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Differential changes in Phytohormones, oxidative damage and yield of wheat Genotypes under drought stress at post anthesis stage

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In this study, we measured the variations of plant hormones under well-watered and drought stress conditions during post anthesis stage in a field experiment. Hormonal levels in the flag leaves of Azar-2, Sardari (tolerant to drought stress), HN7, DH-2049 (moderately tolerant to drought stress), SARA and TEVEE (susceptible to drought stress) genotypes were monitored continuously at 7, 17 and 23 days post anthesis. The contents of Indole-3-acetic acid (IAA) and abscisic acid (ABA) were higher in tolerant genotypes than in susceptible ones at all the stages. The IAA content also increased during the first and second drought stages in wheat flag leaves, but it dropped sharply thereafter under drought stress. ABA content increased dramatically in flag leaves under stress as well as with age, and then the highest levels of ABA were observed at third drought stage. The GA_3 content decreased in wheat flag leaves subjected to drought stress. The effects of water stress on hydrogen peroxide content, electrolyte leakage, glutathione content and two antioxidant enzymes (Glutathione reductase and Ascorbate peroxidase) were also studied. It was observed that drought stress lead to oxidative damage to plant cells that damaging effects of oxidative stress was higher in susceptible than in tolerant and moderately tolerant genotypes. The results suggest that the shortened maturity duration and higher kernel weight in tolerant genotypes than others under drought stress is associated to others phytohormone content in the flag leaves of these genotypes.

Keywords: antioxidants, drought, oxidative stress, phytohormones, wheat, yield

INTRODUCTION

Drought is one of the most important abiotic factors limiting plant growth in arid and semiarid regions (Kramer and Boyer, 1995), and it is mainly caused by high evaporative demand and low water availability (Patakas and Notsakis, 2001). Plants often suffer from drought stress, and the severity of the resulting damage varies depending on its intensity and duration. Drought stress could lead to the disruption of cellular membranes, making them more permeable to ions by increased solubilization and peroxidation of membrane lipids under

stress conditions and thus impairs both membrane structure and function (Saneoka et al., 2004).

Wheat (*Triticumaestivum*L.) is the staple food for more than 35% of the people in the world and is grown on over 95% of the wheat growing area. Wheat which often experiences water-limited conditions, is an attractive study system because of the natural genetic variation in traits related to drought tolerance (Loggini et al., 1999). During grain development of wheat, appropriate soil water status is of key importance for accumulation assimilates in grains and thus formation of grain yield and quality (Ahmadi and Baker, 2001).

Plants respond to drought and adapt to drought stress through various biochemical changes (Monneveux and Belhassen, 1996), including changes of the endogenous hormone levels, especially Abscisic acid (ABA).Plants exposed to drought stress can recruit ABA as an endogenous signal to initiate adaptive responses (Zhu, 2002; Wang et al., 2008). However, the variation of IAA and GA contents under drought stress are contradictory. It was reported that drought resulted in a decrease of IAA content in the leaves of wheat (Xie et al., 2003). However, adaptation to drought was accompanied with an increase in the IAA content (Sakurai et al., 1985; Pustovoitova et al., 2004).

Lur and Setter (1993) observed Indole-3-acetic acid (IAA) concentrations increased in the endosperm of maize (*Zea mays*) kernels at about 10 d after pollination. Kato et al (1993) reported that ABA content in large-size grains was higher than that in small-size grains during rice grain filling. Wang et al (1998) and Yang et al (1999) suggested that the poor grain filling was associated with low IAA and ABA contents in rice grains.

Improving grain filling is important in cases where slow grain filling is a problem, e.g. heavy use of nitrogen fertilizer (Ling et al., 1993; Yang et al., 1996, 2001;Wang et al. 2008), or adoption of lodging-resistant cultivars of which some stay “green” for too long (Yuan, 1997; Zhu et al., 1997; Yang et al., 2001). But yet, our knowledge about the variation of IAA and GA contents in different genotypes of crop plants at under water stress is very rare (Yang et al. 2001; Xie et al., 2003), particularly when the drought occurs during reproductive growth, affecting production whether it is for subsistence or economic gain (Khana-Chopra and Selote, 2004).

Besides these, researchers have linked various physiological responses of wheat plants to drought with their tolerance mechanisms such as membrane stability and oxidative damages to plant cells and antioxidant protection (Loggini et al., 1999; Sairam et al., 2001; Hameed et al., 2011). However, reports regarding variations in these physiological parameters on genotypic basis at reproductive stages such as post anthesis are very rare.

Thus, we undertook an experiment to test the six prevalent wheat genotypes under well watered and drought stress at 7, 17 and 23 days post anthesis (DPA).

MATERIALS AND METHODS

Wheat (*Triticumaestivum*L.) cvs Azar-2 and Sardari (tolerant to drought stress), DH-2049, HN7 (moderately tolerant to drought stress), SARA, TEVEE (susceptible to drought stress) were grown in the field of Agricultural Dryland Research Station, Maragheh, Iran (37º 25´ N, 46º 40´E, 1619 m.s.l.). The seeds were sown on 22 November 2008, in 5 rows with 20 cm row spacing and interplant space of 10 cm adjusting seeding rate of 200 seeds m–2. Soil had an electrical conductivity (EC) 1.4 dSm^{-1}, pH 7.5 and sodium adsorption ratio (SAR) of 1.32. Fertilizer was applied at the rate of 110:65:60 kg ha^{-1} N:P:K as split dose, first at 20 days after sowing (DAS) at the rate of 60:65:60 kg ha^{-1}N:P:K and second at 85 DAS at the rate of 50:0:0 kg ha^{-1}N:P:K. Plants were watered as and when required to keep them fully turgid.

The water stresstreatment was applied at 7, 17 and 23 days after anthesis (DAA) for a uniform period of18 days at each treatment.At all stages of plant growth in controlled conditions, soil moisture was maintained at field capacity. Samples for various assays were taken from flag leaves between 10:30 and 11:30 h at the end of each treatment period. Anthesis in each variety was considered to have occurred when approximately 50 % of main shoot ears showed anther dehiscence (Sairam and Srivastava, 2001). However the duration to anthesis was shortened by 4–5 days due to drought stress in all six genotypes. Total rainfall received by wheat plants from sowing to physiological maturity was 2.2 cm. Rainfall was very low between anthesis and post-anthesis periods. Hydrogen peroxide was assayed with titanium reagent (Teranishi et al., 1974). One gram of titanium dioxide and 10 g of potassium sulphate were mixed and digested with 150 ml of concentrated sulphuric acid for 2 h on a hot plate. The digested mixture was cooled and diluted to 1.5 l with distilled water and used as the titanium reagent. Sample preparation and H2O2 estimation were determined as described by Mukherjee and Choudhuri (1983). Membrane integrity was determined by relative electrolyte leakage (EL) of 2 cm leaf segments floating on distilled water for 24 h at 4 °C (using a conductivity meter), and expressed in percentage of the total leaf electrolyte content obtained after boiling the segments (Nunes and Smith, 2003).

