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Physiological and agronomic assessment of tolerance to drought of perspective breeding lines common winter wheat

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Abstract

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Physiological and agronomic assessments of tolerance to drought of eight breeding lines of common winter wheat were done during period 2015-2017. The aim of the study was to make a comprehensive assessment of the potential of genotypes of common winter wheat to overcome different drought intensities using various types of trials. The highest yield of a micro field trial was recorded for the breeding lines MX 268/1008, MX 270/50, MX 270/28 and MX 270/24. The most intense morphometric indexes of the leaves were taken into account for MX 247/33, MX 270/24 and MX 270/86, while morphological markers for drought resistance were distinguished by the breeding lines MX 268/1008, MX 187/3 and MX 270/24. The most tolerant response to controlled drought in young plants developed in vegetation pot experiments was found in MX 270/50, MX 187/3, MX 268/1008 and MX 270/24. In conclusion the genotypes MX 270/24, MX 187/3 and MX 268/1008 were considered as high yielding and they also showed good physiological performance under drought stress conditions.

Keywords: common winter wheat; physiological assessment; agronomic assessment; drought, yield

Introduction

Wheat is one of the three major cereal crops consumed throughout the world. As sessile organisms, the plants are constantly exposed to various abiotic stress factors in their natural environment. Drought, salt, and temperature stress are major environmental factors that have influence on wheat productivity in agriculture. To survive under these conditions, plants express a various range of responses at the cellular, molecular, and functional levels (Fujita et al., 2006; Sana et al., 2019). To avoid drought stress, wheat cultivars have developed diverse drought tolerance mechanisms, including the formation of deeper roots, regulation of ion homeostasis, improvement of osmoprotective and antioxidant response, and regulation of gene expression (Huseynova, 2012; Zhang et al., 2012). Drought tolerance includes various mechanisms that are generally classified into three main groups: ability to maintain high water potential in the leaves by preventing fading, tolerance to low water potential, tolerance to drought and drought avoidance (Levitt, 1980; Bohner et al., 1995; Nguyen et al., 1997).

Wheat varieties resistant or drought-tolerant are sought after by producers to deal with water shortages. A number of authors point to the link between physiological processes and the yield potential of common wheat. They believe that productivity genes act indirectly through them rather than directly through a certain element of productivity (Evans, 1981; Fisher et al., 1998; Reynolds et al., 2001; Foulkes, 2005).

One of the main tasks of breeders is the creation of adaptive varieties which combining stable yields and tolerance of abiotic and biotic stress. The selection lines from the advanced generations pass through perennial physiological, phytopathological and technological assessments and those combining the sought-after complex of qualities are presented as candidate varieties (Neikov, 2014).

The aim of the study was a comprehensive assessment of the potential of conventional winter wheat selection lines to overcome different drought intensities using various physiological analyzes and field trials.

Material and Methods

Eight lines of common winter wheat (*Triticum aestivum* L.) were sown in an experimental area of Institute of Plant Genetic Recourses, Sadovo, Bulgaria during period 2015-2017. The breeding lines MX 187/3, MX 247/33, MX 219/98, MX 270/28, MX 270/24, MX 270/50, MX 270/86, MX 268/1008 were obtained by the cross-hybridization method and tested in a competitive variety trial with an experimental area of 10 m^2. Additionally, they were tested in micro field competitive trial. The scheme was one row in three replications with an experimental area of 0.6 m^2 for each variety. The length of the rows was 1 m with a spacing of 0.20 m. From the two sides of each parcel there were sown plants that did not participate in the study. Throughout the growing season, all agro-technical procedures were applied and, if necessary, the experimental crop was treated with pesticides and fungicides. Cv. Katya was recognized as a standard for drought tolerance. It was included in 1^{-st} Facultative and Winter Wheat Elite Trial for Rain Fed Conditions, which was performed in SYMMIT-Ankara Turkey and ICARDA-Syria).

