Australian Journal of Crop Science AJCS
AJCS 8(2):200-207 (2014) ISSN:1835-2707

Screening for drought tolerance in Iranian wheat genotypes (*Triticum aestivum* L.) using physiological traits evaluated under drought stress and normal condition

Zeinab Soleimani, Hosseinali Ramshini*, Seyed Mohammad Mahdi Mortazavian, Mahdi Fazelnajafabadi, Behroz Foughi

Department of Agronomy Sciences and Plant Breeding, College of Aburaihan, University of Tehran, Pakdasht, Iran

***Corresponding author: ramshini_h@ut.ac.ir**

Abstract

Cultivation of drought adapted genotypes is the best approach to avoid yield loss under water deficit condition. In order to screen for drought tolerance 82 Iranian wheat genotypes were evaluated by recording relative water content (RWC), days to maturity, leaf rolling and leaf silvering under stress condition in a field trial. In next experiment ten genotypes (six tolerant and four sensitive) selected from field experiment were assessed for drought adaptability measuring RWC, osmotic adjustment (OA), catalase (CAT) and peroxidase (POD) activity and stomatal characteristics. The genotypes were grown under normal and stress condition with three replications. There was high variability among genotypes in response to drought. Azadi and Ohadi showed significant enhancement in CAT activity while in POD activity maximum increase was recorded for Homa. Kohdasht (0.59MPa) and Arta (0.15MPa) had the highest and lowest OA, respectively. Higher OA ability indicates the potential for accumulating osmolytes in cells to absorb water more easily under stress condition. Stomatal closure in response to drought was more significant in Kohdasht and Arta while in Homa almost no change in stomatal closure was detected. Significant negative correlation ($r = -0.65$, $p<0.05$) was obtained between RWC and stomatal opening implying the dominance of stomatal conductance control for water retention in genotypes with higher RWC. The activity of POD in Homa, a drought sensitive genotype, was significantly high under stress condition. Presumably the scavenging of H_2O_2 may be the last defense barrier of this genotype against drought. Kohdasht had the highest ability of water retention under stress condition mostly due to its excellent OA and stomatal closure. These physiological characters can be transferred to high yield genotypes to improve drought adaptability.

Keywords: catalase, peroxidase, relative water content (RWC), stomatal width, *Triticum aestivum* L.
Abbreviations: CAT_catalase, OA_osmotic adjustment, POD_peroxidase, RWC_relative water content.

Introduction

Bread wheat (*Triticum aestivum L.*) is the major source of protein and energy in the world (Harlan, 1998). Drought as one of the most important environmental stresses is considered the main cause of yield loss in many crops (Babu et al., 2004). Climate changes and reduced water resources around the world will force the farmers to apply less water for growing irrigated crops (Rebetzke et al., 2006). As a consequence, even the irrigated crops experience drought stress during plant growth period due to lack of adequate water. Water deficit or drought is the situation in which adequate moisture for normal growth of plants does not exist. With fluctuating drought stress across years and scarce water plants cannot successfully accomplish their life cycle and subsequently grain yield will decline greatly (Kusaka et al., 2005). During drought stress the oxidative stress usually is induced. Although under normal condition the reactive oxygen intermediates (ROIs) are present in cell and consumed in various processes like plant cell death but their generation is tightly regulated. Under drought stress the cellular homeostasis of cell is damaged and the level of RIOs increases substantially. The ROIs such as H_2O_2 can react to membrane lipids, inhibit enzyme activity and causes DNA and RNA damages (Mittler, 2002). Catalase (CAT, EC 1.11.1.6) which remove the hydrogen peroxide resulted from photorespiration and peroxidase (POD, EC 1.11.1.7) which consumes H_2O_2 in vacuoles and cytosol for oxidation of other substrates are key enzymes in defense against RIOs (Simova-Stoilova et al., 2008). Shao et al. (2005) studied the activity of antioxidant enzymes under drought stress in ten wheat cultivars during the whole growth period (i.e. seedling, tillering and maturing). They demonstrated that the ability of plants to adapt themselves to changing environment is closely linked with their growth and development. In their experiment the response of wheat genotypes to drought stress was variable i.e. genotypes had dissimilar stress tolerance threshold due to their individual physiological adaptive mechanism which regulates their redox status. Huseynova (2012) examined catalase activity at different stages of development in varieties of durum and bread wheat cultivars under normal and severe drought stress condition. Their results showed that in durum and bread wheat maximal activity of CAT was observed at the milk ripeness and at the end of flowering. They also showed that enzyme activity enhanced during development and this enhancement was significantly higher in the tolerant cultivars compared to susceptible ones. Osmoregulation is a chief mechanism which has been developed in drought tolerant genotypes (Zhang et al., 1999). In this mechanism plants assimilate solutes in their cell sap to reduce intracellular osmotic potential (Blum, 2011). These solutes are small organic