The glutathione (GSH) content was measured as described by Griffith and Meister (1979). Fresh leaves were homogenized in 2 mL of 2 % metaphosphoric acid and centrifuged at 17,000 x gfor 10 min. The addition of 0.6 mL 10% sodium citrate neutralized the supernatant. One milliliter of assay mixture was prepared by adding 100 µL extract, 100 µL distilled water, 100 µL of 6 mM 5,5–dithio–bis–(2–nitrobenzoic acid) (DTNB) and 700 µL

of 0.3 mM NADPH. The mixture was stabilized at 25 ºC for 3–4 min. Then, 10 µL of glutathione reductase was added, and the absorbance was measured at 412 nm in a spectrophotometer; the GSH content is expressed in µg g^{-1}dry weight (DW).

Antioxidant enzymes assay

The GR activity was determined spectrophotometrically at 30 ◦C as described by Barata et al (2000) in a reaction mixture consisting of 3 ml 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM 5,5''-dithiobis(2-nitrobenzoic acid), 1 mM oxidized glutathione and 0.1 mM NADPH. The reaction was initiated by the addition of 50 µL of plant extract. The rate of reduction of oxidized glutathione was followed by monitoring the increase in absorbance at 412 nm over 2 min. The GR activity of the extract was expressed as GR unit$min^{-1}mg^{-1}$protein. Ascorbate peroxidase (APX) was assayed by recording the decrease in absorbance at 290 nm due to a decrease in ascorbic acid content (Nakano and Asada, 1981). Reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7.8), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.5 mM $H_2O_2$and 0.1 mL enzyme extract. The reaction was started with the addition of H2O2 Absorbance was measured at 290 nm for 3 min. The APX activity of the extract was expressed as APX unit $min^{-1}mg^{-1}$protein.

Determination of plant phytohormones

Five grams of leaf samples were used for ABA, GA_3 and IAA extraction, according to the method of Kelen et al (2004). Briefly, leaf samples were homogenized in methanol 70% and stirred overnight at 4 ◦C. After filtration through a Whatman 0.45 µM filter, the extracts were completely evaporated under vacuum and dissolved in water. The solutions were partitioned with diethyl ether three times and then passed through anhydrous sodium sulphate. After evaporation of the non-polar phase, the dry residue was dissolved in 3 mL of methanol and the solution was used directly for injections. Analysis was performed in three replicates for each treatment.

The high performance liquid chromatography (HPLC) analysis was performed on a Waters HPLC system equipped with Empower software, a pump (Waters 600, USA), and a UV-Vis detector (Waters model 2487). A column, µBondapack^{TM}C18, from Waters (Ireland) was used for the separation. The mobile phase was acetonitrile-water containing 30 mM phosphoric acid at pH 4.0, flow rate 0.8 µL min^{-1}. For each extraction, three different injections in HPLC were performed. Each of the standard solutions was detected at wavelengths of 208, 265 and 280 nm for GA_3, ABA and IAA, respectively. To quantify the GA_3, ABA and IAA contents, known amounts of pure standards (Sigma) were injected into HPLC system and equations, correlating peak area to concentrations of GA_3, ABA and IAA, were formulated.

Statistical analysis

All data were subjected to two analysis of variance (ANOVA) using the GLM procedure in SAS release 9.1.2. The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. The significance of differences among treatment means were compared by Fisher's least-significant difference test (LSD). Values presented in graphs are mean ± SD. In graphs, bars with different alphabets differ significantly from each other.

RESULTS

Hydrogen peroxide content (H_2O_2) in the flag leaves of wheat cultivars increased under water stress (Figure 1). The lowest H_2O_2contents were observed in Azar-2 and Sardari, and the highest in SARA and TEVEE at all stages both under well-watered and under drought stress conditions. DH-2049 and HN7 showed intermediate levels at all stages under both well-watered and under drought stress conditions. The electrolyte leakage (EL) also increased markedly under first drought stage and then showed a declining trend after second stage (Figure 2). The highest EL was observed in SARA and TEVEE and the lowest in Azar-2 and Sardari under well-watered conditions. Similar results were also obtained under drought stress conditions. GSH content decreased under water stress (Figure 3). All the genotypes showed higher GSH content at the first drought stage (7 DPA). Amongst the genotypes, the highest GSH content was observed in Azar-2 and Sardari, and the lowest in SARA and TEVEE at all stages in both environments. The GSH also decreased under drought stress and also showed a declining trend with age.

GR activity increased significantly under drought stress conditions at all the stages (Figure 4). The genotypic response was significant at all stages under both well waterd and drought stress conditions, so the highest GR activity was observed in tolerant genotypes (Azar-2 and Sardari) and the lowest in susceptible genotypes (SARA and TEVEE) under both well watered and drought stress conditions. Moderately genotypes (DH-2049 and HN7) also exhibited an intermediate response. Ascorbate peroxidase (APX) activity increased under drought stress as well as with age (Figure 5). The lowest APX activity were observed in SARA and TEVEE, and the highest in Azar-2 and Sardari at all stages both under well-watered

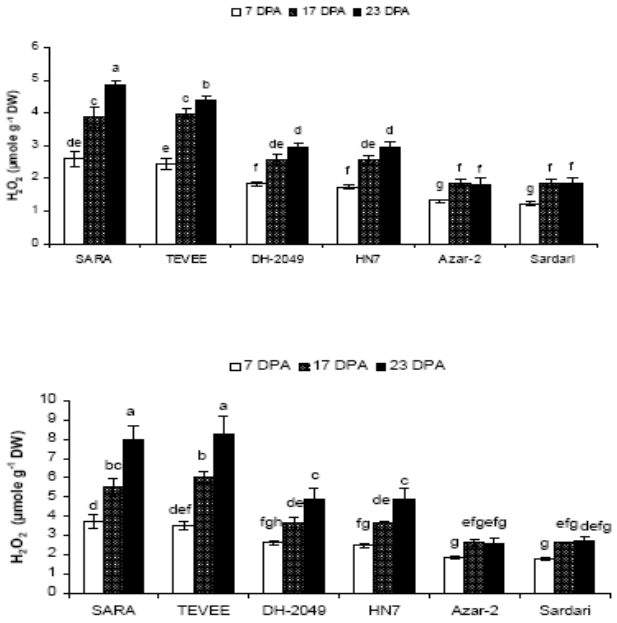

Figure 1. Hydrogen peroxide (H_2O_2) in flag leaves of wheat genotypes grown under well watered (high) drought stress (low) conditions at post anthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

