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Use of water use efficiency and water stress tolerance indices for the comparing of bread wheat (*Triticum aestivum*) genotypes

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ABSTRACT

Bread wheat (*Triticum aestivum* L.) is one of the most important cereals worldwide and is being grown in many areas of the world. However, there is a lack of information about using the proper stress index to determine the tolerance of wheat cultivars to water stress. A two-year field study was conducted with the objective of determining the effect of different rates of nitrogen (N) (control, 80 kg N/ha, and 160 kg N/ha) and two irrigation treatments (non stressed and post anthesis water stressed) on three bread wheat cultivars. Water deficit markedly affected grain yield, straw yield, and harvest index. Under water deficit grain yield was reduced by 58%, showing that wheat cultivars were sensitive to drought stress. Tolerance (TOL) index was lower under water deficit than well watered (WW) conditions in both years. Stress Tolerance Index (STI), Mean Productivity (MP), and TOL indices were efficient in evaluating the sensitivity to drought stress. Grain yield under stress condition showed positive correlation with STI, MP and TOL indices. Water use efficiency and irrigation water use efficiency increased with increasing the N rates in WW treatment but decreased in WD treatment. In general, for the most efficient use on both N and water, the supply of one should be adjusted to that of the other. If irrigation is for maximum yields, fertilization should be too; but if irrigation is limited, the fertilizer supply should be adjusted accordingly to prevent N loss and pollution of the environment.

Key words: Nitrogen, Stress tolerance indices, Water deficit, Wheat, WUE

Drought limits agricultural production in many areas of the world affecting many crops and especially wheat (*Triticum aestivum* L.) which is one of the most important crops worldwide. Water stress can occur at different growing stages of wheat plants but the most important is during grain filling stage (Blum 1998). In many Mediterranean type climates such as the ones that exists in many areas of Iran, rainfall decreases and evaporation and temperature increases in spring, when wheat enters the grain filling stage (Ahmadi and Baker 2001, Fathi 2005). Consequently, wheat plants often experience severe water deficit during grain filling, causing significant decrease in grain yield (Spiertz *et al.* 2006, Barnabas *et al.* 2008, Blum 1998).

The response of different genotypes in stress and non-stress environments was described by Fernandez (1992) who proposed four types of responses for genotypes: 1- Genotypes with similar response in stress and non stress conditions (Class A), 2- Genotypes with suitable response in non-stress conditions (Class B), 3- Genotypes with high yield in stress condition (Class C), 4- Genotypes with poor response in both conditions (Class D). Fernandez (1992) stated that the best standard selection for stress can recognize the class A group than the other groups.

A number of different indices were also developed to determine the stress sensitivity of the different genotypes. Fischer and Maurer (1978) proposed the stress susceptibility index (SSI) and when SSI is < 1 indicates higher tolerance to drought. Relative tolerance or susceptibility of different cultivars can be determined with comparison of their SSI (Andarzian 2002). On the basis of SSI A and B class genotypes can be distinguished more than other groups. Therefore, this index cannot separate genotypes of B and C groups and includes them in the same group. Another index which was introduced by Rosielle and Hamblin (1981) was the tolerance index (TOL) and the mean productivity (MP) index which are the yield difference and mean productivity in stress and non-stress conditions, respectively. High values of TOL show higher sensitivity to drought and therefore, lower rates are

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favorable. In addition, more tolerant cultivars have higher MP rates and using the classification by Fernandez's equation we can separate B and C genotypes groups using MP and TOL indices. However, separation of C group from a group is impossible with these indices. Therefore, Fernandez (1992) suggested stress tolerance index (STI) in order to distinguish the genotypes with high yield both under stress and non-stress conditions. Higher values of STI for a genotype show higher tolerance to drought and more yield potential (Fernandez 1992). In addition, STI can be used to distinguish a group genotypes from B and C groups, and STI is considered as superior index than TOL and MP indices. Fernandez (1992) also introduced another index the geometrical mean productivity (GMP). Those indices showing high correlation with grain yield were introduced as the best indices because of their ability to separate genotypes with high yield, both in stress and non-stress conditions.