molecules such as amino acids, betaeines and sugars. However some inorganic solutes are also involved in osmoregulation. Plants ability for osmolyte accumulation can indirectly be estimated by osmotic adjustment (OA) evaluation. OA is receiving increasing recognition as a major mechanism of drought resistance in crop plants (Zhang et al., 1999). For a reliable estimation it is underlined that the stress should be reached slowly to the specified threshold so that plants have adequate time to generate solutes and achieve OA (Zhang et al., 1999). Significantly positive correlations have been reported between OA and grain yield under drought condition in numerous studies (Blum, 2011; Blum et al., 1999; Subbarao., 2000). Another screening tool of drought tolerance as well as a good indicator of plant water-status is relative water content (RWC). It is a simple and effective method and estimates the volumetric water content of leaf relative to water content at full turgor (Blum, 2011). Several studies have shown that plants lose over 95% of their water through stomata by transpiration (Mackay et al., 2003; Tang et al., 2006). Number of stomata per unit area and their size play a critical role in plant gas exchange (Hetherington and Woodward, 2003). Furthermore, it has been recognized that different varieties of crops at the same growth condition express various abilities in gas exchange which is due to the different number of stomata per leaf unit area and stomata opening size. However as it is dependent on leaf age and position the measurement should be performed at the specified growth stage and leaf. Additionally the response of gas exchange of genotypes toward environmental alterations is not the same (Bkagwat and Bhatia, 1993; Ewers et al., 2001). During past decades, stomata frequency and size as a measure of the rate of water loss by plants have been investigated (Khazaei et al., 2010; Merah et al., 2001). This method affords an easier indirect way for measuring stomatal conductance. There may be genetic variation in stomatal density and in stomatal closure ability which can be discovered and exploited in plant breeding for stress tolerance. The response to oxidative stress has been less investigated in Iranian wheat genotypes. In this view the objective of present study was to screen drought tolerance in Iranian wheat genotypes to discover tolerant varieties to be utilized as gene donor in breeding programs for improving physiologically drought tolerance in high yielding genotypes.

Results

Field experiment

Significant differences among genotypes were detected for RWC. Table 1 shows the mean value of RWC for 82 genotypes examined in this experiment. Each value is an average over nine numbers including three replications and three time points of stress treatment. The main purpose of field experiment was selection of a few genotypes for more investigation in pot experiment and enzyme assays. Genotypes in table 1 have been sorted according to RWC values. Selection of sensitive and tolerant genotypes was carried out not only based on RWC but also other tolerance related indices such as flowering and maturity time, leaf rolling and leaf silvering evaluated under stress condition. Altogether ten genotypes were selected for pot experiment. Six of them were considered as tolerant and four others were sensitive. The genotypes with earlier flowering and maturity and good rolling ability and silvering were considered as tolerant. The data for traits other than RWC of these ten genotypes are shown in Table 2.

Pot experiment

There were significant differences among genotypes grown under stress and normal conditions. Regarding antioxidant enzymes under normal condition Ghods had the highest CAT activity while under stress condition Azadi had the highest value. In general the results of ANOVA revealed significant enhancement in CAT activity under stress condition i.e. the mean CAT activity of normal and stress conditions were 1.8 and 2.13 (Umg^{-1} protein), respectively. However the magnitude of increase varied among genotypes. Under stress condition CAT activity for Azadi and Ohadi increased significantly compared to normal condition (Fig 1). CAT activity for other genotypes remained unaffected under stress condition and was nearly similar to normal condition. Similarly POD activity also was influenced significantly by drought stress ($p \leq 0.01$). Under normal and stress condition the average POD activity was 3.98 and 5.54 (Umg^{-1} protein), respectively. However the difference among genotypes was not as much as CAT activity results. Drought stress did not modify significantly POD activity except in Homa that showed much induced POD activity under stress compared to normal condition. The POD activity increase for this genotype was above two fold i.e. its value under normal condition was 2.3 while it rose to 6.6 (Umg^{-1} protein) under stress condition (Fig 2). Significant differences in OP among genotypes grown under stress and normal conditions were observed according to ANOVA results. The difference between values of normal and stress condition was calculated as osmotic adjustment (OA) for each genotype. Kohdasht had the highest value of OA (0.59) while Arta, Ohadi and Homa had the lowest values (0.15, 0.29 and 0.34, respectively) (Fig 3). The effect of stress was significant ($p \leq 0.05$) and for all genotypes the osmotic potential increased under stress condition. Genetically there was variation at osmotic potentials among genotypes when comparison was carried out across genotypes under each growth condition separately. Significant variation in RWC was found among genotypes ($p \leq 0.01$). Fig 4 shows the results based on mean value over two time points of measuring RWC. Ranking of genotypes at both times was the same. Maximum RWC (0.69) was belonged to Kohdasht while the RWC for Homa was as low as 0.54. The average of RWC for genotypes was obtained as 0.63. Significant variation among genotypes ($p \leq 0.01$) in stomata density of leaf surface was detected using ANOVA. Ghods had the maximum stomata density of 60 followed by Star (59 mm^{-2}) while Arta and Homa had a much lower stomata density (46 and 48 mm^{-2}, respectively). In terms of stomata length there was no significant differences among genotypes under stress and normal condition. In other words drought stress had no influence on the stomata length while stomata width either decreased significantly or remained unaffected under stress condition. However the reduction was not the same for all genotypes (Fig 5). In genotypes Kohdasht, Arta, Bezostaya and Star the reduction was significant ($p \leq 0.05$). It means these genotypes could manage their stomata conductance under stress condition. Drought stress could not alter chlorophyll content relative to normal condition. However in each growth condition differences among genotypes were significant (Fig 6). Star had the maximum value for chlorophyll content under normal condition (0.85 g/m^2). Under stress condition Ghods (0.93 g/m^2) and Moghan3 (0.53 g/m^2) had the maximum and minimum value of chlorophyll content, respectively. Correlation analysis showed some significant associations among traits measured in present study. The correlation between stomatal width and RWC was obtained as -0.65

Table 1. Mean value of relative water content (RWC) of 82 genotypes studied for drought tolerance screening along with standard error (±SE). Each value is a mean over nine numbers (three time cycles of stress and three replications).