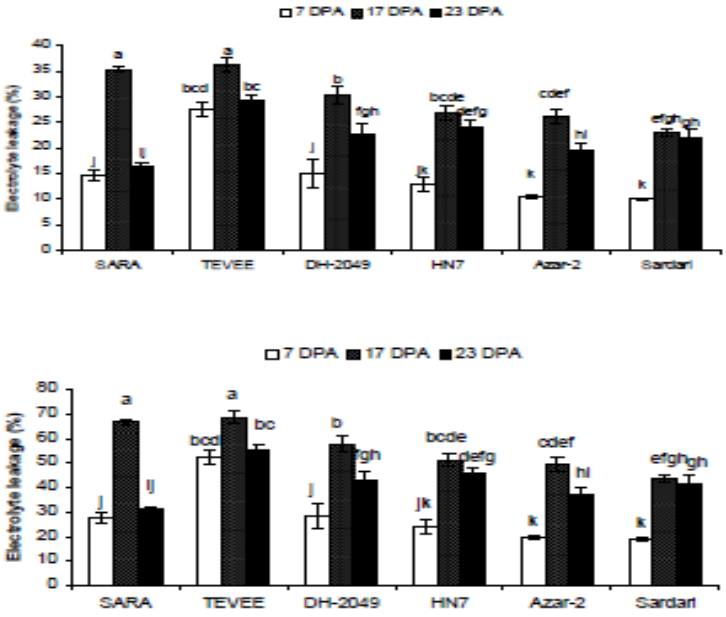

Figure 2. Electrolyte Leakage in flag leaves of wheat genotypes grown under well watered (high) drought stress (low) conditions at postanthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

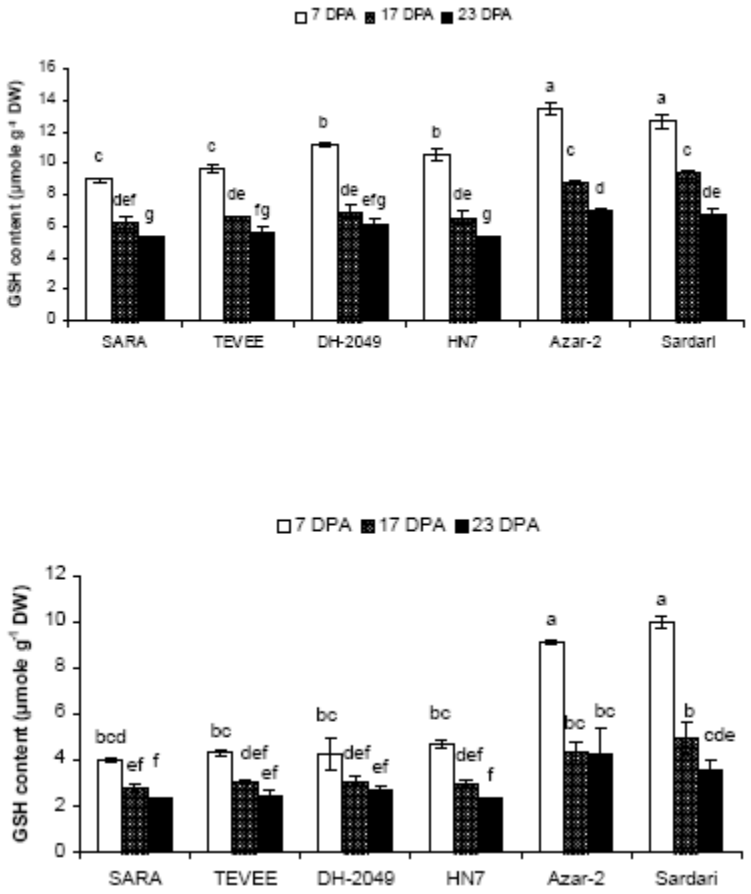

Figure 3. Glutathione content (GSH) in flag leaves of wheat genotypes grown under well watered (high) drought stress (low) conditions at post anthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

and under drought conditions.

DH-2049 and HN7 showed intermediate levels at all stages under both well-watered and drought stress conditions. Under drought stress conditions, endogenous ABA contents were sharply increased under drought stress as well as with age (Figure 6). The lowest ABA contents was observed in SARA and TEVEE, and the highest in Azar-2 and Sardari at all stages both under well watered and under drought conditions. Two moderately tolerant genotypes showed intermediate ABA contents at all stages under both well-watered and drought stress conditions.

The endogenous IAA content showed an increasing trend with age under well-watered conditions (Figure 7). Drought stress notably decreased IAA contents. Under drought stress conditions, IAA content increased from 7 DPA and reached a peak at 17 DPA under water stress; however, it dropped at 23 DPA (Fig. 7). The lowest IAA contents was observed in SARA and TEVEE, and the highest in Azar-2 and Sardari at all stages both under well-watered and under drought conditions. Two moderately tolerant genotypes showed intermediate IAA contents at all stages under both environments. Similar to IAA, GA_3 content displayed an increasing trend with age under well-watered conditions (Figure 8). Under drought stress conditions, the levels of endogenous GA_3 changed inconsistently among genotypes and drought stages. The genotypes TEVEE (susceptible), DH-2049 (moderately tolerant) and Sardari (tolerant) showed a declining trend with age under drought stress. But, the genotypes SARA

