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REGULAR ARTICLE

Evaluation of tolerance to osmotic stress of winter bread wheat genotypes using indirect physiological method

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Abstract

Fifteen winter bread wheat cultivars from Romania and Serbia were evaluated with regard to their tolerance to osmotic stress. Evaluation was made by applying the indirect physiological method, recognizing the growth depression seedling, cultivated in solution with increased osmotic pressure (Atm). Water deficit in most of the genotypes suppresses to a great extent the growth of roots compared to that of the shoot. The average coefficient of root growth depression was 55.77% during the moderate osmotic stress trial and 55.83 % during the strong osmotic stress trial, while with shoots the percentage was 35.76 % and 50.12 %, respectively. The average root length / shoot length ratio (R/Sh ratio) for all genotypes in the control was 1.70; in the 0.5 M and 1 M sample solution sucrose it was 1.14 and 1.55, respectively. In genotypes most tolerant to osmotic stress as Renesansa, Dragana, Izvor and Faur the root/ shoot length ratio is decreased in the highest degree. It was established there is negative regression dependence between the growth of the root/shoot and the solution with increase of osmotic pressure. The equations showed the strong limitation role of osmotic pressure for the growth of the seedling. The results of this study showed that the varieties Renesansa, Dragana, Izvor and Faur had the best ability of osmotic regulation.

Key words: Depression coefficient, Drought tolerance, Osmotic stress, Winter bread wheat

Introduction

The main objective of any wheat breeding program is to create varieties with high yield potential, possessing a complex of biological and agricultural quality, resistant to biotic and abiotic stress factors and suitable for low input (Rachovska et al., 2003; Dimova et al., 2006; Ivanova and Tzenov, 2009b; Tzenov et al., 2009; Bozhanova et al, 2009a). By approaching the limits of biological productivity and as a result of global climate change, the need for new sources material to create new varieties that meet the climate change has greatly increased. The efforts of researchers have been directed to searching for new sources of gene plasm, as carriers of ecological plasticity and stress tolerance in the highest degree. Identification of genotypes with tolerance to drought includes various methods – from physiological to molecular markers (Bruce et al., 2002; Yousufzai, 2007; Maccaferri et al., 2008; Aliyev, 2012; Khavarinejad and Karimov, 2012). In many investigations identification of tolerant and sensitive forms is based on measurements of some physiological parameters related to drought tolerance, such as: reaction of roots and shoots to osmotic stress, intensity of transpiration, relative water content in the leaves (RWC) and gas exchange indices (Bozhanova, 1997; Bozhanova and Dechev, 2002; Bozhanova et al., 2005, 2009a, 2009b, 2010; Luigi et al., 2008, Gonzalez et al. 2010; Ganusheva et al., 2011).

The method of measuring the coleoptiles to water deficit developed by Morgan (1988) is based on the fact, that the genotypes with the better potential for osmoregulation are able to maintain better turgor and associated physiological processes, such as maintaining a more intensive cells increase in water deficit. Genotypic differences in the terms of osmoregulation ability have been reported in various crops. Significant variation in this trait was observed in wheat (Morgan 1983; Blum et al., 1999), sunflower (Jamaux et al., 1997), shorgum (Ackerson et al.,

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1980), millet (Henson et al., 1982), rice (Lilley et al., 1996; Babu et al., 1998), barley (Blum 1989) and wild species from Gramineacea (Bozhanova et al., 2006; Uhr et al., 2007).

The objective of this study was to evaluate the winter bread wheat accessions by their tolerance to osmotic stress, by using the indirect physiological method.

Materials and Methods

The study was conducted in the Physiological laboratory in the Institute of Plant Genetic Resources "Konstantin Malkov"-Sadovo, Bulgaria. Fifteen varieties of winter bread wheat with origin from Rumania (Gruia, Izvor, Delabrad, Litera, Boema, Faur and Golosa) and Serbia (Gordana, Dragana, Zvezdana, Rusiya, Evropa 90, Renesansa, Gora and Rapsodiya), were investigated. The accessions are maintained in the *ex-situ* field collection in IPGR-Sadovo. The Katja variety was used as a standard of drought tolerance, which was defined as a standard variety according to international studies in the drying condition under CIMMYT, Turkey and ICARDA-Syria.

