Original scientific paper
Journal of Central European Agriculture, 2020, 21(2), p.268-274
DOI: /10.5513/JCEA01/21.2.2341

# Changes of morphofunctional traits of *Triticum aestivum* and *Triticum dicoccum* seedlings caused by polyethylene glycol-modeling drought

Oleksandr SMIRNOV (✉), Leila-Anastasiia KARPETS, Andrii ZINCHENKO, Mariia KOVALENKO, Victoriia BELAVA, Nataliya TARAN

Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska Street, Kyiv 01601, Ukraine

✉ Corresponding author: plantaphys@gmail.com

Received: 26 November 2018; accepted: 1 April 2019

## ABSTRACT

Wheat genus belongs to the main grain crops in Ukraine and in the world. An important element of its cultivation technology, which affects productivity, is the use of high yield varieties resistant to biotic and abiotic stressors. Therefore, the aim of a study was evaluation of polyethylene glycol-modeling drought stress adaptive responses of different varieties of wheat germs (*Triticum aestivum* and *Triticum dicoccum*) on such parameters as water shortages, relative water content, roots damage membranes and dehydrogenase activity and photosynthetic pigments content. Studied varieties of common bred wheat and emmer wheat presented resilience-anisohydric response maintaining high relative water content, increasing of root length / shoot length ratio and maintaining the ratio of photosynthetic pigments in response to drought with marked differences among the varieties. According to the result of the research Holikovska variety (*Triticum dicoccum*) is the most drought- resistant variety which makes it promising for use in breeding for drought tolerance. Because of resilience-anisohydric behaviour patterns might be the beneficial strategy for growing under drought stress conditions.

**Keywords:** wheat varieties, *Triticum aestivum*, *Triticum dicoccum*, drought resistance

## INTRODUCTION

Because of unfavorable environmental factors, there is a challenge to maintaining the world production of wheat grain. Up to 50% of the harvest is lost only under the influence of abiotic stressors. Another 10-30% of the crop may be lost due to biotic factors (Thiry et al., 2016). Any type of stress affects the plant's homeostasis, changing the equilibrium of the whole system. Thus, for example drought causes variety of plant’s stresses such as oxidative and osmotic (Iqbal et al., 2016). In addition, it change leaf water potential, create turgor loss and water deficit, induce premature leaf senescence and inhibits cell enlargement and growth.

Effecting different biochemical and physiological processes, like transpiration and photosynthesis drought promotes closure of stomata and changes in the ratio of plant’s photosynthetic pigments (Vassileva et al., 2009). Displacement of balance between chlorophyll *a*, *b* and carotenoids change all of the subsequent metabolic process of the organism. Nevertheless, each species has its own norm of reaction, which allows surviving in changing environments by flexibility of metabolic patterns (Saqib et al., 2013). Better understanding of the morphofunctional traits and physiology of changes under water stress conditions of different varieties of *Triticum sp.* could be used to select or create new varieties of crops according to their sustainability under drought influence (Manschadi et al., 2008).

*Triticum dicoccum*, also known as farro or emmer, served as one of the main food sources for the dwellers of the Old World. It is one of the oldest grain crops with motherland in the Middle East. Precisely from this species was bred by selection *Triticum durum* (Hejtmankova et al., 2010). *T. dicoccum* was displaced from the crop areas by other cultural varieties of wheat, because of its complex process of threshing. Nowadays, cultivation of all species of *Triticum sp.* have been stopped or minimized, with the exception of *Triticum aestivum* L. and T*riticum durum* Desf. This led to a reduction in the diversity of genes that cause resistance to biotic and abiotic stressors (Almansouri et al., 2001). Wheat crops became vulnerable, and production and quality of crops become unstable. Besides, seed areas have reached or exceeded the limits of environmental safety with each passing year.

This situation makes it possible to understand the negative effects of genetic erosion and draws attention to the species and intraspecific diversity of wheat.

Thanks to the well-developed root system *T. dicoccum* grows on podzolic soils, clays. On black soil, it is much more durable than common wheat and durum. It is can be grown on poorly treated and much depleted soils, but its yield than is significantly reduced (Hejtmankova et al., 2010).

Emmer is recommended for nutrition, given the high protein content (23.9%, which is one and a half times higher than that of common wheat), food fibers, B vitamins, iron, and low fat (Cooper, 2015).