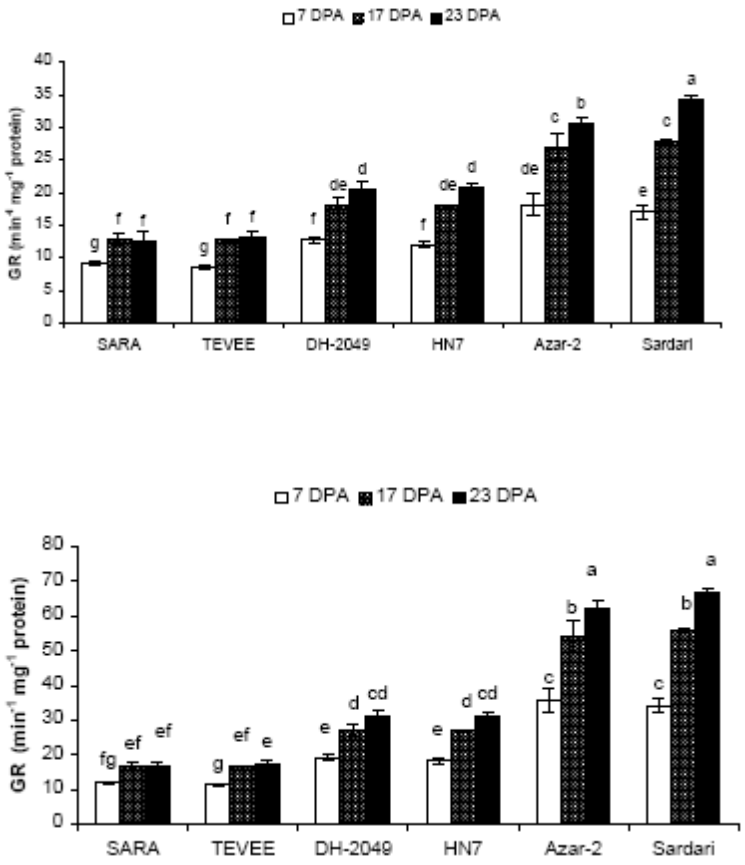

Figure 4. Glutathione reductase activity (GR) in flag leaves of wheat genotypes grown under well-watered (high) drought stress (low) conditions at post anthesis stage. D7- drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

(susceptible) and HN7 (moderately tolerant) showed an increase from 7 DPA to 17 DPA; however, it dropped at 23 DPA. The lowest GA_3 contents was observed in SARA and TEVEE, and the highest in Azar-2 and Sardari both under well-watered and under drought conditions. As shown in Table 1, final grain yield was highest in case of SARA while lowest in DH-2049 in well-watered plants. Under drought stress conditions, HN7 exhibited the highest grain yield, closely followed by Sardari and Azar-2, while SARA and DH-2049 showed the lowest grain yield. Lowest grain yield in all the genotypes were observed at third drought stage (23 DPA), and the highest grain yield in all the cultivars except Sardari were observed under control conditions. The lowest rate of decrease in yield was obtained in tolerant gentypes (0.33 in Sardari), and the highest (0.72 in SARA) was obtained in susceptible genotypes.

Under drought stress conditions, number of kernels per spike and maturity duration (MD) was decreased under drought stress (Table 1). Maturity duration (MD) was calculated from the onset of anthesis stage to physiological maturity in the studied genotypes. The lowest number of kernels and MD was observed in Azar-2 and Sardari (tolerant genotypes), and the highest in SARA and TEVEE (susceptible genotypes) under both wellwatered and under drought stages. Two moderately tolerant genotypes had intermediate number of kernels at all stages under both well-watered and drought stress conditions. Drought stress at 7 DPA led to higher reduction in number of kernels and MD, followed by drought at 17 and 23 DPA (Table 1).

Similar to the number of kernels per spike and MD, 1000 kernel weight was decreased under drought stress (Table 1). But the lowest 1000 kernel weight was

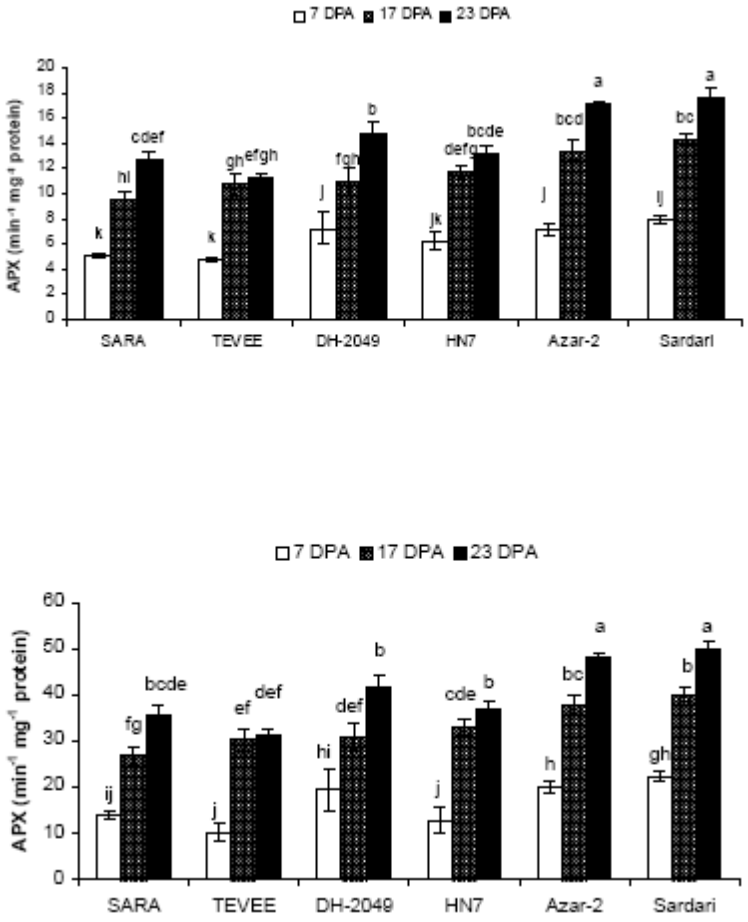

Figure 5. Ascorbate peroxidase activity (APX) in flag leaves of wheat genotypes grown under well-watered (high) drought stress (low) conditions at post anthesis stage. D7- drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

observed in SARA and TEVEE (susceptible genotypes), and the highest in Azar-2 and Sardari (tolerant genotypes) under both well-watered and under drought stages (Table 1).

DISCUSSION

The development of more drought-resistant crops is necessary to alleviate future threats to food availability in the world (Plucknett et al., 1987). However, this requires comprehensive studies of the many potential genetic resources and understanding of the adaptive mechanisms and responses to drought stress that allows survival in arid and semiarid environments (Sairam et al., 2001; Rampino et al., 2006).

Physiological and genetic evidence clearly indicates that the reactive oxygen species (ROS) scavenging systems of crop plants are an important component of the stress protective mechanism (Noctor and foyer, 1998; Noctor et al., 2002; Sairam and Srivastava, 2001). In this study, both the tolerant genotypes had lower H_2O_2 content and electrolyte leakage under drought stress than the susceptible genotypes SARA and TEVEE, while DH-2049 and HN7 (moderately tolerant cultivars) exhibited an intermediate response. This may be due to higher membrane stability in tolerant cultivars (Azar-2 and Sardari), than in susceptible ones (SARA and TEVEE) (Sairam et al., 2001).