During the grain filling phase were performed physiological analyzes of flag leaves as follows:

1. Water exchange of the leaf and leaf morphometry indicators

– fresh weight (FW), absolute dry weight (DW) and relative water content of leaves (RWC) (Turner 1981). The last indicator is determined in percent by the formula:

$$RWC, \% = \frac{W_1 - W_2}{W_2 - W_0} \times 100,$$

where W_1 – initial leaf weight (g); W_2 – leaf weight in full turgor after 24 hours immersed in water (g); W_0 – dry weight of leaf after dried in dry chamber for 8h at 105°C (g).

– intensity of transpiration – the evaporated water from the leaves is determined by the difference IT = T1 – T2, where T1 is initial leaf weight; T2 is evaporated water from the leaves in five minutes end calculated in $gH_2O/gDW/min$. (Valchev & Georgiev, 1991).

– leaf area LA (cm^2) is calculated by the formula: $LA = L \times H \times 0.65$, where L-length of the leaf blade; H-maximum leaf width; 0.65 is a wheat-specific coefficient (Kerin et al. 1997; Chanda & Singh, 2002).

2. Relative leaf chlorophyll content, expressed as CCI index. Measurement was performed with chlorophyll content meter-CCM 200+ manufactured by Opti science, England. From each genotype measurements of 15 flag leaves are made.

3. The biometric measurements of yield elements were as follows: number of spikes, weight of spikes, grain weight and mass per 1000 grains.

4. Vegetative pot trial with young plants subjected to controlled drought in phase third leaf.

In a glass greenhouse of each line were sown 50 seeds in containers filled with 1 kg soil. At the third leaf stage, half of the plants were dried to a stable water deficit and the other half were irrigated until the end of the study. From an average sample, data on water exchange, leaf morphology, and index of damage to plasma lemmas were collected.

– Index of leaf damage (Id, %) of dried 20-day plants is determined by conductivity by the formula

$$Id, \% = [1 - (1 - \frac{T1}{T2})/(1 - \frac{C1}{C2})],$$

where T1 and T2 are the values recorded respectively before and after autoclaving for the treated plants, and C1 and C2 are the respective values for the control (untreated) plants (Blum & Ebercon, 1981; Premachandra et al., 1992; Kocheva et al., 2005).

Statistical analyses included ANOVA, correlations analysis and mean values were performed using the statistical program SPSS 19.0.

Results and Discussion

The results of the physiological analysis for the Katya standard have two values because the all accessions were tested in two groups of four breeding lines for a pot trial (Table 1 and Table 2). Depending on the value of the indicators in the controls, their potential for growth to the third leaf stage was determined under optimal conditions of water supply. With the most fresh and dry mass compared to the standard in the first group was MX 219/98, and in the second group – MX 270/24 (Table 1). No significant difference in values was found, similar results published by Chipilski & Uhr, 2015. The intensity of transpiration was lowest in Katya variety for the first group and in line MX 270/86 for the second group.

After dried in all breeding lines, a reduction in the readings values to the controls was observed. The strongest decrease was found in hydration and transpiration, and the lowest-in dry weight (Table 2). As expected, the variation of the result by genotypes in dried variant was stronger than controls, which fact was previously reported by Chipilski & Georgiev, 2014. Closest values relative to control to the fresh and dry weight were reported on the lines MX 187/3 and MX

Table 1. Average values of 20-days irrigated (control) plants in two years reproduction 2015-2016 and 2016-2017 in pot trial

Breeding lines	FW g	DW g	RWC %	g EW/g DW/min
MX 187/3	0.3116±0.0185	0.0726±0.0290	91.6±3.24	0.0252±0.0050
MX 247/33	0.3539±0.0529	0.0689±0.0113	90.2±2.64	0.0234±0.0099
MX 219/98	0.4137±0.0043	0.0767±0.0018	89.7±3.91	0.0205±0.0112
MX 270/28	0.3769±0.0500	0.0672±0.0114	92.7±1.70	0.0242±0.0056
St. Katya	0.3617±0.0673	0.0652±0.0262	92.6±2.62	0.0149±0.0078
MX 270/24	0.5850±0.0728	0.1058±0.0110	92.9±2.99	0.0246±0.0095
MX 270/50	0.4736±0.0742	0.0909±0.0212	90.9±2.62	0.0189±0.0063
MX 270/86	0.5279±0.0966	0.1017±0.0203	90.3±3.46	0.0146±0.0056
MX 268/1008	0.5058±0.0456	0.0935±0.0111	88.6±2.35	0.0223±0.0045
St. Katya	0.5099±0.0325	0.0912±0.0212	91.2±2.39	0.0160±0.0069

Data are presented as mean values ± SE

270/50. The variety standard Katya performed better in the first group and it is result is comparable to line MX 187/3.

