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Effect of Restricted Irrigation on Water Relation Traits and Antioxidant Activity

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A B S T R A C T

Water relation traits and antioxidant metabolism play a very imperative role in barley growth and productivity when crop imposed under restricted irrigation. Present investigation was planned to study the responses to water status and antioxidant mechanism under normal (irrigated) and drought (restricted irrigation) condition. Twenty barley genotypes were cultivated under irrigated (Two irrigations at tillering and anthesis each) and limited irrigation conditions (one irrigation at tillering) in concrete drought plots during the 2014-15 and 2015-16 growing seasons at the Crop physiology field area, Hisar. The experiments were laid out using RBD with three replications. The results from combined analysis of variance in both normal and drought conditions indicated that there were significant differences among genotypes with regard to all studied traits which were due to high variation among the genotypes. Limited irrigation significantly decreased the plant leaf water potential, leaf osmotic potential and relative water content while increased membrane injury in all barley genotypes studies. It was found that the activity of enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) were increased under drought stress conditions, so that tolerant genotypes had more changes in enzyme activity. Genotypes BH 14-05 and BH-10-30 in present study were found better antioxidant activity and maintained plant water status among all the genotypes under both normal and restricted irrigation condition and vice-versa. These genotypes because of better plant water status and antioxidant activity were found maximum in grain yield.

Introduction

Barley (*Hordeum vulgare* L.) is oldest domesticated cereals crops of world agriculture and stand fourth major ranked crop in the worldwide production of cereals. Barley is an important cereal crop targeted for animal feed (Jacob and Pescatore 2012) malting and industrial purposes while 2% is used for human consumption (Baik and Ullrich 2008). This crop mostly grown under rainfed conditions, where drought and heat stress usually constrain yield during the grain filling period (Dorostkar *et al.* 2015). Water deficit or drought stress is one of the most common environmental stresses that affects growth and development of plants (Abarshahr *et al.*, 2011).

Water status of a crop plant is usually

expressed in terms of relative amount of water content, water potential and osmotic potential (Sabir *et al.* 2008; Javed *et al.* 2011). During water stress reduction was found between osmotic potential (Zhang *et al.* (2015), water potential (Iqubal *et al.* (2014) and relative water content (Vaezi *et al.* 2010) in barley genotypes (Sharma *et al.*, 2016; Aboughadareh *et al.* 2017). When plant expose to adverse environment (drought) reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) are produced (Faize *et al.* 2011). Multiple antioxidant enzymes like superoxide dismutases (SOD), catalases (CAT) and peroxidases (POX) are involved in the enzymatic scavenging of ROS (Apel and Hirt, 2004; de Carvalho, 2008; Farooq *et al.* 2009).

The accumulation of ROS is defend by superoxide dismutase (SOD), which dismutates the O^- radicals to H_2O_2 (de Carvalho, 2008). Catalase (CAT) and ascorbate peroxidase (APX) are two enzymes that scavenge H_2O_2 and prevent its accumulation to toxic levels. Peroxidase on other hand is an oxidoreductase that causes H_2O_2 breakdown (Jiang and Jhang, 2004). In a field study, it was observed that when crop genotypes are subjected to restricted irrigation at different growth stage, the drought-tolerant genotypes acclimatized better than the drought-susceptible cultivar by conserving water relations and accumulation antioxidant defense enzyme in the leaves (Ranjeet *et al.* 2012).

Therefore, the present study aimed to determine the effect drought stress on enzymatic antioxidant systems including superoxide dismutase, catalases and peroxidases and plant water relation traits of barley genotypes.

Materials and Methods

Plant material and experimental site

Twenty barley genotypes *viz.* BH 07-35, BH 10-30, BH 12-17, BH 12-20, BH 12-29, BH 12-46, BH 13-10, BH 13-21, BH 13-22, BH 13-23, BH 14-01, BH 14-05, BH 14-06, BH 14-07, BH 14-08, BH 14-09, BH 14-10, BH 14-11, BH 902 (check) and BH 946 (check) were grown in concrete drought plots (6 m × 45 m) with rainout shelters facilities and filled with dunal sand located at Crop Physiology Field Area of Department of Agronomy, CCS Haryana Agricultural University, Hisar. The plot size for each genotype was 2.5 × 1.5 m (5 rows of 5 m length with 30 cm spacing). The experiment was laid out in RBD (Randomized Block Design) with three replications.

