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Full Length Research Paper

Evaluating spring wheat cultivars for drought tolerance through yield and physiological parameters at booting and anthesis

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Progress in wheat yields under drought conditions is rather a difficult task to achieve. The experiment was conducted in factorial design with 16 spring wheat cultivars grown under two irrigation regimes, non-stress and water-stress imposed at boot and anthesis growth stages. Water-stress significantly influenced the physiological and yield traits in both the growth stages, yet the reductions in most traits were pronounced at anthesis than at boot. Stomatal conductance, relative water content, leaf area (LA), seeds/spike, 1000-grain weight and grain yield/plant were the best drought tolerant indicators. On the basis of physiological and yield traits, the cultivars Moomal, Bhitai, TD-1, and Abadgar proved to be the best performing in water-stress conditions. Stomatal conductance, RWC% and LA were significantly and positively correlated with grain yield/plant. These results suggest that the stomatal conductance, relative water content and leaf area are the most important traits that should be considered while developing drought tolerant wheat genotypes.

Key words: Water stress, boot and anthesis, yield and physiological traits, wheat genotypes.

INTRODUCTION

Although, breeders are continuing to improve the yield potential of wheat, however, progress in increasing wheat yields in drought environments has been more difficult to achieve than many breeding objectives (Blum, 1988). In defining a strategy for wheat breeding under drought stress, Rajaram et al. (1996) suggested that simultaneous evaluation of the germplasm should be carried-out both under near optimum conditions and under stress conditions. Several studies have been conducted where yield was greatly reduced mostly when drought stress occurred during the heading or flowering stages. While drought stress during maturity resulted in about 10% decrease in yield, moderate stress during the early vegetative period had essentially no effect on yield (Bauder, 2001). Pireivatlou and Yazdansepas (2008) and Plaut et al. (2004) evaluated the responses of yield and yield components of 24 advanced bread wheat genotypes to pre-and post-anthesis drought stress conditions and recorded significantly lower spikes numbers, seeds numbers per spike and grain yield under pre-anthesis than in post-anthesis drought stress conditions. Grain filling is maintained by high contribution of assimilates before and immediately after anthesis and remobilization of vegetative reserves during kernel growth (Royo et al., 1999). The growth period most sensitive to drought stress with respect to grain yield would be from double ridge to anthesis due to its negative impact on spikelet numbers and kernels per spike (Shpiler and Blum, 1991). In the same way, water-deficit around anthesis may lead to a loss in yield by reducing spike length and spikelet number (Giunta et al.,

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1993). Besides, drought stress from anthesis to maturity hastens leaf senescence, consequently reduces the duration and rate of grain filling, thus reduces mean kernel weight (Royo et al., 2000) and kernels per spike (Giunta et al., 1993; Zhong-hu and Rajaram,1994; Garcia-del-Moral, 2003). The successful development of cultivars for water-limited environments would involve selection and incorporation of both physiological and morphological mechanisms of drought resistance through traditional breeding programmes (Rauf et al., 2007). However, physiological changes have been considered important criteria for yield progress as breeders and physiologists regularly select for desirable expression of these traits to maintain adaptation and optimal yield of crops in water-stress environments (Barodi et al. 2008 and Richards, 2006). The main objectives of the present study therefore were: (1) to identify the high yielding genotypes tolerant to water stress at boot and anthesis growth stages, (2) to identify morpho-physiological traits to serve as best selection indicators of drought tolerance in spring wheat genotypes, and (3) to work-out the correlations between morphological and physiological traits.

MATERIALS AND METHODS

Sixteen spring wheat genotypes (*Triticum aestivum* L.), with diverse characteristics viz: Anmol, Inqilab, Moomal, TJ-83, Sarsabz, Khirman, SKD-1, TD-1, Kiran, Abadgar, Marvi, Mehran, Bhitai, ZA-77, Pavan, and Imdad were studied. All these varieties are very popular and are cultivated on a wide area of Pakistan. Moreover, these varieties were almost similar in height and earliness. The plastic pots with a capacity of accommodating 14 kg soil were used in this study. Initially, six seeds were sown to each pot. Afterwards, only three plants were maintained to record various observations. The plants were thinned to three per pot to take final observations. The experiment was laid-out with three repeats in randomized complete blocks with factorial arrangement in the greenhouse of the University of Reading, U.K. during 2009. The treatments were two irrigation regimes and considered as main factor while varieties as sub-factor. The control received frequent irrigations without any water stress whereas in the second water regime, plants were stressed at both boot (20 day stress) and at anthesis (20 day stress) growth stages. Thus, watering of stressed plants was stopped at about 40 days after sowing (considered stress at boot) and were re-watered when 80% of the plants reached boot stage. After 75 days of sowing, the stress at anthesis was imposed by withholding water from initiation of flowering to the start of grain formation.

