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European Journal of Experimental Biology, 2013, 3(3):261-266

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of super absorbent polymer application on chemical and biochemical activities in red bean (*Phaseolus volgari*s L.) cultivars under drought stress

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ABSTRACT

Chemical and biochemical activities of bean (phaseoulus vulgaris) cultivars were studied in three red bean cultivars (V1, V2, and V3) at the two levels of irrigation and two different amounts of super absorbent (0 &7%). Field experiment was conducted in 2005 and the experimental design was randomized by complete block in a split plot arrangement with three replications. Our results showed that, the drought stress significantly increased the activities of some antioxidant enzymes (catalase, glutathione redoctase and super oxid dismutase) content. Of course, the content of antioxidant enzymes was more at moderate than extreme water deficit. Lipid and protein oxidation (malondialdehyde and dityrosine contents) also significantly increased in response to water deficit-induced. As it was shown, stress decreases the yield and it can increase biochemical characters and on the other hand, using 7% super absorbent in field situation increases its agricultural characters.

Keywords: water deficit stress, Antioxidant enzymes, Oxidative stress, super absorbent.

INTRODUCTION

Drought is one of the most important factors in reducing the agricultural field productivity, dry and semi dry climate affects most part of the country, especially recent droughts have increased this problem.

Decreasing water loss and increasing irrigation effectiveness have always been noted by agricultural researchers and specialists. One noticeable way introduced by agricultural researchers is the use of soil correctives and additives.

Application and mixture of super absorbent polymer can reserve different amounts of water in itself and so increase the soil ability of water storing and preserving, and at last in water deficiency, produce plant water need and approve its growth. Polymers are synthetic organic compounds and are synthesized manually. These compounds are produced from potassium polyacrylate and polyacrylamide co-polymers and when exposed to water are able to absorb and preserve water fast and several times of their volume. They increase the ability of soil to preserve the water and with decreasing drought stress approve the growth of plant. These materials are odorless, colorless and have no pollution effect on soil or water and plant organs (Roshan, 2005). Drought stress is one of the several environmental factors greatly limiting the crop production and plant distribution worldwide (Zhang et al., 2010). These polymers improve the physical properties of soil, also serve as buffers against temporary drought stress and reduce the risk of plant failure during its establishment (Foyer and Noctor 2003),(Mansour et al., 2005).

The application of super absorbent polymer may effectively increase water and fertilizer use efficiency in crops (Islam et al., 2011 a). When the polymers are incorporated in to the soil, it presumes the retaining of large quantities of water supply (Islamic et al., 2011b). ROS are partially the reduced forms of molecular oxygen, highly reactive and considered inevitable sub products from aerobic metabolism (Gratao et al., 2005). The main forms are superoxide anion $(O_2)^{\bullet}$, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen (O_2); they are produced in different cellular compartments, including chloroplasts, mitochondria, peroxisomes and apoplast (Gratão et al., 2005; Edreva, 2005).These ROS are all toxic (Movahhedy-Dehnavy et al., 2009) and very reactive and cause severe damage to DNA, proteins, lipids and chlorophyll (Clement et al., 2008). Numerous studies have shown the effects of oxidative stress in various crops in response to salinity (Hajiboland and Joudmand, 2009), drought (Shaoyun et al., 2009), pollutants (Zhenmei et al., 2009) as well as the high temperature (Agarwal et al., 2005); play key roles in the formation and degradation of H_2O_2, too. The protective role of catalase has been examined under certain abiotic stress conditions (Dı´az-Rosales et al., 2006). Lovelli et al., (2007) pointed out that drought-tolerant species increased their antioxidant enzymes activities and antioxidant contents in response to drought treatment, whereas drought-sensitive species failed to do so. Glutathione is a cystein-containing peptide found in most forms of aerobic life (Fahey, 2001). The main enzymes involved in the homeostatic control H_2O_2 and O_2 levels in plant metabolism are SOD, CAT, POX, APX and GPX (Gratão et al., 2005).Glutathione has antioxidant properties since the thiol group in its cydtein moiety is a reducing agent and can be reversibly oxidized and reduced. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is considered as one of the most important cellular antioxidants (Meister, 2005).In addition, the aim of the study was to investigate the effect of different levels of irrigation and super absorbent on seed and activities of some antioxidants enzymes (CAT, GPX and SOD), lipid and protein oxidation (MDA and dityrosine content) for three cultivars of bean. (Padman et al., 1994), studied effect of different levels of irrigation, nitrogen and jalashakti polymer on growth and yield of brassica juncea and concluded that seed yield in the treatment with super absorbents polymer is more than in treatment without it.