In the second group, line MX 270/50 exceeded an average of up to 20.0% the percentage of the ratio obtained from the Katya variety. Highest RWC values and respectively the lowest reduction for drought variants were calculated for lines MX 219/97, MX 270/50, and all breeding lines in the second group exceeded the standard Katya result. There is a trend in which the intensity of transpiration is the most reduced indicator. The lines MX 270/50, MX 270/86 and MX 268/1008 have higher ratios between dried and controls plant compare to the standard. The higher transpiration of these lines is normal due to the higher water content of their leaves (Clarke et al., 1991). We are interested in lines that combine high hydration and low transpiration. With such values was lines MX 219/98 and MX 270/86, reacting similarly to the Katya standard (Table 2). The last parameter by which we evaluated genotypes was an index of plasmalemmas damage of cell under short-term drought stress. The results lead us to the conclusion that the selected breeding lines behave in the same way. Interesting is the fact that the standard Katya has the most negative result.

The values of morphometric and physiological parameters of flag leaves recorded during the grain filling phase from the plants in the micro field trial (Table 3) varied more in the morphometric indicators mainly genetics determined. Most distinctive were Katya variety and line MX 187/3, characterized by erect leaves. Due to the lack of drought during the period of heading and grain filling, the values of these indicators are not relevant for the purposes of this study. The comparative analysis of the values of the water exchange parameters (transpiration and relative water content) proved the low level of stress caused by drought. There was a significant difference in transpiration, where Katya variety and line MX 187/3 were with low transpiration, as the water content being at the level of others. A significantly higher transpiration intensity was observed in the line MX 270/28.

The reported chlorophyll content of *in vivo* flag leaves was significantly higher by the lines MX 187/3, MX 219/98, MX 270/50 and MX 270/86, exceeding the result of stand-

Table 2. Average values of 20-days no irrigated plants in two years reproduction 2015-2016 and 2016-2017 in pot trial

Breeding lines	FW g	% of control	DW g	% of control	RWC %	% of control	g EW/g DW/min	% of control	Id index
MX 187/3	0.2833±0.073	90.9	0.0689±0.0096	94.9	53.16	58.0±3.86	0.0085±0.002	33.7	3.05
MX 247/33	0.2663±0.072	75.2	0.0644±0.0017	93.5	51.65	57.3±6.25	0.0061±0.001	26.1	4.00
MX 219/98	0.3464±0.096	83.7	0.0542±0.0060	70.7	68.30	76.1±6.01	0.0070±0.001	34.1	6.04
MX 270/28	0.2725±0.077	72.3	0.0607±0.0027	90.3	51.40	55.4±6.25	0.0063±0.001	26.0	4.65
St. Katya	0.2855±0.068	78.9	0.0631±0.0087	96.8	61.40	66.3±4.63	0.0047±0.001	31.5	–
MX 270/24	0.3664±0.064	62.6	0.0619±0.0092	58.5	66.20	71.3±5.63	0.0108±0.002	43.9	5.89
MX 270/50	0.4323±0.058	91.3	0.0881±0.105	96.9	68.47	75.3±4.73	0.0114±0.002	60.3	3.48
MX 270/86	0.3811±0.033	72.2	0.0818±0.0086	80.4	59.51	65.9±6.52	0.0089±0.001	61.0	1.69
MX 268/1008	0.3698±0.051	73.1	0.0797±0.0065	85.2	62.49	70.5±2.18	0.0152±0.002	68.2	5.51
St. Katya	0.3091±0.076	60.6	0.0711±0.0096	78.0	56.41	61.9±4.00	0.0087±0.001	54.4	9.35

Data are presented as mean values ± SE

Table 3. Average values of physiological parameters of flag leaves of common winter wheat grown in micro field trial during period 2015-2017

