Pak. J. Bot., 45(SI): 151-155, January 2013.

PHYSIOLOGICAL RESPONSES AND DROUGHT RESISTANCE INDEX OF NINE WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS UNDER DIFFERENT MOISTURE CONDITIONS

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Abstract

Nine commercial wheat cultivars of Pakistan viz; Inqulab-91, AS-2002, Sehar-2006, Shafaq-2006, Bhakkar-2002, Auqab-2000, GA-2002, Chakwal-50 and Fareed-06 were evaluated for their physiological performance and drought resistance index under different levels of soil moisture. Germination of these cultivars was tested under PEG induced osmotic stress of -2, -4, -6, and -8 bars. Highest germination percentage was observed for Fareed-06, Chakwal-50, GA-2002 and AS-2002 at -8 bars osmotic stress. To study physiological responses and drought resistance index, plants were grown in clay pots and water equivalent to 75%, 50% and 25% field capacity was applied. Decrease in soil moisture significantly affected relative water content, leaf succulence, chlorophyll content, cell membrane stability index, number of grains/spike and 100 grain weight. Significant variation was found among cultivars. Based on physiological responses and drought resistance index performance of Chakwal-50, GA-2002 and Bhakkar-2002 were found relatively better under limited moisture conditions.

Introduction

Major proportion of global land area (about 60%) belongs to arid and semi-arid zone. About 37 % of the area in developing countries consists of semiarid environments with large fluctuations in the amount and frequency of rainfall (Nakashima *et al*., 2000). Wheat is commonly grown in these drought prone areas. Drought stress often causes serious problems and is a major limitation to productivity of the crop. Grain yield of wheat in these areas is highly fluctuating depending upon the duration and degree of drought stress. In Pakistan, about 20 % of the total wheat acreage is planted under rained conditions. Yield of the crop from these areas is much less than that of irrigated area mainly due to occurrence of drought spells. Insufficient water is the primary limitation to wheat production world-wide. Drought is a multidimensional stress affecting plants at various levels of their organization. Drought affects morphological, physiological and biochemical processes in plants resulting in growth inhibition, stomatal closure with consecutive reduction of transpiration, decrease in chlorophyll content and inhibition of photosynthesis (Demirevska, 2008) making it the largest single factor for yield reduction globally (Narusaka *et al*., 2003). The response of plants to water stress depends on several factors such as developmental stage, severity and duration of stress and cultivar genetics (Beltrano & Marta, 2008, Khan *et al*., 2011).

Development of resistant cultivars, however, is hampered by low heritability for drought tolerance and a lack of effective selection strategies. Effective selection of genotypes to increase the productivity of rainfed wheat may be a convenient and efficient approach to meet this challenge. Several indices based on drought resistance or susceptibility of genotypes have been evaluated and used for selection of drought-tolerant wheat genotypes (Talebi *et al*., 2009). Physiological parameters like tissue water, chlorophyll content and membrane stability may be considered as indicators of good growth and yield under water stress. Plants keeping high relative water content show a positive relation with grain yield (Makoto *et al*., 1990). Decrease in soil water potential causes decline in succulence (Qi *et al*., 2009). Maintaining leaf succulence maybe an adaptation to resist drought. Chlorophyll content may be considered an indicator of dry matter accumulation with least oxidative damage. Zaharieva *et al*., (2001) found positive correlation between grain yield and chlorophyll content. Low chlorophyll content leads to lower photosynthesis and consequently low dry mater production and yields.

Drought resistance is the ability to minimize yield loss under decreasing soil water. Relative yield of a genotype may reflect its performance under drought. Therefore, most widely used criteria for selection are based on yield performance under stress and non-stress conditions (Rashid *et al*., 2003). Drought resistance index (DRI) to assess drought tolerance of a genotype reflects drought resistance and high yield (Hu *et al*., 2007) indicating the performance of wheat cultivars under water deficit conditions (Dong & Lui, 2005). DRI may be useful for selecting a cultivar for sowing in water limited areas. Present study was conducted to evaluate drought tolerance of nine Pakistani wheat genotypes based on physiological responses and drought resistance index.

Materials and Methods

The experiments for estimating drought tolerance of wheat cultivars based on DRI were conducted in Crop Physiology Laboratory, Department of Agronomy, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan. Seeds of Shafaq 2006, AS-2002, Fareed-2006, GA-2002, Auqab-2000, Sehar-2006, Inqulab-91, Chakwal-50 and Bhakkar-2002 were washed with 70 % ethanol for about 2-3 minutes followed by treating the seeds with 20 % solution of sodium hypochlorite for 30 minutes for surface sterilization followed by rinsing with ample amount of distilled water.