The reactions of roots and shoots to two levels of osmotic stress were estimated by applying the method of Bozhanova (1997). The seeds from all genotypes included in the research were sterilized and put for germination on wet filter paper in to Petri dishes with 20 ml distillated water in thermostat for 72 h, at 25ºC, in the dark. After germination the seedlings from every genotype were placed in three variants:

1. Control variant- after germination the seedlings were left in distillated water;
2. Moderate osmotic stress variant- after germination the seedling were transferred in 0.5 M solution of sucrose, which provokes osmotic stress with pressure of 12.23 Atm;
3. Strong osmotic stress variant- after germination the seedling was transferred in 1 M solution of sucrose, which provokes osmotic stress with pressure of 24.45 Atm.

The seedlings from all variants were put on wet filter paper, turned in to rolls. The rolls were put in the thermostat for 48 h, at 25ºC. After that the length of the roots and the shoots were measured in cm, in all three variants. The biometrical measurements were carried out on 20 seedlings per accession.

The osmotic pressure of the sucrose solution was calculated according to the Todd Helmenstine (http://chemistry.about.com/od/workedchemistryproblems/a/Osmotic-Pressure-Example.htm).
Osmotic pressure, Atm= iMRT, where
i- van't Hoff factor of the solute
M- molar concentration in mol/L
R- universal gas constant = 0.08206 L Atm/mol K
T- absolute temperature in K.
The coefficient of depression was calculated according to the Blum et al. (1980):
Coefficient of depression, % = [(A-B)/A] x 100, where
A – average length of the roots /shoots in the control variant, cm
B – average length of the roots/shoots in the osmotic stress variant, cm.

The data was processed by methods of correlation and regression analysis (Lidanski, 1988). T-test was used to establish the significance of the difference between means of the standard (Katja variety) and the other genotypes (Lidanski, 1988). Statistical analyses were performed using the statistical program SPSS 13.0 and Statistica-6.

Results and Discussion

The osmotic stress stimulated by adding of 0.5 M solution of sucrose and 1 M solution of sucrose and applied after germination, inhibits the growth of the seedling in all genotypes included in the experiment. In most genotypes the water deficit suppresses greatly the growth of the roots compared to that of the shoot (Table 1). The average coefficient of root growth depression was 55.77% in the trial with moderate osmotic stress and 55.83% in the trial with strong osmotic stress, while for the shoots these values were respectively 35.76 % and 50.12%. Regardless of the lower depression in the growth of coleoptiles compared with the roots, there is clear tendency to stronger deceleration in their growth in the higher concentration of osmotic. Six varieties make an exception away from the trent (Gruia, Delabrad, Litera, Goloza, Rusiya and Rapsodiya), where in the Delabrad variety the inhibition of the overhead part was significantly greater than that of the root with coefficient of depression-48.34% for roots and 60.75% for shoots (in 1 M solution of sucrose). The fact that the water deficit influences in a greater extent the roots of the young seedlings was also established from Marcheva et al. (2013) for *Triticum durum* Defs.

When applying moderate osmotic stress onto young wheat plants the coefficient of depression of shoot growth ranged from 17.5% for Izvor variety to 68.9% for Rapsodia, as the value of the coefficient of depression in the standard variety Katja was 39.9%. Depression of the root growth ranged from 39.8% for Renesansa to 67.3% for Rapsodiya, as the all accessions except Rapsodiya showed the lower coefficient of depression of the root in comparison of the standard variety (Katja).

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Table 1. Reaction to osmotic stress of winter bread wheat seedlings.