The physiological observations in stress were taken at both the stress stages (boot and anthesis) before watering the plants while most of the growth and developmental data were recorded at post-anthesis and maturity stages. Physiological measurements were made on fully expanded leaf second to flag leaf. Leaf area (cm^{-2}) was measured by Portable Delta-T leaf area meter. Stomatal conductance (mmol m^{-2} s^{-1}) was measured by Porometer AP4, Delta Devices, Cambridge, U.K. Leaf relative water content (RWC %) was calculated according to Schonfeld et al. (1988) with Formula as: RWC% = (fresh weight- dry weight)/ (turgid weight- dry weight) x 100. The statistical analysis, least significant differences between means and correlation coefficients were calculated by using Gentstat. software, 11th edition.

RESULTS

Effect of water stress on physiological traits at boot and anthesis growth stages

Mean squares for treatments, varieties and their interactions were significant for all the physiological traits (Table 1). On average, water-stress caused large reductions in stomatal conductance (mmol $m^{-2}s^{-1}$), nonetheless, declines were slightly higher at boot (86.4%) than at anthesis (83.3%) (Table 2). Since treatment x variety interaction was significant, so the rank order of varieties in water stress at boot was not exactly the same as in anthesis stage. However, varieties Inqilab, TJ-83 and Pavan gave relatively higher stomatal resistance (less conductance) in water-stress conditions at both the growth stages. Relative water content (RWC), though decreased due to water stress at both the stages; yet the decline was much higher at anthesis (14.9%) against boot stage (6.6%). Varieties Moomal, Bhitai, and Pavan maintained higher RWC% at both boot and anthesis with their corresponding values being 88.8, 71.8, 92.8% and 77.5, 84.8 and 69.5% respectively. The reduction in leaf area (LA) was also substantial at both boot and at anthesis. The average LA in non-stress and water stress at boot and anthesis were 25.5 and 20.5 cm^2 and 22.5 and 14.5 cm^2, respectively. However, on average, water stress caused significant reductions of 11.8 and 29.3% cm^2 in LA at boot and anthesis stages respectively (Table 2). Very interestingly in non-stress conditions, cv. Bhitai and Moomal which gave 35.8 and 27.9 cm^2 LA respectively had broader leaves, thus maintained the same rankings in stress conditions at both the growth stages (Table 2)

Effect of water stress on yield traits imposed at boot and anthesis stages

For yield traits, the mean squares for water stress treatments, varieties and treatment x variety interactions were all significant at boot and at anthesis stages except that interactions were non-significant for grain yield per plant (Table 1). The effects of stress were substantial at anthesis than at boot as revealed by the averages. Water stress caused significant effect on yield traits and the varieties also showed significant variability for tillers/plant, seeds/spike, grain yield/plant and 1000-grain weight. Significant variation due to genotypes for almost all the characters suggested that the magnitude of differences in genotypes was sufficient to provide some scope for selecting the traits for improving drought tolerance of wheat genotypes. Water-stress caused significant reductions in tiller numbers, yet the average tillers in non-

Table 1. Mean squares from analysis of variance for morpho-physiological traits of spring wheat cultivars under moisture-stress at boot and anthesis growth stages.

Trait	Stress			
	Treatment (T) D.F. =1	Variety (V) D.F.=15	T x V D.F.=15	Error D.F.= 62
I. Stress at boot stage				
Physiological traits at boot stage				
Stomatal conductance	623152.00**	9576.00**	11120.00**	1834.00
Relative water content	437.98**	124.55**	146.72**	23.78
Leaf area	140.52**	61.70**	10.22**	3.91
II. Stress at anthesis stage				
Physiological traits at anthesis stage				
Stomatal conductance	45587.32**	570.21**	679.80**	33.31
Relative water content	1675.09**	124.85**	146.72**	23.78
Leaf area	569.021**	61.701**	10.217**	3.914
Yield traits after combined stresses				
Tillers per plant	6.674**	1.135**	0.381**	0.158
Seeds/spike	4539.39**	89.25**	40.11**	13.58
Grain yield/plant	98.754**	0.335**	0.128	0.128
1000-seed weight	60.795**	190.560**	26.178**	5.341