In Mediterranean environments, where crop canopy development in winter is slow and rain occurs as frequent and small events, soil water evaporation may account for 30 to 60% of seasonal ET (Cooper *et al.* 1983, French and Schultz 1984, Siddique *et al.* 1990, Zhang *et al.* 1998). Thus, agronomic practices that reduce soil water evaporation via a larger plant canopy and early ground cover and at the same time increase the crop's ability to extract soil water may increase the amount of water transpired and, consequently, WUE. Nitrogen deficiency is another major constraint in canopy development in the Mediterranean region (Anderson 1985). Crop responses to N fertilization depend on the level of water availability (Pala *et al.* 1996). Application of fertilizers not only increases plant shoot and root growth (Brown *et al.* 1987), but also increases ET through a larger root system and greater extraction of stored water (Cooper *et al.* 1987). In addition, a large and earlier canopy cover resulting from the application of N can reduce soil water evaporation and increase crop WUE (Zhang *et al.* 1998). In a study in Kentucky Corak *et al.* (1991) found that WUE increased with the addition of N fertilizer and the use hairy vetch (V*isia villosa* Roth) residues.

Wheat grown in the Mediterranean climates commonly is in the grain filling period when there is soil water deficit which restricts N uptake from the soil. The objectives of this study were (i) to compare response of grain yield, water use, and water use efficiency of three wheat cultivars to different levels of nitrogen and post anthesis water deficit and (ii) to compare the different tolerance indices to water stress using three wheat cultivars to post anthesis water deficit.

MATERIALS AND METHODS

The experiment were conducted at Shiraz Agricultural Research Station, Iran (52°, 36´ E, 29° 33´*N*) during the growing seasons of 2006-2007 and 2007-2008. The different cultivars that were used to determine their response to N fertilization and to water stress were as follows: Shiraz (early maturity), Marvdasht (middle-maturity), and Chamran (middle-maturity). The soil was sampled pre-planting at a depth of 30 cm and before the application of the fertilizers. The soil characteristics were determined according to methods detailed in the Shiraz Soil Testing Laboratory. Briefly, the soil contained 3.2 g/kg organic matter, 0.3 mg/kg N, 11 mg/kg P and 270 mg/kg exchangeable K and had a pH 7.9. Weather data (i.e. rainfall and average temperature) were recorded daily in the experimental site and reported as mean monthly data for the two years of the study together with the thirty-year averages for temperature and rainfall (data not shown).

The experimental design was split-split plot with the irrigation treatments as the main plots, the fertilizer treatments as the split plot and the cultivars as the split-split plots with four replications. The experimental plots were 8 by 1.5 m with 6 rows 0.2 m apart. The irrigation treatments were I1 (non-stressed) and I2 (Post anthesis water stressed plots with 65% FC). The fertilization treatments were as follows: 0, 80, and 180 kg N/ha were applied (pre-planting) in the form of $(NH_4)_2SO_4$ (N-P-K, 20.5-0-0). The N rates that were selected were those that are used in many other countries with similar climate and after many years of experimentation and the recommendation according to soil available N. In addition, P and K were applied at a rate of 60 kg/ha and 100 kg/ha (pre-planting) in the form of superphosphate and K_2SO_4 respectively, following the recommendation of the data of the soil analysis and were incorporated in the soil before sowing. Wheat cultivars were sown on 11 November 2006 and 14 November 2007. Irrigation of each main plot was measured volumetrically by field calibrated gypsum block. Six gypsum blocks were installed in each replication randomly. Measurements were made after anthesis, before each irrigation, during the growing period. The change in soil moisture was measured weekly to a depth of 30 cm. A drip irrigation system was used and was operated avoiding runoff losses.

The following variables were determined: total aboveground biomass at heading was determined by cutting at ground level in 0.3 m^2 quadrants per plot (Zadoks growth stage 60). Following at harvest (Zadoks growth stage 95) the central four rows from each plot were harvested for grain yield and the total biomass was weighted. Harvest index (HI) was determined by dividing grain yield by the total biomass. All samples were dried in an air-forced oven at 70°C for 48 hr.

