

NEW METHOD FOR IDENTIFICATION DROUGHT-TOLERANCE WINTER WHEAT BREEDING MATERIAL

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Abstract

Drought tolerance is one of the key agricultural value traits for arid regions. Our investigation consists of two stages. During fist we worked with well-known by this drought tolerance winter wheat official realized 16 varieties, at the second one we evaluated new 88 mutant lines at M4 – M6 generations, which are potentially higher yielding as compared with the national Ukrainian standard for arid zone (Northern Steppe). It is well known that photosystem-II (PS- II) is less thermal-resistant than photosystem-I (PS-I). Since PS-II is more energy consuming and directly requires water for it active operation, this leads to a conclusion that during drought its activity predominance in the photosynthesizing system is undesirable. We evaluated potential drought tolerance by comparison photosystems activity. The simazine (Sim) 10-4(M) was used as PS-II inhibitor, represses the oxygen release processes in the photosystem. More drought-resistant winter wheat lines showed the predominance of PS-I over PS-II. It was established that the best lines in terms of drought resistance were the wheat lines 133, 157, 157-1, 185, 213, that is the lines with the lowest ratio between PS-II and PS-I were identified as being more drought-resistant.

Key words: *drought tolerance, winter wheat.*

INTRODUCTION

One of the main tasks of creating new genotypes of bread winter wheat, associated with the climate changes, escalation of global environmental problems, is the solution of the problem of adaptation and resistance, the study of environmental stress effect on plants.

With the annual production of about 783 million tons (in 2019) (USDA, 2020), bread wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops. Winter wheat is the world's leading cereal grain and the most important food crop, occupying commanding position in Ukraine. Ukrainian agriculture takes about 48% area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2016b). Consequently, wheat supplies approximately one-fifth of human calories in a variety of forms. Wheat will remain a crucial component of human nutrition, and increasing its production is therefore an important requirement for food security. Wheat consumption has been steadily increasing due to population expansion and urbanization (Halford et al., 2014, Li et al., 2019).

Winter wheat is an important crop, suited to the typical weather conditions in the current climate. In a changing climate the increased frequency and severity of adverse weather events, which are often localized, are considered a major threat to wheat production (Harkness et al., 2020).

Winter wheat is not only a world's leading cereal crop but also the most important food crop in Ukraine, which occupied leading position in the national agriculture (Nazarenko and Lykholat, 2018). As for the quality traits winter wheat is the main stable crop for our country and provides more than 20 % of calories and proteins. Focused on only yield traits we have to understand that any high yield has no sense without proper quality for food and fodder demands (Shewry et al., 2012). Wheat improvement, which bases on the principal of ecological stability of new forms and taking into account special interactions between environment and genotypes, special abilities for agroecological variability under wide ranges of conditions providing us new approaches for formation stable agroecological cereals systems without great losses at productivity (Bordes et al., 2011).

But in spite of increasing total grain productivity tolerance to the special ecological demands of new varieties have been decreased, what, consequently, influencing on the future adaptability and special interactions with environment of winter wheat (Pemental et al, 2014; Nazarenko, 2017; Nazarenko et al, 2020).

Disequilibrium in influence of different nature-agricultural factors and their interactions of region determine distinguishes summarized in different genotypes grain productivity and quality (Essam et al, 2019). Due to this fact we investigated varieties main agricultural-value traits under regional conditions. They determined balance of moisture, character of winter wheat growth and development, differences in seasons conditions, interaction between types of variety development (terms and specify of development stages) (Chope et al, 2014; Amram et al, 2015).

Key attention to pay for main agronomic-value traits such as grain productivity (and formation of this trait) and grain quality (in sense of grain protein content). These traits in interaction actually determine the overall genotypes of wheat by suitability for cultivation (Gepts & Hancock, 2006). Winter wheat yield has the most important and complex character affected directly or indirectly by genome systems present in plant (Rangare et al., 2010) as well as interaction with environment (Tester & Langridge, 2010, Serpolay et al., 2011). Thus, ecological exam (part of evaluation process in breeding program for measurements of adaptation for new lines and varieties under difference regional conditions) of new wheat lines with high yield and quality genetic potential under difference condition, it's components (Slafer & Andrade, 1993) and interaction (Dai et al., 2015) has become a permanent task in the plant farming and breeding programs (Reif et al., 2005, Tuberosa & Salvi, 2006).

