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Selection for Osmoregulation Gene to Improve Grain Yield of Wheat Genotypes under Osmotic Stresses

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Abstract: For identifying osmoreguulation (*or*) differences in bread wheat genotypes, three experiments by using of randomized complete blocks design with five replications were carried out separately on 6 genotypes (Tabasi, Alvand, Shahi, Sabalan, Sardary and Roshan) under normal, drought and salinity stresses. At earring stage, in order to studying osmotic stress on pollen grains, treatments of 30% PEG (control) and 50% PEG with 10 mM KCl (osmotic stress) were applied. All pollen grains of genotypes were been swollen at control treatment, but under osmotic stress except of Roshan pollen grain (due to accumulation of K^+ ion) was been shrinkage. Orthogonal analysis of variance showed significant differences for traits of grain yield and its components, soluble sugars, Na^+, K^+ accumulations. Under stress Roshan genotype had high accumulation of soluble sugars and K^+. Increasing of grain yield due to *or* gene at Roshan than other genotypes under drought and salinity stresses were, respectively 18.14 and 38.80%. So, under drought and salinity stresses, identification of *or* allele and selection based on it in wheat breeding programs are strongly recommended.

Key words: Drought and salinity stresses, pollen selection, wheat

INTRODUCTION

Before physiological traits were used for improving abiotic stresses, it was necessary to demonstrate genetic variation, association with yield and easy of measurement. *or* gene conforms these criteria. During the course of stresses, osmotic potential decreased by the active accumulation of organic and inorganic solutes within the cell sap (Kerepesi and Galiba, 2000). *or* gene contributes through accumulation of these solutes; facilitate maintenance of leaf hydration and turgidity (Guicherd *et al*., 1997). K^+ regulates guard cell turgor and stomatal aperture (Webb *et al*., 1996). Increasing of soluble sugars under stress can be attributed to smaller translocation from the leaf to other parts of the plant. Slower consumption due to decreased growth and other changes such as starch hydrolysis (Kameli and Losel, 1996) and it is effective in stress tolerance mechanism (Moinuddin *et al*., 2005).

It is known an association between *or* and increases in grain yield in wheat (*Triticum aestivum* L.) genotypes (Erdei *et al*., 2002), sunflower (*Helianthus annuus* L.) (Chimenti *et al*., 2002), chickpea (*Cicer arietinim* L.) (Moinuddin and Chopra, 2004) and kentucky bluegrass (*Poa pratensis* L.) (Jiang and Huang, 2001) which is sustained over a wide range of conditions. Yield improvement associated with improvements in biomass, resulting from greater growth (Blum *et al*., 1999), tiller number (Morgan, 2000) and harvest index through increased of seed set (Morgan and Condon, 1986).

Studies showed that the differences in *or* at wheat controlled by a single locus (oror/OROR) on the short arm of chromosome 7A, with the high *or* response recessive (Morgan and Tan, 1996). Therefore, We need more supporting evidence of this gene in different genetic backgrounds of wheat at a wide range of environments before this criterion could be used in breeding programs. The present study evaluates such associations in Iranian landraces.

MATERIALS AND METHODS

Pure Iranian landraces of wheat genotypes including, Tabasi, Alvand, Shahi, Sabalan, Sardary and Roshan used in this study. The experiment was carried out in Western Azerbaijan Agricultural Research Center at the growing season of 2005-06. The experimental field station located in latitude 36° 58′, longitude 46° 6′ and altitude 1371 m, by a typical silty loam texture. The genotypes planted separately at three randomized complete block design, with five replications in saline soil, drought and normal conditions.

To determine irrigation time, the values of evaporation from the Class A Pan measured. Irrigation

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was done after 150±5 and 75±5 mm evaporation from the Class A Pan for drought and (control and salinity) plots, respectively.

Three spike of each genotype sampled at dehiscing anthers and in the laboratory excised. Some of the pollen grains were being rested on two slides. Before sampling we need to have two solutions of polyethylen glycol (PEG) 30% and polyethylen glycol 50% with 10 mM KCl. With pouring a drop of solutions on each slide and putting cover slip on them, they incubated at 20°C for 2 days. Pollens were being screened for *or* gene, with comparing the size of them under microscope withx100 magnifying power (Morgan, 1999).

Six flag leaves from main stem taken from drought stress and normal conditions and Relative Water Content (RWC) determined according to the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Turgid weight obtained after soaking in distilled water at 5°C in darkness over night and dry weight measured after 24 h incubation at 80°C (Gonzalez *et al*., 1999).

0.02 g dry weight of flag leaves extracted in 2 mL of 70% (v/v) ethanol and centrifuged at 2000 g for 10 min. The pellet washed with 2 mL of 70% ethanol and then with 0.8 mL distilled water. The pooled supernatant mixed with an equal volume of chloroform and the aqueous phase recovered and brought to 4 mL with distilled water. Soluble carbohydrates determined by anthrone methods (Sanchez *et al*., 1998). The Na^+ and K^+ contents were measured by flame photometry (Munns *et al*., 2000). Data analyzed by MSTAT-C software.