A number of different drought stress indices were determined to evaluate their use in the selection of wheat cultivars:

Stress susceptibility index (SSI) calculated with the equation described by Fisher and Maurer (1978):

$$SSI = \frac{1-(Ys/Yp)}{SI},\ SI = 1-\left(\frac{\overline{Ys}}{\overline{Yp}}\right)$$

where SI is stress intensity, Yp, potential yield of each genotype in non-stress condition; Ys, potential yield of each genotype in stress condition; Yp, mean yield of all genotypes in non stress condition. Ys, mean yield of all genotypes in stress condition. Tolerance index (TOL) and mean productivity (MP) index which are the yield difference and mean productivity in stress and non-stress conditions, respectively were calculated according to Rosielle and Hambelin (1981).

$$TOL = Yp - Ys,\ Mp = \frac{Yp + Ys}{2}$$

Stress tolerance index (STI) was calculated according to Fernandez (1992)

$$STI = \left(\frac{Yp}{\overline{Yp}}\right)\left(\frac{Ys}{\overline{Ys}}\right)\left(\frac{\overline{Ys}}{\overline{Yp}}\right) = \frac{(Yp)(Ys)}{(\overline{Yp})^2}$$

Geometrical mean productivity (GMP) was calculated according to Fernandez (1992)

$$GMP = \sqrt{(Ys)(Yp)}$$

Water use efficiency (WUE) = Y/ET was calculated according to Aase and Pikul (1995):
Where Y is the yield of the crop, either in total harvestable biomass or marketed yield, and ET is evapotranspiration of water from the soil surface, plant leaves, and through the stomata (transpiration).
Evapotranspiration was determined according to the equation:

$$ETP_{crop} = ETP_{pan} \times K_c$$

where ETP_{crop}, ETP_{pan} and K_c are crop evapotranspiration, Pan evaporation and crop coefficient, respectively.

The data were analyzed by the ANOVA method according to a factorial design with four replications per treatment combination. More specifically, the experiment was set up as a Randomized Complete Block Design for the irrigation treatments (main plots), with N levels as split plots and cultivars as split-split plots. A combined analysis over growing season was carried out according to the aforementioned design Steel *et al.* (1997). Duncan's multiple range test (DMRT) was used for testing the differences between treatment means. The significance level of all hypotheses testing was preset at $P<0.05$. All statistical analyses were performed using the SAS (SAS Institute 1995).

RESULTS AND DISCUSSION

Water deficit affected grain yield, straw yield and harvest index (Table 1). Under water deficit grain yield was reduced by 58%, showing the sensitivity of wheat to drought stress conditions (Table 2). The reduction was because there was a reduction in grain size, but not in grain number (data not shown). The cultivars showed differences in grain yield in the different treatments (Table 2) showing that yield potential of the cultivars was different in well watered and stress condition. TOL index was lower for Chamran than the other cultivars in both years (Table 2), indicating that Chamran

Table 1 Results of analysis of variance combined across years, Irrigation, fertilizer and wheat cultivars to both years

Sources	df	Grain yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)
Year (Y)	1	**	**	NS
Water stress (S)	1	*	*	*
Y×S	1	NS	NS	NS
Cultivar (C)	2	*	**	*
Y×C	2	NS	NS	NS
S×C	2	*	*	NS
Y×S×C	2	NS	NS	NS
CV, %		13	16	17

*,** Significant at 0.05 and 0.01 probability levels, respectively. NS, nonsignificant at P > 0.05.