The aim of the study was to investigate the effect of long-term drought stress interaction on chemical and biochemical characters activities in red bean. The research was aimed also whether a super absorbent polymer supply to plant might be a strategy for increasing the drought tolerance.

MATERIALS AND METHODS

The experiments were carried out at experimental farm of Islamic Azad University in Karaj-Iran during growing season. The region has a semi-arid climate. The site is located at 35º 48′ N latitude, 51º 59′ E longitudes and an altitude of 1313 meter above the sea level. The soil experimental site was of sand loam with low level of nitrogen (0.06%), low in organic matter (0.83-0.72) and alkaline in reaction with a PH of 7.8.

AGRICULTURAL PRACTICES

The experimental design was a RCBD arranged in split plot with three replications. Each replication was divided into three main plots, which differed in severity of imposed water shortage.

Bean Cultivars included V_1, V_2 and V_3 that were arranged in sub plots. Before planting, the soil surface was ploughed during autumn and then disked twice in the spring (at the beginning of April and middle of May). Then 7% concentration of super absorbent was noticed for each plot. After calculation, the super absorbents were poured in the necessary amount of each pail separately and sufficient water was applied to it. Then 30 minutes was left till super absorbents absorb the water completely and then were poured on the whole plot monotonously and accurately. After settling each plot was covered with soil. Irrigation was initiated on 16th Sep. 2005 exactly after culturing with Acequia method. Electrical soil moisture meter system and plaster blocks were used for inducing the stress. In this way before starting the experiment, a plot was prepared and assigned with a 2×3 area beside the farm and plaster blocks were settled in it in 40 cm depth and irrigated. Then the curve carve was depicted with daily electrical humidity measuring and the percentage of humidity calibration. According to this curve the soil weight humidity was 8% when the electrical humidity measurement reached zero. Plaster block was used in the main experiment, according to electrical humidity rate and amount of soil humidity percent and water stress was depicted using calibration curve. That was when the electrical humidity measurement system reaches 60, the herb dejection appearance was revealed and the soil humidity was 12% in this stage. Triple super phosphate fertilizer was applied before sowing at a rate of 150 kg ha^{-1}. The nitrogen fertilizer (15 kg ha^{-1}) in the form of urea was applied before planting (one third of the application). The rest of the nitrogen fertilizer was distributed before starting the first stress treatment. Plots were 5-m long and consisted of 4 rows, 0.6 m apart from each other. Between all main plots, a 3-m wide strip was left bare to eliminate all influences of lateral water movement. Soil surface of cultivated area was thoroughly irrigated 6 days before planting. The bean seeds were inoculated with *Rhizobium japonicum* before planting and hand-planted on 24th May 2005 at the rate of 20 seeds per m^2 of row and then were thinned to achieve a density of approximately 333,333 ha^{-1}. Weeds and insects were effectively controlled during the whole growth season.

MEASURMENT of CHEMICAL and BIOCHEMICAL PROPERTIES

Fifteen leaves were taken from each plot randomly and placed in liquid N_2, then stored at -80°C pending biochemical analysis to quantify antioxidant's enzymatic activity. In order to prepare samples for enzyme assays and protein measurement, Leaves were washed from each plant by distilled water and homogenized in 0.16M Tris buffer (pH=7.5) at 4°C. Then, 0.5 ml of total homogenized solution was used for protein determination by the Lowry et al., (1951) method. The following enzymes were assayed in the volume containing a known protein concentration in order to calculate the specific activities of the enzymes based on the amount of protein per volume of homogenized solution.

Measurement of Antioxidant enzymes (SOD, CAT, GPX)

Catalase activity was estimated by the method of Cakmak and Horst (1991).The reaction mixture contained 100 crude enzyme extract, 500 µL, 10 mM H2O2 and 1400 µL 25 mM sodium phosphate buffer.