Water relation traits

Relative water content (RWC) of fully expended flag leaf was measured by the used the method of Barrs aand Weatherley (1962).

Water potential (Ψ_w) in flag leaf was measured with the help of pressure chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), between 8 to 10 AM. The osmotic potential of leaf was estimated by the method of Morgan (1980) with psychrometric technique using vapour pressure osmometer (Wescor INC., Lorganan, Utah, USA).

Antioxidant enzymes

Preparation of enzyme extract

One-gram leaf sample were homogenized in 4 ml of 0.1M phosphate buffer (pH 7.0) contains 1% polyvinyl pyrrolidine and centrifuged at 10,000 x g for 20 min. in a refrigerated centrifuge at 4^0C. The supernatant was used for determining the activity of superoxide dismutase (SOD), catalase (CAT) and

peroxidise (POX).

Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) adopting the method of Beauchamp and Fridovich (1971). Per cent inhibition was calculated by following formula of Asada *et al.* (1974):

$$\text{Percent inhibition} = \frac{(V - v)}{v} \times 100$$

Where,
V= Rate of assay reaction in absence of SOD, v = Rate of assay reaction in presence of SOD.

One enzyme unit is defined as the amount of enzyme that inhibits the nitro blue tetrazolium photoreduction by 50%.

Catalase activity was measured according to the method of Sinha, (1972). One unit of enzyme activity is defined as the amount of enzyme which catalyzed the oxidation of 1 µ mole H_2O_2 per min. under assay conditions.

Peroxidase activity was assayed by adopting the method of Shannon *et al.* (1966). One unit of peroxidase represents 1.0 O.D. change per min.

Results and discussion

Water relation traits

Leaf water potential, osmotic potential and relative water content showed a decreasing trend under restricted irrigation condition among all genotypes as compared to normal irrigation condition at both stages (flag leaf and anthesis) of observation (Table 1).

Water potential (-MPa)

Drought at flag leaf and anthesis stage reduced water potential in flag leaf. Reduction in water potential was more at anthesis as compare to flag leaf stage under normal and restricted irrigation condition respectively. Average water potential for different genotypes for normal irrigation ranged from -1.44 to -1.88 MPa (flag leaf) and -1.44 to -1.87 MPa (anthesis) whereas, for restricted irrigation water potential value varied between -1.65 to -2.25 MPa (flag leaf) and -1.95 to -2.68 MPa (anthesis). Average water potential at flag leaf was -1.64 and -1.65 MPa and at anthesis -1.90 and -2.33 MPa under normal and restricted irrigation condition respectively. Genotype BH 14-08 and BH 13-10 had maximum water potential at both growth stage flag leaf and anthesis respectively. Our finding is accordance with those of Ahmed *et al.* (2013) and Liu *et al.* (2015) observed reduction in water potential when crop was imposed under drought environment. Water stressed plants showed a significant reduction in leaf water potential than non-water stressed plants (Pang *et al.* 2016).

Osmotic potential (-MPa)

Results presented in table showed of osmotic potential flag leaf and anthesis under normal and restricted irrigation condition. Average osmotic potential for different genotypes for normal irrigation ranged from -0.29 to -1.67 MPa (flag leaf) and -0.30 to -1.67 MPa (anthesis) whereas, for restricted irrigation OP value varied between -0.63 to -1.72 MPa (flag leaf) and -1.51 to -2.01 MPa (anthesis). Average osmotic potential at flag leaf was -0.87 and -0.90 MPa and at anthesis -1.17 and -1.75 MPa under normal and restricted irrigation condition respectively. Genotype BH 14-08 and BH 13-10 had maximum osmotic potential at both growth stage flag leaf and anthesis respectively.
Our results are in agreement with those of