Genotype	origin	RWC	Genotype	origin	RWC	Genotype	origin	RWC
Star	*	0.79±0.03	Sarda101	*	0.71±0.04	Bahar	ICARDA	0.66±0.03
Bezostaya	Russia	0.78±0.03	Azar2	Iran	0.71±0.02	Shiraz	Iran	0.66±0.04
Gaskojen	*	0.78±0.02	Ghafghaz	*	0.71±0.03	Akbari	Iran	0.66±0.03
Gamptus _R	USA	0.77±0.01	Zagros	ICARDA	0.71±0.02	S_83_3	Iran	0.66±0.02
Vee/Nac	CIMMYT	0.77±0.04	Darya	CIMMYT	0.71±0.02	Ghods	Iran	0.66±0.03
Kohdasht	Iran	0.76±0.02	Moghan2	India	0.70±0.03	Sayson	*	0.66±0.03
Arta	CIMMYT	0.76±0.01	C_F_H	CIMMYT	0.70±0.03	Roshan	Iran	0.65±0.04
Karkhe	ICARDA	0.76±0.03	Moghan1	CIMMYT	0.70±0.03	Shahpasa	Iran	0.65±0.04
R_BC_S	Iran	0.75±0.02	C_Alborz	CIMMYT	0.70±0.02	Bolani	*	0.65±0.03
Moghan3	CIMMYT	0.75±0.02	Atrak	CIMMYT	0.70±0.02	Chamran	CIMMYT	0.64±0.04
Zarin	CIMMYT	0.74±0.02	Arvand_M	*	0.70±0.03	Shirodi	CIMMYT	0.64±0.03
Navid	Iran	0.74±0.02	Gamptus_S	USA	0.70±0.02	Niknejad	ICARDA	0.64±0.04
Shahryar	Iran	0.74±0.04	Sabalan	Iran	0.70±0.02	Kavir	Iran	0.63±0.04
Somali3	*	0.74±0.02	Frontana	*	0.70±0.02	Azadi	Iran	0.63±0.03
Sepahan	Iran	0.73±0.04	Rasad	Iran	0.70±0.03	Dez	CIMMYT	0.63±0.05
Rasol	CIMMYT	0.73±0.03	DN_11	Iran	0.70±0.02	Inia	CIMMYT	0.63±0.03
Marvdash	Iran	0.73±0.03	Maron	Iran	0.70±0.03	Bayat	Iran	0.63±0.04
MV_17	Hungary	0.73±0.02	Mahdavi	Iran	0.69±0.03	Tabasi	Iran	0.62±0.03
Alamot	*	0.73±0.02	Pishtaz	Iran	0.68±0.03	Hamon	Iran	0.62±0.04
WS_82_9	Iran	0.73±0.01	Hirmand	Iran	0.68±0.03	Ovhadi	Iran	0.61±0.02
Omid	Iran	0.73±0.03	Unkwon	Iran	0.68±0.04	Azar1	Iran	0.61±0.03
Tos	USA	0.72±0.02	Sistan	CIMMYT	0.67±0.03	Darab2	CIMMYT	0.61±0.05
Gaspard	Franc	0.72±0.02	Weebli_1	*	0.67±0.02	Shole	*	0.60±0.04
R_BC_W	Iran	0.72±0.03	Naz	CIMMYT	0.67±0.03	Homa	Iran	0.59±0.04
Alborz	CIMMYT	0.72±0.03	Shahi	Iran	0.67±0.02	Tajan	CIMMYT	0.57±0.05
Fong	*	0.72±0.02	Tipik	*	0.67±0.04	Sardari	Iran	0.55±0.03
Excalibu	Australia	0.72±0.05	Alvand	Iran	0.67±0.03			
Karaj3	Iran	0.72±0.02	Karaj2	Iran	0.67±0.02			

* origin of these genotypes are not clear although they are cultivated as common varieties in Iran or are used as gene donor in breeding programs at research institutes.

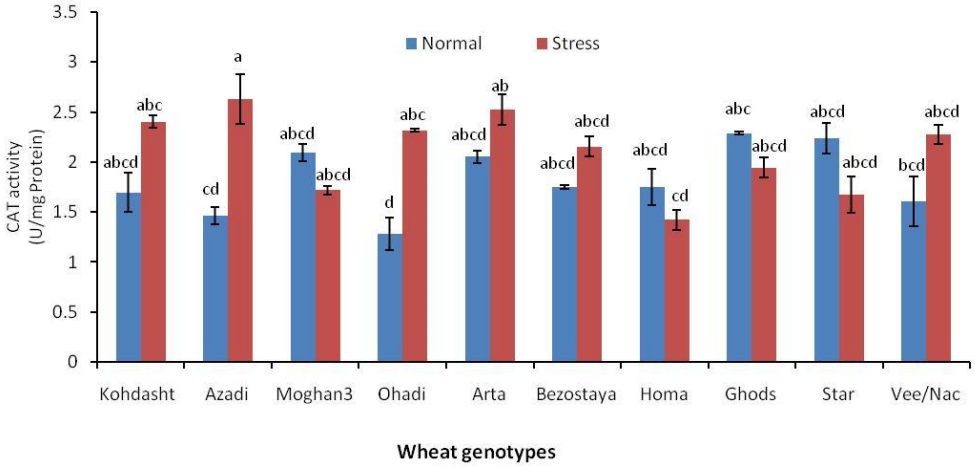

Fig 1. CAT activity in flag leaves of wheat genotypes grown under stress and normal condition. Means with the same alphabetic letters are not different statistically from each other.