Polyethylene glycol (PEG-6000) was used for induction of stress to test the germination. Different solutions of PEG were prepared in order to induce osmotic stress of -2, -4, -6, and -8 bars, respectively. Seeds were subjected to osmotic stress by placing in blotters saturated with PEG-6000 solutions for germination test. Distilled water was used for control.

A pot experiment was conducted to study the effect of different moisture levels on wheat growth. Seeds were germinated in clay pots. After 6 weeks of plant growth in pots water equivalent to 75 %, 50 % and 25 % of field capacity measured by gravimetric method was applied. The experiment was arranged according to CRD factorial. Data on physiological parameters and drought resistance index as described below was recorded and analyzed statistically by using MSTATC software.

To determine relative water content (RWC) second leaf of plants was taken from each pot. Fresh weight (FW) immediately recorded, and then leaves were soaked for 4 hours in distilled water at room temperature under a constant light and turgid weight (TW) was recorded followed by drying for 24 hours at 80 ºC for dry weights (DW).

$$RWC = \{(FW - DW) / (TW - DW)\} \times 100$$

Where FW = Fresh weight, DW = Dry weight, TW = Turgid weight

Leaf area of the selected leaves was recorded to determine succulence as below:

Succulence = [Fresh weight – Dry weight] / leaf area

Chlorophyll contents were measured by chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc. Japan).

For cell membrane stability index (CMSI) leaf strips (0.2 g) were taken in test tubes containing 10 ml of double distilled water in two sets. Test tubes in set one were kept at 40 °C in a water bath for 30 min and test tubes in set two were incubated at 100 °C in the boiling water bath for 15 minutes. CMSI was calculated using the formula given below:

$$CMSI = [1- (C1/C2)] \times 100$$

C1 = electrical conductivity of the water containing the sample in set one.
C2 = electrical conductivity of the water containing the sample in set two.

Yield attributes including number of spikelets/spike, number of grains/spike, 100 grain weight were measured.

Drought resistance index is defined as DC (drought resistance coefficient) multiplied by variety's minimum yield, and then divided by average minimum yield of the varieties used in the experiment (Dong & Liu, 2005).

$$DRI = DC \times (Ya / \check{Y}) \text{ where } DC = Ya / Ym \text{ and}$$

$\check{Y}$ is the average yield of all varieties under stress.
Ya is yield of the variety under observation under stress.
Ym is maximum yield of variety under observation without stress.

Results and Discussion

Results of experiments conducted to determine the effect of drought stress on nine wheat cultivars are described and discussed below:

Seed Germination: The data on germination percentage of different cultivars as affected by different levels of osmotic stress are depicted in Table 1. Data showed that all treatments differ significantly from each other at 5 % probability level. On the average, maximum germination (62.2 %) was observed for Chakwal-50 followed by GA-2002 and minimum (43.3 %) for Fareed-06. Increased water stress was accompanied by a rapid and significant reduction in germination percentage. Germination decreased from 89.7 % in control to 24.1 % at -8 bars, on the average. Declining trend of germination with increase in osmotic stress was recorded in all cultivars under study. At highest level of water stress (-8 bars) a tremendous reduction in germination percentage was found in all varieties compared to control. Maximum germination at -8 bars stress was recorded for Fareed-06, GA-2002, Chakwal-50 and AS-2002 and Auqab-2000 whereas minimum germination was found in case of Auqab-2000, Bhakkar-2002 and Inqulab-91. Farooq & Azam (2006) also found significant decrease in germination of different wheat varieties subjected to water stress in laboratory experiment. Differential germination response of wheat cultivars under water stress was also reported by Alaei *et al*., (2010) & Jajarmi (2009). Establishment of seedling is extremely important in determining the yield of crop under rainfed conditions with limiting moisture (Misra *et al*., 2002). Soil moisture is one of the factors limiting crop germination, especially in rainfed areas. The cultivars, which germinate and produce vigorous stands in soils with limited moisture contribute to successful wheat production and good yield. Based on germination percentage at -8 bars Fareed-06, Chakwal-50, GA-2002 and AS-2002 seem to be good cultivars for areas with limited moisture at sowing time. However, only germination percentage cannot be considered a good index to identify high performing cultivars under water deficit because the germination is less affected by drought stress than the other traits and is strongly influenced by seed age, storage conditions and the environment in which the seed developed (Jajarmi, 2009; Perez *et al.,* 2007).

