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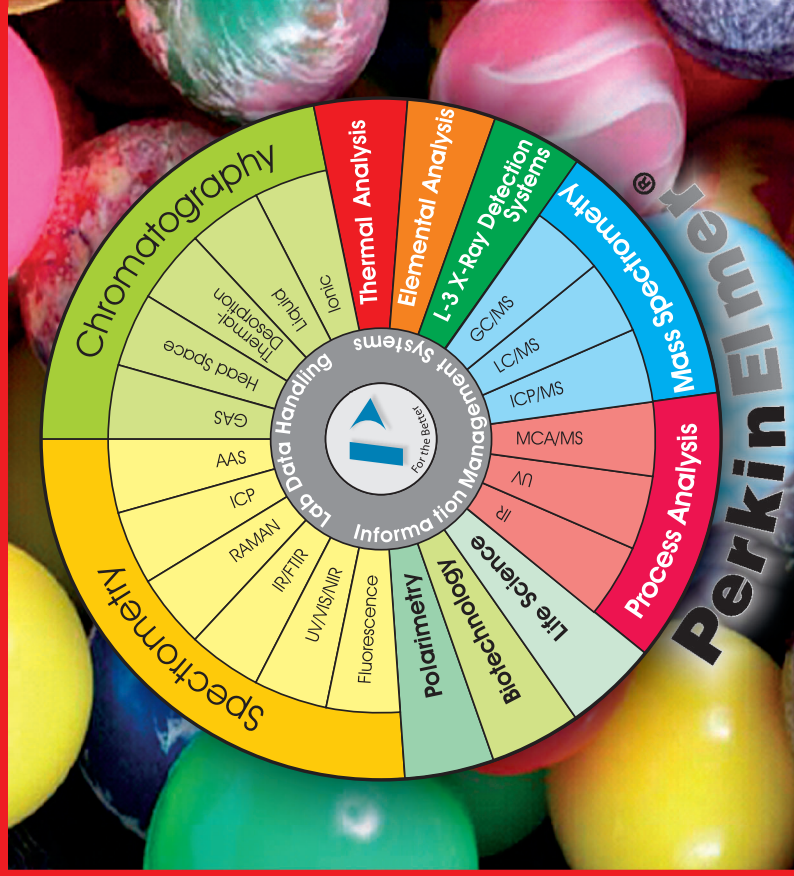
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Genetics and Breeding

Heritability of osmoregulation ability at durum wheat

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Abstract. The osmoregulation is one of the most important cell adaptation mechanisms occurred at beginning of drought. The inheritance of osmoregulation ability in durum wheat by using indirect method for trait measuring was studied. Five durum wheat genotypes with different level of tolerance to osmotic stress were included in diallel crossing scheme. Among the parents genotypes the old Bulgarian cultivar A-223 distinguishes with the lowest seedlings growth depression coefficient of 32,5 %, i.e. with best ability of osmotic regulation. The lowest osmotic regulation ability shows cultivar Gergana. The hybrid combinations including the most tolerant parent A-223 show the lowest coefficient of depression among the F₁ hybrids. The conducted diallel analyses shows the high significance of the effects of the general (GCA) and specific combinative ability (SCA) during the all 3 years of the investigation. The inheritance of high levels of osmoregulation is determined by both the additive and non-additive gene effects. The non-additive gene effects (SCA) strongly predominate over the additive and have a significant role in the observed big heterosis effect. The significance of variances of both GCA and SCA requires the selection of single plants by investigated traits to be shifted in the later segregating progenies.

Keywords: durum wheat, drought resistance, osmoregulation ability, inheritance

Abbreviations: GCA-general combinative ability, SCA-specific combinative ability

Introduction

The osmoregulation is one of the most important cell adaptation mechanisms occurred only when dehydration begins. To minimize the loss of water from the cells and to sustain the cell's functions in case of water deficit, soluble materials are being stored in them. By this main cell response, occurring during drought, dehydration of cells and decrease of the yields are being avoided (Blum, 2005). Plants with better osmoregulation ability show better growth and higher yields in drought conditions. Genotype variation in the ability of osmoregulation in different species have been reported up to now. Considerable variability of this trait in wheat – common and durum (Blum et al., 1999; Morgan et al., 1986), sunflower (Jamaux et al., 1997), sorghum (Ackerson et al., 1980), millet (Henson et al., 1982), rice (Babu et al., 1998; Lilley et al., 1996) and barley (Blum, 1989) was observed.