Holikovska is one of the new and unstudied varieties of emmer. Average annual rate of wheat production is far behind the growth rate of the planet's population. Inequality must be reduced by increasing grain production. Accordingly, all these advantages of the farro make it possible to assume its use as the main sustainable and useful type of wheat in changing conditions of our climate (Babenko et al., 2018).

So the aim of the study was to conduct a comparative analysis of polyethylene glycol induced drought stress adaptive responses of common bred wheat varieties (*Triticum aestivum* L.) with emmer wheat (T*riticum dicoccum* Schrank.).

## MATERIALS AND METHODS

### *Plant materials*

Seeds of *Triticum dicoccum* (Holikovska variety) and *Triticum aestivum* (Podolianka, Favorytka varieties) were sterilized with potassium permanganate for 30 minutes and then germinated in the dark at 25 °C in Petri dishes with distilled water for 24 hours. Containers for further germination by aquaculture were treated with hydrogen peroxide. Stress conditions were caused using polyethylene glycol 6000 to achieve osmotic pressure -0.3 MPa. As a control was used distilled water. For next 7 days, seedlings were grown in controlled conditions: temperature – 25 °C, photoperiod of 16 hours at a photosynthetic photon flux density of ≈ 200 μmol photons/m$^2$s. For investigation, 7-day roots and leaves of controlled and treated plants were used. Spectrophotometric assays were measured using spectrophotometer UV-1800 (Shimadzu, Japan).

### *Morphological parameters*

Growth parameters, the length of the underground and aboveground parts of seedlings, the mass of fresh and dry material were measured by standard methods (Poorter H. and Gamier E., 1996). The water status of the plants was assessed by the indicators of water deficit and relative water content (RWC) of the roots and leaves (Hassanzadeh et al., 2009).

### *Photosynthetic pigments content*

Leaves were grind with sodium sulfate and glass sand, and then extracted by using 96% ethanol. Pigments content were measured by using spectrophotometer with different wavelength for each group of pigments (chlorophyll *a* (Chl. *a*) λ = 665 nm, chlorophyll *b* (Chl. *b*) λ = 649 nm, carotenoids (Car.) λ = 440.5 nm) (Hassanzadeh et al., 2009).

***Root cells membranes damage***

Roots were colored by 0.025% solution of trypan blue in 100 ml 100 µM $CaCl_2$ (pH 5.6) for 10 minutes. Then the roots were washed three times in 100 µM $CaCl_2$ (pH 5.6) and roots damage membranes were evaluated by intensity of blue roots color (Vijayaraghavareddy et al., 2017).

***Total dehydrogenase activity of root tissues***

Roots were colored by 0.01% solution of triphenyltetrazolium chloride for 24 hours in the dark and temperature - 25 °C. Total dehydrogenase activity was evaluated by reduction's intensity of triphenyltetrazolium hloride to formazan in roots (Ji et al., 2014).

***Statistical analysis***

The results were processed by comparison of the mean ± standard deviation (M ± SD) with using Student's t-test. Differences were accepted as significant for $P \le 0.05$. All results are compared with control plants.

## RESULTS AND DISCUSSION

As known drought stress occurs when transpiration exceeds the absorption of water by roots. The most sensitive to the effects of drought are growth processes (Fahad et al., 2017). Analysis of morphological traits showed that under the conditions of water shortage the length of the aboveground part of the seedlings increased by 25% of the Podolianka and Favorytka varieties, while as for the Holikovska variety decreased by 25% (Table 1). It can be interpreted as a stress activation of cell division in two varieties of wheat and as the beginning of protective mechanisms of Holikovska variety, for example the accumulation of stress-related phytohormones, which leads to slowing down the processes of cell growth (Bengough et al., 2011). The length of roots of seedlings has increased in 3 times (Podolianka), 1.5 times (Favorytka) and (Holikovska). Under the conditions of the water deficit elongation of the root system may be explained by search for a water sources (Table 1).

Under the conditions of the PEG-modeling drought stress elongation of the root system may be explained by search for a water sources. Some authors considered an increasing of root length to shoot length ratio as a drought tolerance potential parameter (Gazal et al., 2004). They have emphasized that the first and the most common feature of plant growth is increasing of root to shoot ratio in arid regions (Akhzari et al., 2016). Analysis of morphological traits showed that root to shoot ratio increased by 2 times in all three studied varieties.

