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Effect of Brassinosteroid on Antioxidant Enzymes Changes of Cowpea (*Vigna un guiculata*) under Water Stress Conditions

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Absract: In order to study the effects of water stress and the application of brassinosteroide on growth and seed yield of Cowpea cv. Kamran, an experiment was carried out. The experiment was conducted in the form of split-plot at the basis of Randomized Complete Block Design (RCBD) with three replications. The main plots were comprised of 2 irrigation levels including irrigation after 60 mm evaporation from class A evaporation pan as normal condition (I0) and irrigation after 120 mm evaporation from class A evaporation pan as water stress conditions (I1). The sub plots were comprised of five level of application of brassinosteroide including the control (B0), seeds soaking in brassionsteroid 2 μM concentration (B1), seeds soaking in brassionsteroid 4 μM concentration (B3), spraying of brassinostroid 2 μM concentration (B4) and spraying of brassinostroid 4 μM concentration (B5). The results showed water stress increased SOD (113%), POD (54%), CAT (93%), GPX (24%) activities, MDA content (109%). In this study, water stress had no significant effect on APX activity. In the other hand exogenous brassinosteroid affected allstudied traits except POD activity. In the most studied traits spraying of brassinostroid with 2 μM concentration was more effective but in the case of seed yield seeds soaking in brassionsteroid with 4 μM concentration produced higher seed yield, although, it had not significant difference with spraying of brassinostroid with 2 μM concentration. The results of this study suggest that in order to mitigate the adverse effects of water stress in cowpea plants can be used the spray of brassinostroid with 2 μM concentration or seeds soaking in brassionsteroid with 4 μM concentration.

Key words: Brassinosteroid, cowpea, antioxidant enzyme, water stress, yield, Iran

INTRODUCTION

Population growth and economic and social development of the country in the past two decades has led to a dramatic increase in the consumption of protein, especially red meat. Accordingly, increase of protein production is unavoidable, especially plant proteins that resources are valuable in nutrition. Today, Lack of protein in feeding millions of people in underdeveloped countries one of the acute problems feeding are considered. Beans as an important plant source of protein-rich after cereal and the second most important source of food is considered. Among the beans, cowpea (*Vigna unguiculata*) is a 1 year Summer legume with trifoliate leaves that in a wide range of soil textures from clay to sandy grows. In the bean, cowpea is grown in tropical and subtropical countries and is an important source of nutrition (Singh *et al*., 1970). Water stress is one of the main barriers in crop production in many parts of the world especially in arid and semi-arid Iran. Water deficit is one of the important environmental factors that affect plant growth and plant production limits (Bohnert *et al*., 1995). Since about 50% of the crop due to lack of water and inappropriate distribution rainfall in Iran will be affected (Vaezi and Ahmadi, 2011). The use of plant growth regulators such as Brassinosteroids can be a good way to reduce damage from drought stress. Brassinosteroids are steroid plant compounds with a lot of biological activities that have the ability to increase plant performance through changes in plant metabolism and protection against environmental stresses (Eskandari, 2011). Therefore, it seems necessary to determine the appropriate concentration of Brassinosteroid in cowpea various stages of growth that would reduce the adverse effects of drought. Hayashi and Hanada (1985) to study the effects of water stress during the growing season on yield, yield components of safflower concluded that lack of water prevents the growth of internodes of the main stem and lateral buds. Siddique *et al*. (1999) reported that the drought as the most important factor controlling the performance were effective on all plant growth processes. It is reported that Brassinosteroids are lead to reduce the

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adverse effects of cold stress, high temperatures, heavy metals, Salinity (Ozdemir *et al*., 2004) and water stress (Jaisingh and Ota, 1993). Brassinosteroied regulates one of stomatal development plant pathways, this pathway in plants is well known (Fridman and Goldstein, 2013). In the report changes in the expression and activity of antioxidant enzymes has been evident in several species of plants in response to adverse environmental conditions such as lack of water as well as other abiotic stresses, biotic stresses and growth provocations. The synthesis of enzymes such as catalase against oxidative stress is a response adapted (Mittler, 2002). It is reported that high antioxidant capacity under stress conditions can prevent damage and is associated with stress tolerance (Khan *et al*., 2004).

MATERIALS AND METHODS

The study was conducted in the spring of 1392 in the field of education in Islamic Azad University Yadegar Imam Khomeini of Reye town, located in the South Tehran. Farm height of 1000 m above sea level and in terms of geographical location, field of geographical latitude 35°51 and longitude 51°29 min is located. To measure physical and chemical properties of field soil, the sampling was performed of soil before conducting the experiment. An experiment split plot in randomized complete block design with three replications was conducted. The main plots included two irrigation levels were as follows: I0) irrigation after 60 mm evaporation from evaporation pan class A as a normal condition I1) irrigation after 120 mm evaporation from pan class A as drought conditions. Subplots also includes five levels of with different concentrations as follows: b0: non-application of (seeds soaking in distilled water) b1: seeds soaking in brassinisteroid solution with concentration 2 μM b2: seeds soaking in brassinisteroid solution with concentration 4 μM b3: Spraying brassinisteroid solution with concentration 2 μM b4: Spraying brassinisteroid solution with concentration 4 μM.