($p \leq 0.05$) (Fig. 7). A negative and strong correlation ($r = -0.81$, $p \leq 0.01$) was calculated between POD activity and chlorophyll content under stress condition. The maximum correlation was detected between stomatal density and OA ($r = 0.86$, $p \leq 0.01$).

Discussion

Large scale screening is imperative to detect novel genetic resource for drought tolerance. For this reason here we attempted to examine a large collection of genotypes. Sardari, Tajan, Homa, Shole and Darab 2 had the lowest value for RWC in field experiment of screening 82 genotypes. Based on yield stability experiment under drought stress and normal condition done by Abdolshahi et al. (2012) these genotypes were classified as drought sensitive ones. However the genotypes with highest RWC in our experiment are missing in stable yield cluster in their report probably because water retention ability in plant is only one component of tolerance mechanism and stable genotypes should be equipped with several components to keep on their normal growth and generate satisfactory yield. In pot experiment we focused on evaluation of genotypes selected from the field experiment based on RWC and some other physiological characters. To avoid large experimental error plants were grown in pots to achieve a uniform development of water deficit in soil. In pot

Table 2. Maturity time, rolling ability and silvering score for selected genotypes in field experiment. Maturity time has calculated from day of sowing. Values are mean of three replications of stress experiment along with standard errors (±SE). Rolling score has recorded in a scale ranging from 0(no rolling) to 3(full rolling) under stress condition. Silvering scores ranged from 0 (no silvering) to 3 (complete silvering) recorded at the same time as rolling score was recorded.

Genotype	Days to maturity	Rolling score	Silvering score
Star	66±2.6	2.3	3
Bezostaya	67±1.2	1	3
Vee/Nac	59±0.05	3	1.3
Kohdasht	63±2	2.3	2
Arta	65±1.2	2	1
Moghan3	62 ±2.4	1	2
Ghods	64±1.8	1	0
Azadi	66±1.3	1	0
Ovhadi	66±0.7	1	1
Homa	66±1.3	1	1

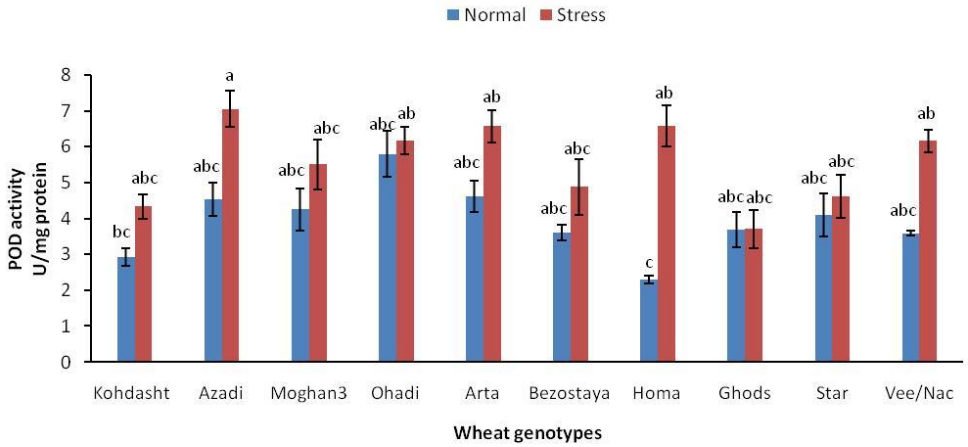

Fig 2. POD activity in flag leaves of wheat genotypes grown under stress and normal condition. Means with the same alphabetic letters are not different statistically from each other.

experiment RWC decrease throughout stress treatment was monitored indirectly as a stress index. Watering carried out for all plants whenever RWC was approximately 55% for the most sensitive genotype as well as wilting was severe. CAT and POD are key enzymes in scavenging and detoxification of hydrogen peroxide, a hazardous byproduct of photorespiration (Hameed et al., 2011). In present experiment the activity of these enzymes rose by stress treatment. The increase in activity was higher in POD across genotypes responding to stress. Induced activity of these enzymes under stress condition has been reported previously (Hameed et al., 2011; Khanna- Chopra and Selote, 2007). The highest difference in activity of CAT in response to drought was observed in Azadi and Ohadi and the highest response in POD activity under stress condition was recorded in Homa. Ohadi and Azadi are among sensitive genotypes in field experiment as they had low RWC under stress condition. In pot experiment these data were confirmed as they had the minimum RWC. It means these genotypes experienced oxidative stress and their antioxidant enzymes triggered to detoxify cells. These results are in consistence with Loggini et al. (1999) and Simova-Stoilova et al. (2009) in which the antioxidant enzyme activity were reported higher in sensitive wheat cultivars. It has been reported that higher OA ability is coincided with higher plant production under stress condition (Blum et al., 1999). During this experiment stress intensity accumulated gradually in six days and ultimately RWC reached to 0.50 - 0.60. For measuring osmotic adjustment plants should have sufficient time to accumulate solutes in their cell to reduce osmotic potential (Blum, 2011). In this experiment Kohdasht had the highest OA. Based on RWC measured in both field and pot experiment it is a tolerant genotype considering at least the water retention ability. In contrast, Arta, Ohadi and Homa had the lowest value of OA and they are classified as sensitive genotypes. Higher OA have been reported to be associated with higher RWC and drought tolerance of wheat genotypes (Bajji et al., 2001). Bajji et al. (2001) reported OA of 0.27-0.31 MPa for tolerant durum wheat genotypes while in sensitive genotype it was as low as 0.1MPa. In our experiment its range was 0.15-0.59 Mpa. Moinuddin et al. (2005) reported OA in a range of 0.37-0.59 MPa for eight bread wheat genotypes. Moreover they reported a highly significant correlation between OA and yield under stress condition. There was a high variability among genotypes in terms of stomata traits measured in adaxial epidermis. Stomata width in most genotypes decreased under stress condition. Plants usually close their stomata in response to drought to avoid water loss. When the guard cells are far from turgor state their width decreases. The decrease in stomatal width was significant in Kohdasht, Arta, Bezostaya and Star. In other words these genotypes responded to drought by closing their stomata. These four genotypes were classified as drought tolerant in field experiment. Moreover Kohdasht showed the highest RWC in pot experiment. Decrease in stomatal width in response to drought has been reported previously in olive (Bosabalidis and Kofidis, 2002). Open stomata causes more transpiration and subsequently the RWC of plants reduce. Under this