Physiological Responses: Data on average physiological responses of wheat cultivars to water stress is presented in Table 2. Cultivars differed significantly at 5 % probability in their response to drought for all parameters studied. Chakwal-50 followed by GA-2002 and Bhakkar-2002 were able to maintain significantly higher relative water content, leaf succulence, chlorophyll content and cell membrane stability. Other cultivars exhibited inconsistent trend for these parameters. Maximum and significantly higher 100 grain weight was recorded for Chakwal-50 followed by GA-2002. There was non-significant difference in 100 grain weight of all other cultivars. All cultivars produced statistically similar number of grains/spike.

Table 1. Germination (%) of wheat cultivars as affected by different levels of osmotic stress.

Cultivars	Stress levels				
	0 Bars	**-2 Bars**	**-4 Bars**	**-8 Bars**	**Means**
Shafaq-2006	96.7 a	50.0 ghi	36.7 jkl	**23.3 mn**	50.7 E
AS-2002	96.7 a	60.0 efg	40.0 ijk	**30.0 klm**	56.7 C
Fareed-06	90.0 ab	66.7 de	53.3 fgh	**36.7 jkl**	61.6 B
GA-2002	86.7 ab	53.3 fgh	46.7 hij	**30.0 klm**	54.2 D
Auqab-2000	73.3 cd	50.0 ghi	36.7 jkl	**13.3 no**	43.3 H
Sehar-2006	96.7 a	63.3 def	40.0 ijk	**26.7 lm**	56.7 C
Inqulab-91	83.3 bc	46.7 hij	40.0 ijk	**10.0 o**	45.0 G
Chakwal-50	93.3 ab	60.0 efg`	50.0 ghi	**33.3 klm**	62.2 A
Bhakkar-2002	90.0 ab	50.0 ghi	40.0 ijk	**13.3 no**	48.3 F
Means	**89.7 A**	**55.6 B**	**41.7 C**	**24.1 D**	

Values sharing similar letter are non-significant at P < 0.05

Table 2. Average physiological responses and drought resistance index (DRI) of wheat cultivars.

Cultivars	Relative water content (%)	Leaf succulence (mg/m^2)	Chlorophyll content (SPAD value)	Cell membrane stability index (%)	Number of grains/spike	100 grain weight (g)
Shafaq-2006	68.63 ab	9.97 cde	30.14 d	61.73 d	42.22 **	2.52 c
AS-2002	61.07 d	9.47 ef	30.50 d	62.34 d	43.22 **	2.54 c
Fareed-06	64.69 c	9.79 def	27.36 e	63.31 cd	43.00 **	2.44 c
GA-2002	68.47 ab	11.53 ab	34.64 ab	65.09 a	42.67 **	2.71 b
Auqab-2000	52.96 e	9.31 ef	33.23 bc	63.47 cd	42.89 **	2.48 c
Sehar-2006	63.84 cd	10.43 cd	32.74 bc	64.01 bc	43.44 **	2.46 c
Inqulab-91	65.19 bc	9.01 f	31.52 cd	64.79 bc	43.33 **	2.44 c
Chakwal-50	69.10 a	11.81 a	35.37 a	65.09 a	44.44 **	3.00 a
Bhakkar-2002	68.63 ab	10.68 bc	33.58 ab	64.90 ab	43.11 **	2.57 c

Values sharing similar letter are non-significant at p<0.05
** Non-significant difference

Effect of different levels of water levels on physiological performance of wheat cultivars is shown in Table 3.All cultivars manifested a significant decline in leaf relative water content with decrease in moisture level from 75 % to 25 % field capacity (FC). Nonetheless, the cultivars differed significantly in their response at different stress levels. Highest RWC was observed in Chakwal-50 at 25 % FC followed by Shafaq-2006, Bhakkar-2002 and GA-2002. Succulence was highest in Chakwal-50 and GA-2002 whereas chlorophyll content was highest in Chakwal-50 and Bhakkar-2002. Effect of lowest moisture level (25 % FC) on cell membrane stability was almost similar in all cultivars. Number of grains/spike in all cultivars reduced in similar fashion at all levels of FC. GA-2002, Inqulab-91, Chakwal-50 and Bhakkar-2002 produced statistically equal but relatively higher 100 grain weight than other varieties at lowest moisture level.

