of drought tolerant varieties.

The cytoplasm area of durum wheat pollen grains subjected to different levels of osmotic stress differentiates depending both on the genotype and on the conditions of pollen cultivation (Table 1). This is confirmed by two-way analysis of variance ($F_{genotypes} = 13.02 > F_{0,05} = 2.76$; $F_{conditions} = 3,98 > F_{0,05} = 3.74$).

Table 1. The projected area of spring durum wheat pollen grains under conditions of *in vitro* osmotic stress, pxl^2 (mean ± SE)

Genotype	30% PEG (A)	55% PEG (B)	55% PEG + 10 mM KCl (C)
Solnechnaya 573	56824.47±4467.5	60323.32±3019.3	56036.94±723.2
Pamyati Yanchenko	55861.76±2198.2	56305.41±4452.7	56489.54±507.7
Bezenchukskaya 210	55595.15±832.7	64724.56±9801.9	56042.11±128.2
Oazis	50342.31±533.6	54818.37±726.9	57016.49±626.3
Zhemchuzhina Sibiri	50206.27±2328.9	50268.12±838.0	51399.17±395.4
Omskaya stepnaya	48386.25±553.3	54114.92±355.0	51082.20±606.9
12S2-24	46364.25±534.3	40386.25±1013.3	40956.21±2021.2
12S1-14	45943.25±423.3	41869.25±343.3	40023.25±335.8
$LSD_{0.05}$	**7220.9**	**12946.8**	**1596.8**

To avoid the influence of the initial pollen size, we calculated the relative index for estimating the pollen grain areas under conditions of induced stress, expressed as a percentage of the cytoplasm area of pollen exposed to 30% PEG 6000 solution. As it turned out, the studied genotypes are characterized by different behavior scenarios under different models of osmotic stress. So in Zhemchuzhina Sibiri and Pamyati Yanchenko, the cytoplasm area of pollen after immersion in 55% PEG 6000 has not changed. Four genotypes showed an increase in the index, while the lines 12S1-14 (Fig. 1A) and 12S2-24 did a decrease by more than 10% in the cytoplasm area of stressed pollen (Fig. 2).

Morgan (1999) found that the mechanism of osmotic adjustment in wheat pollen grains under induced stress conditions occurred only after the addition of a small amount of potassium chloride. The greatest response was observed at a concentration of 0.2 to 10 mM. He suggested that the before identified gene *or*, which conditions differences in the OA of wheat leaves, is responsible for the potassium transport. In our experiment, the reaction of pollen to the addition of potassium chloride as an osmolyte varied in different genotypes. In some varieties, like Pamyati Yanchenko and Zhemchuzhina Sibiri, the projected area of the cytoplasm in pollen subjected to osmotic stress remained at the control level, while in others we observed a decrease in the index relative to non-stressed pollen (Solnechnaya 573). The surface of the cytoplasm has changed. Clear cavities have appeared (Fig. 1B). In contrast, the Oazis variety showed active accumulation of osmolyte, increasing the area of pollen grains by 13%. Besides, we did not observe any plasmatic phenomena, and the pollen remained round with a uniformly distributed cytoplasm in the cell (Fig. 1C). Various durum wheat genotypes probably use different types of osmotic adaptation with different levels of inorganic cation involvement.

We tried to determine the mechanisms of OA, working in the accessions we studied. The identification of such mechanisms is based on the reasoning presented by Patil & Ravikumar (2011) and by David (2012). The detection of the type of pollen reaction on osmotic stress was carried out according to the ratio of the parameters of the cytoplasm (A, B, C). The so-called "intrinsic" OA is defined as the ratio B/A ≅ 1. When B/A < 1, there is no intrinsic OA. The reaction of the pollen cytoplasm to the external supply of an osmolyte in the culture medium is treated as an "induced" OA, which is found at the ratio C/B ≅ 1. The ratio C/B < 1 indicates the absence of induced OA. The total OA is composed of internal and induced ones. It is determined by the ratio C/A.