The study of drought-resistance represents one of the fundamental tasks in phytophysiology. Draughts that differ in character and duration occur annually in all regions of Ukraine. The stressing influence of the drought induces substantial reduction in yielding capacity and quality of winter grains. Potential effect of stress on the plants is constantly growing,

which is caused by a severe shortage for water, rise in air temperature and pollution of environment with toxic chemicals (Desfeld et al, 2014; Daryanto et al, 2017; Jaradat, 2018). Adaptation is only possible when an organism is capable of resistance on any level (from cell level to population level) and is able to adapt to the new environment conditions (Forsman, 2015; Pilbean, 2015; Prabhu, 2019).

The key part in field crops yield generation is played by photosynthesis. This is the only natural process resulting in growth of free energy of biological environment on account of external source – the sun, and ensures the existence both of plants and of all heterotrophs, including a human. The photosynthesis process is the biological basis of yielding capacity of agricultural crop, during which up to 95 % of dry biomass of plants is generated (Xu, 2016; Quintero et al, 2018).

It is generally thought that it is the photosynthesis that suffers most from drought: ATP synthesis decreases, while the synthesis of promoting agent grows, which causes excessive reducibility of electron-distribution chain and the decomposition of pigment-protein complex of chloroplasts (Mba et al, 2012; Richardson et al, 2017; Khalili et al, 2018).

It is well known that in the process of cells generation, the chloroplasts may be of two types according to the ratio of content of PS-I and PS-II –1:3 and 1:2 respectively, PS-II is less thermal-resistant than PS-I, which may result in changes to the electron-distribution chain (Resende, 2016; Tokalidis, 2017). The excessive content of PS-II increases the photochemical activity of chloroplasts, and under conditions of drought it causes their destruction of components of PS-II followed by the chlorophyll burn-out. Functional impairment in plants further leads to decrease of yielding capacity (Liu et al, 2016; Klcova et al, 2019; Le Gouis et al, 2020).

Non-uniform resistance of particular elements of photosynthesis system allows an assumption that the photosynthesis reaction may in general be dependent on the inhibition of the most sensitive link of the electron-distribution chain. The currently known specific inhibitors of the PS-II reaction are the monodiurons, hydroxylamine and other enzyme poisons

(Milev et al, 2014; Mickelbart et al, 2015; Nutall et al, 2017).

The enhancement of methods of winter wheat breeding material assessment in terms of drought resistance, discovering the ability of the plants to provide the acceptors with the assimilators within the system of donor and acceptor relations and the ability for cells self-maintenance in the environment of increasing water deficiency or high temperature, provide an opportunity to reasonably describe the level of drought-resistance of new lines and varieties and to forecast their behavior in corresponding environmental conditions (Tribo et al, 2003; Tsenov et al, 2015; Tencgong et al, 2020).

We suggest one of the methods of breeding material assessment in terms of drought-resistance by photosystem (PS) activity.

MATERIALS AND METHODS

Winter wheat varieties and M₄–M₅ generations of mutation lines have been sown at the end of September, at a depth of 4-5 cm. The controls were national standard by productivity ‘Podolyanka’ and initial variety. The working-methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 10 m² in 2–3 replications.

Experiments were conducted on the experiment field of Dnipro State Agrarian and Economic University (village Oleksandrovka, Dnipropetrovsk district, Dnipro region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Evolution was conducted during 2011 – 2018 years (Nazarenko, 2016; Nazarenko and Lykholat, 2018).