Several investigators have described the effect of water deficit on various physiological attributes of growth in wheat which were studied in this experiment. In general, soil water deficit results in decrease in RWC (Tas & Tas, 2007), leaf succulence (Qi *et al*., 2009), chlorophyll content (Paknejad *et al*., 2007), cell membrane stability index (Farooq & Azam, 2006; Tas & Tas, 2007), number of grains/spike (Sanjari-Pirevatlou *et al*., 2011) and grain weight/grain yield (Paknejad *et al*., 2007; Sanjari-Pirevatlou & Yazdansepas, 2008). These physiological traits definitely contribute towards growth and productivity of plants under water deficit conditions. These are adaptive mechanisms of plants under drought. Maintenance of high RWC and succulence under drought may be due to relatively more growth of the roots than shoots thereby tending to sustain water supply. In addition, this may also be due to ABA (abscisic acid) induced reduction in stomatal opening thus decreasing the loss of water to help maintain RWC which has positive relationship with yield (Makoto *et al*., 1990). Chakwal-50, in this study, was found to maintain its RWC and succulence relatively higher than other cultivars at 25 % FC and may be considered productive under low level of soil moisture. Similarly, positive correlation exists between chlorophyll content and grain yield (Zaharieva *et al*., 2001) and may be used as an important criterion for evaluating drought resistance of wheat. Chakwal-50 and Bhakkar-2002 were able to maintain their chlorophyll content significantly higher than other cultivars at lowest level of soil moisture (25 % FC) suggesting their high yield potential under drought. Stability of cell membrane under drought is an important mechanism to resist drought. Cell membrane stability index (CMSI) indicates the functionality of plasma lemma and in turn the normal functioning of cellular machinery. High CMSI certainly means ability of the plants to resist drought as postulated by Sairam *et al*., (2002). Therefore, this may also serve as a useful tool for screening drought tolerant genotypes. However, the results we obtained for CMSI were not conclusive for any of the cultivars under study. Number of grains/spike and grain weight may also add to selection criteria. Satisfactory yield under water deficit condition is the ultimate goal of research for drought tolerance. Cultivars used in this study did not differ in number of grains/spike. GA-2002, Inqulab-91, Chakwal-50 and Bhakkar-2002 were found equivalent in 100 grain weight but relatively better than other cultivars. Consequently, performance of Chakwal-50, GA-2002 and Bhakkar-2002 may be considered better than other cultivars under water deficit conditions.

Table 3. Physiological responses of wheat cultivars under different levels of soil moisture.