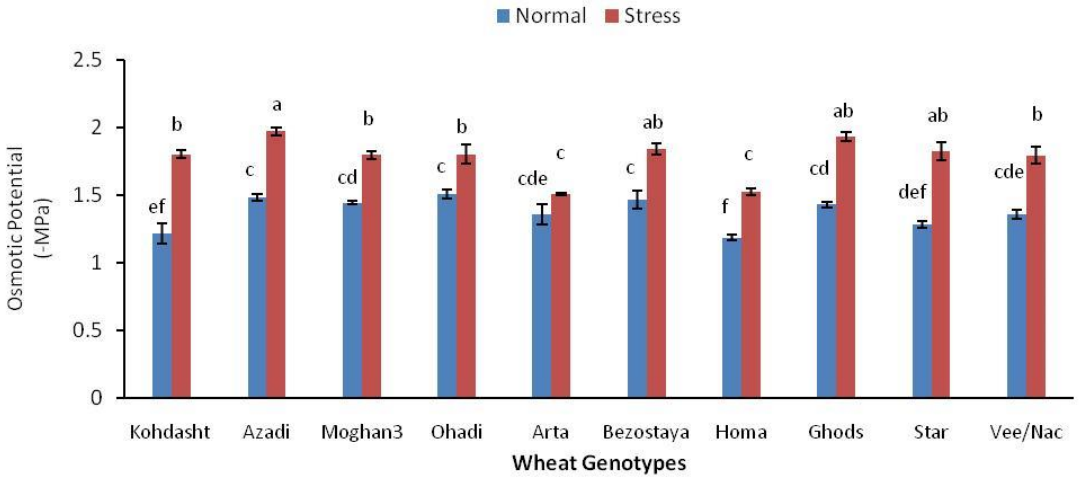

Fig 3. Osmotic potentials of flag leaf extract of ten wheat genotypes grown under stress and normal condition. Means with the same alphabetic letters are not different statistically from each other.

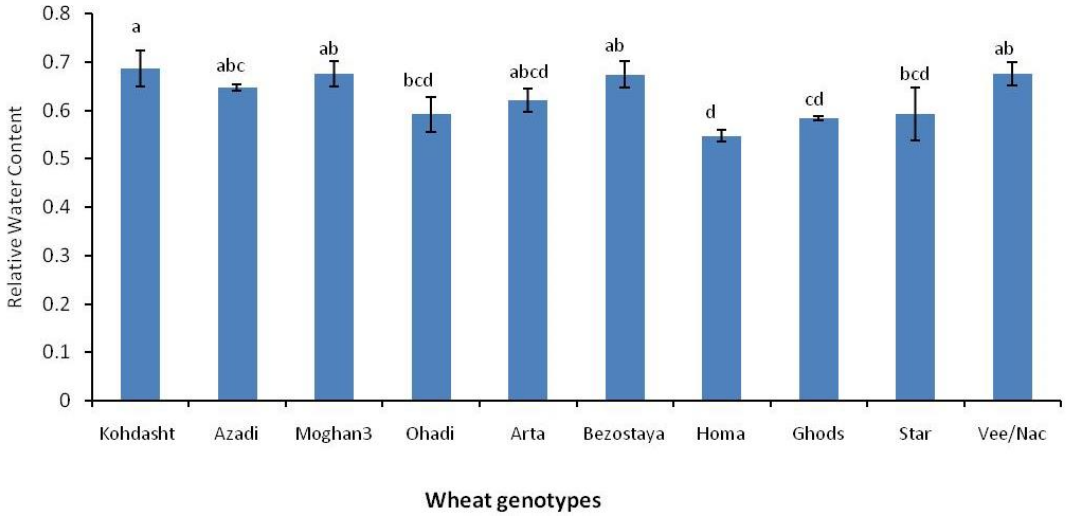

Fig 4. Relative water content (RWC) of flag leaves measured in ten wheat genotypes grown under stress condition. Means with the same alphabetic letters are not different statistically from each other.

condition the genotype loses a lot of water and particularly if drought prolonged for a specified time plant recovery is impossible and death will occur. There was a significant negative correlation between stomatal width (as an indirect measure of stomata conductance) and RWC under stress condition (Fig 7). This result showed that across genetic material used in present experiment water state in plants is managed mainly by stomata closure regulation. Tolerant genotypes maintain water in their leaves by stomatal closure and consequently reduction in leaf gas exchange (Thameur et al., 2012).