**,*, = Significance at 1 and 5% probability levels respectively.

stress and water-stress were 3.01 and 2.37 respectively. Thus, water stress caused 45.2% decline in seeds/spike, yet in non-stress and in stress conditions, the rank order of cvs. Bhitai and Moomal were similar, hence showing drought tolerance (Table 3). A sizeable decrease in grain yield/plant was noticed due to water stress. On average, grain yield declined by 61.5% in water stress (Table 3).

It is very surprising, yet interesting to note that 1000-grain weight (g) increased rather declined in water stress conditions (Table 3). This unusual trend is rather hard to explain but our visual observation clearly showed very high leaf senescence in non-stress treatment at grain filling, hence declined 1000-grain weight. Nonetheless, 1000-grain weight of cvs. Abadgar, Imdad, and TD-1 were higher than the other varieties in both treatments.

Correlations between physiological *vs.* morphological/yield traits

Significant and positive correlations of stomatal conductance with seeds per spike (r = 0.82**) and grain yield per plant (r=0.78**); RWC with seeds per spike (0.45**) and grain yield per plant (r = 0.47**) and LA with seeds per spike (0.51**) and grain yield per plant (r = 0.56**) were recorded (Table 4). These positive correlations suggest that when stomatal conductance, RWC and LA increased, they caused corresponding increase in yield traits also. However, none of the physiological attributes were significantly correlated with 1000-grain weight. Among the yield traits, tillers/plant, seeds/spike, grain yield per plant and 1000-grain weight were positively correlated except that 1000-grain weight was negatively correlated with seeds per spike.

DISCUSSION

Effect of water stress on physiological parameters at boot and anthesis growth stages

Drought stress significantly reduced stomatal conductance of wheat varieties. This decline was more prominent at boot than at anthesis. The significant treatment x variety interaction reflected differential behavior of wheat varieties at different growth stage. The varieties Inqilab, TJ-83 and Pavan had the highest stomatal resistance under drought stress. Earlier workers also reported higher leaf diffusive resistance under drought condition at anthesis compared to booting (Gupta et al., 2001). Relative water content is an important indicator of leaf water stress (Merah, 2001) that is closely related to cell volume and it closely reflects the balance between leaf water supply and transpiration rate

Table 2. Mean performance of physiological traits of wheat varieties grown in non-stress and water-stress conditions at boot and anthesis stages of plant development.

Cultivar	Stomatal conductance (mmol m^{-2} s^{-1})				Relative water content (%)				Leaf area (cm^{-2})			
	Boot stage		Anthesis stage		Boot stage		Anthesis stage		Boot stage		Anthesis stage	
	Non-stress	Water stress	Non-stress	Water stress	Non-stress	Water stress	Non-stress	Water stress	Non-stress	Water stress	Non-stress	Water stress
Anmol	167.2	22.0	25.0	5.5	77.5	70.9	67.5	55.9	26.8	25.5	21.8	17.5
Inqilab	257.0	19.9	53.5	3.7	73.1	81.4	63.1	66.4	24.8	19.3	19.8	11.3
Moomal	326.2	7.5	43.0	16.3	75.2	86.8	65.2	71.8	27.9	27.4	22.9	19.4
TJ-83	340.2	16.0	50.0	6.4	75.3	66.4	65.3	51.4	24.1	19.3	19.3	11.3
Sarsabz	102.5	45.6	79.0	16.6	67.5	63.8	57.5	48.8	24.3	23.7	18.6	15.7
Khirman	115.5	30.8	91.8	7.2	82.1	55.6	72.1	40.6	23.6	15.2	16.5	7.2
SKD-1	137.5	37.1	52.5	5.1	73.6	75.8	63.6	60.8	21.5	25.3	16.7	17.3
TD-1	147.8	18.4	38.4	12.2	80.6	79.1	70.6	64.1	21.7	16.4	19.4	8.4
Kiran	257.5	22.6	43.5	28.6	82.1	63.7	72.1	48.7	24.4	17.3	19.8	9.3
Abadgar	180.5	54.5	94.8	8.9	81.3	60.9	71.3	45.8	26.9	25.3	21.9	17.3
Marvi	249.0	56.4	45.4	2.8	78.1	74.3	68.1	59.3	25.5	24.8	20.4	16.8
Mehran	458.8	13.5	80.8	25.8	82.1	61.4	71.9	46.4	28.4	21.2	23.4	13.2
Bhitai	332.5	75.0	91.8	2.5	78.9	92.5	68.9	77.5	35.8	34.9	30.8	26.9
ZA-77	116.7	34.0	54.8	18.6	83.9	82.9	73.9	67.9	24.4	21.8	19.4	13.8
Pavan	224.0	20.7	95.8	1.5	79.1	84.5	69.1	69.5	26.6	23.2	21.6	15.2
Imdad	242.0	23.4	84.8	9.1	83.2	69.9	73.2	54.9	21.5	19.9	16.5	11.9
Average	228.5	31.1	64.0	10.7	78.3	73.1	68.3	58.1	25.5	22.5	20.5	14.5
R. D. %		-86.4		-83.3		-6.6		-14.9		-11.8		-29.3
C.D. Trt. 5%	21.8		2.9		2.5		2.5		1.0		1.0	
C.D. Var. 5%	61.8		8.3		7.0		7.0		2.8		2.8	
C.D. T x V 5%	87.3		11.8		9.9		7.0		4.0		4.0	