Usually the genotypes are characterized in respect of osmotic regulation by the traits osmotic potential (OP) and relative water content (RWC) in the leaves. These physiological indexes are very expensive, besides time they require routine and are not applicable for evaluation of large numbers of breeding lines. (Bozhanova et al., 2009). Two indirect methods for osmoregulation measuring suggested by Morgan: method of measuring the coleoptiles length in seedlings expose to osmotic stress and the method of measuring of the osmotic regulation in the pollen grains (Morgan, 1988; Morgan, 1999) have potential to substitute the complicated physiological methods. The above maintained indirect methods are used recently in the drought resistance studies (Moud and Yamagishi, 2005; Eivazi et al., 2007; Moud and Maghsoudi, 2008).

The detected relationships between osmoregulation ability and productivity at conditions of water deficit (Moinuddin et al., 2005)

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determines the increasing interest to investigate heritability of this trait aiming to its further genetic improvement (Basnayake et al., 1995; Zhang et al., 1999; James et al., 2008).

The goal of the present work is to ascertain the inheritance of osmoregulation ability in durum wheat by using indirect method for trait measuring and diallel crossing scheme.

Materials and methods

The investigation has been conducted during 2006-2009 years. Five durum wheat genotypes - the old Bulgarian variety A-233, varieties Gergana, Vazhod and Beloslava and breeding line D-6189, previously manifested variability of the trait tolerance to osmotic stress (Bozhanova et al., 2005) were included in the investigation. A diallel crossing schemes Model 1 and Method 4 (Greiffing, 1956) were applied.

The hybridization has been made in field conditions. Indirect method for measuring of osmoregulation ability, based on the Morgan's method for coleoptiles length measuring at osmotic stress (Morgan, 1988), was used. The seeds of all included in the research genotypes (parents and hybrids) were sterilized and put for germination on wet filter paper, turned into a rolls. The rolls were put in plastic boxes filled with 100 ml destilated H₂O in termostate for 48 hours, at 26 °C in the dark. Six rolls from each genotype were made. After the germination the half of the rolls were put again in destilated water (control variant) and the other half were transferred in 1M solution of sucrose, which provokes osmotic stress with pressure of 22 Atm and by this way water deficit is simulated. After 3 days the length of the roots and shoots were measured in mm, in both

variants - the control one and the one with the osmotic stress. The experiment was conducted in three replications for each variant and genotype and 20 seedlings were measured for each replication. The coefficient of depression was calculated according to the Blum et al. (1980):

$$\% \text{ of depression} = [(A-B)/A] \times 100;$$

A - average length of the roots/shoots in the control variant, mm

B - average length of roots/shoots in osmotic stress, mm

The results were processed via two-factor analysis of variance, Duncan's multiple test for significance by means of a program package Statistica-7 (2004). A diallel analysis of general (GCA) and specific (SCA) combination ability was carried out by Griffing, 1956 with computer program DIALLEL, kindly provided by the authors Burrow and Coors, 1994.

Results and discussion

The method of coleoptiles growth measuring at water deficit is developed by Morgan (Morgan, 1988) and is based on the fact that genotypes possessing better potential for osmoregulation are in a position to support better turgor and related with it physiological processes, such as maintaining more intense cells growth during the water deficit. The method we have used to measure the seedlings growth depression is based on the above mentioned method of Morgan. The osmotic stress, stimulated by adding of 1M sucrose solution and applied after the germination, inhibates the growth of the seedlings at all genotypes included in the experiment. In most of the genotypes the water deficit depresses greatly the growth of the shoots than the growth of the roots. The average values of the traits:

length of roots, length of shoots, ratio between the roots and the shoots and depression coefficients in seedlings as an expression of the ability of osmotic regulation at a whole plant level of the parent genotypes and the hybrid combinations are presented in Table 1. Among the parent genotypes the old Bulgarian cultivar A-223 distinguishes with the lowest coefficient of depression 32,5 %, i.e. with best ability of osmotic regulation. The highest coefficient and lowest osmotic regulation ability shows cultivar Gergana - 52 %. The other three parent genotypes arrange between both cultivars with similar coefficients of depression. The cultivar Beloslava approximates to A-223. The hybrid combinations including the most tolerant parent A-223 show the lowest coefficient of depression among the F_1 hybrids. With highest depression coefficient and lower osmoregulation ability are F_1 lines from hybrid combination Gergana x Vazhod, Gergana x 6189, Beloslava x Vazhod and Vazhod x 6189.