Table 2 Stress susceptibility indices of the different cultivars used in this study and water deficit treatment for grain yield during the two growing seasons (2006-2007 and 2007-2008)

Cultivars	Grain yield (kg/ha)			Stress tolerance indices			
	Yp	Ys	TOL	MP	SSI	STI	GMP
				2006-2007			
Shiraz	8 857	3 253	5 503	6 053	1.07	0.50	5 380
Marvdasht	7 482	3 170	4 211	5 326	0.97	0.40	4 879
Chamran	6 649	3 066	3 482	4 858	0.91	0.34	4 523
Mean	7 662	3 163	4 398	5 412	0.98	0.41	4 927
LSD	1 021	316	439	636	0.08	0.04	418
				2007-2008			
Shiraz	7 066	2 582	4 383	4 824	1.06	0.49	4 284
Marvdasht	5 966	2 515	3 349	4 241	0.97	0.40	3 884
Chamran	5 300	2 432	2 766	3 866	0.91	0.34	3 597
Mean	6 110	2 509	3 499	4 310	0.98	0.41	3 921
LSD	678	334	507	763	0.09	0.04	522

was more tolerant to drought stress than Marvdasht and Shiraz. Between cultivars MP index was higher for Shiraz than the other cultivars.

The highest SSI value obtained in WD treatment (Table 2). Therefore, SSI like MP and TOL showed to be suitable in evaluating the sensitivity to drought stress. Chamran showed the lowest SSI index compared with the other cultivars and had the lowest sensitivity to drought stress in both years. Therefore, it can be said that this index was not efficient in the separation of tolerant cultivars to drought stress.

Since there was a significant interaction between cultivar and water regime (Table 1) it was expected that the different cultivars can show different sensitivity to water stress, which was not observed in other indices. In addition, the use of STI showed that there was a different response in the different cultivars. As, in TOL and SSI indices Chamran showed lower sensitivity but using the STI and MP indices this cultivar showed to be more sensitive to drought stress. Therefore, STI was more appropriate for the selection of cultivars to drought tolerance. The results of GMP were similar to STI. Therefore, it can be concluded that STI and GMP indices are more important for the selection of tolerant cultivars under drought condition.

Grain yield in stress condition showed positive correlation with STI, MP and TOL indices (Table 3). The highest correlation was between grain yield in stress condition and STI index. In addition, the high correlation between TOL and MP, with grain yield under stress condition, indicates that tolerant cultivars can be found with these indices. Also, it was observed that there was a relationship among GMP, STI and MP. However, STI is more effective than the other indices in separation of stable and more productive cultivars.

Water use efficiency: Water use efficiency was increased with increasing the N rates in WW treatments but decreased in WD treatments (Table 4). On WW treatments the 160 kg N/ha rate was sufficient for maximum WUE while the 80 kg N/ha rate was sufficient for WD treatment. On WD treatment the N fertilizer treatment did not affect WUE in 2007-2008; but in 2006-2007, when there was a trend towards increasing grain yield up to 160 kg N/ha treatment, this treatment resulted in WUE significantly higher than that of the 0-N treatment. In 2006-2007, on adequately fertilized treatments, WUE was highest on WW and lowest on WD treatment. In 2007-2008, however, efficiencies were highest on WD treatment and lowest on WW treatment. The differences in WUEs in WD treatment between the two growing seasons resulted from less water use on this treatment in 2007-2008 than in 2006-2007. The higher WUE in 2006-2007 compared with the 2007-2008 may have been at least partially due to differences in temperature and rainfall distribution during the two seasons. Temperatures during grain filling and maturity periods were above normal in 2007-2008 and below normal in 2006-2007. The cooler, moist conditions in 2006-2007 allowed normal grain filling and maturity on WD treatment and reduced the effects of stress during that period. Drought conditions hastened maturity on this treatment in 2007-2008, resulting in reduced grain and test weights and, consequently, lower WUE. WUE for Chamran increased when soil moisture and/ or N were in limited supply during the grain filling period. When N and soil moisture were probably abundantly available, no significant difference in WUE were observed between Shiraz and Marvdasht.

Table 3 Correlation coefficients of stress tolerance indices with grain yield under normal and stress condition

	STI	SSI	MP	TOL	Ys	Yp
SSI	0.39 *					
MP	0.95**	0.55*				
TOL	–0.44*	0.52*	–0.19 NS			
Ys	0.90**	0.09 NS	0.78**	–0.76**		
Yp	0.39*	0.89**	0.61**	0.59*	0.08NS	
GMP	0.98**	0.34 NS	0.95**	0.49*	0.90**	0.32 NS

*,** Significant at 0.05 and 0.01 probability levels, respectively.