The seeds 24 h before sowing for 8 h in a solution of distilled water, 2 and 4 μM brassinosteroid solution were soaked and after 8h out of solution and after drying, it was placed inside the bags until the next morning to be ready for planting. At the vegetative stage (6 leaves) and flowering different plots, twice with brassinisteroid solution was sprayed. In this experiment, the cultivar of Kamran cowpea was used. For culture on the stack using Foca furrows to the depth of 4-3 cm was created. Then the seeds were planted by hand. According to soil test results equivalent to 100 kg ha pure nitrogen (urea) in two sets (50 kg ha before planting and 50 kg per hectare after planting) and 100 kg of phosphorus fertilizer (superphosphate Triple) before planting and when the disc into ground was added.

Malondialdehyde (MDA) measurement: Amount of MDA with absorption measurements at wavelengths of 532 and 600 nm and using the extinction coefficient (μM -1cm 155 = ε)was calculated (DeVos *et al*., 1991).

Catalase (CAT) enzyme activity assay: Measurement of catalase activity was performed by Cakmak and Horst (1991).

Superoxide dismutase (SOD) enzyme activity measurement: Samples absorption amount at wavelengths of 560 nm using a spectrophotometer was measured.

Peroxidase enzyme activity measurement: Peroxidase activity at a wavelength of 470 nm was measured.

Ascorbate peroxidase enzyme activity measurement: Activity level this enzyme was measured by methods Ranieri.

Glutathione peroxidase enzyme activity measurement: For measuring glutathione peroxidase enzyme was performed according to Paglia and Valentine (1967).

Statistical analysis: Data obtained using the Softwares Mstat-C, SPSS V.19 and Minitab V.16 were analyzed. Determine the correlation level between traits using SPSS Software was performed. Regression equations calculated using Minitab software and diagrams were drawn by the software Excel 2007.

RESULTS AND DISCUSSION

In this experiment, the enzyme activity of superoxide dismutase, peroxidase, catalase ascorbate peroxidase, glutathione peroxidase and eventually the amount of malondialdehyde under drought conditions and brassinosteroid application were studied and analysis of variance, mean comparison and characteristics correlation was performed and results were presented as follows (Table 1-3).

Superoxide dismutase: Effect of drought stress on the activity of superoxide dismutase analysis of variance showed that the effect of drought stress on superoxide dismutase activity was significant at level 1%. As

Table 1: Analysis of variance the enzymes activity of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione peroxidase malondialdehyde under drought conditions and brassinosteroid application

Mean of squars							
Malondialdehyde	Glutathione peroxidase	Ascorbate peroxidase	Catalase	Peroxidase	Superoxide dismutase	df	Source of variation
04.207^{ns}	38.022*	1.266^{ns}	5.753*	1.717^{ns}	15.887^{ns}	2	Replication
7985.051	463.661**	10.538$_{ns}$	2373.453**	788.276*	12659.21**	1	Drought stress
2.174	1.503	0.933	0.133	9.792	25.689	2	Error
297.042**	63.444**	13.292*	131.091**	22.731$_{ns}$	908.380**	4	Brassinosteroiddrought
562.766**	17.058^{ns}	0.366^{ns}	8.985^{ns}	83.921**	68.257^{ns}	4	Brassinosteroid
26.583	9.099	1.490	6.152	13.040	32.017	16	Error
11.20	8.39	10.59	8.56	15.17	9.96)	------	%(CD

ns, *,**: respectively non-significant and significant at the level of 5 and 1%

Table 2: Mean comparison of drought stress and brassinosteroid application effects on enzymes activity of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione peroxidase malondialdehyde

Malondialdehyde (µmol/gram of fresh weught)	Glutathione peroxidase absorbance units per mg protein per (min)	Ascorbate peroxidase absorbance units per mg protein per (min)	Catalase absorbance units per mg protein per (min)	Peroxidase absorbance units per mg protein per (min)	Superoxide dismutase absorbance units per mg protein per (min)	Treatment
						Drought stress
29.72^{b}	32.03^{b}	10.93^{a}	20.08^{b}	18.68^{b}	36.27^{b}	Non drought stress
62.35^{a}	39.89^{a}	12.12^{a}	37.87^{a}	28.93^{a}	77.35^{a}	drought stress
52.64^{a}	32.78^{c}	9.88^{c}	21.67^{c}	22.75^{a}	40.76^{c}	control
46.85^{a}	35.52^{bc}	11.26^{bc}	28.52^{b}	22.71^{a}	52.60^{b}	Seedsoaking (2 µM)
39.57^{b}	38.50^{ab}	11.95^{b}	32.86^{a}	25.72^{a}	68.95^{a}	Seedsoaking (4 µM)
38.08^{b}	40.04^{a}	13.82^{a}	33.29^{a}	26.06^{a}	69.53^{a}	Spraying (2 µM)
53.01^{a}	32.98^{c}	10.71^{bc}	28.54^{b}	21.78^{a}	52.22^{b}	Spraying (4 µM)