As a result of the dehydration the ratio between the lengths of the root and the shoots changes, too. In the most of the studied genotypes during the osmotic stress these ratio grows, but in different degree (Figure 1). There is tendency for greater increases in the more drought sensitive genotypes, that was ascertained from other researches at different species (Dhanda et al., 2004). This index can be used for fast screening of genotypes with higher potential for osmoregulation, too. The information for the inheritance of indirect physiological traits is important for the preparation of adequate breeding strategy for improvement of drought resistance, having in mind the low inheritance of the yield in different environment due to the great interaction between genotypes and environments (Cattivelli et al., 2008).

The analysis of variance applied for trait length of seedlings, cultivated in different condition of water supply, separately for each year of the experiment reveals that influence of genotype,

Table 1. Tolerance to osmotic stress at seedling stage of durum wheat genotypes in diallel cross, average for 3 years

Genotypes	Length, cm						Coefficient of depression, %		
	Normal water supply			Osmotic stress					
	roots	shoots	roots/ shoots	roots	shoots	roots/ shoots	roots	shoots	seedlings
A-233	47.7	50.4	0.9	38.6	34.0	1,1	19.1	32.5	24.7 ^{ab}
Gergana	54.4	49.4	1.1	37.2	23.9	1,6	31.6	52.0	39.7 ^{cde}
Beloslava	54.0	54.7	1.0	41.0	34.8	1,2	24.1	36.4	28.0 ^{abc}
Vazhod	61.4	45.5	1.4	34.1	26.4	1,3	44.5	40.9	36.7 ^{bode}
D-6189	69.8	58.0	1.2	38.5	34.0	1,1	44.8	41.4	42.1 ^{de}
233 x Gergana	48.9	33.3	1.5	40.3	23.9	1,7	17.6	28.2	22.7 ^a
233 x Beloslava	58.7	45.3	1.3	42.9	32.5	1,3	26.9	21.6	24.8 ^{ab}
233 x Vazhod	55.4	45.5	1.1	43.6	29.0	1,5	21.3	36.3	25.4 ^{ab}
233 x 6189	50.8	43.0	1.2	36.9	28.9	1,3	27.4	32.7	28.5 ^{abcd}
Gergana x Beloslava	60.6	50.9	1.2	46.8	31.0	1,5	22.8	39.2	29.9 ^{abcd}
Gergana x Vazhod	56.4	44.2	1.3	32.5	21.2	1,5	42.4	52.0	41.8 ^{de}
Gergana x 6189	48.8	45.6	1.1	30.8	23.0	1,3	38.5	49.6	47.7 ^e
Beloslava x Vazhod	60.5	45.3	1.3	39.3	27.9	1,4	35.0	49.9	35.2 ^{bcd}
Beloslava x 6189	48.5	32.6	1.5	29.6	19.8	1,5	38.9	39.3	39.4 ^{cde}
Vazhod x 6189	55.6	38.9	1.4	33.9	21.7	1,6	39,0	44.2	36.6 ^{bode}

a,b, c, d- values within a column having a different superscript letter differ significantly ($p < 0.05$)