Hydrogen peroxide is a toxic compound produced as a result of the dismutation of the superoxide radical, and a higher concentration is injurious to the plant cells,

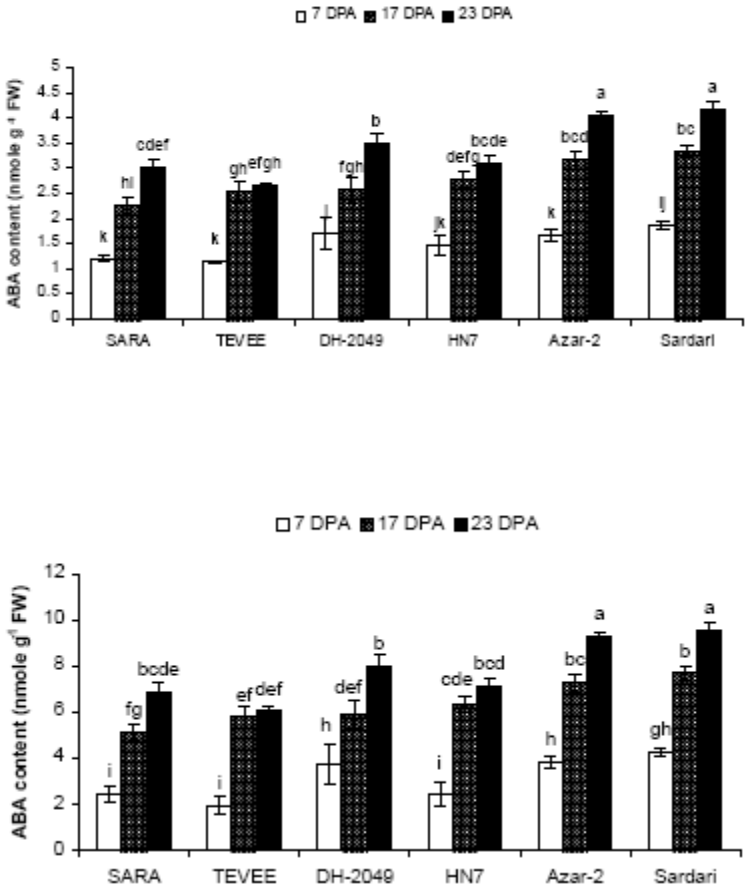

Figure 6. Abscisic acid content (ABA) in flag leaves of wheat genotypes grown under well watered (high) drought stress (low) conditions at post anthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

resulting in lipid peroxidation and membrane injury (Menconi et al., 1995, Loggini et al., 1999; Sairam et al., 2001).

Tolerant genotypes, which had lower H_2O_2content and electrolyte leakage under drought stress, also showed higher GR and APX activity as compared to susceptible and moderately tolerant genotypes under drought stress as well as well-watered conditions at all the stages. These two antioxidant enzymes are involved in the scavenging of the products of oxidative stress, such as hydrogen peroxide generated in the chloroplast (Gamble and Burke, 1984; Gillham and Dodge, 1986; Moran et al., 1994; Jagtap and Bhargava, 1995; Sairam and Srivastava, 2001), and thus help in ameliorating the damaging effects of oxidative stress. Elevated GR activity during stomatal closure in response to drought stress may also serve to ensure the availability of NADP to accept electrons derived from photosynthetic electron transport, thereby directing electrons away from oxygen and minimizing the chances of production of superoxide radicals (Egneus et al., 1975; Foster and Hess, 1982;Elstner, 1987; Sairam and Saxena, 2001). In this study tolerant genotypes also showed higher GSH content activity as compared to susceptible and moderately tolerant genotypes. Increased GR and APX activity in drought–tolerant genotypes of maize (Pastori and Trippi, 1992), tomato (Walker and McKersie, 1993), tobacco (Van Rensburg and Kruger, 1994) and wheat (Sairam and saxena, 2001) has also been reported. Lesser oxidative damage in the tolerant wheat cultivar during osmotic stress has been attributed to higher AsA and GSH content, and induction of AsA_GSH cycle

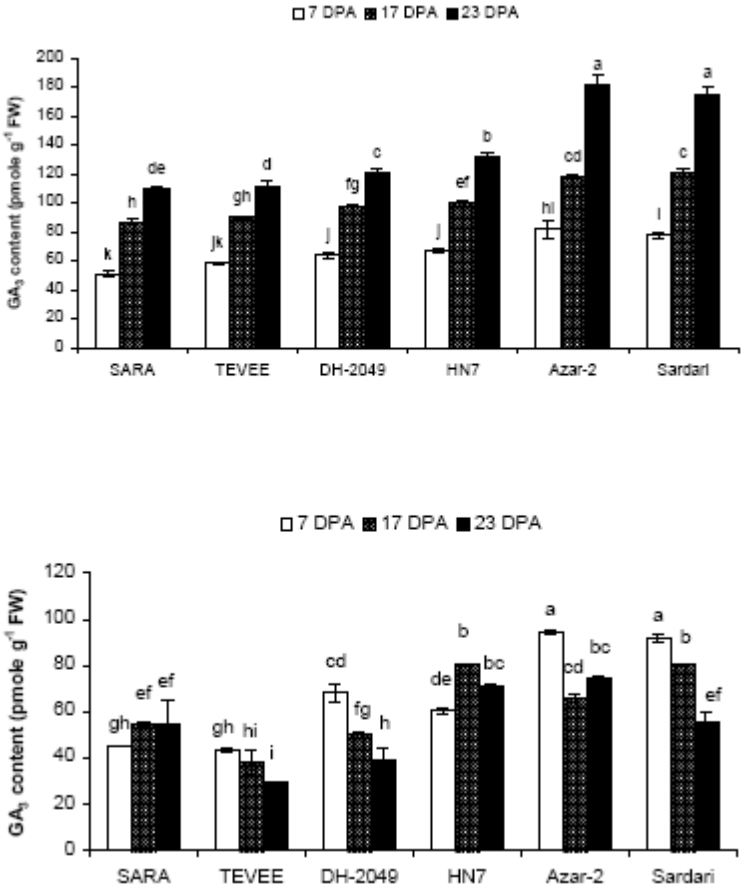

Figure 7. Gibberellic acid content (GA3) in flag leaves of wheat genotypes grown under well-watered (high) drought stress (low) conditions at post anthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

enzymes (Lascano et al., 2001; Sairam and saxena, 2001).