R.D. % = Relative difference: percentage increase (+) or decrease (-) due to water-stress.

(Farquhar et al. 1989). It helps plants to recover from stress and thus affects grain yield and yield stability. Drought stress decreased relative water content at both the stages but this decline was more at anthesis (Table 2). These results are similar as obtained for stomatal conductance. Interestingly, varieties Moomal, Bhitai, and Pavan maintained higher relative water content at both stages. Similar results were obtained earlier where relative water content decreased from 88 to 45% due to drought, nonetheless, some cultivars also resisted this decline (Siddique et al., 2000). In this study, drought stressed plants had highly cooler foliage at boot against warmer foliage at anthesis (Table 2). This is a common phenomenon caused by the check to transpiration exerted by stomatal closure (Kumar and Sankhola, 1983). The leaf area was also substantially reduced at both growth stages. However, the cultivars Bhitai and Moomal had similar ranking for leaf area at both the growth

Table 3. Mean performance of 16 spring wheat cultivars for tillers per plant, seeds per spike, grain yield per plant and 1000-grain weight under non-stress and water-stress conditions.

Cultivars	Tillers/ plant		Seeds/spike		Grain yield/plant (g)		1000-grain weight (g)	
	Non stress	Water stress	Non stress	Water stress	Non stress	Water stress	Non stress	Water stress
Anmol	2.8	2.3	43	22	4.03	1.67	55.4	61.7
Abadgar	3.0	1.7	32	12	3.09	1.08	63.2	71.8
Marvi	1.8	1.2	41	22	3.59	1.55	61.0	67.6
Mehran	2.8	2.3	43	15	3.54	1.35	55.7	61.6
Bhitai	2.3	1.7	44	28	4.17	1.89	58.4	58.9
ZA-77	3.2	3.3	33	15	3.74	1.10	51.5	56.1
Pavan	3.5	1.8	37	25	4.33	1.47	46.8	39.5
Imdad	2.8	1.7	29	23	4.33	1.86	67.6	70.0
Inqilab	3.0	2.3	34	23	4.44	1.53	59.6	55.0
Moomal	3.3	1.7	45	26	4.30	1.73	58.2	63.8
TJ-83	3.2	3.5	38	21	3.64	1.80	51.4	53.6
Sarsabz	2.8	3.0	48	21	4.29	1.48	54.6	59.6
Khirman	3.2	3.2	37	15	4.22	1.47	60.5	64.2
SKD-1	3.2	2.5	32	19	4.71	1.62	64.7	61.7
TD-1	4.2	3.2	27	12	3.97	1.36	68.9	71.9
Kiran	3.0	2.5	33	27	4.11	1.79	57.6	49.5
Range	1.8-4.2	1.2-3.5	27-48	12-28	3.09-4.71	1.1-1.9	46.8-68.9	39.5-71.9
Mean	3.01	2.37	37.22	20.38	4.03	1.55	58.44	60.41
R.D.%	-21.3		-45.2		-61.5		+3.2	
CD. Trt. (5%)	0.20		1.88		0.18		1.2	
CD. Var. (5%)	0.57		5.31		0.50		3.3	
CD TxV (5%)	0.81		7.5		ns		4.7	

R.D. % = Relative difference: percentage increase (+) or decrease (-) due to water-stress, ns = non-significant.