Winter wheat plants of different lines and varieties were studied. Photosynthesis of plants was determined in a gasometric device developed in the laboratory of plant physiology and biochemistry MIP NAASU on the basis of the manometric method of Warburg. Simazine (Sim) 10-4 (M) was used as an inhibitor of PS-II, which sharply inhibits the processes of oxygen release in the photosystem. The

intended site of action of simazine is the ETL link between the primary PS-II acceptor (Q) and the inclusion of plastoquinone. Drought resistance of the lines was determined by the advantages in the activity of individual photosystems.

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, the grouping mutants cases was performed by factor analyses. In all cases standard tools of the program Statistic 8.0 were used.

RESULTS AND DISCUSSIONS

The assessment of energy state of the photosynthesis system is essential for the development of methods of diagnostics of plants’ drought-resistance. The goal of our study was to assess the photosynthesis system by means of photosystems analysis using the inhibitor method for determination of winter wheat drought-resistance.

Plants of different lines were studied. For reconnaissance research, we deliberately selected varieties with low drought resistance in our region.

The addition of the inhibitor caused a peak decrease in the intensity of photosynthesis. All plants had a predominant activity of PS-II (Table 1) under the drought conditions of 2018 year.

In October–November 2017, when the photosynthetic apparatus was formed, the air temperature fluctuated sharply. It is known from the literature that in the conditions of significant daily fluctuations of air temperature in the leaves a photosynthetic apparatus with a predominant content of PS-I is formed.

Table 1. Photosynthesis winter wheat plant after treatment of PS-II inhibitor

N	Date	O ₂ , mkl/hour							
		Mironivska 808		Mirleben		Mironivsk a 33		Mironivsk a 65	
		Check	Treated	Check	Treated	Check	Treated	Check	Treated
1	28.10	1494	3360	2935	3002	3151	1867	3024	3472
2	25.11	4343	3394	4141	2919	4005	4005	5023	3940
3	07.05	4611	3425	-	-	3564	2828	2511	3733

Determining the intensity of photosynthesis of winter wheat plants of different lines of ecological testing showed that the crops of the 2018 harvest had almost the same number of varieties with a predominant activity of both PS-II and PS-I. The data show that in the electron transport chain, the ability of plants to withstand droughts is directly proportional to the ratio of photosystems.

According to the activity of photosynthesis in winter wheat plants, both PS-I and PS-II worked in all ecological varieties, because their ratio was about one, except for Kolos Mironivschiny, where it was equal to 1:3 (Table 2). In such ecologically plastic varieties as Podolyanka, Mironivska 808, Leganda, Pamyati Remesla and Mironivska 65 during the autumn-spring growing season the activity of PS-II slightly prevails.

Table 2. Quality of photosynthesis of winter wheat plants under ecological exam

Copr	Intensity of photosynthesis, mkl/hour		PS-I:PSII	PS
	Check (H ₂ O)	Simasin		
Podolyanka	6003	4793	1,25	II
Mironivska 808	5873	4973	1,18	II
Mironivska 65	5645	6362	0,89	I
Vesta	7034	6720	1,05	II
Snizhana	5197	6720	0,77	I
Krizhinka	8064	7392	1,09	I-II
Mironivska rannostigla	1930	2726	0,71	I
Remeslivna	2334	2550	0,91	I
Demetra	8960	8064	1,11	II
Kalinova	3733	2715	1,37	II
Kolos Mironivschiny	985	2867	0,34	I
Pamyati Remesla	6630	5960	1,16	II
Legenda	8467	7302	1,16	II

Various lines of winter wheat were studied. The simazine (Sim) 10⁻⁴(M) was used as PS-II inhibitor, dramatically represses the oxygen release processes in the photosystem. An envisaged center of simazine action is the link of the electron-distribution chain between the PS-II (Q) primary acceptor and the plastoquinone inclusion. For the reconnoitering study we deliberately selected the breeds of low-level drought resistance, which had been created in our region environment.

The inhibitor added caused a dramatic photosynthesis inhibition. All the plants were basically active in terms of PS-II under conditions of the droughty year 2012.