Table 3: Mean comparison of interactions drought stress and brassinosteroid application On enzymes activity of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione peroxidase malondialdehyde

Treatment		Malondialdehyde (µmol/gram of	Glutathione	Ascorbate			Superoxide
Irrigation	Brassinosteroid	fresh weight)	peroxidase	peroxidase	Catalase	Peroxidase	dismutase
Non-drought stress	Control	20.16^{e}	2.28^{e}	8.907^{e}	14.27^{e}	11.28^{e}	2.08^{d}
	Seed Soaking (2 uM)	39.95^{d}	2.92^{de}	10.74^{cde}	19.91^{d}	18.14^{cd}	3.38^{d}
	Seed Soaking (4 uM)	25.76^{e}	3.32^{bcd}	11.35^{bcd}	22.99^{d}	23.54^{bc}	4.73^{c}
	Spraying (2 uM)	36.59^{d}	3.80^{cde}	10.41^{de}	20.34^{d}	16.90^{de}	3.88^{d}
Drought stress	Control	85.12^{a}	3.28^{ab}	10.87^{cde}	29.08^{c}	34.21^{a}	5.44^{c}
	Seed Soaking (2 uM)	35.76^{c}	4.12^{a}	11.78^{bcd}	37.12^{b}	27.28^{b}	7.82^{b}
	Seed soaking (4 uM)	53.39^{c}	4.68^{a}	12.56^{abc}	42.72^{a}	27.89^{b}	9.16^{a}
	Spraying (2 uM)	50.03^{c}	4.24^{a}	14.38^{a}	43.69^{a}	28.59^{ab}	9.78^{a}
	Spraying (4 uM)	69.44^{b}	3.16^{bc}	11.01^{cde}	36.74^{b}	26.67^{b}	7.57^{b}

The numbers in each column in each treatment with are similar letters, no significant differences according to Duncan test at level of 5%

antioxidant enzymes other such as catalase and peroxidase, superoxide dismutase activity increased as a result of drought and non-drought treatment from 36.27 unit to 77.35 unit came under drought stress (Fig. 1). The researchers said that in order to inhibit the production of oxygen free radicals in plants under drought stress, antioxidant defense systems are activated in the plant that is one of the most important enzymes Super Oxide Dismutase (SOD) (Sirhindi *et al*., 2009). It is reported that antioxidant enzyme activity in conditions of stress doubles, that lead to increased resistance to oxidative stress (Luna *et al*., 2005).

Effects of Brassinisteroid application on activities of superoxide dismutase enzyme: Results of variance analysis showed that application of brassinosteroid significant effect on the activity of superoxide dismutase was at the level of 1% (Table 1). Impact of

Table 4: Simple correlation some of the traits tested with all the tested attributes

Enzymes	1	2	3	4	5	6
Superoxide dismutase	-0.33	-0.231	-0.232	-0.262	-0.543	0.198
Peroxidase	-0.489	-0.235	-0.27	-0.27	-0.552	0.040
Catalase	-0.38	-0.299	-0.27	-0.312	-0.587	0.164
Ascorbate peroxidase	0.238	0.386	0.174	0.273	0.047	0.451
Glutathione peroxidase	-0.271	-0.108	-0.136	-0.13	-0.421	0.233
Malondialdehyde	-0.781	-0.628	-0.652	-0.689	-0.800	-0.391

Table 5: Simple correlation some of the traits tested with all the tested attributes

Enzymes	7	8	9	10	11	12
Superoxide dismutase	0-0.59	0-0.661	0-0.783	-0.43	-0.63	0.927
Peroxidase	0-0.700	0-0.653	-0.556	-0.467	0-0.735	0.867
Catalase	0-0.637	0-0.688	0-0.791	-0.454	0-0.663	0.911
Ascorbate peroxidase	0-0.039	0-0.09	-0.217	0.167	-0.107	0.794
Glutathione peroxidase	0-0.507	0-0.552	-0.559	-0.314	-0.579	0.919
Malondialdehyde	0-0.893	0-0.794	0-0.696	0-0.738	0-0.888	0.561