Accession	Root length, cm			Shoot length, cm			Depression coefficient, %				Root length/shoot length ratio, cm		
	0 M sol. sucrose	0.5 M sol. sucrose	1 M sol. sucrose	0 M sol. sucrose	0.5 M sol. sucrose	1 M sol. sucrose	0.5 M sol. sucrose		1 M sol. sucrose		0 M sol. sucrose	0.5 M sol. sucrose	1 M sol. sucrose
							Root	Shoot	Root	Shoot			
Katja St	8.46	2.95	3.13	5.24	3.15	3.03	65.13	39.89	63.00	42.18	1.61	0.94	1.03
Gruia	9.73	3.99***	3.49	5.62	3.70**	1.94***	58.99	34.16	64.13	65.48	1.73	1.08	1.80
Izvor	9.09	4.09***	4.52***	4.40*	3.63	2.91	55.01	17.50	50.17	33.86	2.07	1.13	1.55
Delabrad	6.04***	3.61**	3.12	4.28**	2.80	1.68***	40.23	34.58	48.34	60.75	1.41	1.29	1.86
Litera	10.78***	4.2***	3.97*	6.46*	3.50	2.34*	61.04	45.82	63.17	63.78	1.67	1.20	1.70
Boema	9.55	3.85**	3.48	5.25	3.15	2.03***	59.69	40.00	63.56	61.33	1.82	1.22	1.71
Faur	9.51	3.96***	4.38***	4.58	3.36	3.1	58.36	26.64	53.94	32.31	2.08	1.18	1.41
Golosa	10.58**	4.32***	5.06***	5.57	3.46	2.53**	59.17	37.84	52.17	54.58	1.90	1.25	2.00
Gordana	10.15**	4.26***	4.23***	6.21*	3.61	2.63	58.03	41.87	58.33	57.65	1.63	1.18	1.61
Dragana	8.4	4.01***	4.26***	4.81	3.89***	3.31	52.26	19.13	49.29	31.19	1.75	1.03	1.29
Zvezdana	8.38	3.84**	3.76**	4.24**	3.18	2.53***	54.18	25.00	55.13	40.33	1.98	1.21	1.49
Rusiya	9.56	4.31***	3.90**	7.45***	4.13***	2.52	54.92	44.56	59.21	66.17	1.28	1.04	1.55
Evropa 90	8.92	4.21***	4.08***	7.15***	4.28***	3.56***	52.80	40.14	54.26	50.21	1.25	0.98	1.15
Rapsodiya	8.59	2.81	3.36	5.42	2.67*	1.93***	67.29	50.74	60.88	64.39	1.58	1.05	1.74
Renesansa	9.25	5.57***	5.38***	5.35	4.26***	3.76***	39.78	20.37	41.84	29.72	1.73	1.31	1.43
Average	9.13	4.00	3.99	5.47	3.52	2.65	55.79	34.55	55.83	50.26	1.70	1.14	1.55

*the mean difference is significant at the 0.05 level, **the mean difference is significant at the 0.01 level, ***the mean difference is significant at the 0.001 level

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When applying strong osmotic stress, the coefficient of depression of the shoot growth ranged from 29.7% for Renesansa to 66.17% for Rusiya, and for the root from 41.94% for Renesansa to 64.13% for Gruia. The values of the coefficient of depression for the Standard –Katja for shoots and roots were respectively 42.2% and 63.0%.

The average values of the depression coefficients in the seedling as an expression of the proneness to osmotic regulation at the whole plant level of the genotypes is presented in Figure 1. Based on the obtained results we could concluded that Renesansa, Dragana, Izvor and Faur varieties are the least affected in both variants of osmotic stress. These varieties demonstrated best ability of osmotic regulation.