The mass of fresh material in all three varieties changes within the limits of the error. The mass of dry material of leaf of the Podolianka and Favorytka varieties increased by 25%, while Holikovska - within the limits of error. The mass of dry material of the roots increased 3 times, 2.5 times respectively (Table 1). To maintain a balance caused by osmotic stress, plants have developed a number of special mechanisms such as an accumulation of free proline. It is a result of changes in the cell water potential. It's considered that proline acts as a regulator of intracellular osmotic potential, stabilizer of cellular structures and biopolymers and participates in overcoming oxidative stress. Thus, increasing of dry metter can be explained (Konotop et al., 2017).

Water deficit known as the percentage shortage of water from its total amount in plant tissues at its full absorption. It can occur by the disturbance of the water supply of the plant and cause changes in the intensity of physiological and biochemical processes, affecting the productivity of the plant. In this experiment water content in leaves and roots of seedlings has decreased by 25%, and the water deficit has increased threefold (Podolianka, Favorytka), while in Holikovska water content has decreased by 6%, and the water deficit has increased by 25% (Figure 1).

Relative water content (RWC) shows percentage content of the initial amount of water in the leaves from the contents of it in a state of saturation. It is well known that drought restricts water supply which results in a reduction of leaf water content.

**Table 1.** Morphological traits of different wheat varieties under the polyethylene glycol-modeling (PEG) drought stress

| Variety | Podolianka | | Favorytka | | Holikovska | |
|---|---|---|---|---|---|---|
| n=100 | Control | PEG | Control | PEG | Control | PEG |
| Leaves length, cm | 6.2±0.3 | 7.5±0.2* | 5.1±0.2 | 6.9±0.1* | 9.1±0.4 | 7.2±0.2* |
| Root length, cm | 2.9±0.2 | 7.6±0.2* | 3.5±0.2 | 8.4±0.2* | 4.8±0.3 | 7.4±0.1* |
| Root / Shoot ratio | 0.46 | 1.01 | 0.68 | 1.21 | 0.52 | 1.02 |
| Leaves fresh mass, mg | 57±2.9 | 64±2.4* | 62±2.03 | 64±2.10 | 65±2.12 | 63±3.10 |
| Root fresh mass, mg | 47±2.1 | 48±3.2 | 54±1.01 | 51±1.9* | 53±1.8 | 52±2.3 |
| Leaves dry mass, mg | 4.2±0.6 | 6.4±0.8* | 4.8±1.1 | 6.6±0.9* | 5.1±0.7 | 5.4±0.9 |
| Root dry mass, mg | 2.1±0.9 | 5.4±1.2* | 2.6±1.3 | 8.1±1.1* | 3.1±1.2 | 3.9±1.3 |

* Data represent mean values with standard deviation; significant difference at P≤0.05 comparing to control level for each variety