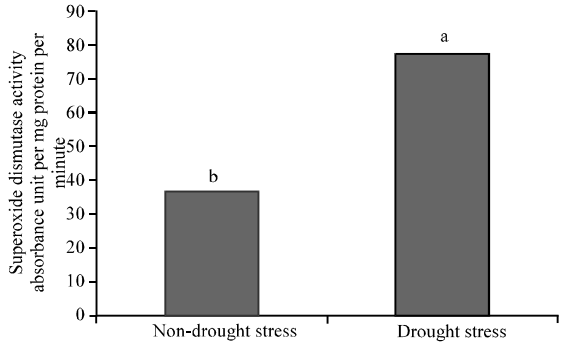

Fig. 1: Effect of drought stress on superoxide dismutase activity

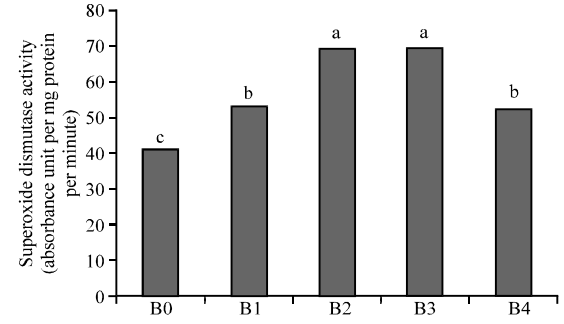

Fig. 2: Effect of brassinisteroid application on superoxide dismutase enzyme activity

brassinosteroid on the activity of superoxide dismutase, was similar to its effect on catalase. So that minimumactivities are related to non-application treatment of brassinosteroid and brassinosteroid spraying treatment with concentration 2 µM, respectively with an average of 40.76 and 69.53 units had the highest activity (Fig. 2). Increase the activity of superoxide dismutase treated brassinosteroid in tomato plants (Ahammed *et al*., 2012), corn seedlings under drought stress and rice buds by salinity were reported.

The interaction of drought stress and brassinosteroid application on activities of superoxide dismutase: Results of variance analysis showed that the interaction of drought stress and use brassinosteroid application on superoxide dismutase activity was not significant. Generally, the highest superoxide dismutase enzyme activities related to drought stress treatment along with seeds soaking in brassinisteroid with concentration of 4 µM (an average 92.16) was obtained that with drought stress along with brassinosteroid spray with concentration of 2 µM, no significant difference showed. The minimum superoxide dismutase enzyme activity is also related to treatment non drought stress and brassinosteroid non application with average 8.26 units were obtained. Despite the lack of treatments significant interaction between them significant difference be seen. brassinosteroid application both stress conditions and non-drought stress conditions lead to increase the activity of superoxide dismutase enzyme with the same slope became. Behnamnia *et al.* (2009) reported under drought stress conditions, the activities of antioxidant enzymes such as ascorbate peroxidase, peroxidase, catalase and superoxide dismutase in plants under drought stress increased after treatment with brassinosteroid. Superoxide dismutase had a negative correlation with grain yield (r = -0.262). The highest correlation was superoxide dismutase with catalase (r = 0.990**) (Table 4-7).

Peroxidase

Impact of drought stress on peroxidase enzyme activity: Results of variance analysis showed that the effect of drought stress on peroxidase activity was significant at 5% level (Table 1). With increasing irrigation distances (Creation drought stress) significantly peroxidase activity increased and from 18.68-28.93 units reached (Fig. 3). Plants when exposed to various environmental stresses such as drought, salinity and etc. are placed, active forms of oxygen such as hydrogen peroxide and hydroxyl

Table 6: Simple correlation some of the traits tested with all the tested attributes

Enzymes	13	14	15	16	17	18
Superoxide dismutase	1	0.748*	0.990**	0.715*	0.900**	0.451
Peroxidase	0.748*	1	0.757*	0.586	0.847**	0.604
Catalase	0.990**	0.757*	1	0.686*	0.882**	0.509
Ascorbate peroxidase	0.715*	0.586	0.686*	1	0.820**	-0.13
Glutathione peroxidase	0.900**	0.847**	0.882**	0.820**	1	0.362
Malondialdehyde	0.541	0.793**	0.605	0.123	0.493	0.885**

Table 7: Simple correlation some of the traits tested with all the tested attributes

Enzymes	19	20	21	22
Superoxide dismutase	0.541	0.902**	986**	0.971**
Peroxidase	0.793**	0.747*	0.699*	0.849**
Catalase	0.605	0.934**	981**	0.984**
Ascorbate peroxidase	0.123	0.493	0.663*	0.751*
Glutathione peroxidase	0.649*	0.826**	863**	0.888**
Malondialdehyde	1	0.57	0.489	0.715*