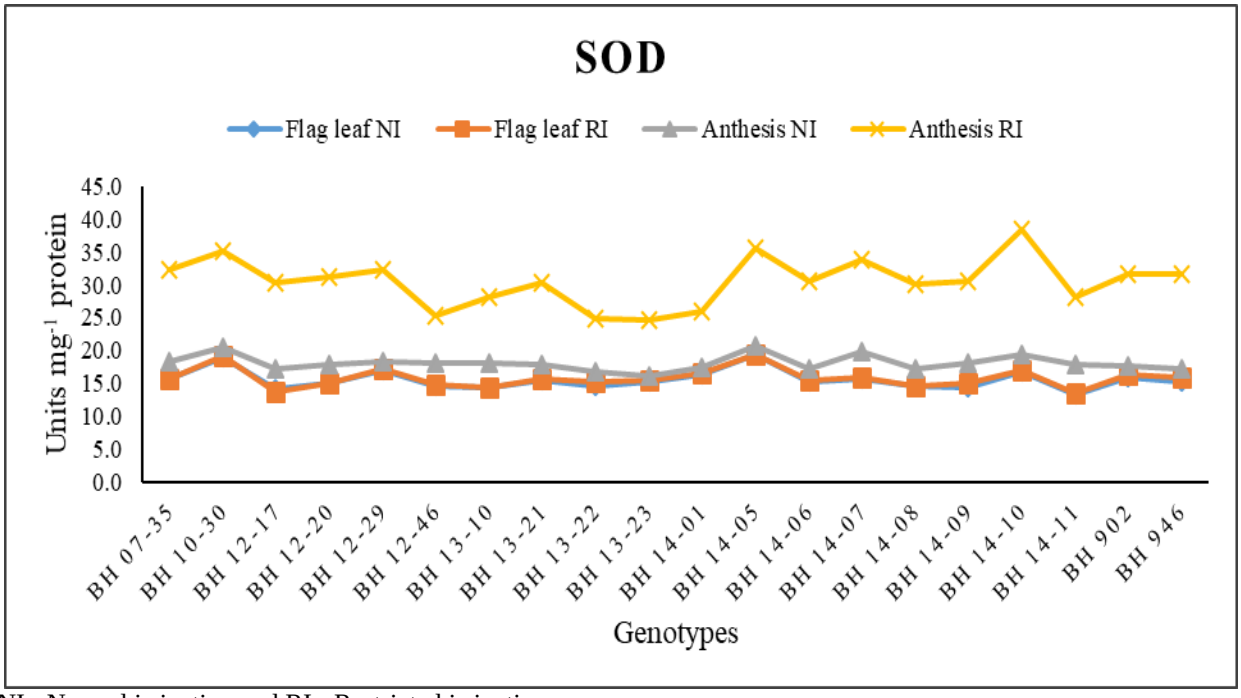
Sharma *et al.* (2016) in barley and Ram *et al.* 2017 in wheat found decrease in osmotic potential on the onset of stress. Hein *et al.* (2016) also observed significant difference in osmotic potential between drought and in control plants, OP was lowest in the flag leaf, whereas after the 4-days drought treatment, OP had dropped subsequently in the flag leaf.

Table.1 Effect of restricted irrigation on leaf water potential, osmotic potential and relative water content at flag leaf and anthesis in barley genotypes

