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EVALUATION OF DROUGHT STRESS TOLERANCE IN SPRING WHEAT ACCESSION BASED ON SELECTION INDICES

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Abstract

To assess the effect of salicylic acid (SA) and GA_3 on wheat grown under drought and control conditions four factorial experiment was done in a RCBD across two locations with three replications. The aim of this experiment was also to study the effect of priming on antioxidant behavior, physiological and biochemical indices. In parallel study the drought resistance of different wheat accessions. The results showed that there was a considerable increase in antioxidant under water stress. In general, SA and GA_3 treated seed plants showed, a higher accumulation of assimilatory pigments and antioxidants compared to those of untreated seed plants. It was observed that GA_3 or SA improved wheat performance under normal condition. Under drought condition GA_3 and SA increased tolerance in stressed wheat by significantly activated catalase, per-oxidase and ascorbate peroxidase. Increased assimilatory pigments and enhancing the accumulation of nontoxic metabolites as a protective adaptation mechanism. Results signify the role of SA and GA_3 in regulating the drought response of plants. It was suggested that SA and GA could be used as a potential growth regulator, for improving plant growth under water stress.

Introduction

Secondary metabolites are increased in plants under water stress. Plants can react and adjust them by altering their cellular mechanism and increased the secondary metabolites which act as a defense mechanism (El-Tayeb 2006). Wheat (*Triticum aestivum* L.), one of the most central food crops, can be grown in a diverse range of environments. Wheat production in low rainfall and poorly irrigated areas was limited because of available water is inadequate to cope with wheat crop production. Water deficit was one of the most important environmental factors that restrict plant survival and crop productivity in arid regions. To reduce the unfavorable effect of drought stress and make sure crops under optimal growth conditions protect themselves adopting some naturally defense mechanisms such as an increased content of reactive oxygen species (ROS) (Miller *et al*. 2010, Zhang *et al*. 2012). Oxygen is important for life, however toxic reactive oxygen species (ROS), including the superoxide anion radical, the hydroxyl radical and hydrogen peroxide, tend to increase when the plants were exposed to stress. Seed priming is an economic technique which is widely used to overcome the germination related problems or to ameliorate the stress tolerance in different crops. Several priming techniques have been reported to have a numerous beneficial effects (Farooq *et al.* 2008). However, priming induced changes in the seed biochemistry that are actually responsible for beneficial effects of treatments. Based on above findings, proposed research work was focused on drought and effect of priming under drought and control conditions as well. The general objective of this study was to investigate the sensitivity and tolerance of wheat genotypes to drought stress. Another objective was to investigate the biochemical changes in the seeds induced by different pre-sowing treatments. In parallel special emphasis was given on antioxidant enzyme alteration and assimilatory pigments under control and drought stress conditions.

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Material and Methods

A four factorial experiment based on RCBD with three replications were conducted in 2014 - 2015 in the field area of University of Azad Jammu and Kashmir, Muzaffarabad and Rawalakot of Pakistan. Five wheat (*Triticum aestivum* L.) accessions *viz.*, AARI-11(A1), Chakwal-50 (A2), Shahkar (A3), Pakistan-13(A4) and FSD-08 (A5) were used. Seeds were socked in 10 - 4 M solution of different growth regulators *viz.*, GA_3 and salicylic acid (SA) for 8 hrs prior to sowing and continuous aeration was provided by aquarium pumps. Under experimental units main plot was subdivided into two sub-plots, i.e., control and drought. Sub-plots were further splited into three sub-sub-plots which were considered as replications. Within sub-sub-plots priming treatments and five cultivars were completely randomized as experimental treatments. Fifteen sub-plots served as a control with proper irrigation whereas the group of other 15 sub-plots was considered as stress group giving no irrigation as preventing irrigation with water protected sheet. Five accessions in sub-plots were tested and three priming levels such as non primed, salicylic acid and GA_3 (Np, SA and GA_3, respectively) were used. Single row hand drill method was used for seed sowing with row to row and plant to plant spacing of 20 cm and 1.5 cm, respectively. Flag leaf was used for the analysis of antioxidant and assimilatory pigments. Catalase (CAT) was estimated by following method described by Beers and Sizer. For measurement of CAT activity, assay solution (3 ml) containing 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 ml enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 sec. An absorbance change of 0.01 unit/min 1 was defined as one unit CAT activity. Enzyme activities were expressed on fresh weight basis. Ascorbate peroxidase (APX) activity was described by the method (Dixit *et al.* 2001). For assay of the enzyme activity, the rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in a reaction mixture that contained 50 mM potassium phosphate buffer, pH 7.0, 0.6 mM ascorbic acid and enzyme extract (Chen *et al.* 1989). The reaction was initiated by addition of 10 ml of 10% (v/v) H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 3 min. Assimilatory pigments i.e. chlorophyll a, b, carotenoid and anthocyanine analysis was measured by the procedures of Sims and Gamon (2002). ANOVA was performed on the basis of factorial experiment and DMRT at 0.05 level of significance was used to compare the means. Values presented in graphs are means. Bars with different alphabets differ significantly from each other.