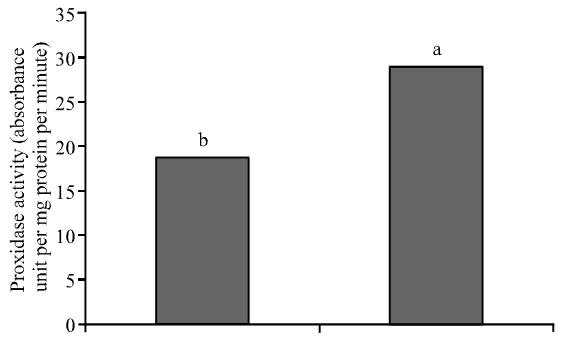

Fig. 3: Effect of drought stress on peroxidase enzyme activity

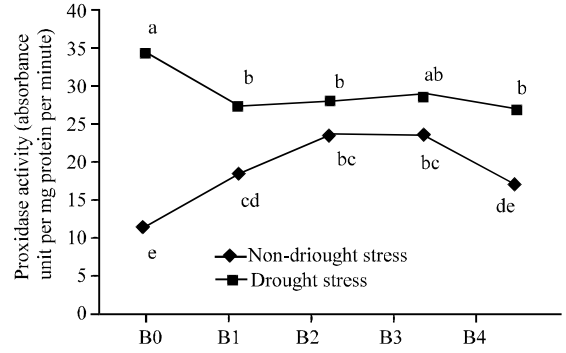

Fig. 4: The interaction drought stress and brassinosteroid application on proxidase enzyme activity

radicals in them are produced. Plants to eliminate free radicals from enzymatic and non-enzymatic mechanisms are used that one of the most important mechanisms is peroxidase also studies showed that drought stress leading to peroxidase activity was stimulated in wheat and peas (Alexieva *et al*., 2001).

Impact of brassinosteroid application on peroxidase enzyme activity: Analysis of variance showed that use of brassinosteroid had no significant effect on peroxidase activity. Although, use of brassinosteroid somewhat increased peroxidase activity but this increase was not significant.

The interaction of drought stress and brassinosteroid application on peroxidase enzyme activity: Results of variance analysis showed that interaction of drought stress and use of brassinosteroid on peroxidase activity was significant at level of 1%. Overall, the highest peroxidase enzyme activity related to irrigation treatment after 120 mm evaporation (drought stress) and non-application of brassinosteroid with an average 34.21 unit and the lowest amount of peroxidase enzyme activity related to irrigation treatment after 60 mm evaporation (non- drought stress) and non-application brassinosteroid with an average 11.28 unit obtained (Fig. 4). Brassinosteroid application in drought stress lead to decreased peroxidase enzyme activity and normal irrigation condition was increased peroxidase enzyme activity. Peroxidase was non-significant and negative correlation with seed yield (r = -0.270). Seed protein percentage had correlation highest with peroxidase enzyme (r = 0.867).

Catalase

Effect of drought stress on catalase enzyme activity: Results of variance analysis showed that effect of drought stress on catalase enzyme activity was significant at level of 1%. Like peroxidase, catalase enzymeactivity also increased under drought conditions, this increase activity was from 20.8 unit in non-drought stress to 37.87 unit under drought stress (Fig. 5). In the study of enzymatic activity of safflower under water deficit conditions, appeared that drought stress on activity of catalase and peroxidase enzymes was significant and drought stress leads to an increase activity of these enzymes became. The balance between the production of

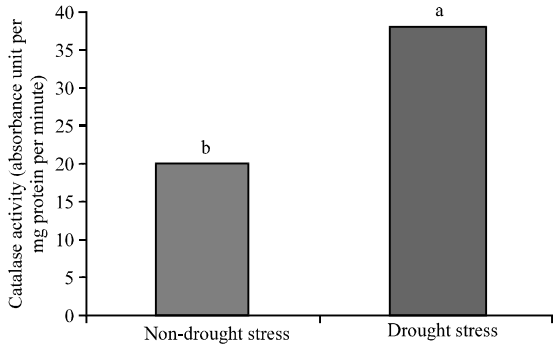

Fig. 5: Effect of drought stress on catalase enzyme activity

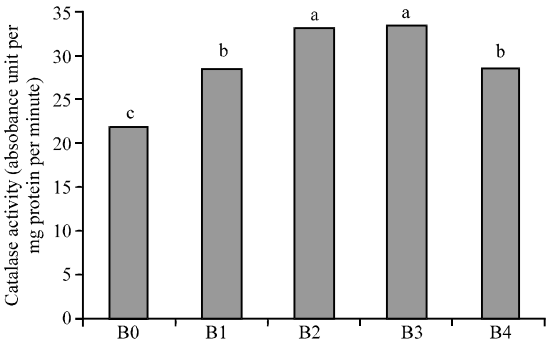

Fig. 6: Effect of brassinosteroid application on catalase enzyme activity

reactive oxygen species and silencing under stress conditions normally carried out through antioxidant systems such as catalase (Harinasut *et al*., 2003).