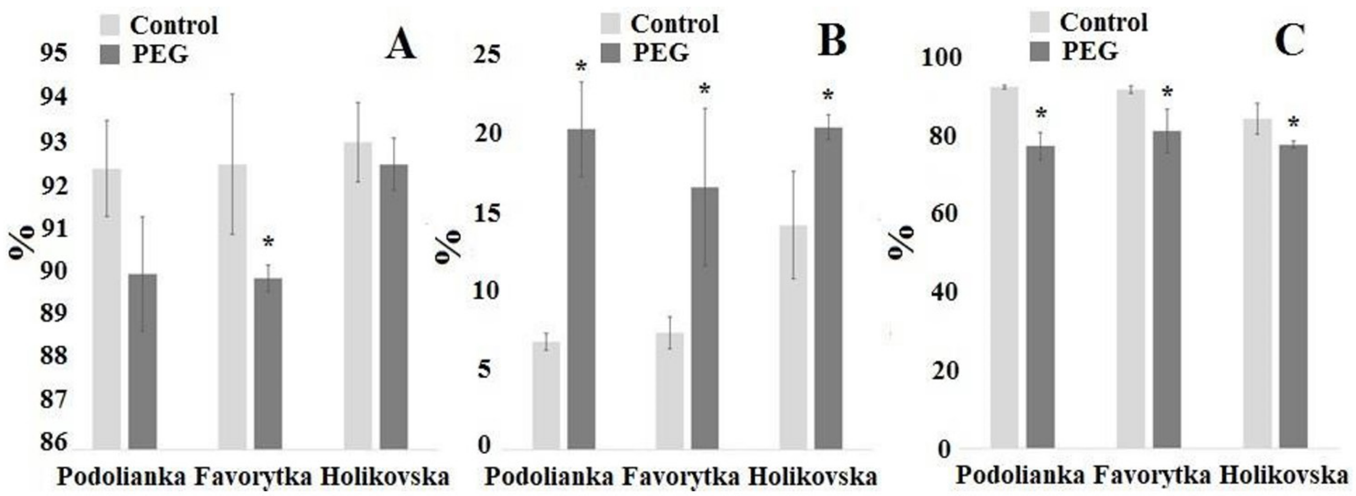


**Figure 1.** The water status of the plants: water content in seedlings (A), water deficit (B), relative water content (C) under the drought stress; n=100 (PEG – polyethylene glycol treatment variant; *data represent mean values with standard deviation; significant difference at P≤0.05 comparing to control level for each variety)

The decreasing of RWC by more than 20% can lower leaf water potentials, leading to reduced turgor, stomatal conductance, and photosynthesis, and thus eventually to reduce grain yield (Amini et al., 2014). RWC of the leaves of seedlings Podolianka and Favorytka decreased by 10%, in Holikovska - by 6% (Figure 1). These changes of RWC characterized varieties as resilience-anisohydric with slight decrease of RWC and indicated resilience-anisohydric strategy for how closely related biomass species deal with water deficit (Attia et al., 2015).

Various abiotic stressors including drought, water deficit, and hyperthermia caused destruction ultrastructure of mesophyll cells and chloroplasts of the leaf and the content of photosynthetic pigments composition (Babenko et al., 2019). The photosynthetic apparatus is highly sensitive to changes in various environmental factors. Thereby, the quantity and activity of photosynthetic pigments characterizes the efficiency of all pathways of plant metabolism. In addition, it can be used as a sensible indicator of the general physiological state of the organism (Frosi et al., 2017). Plant tissues under optimal conditions have more chlorophyll *a* than chlorophyll *b*. The ratio of these pigments of healthy plants is higher than 1.0. Changes in the ratio of these pigments indicate a malfunction of the physiological state of plants, on the other hand decreasing of this ratio lower than 1.0 indicates the prevalence of processes of destruction of substances over their production (Genty et al, 1989).

The content of all pigments in the Favorytka variety decrease by 25%, in the Podolianka variety changes within the range of error, and in the variety Holikovska increase by 10%. The sum of chlorophyll *a* and *b* and their ratio in Favorytka and Podolianka varieties increased, while the ratio of chlorophyll to carotenoids decreased. Simultaneously, the ratio of chlorophylls in the Holikovska variety decreased 3-fold, and the ratio of chlorophylls to carotenoids increased by 40% (Table 2).

The ratio of chlorophylls *a* and *b* usually varies in the range 2.2-4.0 and is used as a marker of the physiological state of the plant organism. Changes in the ratio of chlorophyll *a:b* may indicate a stoichiometry disturbance between the reaction center complexes and the light-harvesting complex.

These photosynthetic pigments changes could affects the functional activity of roots – the tissues of which are the first to encounter PEG-modeling drought and are responsible for the supply of water to the aboveground part of the plant. The coloring of trypan blue showed that under conditions of the drought stress, the membrane of root cells of all three varieties had damage (Figure 2, A).

The coloring of the roots with triphenyltetrazolium chloride showed that root apexes of stressed variants evaluated the highest activity of dehydrogenases in the roots of the seedlings of Podolianka and Favorytka varieties (Figure 2, B). It was recorded in the meristematic zone, but in the roots of the Holikovska variety – throughout the length, which may indicate more active breathing processes in Holikovska compared with other varieties under stress condition (Ji et al., 2014).


**Figure 2.** Root membranes damage of different varieties of wheat under the drought stress (A); and root total dehydrogenase activity of different varieties of wheat under the drought stress (B); n=30 (PEG – polyethylene glycol treatment variant)