Results and Discussion

When the authors observed the mean estimations of catalase activity of various accessions, it was found that every accession was altogether not quite the same (Fig. 1). The highest catalase activity was found in FSD-08 while the lowest activity was found in AARI-11. Priming with GA_3 demonstrated most noteworthy difference with contrasted to SA and control. More catalase was gathered under dry stress condition in contrast to well-watered condition. Muzaffarabad location indicated the most astonishing as compare to Rawalakot. Interaction of all factors demonstrated significant and non significant impact. Present results were in conformity with Zhang *et al*. (2007) who reported that activities of antioxidant enzymes i.e. catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) increased in Victoria and Victor seedlings after priming treatments. Catalase is the key enzyme for removal or detoxification of excessive hydrogen peroxide in the seeds. In fact, increased level of antioxidative enzymes protects the cell against the oxidative damage by removal of free radicals or reactive oxygen species.

Significant differences were found in all accessions in case of ascorbate pexoxidase (APX) activity (Fig. 2). The highest activity of ascorbate peroxidase was observed in FSD-08 while the lowest was observed in Chakwal-50. Salicylic acid and GA_3 showed the highest activity followed by control. Under drought stress and under control condition highly significant differences were observed. Under drought high activity of APX was observed. Interactive effect was also significant with and without drought. Rawalakot location showed the highest activity of ascorbate peroxidase as compare to Muzaffarabad. Our findings were similar to previous researchers who found that plants usually increased the activity of anti-oxidative peroxidases or glutathione reductase in response to the drought induced oxidative stress (Miller *et al*. 2010). Therefore, these enzymes are good biochemical markers of stress and their increased activity may attest to a potential for remediation.

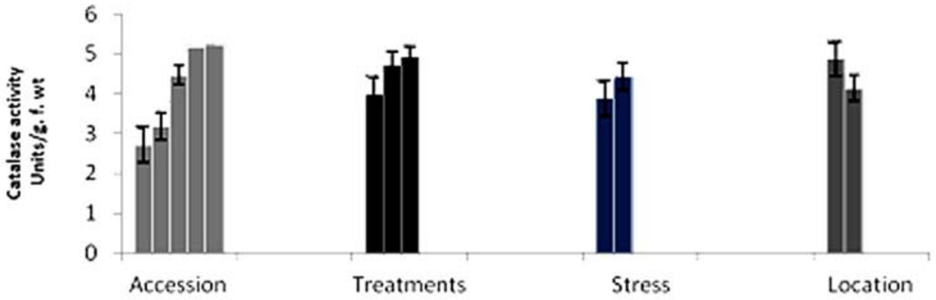


Fig. 1. Variation in catalase activity among accessions, priming, stress and localities. (A) Accession: AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (B) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.