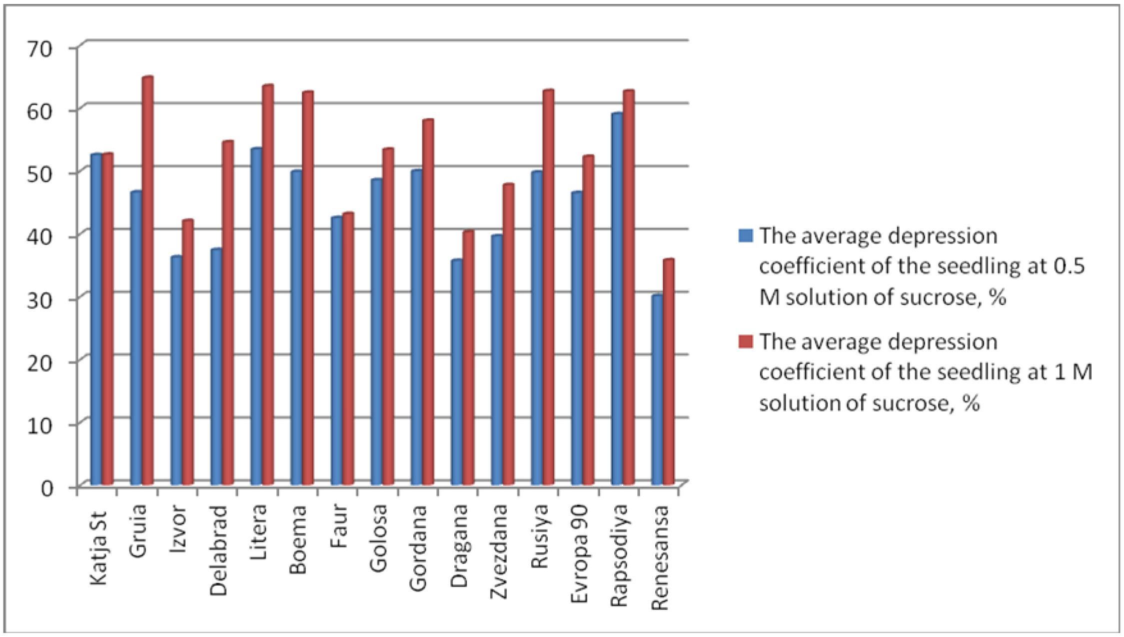

Figure 1. The ability of osmotic regulation of 15 winter bread wheat genotypes expressed through the average values of depression coefficient of seedling at two levels of osmotic stress.

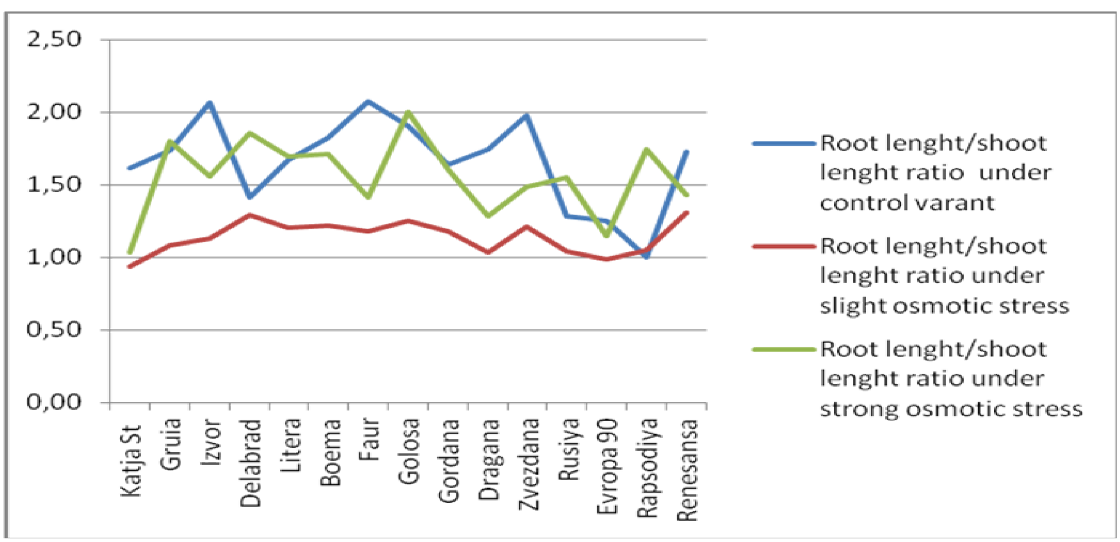

Figure 2. Root length to shoot length in winter bread wheat seedlings under normal water providing and under osmotic stress.

As a result of dehydration the ratio between the lengths of the root and that of shoots changes, too (R/Sh ratio) (Table 1). The average root/ shoot length ratio (R/Sh ratio) for all genotypes in the control variant was 1.70; in the variants with 0.5 M and 1 M solution sucrose it was 1.14 and 1.55, respectively. In the trial under moderate osmotic stress all accessions included in the study showed a lower ratio (R/Sh), compared the control variant (Table 1, Figure 2). This result is due to the strong roots growth depression compared to that of shoots. The strong osmotic stress trial showed a steady tendency of R/Sh ratio decrease. Exceptions were varieties: Delabrad, Rusiya, Gruia, Litera, Goloza and Rapsodiya, where the R/Sh growth ratios were higher than these in the trial with 0 M solution of sucrose. In them the growth of the shoot is to the greatest extent inhibited the, i.e. they hardly tolerate dehydration and are more sensitive. In the genotypes most tolerant of osmotic stress, such as Renesansa, Zvezdana, Dragana, Izvor and Faur the root/ shoot length ratio decreases to the highest degree. This trend was ascertained by other researchers in thetraploid and in hexaploid wheat (Bajji et al., 2000; Dhanda et al., 2002; Bozhanova et al., 2006) and can be used as an indirect indicator of the screening of drought tolerant genotypes.

