Role of abscisic acid and drought stress on the activities of antioxidant enzymes in wheat

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ABSTRACT

The effect of drought stress and abscisic acid (ABA) applied at tillering stage (55 days after sowing) was compared in 2 wheat cultivars differing in drought tolerance. The activities of superoxide dismutase (SOD) and peroxidase (POD) and contents of endogenous ABA in plants were measured at 3 days of drought stress in cv. Chakwal-97 (drought tolerant) and cv. Punjab-96 (drought susceptible). ABA was applied at 10^{-6} mol/L as presowing seed treatment for 18 h. Drought tolerant cultivar has a more efficient mechanism to scavenge reactive oxygen species as shown by a significant increase in the activity of antioxidant enzyme SOD. Under drought stress, ABA significantly increased the activities of SOD and POD, showing a significant decline on rewatering. The relative water content was significantly increased by ABA priming under drought stress in both wheat cultivars. The sensitive cultivar exhibiting lower endogenous ABA content was more responsive to ABA priming. On rewatering, the magnitude of recovery from drought stress was greater in tolerant cultivar. ABA was highly effective in improving grain weight of tolerant cultivar under drought stress.

Keywords: water stress; phytohormone; plant defense system

Drought stress is among the most damaging abiotic stresses that affect agriculture these days. Due to current climate change scenario, plants suffer from erratic water stress (Zhang et al. 2006).

Wheat (*Triticum aestivum* L.) is an important staple food crop of many countries including Pakistan. It is grown under diversified environments. However, the lack of adequate moisture is common to wheat in rain fed and poorly irrigated areas and results in a significant reduction of yield. Among different growth stages, tillering stage is considered as highly sensitive to water stress.

Understanding the biochemical and physiological basis of water stress tolerance in plants is vital to select and breed plants for improving crop water stress tolerance (Chaves et al. 2003). The production of reactive oxygen species is a common phenomenon in plants under drought stress. These reactive oxygen species (ROS) generations led to lipid peroxidation (Sreeni-Nivasuhu et al. 1999, Chen et al. 2000), protein degradation (Jiang and Zhang 2001) and nucleic acid damages (Hagar et al. 1996). To alleviate adverse effects of reactive oxygen species, plants have evolved an antioxidant defense system that include enzymes like superoxide dismutase, peroxidase and catalase etc. (Agarwal and Pandey 2004).

Abscisic acid (ABA) is a plant stress hormone that is observed to accumulate under drought stress and mediates many stress responses. It is reported to gradually degrade upon removal of stress (Zhang et al. 2006). Pospíšilová et al. (2005) reported that ABA pre treatment further increased the endogenous ABA level in maize seedling. Pre-soaking seed treatment with ABA was reported to significantly enhance the antioxidant enzymes activity in maize seedlings subjected to water stress (Jiang and Zhang 2002). Similarly, Boominathan et al. (2004) found that relative water content of ABA treated plants was higher under drought stress. Moreover, exogenous application of ABA under water stress increased the grain weight in susceptible wheat cultivars (Nayyar and Walia 2004).

The aim of the present investigation was to compare the effects of exogenously applied ABA (10^{-6} mol/L) and drought stress imposed during tillering in drought susceptible and tolerant wheat cultivars.

MATERIAL AND METHODS

The experiment was carried out in complete randomized design (CRD) with four replicates in greenhouse of the Department of Plant Sciences Quaid-i-Azam University, Islamabad during the wheat growing season. The seeds of the two wheat cultivars cv. Chakwal-97 (drought resistant) and cv. Punjab-96 (drought susceptible), obtained from National Agriculture Research Centre Islamabad were surface sterilised with 10% chlorox for five minutes with intermittent shaking and thoroughly rinsed with sterile water. Autoclaved distilled water (in case of control) or aqueous solution of ABA [RS (±) ABA (Sigma Chemicals Company Ltd. USA)] was applied at 10^{-6} mol/L as seed soaking treatment prior to sowing for 18 h. The seeds were sown in earthen pots measuring 27 × 30 cm^2 filled with soil and farmyard manure in the ratio of 6:1. Recommended irrigation practices were carried out throughout the growing season. Drought was induced at tillering stage (55 DAS) for a period of three days. While non-stressed plants continued to receive daily irrigation. One set of the plants was harvested 3 day after drought stress; other set was irrigated and harvested after 24 h of rewatering.