Materials and Methods

Field experiment

Plant material and stress treatment

82 wheat genotypes originated from different regions were selected for screening drought tolerance (Table 1). Most of the genotypes were cultivating in Iran through past years and some of them are the most common varieties in the country now. Seeds were sown in field in two separate sites of normal and stress. Experiment had three replications in each site and conducted in a RCBD experimental design. Each genotype was planted in four rows that were 25 cm apart. Seeds were sown at 3 cm space from each other in each row. Stress was applied at the onset of flowering. Soil texture was sandy loam. The wilting point of soil was the moisture equal to 0.14 (w/w) determined by pressure plates. Soil sampling at depth of 30 cm was carried out at stress site every day. Whenever the soil moisture reached to 0.14 then re-watering occurred. Prior to maturity three cycles of stress were applied. At each cycle relative water content (RWC) was measured for all genotypes (Barrs and weatherley, 1962). Days to flowering and maturity was recorded for all genotypes. Date of flowering was recorded whenever half of plants of each plot reached to flowering. Leaf rolling was recorded as a range of 0 (no rolling) to 3 (complete rolling) at the last day of stress treatment. Silvering was recorded as scores ranging from 0 (no silvering) to 3 (complete silvering) at the same time as rolling was recorded. RWC, leaf rolling and leaf silvering was averaged over three time points of stress treatments. Analysis of variance was performed using SAS (SAS Institute Inc., Cary, NC USA).

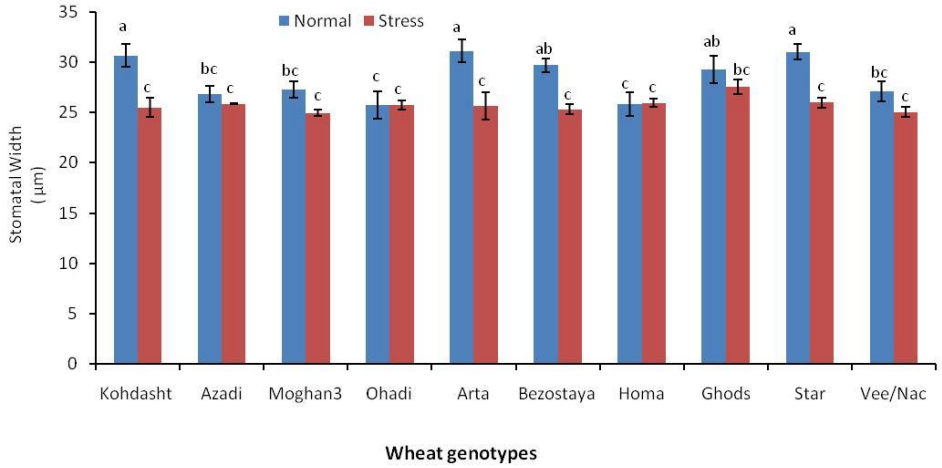

Fig 5. Stomatal width of flag leaves measured in ten wheat genotypes grown under stress and normal condition. Means with the same alphabetic letters are not different statistically from each other.

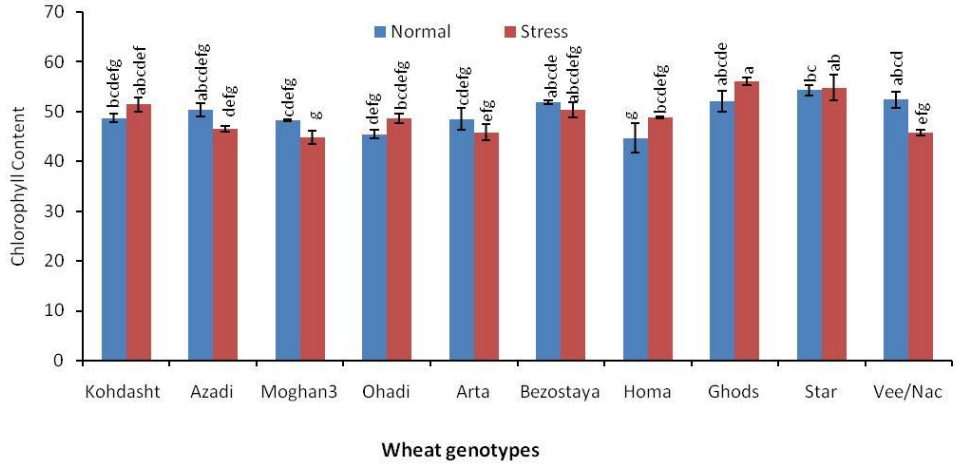

Fig 6. Chlorophyll content in flag leaves of ten wheat genotypes grown under stress and normal condition. Means with the same alphabetic letters are not different statistically from each other.

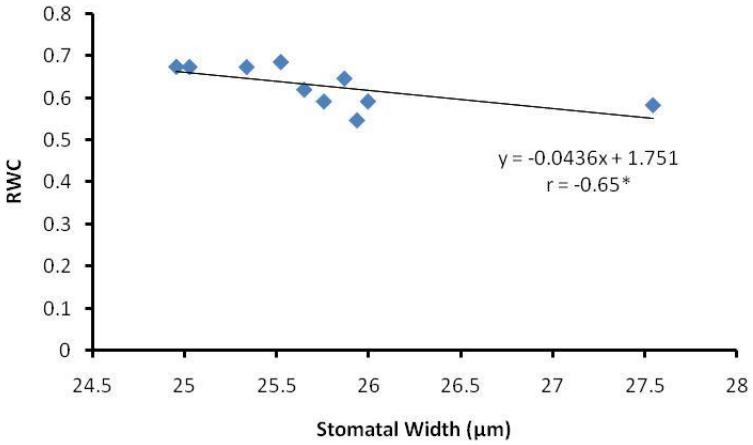

Fig 7. Relationship between stomatal width (µm) and RWC of ten wheat genotypes grown under stress condition. Correlation was significant at 0.05 probability value.