In October to November 2014, when the photosynthesizing system was being formed,

the air temperature fluctuated greatly. Literature references state that under conditions of significant fluctuations of air temperature during 24 hours, a photosynthesizing system with the PS-I predominant content is formed in the leaves.

The study of photosynthesis intensity in various breeds of winter wheat showed that the winter wheat seeds of 2014 – 2016 yield had were almost equal in terms of amount of breeds with predominating activity both of PS-II and PS-I. The data proves that in the electron-distribution chain, the photosystems relation directly influences the plants' ability to resist the droughts.

As to the photosynthesis intensity in winter wheat of a previous study, in all strains both PS-I and PS-II were active, since their relation was around figure of one, except for the strains 133, 157, 185, 213 (Table 3), where this figure was much less than one. Such strains were referred to environmentally adaptive, where during the spring and autumn vegetation period the PS-II activity prevails to a small extent.

Since PS-II is more energy consuming and directly requires water for it active operation, this leads to a conclusion that during drought its activity predominance in the photosynthesizing system is undesirable.

As stated above, 133, 157, 157-1, 185, 213 showed quite good results in terms of yielding capacity, and the ratio between PS-II and PS-I indicated the predominance of the PS-I, so those strains declared themselves as drought-resistant. This is the fifth paragraph from Results and Discussions that should be replaced with your content. It only contains example text and proper formatting.

Table 3. Quality of photosynthesis of winter wheat plants under lines exam

Line	Intensity of photosynthesis, mkl/hour		PS-I:PSII	PS
	Check	Treated		
Podolyanka	5513	4276	1,29	II
130	985	2867	0,34	I
133	4553	4002	1,14	II
142-1	3017	2906	1,04	II
156	1217	1045	1,16	II
157	2715	4114	0,66	I
157-1	2334	2550	0,92	I
172	3508	3400	1,03	II
174	3300	2940	1,12	II
179	2452	1856	1,32	II
185	1234	1896	0,65	I
186	1214	1059	1,15	II
211	627	627	1,00	I
213	947	1888	0,50	I

Due to the increase of bioclimatic potential, it seems economically viable to replace the current breeds by the breeds with the photosynthesizing system that stay active for a longer period, with no destructive irregularities in various climatic conditions.

Thus, more drought-resistant strains show the predominance of PS-I over PS-II.

This is suggested as an express method of assessment of drought-resistance ability of breeds and strains of winter wheat by the ratio of photosystems activity, which enables fast and reliable determination of starting material for drought-resistance breeding.

It was established that the best strains in terms of drought resistance were the wheat strains 133, 157, 157-1, 185, 213, that is the strains with the lowest ratio between PS-II and PS-I of soft winter wheat were described as being more drought-resistant.

CONCLUSIONS

In the context of climate change associated with global warming, and possible risks of loss of winter wheat yield, the proposed method makes it possible to reliably identify drought-resistant forms already at the early stages of the breeding process on limited winter wheat plants material. It was discovered the best drought-resistant wheat lines were the 133, 157, 157-1, 185, 213 strains, that is the strains with the lowest ratio between PS-II and PS-I of soft winter wheat were described as being more drought-resistant. The method is simple enough for field diagnostics, while being based on fundamental physiological processes and fully substantiated from a theoretical basis. The use of basic patterns makes it possible to directly establish a relationship between the processes of photosynthesis and the formation of such a complex trait as drought resistance.

