Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Original Research

Consequences of ultrasonic waves radiation and 24-epi-brassinolid foliar application for reduction of water deficit stress on qualitative properties of red beans (Akhtar)

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ABSTRACT:

This experiment was conducted to study the possible alleviation of drought stress effects on red bean (Akhtar) by ultrasonication and 24-epi-brassinolid foliar application simultaneously at two sites, one in the research field located in Agricultural Research Center in Shahrood and the other in bean farm, 40 km of Shahrood city in 2015 as split factorial in complete randomized block design with three replications. Experimental factors included irrigation of main terrace at three levels of normal irrigation, mild stress and severe stress respectively (60, 90 and 120 mm evaporation from evaporation pan). Stress levels were applied after 4-leaf stage and ultrasound waves treatments (in two levels of nonuse of seeds irradiation and use of irradiation for 3 minutes at 32°C) and 24-epi- brassinolid foliar application (in two levels of non-use of foliar application and foliar application at a rate of 0.1 mg/L at 50% flowering during two stages) which were located in sub-terraces. The evaluated properties included grain yield, qualitative traits of superoxide dismutase content, peroxidase, catalase, proline and ascorbate. The results showed that with severe water stress (comparison of severe stress and lack of stress), grain yield showed a significant decrease in both the experiment sites however 24-epi-brassinolid foliar application and use of ultrasonic waves at both normal and stress conditions increased the seed yield. The same condition was established for evaluated enzymes. Thus it could be stated that irradiation of ultrasonic waves and 24-epi-brassinolid foliar application for cultivating beans play an important role in the increment of competitive strength of plant at water deficit conditions.

Keywords:
Water deficit, irrigation, ultrasonic waves, brassinolide, grain yield.

Article Citation:
Amir Younesian, Hossein Ajam Norouzi, Manouchehr Gholipoor and Afshin Soltani
Consequences of ultrasonic waves radiation and 24-epi-brassinolid foliar application for reduction of water deficit stress on qualitative properties of red beans (Akhtar)
Journal of Research in Ecology (2017) 5(1): 686-699

Web Address:
http://ecologyresearch.info/documents/EC0350.pdf

Dates:
Received: 06 Apr 2017 **Accepted:** 17 Apr 2017 **Published:** 23 May 2017

INTRODUCTION

Red bean (*Phaseolus vulgaris.* L) is an annual plant of legume family with upright, indeterminate bus and indeterminate climbing varieties. The area under cultivation of cereal products in Iran from 2000-2006 indicated that in terms of average and rate of production in Iran, pea is in the first place and varieties of dry beans are in the second place (Mohammadzadeh, 2011). Water deficit is one of the abiotic environmental stresses. This stress in addition to its direct effect on reducing photosynthesis plants decreased absorption of nutrients from the soil (Mohammadzadeh, 2011).

An important factor in increasing the beans yield is selection of high yielding variety which is tolerant to biotic and abiotic stresses. Ghanbari and Mazandarani (2004) said that drought and related stress are the most common environmental stresses which restrict agricultural production which reduce the efficiency of using arid and dry areas. Szilagyi (2003) stated that drought stress is the most important factor limiting beans production around the world.

Sing (2007) examined the effects of drought stress on beans and reported that average yield reduction under drought stress was 60% and grain weight loss was 14% while the property of number of days to maturity reduced at drought condition. Yield, weight and maturity had positive correlation in normal and stress conditions. German and Tran (2006) stated that drought results in the reduction of biomass, grain yield, harvest index, and grain weight. They also reported that grain yield under normal and drought stress conditions had positive correlation. Brassinosteroids (BRs) have been recognized as a phytohormones that play essential roles in plant development. BRs are essential for normal plant growth, reproduction and development. They play critical roles in a variety of physiological responses in plants, including stem elongation, pollen tube growth, leaf bending and epinasty, root growth inhibition, ethylene biosynthesis, proton pump activation, vascular differentiation, nucleic acid and protein synthesis and photosynthesis (Hayat *et al.,* 2010). BRs play a significant role in amelioration of various abiotic and biotic stresses, such as cold stress, water deficit, salt injury, oxidative damage, thermal stress, heavy metal stress and pathogen infection. Despite the correlation between oxidative stress and BR level in plants, the physiological rationale for such alteration in BR level is little known (Bajguz and Hayat, 2009).

In brassinolide treatment of maize were subjected to water stress, the activities of Superoxide Dismutase (SOD), Ascorbate Peroxidase (APOX) and Catalase (CAT) as well as ascorbic acid and carotenoid contents increased (Li *et al.,* 1998). However, brassinolide enhanced the activity of CAT and reduced the activities of peroxidase and ascorbic acid oxidase under osmotic stress conditions (Vardhini and Rao, 2003).