Pot experiment

Plant material and stress treatment

Ten wheat (*Triticum aestivum L.*) genotypes i.e. Kohdasht, Azadi, Moghan3, Ohadi, Arta, Bezostaya, Homa, Ghods, Star and Vee/Nac were used in this experiment. The genotypes were selected based on field experiment. The experiment was conducted in 2011-12 growth season. Plants were grown in pots of 25cm diameter and height. Pots were filled by soil and manure in 3:1 volume ratio, respectively. Soil texture was sandy loam (50% sand, 25% silt and 25% clay) prepared from local research field. It had an electrical conductivity (EC) of 5.3dSm^{-1} and pH of 7.9. Experiment was carried out under normal and stress conditions. The experiment had three independent replications for each treatment and the experiment design for each condition was RCBD. In each pot ten seeds were sown uniformly and thinned to eight after plant emergence to achieve final seed rate of around 160 seeds m^{-2}. Plants were irrigated normally until the onset of flowering. Subsequently under normal condition pots were well watered every three days while under stress condition water withholding prolonged until visual wilting was recorded. To ensure the application of drought stress plants were watered whenever the RWC for the most wilted genotype reached to 0.55±0.01. In average it took six to eight days to detect severe wilting in plants. Pots were placed in wide area and moveable shelters were used whenever the rainfall was predicted. The EC of irrigated water was 2dSm^{-1}. Weeds were controlled manually.

Enzyme assay

For enzyme assay flag leaves were collected. Leaves (0.1 g) were ground in liquid nitrogen and transferred to microtubes. Total soluble protein was measured by Bradford method (Bradford, 1976). CAT activity was determined by the method of Aebi (1984). Briefly, the assay solution (2 mL) contained 50 mM sodium phosphate buffer (pH = 7), 15 mM H_2O_2 and 0.040 mL enzyme extract (Zhang and Kirkham, 2006). POD activity was measured by the method of Chance and Machly (1995). Assay solution (2 mL) contained 25 mM sodium phosphate buffer (pH=7), 2.5 mM guaiacol, 70 mM H_2O_2 and 0.04 mL enzyme extract. The enzyme activity was measured as UmL^{-1} and reported in Umg^{-1} protein (Kim et al, 2012)

Physiological traits

Osmotic adjustment (OA) was calculated as the difference in measured osmotic potential (OP) between non-stressed and rehydrated stressed plants (Babu et al., 1999). Plants experiencing drought at RWC of about 55% were irrigated in the evening and leaves were sampled the next morning for measurement OP. Cut leaves were placed in 1.5-ml microtubes, frozen immediately in liquid nitrogen and stored at -20˚C. To extract sap, samples were transferred to room temperature to be thawed and then a fine hole was bored in the base of 1.5- ml microtube and pushed firmly into a 2-ml microtube. Two ball-bearings with combined mass of 1.5 g rested above each sample. Then microtubes were centrifuged (Sigma, Germany) for 10 min at 10000 rpm. To get rid of small solid particles the extracted sap was centrifuged again for 5 min at 5000 rpm and clear sap was transferred to new microtubes to measure OP. The measurement of OP (mmolkg^{-1}) was performed using osmometer (GONOTEC GmbH, Berlin, Germany). The unit of OP changed to MPa using vant's Hoff equation. This measurement was compared to OP values of leaves that never experienced drought and were well watered. RWC was calculated using Barrs and Weatherley (1962) method.

The chlorophyll content of flag leaves was measured using the chlorophyll meter (SPAD-502). Udding et al. (2007) reported following equation for relationship between SPAD-502 value and chlorophyll concentration in wheat:

$$y = 0.0599e^{0.0493x} \quad (1)$$

where y is chlorophyll concentration (g m^2 projected leaf area) and x is SPAD-502 value.

Stomatal density and their dimension were examined by light microscopy using nail polish imprints (Berger and Altmann, 2000). To obtain a reliable estimate of stomata density and dimensions 15 photos were taken from various areas of a leaf and the average value was reported.

Statistical analysis of data

Analysis of variance and correlation were performed using SAS (SAS Institute Inc., Cary, NC USA) and Microsoft Office Excel 2007, respectively. Means of treatments were compared using Duncan's multiple range test at $p \leq 0.05$. Means of treatments are presented in graphs with standard error bars. Means with different alphabetic letters differ statistically from each other at $p<0.05$.

Conclusion

Under moisture limited environment plant production is influenced by some adaptive traits which contribute to water maintenance in leaves or protect cells against oxidative stress injuries. In conclusion genotype Kohdasht was the most tolerant genotype in present study. It had the maximum OA and the most reduction in stomatal width in response to drought. Due to these two mechanisms which are critical in water retention it showed the maximum RWC. Homa was the most sensitive genotype. It exhibited neither of the mentioned two mechanisms for water retention (i.e. it had low OA and nearly no change in stomatal width in response to drought). Nevertheless it had a significantly higher POD activity in response to drought probably because this is the last mechanism for avoiding cell damage. The results of present research may contribute toward choosing parents for stress tolerance breeding in Iranian wheat cultivars.