Genotypes	Water potential (-MPa)				Osmotic potential (-MPa)				Relative water content (%)			
	Flag leaf		Anthesis		Flag leaf				Flag leaf		Anthesis	
	NI	RI	NI		NI	RI	NI		NI	RI	NI	
BH07-35	1.53	1.55	1.68	**BH07-35**	1.53	1.55	1.68	**BH07-35**	1.53	1.55	1.68	**BH07-35**
BH10-30	1.48	1.49	1.65	**BH10-30**	1.48	1.49	1.65	**BH10-30**	1.48	1.49	1.65	**BH10-30**
BH12-17	1.60	1.60	1.93	**BH12-17**	1.60	1.60	1.93	**BH12-17**	1.60	1.60	1.93	**BH12-17**
BH12-20	1.61	1.61	1.94	**BH12-20**	1.61	1.61	1.94	**BH12-20**	1.61	1.61	1.94	**BH12-20**
BH12-29	1.49	1.50	1.67	**BH12-29**	1.49	1.50	1.67	**BH12-29**	1.49	1.50	1.67	**BH12-29**
BH12-46	1.72	1.72	1.95	**BH12-46**	1.72	1.72	1.95	**BH12-46**	1.72	1.72	1.95	**BH12-46**
BH13-10	1.80	1.80	2.04	**BH13-10**	1.80	1.80	2.04	**BH13-10**	1.80	1.80	2.04	**BH13-10**
BH13-21	1.70	1.71	2.02	**BH13-21**	1.70	1.71	2.02	**BH13-21**	1.70	1.71	2.02	**BH13-21**
BH13-22	1.72	1.72	1.94	**BH13-22**	1.72	1.72	1.94	**BH13-22**	1.72	1.72	1.94	**BH13-22**
BH13-23	1.74	1.74	2.05	**BH13-23**	1.74	1.74	2.05	**BH13-23**	1.74	1.74	2.05	**BH13-23**
BH14-01	1.75	1.76	1.98	**BH14-01**	1.75	1.76	1.98	**BH14-01**	1.75	1.76	1.98	**BH14-01**
BH14-05	1.49	1.49	1.65	**BH14-05**	1.49	1.49	1.65	**BH14-05**	1.49	1.49	1.65	**BH14-05**
BH14-06	1.76	1.77	1.98	**BH14-06**	1.76	1.77	1.98	**BH14-06**	1.76	1.77	1.98	**BH14-06**
BH14-07	1.47	1.49	1.70	**BH14-07**	1.47	1.49	1.70	**BH14-07**	1.47	1.49	1.70	**BH14-07**
BH14-08	1.80	1.79	2.12	**BH14-08**	1.80	1.79	2.12	**BH14-08**	1.80	1.79	2.12	**BH14-08**
BH14-09	1.88	1.87	2.11	**BH14-09**	1.88	1.87	2.11	**BH14-09**	1.88	1.87	2.11	**BH14-09**
BH14-10	1.44	1.44	1.69	**BH14-10**	1.44	1.44	1.69	**BH14-10**	1.44	1.44	1.69	**BH14-10**
BH14-11	1.73	1.75	2.25	**BH14-11**	1.73	1.75	2.25	**BH14-11**	1.73	1.75	2.25	**BH14-11**
BH902	1.59	1.57	1.83	**BH902**	1.59	1.57	1.83	**BH902**	1.59	1.57	1.83	**BH902**
BH946	1.56	1.56	1.86	**BH946**	1.56	1.56	1.86	**BH946**	1.56	1.56	1.86	**BH946**
Mean	**1.64**	**1.65**	**1.90**	**Mean**	**1.64**	**1.65**	**1.90**	**Mean**	**1.64**	**1.65**	**1.90**	**Mean**
CD at 5%	**E**= 0.014	**ExS**= 0.021			**E**= 0.008	**CD at 5%**			**E**= 0.014	**ExS**= 0.021		
	S= 0.020	**ExG**= 0.063			**S**= 0.008				**S**= 0.020	**ExG**= 0.063		
	G= 0.045	**SxG**= NS			**G**= 0.025				**G**= 0.045	**SxG**= NS		
	ExSxG= NS				**ExSxG**= 0.050				**ExSxG**= NS			

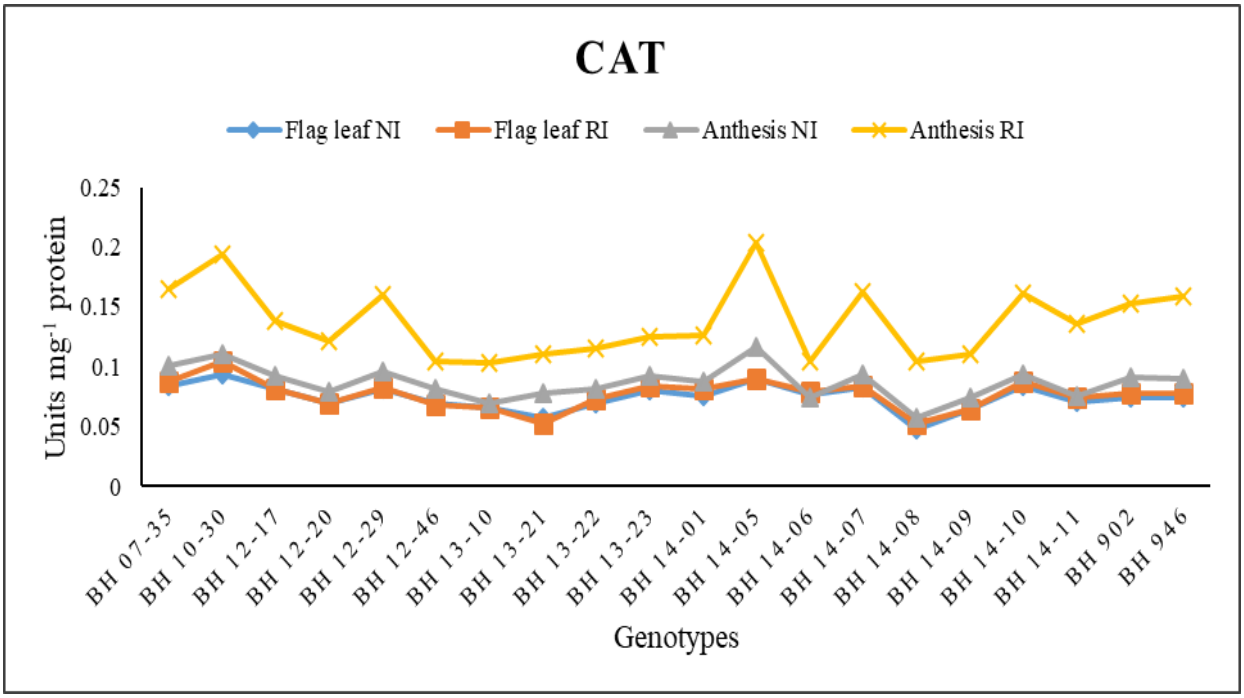
NI= Normal irrigation, RI= Restricted irrigation, E= Environment, S= Stage and G= Genotypes