The decrease in the absorbance at 240 nm was recorded for 1 min. by spectrophotometer, model cintra 6 GBC (GBC scientific equipment Dandenong, Victoria, Austeralia). CAT activity of the extract was expressed as CAT units per milligram of PROT. Superoxide dismutase activity was determined with the reaction mixture contained 100 µl 1 mM riboflavin, 100 µL 12 mM L- methionine, 100 MM 0.1 mM EDTA (PH 7.8), 100 µL 50 mM Na2Co3 (PH 10.2) and 100 µL 75 µM Nitroblue tetrazolium (NBT) in 2300 µL 25 mM sodium phosphate buffer (Ph 6.8) 200 µL crude enzyme extract in a final volume of 3 mL.SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photo chemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W), identical tubes that were not illuminated served as blanks. After illumination for 15 min, The absorbance was measured at 560 nm after illuminating for 15 minutes.

One unit of SOD was defined as the amount of enzyme activity that was of enzyme activity able to inhibit by 50% of NBT the photo reduction to blue Formosan. The SOD activity of the extract was expressed as S units per milligram of PROT. The peroxides' activity was determined by the oxidation of guaiacol in the presence of H2O2. The increase in absorbance was recorded at 470 nm (Hernandez et al., 2000). The reaction mixture contained 100 µL crude enzymes, 500 µL H2O2 5 mM, 500 ml of guaiacol 28 mM and 1900 µL potassium phosphate buffer 60 mM (PH 6.1). POX activity of the extract was expressed as POX units per mg.

LIPID PERDOXIDATION (MALONDIALDEHYDE CONTENT)

The level of lipid peroxidation was measured in terms of MDA content using thiobarbituric acid (TBA)-reactive substances following the protocol of Sairam et al. (1998). Leaf samples of 0.5 g were homogenized in 10mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 5 min. to 2mL of aliquot of the supernatant, 4mL of 0.5% TBA was added in 20% TCA. The mixture was heated at 100 1C for 30 mins. and then quickly cooled in an ice bath. After centrifugation at 10,000g for 10 mins to remove suspended turbidity, the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption was subtracted at 600 nm.

PROTEIN DAMAGE (DITYROSINE CONTENT)

Fresh tissue material (1.2 g) was homogenized with 5 ml of ice-cold 50mM HEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM DL-norleucine and 100 mg polyclar AT. The plant tissue homogenate was centrifuged at 5000 g for 60 min to remove its debris. o,o-dityrosine was recovered by gradient elution from C-18 column (Econosil C18, 250mm × 10 mm) and was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm). A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 mins. A standard dityrosine sample was prepared according to Amado et al. (1984). Dityrosine was quantified by assuming that it's generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient $e_{315} = 4.5\ mM^{-1}\ cm^{-1}$ at pH 7.5).

STATISTICAL ANALYSIS

The whole data was analyzed using MSTAT-C, Duncan's multiple range test was applied to compare the means at $p < 0.05$ Zhang and Kirkham (1995).

RESULTS

SEED YIELD

Analysis of variance for Seed yield, indicated significant differences (P<0.01) among irrigation levels, bean cultivars, super absorbent content and their interactions (Table 1).

In all of bean cultivars, the seed yield was decreased from first level of irrigation (S_1) to S_2, significantly. The highest and lowest seed yields at the optimum conditions of irrigation, were measured by cultivars of V3 and V1, at the drought stress conditions, as well as the highest and lowest seed yields were observed in cultivars of V3 and V1 (Table 2). Super absorbent (7%) could increase the seed yield in all of the cultivars.

CHEMICAL AND BIOCHEMICAL CHARACTERS:
ANTIOXIDANT ENZYMES (SOD, CAT, GPX)

The main effect of water deficit, cultivars, super absorbent and the interaction of irrigation levels×superabsorbent were significant in all of the cultivars (Table 1). The drought stress increased the biochemical characters (Antioxidant enzymes and biomarkers) level compared to optimum conditions of irrigation. In the normal irrigation (S_1) and drought stress (S_2), the highest and lowest (SOD, CAT and GPX) contents were obtained from cultivar of V3 and V1 respectively (Tables 2). This study showed that the most the of cultivar resistance was V3 (NAZ cultivar). The use of polymer causes decrease of antioxidant content in all of the cultivars and irrigation conditions. There was a positive and significant correlation between (GPX, SOD and CAT) levels and (MDA and Dityrosin) levels, but there was a negative and significant correlation with seed yield (Tables 3).