Acknowledgments

We thank University of Tehran for financial support of this research. Also we thank Dr Godarz Najafian from Seed and Plant Improvement Institute and Dr Mozafar Rostaii from Dryland Agriculture Research Institute for providing bread wheat seed samples.

References

Abdolshahi R, Safarian A, Nazari M, Pourseyedi S, Mohamadi-Nejad G (2012) Screening drought-tolerant genotypes in bread wheat (*Triticum aestivum L.*) using different multivariate methods. Arch Agron Soil Sci. 59(5): 685-704

Aebi H (1984) Catalase in vitro. Method Enzymol. 105:121-126

Babu RC, Pathan MS, Blum A, Nguyen HT (1999) Comparison of measurement methods of osmotic adjustment in rice cultivars. Crop Sci. 39:150-158

Babu RC, Zhang J, Blum A, Ho T-HD, Wu R, Nguyen HT (2004) HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa L.*) via cell membrane protection. Plant Sci. 166:855–862

Bajji M, Lutts S, Kinet J-M (2001) Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum Desf.*) cultivars performing differently in arid conditions. Plant Sci. 160:669-681

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci. 15:413-428

Berger D, Altmann T (2000) A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in Arabidopsis thaliana. Gene Dev. 14:1119-1131

Bkagwat SG, Bhatia CR (1993) Selection for flag leaf stomatal frequency in bread wheat. Plant Breed. 110:129-136

Blum A (2011) Plant Breeding for Water-Limited Environments. Springer Verlag, New York

Blum A, Zhang J, Nguyen HT (1999) Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crop Res. 64:287-291

Bosabalidis AM, Kofidis G (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Sci. 163:375-379

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-254

Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Method Enzymol. 2:764-775.

Ewers BE, Oren R, Johnsen KH, Landsberg JJ (2001) Estimating maximum mean canopy stomatal conductance for use in models. Can J Forest Res. 31:198-207

Hameed A, Bibi N, Akhter J, Iqbal N (2011) Differential changes in antioxidants, proteases, and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiol Bioch. 49:178-185

Harlan JR (1998) The living fields: our agricultural heritage. Cambridge University Press, UK

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature. 424:901-908

Huseynova IM (2012) Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. BBA-Bioenergetics. 1817:1516-1523

Khanna-Chopra R, Selote DS (2007) Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions. Environ Exp Bot. 60:276-283

Khazaei H, Monneveux P, Hongbo S, Mohammady S (2010) Variation for stomatal characteristics and water use efficiency among diploid, tetraploid and hexaploid Iranian wheat landraces. Genet Resour Crop Ev. 57:307-314

Kim HG, Lee JS, Lee JS, Han JM, Son CG (2012) Hepatoprotective and antioxidant effects of Myelophil on restraint stress-induced liver injury in BALB/c mice. J Ethnopharmacol. 142:113-120

Kusaka M, Lalusin AG, Fujimura T (2005) The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum [L.] Leeke*) cultivars with different root structures and osmo-regulation under drought stress. Plant Sci. 168:1-14

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol. 119:1091-1100

Mackay DS, Ahl DE, Ewers BE, Samanta S, Gower ST, Burrows SN (2003) Physiological tradeoffs in the parameterization of a model of canopy transpiration. Adv Water Resour. 26:179-194

Merah O, Monneveux P, Deleens E (2001) Relationships between flag leaf carbon isotope discrimination and several morpho-physiological traits in durum wheat genotypes under Mediterranean conditions. Environ Exp Bot. 45:63-71

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410

Moinuddin, Fischer RA, Sayre KD, Reynolds MP (2005) Osmotic adjustment in wheat in relation to grain yield under water deficit environments. Agron J. 97:1062-1071

Rebetzke GJ, Richards RA, Condon AG, Farquhar GD (2006) Inheritance of carbon isotope discrimination in bread wheat (*Triticum aestivum L.*). Euphytica. 150:97-106

Shao HB, Liang ZS, Shao MA, Wang BC (2005) Changes of anti-oxidative enzymes and membrane peroxidation for soil water deficits among 10 wheat genotypes at seedling stage. Colloid Surface B. 42:107-113

Simova-Stoilova L, Demirevska K, Petrova T, Tsenov N, Feller U (2008) Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. Plant Soil Environ. 54:529-536

Simova-Stoilova L, Demirevska K, Petrova T, Tsenov N, Feller U (2009) Antioxidative protection and proteolytic activity in tolerant and sensitive wheat (*Triticum aestivum L.*) varieties subjected to long-term field drought. Plant Growth Regul. 58:107-117

Subbarao GV, Chauhan YS, Johansen C (2000) Patterns of osmotic adjustment in pigeonpea - its importance as a mechanism of drought resistance. Eur J Agron. 12:239-249

Tang J, Bolstad PV, Ewers BE, Desai AR, Davis KJ, Carey EV (2006) Sap flux-upscaled canopy transpiration, stomatal conductance, and water use efficiency in an old growth forest in the Great Lakes region of the United States. J Geophys Res. 111:1-12

Thameur A, Lachiheb B, Ferchichi A (2012) Drought effect on growth, gas exchange and yield, in two strains of local barley Ardhaoui, under water deficit conditions in southern Tunisia. J Environ Manage. 113:495-500

Udding J, Gelang-Alferdsson J, Piikki K (2007) Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth Res. 91:37-46

Zhang J, Kirkham M B (2006) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytolo. 132:361-373

Zhang J, Nguyen HT, Blum A (1999) Genetic analysis of osmotic adjustment in crop plants. J Exp Bot. 50:291-302