Table 2 shows the correlation coefficients between some traits (length of the root, length of the shoot, depression of the root length and depression of the shoot length) calculated for all molar concentrations. A positive correlation between traits: length of root and length of shoot, more pronounced and statistically proven at 0.5 M and 1 M solution of sucrose, respectively r=0.771 and r=0.639. Induced osmotic stress causes genotypic differences by reducing the intensity of growth of the seedling. This is confirmed from the results on depressions of the root and shoot growth, where strong positive and significant at 0.01 level correlations between the two osmotic concentrations as the root (r=0.811) and the shoot (r=0.846) were observed.

Table 2. .Correlation between traits inclusions in the study at two levels of osmotic stress.

	Shoot length 0 M sol. sucrose	Shoot length 0.5 M sol. sucrose	Shoot length 1.0 M sol. sucrose	Depression of root at 1 M sol. sucrose	Depression of shoot at 1 M sol. sucrose
Root length 0 M sol. sucrose	0.505 [n.s.]	-	-	-	-
Root length 0.5 M sol. sucrose	-	0.771**	-	-	-
Root length 1.0 M sol. sucrose	-	-	0.639*	-	-
Depression of root at 0.5 M sol. Sucrose	-	-	-	0.811**	-
Depression of shoot at 0.5 M sol. sucrose	-	-	-	-	0.846**

n.s. no significance correlation; *- Correlation is significant at the 0.05 level; **- Correlation is significant at the 0.01 level

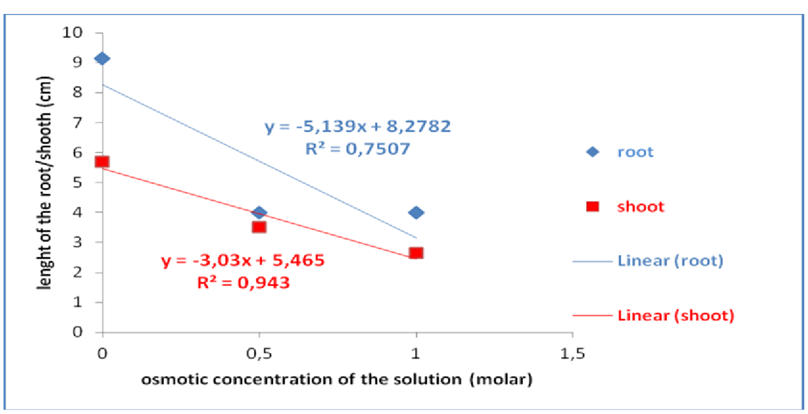

Figure 3. The linear relationship between the intensity of the growth of the seedling and the osmotic concentration of the solution.

Figure 3 shows the linear relationship between the intensity of the growth of the seedlings and the osmotic concentration of the solutions. It was established there is negative regression dependence between the growth of the root/shoot and the solution while osmotic pressure increases. The equations show the strong limiting role of osmotic pressure onto the seedling's growth.

Conclusion

Water deficit suppress in a greater extent the growth roots compared to that of shoots. As result of dehydration the ratio between the lengths of the root and the shoots changes, too. In genotypes most tolerant of osmotic stress the root/ shoot length ratio decreases in the highest degree. A positive correlation between traits: the length of root and length of shoot, more pronounced and statistically proven at 0.5 M and 1 M solution of sucrose was established. Induced osmotic stress causes genotypic differences by reducing the intensity of growth of the seedling. It was established there is negative regression dependence between the growth of the root/shoot and the solutions while osmotic pressure increases. The equations showed the strong limitating role of osmotic pressure over the growth of the seedling. The varieties Renesansa, Dragana, Izvor and Faur showed the best ability of osmotic regulation. Initial screening needed by studying more physiological and agronomical characteristics connected to growth and productivity of plants to drought.

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