Table 1 : The mean squares of ANOVA for super oxid dismutase (SOD),glutathione proxidase (GPX), catalase (CAT), malondialdehyde (MDA) content, dityrosine content, relative water content (RWC), seed yield in different bean cultivars and irrigation treatments under super absorbent polymer.

Features	Mean Square						
	df	SOD	GPX	CAT	MDA	Dityrosine	Seed yield
Irrigation	1	**	**	**	*	*	**
Cultivars	2	**	**	**	**	ns	**
Cultivars *Irrigation	2	ns	ns	ns	**	**	**
Super absorbent	1	**	**	**	ns	ns	**
Irrigation*Superabsorbent	1	**	**	**	*	**	**
Super absorbent* cultivars	2	ns	ns	ns	ns	ns	**
Cultivars*Irrigation*superabsorbent	2	ns	ns	ns	ns	ns	ns

Table 2: Effects of irrigation levels on glutathione redoctase (GPX), catalase (CAT), malondialdehyde (MDA) content, dityrosine content, relative water content (RWC) and seed yield in bean cultivars

Treatments			SOD (u mg^{-1} protein)	GPX (u mg^{-1} protein)	CAT (u mg^{-1} protein)	MDA (nmol mg protein)	Dityrosine (nmol mg protein)	Seed yield (kg ha^{-1})
S_1*V	S_1	V1	1152 a	11.70a	80.57a	17.71bc	2.81d	1120c
	S_1	V2	1300 a	11.52a	76.72a	15.94b	2.66c	1537b
	S_1	V3	1620 a	13.69a	87.35a	15.23bc	2.22c	2002a
S_2*V	S_2	V1	2139 a	17.42a	121.80a	18.00bc	2.81c	471e
	S_2	V2	2392 a	17.08a	131.30a	19.81b	2.90b	630d
	S_2	V3	2697 a	20.03a	150.39a	26.52a	3.64a	1074c
S_1*P	S_1	P1	1399 c	12.42c	79.75c	15.56c	2.41c	1323b
	S_1	P2	1316 c	12.19c	83.35c	16.94c	2.71c	1783a
S_2*p	S_2	P1	2689 a	20.20a	141.17a	22.68a	3.37a	587d
	S_2	P2	2130 b	16.15b	121.82b	20.20b	2.86b	863c
P*v_1	P1	V1	1773 a	15.30a	104.92a	17.08a	2.64a	649d
	P2	V1	1519 a	13.82a	97.45a	18.64a	2.98a	941c
P*v_2	P1	V2	1988 a	15.04a	109.32a	18.93a	2.98a	918c
	P2	V2	1704 a	13.56a	98.70a	16.81a	2.58a	1250b
P*v_3	P1	V3	2371 a	18.59a	126.14a	21.46a	3.05a	1299b
	P2	V3	1947 a	15.13a	111.60a	20.27a	2.80a	1778a

Levels of irrigation: S_1; optimum condition of irrigation, S_2; drought stress level. Cultivars: v1: Derakhshn, V2:D8182,V3:Naz content polymer: P1,0% P2,7%

For a given means within each column of each section followed by the same letter are not significantly different ($P < 0.05$).

u mg^{-1} protein : International Units of activity per milligram protein

PROTEIN DAMAGE (DITYROSINE), LIPID PEROXIDATION (MDA CONTENT)

Analysis of variance for DITYROSINE and MDA contents showed that, there were significant differences among irrigation levels, super absorbent and the interaction of irrigation levels×superabsorbent (Table 1). Analysis of variance for MDA content showed that, there were significant differences of ($P<0.01$) among cultivars too (Table 1). The dityrosine levels were found to be significantly higher in the drought stress than that of optimum conditions of irrigation (S_1) (Table 2). Among cultivars and at the optimum conditions of irrigation, the highest and lowest dityrosine levels were observed in V1 and V3, respectively. Assessment of correlation tables indicated that, there was a positive and significant correlation between protein damage (dityrosine level) and GPX, CAT and SOD at two conditions irrigation. (Tables 3). The drought stress significantly increased the MDA contents in all of the cultivars (Table 2). Assessment of interaction between irrigation levels×cultivars indicated that, leaves of all cultivars suffered more oxidative damage at moderate and extreme drought stress levels. According to consequences, cultivars of V3 and V1 had the highest and lowest MDA content at the optimum and drought stress conditions of irrigation, respectively. In the present study results observed between MDA content with antioxidant enzymes activity (CAT, GPX and SOD content) were significant and positively correlated. A negative and significant correlation among lipid peroxidation (MDA content) was observed with seed yield in these conditions (Tables 3).