RESULTS AND DISCUSSION

Since wheat genotypes self-pollinated and *or* gene controlled with recessive allele (oror); therefore at haploid stage (pollen grain) didn't influence with dominant allele (*Or*). Pollen grains in 30% PEG (control) was swollen, but at 50% PEG with 10 mM KCl (osmotic stress) except of Roshan genotype were shrinkage. In addition some pollen grains at 30% PEG germinated (Fig. 1).

According to the orthogonal analysis of variance (Roshan vs. other genotypes), except of relative water content were significantly different. Comparison of means between two groups showed that Roshan had higher values of soluble sugars and K^+ accumulations under stresses (Table 1).

Grain yield of Roshan genotype were higher than others. Differences in *or* gene found in yield under stresses but at normal condition there were not significant between two groups. Standard deviation and range of variation for Roshan grain yield at three conditions (245.17 and 450 g^{-2}), had lower than other genotypes (247.46 and 455.60 g^{-2}). Increasing grain yield ratio for Roshan to other genotypes under drought and salinity stresses were 18.14 and 38.80%, respectively. In addition grain spike and spike m^{-1} for Roshan genotype had more values. Under salinity stress 1000-kernel weight at Roshan genotype had low value. Decreasing of 1000 kernel weight at this genotype was due to producing of more grains and reduced sterile floret at spike under stress. Except of Roshan genotype amount of Na^+ accumulation were high and inversely K^+ and K^+/Na^+ ratio had low values. Under normal condition accumulation of soluble sugars between two groups didn't significant but at drought stress in Roshan genotype was more obvious.

Differences in expressed *or* gene at pollen grains investigated in 6 winter wheat genotypes. It demonstrated by difference in relative size, after osmotic stress by using of polyethylene glycol 50% with 10 mM KCl. It suggests that *or* gene in the Roshan genotype may have high affinity potassium transport. The pollen response is suitable as a routine test for selecting such genotypes in plant breeding programs. Morgan (1999) with this method could segregate many lines for expressing this gene. Utility of the technique would depend on the existence of allelic variation. K^+ ion had a main role under osmotic stress and induced tolerance (Jiang and Huang, 2001). Morgan (1999) found that pollen expression had associated with leaf responses (accumulations of K^+ and soluble sugars) and it was similar with our studies. Roshan genotype had high value of K^+/Na^+ under salinity stress and low accumulation of Na^+ ion.

More increasing of soluble sugars, induced by drought stress, at genotypes with *or* gene correlated with osmotic adjustment and turgor maintenance (Ingram and Bartels, 1996). An additional aspect of *or* was delaying leaf rolling, therefore these genotypes were higher relative

Table 1: Comparison of means between two groups of winter wheat genotypes

Treatments	Groups	Grain yield ($g\ m^{-2}$)	1000 KW (g)	Spike (m^2)	Grain $spike^{-1}$	RWC (%)	Soluble sugars (mM)	Na^+ (mM)	K^+ (mM)	K^+/Na^+ ratio
Normal condition	Roshan	892.00	40.91	850.80	60.28**	80.74	9.05	0.37	11.11*	34.74
	Others	772.80	43.10	764.52	48.70	75.65	9.48	0.43	9.15	22.63
Drought stress	Roshan	836.00**	35.51	664.00*	48.28**	74.41	14.95*			
	Others	707.60	36.26	557.32	36.96	70.83	13.32			
Salinity stress	Roshan	440.00**	26.44	390.80	43.12*			1.33	9.16*	6.96*
	Others	317.00	33.77**	358.36	34.83			2.45**	8.17	3.83

* and **: Indicate significant differences at probability levels of 0.05 and 0.01, respectively