Irrigation water use efficiency: Irrigation water use efficiency increased with increasing the N rates up to 160 kg N/ha on WW treatment in 2006-2007 (Table 4). In WD treatment, applied N had little effect on yield, because the soil supplied enough N for yields as high as water supply would allow. Without applied N, N was limiting when water was applied. Both applied N and water were required for substantial yield increases from either variable. For the most efficient use on both N and water, the supply of one should be adjusted to that of the other. If irrigation is for maximum yields, fertilization should be too; but if irrigation is limited, the fertilizer supply should be limited accordingly.

Post anthesis water stress affected grain yield, straw yield and harvest index which agree with several other studies where the effect of water stress was studied and was also similar with the water stress applied during the growing period of the crop (Nouri et al., 2011). One of the objectives of the present study was to determine the effect of post anthesis water stress on the different stress tolerance indices that have been proposed to describe the behavior of a given genotype under stressed and non-stressed conditions while most of the previous studies focus on water stress during the growth period of the crop (Mohammadi *et al.* 2010, Nouri *et al.* 2011).

On the basis of TOL more tolerant cultivars show low values of this index (Fischer and Wood 1979). TOL index was lower for Chamran than the other cultivars in both years, indicating that Chamran was more tolerant than the other cultivars. On the other hand MP index showed that Shiraz had lower sensitivity to water deficit compared with Marvdasht and Chamran. Therefore, the MP index is better than TOL for evaluating tolerant cultivars to water stress as suggested by others (Sanjari *et al.* 2005). SSI index was lower in Charman compared with the other two cultivars.

Table 4 Water use, water use efficiency, and irrigation water use efficiency of three wheat cultivars as affected by N fertilizer and irrigation treatment in 2006-2008

Treatment		Water use Irrigation treatment		Water use efficiency Irrigation treatment			Irrigation water use efficiency Irrigation treatment		
Nitrogen (kg/ha)	cultivars	WW	WD	WW	WD	Avg.	WW	WD	Avg.
		mm		kg grain/m					
				2006-2007					
0	Shiraz			0.56	0.51	0.53	0.20	0.12	0.16
	Marvdasht			0.58	0.57	0.57	0.24	0.15	0.20
	Chamran			0.65	0.61	0.63	0.28	0.21	0.25
	Avg.	562	364	0.59	0.56		0.24	0.16	
80	Shiraz			0.70	0.54	0.62	0.70	0.50	0.60
	Marvdasht			0.76	0.60	0.68	0.74	0.52	0.63
	Chamran			0.85	0.64	0.74	0.84	0.61	0.73
	Avg.	631	400	0.77	0.59		0.76	0.54	
160	Shiraz			0.81	0.49	0.65	0.90	0.70	0.80
	Marvdasht			0.90	0.55	0.72	0.95	0.75	0.85
	Chamran			0.99	0.59	0.79	1.18	0.80	0.99
	Avg.	701	490	0.90	0.54		1.01	0.75	
LSD (0.5)				0.16	0.10		0.13	0.11	
				2007-2008					
0	Shiraz			0.45	0.40	0.48	-0.03	-0.13	-0.06
	Marvdasht			0.48	0.44	0.46	0.10	0.02	0.05
	Chamran			0.55	0.50	0.53	0.18	0.10	0.14
	Avg.	620	320	0.49	0.45		0.10	0.01	
80	Shiraz			0.67	0.59	0.63	0.25	0.09	0.12
	Marvdasht			0.70	0.63	0.67	0.29	0.13	0.21
	Chamran			0.80	0.71	0.76	0.35	0.20	0.28
	Avg.	634	394	0.72	0.64		0.30	0.11	
160	Shiraz			0.74	0.60	0.67	0.59	0.39	0.49
	Marvdasht			0.80	0.65	0.73	0.67	0.56	0.61
	Chamran			0.92	0.72	0.82	0.98	0.71	0.85
	Avg.	719	400	0.82	0.66		0.75	0.55	
LSD (0.5)				0.15	0.12		0.07	0.09	

*WW, Well watered treatment and WD, post anthesis water deficit; *, ** significant at 0.05 and 0.01 probability levels, respectively.