Effects of brassinosteroid application on catalase enzyme activity: Results of variance analysis showed that effect of brassinosteroid application on catalase enzyme activity was significant at level of 1% (Table 1). Unlike peroxidase enzyme, brassinosteroid application on catalase enzyme activity had significant effect and leading to increase it significantly. The highest catalase activity from treatments of seeds soaking in brassinosteroid in concentration of 4 μM and spray brassinosteroid with concentration of 2 μM, respectively with an average of 32.86 and 33.29 units were obtained (Fig. 6). The least catalase enzyme activity of the control treatment (non-application of brassinosteroid) with an average of 21.67 was obtained. It has been reported that brassinosteroid catalase, peroxidase andsuperoxide dismutase enzymes activity in response to high temperatures in the leaves of tomato increased (Mazorra *et al*., 2002).

The interaction of drought stress and brassinosteroid application on catalase enzyme activity: Results of variance analysis showed that interaction of drought stress and brassinosteroid application on catalase enzyme activity was not significant (Table 1). The results showed that brassinosteroid of different treatments effects on the amount of catalase in both drought and non-drought were same. In total, the highest catalase activity related to drought stress and brassinosteroid spray with concentration of 2 μM with an average of 43.69 unit was obtained and the lowest of catalase activity with an average 14.27 unit from non-drought stress and non-application of brassinosteroid was obtained (Table 3). Catalase enzyme had negative and non-significant correlation with grain yield (r = -0.312). Like peroxidase, also catalase enzyme was high correlation with grain protein content (r = 0.911) but the highest correlation was catalase with superoxide dismutase (r = 0.990) (Table 6).

Ascorbate peroxidase

Effect of drought stress on the activity of Ascorbate peroxidase enzyme: Results of variance analysis showed that drought stress on Ascorbate peroxidase enzyme activity was not significant (Table 1). Drought stress increased the activity of ascorbate peroxidase enzyme from 10.93-12.12 units but this increase was not significant (Table 2). Ascorbate peroxidase enzyme activity increased under drought stress by other researchers in plants such as maize and tomatoes (Behnamnia *et al*., 2009) is reported.

Effects of brassinosteroid on Ascorbate peroxidase enzyme activity: Results of variance analysis showed that effect of brassinosteroid application on Ascorbate peroxidase enzyme activity was significant at level of 1% (Table 1). With brassinosteroid application of ascorbate peroxidase enzyme activity Significantly increased. The highest level of Ascorbate peroxidase enzyme activity from brassinosteroid spray treatment with concentration of 2 μM units was achieved with an average 13.82 and the least activity of ascorbate peroxidase enzyme as well as the control treatment (non-application of brassinosteroid) with an average of 9.88 unit was achieved (Fig. 7). It has been reported that ascorbate peroxidase enzyme activity in corn seedlings treated with drought stress had increased.

The interaction of drought stress and the use of brassinosteroid on ascorbate peroxidase enzyme activity: Results of variance analysis showed that interaction drought stress and use of brassinosteroid on Ascorbate peroxidase enzyme activity was not significant (Table 1). Due to non-significant interaction of drought stress and brassinosteroid applying was different treatments same effects of brassinosteroid on the activity of Ascorbate

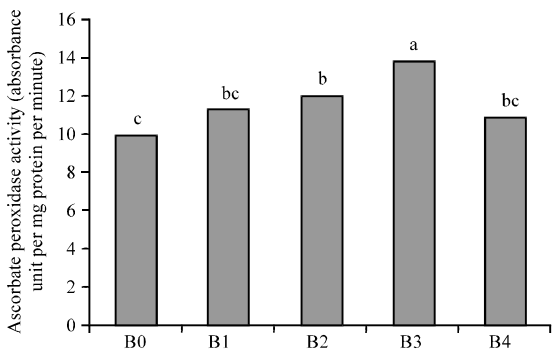

Fig. 7: Effect of brassinosteroid application on ascorbate peroxidase enzyme activity

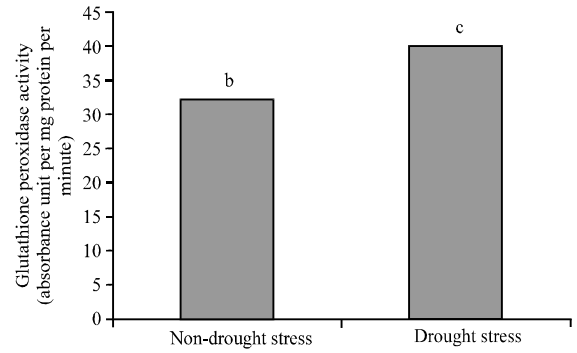

Fig. 8: Effect of drought stress on glutathione peroxidase enzyme activity

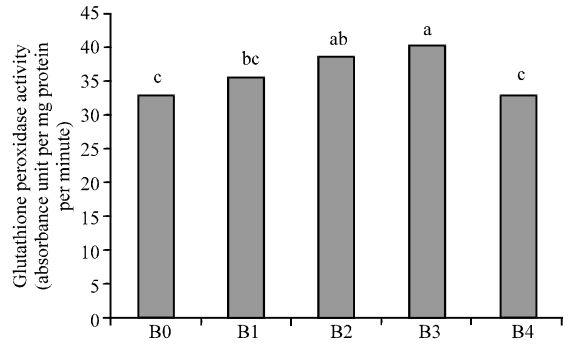

Fig. 9: Effect of brassinosteroid application on glutathione peroxidase enzyme activity