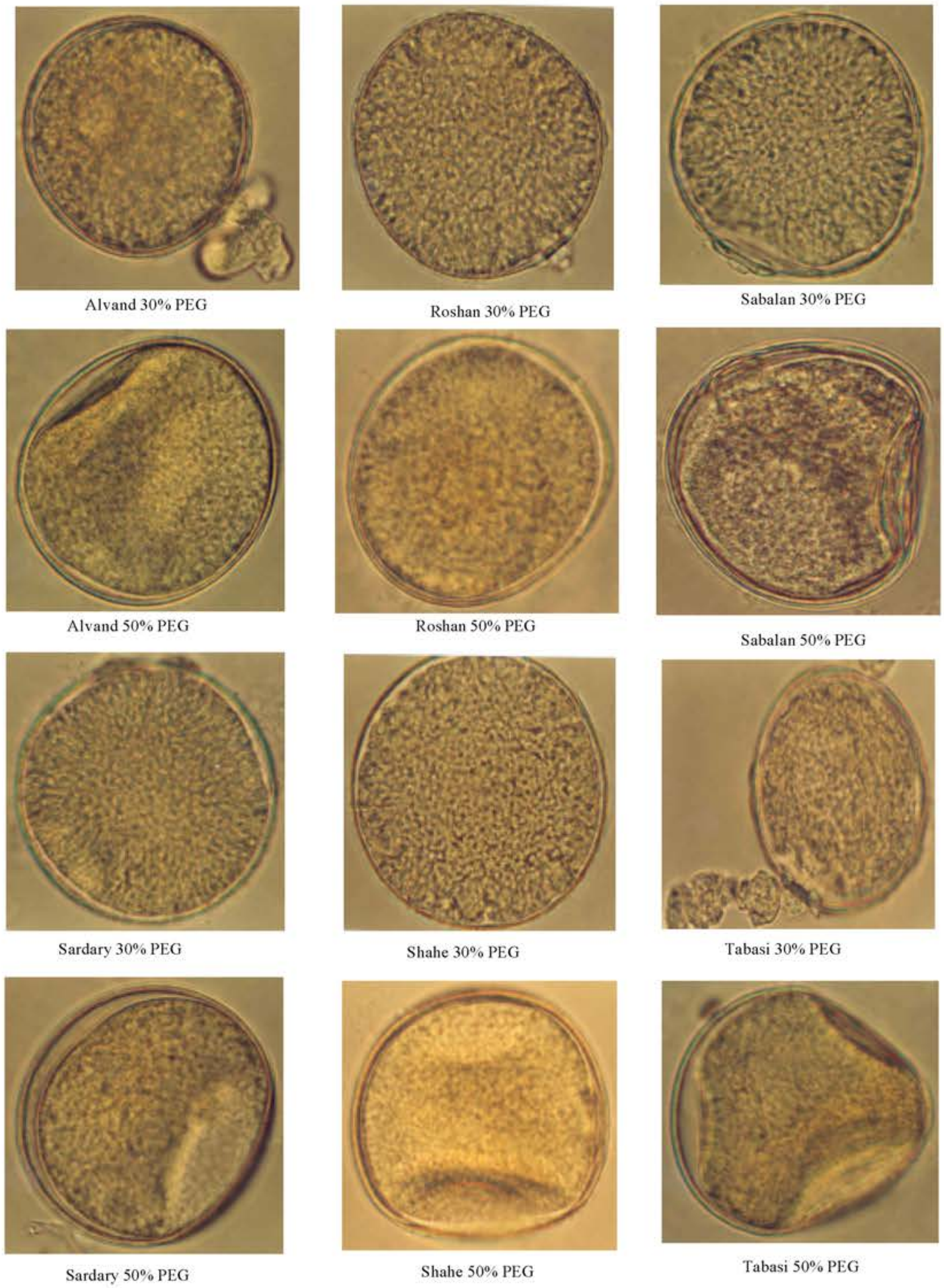

Fig. 1: Wheat pollen grains of Alvand, Roshan, Sabalan, Sardary, Shahe and Tabasi at polyethelon glycol (PEG) 30 and 50% with 10 mM KCl . Pollen grains of all genotypes at 30% PEG were swollen and at 50% PEG with 10 mM KCl except of Roshan were shrunken

water content and it was one of the important response at production processes. In this study Roshan had high relative water content but did not significant.

Screening genotypes for high osmoregulation using the pollen grain technique increased grain yields under stresses. Increasing of grain yield, At Morgan *et al*. (1986) results were found 7 and 11% for durum and bread wheat genotypes, respectively.

Increasing of grain yield at genotypes with high *or* could be justified as follow: Genotypes with *or* gene produced more grain yield due to decreasing number of sterile spikelet under stress. Maintenance of supply assimilations at grain filling period due to delaying leaf desiccation percent and not leaf rolling that caused maintenance photosynthetic activity. Increasing retranslocation of assimilates to grains at filling period and opening of stomatal and gas exchange at genotype with *or* gene caused the yield was increased under stresses. Tangpremsri *et al*. (1995) at evaluating of sorghum lines to drought stress found that with adopting genotypes with high *or* capacity effects of injurious stresses diminished. They expressed the most profit of this gene prevent of damaging effects of stresses on evolution and growth spike.

Genotype with high *or* was low 1000 kernel weight due to high seed set. In our studying Roshan genotype had the low value of 1000 kernel weight at three conditions. Morgan (1980) reported that under stress endogenous ABA increased and distorted pollen grains devoid of starch granules and anthers, which were reduced in size, pale yellow and often shriveled, Furthermore, drought stress produced a few tillers, which failed to develop beyond the early boot swell stage and at final reduced seed set. Subbarao *et al*. (2000) found a negative relationship between osmotic adjustment and grain yield under drought stress in pigeopea.

In conclusion, with enhancing drought and salinity stresses, it related to identification of *or* alleles and selection based on it in wheat breeding programs is strongly recommended. K^+ and soluble sugars were the major solutes to contributing *or* in wheat genotypes, therefore application of exogenous compatible solutes (such as K+), under stress not only increased tolerance of plant, but also it seems that had beneficial effects on quality of production.

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