Also, SSI was not an efficient index which can be used to separate tolerant cultivars to drought stress. In agreement with these findings Moghadam and Hadi Zadeh (2002) reported that SSI index did not show great efficiency for the selection of tolerant cultivars and it must be used only when severe stress occurs. In other words, the SSI index must be used for determining the sensitive cultivars and not for the selection of tolerant cultivars to stress. The index GMP showed similar response with STI which indicates that STI and GMP indices are more important for the selection of tolerant cultivars under drought condition. These results are similar to the results from other studies (Moghadam and Hadizadeh 2002, Sanjari *et al.* 2005, Samizadeh 1997, Mozafari 1996, Nouri *et al.* 2011). Nikhkhah (1999) also introduced GMP, MP and STI as the best indices for the selection of tolerant genotypes under stress condition which was confirmed by other authors (Nouri *et al.* 2011, Sio-Se Mardeh *et al.* 2006).

Grain yield in stress condition showed positive correlation with STI, MP and TOL indices. In addition, it was observed that there was a relationship among GMP, STI and MP. Similarly, Fernandez (1992) found correlation between STI and GMP. Since, there was a significant correlation among GMP, STI, and MP these indices can be used for the selection of tolerant cultivars to drought and STI and MP are more efficient (Mohamadi *et al.* 2005, 2010; Sanjari *et al.* 2005, Nouri *et al.* 2011). Moghadam and Hadizadeh (2002) reported that STI was correlated with grain yield under both stress and optimum conditions (0.96 in stress and 0.5 in non stress condition). Tari Nezhad (1999) also found that there was no correlation between TOL and GMP and the use of MP is more effective than TOL in the selection of tolerant genotypes. However, STI is more effective than the other indices in separation of stable and more productive cultivars. Nik Khah (1999) also introduced GMP, MP and STI as the best indices for finding tolerant genotypes under drought stress condition and also reported that these indices have positive and significant correlation with grain yield in stress and non-stress conditions. However, Hohls (2001) proposed that MP cannot be used to select high yielding genotypes in both stressed and non-stressed environments, if the correlation of grain yield in contrasting environments is highly negative. Also MP is related to yield under drought stress if it is not too severe and the difference between YR and YI is not too large.

Water use efficiency: Water use efficiency was increased with increasing the N rates in WW treatments but decreased in WD treatments. According to several researchers (van Herwaarden *et al.* 1988, Angus and Herwaarden 2001, Halvorson *et al.* 2004), N fertilization can improve WUE; however, high N levels can reduce yields through "haying off" due to excess WU in the preanthesis period, leaving insufficient water for the postanthesis grain filling period. It is therefore important to balance N fertilization with the available seasonal water supply. Van den Boogaard (1996) and El Hafid *et al*. (1998) concluded that drought did not increase WUE.

Irrigation water use efficiency: Irrigation water use efficiency increased with increasing the N rates up to 160 kg N/ha on WW treatment in 2006-2007. In WD treatment, applied N had little effect on yield, because the soil supplied enough N for yields as high as water supply would allow. For the most efficient use on both N and water, the supply of one should be adjusted to that of the other. If irrigation is for maximum yields, fertilization should be too; but if irrigation is limited, the fertilizer supply should be limited accordingly.

In conclusion, GMP, STI, and MP were better indices for the distinction of the tolerance of the different cultivars to drought. Also grain yield under stress condition showed positive correlation with STI, MP, and TOL indices. Thus the different tolerance indices can be classified into distinct groups considering different concepts of drought tolerance, resistance and susceptibility under mild drought stress. Water use efficiency and irrigation water use efficiency increased with increasing the N rates in WW treatment but decreased in WD treatment. In general, for the most efficient use on both N and water, the supply of one should be adjusted to that of the other. If irrigation is for maximum yields, fertilization should be too; but if irrigation is limited, the fertilizer supply should be adjusted accordingly to prevent N loss and pollution of the environment.

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