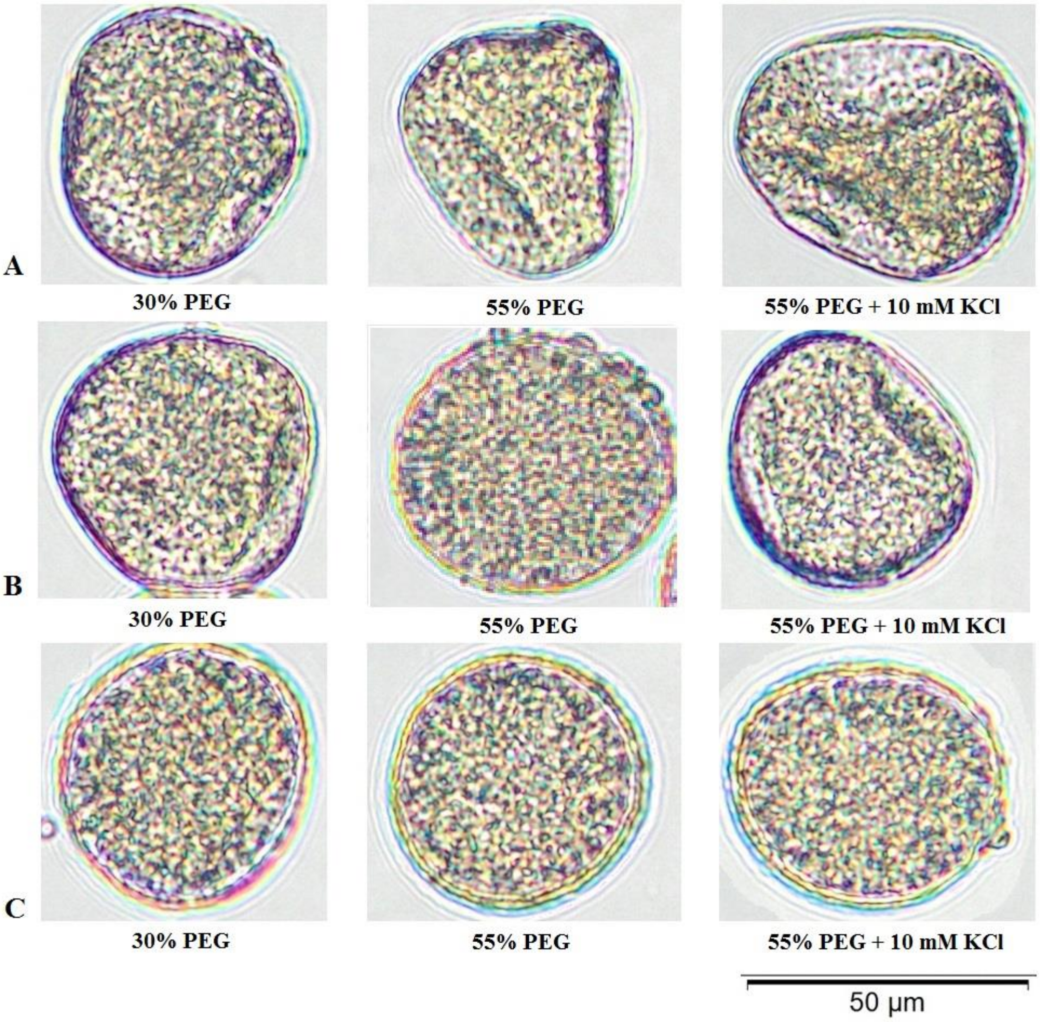

Figure 1. Modification of projected area of pollen grain cytoplasm in durum wheat genotypes under different models of *in vitro* osmotic stress induced by PEG 6000: A – 12S1-14; B – Solnechnaya 573; C – Oazis

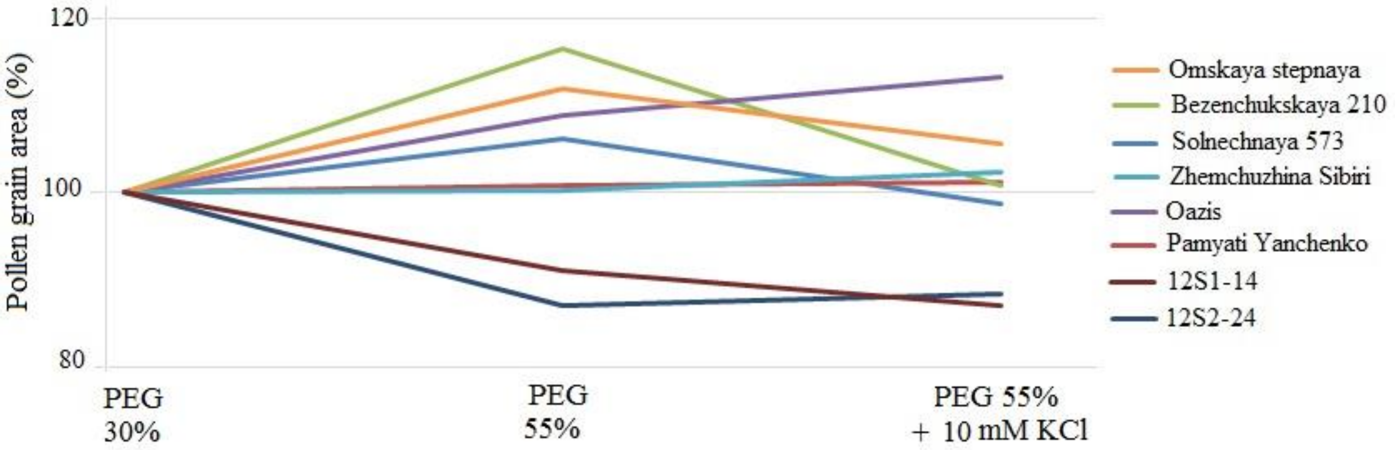

Figure 2. The reaction of durum wheat pollen cytoplasm under different models of *in vitro* osmotic stress induced by PEG 6000, % to control