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REFERENCES

- Amram, A., Fadida-Myers, A., Golan, G., Nashef, K., Ben-David, R., Peleg, Z. (2015). Effect of GA-sensitivity on wheat early vigor and yield components under deep sowing. *Frontier Plant Science*, 6, 487.
- Bordes, J., Ravel, C., Le Gouis, J., Lapierre, A., Charmet, G., Balfourier, F. (2011). Use of a global wheat core collection for association analysis of flour and dough quality traits. *Journal of Cereal Science*, 54, 137–134.
- Chope, G.A., Wan, Y., Penson, S.P., Bhandari, D.G., Powers, S.J., Shewry, P.R., Hawkesford, M.J. (2014). Effects of genotype, season, and nitrogen nutrition on gene expression and protein accumulation in wheat grain. *Journal of Agricultural Food Chemistry*, 62, 4399–4407.
- Dai, Z., Plessis, A., Vincent, J. (2015). Transcriptional and metabolic alternations rebalance wheat grain storage protein accumulation under variable nitrogen and sulfur supply. *Plant Journal*, 83, 326–343.
- Daryanto, S., Wang, P., Jacinthe, P. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review, *Agricultural Water Management*, 179, 18–33.
- Destelfeld, A., Avni R., Fischer, A. (2014). Senescence, nutrient remobilization, and yield in wheat and barley. *Journal of Experimental Botany*, 65, 3783–3798.
- Essam, F., Badrya, M., Aya, M. (2019). Modeling and forecasting of wheat production in Egypt. *Advances and Applications in Statistics*, 59(1), 89–101.
- Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individual, population and species. *Heredity*, 115, 276–284.
- Jaradat, A. (2018). Simulated climate change differentially impacts phenotypic plasticity and stoichiometric homeostasis in major food crops. *Emirates Journal of Food and Agriculture*, 30(6), 429–442.
- Gepts, P., Hancock, J. (2006). The future of plant breeding. *Crop Science*, 46, 1630–1634.
- Halford, N., Curtis, T, Chen, Z., Huang, J. (2014). Effects of abiotic stress and crop management on cereal grain composition: Implications for food quality and safety. *Journal of Experimental Botany*, 66, 1145–1156.
- Harkness, C., Semenov, M. A., Areal, F. (2020). Adverse weather conditions for UK wheat production under climate change. *Agricultural and Forest Meteorology*, 1078622, 282–283.
- Khalili, M., Naghavi, M., Yousefzadeh, S. (2018). Protein pattern analysis in tolerant and susceptible wheat cultivars under salinity stress conditions. *Acta agriculturae Slovenica*, 111(3), 545–558.
- Klčová, L., Ondreichková, K., Mihálik, D., Gubišová, M. (2019). The choice of suitable conditions for wheat genetic transformation. *Agriculture (Polnohospodárstvo)*, 65 (1), 30–36.
- Le Gouis, J., Oury, F.-X., Charmet, G. (2020). How changes in climate and agricultural practices