peroxidase enzyme under drought stress and normal irrigation. The highest activity of Ascorbate peroxidase enzyme in drought stress treatment altogether with brassinosteroid spray in concentration of 2 μM with an average of 14.38 was obtained (Table 3). The least activity of Ascorbate peroxidase enzyme as well as the treatment of non-drought stress and non-application of brassinosteroid with an average of 8.90 unit was achieved. ascorbate peroxidase was positively correlated with grain yield but this correlation was non-significant and weak (r = 0.273). The highest levels of Ascorbate peroxidase correlation with glutathione peroxidase (r = 0.820**) and after was grain protein content (r = 0.794**) (Table 6).

Glutathione peroxidase

Effects of drought stress on glutathione peroxidase enzyme activity: The results of analysis of variance showed that the effect of drought stress on glutathione peroxidase activity was significant at level of 1% (Table 1). Drought stress leading to significant increase in glutathione peroxidase enzyme activity became. The highest glutathione peroxidase enzyme activity with an average 39.89 unit from 120 mm treatment of evaporation (drought stress) and the lowest glutathione peroxidase enzyme activity of irrigation after 60 mm evaporation (non-drought) with an average of 32.02 unit were obtained (Fig. 8). The researchers showed that antioxidant enzymes activity in wheat plant doubled under drought stress, therefore resistance to oxidative stress increased (Luna *et al*., 2005). Other researchers Glutathione peroxidase enzyme activity increased under drought stress also have been reported (Ahammed *et al*., 2012).

Effects of brassinosteroid application on glutathione peroxidase enzyme activity: The results of analysis of variance showed that brassinosteroid application on glutathione peroxidase enzyme activity was significant at level of 1% (Table 1). Brassinosteroid application glutathione peroxidase enzyme activity significantly increased and from 32.78 unit in control treatment to 40.04 unit in brassinosteroid spray treatment with concentration of 2 μM increased (Fig. 9). Behnamnia *et al.* (2009) reported that lipid peroxidation and H_2O_2 content Reduced in tomatoes treated with brassinosteroid this decrease related to activity of some antioxidant enzymes such as glutathione peroxidase was in plants under Drought stress and treated with brassinosteroid. Also Ahammed *et al.* (2012) also increased glutathione peroxidase activity in tomato with use of brassinosteroid were reported.

The interaction of drought stress and brassinosteroid application glutathione peroxidase enzyme activity: Results of variance analysis showed that interaction of drought stress and brassinosteroid application on glutathione peroxidase enzyme activity was not significant (Table 1). The highest glutathione peroxidase activity of irrigation treatment after 120 mm

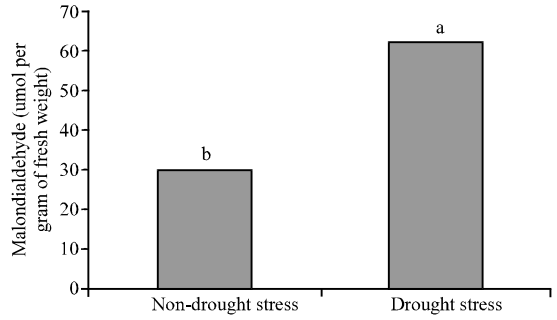

Fig. 10: Effect of drought stress on Malondialdehyde concentration

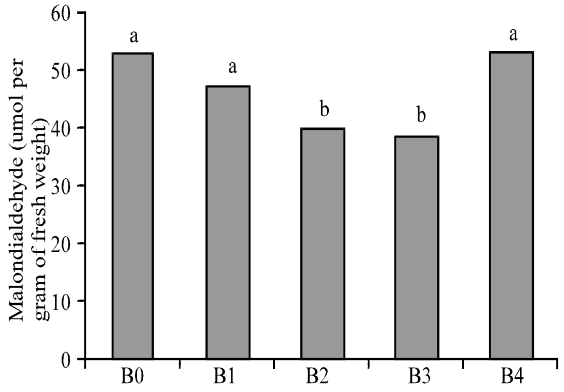

Fig. 11: Effect of brassinosteroid application on malondialdehyde concentration

evaporation (drought stress) altogether with seed soaking brassinosteroid with concentrations 4 μM and an average of 42.68 unit was obtained. The least of glutathione peroxidase activity in the treatment of non-drought stress and non-application of brassinosteroid with an average of 27.28 unit was achieved (Table 3). Results showed that drought stress conditions could as an antioxidant enzyme, increased glutathione peroxidase enzyme activity and Was prevented from oxidative stress. Glutathione peroxidase was very weak and non-significant negative correlation with grain yield (r = -0.130). The highest levels of correlation were with grain protein content trait (r = 0.919**) (Table 6).