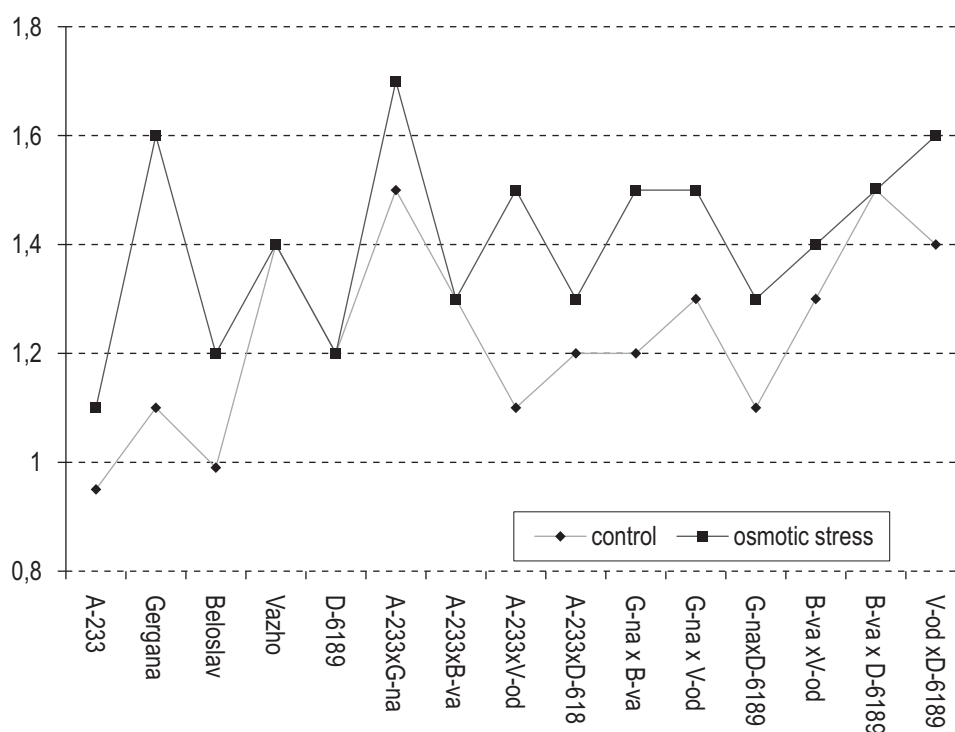


Figure 1. Roots to shoots length ratio

environment (control and osmotic stress) and the interaction between them on the variation of this trait is significant. The variation of the roots and shoots length depends in greatest degree on genotype following on conditions for germination (different level of water supply) and least on interaction between them. The fact that the phenotype variation on the trait depression of the seedling growth is determined mostly by the genotype, shows a high level of inheritance of the trait in broad sense. This variability can be effectively used for screening of genotypes with high potential of osmoregulation, but the determination of the applied breeding strategy depends on the inheritance in the narrow sense. The analysing of combinative ability reveals the gene actions. The effects of general combinative ability cover the additive effect of genes,

while the effects of the specific combinative ability represent only the non-additive gene effects.

The conducted diallel analysis on the examined trait, as a indirect index for the osmoregulation ability, shows the high significance of the effects of the general and specific combinative ability during the all 3 years of the investigation. Therefore the inheritance of high levels of osmoregulation is determined by both the aditive and non-aditive gene effects (Table 2). Besides the non-aditive gene effects (SCA) strongly predominate over the aditive and have a significant role in the observed big heterosis effect, leading to decrease in the depression coefficient, i.e. to higher levels of osmoregulation. Among the included in the investigation genotypes, cultivar A-223 is the proven positive common combinator on the trait

Table 2. Analysis of variance of seedlings length at different water regimes

Source of variation	Roots length			Shoots length		
	MS	SS	η^2 %	MS	SS	η^2 %
Total		14218.58			16781.0	
Environment	4167.5***	4167.45	29.3	3744.3	3744.3***	22.3
Genotype	503.4***	7048.11	49.6	788.8	11043.5***	65.8
Interactions	163.1***	2283.17	16.1	116.8	1643.9***	9.7
Error	12.1	3129.90	4.9	5.6	326.6	1.9

*** $p \leq 0.1$ %

of high osmotic regulation and Gergana is the proven negative one. This cultivar's characteristics should be taken in account in future

Table 3. Significance of GCA and SCA and ratio between the variances of GCA and SCA

Depression coefficient	General Combining Ability GCA	Specific Combining Ability SCA	GCA/SCA
Roots	***	***	≤1
Shoots	***	***	≤1

breeding programs for improving the osmoregulation ability of the durum wheat. The significance of variances of both GCA and SCA (Table 3) requires the selection of single plants by investigated traits to be shifted in the later segregating progenies. Then the efficiency of selection will be vastly higher due to decrease of SCA effects as results of the natural increase of homozygosity in every next generation at self pollination. The ascertained in our investigation prevalence of non-additive genes effects supports the finding of many authors at other cereals species that relative little numbers of locus are responding for a big part of the variability of this trait (Lilley et al., 1996; Morgan, 1991; Teulat et al., 1998).

Conclusion

The observed relatively high heritability of the studied trait reveals that it is possible a breeding progress in the improvement of the osmoregulation ability and thus drought resistance to be achieved. The determined genes effects permit an adequate breeding strategy for it to be chosen.

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