**Table 2.** Pigments composition of different wheat varieties under the polyethylene glycol-modeling (PEG) drought stress

| Variety | Variant (n=15) | Chl. *a*, mg/g FW | Chl. *b*, mg/g FW | Car., mg/g FW | Chl. *a*/Chl. *b* ratio | Chl. *a*+*b*/Car ratio |
|---|---|---|---|---|---|---|
| Podolianka | Control | 2.40±0.37 | 1.19±0.20 | 0.55±0.09 | 2.01±0.02 | 6.47±0.02 |
| | PEG | 1.53±0.26* | 0.69±0.15* | 0.35±0.07* | 2.26±0.29 | 6.47±0.12 |
| Favorytka | Control | 2.04±0.21 | 1.02±0.26 | 0.52±0.13 | 1.98±0.01 | 5.84±0.03 |
| | PEG | 1.75±0.11* | 0.76±0.12* | 0.42±0.10* | 2.39±0.37* | 6.07±0.30* |
| Holikovska | Control | 2.03±0.42 | 0.98±0.20 | 0.57±0.12 | 2.07±0.01 | 5.22±0.04 |
| | PEG | 2.11±0.17 | 1.10±0.10 | 0.56±0.10 | 2.09±0.02 | 5.51±0.06* |

* - data represent mean values with standard deviation; significant difference at P≤0.05 comparing to control level for each variety

## CONCLUSIONS

Therefore during the cultivation of wheat seedlings in the environment without the addition of nutrients (only internal seed reserves were used) under conditions of drought. Studied varieties of common bred wheat (*Triticum aestivum* L.) and emmer wheat (*Triticum dicoccum* Schrank.) presented resilience-anisohydric response maintaining high relative water content, increasing of root length / shoot length ratio, maintaining the ratio of photosynthetic pigments in response to drought with marked differences among the varieties. The obtained data allow concluding that drought influences on parameters of wheat varieties in different ways. Holikovska variety is the most drought-resistant among the analyzed varieties and is promising for use in selection for drought tolerance. Because of resilience-anisohydric behaviour patterns might be the beneficial strategy for growing under drought stress conditions.

## FUNDING

The publication contains the results of studies conducted by President's of Ukraine grant for competitive projects №F75/170-2018 of the State Fund for Fundamental Research.

## REFERENCES

Akhzari, D., Pessarakli, M. (2016) Effect of drought stress on total protein, essential oil content, and physiological traits of *Levisticum officinale* Koch. Journal of Plant Nutrition, 39 (10), 1365-1371. DOI: https://doi.org/10.1080/01904167.2015.1109125

Almansouri, M., Kinet, J. M., Lutts, S. (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and soil, 231 (2), 243-254. DOI: https://doi.org/10.1023/A:1010378409663

Amini, H., Arzani, A., Karami, M. (2014) Effect of water deficiency on seed quality and physiological traits of different safflower genotypes. Turkish Journal of Biology 38, 271-282. DOI: https://doi.org/10.3906/biy-1308-22

Attia, Z., Domec, J. C., Oren, R., Way, D. A., Moshelion, M. (2015) Growth and physiological responses of isohydric and anisohydric poplars to drought. Journal of Experimental Botany, 66, 4373-4381. DOI: https://doi.org/10.1093/jxb/erv195

Babenko, L. M., Hospodarenko, H. M., Rozhkov, R. V., Pariy, Y. F., Pariy, M. F., Babenko, A. V., Kosakivska, I. V. (2018) *Triticum spelta*: Origin, biological characteristics and perspectives for use in breeding and agriculture. Regulatory Mechanisms in Biosystems, 9 (2), 250-257. DOI: https://doi.org/10.15421/021837

Babenko, L. M., Vodka, M. V., Akimov, Yu. N., Smirnov, A. E., Babenko, A. V., Kosakovskaya, I. V. (2019) Specific features of the ultrastructure and biochemical composition of leaf mesophill cells of *Triticum spelta* L. in the initial period of stress temperature action. Cell and Tissue Biology, 13 (1), 70-78. DOI: https://doi.org/10.1134/S1990519X19010024

Bengough, A. G., McKenzie, B. M., Hallett, P. D., Valentinem, T. A. (2011) Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. Journal of Experimental Botany, 62, (1), 59-68. DOI: https://doi.org/10.1093/jxb/erq350

Cooper, R. (2015) Re-discovering ancient wheat varieties as functional foods. Journal of traditional and complementary medicine, 5 (3), 138-43. DOI: https://doi.org/10.1016/j.jtcme.2015.02.004

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., Huang, J. (2017) Crop Production under Drought and Heat Stress: Plant Responses and Management Options. Frontiers in plant science, 8, 1147. DOI: https://doi.org/10.3389/fpls.2017.01147