Cultivars	Moisture level	Relative water content (%)	Leaf succulence (mg/m^2)	Chlorophyll content (SPAD value)	Cell membrane stability index (%)	Number of grains/spike	100 grain weight (g)
	75 % FC*	80.53 ab**	13.43 d	39.70 def	71.07 d	60.67 a	3.37 b
Shafaq-2006	50 % FC	69.83 de	10.03 g	31.67 jk	62.03 g	42.67 b	2.23 gh
	25 % FC	**55.53 ijk**	**6.43 jkl**	**19.07 mn**	**52.10 i**	**23.33 c**	**1.97 ij**
	75 % FC	73.73 cd	11.90 ef	41.60 cde	72.13 cd	61.00 a	3.20 bc
AS-2002	50 % FC	60.83 hi	10.60 fg	33.27 ijk	62.23 g	41.67 b	2.47 f
	25 % FC	**48.63 lm**	**5.90 kl**	**16.63 n**	**52.67 hi**	**27.00 c**	**1.97 ij**
	75 % FC	78.57 abc	13.00 de	38.73 efg	73.17 bcd	61.00 a	3.37 b
Fareed-06	50 % FC	63.40 fgh	10.07 g	24.67 l	63.13 fg	44.67 b	2.20 gh
	25 % FC	**52.10 kl**	**6.30 jkl**	**18.67 mn**	**53.63 hi**	**23.33 c**	**1.77 j**
	75 % FC	83.93 a	16.67 ab	47.53 b	74.93 ab	59.67 a	3.33 b
GA-2002	50 % FC	67.33 efg	10.73 fg	35.80 ghi	65.20 ef	43.00 b	2.70 e
	25 % FC	**54.13 jkl**	**7.20 ijk**	**20.60 m**	**55.13 h**	**25.33 c**	**2.10 hi**
	75 % FC	65.40 fgh	14.37 cd	51.53 a	74.20 abc	60.33 a	3.00 cd
Auqab-2000	50 % FC	50.87 kl	7.77 ij	30.67 k	63.60 fg	44.33 b	2.47 f
	25 % FC	**42.60 m**	**5.80 kl**	**17.50 mn**	**52.60 hi**	**24.00 c**	**1.97 ij**
	75 % FC	77.40 bc	13.77 d	43.17 cd	75.80 a	58.67 a	3.20 bc
Sehar-2006	50 % FC	62.47 gh	10.87 fg	35.10 hij	63.50 fg	43.67 b	2.37 fg
	25 % FC	**51.67 kl**	**6.67 jkl**	**19.97 mn**	**52.73 hi**	**28.00 c**	**1.80 j**
	75 % FC	77.63 bc	13.03 de	41.20 cde	75.03 ab	60.33 a	3.03 cd
Inqulab-91	50 % FC	67.77 efg	8.43 hi	33.83 hijk	65.17 ef	41.33 b	2.23 gh
	25 % FC	**50.17 kl**	**5.57 l**	**19.53 mn**	**54.17 hi**	**28.33 c**	**2.07 hi**
	75 % FC	77.97 abc	17.50 a	43.77 c	75.50 ab	60.33 a	4.07 a
Chakwal-50	50 % FC	69.13 def	10.67 fg	36.83 fgh	65.27 ef	44.00 b	2.90 de
	25 % FC	**60.20 hij**	**7.27 ijk**	**25.50 l**	**53.93 hi**	**29.00 c**	**2.03 hi**
	75 % FC	82.70 ab	15.33 bc	44.47 bc	74.97 ab	60.67 a	3.27 b
Bhakkar-2002	50 % FC	68.90 def	9.90 gh	31.53 k	66.73 e	41.33 b	2.40 fg
	25 % FC	**54.30 jkl**	**6.80 jkl**	**24.73 l**	**53.57 hi**	**27.33 c**	**2.03 hi**

* FC stands for field capacity

** Means sharing similar letters in a column do not differ significantly at 5% probability

Wheat cultivars respond differently to water stress in the form of physiological and biochemical changes (Yagmur & Kaydan, 2008; Paknejad *et al*., 2007; Sanjari-Pireavatlou *et al*., 2011) indicating existence of genotypic variation. We also found differential response of wheat cultivars to water stress manifesting presence of genetic differences among cultivars. These differences need to be explored and incorporated into new cultivars for better productivity from drought prone environments. Chakwal-50, GA-2002 and Bhakkar-2002 seem to have good potential to be used as parent material for breeding new drought resistant varieties.

Drought resistance index (DRI): DRI values calculated for all the cultivars used in this study are given in Table 2. Highest DRI (0.622) was obtained for Chakwal-50 followed by Fareed-06 and GA-2002 with DRI value of 0.610 and 0.604, respectively. DRI of these three cultivars do no differ significantly at 5 % level of probability. Bhakkar-2002 was the fourth cultivar with significantly higher DRI than the remaining cultivars. Lowest DRI (0.442) was obtained for Sehar-2006. Cultivars showing greater value of DRI are considered to have more resistant to drought. Dong & Liu (2005) calculated DRI values for seven wheat cultivars grown under irrigated and non-irrigated conditions and found that cultivars having more DRI values were more resistant to drought.

DRI is considered the best criterion for evaluation of resistant cultivars (Hu *et al*., 2007) and provides information about drought resistance and yield of a variety under water deficit condition. Agronomic traits and yield of wheat cultivars are related with DRI. Flag leaf area is negatively related with DRI (Dong & Liu, 2005) but positively with coleoptile length (Wang *et al*., 1999). It can be used to evaluate drought tolerant wheat genotypes and has become a standard in identification of drought resistance of wheat and rice in China (Li *et al*., 2006). However, drought resistance is not a simple phenomenon. A single trait cannot make a plant resistant to water stress. Combination of physiological traits and DRI may help assess drought tolerance of wheat. In our experiments cultivars with high DRI values also have better physiological attributes approving drought resistance of these genotypes. Consequently, Chakwal-50, GA-2002 and Bhakkar-2002 may be considered relatively suitable for moisture limited conditions as per results of this study.

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(Received for publication 1 September 2012)