Table 2 represents the parameters described above which we calculated for an individual accession. Statistically significant differences between genotypes for various types of osmotic regulation have been established. The range of variation of the intrinsic osmotic adjustment was from 0.871 in 12S2-24 to 1.164 in Bezenchukskaya 210. Under induced osmotic stress, the cytoplasm area of the two wheat lines (12S1-14 and 12S2-24) significantly decreased relative to the control value (B/A = 0,911 and 0,871, respectively). The remaining varieties retained the indicator in comparison with non-stress conditions, revealing intrinsic mechanism of OA. The observed retention of the cytoplasm sizes as well as the absence of appreciable cytoplasm plasmolysis in pollen cells incubated in 55% PEG 6000 solution are probably due to synthesis of organic solutes and (or) accumulation of inorganic cations through their release from membranes. In wheat, soluble sugars and K^+ are shown to play an important role in this process (Morgan, 1999; Eivazi et al., 2007). The efficiency of cation accumulation due to transmembrane migration from the external environment can be assessed by adding osmolyte (KCl) to the pollen cultivation medium containing 55% PEG 6000. According to Table 2, the indices of induced OA (C/B) in four accessions (Bezenchukskaya 210, Omskaya stepnaya, Solnechnaya 573, and 12S1-14) were less than one, indicating that their adaptation to osmotic stress is not related to the external uptake of K^+. In Pamyati Yanchenko, the pollen cytoplasm has not changed, and the remaining genotypes either partially restored, or even increased this parameter. The index of total osmotic adjustment (C/A) is an integration coefficient, summarizing the final effect of “partnership” of various OA mechanisms. It should be concluded that the only variety Oazis, among the studied ones, increased this parameter in comparison with the intrinsic OA index.

Table 2. Types of osmotic adjustment in durum wheat pollen grains under osmotic stress *in vitro* induced by PEG 6000

Genotype	Types of osmotic adjustment		
	intrinsic OA (B/A)	induced OA (C/B)	total OA (C/A)
Bezenchukskaya 210	1.164	0.866	1.008
Omskaya stepnaya	1.118	0.944	1.056
Solnechnaya 573	1.062	0.929	0.986
Oazis	1.089	1.041	1.133
Pamyati Yanchenko	1.008	1.003	1.011
Zhemchuzhina Sibiri	1.001	1.023	1.024
12S1-14	0.911	0.956	0.871
12S2-24	0.871	1.014	0.883

Comparison of different types of osmotic adjustment in pollen grains by regression analysis showed no correlation between induced and total adaptation (Fig. 3A). As a result, 50% of varieties possessing total OA lacked the induced one. However, total osmotic adjustment was found to be highly correlated with intrinsic OA, which was confirmed by a high coefficient of determination equal to 58% (Fig. 3B). In this case, the presence of total osmotic adjustment, as a rule, was accompanied by the presence of the intrinsic OA mechanism. We came to the conclusion that the mechanism of intrinsic adaptation to the osmotic stress induced by PEG 6000 prevails in the studied plant material.

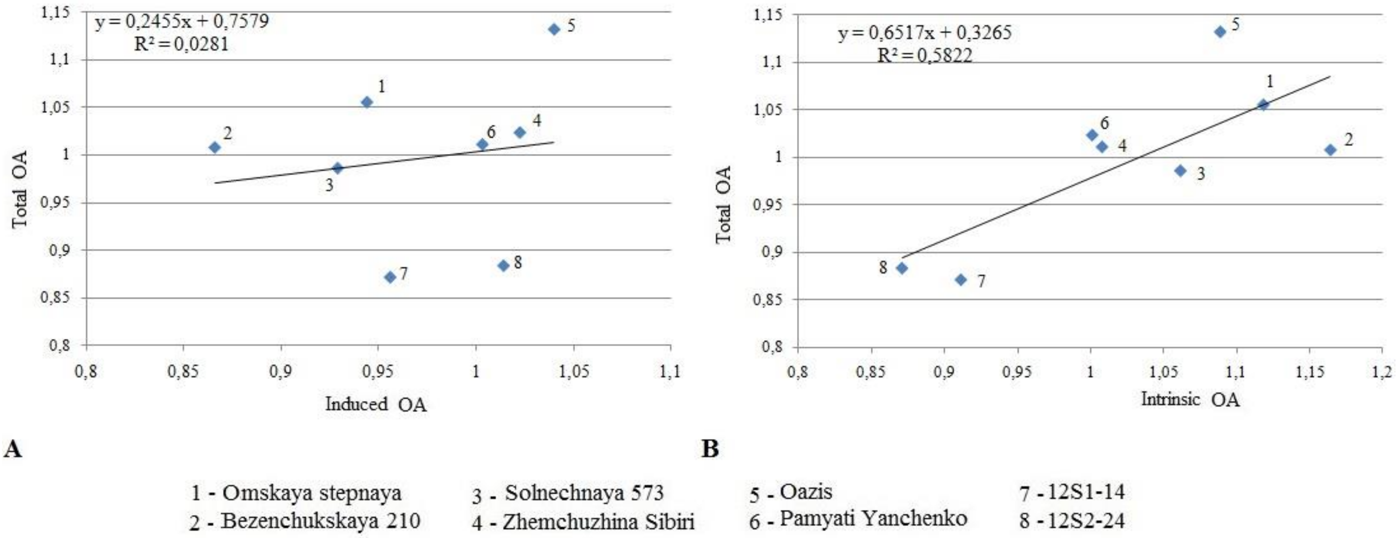

Figure 3. The relationship between induced (A), intrinsic (B) and total osmotic adjustment in spring durum wheat genotypes