Table 4. Correlation coefficients (r) of physio-yield traits of wheat cultivars under non-stress and combined stresses at boot and anthesis stages of plant development.

Parameter	Combined water stress at boot and anthesis stages		
	Seeds per spike	Grain yield per plant	1000 grain weight
Stomatal conductance	0.82**	0.78**	-0.14ns
Relative water content	0.45**	0.47**	-0.18ns
Leaf area	0.51**	0.56**	-0.15ns
Tillers per plant	0.53**	0.43*	-0.14ns
Seeds/spike	-	0.84**	-0.39**
Grain yield/plant	0.90**	-	0.20**
1000-grain weight	0.75**	0.76**	-

**,*Significance at 1 and 5 % probability levels, respectively; ns = non-significant.

stages and were drought resistant. In contrast to this, Balota et al. (2008) found that drought tolerant wheat genotypes had smaller, narrower but thicker leaves with higher photosynthetic activity and in turn produced more grain yield. This discrepancy might be due to the differences of cultivars used in two studies.

Effect of water stress on yield traits at boot and anthesis growth stages

Water-stress significantly reduced number of tillers of wheat cultivars (21%). Drought stress resulted in un-productive tillers at both stages, therefore, grain yield was not predicted solely on the basis of tillers produced by the varieties. Zhang-Hu and Rajaram (1994) reported that spikes per m^2 were most sensitive to drought. Drought stress badly affected seeds per spike, nonetheless, the cultivars Bhitai and Moomal maintained this trait even under drought stress. Higher number of seeds in both these varieties also reflected their drought tolerance at anthesis. Garcia-del Moral (2003) found that drought affected the numbers of kernels/m^2 and kernels/spike. While reviewing last 10 year research, Fischer (2007) found that increased kernel number/m^2 still remains strongly associated with genetic progress in grain yield. Indeed, drought affects grain yield depending on the developmental stage (Agboma et al., 1997). Grain yield plant^{-1} decreased due to water stress by 61.5% (Table 3). Praba et al. (2009) noted that water stress reduced number of grains by 44% compared with non-stress control. Villegas et al. (2007) found grains per spike most affected by water stress. The cultivars Anmol, Bhitai, Moomal, Imdad, TJ-83 and Kiran maintained grain yield even under drought stress. Pireivatlou and Yazdansepas (2008) noted that post-anthesis drought stress significantly decreased grain yield whereas Praba et al. (2009) recorded 32% reduction in grain yield against control due to drought. Interestingly, drought stress increased 1000-grain weight (Table 3) which is rather hard to explain. However, we noticed high leaf senescence in controlled plants during grain filling. This loss of assimilates might be responsible for the lower 1000-grain weight under controlled condition. Nonetheless, 1000-grain weight of cultivars Abadgar, Imdad, and TD-1 were higher than for other varieties. Plaut et al. (2004) reported that 1000-kernel weight and weight of kernels per spike were more severely decreased by water-deficit in the two wheat varieties, yet less in Batavia than in Suneca cultivars.

The significant positive correlations among various parameters (Table 4) suggest that an increase in stomatal conductance, relative water content and leaf area increases yield traits. Very surprisingly, none of the physiological attributes were significantly correlated with 1000-grain weight. Among the yield traits, tillers/plant, seeds spike $^{-1}$, grain yield plant^{-1} and 1000-grain weight were positively correlated with each other. However, 1000-grain weight was negatively correlated with seeds per spike. Foulkes et al. (2007) also noted that genetic trait which showed the clearest correlation with ability to maintain yield under drought was green flag-leaf area persistence and suggested the potential use of leaf area as a selection criterion for yield under drought. Gupta et al. (2001) reported positive correlations of leaf area with grain yield; shoot dry weight with grain yield and seeds/spike.

Pasquale De-Vita et al. (2007) found that genetic gain in grain yield was most clearly associated with a higher number of kernels m^{-2}.

Conclusion

The present study concludes that the stomatal conductance, relative water content and leaf area are the most important traits that should be considered while developing drought tolerant wheat genotypes.

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