Table 3. Correlation coefficient between contents of glutathione redoctase (GPX), catalase (CAT), malondialdehyde (MDA), Dityrosine content, relative water content (RWC) and seed yield.

Features	Seed yield	SOD	GPX	CAT	MDA	Ditirosine
Seed yield	1.000					
SOD	-0.285**	1.000				
GPX	-0.302**	0.942**	1.000			
CAT	-0.349**	0.930**	0.927**	1.000		
MDA	-0.132**	0.528**	0.564**	0.703**	1.000	
Ditirosine	-0.176**	0.401*	0.444**	0.548**	0.855**	1.000

**p< 0.05 and **p<0.01 : significant at the 5% and 1% levels of probability, respectively. Numbers without symbols are non significant.*

DISCUSSION

The result showed that the responses of related enzymes strongly depend on the severity of drought stress. They did differ significantly for drought stress injury in their seed, lipid and protein oxidation (MDA and dityrosine contents), antioxidant enzymes (CAT, SOD and GPX), at moderate and extreme drought stress.

Under water deficit conditions, seed decreased in all of the assessed cultivars. Reductions in yield of bean cultivars were also reported to take place under drought stress, it is due to the reduction in the number of pods per plant (Lee et al., 2008). In our study, cultivar of V3 (Naz) showed the highest seed and yield as well as smaller reduction in these parameters during the drought stress period as compared to other cultivars.

Our study results showed that, the activities of measured antioxidant enzymes (SOD,CAT, GPX) were increased in all of the cultivars and drought stress but, antioxidants levels were higher at moderate than extreme cultivars (v3>v2>v1). This finding can be related to the ability of the crops against different intensities of drought stress. Increase in the level of antioxidants under drought stress in canola and soybean was reported by Tohidimoghadam et al. (2009) and Masoumi (2009).

A positive and significant correlation between antioxidant enzymes and ascorbate peroxidase (APX) was reported under drought stress conditions by Lee et al. (2009). Result showed that, the V3 cultivar, had the highest antioxidant contents and seed yield in both drought stress and normal conditions, it seems that this cultivar has more effective alternative mechanisms for defense against free radicals and oxidative stress.

The MDA and dityrosine contents in leaves increased in all of the cultivars at both moderate and extreme drought stress. The lowest MDA and dityrosine contents were observed in V3. This might explain lower lipid and Protein Oxidation of this cultivar relative to other cultivars. On the other hand the V3 cultivar appeared to has experienced less oxidative damage as compared to V2 and V1 cultivars, which perhaps may be due to its superior capacity to counter the oxidative stress as well as higher water content.

The same results were reported by Masoumi et al. (2008), who showed that drought stress increased lipid peroxidation (MDA content) in soybean cultivars.

On the other hand the study of material effects of irrigation for polymer showed that in both conditions (normal and stress) this polymer has increased (yield and antioxidant enzymes rate) and decreased the biomarkers of (MDA, Ditirosine) in every variety but in Naz its effect was more appropriate, so this variety has used its water and nutrition better than others. Mikkelsen et al, (1993) found that addition of polymer to the fertilizer solutions reduced N leaching losses from soil columns as much as 45% during the first four weeks during heavily leached conditions as compared with N fertilizer alone.

Differences in the responses of bean subjected to superabsorbent polymer application were evident during our observation. This study has shown that application of SAP can increase the resistance of bean under water deficiency.

CONCLUSION

The application of super absorbent polymer could conserve the soil's capacity for water storage, ensuring more available water, thus the RWC content in leaves, as well as plant growth and yield increased under water stresses.

Although laboratory exams. show that the drought stress increases the destructive biomarkers, on the other hand the use of 7% super absorbent polymer decreases these characters by decreasing drought stress. Finally, the present findings revealed that cultivar of V3 is more suitable than other cultivars for sowing and the application of superabsorbent polymer could reserve different amounts of water in it and so increases the soil ability of water storing and preserving and last in water deficiency.

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