The conclusion is confirmed by Fig. 4, which indicates a statistically significant negative correlation between the intrinsic and induced OA. Such a negative correlation can create certain difficulties for combining these mechanisms in breeding for osmotic tolerance. However, studies carried out using a larger collection of wheat showed no correlation between the mechanisms of intrinsic and induced OA (David, 2012), creating reasonable optimism for their integration by breeding for drought tolerance.

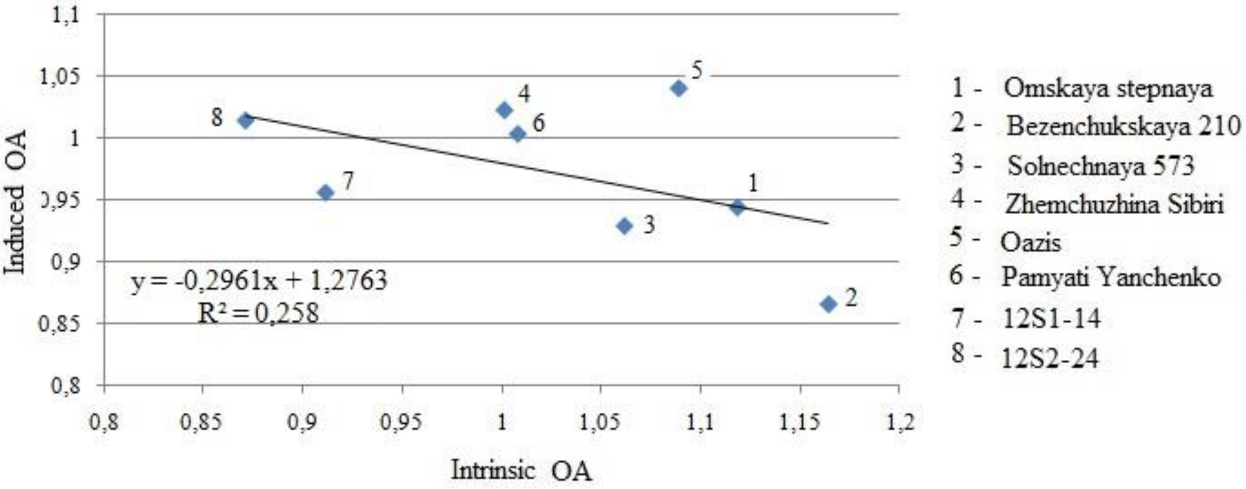

Figure 4. The relationship between intrinsic and osmolyte induced osmotic adjustment in spring durum wheat genotypes

Thus, Bezenchukskaya 210, Omskaya stepnaya, and Solnechnaya 573 successfully resist osmotic stress through the intrinsic adjustment mechanism. Oazis, Pamyati Yanchenko, and Zhemchuzhina Sibiri are likely to have both intrinsic and induced OA. It can be assumed that these accessions contain the *or* gene, which determines one of the OA mechanisms. In 12S2-24 line, only the mechanism of induced adjustment acts, but it does not compensate for the negative changes in the cell cytoplasm that occur under induced osmotic stress. Line 12S1-14 does not withstand osmotic stress and its pollen grains are most exposed to dehydration and, as a result, lose turgor.

According to Morgan (1999), the expression of the *or* gene, responsible for osmotic adjustment in wheat pollen, affects the transport of K^+ cations sucking up from the environment. However, in our experiment, some genotypes coped with osmotic stress by intrinsic OA during the incubation of pollen in a culture medium that does not contain potassium ions (K^+). It should be noted that this fact does not exclude the participation of K^+ as an osmolyte, which can be mobilized from internal cell reserves. Similar results were obtained by David (2012) when screening an extensive collection of bread and durum wheat. Also, it is important to take into account that in many plant species, individual solutes do not contribute much to OA. This mechanism is realized by the accumulation of a variety of substances (Sanders & Arndt, 2012).

The agronomic criterion of drought tolerance of crops is the yield in drought conditions. Blum (2017) analyzed the data of various researchers on the relationship of OA with the yield of plants exposed to drought. He concluded that in most studies a positive association between OA and yield under stress was found. Such an effect was shown to be in wheat, barley, sorghum, maize, sunflowers, chickpeas, pea, pigeon-pea and peanuts (Blum, 2017). There is also an alternative point of view, according to which the value of OA as a component contributing to higher yields under drought conditions is questioned. On the contrary, OA is believed to be associated with “costs” in terms of yield. Probably, it just supports survival (Munns, 1988; Serraj & Sinclair, 2002; Turner, 2017). Challenging skepticism about the role of OA, Blum (2011, 2017) views this discussion as very important, as it stimulated further research in this direction and contributed to the emergence of new ideas. With regard to wheat, the agronomic value of OA in yield stabilization during drought is confirmed by the breeding a drought-resistant variety Mulgara, which is characterized by a high capacity for OA (Richards, 2006). Ciucă et al. (2010) believe that osmotic adjustment plays an important role in the formation of the wheat yield in dry years in Romania, as confirmed by the Izvor variety, which shows an excellent performance under drought and at the same time has a high capacity for OA (Bănică et al., 2008).