- influenced wheat production in Western Europe. *Journal of Cereal Science*.
- Li, H.J., Timothy, D. M., Mc Intosh, R.A., Zhou Y. (2019). Wheat breeding in northern China: achievements and technical advances. *The Crop Journal*, 7(6), 718–729.
- Liu, Q., Wu, X., Ma, J., Xin, C. (2016). Effects of cultivars, transplanting patterns, environment, and their interactions on grain quality of Japonica rice. *Cereal Chemistry*, 92. 284–292.
- Mba, C., Guimaraes, E.P., Ghosh, K. (2012). Re-orienting crop improvement for the changing climatic conditions of the 21st century. *Agriculture & Food Security*, 7. 1–17.
- Mickelbart, M., Hasegawa, P., Bailey-Serres, J. (2015). Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Natural Reviews*, 16. 237–251.
- Milev, G., Nankov, N. Iliiev, I., Ivanova, A., Nankova, M. (2014). Growing Wheat (*Triticum aestivum* L.) by the Methods of Organic Agriculture Under the Conditions of Dobrudzha Region, Bulgaria. *Turkish Journal of Agricultural and Natural Sciences*, 1. 849–857.
- Nazarenko, M. (2016). Identification and characterization of mutants induced by gamma radiation in winter wheat (*Triticum aestivum* L.). *Scientific Papers. Series A. Agronomy*, LIX. 350–353.
- Nazarenko, M. (2016). Characteristics of action of nitrosoalkylureas on cell level in winter wheat. *Visnyk of Dnipropetrovsk University. Biology, ecology*, 24(2), 258–263.
- Nazarenko, M.M. (2017). The influence of radio-mimetic chemical mutagen on chromosomal complex of winter wheat cells. *Regulatory Mechanisms in Biosystems*, 8(2), 283–286.
- Nazarenko, M., Lykholat, Y. (2018) Influence of relief conditions on plant growth and development. *Dnipro university bulletin. Geology. Geography*, 26(1), 143–149.
- Nazarenko, M., Gorschar, V., Lykholat, Yu., Kovalenko, I. (2020). Winter wheat mutations by plant height and structure caused by chemical supermutagens. *Scientific Papers. Series A. Agronomy*, LXIII(1), 443–449.
- Nuttall, J., O'Leary, G., Panozzo, J., Walker, C., Barlow, K., Fitzgerald, G. (2017). Models of grain quality in wheat - A review. *Field Crops Research*, 202. 136–145.
- Pilbeam, D. (2015). Breeding crops for improved mineral nutrition under climate change conditions. *Journal of Experimental Botany*, 66. 3511–3521.
- Pementel, A., Guimarães, J., de Souza, M., Resende, M., Moura, L., Carvalho, J., Ribeiro, G. (2014). Estimation of genetic parameters and prediction of additive genetic value for wheat by mixed models. *Pesquisa Agropecuária Brasileira*, 49. 882–890.
- Prabhu, B. (2019). The Green Revolution and Crop Biodiversity. In: *Biological Extinction. New Perspectives*. Cambridge University Press, Cambridge.
- Quintero, A., Molero, G., Reynolds, M., Calderini, D. (2018). Trade-off between grain weight and grain number in wheat depends on G × E interaction: A case study of an elite CIMMYT panel (CIMCOG). *European Journal of Agronomy*, 92. 17–29.
- Rangare, N.R., Krupakar, A., Kumar, A., Singh, S. (2010). Character association and component analysis in wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding*, 1. 231–238.
- Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L. (2005). Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics*, 110. 859–864.
- Resende, M. (2016). Software Selegen-REML BLUP: A useful tool for plant breeding. *Crop Breeding and Applied Biotechnology*, 16. 330–339.
- Richardson, B., Chaney, L., Shawn, N., Still, S. (2017). Will phenotypic plasticity affecting flowering phenology keep pace with climate change? *Global Change Biology*, 23. 2499–2508.
- Serpolay, E., Dawson, J.C., Chable, V., Van Bueren, L., Osman, A., Pino, S., Silveri, D., Goldringer, I. (2011). Phenotypic responses of wheat landraces, varietal associations and modern varieties when assessed in contrasting organic farming conditions in Western Europe. *Organic Agriculture*, 3. 12–18.
- Shewry, P.R., Mitchell, R.A.C., Tosi, P. (2012). An integrated study of grain development of wheat (cv. Hereward). *Journal of Cereal Science*, 56. 21–30.
- Slafer, G.A., Andrade, F.H. (1993). Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. *Field Crops Research*, 31. 351–367.
- Tester, M., Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, 327. 818–822.
- Tsenov, N., Atanasova, D., Stoeva, I., Tsenova, E. (2015). Effects of drought on grain productivity and quality in winter bread wheat. *Bulgarian Journal Agricultural Sciences*, 21. 592–598.
- Tengcong, J., Jian, L., Yujing, G., He, J. (2020). Simulation of plant height of winter wheat under soil water stress using modified growth functions. *Agricultural Water Management*, 232. 106066.
- Tokatlidis, I. (2017). Crop adaptation to density to optimize grain yield: Breeding implications. *Euphytica*, 213. 92.
- Tribo, E., Martre, P., Tribo-Blondel, A.M. (2003). Environmentally induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *Journal of Experimental Botany*, 54. 1731–1742.
- Tuberosa, R., Salvi, S. (2006). Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science*, 11, 405–412.
- United states department of agriculture. 2020. World Agricultural Production. Retrieved January 31, 2021, from <https://apps.fas.usda.gov/psdonline/circulars/production.pdf>
- Xu, Y. (2016). Envirotyping for deciphering environmental impact. *Theoretical and Applied Genetics*, 129. 653–673.