Soil moisture content (%) was determined before and after induction of drought stress.

% soil moisture = fresh weight of soil – dry weight of soil/dry weight of soil × 100

For determination of relative water content (RWC), second leaf from the top of the plants was harvested, weighed and soaked in distilled water for 24 h. The fully turgid leaves were weighed again. Thereafter leaves were dried in oven for 72 h at 70°C. Relative water content was calculated according to the following formula:

RWC% = FW – DW/FTW – DW × 100

Where: RWC – relative water contents; FW – fresh weight; DW – dry weight; FTW – fully turgid weight.

Determination of superoxide dismutase and peroxidase activity. The frozen leaves (0.5 g) were homogenized in 3 mL of cold solution containing 50 × 10^{-3} mol/L Na phosphate buffer (pH 7.8), 1 × 10^{-3} mol/L EDTA and 2% (w/v) PVP. The homogenate was centrifuged at 40°C for 20 min at 13 000 rpm. The spectrophotometric analysis was conducted on spectrophotometer. The assay for SOD and POD were based on the method described by Beauchamp and Fridovich (1971) and Herzog and Fahimi (1973), respectively. One unit of SOD was defined as the amount of enzyme that inhibits the nitro blue tetrazolium chloride (NBT) photo reduction by 50%. For POD, the activity was defined as the changes in absorbance per minute for 1 g FW of flag leaves.

Determination of endogenous abscisic acid (μg/g). The extraction and purification of ABA was made following the method of Kettner and Doerffling (1995). The plant leaves (1 g) were ground in 80% methanol, at 4°C with an antioxidant butylated hydroxy toluene (BHT). The leaf was extracted at 4°C for 72 h with subsequent change of solvent. The extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary film evaporator. The pH of aqueous phase was adjusted to 2.5–3.0 and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 1 mL of methanol (100%). Samples were analyzed on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller, UK) using U.V. detector and C-18 column. For identification of hormones, plant samples were passed through millipore filters (0.45 μ). Pure ABA [RS (±) ABA (Sigma Chemicals Company Ltd. USA)] was used as standard for identification and quantification of plant hormone. ABA was identified and quantified on the basis of retention time and peak area of the standards at 254 nm. Methanol, acetic acid and water (30:1:70) were used as mobile phase.

Yield parameters like length of spike (cm), number of grains per spike, 100 grain weight (g), and number of grains per spike were determined.

Treatments. The following treatments were made: T1 – drought; T2 – rewatering; T3 – D + ABA; T4 – D + ABA + rewatering; T5 – ABA; T6 – control (pre soaked in water).

Statistical analysis. Data were subjected to Analysis of Variance (Steel and Torrie 1980) and significance of mean values was tested by Duncan's Multiple Range Test (Duncan 1955) utilizing MSTAT-C.

RESULTS AND DISCUSSION

Effect of drought and ABA on soil moisture, relative water content (RWC) and endogenous ABA. Table 1 showed that soil moisture was significantly decreased upon the imposition of drought stress in the rhizospheric soil of both the cultivars. Under drought stress, ABA did not significantly affect the soil moisture content. After rewatering, the soil moisture was completely recovered.

The drought stress significantly decreased the leaf relative water (LRWC) content of both the cultivars (Table 2). In the susceptible cv. Punjab-96, the % decrease in RWC under drought was greater than tolerant cv. Chakwal-97. The presowing seed treatment with ABA significantly ameliorated the adverse effects of drought stress on LRWC. After

Table 1. Soil moisture content (%) measured before and after induction of 3 day drought. Soil moisture content was determined on dry weight basis

Treatments	Punjab-96	Chakwal-97
Control	17[a]	17[a]
Drought	9[b]	8.6[b]
Rewatering	17[a]	16.9[a]
Drought + ABA	9.6[b]	9.4[b]
Drought + ABA + rewatering	17[a]	17.2[a]
ABA	17[a]	17.16[a]

All such means which share a common English letter in the column are similar otherwise differ significantly at $P < 0.05$

Table 2. Effect of drought stress and abscisic acid (ABA) on relative water content (%) and endogenous ABA content (μg/g) of cv. Punjab-96 and cv. Chakwal-97 subjected to 3 days drought at tillering stage. The ABA (10^{-6} mol/L) was applied as seed pre sowing for 18 h