In the south of Western Siberia, the conditions for vegetation of spring crops develop in different ways. They can be both very favorable moistened and sharply arid. The probability of droughts is about 30%. Both early summer droughts of varying duration and intensity and drought throughout the growing season are possible (Rozova & Ziborov, 2016). We compared the capability of pollen grains to OA under *in vitro* induced osmotic stress with field drought tolerance of durum wheat genotypes. The field drought tolerance index (TI) was calculated for the genotypes as a ratio between the mean yield obtained under drought stress and the corresponding trait value in favorable conditions. In 2012 there was a severe drought throughout the entire vegetation (with a short-term discharge of the moisture deficit at the end of the first decade of July), accompanied by high average daily temperatures, significantly exceeding the mean annual values, especially in the first half of the vegetation. Weather conditions in 2009, on the contrary, were characterized as very favorable for the growth and development of plants throughout the growing season.

The total OA slightly correlated with field drought tolerance. The determination index was 20% (Fig. 5A). However, a high significant correlation between TI and intrinsic adjustment was established (Fig. 5B). More than 40% of the variation was determined by the influence of the intrinsic OA mechanism. The relationship between induced OA and field drought tolerance of durum wheat genotypes has not been found. This type of osmotic adaptation, based on the expression of the gene regulating the transport of external osmolytes, is poorly involved in the material studied and could serve as a reserve for increasing the drought resistance of local varieties. The search for sources of induced osmotic adjustment and their involvement in the breeding program can be considered one of the ways to create drought resistance genotypes.

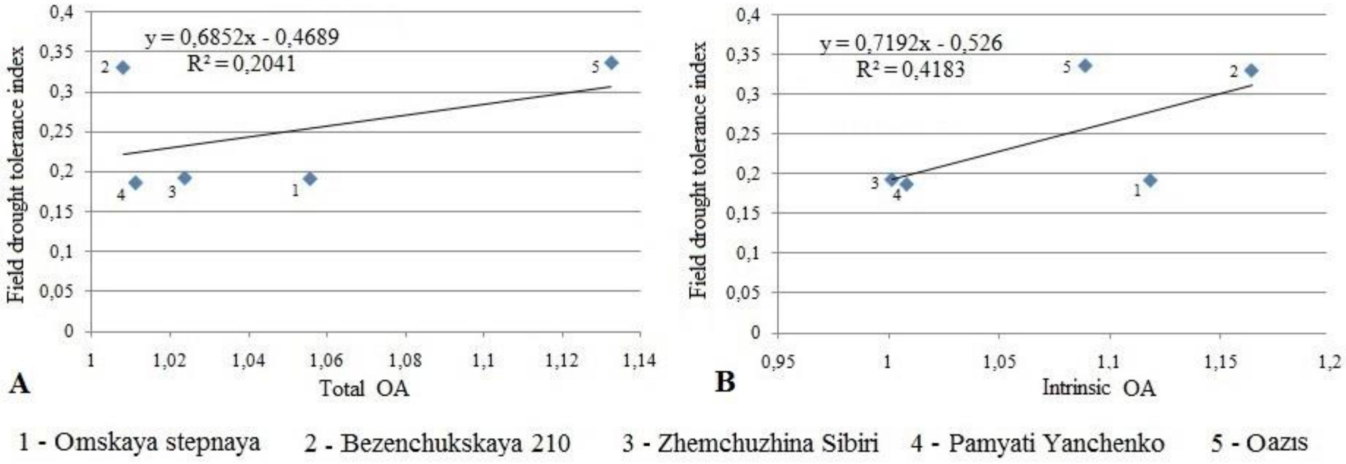

Figure 5. Relationship between total OA (A), intrinsic OA (B) and grain yield of spring durum wheat genotypes cultivated under field drought conditions

It should be noted that a low correlation (or lack of correlation) between the index of field drought tolerance and total OA in the presence of a significant genetic variation of the trait is quite possible because other mechanisms of resistance to stress may act as dominant ones under certain drought scenarios. Such examples are known in the scientific literature (Blum, 2017). So, OA in chickpea at the stage of grain filling did not correlate with the yield under drought, while this mechanism showed such a link at an earlier growth stage. Probably, under the water deficit, the stem reserve utilization for grain filling might have had a greater contribution to the yield (Leport et al., 1999). In our experiment, the mechanism of intrinsic adjustment in genotypes prevailed during the adaptation of pollen under induced osmotic stress, which probably caused a close correlation of this trait with yield under drought. There was no correspondence between TI and the induced OA, despite the fact that some genotypes had this mechanism.