Malondialdehyde

Effects of drought stress on concentration of malondialdehyde: The results of analysis of variance showed that drought stress on concentration of malondialdehyde was significant at level of 1% (Table 3). MDA under stress conditions significantly increased compared to the normal condition and from 29.72 μM per gram of fresh weight to 62.35 μmol per gram of fresh weight reached (Fig. 10). This result associated with reports Sairam and Saxena based on the increase MDA in drought stress. They believed that when antioxidant defenses decrease or increase free radical's formation,oxidative stress occurs which can lead to an increase in unsaturated fatty acids peroxidation, lipid peroxidation, membrane damage and as a result, leading to departure of different aldehydes such as Malondialdehyde (MDA). Also Jingxian and Kirkham (2006) reported that levels of enzymes and Malondialdehyde (MDA) increased under drought stress than under normal irrigation.

Effects of brassinosteroid application on malondialdehyde concentration: The results of analysis of variance showed that brassinosteroid application on malondialdehyde concentration was significant at level of 1% (Table 3). So that highest concentration of MDA from brassinisteroid spray treatment in concentration of 4 μM with average of 53.01 μmol per gram of fresh weight was achieved and of course between control and seed soaking treatment with brassinosteroid in a concentration of 2 μM was no significant difference. The least amount of malondialdehyde also from spray treatments with brassinosteroid in a concentration of 2 μM and seed soaking at a concentration of 4 μM, respectively with an average of 38.08 and 39.57 μmol per gram of fresh weight was obtained (Fig. 11). As regards, increased levels of malondialdehyde indicating damage to the membrane (Sairam and Saxena, 2002), so it can be seen that the use of brassinosteroid decreased damage to the membrane and thus reduced the amount of malondialdehyde. Ahammed *et al.* (2012) also reduce the amount of MDA were reported with application of brassinosteroid especially spraying.

The interaction of drought stress and brassinosteroid application on malondialdehyde concentration: The results of analysis of variance showed that interaction of drought stress and brassinosteroid application on malondialdehyde concentration was significant at level of 1% (Table 3). In total, the highest concentrations of malondialdehyde from irrigation after 120 mm evaporation (drought stress) with non-application of brassinosteroid with an average of 12/85 μmol per gram fresh weight was obtained. The least concentrations of malondialdehyde also from irrigation after 60 mm evaporation (normal conditions) and non-application of brassinosteroid with an average of 20.16 μmol per gram fresh weight was obtained (Fig. 12). It was observed that the application of brassinosteroid especially under drought stress malondialdehyde concentration reduced and Under normal irrigation slightlyincreased the amount of MDA in some treatments. Based on these results we can say that

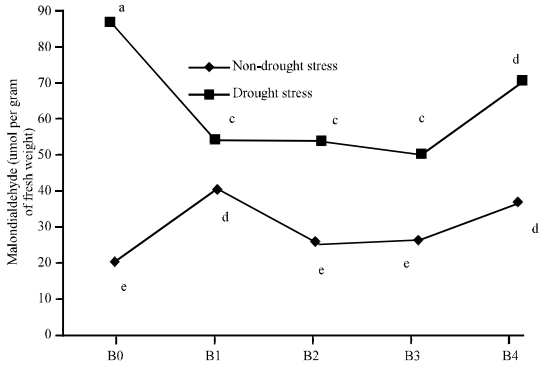

Fig. 12: The interaction of drought stress and Brassinosteroid application on malondialdehyde concentration

brassinosteroid is a role for regulators to MDA. MDA with grain yield had significant and negative correlation (r = -0.689*). The highest levels of MDA negative correlation related to plant height (r = -0.893**) and then for relative water content was (r = -0.888**) (Table 7). As regards, plant height had direct relationship with relative water content can be said that RWC was most important factor in changes in metabolites associated with stress such as Malondialdehyde (MDA).

CONCLUSION

The results showed that drought stress traits such as superoxide dismutase, peroxidase, catalase, glutathione peroxidase, malondialdehyde increased. In this study, drought stress was not significantly affected by ascorbate peroxidase. use of brassinosteroid hormone not affected all studied traits except peroxidase. Brassinosteroid affected all these indices to reduce the adverse effects of Drought stress. According to the results of this study, it is suggested to reduce the adverse effects of drought stress on cowpea plant can be used brassinosteroid spray with a concentration of 2 μM or seed soaking with 4 mM concentration.

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