Treatments	Punjab-96		Chakwal-97	
	RWC	ABA	RWC	ABA
Control	78[ab]	18.14[e]	87[b]	29[e]
Drought	58[d]	34[d]	70[e]	55[b]
Rewatering	61[d]	33[d]	76[d]	35[d]
Drought + ABA	76[bc]	146[a]	81[c]	152[a]
Drought + ABA + rewatering	77[bc]	81[b]	86[b]	42[c]
ABA	74[c]	45[c]	83[c]	37[d]

All such means which share a common English letter in the column are similar otherwise differ significantly at $P < 0.05$

rewatering, the recovery of RWC was greater in cv. Chakwal-97.

The basal level of ABA significantly differed in the two cultivars (Table 2). The sensitive cv. Punjab-96 had low endogenous ABA. On imposition of drought stress, the increase in endogenous ABA was correspondingly lower in the former cv., Punjab-96. However, pre-sowing seed treatment with ABA elevated endogenous ABA in both the cultivars, the magnitude of increase being greater in cv. Punjab-96. When ABA treated plants were exposed to drought stress, endogenous ABA concentration increased significantly, the ABA level being higher in cv. Chakwal-97. On rewatering, the endogenous ABA level dropped significantly within one day in both the cultivars, the % decrease was higher in Chakwal-97. In ABA non-treated plants, ABA decreased upon rewatering only in Chakwal-97.

In cereals, RWC is a relevant attribute for screening drought tolerance (Teulat et al. 2003). ABA priming ameliorated the drought induced decrease in RWC of both cultivars. These results are in agreement with those of Agarwal et al. (2005) who reported that exogenous application of ABA increased RWC in wheat under water stress.

The increase in the endogenous abscisic acid content under water stress was previously reported; the tolerant wheat cultivars were found to posses higher endogenous ABA content (Nayyar and Walia 2004). Moreover, the extent of ABA response is greatly dependent on type of variety and is more common in winter wheat (Wrightman 1979).

Effect of drought and ABA on SOD and POD activity. In the drought susceptible cv. Punjab-96, the SOD activity was not affected by drought stress as compared with the control. ABA treatment resulted in a significant increase in SOD activity (33%) over control (untreated) plants and even more (100%) under drought stress. Rewatering increased the SOD activity in drought stressed plants but not in ABA treated plants exposed to drought stress. In the tolerant cv. Chakwal-97, a significant increase in the SOD activity was recorded under drought stress, which remained high even after 24 h of rewatering. ABA treatment under drought further augmented the SOD activity over control. Rewatering significantly decreased the SOD activity to basal level in the ABA treated plants previously exposed to drought stress (Table 3).

In the sensitive cv. Punjab-97, the drought has no significant effect on POD activity. In contrast, the leaves of ABA treated plants showed a significant increase in POD activity under drought stress, declining to basal level on rewatering. The tolerant cultivar Chakwal-97 showed no marked increase in POD activity on imposition of drought stress but a significant decrease on rewatering. ABA pretreatment resulted in a significant increase under drought stress being non-effective under unstressed conditions (Table 3).

Increased superoxide dismutase and peroxidase activities in response to water stress were reported (Kukreja et al. 2005). The SOD activity which is responsible for scavenging O^{-2} radical to produce H_2O_2 (Smirnoff 1993), increased within three days of drought at tillering stage in both drought susceptible and drought tolerant cultivar. However,

Table 3. Effect of drought stress and abscisic acid (ABA) on superoxide dismutase (SOD) (units/g FW) and reroxidase (OD/min/g FW) activity of cv. Punjab-96 and cv. Chakwal-97 subjected to 3 days of drought at tillering stage. The ABA (10^{-6} mol/L) was applied as seed pre sowing for 18 h

Treatments	Punjab-96		Chakwal-97	
	SOD	POD	SOD	POD
Control	1.2^{efg}	0.09^{e}	1.0^{g}	0.1^{de}
Drought	1.5^{cde}	0.1^{e}	1.5^{def}	0.12^{de}
Rewatering	1.8^{cde}	0.45^{cd}	1.4^{def}	0.02^{e}
Drought + ABA	2.4^{b}	0.8^{ab}	1.6^{def}	0.63^{bc}
Drought + ABA + rewatering	1.3^{fg}	0.2^{de}	1.0^{g}	0.13^{de}
ABA	1.6^{c}	0.1^{de}	1.5^{def}	0.07^{e}