Fig.1 Effect of restricted irrigation on superoxide dismutase specific activity at flag leaf stage and anthesis in barley genotypes



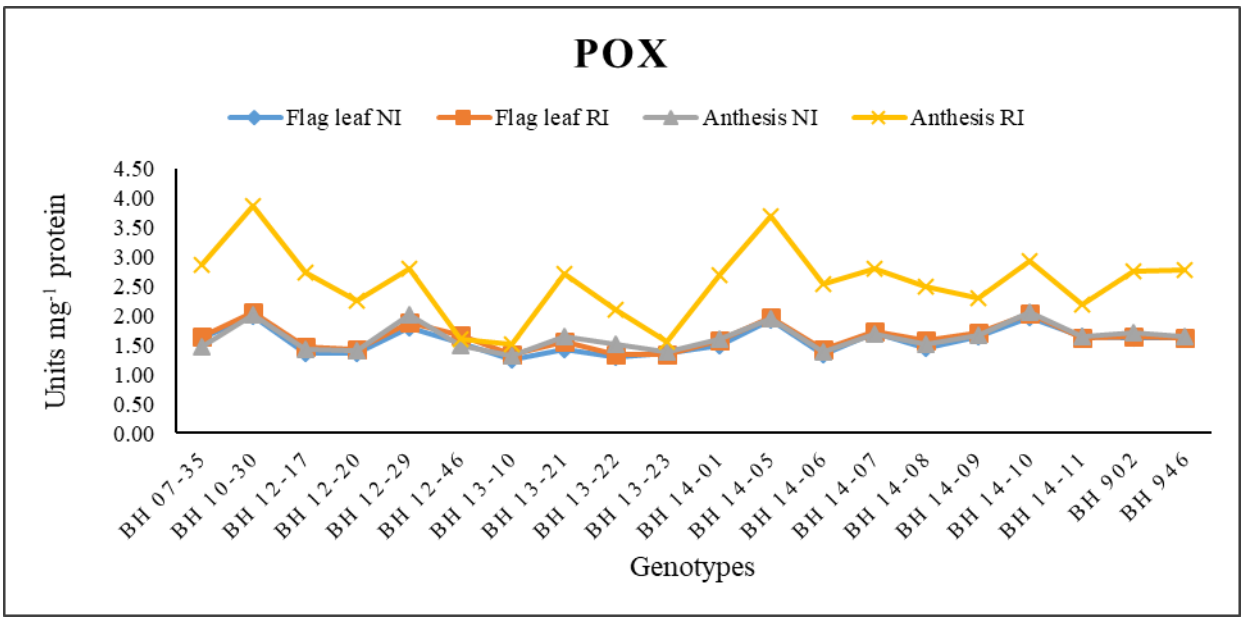
NI= Normal irrigation and RI= Restricted irrigation

Fig.2 Effect of restricted irrigation on catalase specific activity at flag leaf stage and anthesis in barley genotypes



NI= Normal irrigation and RI= Restricted irrigation

Fig.3 Effect of restricted irrigation on peroxidase specific activity at flag leaf stage and anthesis in barley genotypes



NI= Normal irrigation and RI= Restricted irrigation

Relative water content (%)