Conclusions

Thus, we have established significant differences among varieties of spring durum wheat relative to OA mechanisms responsible for dehydration avoidance. The response of pollen grains to *in vitro* induced osmotic stress reflected various strategies for the behavior of genotypes involving or not transporting K^+ cations from the cultivation medium. Most of the accessions tested are capable of supporting the cellular turgor, using only intrinsic adjustment mechanism, or combining it with osmolyte induced OA. One line has been shown to exhibit the induced osmotic adaptation, which caused some restoration of the projected cytoplasm area. Finally, one line did not resist osmotic stress, reducing the pollen turgor both under stress and in the presence of osmolyte. We succeeded in revealing a good correspondence between the pollen reactions under induced *in vitro* osmotic stress and the field drought tolerance index of genotypes. This made it possible to test the diversity of the accessions for tolerance to drought by scanning the pollen surface. Assessment of the behavior of the male gametophyte population of wheat under conditions of induced stress allows screening and determining drought-tolerant genotypes.

Acknowledgements

We are sincerely grateful to Dr. N.Yu. Speranskaya for help and valuable advice in conducting microscopic studies.

References

Ahmed, A.A.S., Uptmoor, R., El-Morshidy, M.A., Kheiralla, K.A., Ali, M.A., Naheif, E.M.M. (2014). Some physiological parameters as screening tools for drought tolerance in bread wheat lines (*Triticum aestivum* L.). World Journal of Agricultural Research, 2 (3), 109–114. doi: 10.12691/wjar-2-3-4

Anjum, S.A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M.F., Ali, I., Wang, L.C. (2017). Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. Front. Plant Sci, 8, 69. doi: 10.3389/fpls.2017.00069

Ashraf, M., Foolad, M. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59(2), 206–216. DOI: 10.1016/j.envexpbot.2005.12.006

Babu, R.C., Pathan, M.S, Blum, A., Nguyen, H.T. (1999). Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Sci., 39, 150–158.

Bacelar, E.V.A., Moutinho-Pereira, J.M., Gonçalves, B.M.C., Brito, C.V.Q., Gomes-Laranjo, J., Ferreira, H.M.F., Correia, C.M. (2012). Water use strategies of plants under drought conditions. In: Aroca R. (ed). Plant responses to drought stress. Berlin Heidelberg: Springer, 145–170. doi:10.1007/978-3-642-32653-0_6

Bănică, C., Petcu, E., Giura, A., Săulescu, N.N. (2008). Relationship between genetic differences in the capacity of osmotic adjustment and other physiological measures of drought resistance in winter wheat (*Triticum aestivum* L.). Romanian Agricultural Research, 25, 7–11.

Beyaz, R., Kaya, G., Cocu, S., Sancak, C. (2011). Response of seeds and pollen of *Onobrychis viciifolia* and *Onobrychis oxyodonta* var. *armena* to NaCl stress. Scientia Agricola, 68(4), 477–481. https://dx.doi.org/10.1590/S0103-90162011000400013

Bi, H., Kovalchuk, N., Langridge, P., Tricker, P.J., Lopato, S., Borisjuk, N. (2017). The impact of drought on wheat leaf cuticle properties. BMC Plant Biology, 17(1), 85. DOI 10.1186/s12870-017-1033-3

Blum, A. (2011) Plant breeding for water-limited environments. Springer, New York. DOI 10.1007/978-1-4419-7491-4_3

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, Cell and Environment, 40, 4–10.

Blum, A., Jordan, W.R. (1985). Breeding crop varieties for stress environments. Crit. Rev. Plant Sci., 2, 199–238.

Blum, A., Zhang, J., Nguyen, H.T. (1999). Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crops Research,, 64(3), 287–291.

Boopathi, N.M. (2013). Genetic mapping and marker assisted selection: Basics, practice and benefits. New Deli: Springer.

Chimenti, C.A., Marcantonio, M., Hall, A.J. (2006). Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. Field Crops Res, 95, 305–315.

Chimenti, C.A., Pearson, J., Hall, A.J. (2002). Osmotic adjustment and yield maintenance under drought in sunflower. Field Crop Res, 75, 235–246.

Clarke, H.J., Khan, T.N., Siddique, K.H.M. (2004). Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. Euphytica, 139, 65–74.

David, M. (2009). Osmotic adjustment capacity and cuticular transpiration in several wheat cultivars cultivated in Algeria. Romanian Agricultural Research, 26, 29–33.

David, M. (2012). Pollen grain expression of intrisic and osmolyte induced osmotic adjustment in a set of wheat cultivars. Romanian agricultural research, 29, 45–52.

De Micco, V., Aronne, G. (2012). Morpho-anatomical traits for plant adaptation to drought. In: Aroca R. (ed). Plant responses to drought stress. Berlin Heidelberg: Springer, 37–61 DOI 10.1007/978-3-642-32653-0_2

Eivazi, A., Talat, F., Saeed, A., Ranji H. (2007). Selection for osmoregulation gene to improve grain yield of wheat genotypes under osmotic stresses. Pakistan Journal of Biological Sciences, 10, 3703–3707. DOI: 10.3923/pjbs.2007.3703.3707

Farooq, M., Bramley, H., Palta, J.A., Siddique, K.H.M (2011). Heat stress in wheat during reproductive and grain-filling phases. Crit. Rev. Plant Sci., 30, 491–507.