All such means which share a common English letter in the column are similar otherwise differ significantly at $P < 0.05$

the increase in SOD activity was higher in drought tolerant cultivar. The results suggested that after rewatering, the SOD activity remained higher than control in drought susceptible cultivar but slowly declined in drought tolerant cultivar. Higher SOD activity is required for scavenging the ROS produced during drought stress and to protect the biomolecules. Jiang and Zhang (2001) and Hu et al. (2005) reported enhanced SOD activity under drought stress by ABA. As compared to drought stress alone, the exogenous application of ABA (10^{-6} mol/L) enhanced the SOD activity of drought susceptible wheat cultivar whereas no significant effect of ABA was observed under drought stress as compared with drought stress alone in cv. Chakwal-97. The increase in the activities of antioxidant enzymes by ABA application was reported previously (Agarwal et al. 2005). Rapid decrease in the level of POD was observed after withdrawal of drought stress. Previous studies showed that ABA gradually degrades upon removal of drought stress (Zhang et al. 2006). The results showed that POD activity remained higher in susceptible cv. Punjab-96 even after rewatering of 24 h. Similar results for increased POD activity after rewatering were reported in *Reaumuria soongorica* (Bai et al. 2009). However, plants treated with ABA and subjected to drought showed a rapid decline in POD activity after rewatering.

Effect of drought and ABA on yield. Drought stress caused significant reduction in yield components of cv. Punjab-96 but the yield of cv. Chakwal-97 was affected much less (Table 4).

The spike length, number of grains per spike and 100 grain weight were significantly decreased under drought stress in cv. Punjab-96. ABA priming completely ameliorated the drought induced inhibition in grain number and 100 grain weight. In cv. Chakwal-97, mild effect of drought stress was observed on the spike length and number of grains per spike.

The correlation between SOD and POD was positive and highly significant ($r = 0.919$) in susceptible cv. Punjab-96 but all the yield parameters including spike length, number of grains per spike and 100 grain weight showed non-significant correlation with SOD and POD. There was found a negative correlation between RWC and SOD ($r = –0.064$). The correlation between spike length and number of grains per spike was positive and significant ($r = 0.949$).

In tolerant cv. Chakwal-97, correlation between SOD and POD was non-significant. However, the correlation between ABA and POD was positive and highly significant ($r = 0.978$). There was found non-significant negative association between SOD and RWC ($r = 0.639$).

Table 4. Effect of drought stress and abscisic acid (ABA) on spike length (cm), number of grains per spike and 100 grain weight (g) of cv. Punjab-96 and cv. Chakwal-97 subjected to 3 days drought at tillering stage. The ABA (10^{-6} mol/L) was applied as seed pre sowing for 18 h

Treatments	Punjab-96			Chakwal-97		
	length of spike (cm)	number of grains per spike	100 grain weight (g)	length of spike (cm)	number of grains per spike	100 grain weight (g)
Control	7^{a}	35^{a}	6^{b}	8^{a}	31^{ab}	5^{bc}
Drought	5^{b}	24^{b}	5^{c}	7^{b}	26^{b}	5^{c}
Drought + ABA	8^{a}	35^{a}	6^{ab}	8^{a}	39^{a}	6^{a}
ABA	8^{a}	36^{a}	7^{a}	8^{a}	38^{a}	6^{a}

All such means which share a common English letter in the column are similar otherwise differ significantly at $P < 0.05$

From the results it can be inferred that the two wheat cultivars (Punjab-96 and Chakwal-97) differ in response to drought stress at tillering stage as well as their response to applied ABA. The drought tolerant cultivar showed lower decrease in the RWC than the susceptible cv. Punjab-96. Chakwal-97 exhibited increase in SOD and POD activity. The presowing seed treatment with ABA (10^{-6} mol/L) solution was found to ameliorate the adverse effects of drought stress in susceptible cultivar of wheat. Positive correlation occurs between endogenous ABA concentration and antioxidant activities of SOD and POD.

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