A number of studies have described the ABA content in tolerant and susceptible wheat genotypes and the relationship between ABA content and the grain-filling rateWhich corresponded with the findings of this study (Yang et al., 2001; Wang et al., 2008; Yang et al., 2006). Here, we found that the maturity duration (MD) of all the genotypes, under either the well-watered or drought stress condition, were closely associated with ABA contents in flag leaves (Fig 6, Table 1). The tolerant genotypes had higher ABA concentrations and shorter MD than the susceptible genotypes. In spite of MD, drought stress led to a reduction in number of kernels per spike in both tolerant and susceptible genotypes, and this increase was also accompanied by an increase in ABA content in these genotypes. These results imply that ABA was responsible for senescence acceleration and grain-filling shortening owing to the lasting water stresses (Nooden, 1988; Yang et al., 2006).

It has also been proposed that ABA has a major role in relation to sugar-signaling pathways and enhances the ability of plant tissues to respond to subsequent sugar signals (Rook et al., 2001; Davies, 2004). There are many reports that ABA can enhance the movement of photosynthetic assimilates towards to developing seeds (Dewdney and McWha, 1979; Ackerson, 1985; Brenner and Cheikh, 1995). The present results showed that endogenous GA3 contents under well-watered conditions changed inconsistently at the postanthesis stage, but markedly decreased under drought stress conditions. The present finding suggests that the reduced GA3 contents

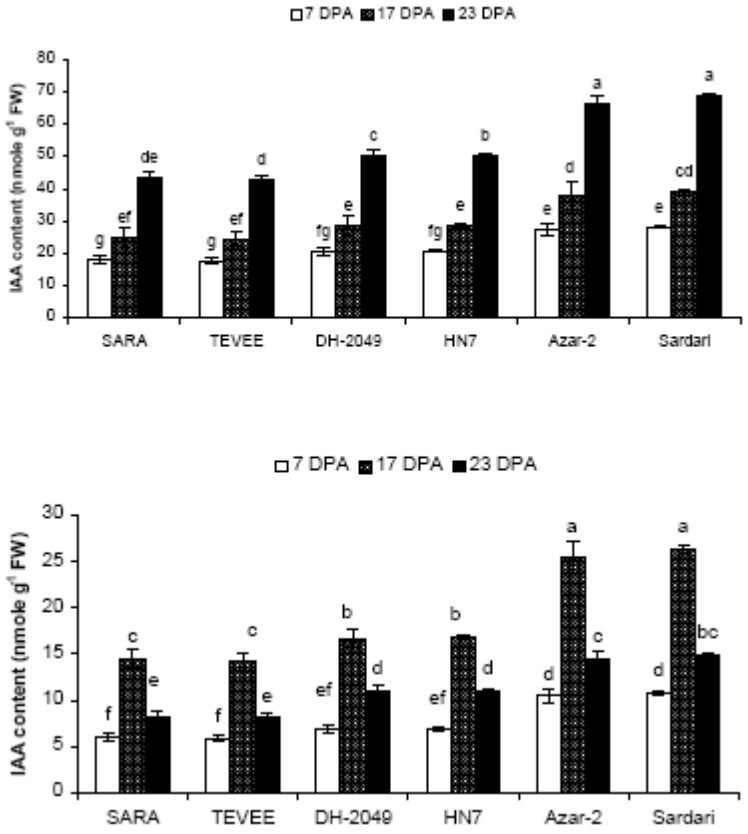

Figure 8. Indole-3-acetic acid content (IAA) in flag leaves of wheat genotypes grown under well-watered (high) drought stress (low) conditions at post anthesis stage. D7-drought at 7 days after anthesis, D17-drought at 17 days after anthesis, D23-drought at 23 days post anthesis.

under drought stress may be attributed to the depressed GA synthesis resulting from the accelerating senescence of the organs (Xie et al., 2003; Yang et al., 2006). Amongst the genotypes, tolerant genotypes had higher GA3 contents under both well watered and drought stress conditions. As a result, GA3 contents in the flag leaves at postanthesis stage under drought stress conditions were positively related to 1000 kernel weight, suggesting that GA_3 plays an important role in accumulation of assimilates in wheat grains.

It has been reported that IAA level in wheat grains increased at the postanthesis stage, and played an important role in regulation of grain filling (Brenner and Cheikh, 1995; Xie et al., 2003). The positive effects of IAA on photo assimilate translocation within developing wheat grains have also been reported in other studies (Bangerth et al., 1985; Darussalam and Patrick, 1998). These results are consonant with the present study, indicating that IAA may be involved in photosynthetic translocation into grains. Here, we also observed that the 1000 kernel weight of all the genotypes, under either the well-watered or drought stress condition, were closely associated with IAA contents in flag leaves (Fig. 7, Table 1). The tolerant genotypes (Azar-2 and Sardari) had higher IAA contents and higher 1000 kernel weight than the susceptible genotypes that may be due to the higher grain filling rate in tolerant genotypes than that of susceptible ones. Davies (1987) stated that auxin also stimulates cell division. It has been reported that IAA can increased the transpiration rate by inducing stomatal opening, with a concomitant increase in transpiration and photosynthesis rate (Wittenbach, 1983; Nawadkar and Anserwadenkar, 1989). In addition, since the high IAA content of flag leaves can lead to the enhancement of ethylene synthesis (Mckeon and Yang, 1988; Wang et al., 2008), the higher IAA contents in tolerant genotypes under drought stress at postanthesis stage may be related to the stress induced shortening in grain-filling

Table 1. Effects of drought stress on final grain yield, Kernels per spike, 1000 kernel weight and Maturity duration of different wheat genotypes.