Reduction in leaf relative water content at flag leaf and anthesis was observed under restricted irrigation condition as compare to normal irrigation. Maximum reduction noticed at anthesis compared to flag leaf at restricted irrigation condition. Average relative water content for different genotypes for normal irrigation ranged from 72.2 to 80.9 % (flag leaf) and 70.7 to 79.2 % (anthesis) whereas, for restricted irrigation relative water content value varied between 78.1 to 84.1 % (flag leaf) and 58.8 to 72.9 % (anthesis). Average relative water content at flag leaf was 76.4 and 75.2 % and at anthesis 80.9 and 65.6 % under normal and restricted irrigation condition respectively. Genotype BH 14-08 and BH 13-10 had maximum relative water content at both growth stage flag leaf and anthesis respectively. Our finding is accordance with those of Kumar and Sharma, (2010) and Sharma *et al.* (2016) observed reduction in relative water content when crop was imposed under drought environment. Karami *et al.* 2013 find genotype whose maintain water status had more in antioxidant activity under drought condition.

Behbahanizadeh, *et al.* (2014) and Ram *et al.* (2017) also reported that under drought stress conditions (moderate stress and severe stress) RWC, osmotic potential and water potential reduced in contrast to control condition and cultivars had a significant difference together in water relation traits.

Antioxidant enzymes

Superoxide dismutase specific activity

Figure 1 showed superoxide dismutase (SOD) specific activity under normal and restricted irrigation condition at flag leaf and anthesis in graphical representation. SOD activity significantly increased from flag leaf stage to

anthesis stage. At flag leaf maximum increase in SOD activity was found in BH 14-09 (4.4%) and BH 13-09 (3.6%) and at anthesis in BH 946 (41.4%) and BH 14-06 (38.4%) under restricted irrigation condition, whereas, minimum increase in BH 12-46 (19.7%) and BH 14-01 (24.9%) at anthesis. Genotypes BH 14-05 and BH 10-30 had maximum in specific SOD activity at flag leaf and anthesis under both environments. The outcome from this study is lined with the results of Acar *et al.* (2001) and Salekjalali *et al.* (2012) in barley, SOD activity was increased in drought-resistant genotypes under progressive drought.

Catalase specific activity

The CAT activity was found to be higher in genotypes BH 10-30 and BH 14-05, respectively, at both the growth stages under normal irrigation condition (Figure 2). Minimum CAT activity was observed in BH 14-08 at both the stages of growth observation under normal and restricted irrigation. Maximum increase in CAT activity was observed in BH 13-23 (133.4%) and BH 14-08 (80.3%) at flag leaf and at anthesis in BH 10-30 (12.9%) and BH 07-35 (11.7%) under restricted irrigation. Minimum increase in CAT activity was observed in BH 12-46 (27.6%) and BH 07-35 (38.7%) at anthesis under restricted irrigation condition. Our results are consistent with Kolarovic *et al.* (2009); Luna *et al.* (2004) and Sharma and Dubey (2005) reported a decrease in CAT activity under drought stress in different cereal crops.

Superoxide dismutase specific activity

The POX specific activity increased from 3.56% to 31.73% from flag leaf to anthesis stage under restricted irrigation condition (Figure 3). Among the genotypes higher POX activity was observed in BH 10-30, BH 14-10 and BH 14-05 in both irrigation environments and growth stage. Under restricted irrigation condition maximum increase in enzyme specific activity among genotypes was observed in BH 14-08 (9.1%) and BH 13-21 (9.0%) at flag leaf and BH 07-35 (94.8%), BH 12-17 (92.9%) and BH 10-30 (91.9%) at anthesis while minimum increase in BH 12-46(0.6%) and BH 14-11(34.2%) at anthesis stage of observation. The interaction among all factors taken into consideration was found to be significant at each growth stage. Our result is accordance with the study of Sairam and Saxena (2000); Shao *et al.* (2005) and Chugh *et al.* (2011). Cultivar exhibited a higher CAT activity compared to another found tolerant under drought condition (Valifard *et al.* 2012).

In conclusion selection for best and promising genotype for adverse environment using physiological and biochemical traits is the best way of selection. The genotype BH 14-05 and BH 10-30 had higher antioxidant activity (SOD, CAT and POX) and maximum in water relation traits compared to among all genotypes. These genotypes further used as to improved physiological and biochemical processes to produced more grain yield.

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