Fleta-Soriano, E., Munné-Bosch, S. (2016). Stress memory and the inevitable effects of drought: A physiological perspective. Front. Plant Sci., 7, 143. doi: 10.3389/fpls.2016.00143

Ciucă, M., Bănică, C., David, M., Săulescu N.N. (2010). SSR markers associated with the capacity for osmotic adjustment in wheat (Triticum aestivum L.). Romanian agricultural research, 27, 1–5.

Gonzalez, A., Martin, I., Ayerbe, L. (1999). Barley yield in water-stress conditions. The influence of precocity, osmotic adjustment and stomatal conductance. Field Crops Res, 62, 23–34.

González, A., Martín I., Ayerbe, L. (2008). Yield and osmotic adjustment capacity of barley under terminal water-stress conditions. J. Agronomy & Crop Science, 194, 2, 81–91.

Gursoy, M., Balkan, A., Ulukan, H. (2012). Ecophysiological responses to stresses in plants: A general approach. Pakistan Journal of Biological Sciences, 15, 506–516.

Honys, D., Twell, D. (2004). Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol., 5(11), R85. DOI: 10.1186/gb-2004-5-11-r85

Hormaza, J.I., Herrero, M. (1996). Male gametophytic selection as a plant breeding tool. Sci. Hortic., 65, 321–333. doi: 10.1007/BF00226682

IPCC, 2007: Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K., Reisinger, A. (eds)]. IPCC, Geneva, Switzerland.

Jarzyniak, K.M., Jasinski, M. (2014). Membrane transporters and drought resistance – a complex issue. Front. Plant Sci., 5, 687. doi:10.3389/fpls.2014.00687

Javadi, T., Arzani, K., Ebrahimzadeh, H. (2008). Study of proline, soluble sugar, and chlorophyll a and b changes in nine Asian and one European pear cultivar under drought stress. Acta Hortic, 769, 241–246.

Karpets, Yu.V., Kolupaev, Yu.E., Grigorenko, D.O., Firsova, K.M. (2016). Response of barley plants of various genotypes to soil drought and influence of nitric oxide donor. The bulletin of Kharkiv national agrarian university. Series: biology, 2(38), 94–105.

Leport, L., Turner, N.C., French, R.J., Barr, M.D., Duda, R., Davies, S.L., Siddique, K.H.M. (1999). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. European Journal of Agronomy, 11, 279–291.

Marcińska, I., Czyczyło Mysza, I., Skrzypek, E., Filek, M., Grzesiak, S., Grzesiak, M.T., Janowiak, F., Hura, T., Dziurka, M., Dziurka, K., Nowakowska, A., Quarrie, S.A. (2013). Impact of osmotic stress on physiological and biochemical characteristics in drought susceptible and drought resistant wheat genotypes. Acta Physiol Plant, 35, 451–461. DOI 10.1007/s11738-012-1088-6

Mascarenhas, J.P. (1990). Gene activity during pollen development. Annu. Rev. Plant Physiol. Plant Mol. Biol., 41, 317–338. doi: 10.1146/annurev.pp.41.060190.001533

Moinuddin, K.C.R., Fischer, R.A., Sayre, K.D. et al (2005). Osmotic adjustment in wheat in relation to grain yield under water deficit environments. Agron J, 97, 1062–1071.

Moinuddin, K.C.R., Khanna-Chopra, R. (2004) Osmotic adjustment in chickpea in relation to seed yield and yield parameters. Crop Sci, 44, 449–455.

Morgan, J.M. (1992). Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol., 19, 67–76.

Morgan, J.M. (1999). Pollen grain expression of a gene controlling differences in osmoregulation in wheat leaves: a simple breeding method. Australian Journal of Agricultural Research, 50, 953–962.

Morgan, J.M., Tan, M.K. (1996). Chromosomal location of a wheat osmoregulation gene using RFLP analysis. Australian Journal of Plant Physiology, 23, 803–806.

Munns, R. (1988). Why measure osmotic adjustment? Australian Journal of Plant Physiology, 15, 717–726.

Nezhadahmadi, A., Prodhan, Z.H., Faruq, G. (2013). Drought tolerance in wheat. The Scientific World Journal, 2013, Article ID 610721. http://dx.doi.org/10.1155/2013/610721

Obata, T., Witt, S., Lisec, J., Palacios-Rojas, N., Florez-Sarasa, I., Yousfi, S., Araus, J.L., Cairns, J.E., Fernie, A.R. (2015). Metabolite profiles of maize leaves in drought, heat, and combined stress field trials reveal the relationship between metabolism and grain yield. Plant Physiology, 169(4), 2665–2683. http://doi.org/10.1104/pp.15.01164

Passioura, J. (2007). The drought environment: physical, biological and agricultural perspectives. J Exp Bot, 58(2), 113–117. DOI: https://doi.org/10.1093/jxb/erl212

Patil, B.S., Ravikumar, R.L. (2011). Osmotic adjustment in pollen grains: a measure of drought adaptation in sorghum? Current science, 100, 3, 377–382.