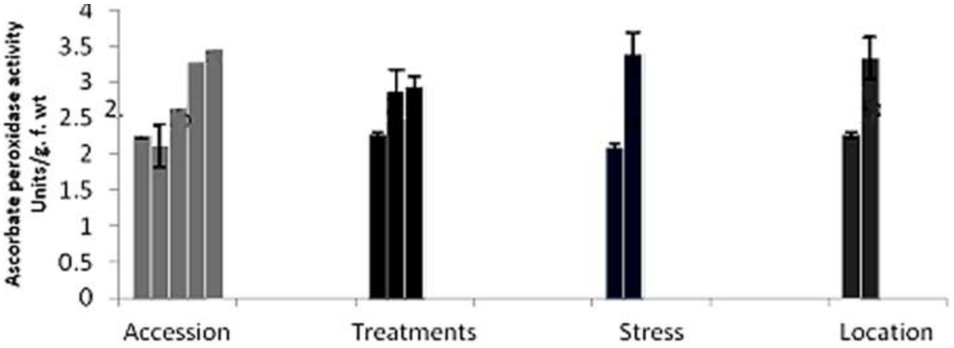


Fig. 2. Variation in ascorbate peroxidase activity among accessions, priming, stress and localities. (A) Accession: AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (b) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.

Chlorophyll *a* pigment indicated distinctive fixation among accessions (Fig. 3). Priming with SA and GA_3 has high allomerative effect that was high compared to control. Interactive effect between drought and no drought was also significant. Under drought stress chlorophyll *a* pigment was reduced. Muzaffarabad and Rawalakot locations showed different response in pigment concentration.

When chlorophyll *b* were examined all accession showed significant differences (Fig. 4). The highest concentrations were seen in Pakistan-13 while the lowest was found in Chakwal-50. In case of chlorophyll *b* in comparison with growth regulators the highest chlorophyll *b* contents were seen in samples treated with GA_3. With and without drought stress significant differences were found. Under drought stress chlorophyll *b* contents reduced. Both locations showed minor change. All variables and their interactions demonstrated significant results. Many workers, such as Shakirova *et al*. (2003) and Iqbal *et al*. (2006) on wheat plants and Abdel-Wahed *et al*. (2006) on maize plants and found that SA caused significant increased in chlorophyll content. This accumulation of photosynthetic pigments as a result of exogenous application of SA may be due to increased in photosynthetic efficiency as reflected by increasing in chlorophyll *a*, chlorophyll *b* and carotenoids content in the leaves of stressed wheat plants.

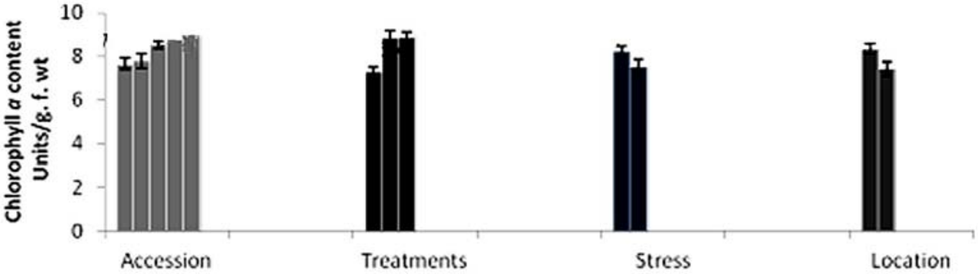
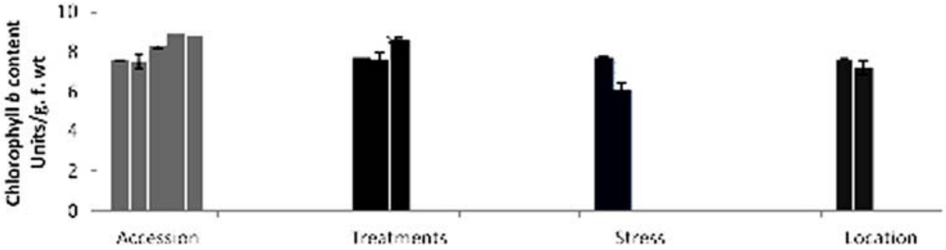


Fig. 3. Variation in chlorophyll *a* among accessions, priming, stress and localities. (A) Accession: AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (B) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.



F 4. Variation in chlorophyll *b* contents among accessions, priming stress and localities. (A) Accession: AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (B) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.

Significant variations were seen in anthocyanin pigment among accession (Fig. 5). Anthocyanin accumulation was lowest in normal condition as compared to SA and GA_3 treatment. Drought reduced the anthocyanine as compared to without drought. In comparison of two area most noteworthy were seen in Muzaffarabad. Some researchers e.g. (Khan *et al*. 2003) showed that SA increased photosynthetic rate in corn and soybean. They concluded that seeds pretreated with 10^{-4} mol/l SA solution exhibited higher chlorophyll and anthocyanin content. These results were also in agreement with those obtained by other authors, showing that SA significantly increased the pigment content under salt stress (El-Tayeb 2005).