Frosi, G., Harand, W., Oliveira, M. T., Pereira, S., Cabral, S., Pereira, M., Abelardo, A. A., Santos, M. G. (2017) Different physiological responses under drought stress result in different recovery abilities of two tropical woody evergreen species. Acta Botanica Brasilica, 31 (2), 153-160. DOI: https://dx.doi.org/10.1590/0102-33062016abb0375

Gazal, R. M., Kubiske, M. E. (2004) Influence of initial root length on physiological responses of cherry bark oak and shumard oak seedling to field drought conditions. Forest Ecology and Management, 189, 295-305. DOI: https://dx.doi.org/10.1016/j.foreco.2003.08.017

Genty, B., Briantais, J. M., Baker, N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA)-General Subjects, 990 (1), 87-92. DOI: https://doi.org/10.1016/S0304-4165(89)80016-9

Hassanzadeh, M., Ebadi, A., Panahyan-e-Kivi, M., Eshghi, A. G., Jamaati-e-Somarin, Sh., Saeidi M., Zabihi-e-Mahmoodabad, R. (2009) Evaluation of Drought Stress on Relative Water Content and Chlorophyll Content of Sesame (*Sesamum indicum* L.) Genotypes at Early Flowering Stage. Research Journal of Environmental Sciences, 3, 345-350. DOI: https://doi.org/10.3923/rjes.2009.345.350

Hejtmánková, K., Lachman, J., Hejtmánková, A., Pivec, V., Janovská, D. (2010) Tocols of selected spring wheat (*Triticum aestivum* L.), einkorn wheat (*Triticum monococcum* L.) and wild emmer (*Triticum dicoccum* Schuebl [Schrank]) varieties. Food chemistry, 123 (4), 1267-1274. DOI: https://doi.org/ 10.1016/j.foodchem.2010.05.064

Ji, H., Liu, L., Li, K., Xie, Q., Wang, Z., Zhao, X., Li, X. (2014) PEG-mediated osmotic stress induces premature differentiation of the root apical meristem and outgrowth of lateral roots in wheat. Journal of Experimental Botany, 65 (17), 4863–4872. DOI: https://doi.org/10.1093/jxb/eru255

Iqbal, M, Raja, N. I, Yasmeen, F., Hussain, M., Ejaz, M. (2017) Impacts of Heat Stress on Wheat: A Critical Review. Adv Crop Sci Tech, 5, 251. DOI: https://doi.org/ 10.4172/2329-8863.1000251

Konotop, Y., Kovalenko, M., Matušíková, I., Batsmanova, L., Taran, N. (2017) Proline application triggers temporal redox imbalance, but alleviates cadmium stress in wheat seedlings. Pakistan Journal of Botany, 49 (6), 2145-2151.

Manschadi, A. M., Hammer, G. L., Christopher, J. T. (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant and Soil, 303 (1-2), 115-129. DOI: https://doi.org/10.1007/s11104-007-9492-1

Poorter, H., Gamier, E. (1996) Plant growth analysis: an evaluation of experimental design and computational methods. Journal of Experimental Botany, 47, 1343–1351. DOI: https://doi.org/10.1093/jxb/47.9.1343

Saqib, M., Akhtar, J., Abbas, G. (2013) Salinity and drought interaction in wheat (*Triticum aestivum* L.) is affected by the genotype and plant growth stage. Acta Physiol Plant, 35, 2761. DOI: https://doi.org/10.1007/s11738-013- 1308-8

Thiry, A. A., Chavez Dulanto, P. N., Reynolds, M. P., Davies, W. J. (2016) How can we improve crop genotypes to increase stress resilience and productivity in a future climate? A new crop screening method based on productivity and resistance to abiotic stress. Journal of experimental botany, 67 (19), 5593-5603. DOI: https://doi.org/10.1093/jxb/erw330

Vassileva, V., Simova-Stoilova, L., Demirevska, K. (2009) Variety-specific response of wheat (Triticum aestivum L.) leaf mitochondria to drought stress. J Plant Res 122, 445. DOI: https://doi.org/10.1007/s10265-009-0225-9

Vijayaraghavareddy, P., Adhinarayanreddy, V., Vemanna, R. S., Sreeman, S., Makarla, U. (2017) Quantification of Membrane Damage/Cell Death Using Evan's Blue Staining Technique. Bio-protocol, 7 (16), e2519. DOI: https://doi.org/10.21769/BioProtoc.2519