Patil, B.S., Ravikumar, R.L., Salimath, P.M. (2006). Effect of pollen selection for moisture stress tolerance on progeny performance in Sorghum. Journal of Food, Agriculture and Environment, 4, 201–204.

Pawar, V.V., Lokhande, P.K., Dalvi, U.S., Awari, V.R., Kale, A.A., Chimote,V.P. et al. (2015). Effect of osmotic stress on osmolyte accumulation and ammonia assimilating enzymes in chickpea cultivars. Indian J. Plant Physiol., 20, 276–280. doi: 10.1007/s40502-015-0159-2

Pedersen, S., Simonsen, V., Loeschcke, V. (1987). Overlap of gametophytic gene expression in barley. Theoretical and Applied Genetics, 75(1), 200–206. DOI: 10.1007/BF00249164

Rani, T.S., Ravikumar, R.L. (2006). Sporophytic and gametophytic recurrent selection for improvement of partial resistance to Alternaria leaf blight in sunflower (*Helianthus annuus* L.). Euphytica, 147, 421–431.

Ramakrishna, A., Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior, 6(11), 1720–1731. http://doi.org/10.4161/psb.6.11.17613

Ravikumar, R.L., Patil, B.S., Soregaon, C.D., Hegde, S.G. (2007). Genetic evidence for gametophytic selection of wilt resistant alleles in chickpea. Theor Appl Genet, 114, 619–625. doi:10.1007/s00122-006-0462-4

Reynolds, M.P., Quilligan, E., Aggarwal, P.K. et al. (2016). An integrated approach to maintaining cereal productivity under climate change. Global Food Security, 8, 9–18. http://dx.doi.org/10.1016/j.gfs.2016.02.002

Richards, R.A. (2006). Physiological traits used in breeding of new cultivars for water-scarce environments. Agricultural Water Management, 80, 197–211.

Richards, R.A., Rebetzke, G.J., Watt, M., Condon, A.G., Spielmeyer, W., Dolferus, R. (2010). Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. Funct. Plant Biol., 37, 85–97. doi:10.1071/FP09219

Rozova, M.A., Ziborov, A.I. (2016). The productivity of spring durum wheat collection accessions under diverse weather conditions in the Priobskaya (the Ob River) forest-steppe of the Altai Region. Bulletin of Altai State Agricultural University, 5(139), 9–15.

Rybka, K., Nita, Z. (2015). Physiological requirements for wheat ideotypes in response to drought threat. Acta Physiol Plant, 37, 97. DOI 10.1007/s11738-015-1844-5

Sanders, G., Arndt, S. (2012). Osmotic adjustment under drought conditions. In: Aroca R. (ed). Plant responses to drought stress. Berlin Heidelberg: Springer, 199–229. doi:10.1007/978-3-642-32653-0_8

Santamaria, J.M., Ludlow, M.M., Fukai, S. (1990). Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) under water-limited conditions. I. Water stress before anthesis. Aust. J. Agric. Res., 41, 51–65.

Sari-Gorla, M., Frova, C., Binelli, G., Ottaviano E. (1986). The extent of gametophytic-sporophytic gene expression in maize. Theoret. Appl. Genetics, 72(1), 42–47. doi:10.1007/BF00261452

Serraj, R., Sinclair, T.R. (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant, Cell and Environment, 25, 333–341.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savouré, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann Bot, 115(3), 433–447. doi.org/10.1093/aob/mcu239

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R. (2014). Abiotic and biotic stress combinations. New Phytol, 203, 32–43. doi:10.1111/nph.12797

Tester, M., Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. Science, 327(5967), 818–822. http://dx.doi.org/10.1126/ science.1183700

Totsky, I.V., Lyakh, V.A. (2015). Pollen selection for drought tolerance in sunflower. Helia, 38(63), 211–220. DOI: 10.1515/helia-2015-0012

Turner, N.C. (2017). Turgor maintenance by osmotic adjustment, an adaptive mechanism for coping with plant water deficits. Plant, Cell and Environment, 40, 1–3. doi: 10.1111/pce.12839

Zhang, J., Nguyen, H.T., Blum A. (1999). Genetic analysis of osmotic adjustment in crop plants. Journal of Experimental Botany, 50(332), 291–302.

Zivcak, M., Brestic, M., Sytar, O. (2016). Osmotic adjustment and plant adaptation to drought stress. Chapter 5. In: Hossain M.A. et al. (eds). Drought Stress Tolerance in Plants, Vol. 1. Switzerland: Springer International Publishing, 105–144. DOI 10.1007/978-3-319-28899-4_5

Citation: Khlebova, L.P., Bychkova, O.V., Titova, A.M., Rozova, M.A., Ziborov, A.I. (2018). Osmotic adjustment in spring durum wheat pollen grains under induced drought stress. *Ukrainian Journal of Ecology, 8*(4), 213-221.