Genotype	Treatments	Grain yield (kg hec^{-1})	Kernels spike^{-1}	1000 kernel weight (gr)	Maturity duration (day)
SARA	Control	3779 ± 101 a	29.73 ± 0.74 ab	28.28 ± 0.73 hij	38.92 ± 1.08 a
	Drought 7 DPA	2186 ± 72 lmn	19.29 ± 0.91 g	20.96 ± 1.33 n	31.85 ± 0.57 efgh
	Drought 17 DPA	1865 ± 146 op	22.16 ± 0.65 ef	24.16 ± 0.77 lm	34.26 ± 0.55 cde
	Drought 23 DPA	1446 ± 18 q	25.19 ± 0.44 cd	25.88 ± 0.92 kl	36.24 ± 0.35 bc
TEVEE	Control	2519 ± 67 hij	30.34 ± 0.83 a	28.56 ± 0.78 ghij	38.72 ± 0.51 ab
	Drought 7 DPA	2191 ± 48 lm	18.61 ± 0.45 g	22.11 ± 0.37 mn	28.26 ± 2.34 ij
	Drought 17 DPA	1878 ± 110 op	22.12 ± 0.91 ef	24.91 ± 0.58 l	36.39 ± 0.45 abc
	Drought 23 DPA	1517 ± 10 q	25.02 ± 0.55 cd	26.39 ± 0.58 jkl	37.54 ± 10 ab
DH-2049	Control	2125 ± 65 lmn	27.95 ± 1.14 b	33.96 ± 0.81 cd	36.28 ± 1.64 bc
	Drought 7 DPA	2170 ± 32 lmn	16.12 ± 2.37 h	25.01 ± 1.02 l	27.36 ± 1.52 jk
	Drought 17 DPA	1839 ± 93 p	22.11 ± 0.21 ef	27.72 ± 0.20 hijk	32.06 ± 0.27 efgh
	Drought 23 DPA	1461 ± 30 q	24.06 ± 0.35 cde	29.90 ± 0.83 fgh	33.56 ± 0.13 defg
HN7	Control	3244 ± 87 bc	25.70 ± 0.28 c	33.19 ± 0.94 de	38.23 ± 0.15 ab
	Drought 7 DPA	2821 ± 61 efg	18.84 ± 0.33 g	24.51 ± 0.91 l	28.37 ± 0.60 ij
	Drought 17 DPA	2460 ± 125 ijk	20.75 ± 0.27 fg	27.45 ± 0.22 ijk	31.07 ± 0.75 gh
	Drought 23 DPA	1954 ± 63 nop	23.31 ± 0.79 de	29.62 ± 0.69 fghi	33.52 ± 0.27 defg
Azar-2	Control	3464 ± 92 b	25.02 ± 0.08 cd	42.60 ± 1.21 a	33.95 ± 0.80 cdef
	Drought 7 DPA	2713 ± 66 fgh	15.37 ± 0.97 h	31.29 ± 0.69 ef	26.03 ± 1.00 jk
	Drought 17 DPA	2827 ± 133 efg	18.92 ± 0.28 g	33.86 ± 0.56 d	30.06 ± 0.95 hi
	Drought 23 DPA	2087 ± 14 mno	22.29 ± 0.37 ef	37.21 ± 0.80 b	31.56 ± 0.57 fgh
Sardari	Control	2449 ± 60 hij	23.82 ± 0.36 cde	41.67 ± 1.21 a	34.80 ± 0.31 cd
	Drought 7 DPA	2739 ± 121 fgh	16.29 ± 0.95 h	30.84 ± 0.76 fg	25.39 ± 0.34 k
	Drought 17 DPA	2388 ± 73 jkl	19.32 ± 0.21 g	33.35 ± 0.55 de	30.09 ± 1.59 hi
	Drought 23 DPA	1897 ± 73 op	22.07 ± 0.24 ef	36.25 ± 0.81 bc	31.66 ± 0.87 efgh

Values are means ±S.E. (n = 8) and differences between means were compared by Fishers least significance test. Different letters indicate significant differences at $P < 0.05$.

period and the acceleration of plant senescence. Generally, our results showed that oxidative stress as a result of drought stress conditions, increases H2O2 contents and electrolyte leakage, resulting in greater membrane injury. The higher antioxidant enzyme activity in tolerant genotypes was associated with the lower H_2O_2contents and electrolyte leakage. The finding of higher GSH content, and higher GR and APX activity in tolerant genotypes compared with susceptible genotypes, provide better protection from oxidative stress, which otherwise could cause damage to the cell membrane and organelles, protein and DNA structure and inhibit photosynthesis and other enzyme activities. The changes in oxidative damage, grain yield, 1000 kernel weight, kernels per spike and maturity duration under drought were associated with the reduced contents of IAA and $GA_3$and elevated content of ABA in different wheat genotypes. However, the complex regulatory network of plant hormone signaling in plants subjected to drought stress needs to be explored, and the stress-related genes involved in the network of plant hormone signaling await identification. The changes of endogenous hormones in the flag leaves of wheat plants subjected to drought stress were detailed, which would allow a better understanding of the drought response and improvement strategy for drought tolerance of crop plants.

REFERENCES

Ackerson RC (1985). Invertase activity and abscisic acid in relation to carbohydrate status in developing soybean reproductive structures. Crop Sci., 25:615–618.

Ahmadi A, Baker DA (2001).The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Growth Regul., 35: 81–91.

Bangerth F, Aufhammer W, Baum O (1985). IAA level and dry matter accumulation at different positions with a wheat ear. Physiol Plant., 63:121–125.

Barata RM, Chapparro A, Chabregas SM, Gonzalez R,

Labate CA, Azevedo RA, Sarath G, Lea PJ, Silva–Filho MC (2000). Targeting of the soybean leghemoglobin to tobacco chloroplasts: Effects on aerobic metabolism in transgenic plants. Plant Sci., 155:193–202.

Brenner ML, Cheikh N (1995).The role of hormones in photosynthate partitioning and seed filling. In: DaviesPJ, ed. Plant hormones, physiology, biochemistry and molecular biology. Dordrecht, the Netherlands: Kluwer Academic Publishers.649–670.

Darussalam Cole MA, Patrick JW (1998).Auxin control of photoassimilate transport to and within developing grains of wheat. Aust J Plant Physiol., 25:69–77.

Davies KJA (1987).Protein damage and degradation by oxygen radicals. I. General aspects. J Biol Chem., 262:9895–9901.

Davies PJ (2004).Introduction. In: DaviesPJ, ed. Plant hormones, biosynthesis, signal transduction, action! Dordrecht, the Netherlands: Kluwer Academic Publishers.1–35.

Dewdney SJ, McWha JA (1979).Abscisic acid and the movement of photosynthetic assimilates towards developing wheat (*Triticumaestivum* L.) grains. Z Pflanzenphysiol., 92:186–193.

Egneus H, Heber U, Kirk M (1975).Reduction of oxygen by the electron transport chain of chloroplasts during assimilation of carbon dioxide.BiochimBiophysActa., 408:252– 268.

Elstner EF (1987).Metabolism of activated oxygen species.The Biochemistry of Plants, Biochemistry of Metabolism, Academic Press, San Diego, USA.,11:253 315.

Foster JG, Hess JL (1982). Oxygen effects on maize leaf superoxide dismutase and glutathione reductase. Phytochemistry., 21:1527–1532.

Gamble PE, Burke JJ (1984). Effect of water stress on the chloroplast antioxidant system. 1. Alterations in glutathione reductase activity. Plant Physiol., 76:615–621.

Gillham DJ, Dodge AD (1987). Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: seasonal variations. Plant Sci., 50:105–109.