Breeding lines	FW g	DW g	RWC %	Transpiration g H_2O/g DW/min	LA cm^2	CCI index
St.-Katya	0.4022	0.1469	84.0	0.0131	18.7	40.2
MX 187/3	0.2750	0.0937	81.5	0.0149	13.6	46.1
MX 247/33	0.5245	0.1893	84.0	0.0173	25.5	39.7
MX 219/98	0.4937	0.1785	84.1	0.0161	22.4	46.6
MX 270/24	0.5950	0.2210	83.0	0.0134	27.2	43.0
MX 270/28	0.4449	0.1610	84.1	0.0241	20.9	33.8
MX 270/50	0.4567	0.1686	82.9	0.0171	20.9	45.2
MX 270/86	0.5207	0.1790	86.2	0.0171	22.2	44.0
MX 268/1008	0.4432	0.1670	82.2	0.0120	20.5	35.3

ard Katya. The line MX 187/3 combines higher ratio of relative chlorophyll content to dry weight unit. A similar result was obtained for Gizda variety and foreign varieties Avenue, Andino and Exotic in a comparative study of Bulgarian and foreign varieties in the field of IPGR.

With the combined use of indirect methods and micro field trial, data are more representative because they cover a greater number of plants per unit area and have a stronger relationship between them. In the specific case (Table 4), average value of grain weight, number of spikes, weight of spikes and 1000 grains weight were recorded, but since had more precipitation, i.e. lack of soil and atmospheric conditions drought there was no significance difference between biometric parameters. With the highest values for all biometric parameters were the lines MX 268/1008, MX 270/24, MX 270/50, MX 270/28 and MX 247/33. Only the 1000 grains weight had some differences-for example, the higher yield line MX 270/24 had one of the lowest absolute grain weight. For determination of significant differences in the yield elements between the standard Katya and the breeding lines a dispersion analysis were used. Significant differences were calculated for the 1000 grains weight, as most breeding lines have a proven higher grain weight than the dry resistance standard Katya. For the other indicators, the breeding lines were in the same group with the values reported for Katya. Only line MX187/3 had proven significantly lower values of the yield elements compared to the standard Katya.

The results of the ANOVA analysis showed a significant influence between the genotypes, the environment conditions and the interaction between them (Table 5). The casual factors that have not been taken into account constitute the largest part of total variation – 42.5%. In other our studies, the results of the ANOVA showed that residual influence was as low as about 6% (Uhr, 2015; Uhr & Chipilski, 2017) or close to that of other factors (Kostov et al., 2011). The breeding lines MX 187/3, MX 270/86 and MX 268/1008 were characterized with significance difference of average yield in compared the standard Sadovo 1 (Table 6).

The correlation between the average yields of the breeding lines from the parallel field trial and microfield trial over the two vegetation years were calculated. An no significant mean positive relationship exists only between the yields of field trial and microfield trial in 2017.

Table 4. Average values of yield elements of common winter wheat plants grown in micro field trial during period 2015-2017

Breeding lines	Grain weight g	Number of spikes	Weight of spikes g	1000 grains weight g
St. Katya	150.5	93.7	199.8	43.3
MX 187/3	122.6*	73.6**	163.6*	37.4***
MX 247/33	150.7 n.s.	96.1 n.s.	202.2 n.s.	49.3***
MX 219/98	139.8 n.s.	74.2**	183.0 n.s.	45.4 n.s.
MX 270/24	152.1 n.s.	89.3 n.s.	198.1 n.s.	45.1 n.s.
MX 270/28	155.3 n.s.	91.4 n.s.	202.6 n.s.	48.3***
MX 270/50	153.0 n.s.	89.0 n.s.	189.2 n.s.	46.6***
MX 270/86	143.7 n.s.	89.6 n.s.	188.7 n.s.	49.5***
MX 268/1008	175.8 *	103.5 n.s.	212.5 n.s.	50.2***

n.s. no significance difference * – significance difference at 5 %; **– significance difference at 1 %; *** – significance difference at 0.1%

Table 5. ANOVA analysis of interaction between genotype and environment conditions during period 2015-2017