When the authors observed the results of carotenoid in different asseccions significant differences were found among accessions (Fig. 6). Highly significant differences were recorded in and control, SA and GA_3 growth regulator treatment. Priming with GA_3 showed the highest contents of carotenoid as compared to SA and control. Carotenoid was decreased under drought as compared to no drought. Both localities were significantly different to each other. Interactive effects of different factors were also significant. Hassanein *et al*. (2009) reported that in salt stressed wheat high salinity caused reduction in growth, lipid peroxidation, hydrogen peroxide accumulation, Chl *a*, *b*, carotenoids and total pigment contents.

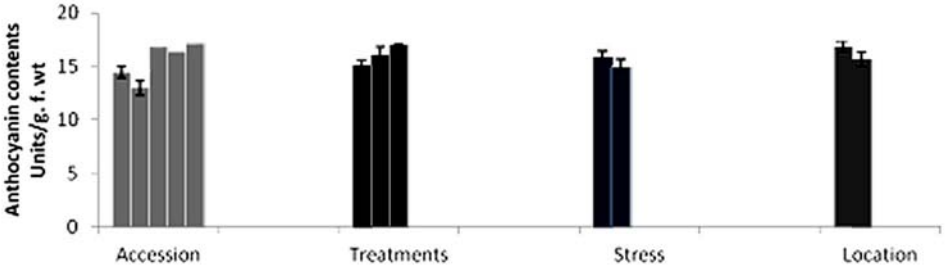


Fig. 5. Variation in anthocyanin contents activity among accessions, priming, stress and localities. (A) Accession: AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (B) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.

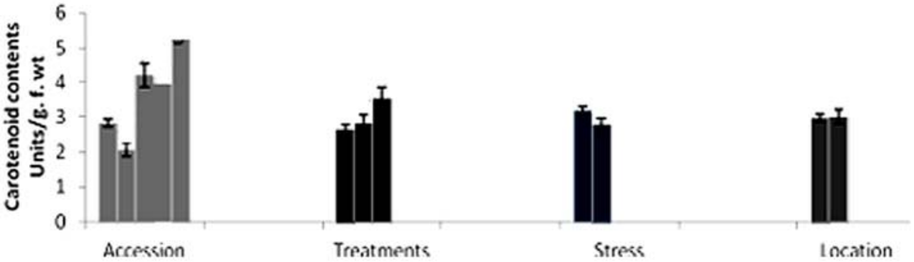


Fig. 6. Variation in carotenoid content among accessions, priming, stress and localities. (A) Accession: *viz.*, AARI-II, Chakwal-50, Shahkar, Pakistan-13 and FSD-08. (B) Treatments: Control, SA and GA_3. (C) Stress: Control and drought stress. (D) Location: Muzaffarabad and Rawalakot.

Table 1. Analysis of variance of selection indices.

SOV	Df	MS catalase	Ascorbate peroxidase	Chlorphyll *a*	Chlorophyll *b*	Anthocyanine	Caroteniod
Replication	2	0.008	0.006	0.26	0.17	0.69	0.09
Accession	4	47.42***	0.06**	12.41***	2.16***	31.20***	37.25***
Hormones	2	3.78***	0.07**	0.27	0.05	20.12***	14.27***
Drought	1	13.56***	2.52***	2.98**	0.30*	36.09***	8.04***
Locations	1	0.047*	0.19**	1.53*	0.018	14.90***	0.04
Cv%		3.12	7.06	6.26	4.41	4.74	6.51

,**,** indicate significance at 5%, 3% and 1% probability level respectively.

From the preceding results and discussion, it can be concluded that priming of wheat (*Triticum aestivum* L.) with both GA_3 and SA as compared to control individually or in combined with drought induced plants stimulate the drought tolerance of wheat plants. This can be done via improving the activities of antioxidant enzymes, enhancement of the biosynthesis of photosynthetic pigments and thereby increasing the general growth rate; as well as enhancing the accumulation of nontoxic metabolites.

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