Griffith OW, Meister A (1979). Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-n-butyl homocysteine sulfoximine). J Biol Chem., 254:7558– 7560.

Hameed A, Bibi N, Javed 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 Biochem., 49:178–185.

Jagtap V, Bhargava S (1995). Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of Sorghum bicolor (L) Moench, exposed to high light, low water and high temperature stress. J Plant Physiol., 145:195–197.

Kato T, Sakurai N, Kuraishi S (1993).The changes of endogenous abscisic acid in developing grains of two rice cultivars with different grain size.Jpn J Crop Sci., 62:456– 461.

Kelen M, CubekDemiralay E, Sen S, Ozkan G (2004). Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (Vitis berlandieri×Vitis rupestris) and Rose Oil (*Rosa damascena* Mill.) by Reversed

Phase Liquid Chromatography. Turk J Chem., 28:603–610.

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.

Kramer PJ, Boyer JS (1995). Water Relations of Plants and Soils. San Diego: Academic Press.

Lascano HR, Antonicelli GE, Luna CM, Melchiorre MN, Gomez LD, Racca RW, Trippi VS, Casano LM (2001). Antioxidant system response of different wheat cultivars under drought: field and in vitro studies. Aust J Plant Physiol., 28:1095–1102.

Ling Q, Zhang H, Cai J, Su Z (1993). Population quality and its approaches for the high yielding of rice. Sci Agric Sin., 6:1–11.

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999). Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol., 119: 1091–1099.

Lur HS, Setter TL (1993). Role of auxin in maize endosperm development: timing of nuclear DNA endoreduplication, zein expression, and cytokinins. Plant Physiol., 103: 273– 280.

Mckeon TA, Yang SF (1988). Biosynthesis and metabolism of ethylene In: Davies P.J. (ed.), Plant Hormones and their Role in Plant Growth and Development. Kluwer Academic Publishers, Dordrecht., 94–112.

Menconi M, Sgherri CLM, Pinzino C, Navari-Izzo F (1995). Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. J Exp Bot., 46:1123–1130.

Monneveux P, Belhassen E (1996). The diversity of drought adaptation in the wild. Plant Growth Regul., 20:85–92.

Moran J, Becana F, Iturbe-Ormaetxe I, Frenhilla S, Klucas RV, Aparicio-Teju P (1994). Drought induces oxidative stress in pea plants. Planta., 194:346–352.

Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant., 58:166–170.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22:867–880.

Narwadkar PR, Ansewadekar KW (1989).Effect of growth regulator on the success of epicotyl grafting in mango. Acta Horticult., 231:175–178.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol., 49:249–279.
Noctor G, Gomez L, Vanacker H, Foyer CH (2002). Interactions between biosynthesis comportmentation and transport in the control of glutathione homeostasis and signaling. J Exp Bot., 53:1283–1304.
Nooden LD (1988). Abscisic acid, auxin, and other regulators of senescence. In: Nooden L. and Leopold A. (eds), Senescence and Aging in Plants. Academic Press, San Diego., 329–368.
Nunes MES, Smith GR (2003). Electrolyte leakage assay capable of quantifying freezing resistance in rose clover. Crop Sci., 43:1349-1357.
Pastori GM, Trippi VS (1992). Oxidative stress induces high rate of glutathione reductase synthesis in a drought resistant maize strain. Plant Cell Physiol., 33:957–961.
Patakas A, Notsakis B (2001). Leaf age effects on solute accumulation in water–stressed grapevines. J Plant Physiol., 158:63–69.
Plucknett DL, Smith NJH, Williams JT, Anishetty NM (1987).Gene Banks and the World's Food. Princeton University Press, New York., 267-277.
Pustovoitova TN, Zhdanova NE, Zholkevich VN (2004). Changes in the levels of IAA and ABA in cucumber leaves under progressive soil drought. J Plant Physiol Russian., 51:513–517.
Rampino P, Pataleo S, Gerardi C, Mita G, Perrotta C (2006). Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell and Environ ., 29:2143–2152.
Rook F, Corke F, Card R, Munz G, Smith C, Bevan MW (2001). Impaired sucroseinduction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signaling. J Planta., 26:421–433.
Sairam RK, Saxena DC (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. J Agron Crop Sci., 184:55–61.
Sakurai N, Akiyama M, Kuraishi S (1985). Role of abscisic acid and indoleacetic acid in the stunted growth of water-stressed, etiolated squash hypocotyls. Plant Cell Physiol., 26:15–24.
Saneoka H, Moghaieb REA, Premachandra GS, Fujita K (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in AgrostispalustrisHuds. Environ. Exp Bot., 52:131–138.
Teranishi Y, Tanaka A, Osumi M, Fukui S (1974). Catalase activity of hydrocarbon utilizing candida yeast. AgricBiol Chem., 38:1213–1216.
Van Rensburg L, Kruger GHJ (1994). Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of Nicotianatobacum L. J Plant Physiol.,143:730–736.
Walker MA, McKersie BD (1993).Role of the ascorbate-glutathione antioxidant system in chilling resistance of tomato. J Plant Physiol., 141:234–239.
Wang C, Yang A, Yin H, Zhang J (2008). Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. J Integr Plant Biol., 50:427–434.
Wang Z, Yang J, Zhu Q, Zhang Z, Lang Y, Wang X(1998). Reasons for poor grain filling in intersubspecific hybrid rice. Acta Agron Sin., 24:782–787.
Wittenbach VA (1983). Effect of pod removal on leaf photosynthesis and soluble protein composition of field-grown soybeans. Plant Physiol., 73:121–124.
Xie ZJ, Jiang D, Cao WX, Dai TB, Jing Q (2003). Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. Plant Growth Regul., 41:117–127.
Yang J, Wang Z, Zhu Q (1996). Effect of nitrogen nutrition on grain yield of rice and its physiological mechanism under different soil moisture. Sci Agric Sin 4:7–14.
Yang J, Wang Z, Zhu Q, Lang Y (1999) Regulation of ABA and GA to rice grain filling. Acta Agron Sin., 25:341–348.
Yang J, Zhang J, Liu K, Wang Z, Liu L (2006). Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. New Phytol., 271:293–303.
Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001). Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol., 127:315–323.
Yuan LP (1997). Hybrid rice breeding for super high yield. Hybrid Rice., 12: 1–6.
Zhu JK (2002). Salt and drought stress signal transduction in plants. Annu Rev Plant Biol.,53:247–273.
Zhu Q, Zhang Z, Yang J, Cao X, Lang Y, Wang Z (1997). Source-sink characteristics related to the yield in intersubspecific hybrid rice. Sci. Agric Sin., 4: 52–59.