Source of variation	SS	df	MS	F exp.	F crit.	Influence **μ**, %
Genotype	106513.1	10	10651.3	2.8**	2.6	18.2
Year	74822.2	1	74822.	19.8***	11.9	12.8
Interaction	155672.5	10	15567.2	4.1***	3.5	26.5
Error	249470.9	66	3779.9			42.5
Total	586478.7	87				100

**significance at 5%; ***significance at 1%

Table 6. Average yield of breeding line from field experiment from 10 m² during period 2015-2017

№	Genotype	Average, kg/da	Difference, kg/da	Significance
1	Sadovo 1– st.	632.7	0.0	n.s.
2	MX 247/33	645.3	12.7	n.s.
3	MX 187/3	750.0	117.3	++
4	MX 270/24	719.0	86.3	n.s.
5	MX 270/28	714.8	82.1	n.s.
6	MX 270/50	718.9	86.3	n.s.
7	MX 270/86	741.5	108.9	+
8	MX 268/1008	725.8	93.2	+
9	MX 219/98	706.3	73.6	n.s.
10	Katya	703.8	71.1	n.s.

GD 5% = 86.8; GD 1% =115.3; GD 0.1% = 149.7

Tables 7 and 8 showed the correlation between the mean yields and the physiological parameters reported by the micro field trial (Table 7) and the pot trial with young plants (Table 8). The interrelationships between the yields obtained from field trial and the values of most physiological parameters are not significant and in negative direction.

At the correlations between average yield from micro field trial and physiological parameters, there are some differences. The value of the morphophysiological paremeters of leaves, dry weight, fresh weight and leaf surface had not significant mean positive relationship to yield (Table 7). They are one of the most stable indirect indicators of yield under optimal conditions (Cruz-Aguadoa et al., 2000).

Correlation coefficients between the reported average yields of field trial and micro field trial with physiological parameters from pot trial in the control plants were in one direction of interaction. Difference was observed in the RWC, where the correlation was positive for field trial and negative for the micro field trial (Table 8). Similar results are also reported in dried plants, where the results of RWC and transpiration are most distinctive, although there were not positive correlations proven. The breeding lines with more tolerant leaf reaction in younger age, tested in the pot tial experiment have a higher yield. Morphometric parameters also showed a low to medium correlation From this result, we can conclude that such parallel studies are useful for breeding despite the lack of complete similarity.

Table 7. Correlation coefficients between parameters average yield of field trial, micro field trial and physiological estimate made from field trial during period 2015-2017

Parameters	FW, g	DW, g	RWC %	Transpiration g EW/g DW/min	LA,cm²	CCI index
Average yield of field trial	-0.476	-0.478	-0.245	-0.202	-0.617	0.239
Average yield of field microtrial	0.426	0.510	-0.008	-0.094	0.441	-0.730*

significance difference at 5 %

Table 8. Correlation coefficients between parameters average yield of field trial, micro field trial and physiological estimate made from pot trial during period 2015-2017

Parameters	FW, g	DW, g	RWC, %	Transpiration g EW/g DW/min	Id, %
Control					
Average yield of field trial	0.249	0.401	0.159	-0.170	–
Average yield of field microtrial	0.496	0.306	-0.317	-0.030	–
Stress					
Average yield of field trial	0.391	0.360	0.241	0.443	-0.226
Average yield of field microtrial	0.304	0.279	0.204	0.586	0.413

Conclusions

- The highest yield of a micro field trial was reported for the lines MX 268/1008, MX 270/50, MX 270/28 and MX 270/24.
- The most intense morphometric indexes of the leaves were obtained for the lines MX 247/33, MX 270/24 and MX 270/86, while morphological markers for drought resistance were distinctived for the lines MX 268/1008, MX 187/3 and again MX 270/24.
- The most tolerant reaction to controlled dry up of young plants developed in pot trials was found in the lines MX 270/50, MX 187/3, MX 268/1008 and MX 270/24, which exceeded the most of the physiological parameters of Katya standard.
- The genotypes MX 270/24, MX 187/3 and MX 268/1008 combined high yield and good physiological and agronomic indicators under drought stress conditions.

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