Ultrasound (US) treatment to stimulate germination has been investigated in many seed types including carrot, radish, maize, barley, rice and sunflower (Fl´orez *et al.,* 2007; Yaldagard *et al.,* 2008). Results of these investigations indicated that the effects ofuson seed germination depend on frequency and exposure time and appear to vary widely between the different species and cultivars. Yaldagard *et al.* (2008) indicated that mild US irradiation on barley seeds produced an accelerated germination proportional to an increase in α-amylase activity. Another possible mechanism for US enhancement of seed germination is the mechanical or shear effects due to the large and rapid oscillations in bubble size (microstreaming), which leads to disruption of plants cell walls, thereby increasing water uptake by the cell or seed (Gaba *et al.,* 2008).

Machikowa *et al.* (2013) showed that seeds irradiation with ultrasonic waves will strengthen the seedling of sunflower. So, the present paper aims to evaluate the consequences of ultrasonic wave radiation and 24-epibrassinolid foliar application for reduction of water deficit stress on qualitative properties of red beans (Akhtar).

MATERIALS AND METHODS

To evaluate the consequences of ultrasonic waves radiation and 24-epi-brassinolid foliar application for reduction of water deficit stress on qualitative properties of red beans (Akhtar), a trial was conducted simultaneously in two areas, one in the research field located in Agricultural Research Center in Shahrood and the other in bean farm, 40 km off Shahrood city in 2005 as split factorial in complete randomized block design with three replications. Experiment factors included irrigation of main terrace in three levels of normal irrigation (60 mm evaporation from evaporation pan), mild stress (90 mm evaporation from evaporation pan) and severe stress (120 mm evaporation from evaporation pan).

Stress levels were applied after 4-leaves stage and ultrasound wave treatments (in two levels of nonuse of seeds irradiation and use of irradiation for 3 minutes at 32°C) using ultrasonic bath (digital ultrasonic, model 4820-CD) with constant frequency of 24 kHz. After voicing, the seeds were moved to the farm for planting and 24 -epi-brassinolid foliar application (in two levels of non-foliar application and foliar application at a rate of 0.1 mg/L at 50% flowering during two stages) while waving and foliar application were located in secondary terraces as factorial. Preparing land procedure includes plow, disc, land leveling and furrows in pre-planting stages in both planting sites. Seeds planting was implemented manually in May. Each terrace consisted of four planting lines with length of four meters planting and distance of 60 centimeters and distance between bushes was considered as five centimeters. It should also be noted that Farm and Field Crops Research Center have not cultivated any crops in the previous crop years. Type of loam was determined according to soil texture of experiment sites due to physical analysis and the percentage of each components of soil. The evaluated properties included grain yield, qualitative traits of superoxide dismutase content, peroxidase, catalase, proline and ascorbate. For measuring the quality of post-harvest plant samples, they were quickly transferred to the laboratory and washing of plant organs was completed as carefully as possible (Mohammadzadeh, 2012).

Enzyme Measurement

In order that measuring the antioxidants power of used vegetative extracts to reduce proxidase activity, this enzyme (peroxidase) activity is accomplished at 25ºC using spectrophotometer at 470 nm, guaiacol as the substrate and H_2O_2 as the hydrogen donor during the following process:

1. Procurement and preparation of extract (peroxidase origin)
2. Preparation of substrate mixture
3. Reaction cuvette (Ponce *et al.,* 2004).

Extracts were prepared using 10 grams of leafy vegetables which was chopped and then 30 ml of distilled water added during homogenization. The slurry was centrifuged (SIGMA-3K30) at 10000g for 15 min at 4°C. The supernatant, which contained peroxidase activity, was used as the enzyme source for the experiment. The substrate mixture contained 10 ml of 1% guaiacol, 10 ml of 0.3% hydrogen peroxide and 100 ml of 0.05M sodium phosphate (pH 6.5) buffer. The reaction cuvette contained 2.87 ml substrate mixture, 0.1 ml crude extract, and 0.03 ml treatment solution (essential oils, ascorbic acid and water) in a total volume of 3 ml. (Ponce *et al.*, 2004).

Superoxide dismutase activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium at 560 nm (Becana *et al.,* 1986). Catalase activity was assayed by mixing 2.5 ml potassium phosphate buffer (pH 7), 0.05 M and 0.3 ml H_2O_2 3% in an ice bath primarily, then 0.2 ml enzyme extract was added immediately. Changes in absorbance curve was assessed at 240 nm for 3-4 minutes. One unit of enzyme was descripted for decomposed µmol ml-1 H_2O_2 per min at 25ºC, then enzyme activity was computed according to unit changes per min for protein (mg).

Table 1. Analysis of variance (square mean) for treatment on some qualitative criteria and yield of red bean in agriculture research center of Shahrood

Source of variance 1	Grain yield (kg/ha)	SOD (U/g Protein)	Peroxidase (U/g Protein)	Catalase (U/g Protein)	Proline (µmol g^{-1})	Ascorbate (µmol APX min^{-1} mg^{-1} protein)
r	1078228.16**	25247.57**	1587.68**	1107.76**	125.11**	205.77
a	3865806.29**	41941.39**	2271.44**	2280.28**	401.96**	70246.51**
ra	456958.35	3415.27	48.58	95.21	2.94	860.45
b	1113499.80**	20380.89**	1585.23**	1185.99**	540.56**	2611.00*
c	999370.09**	8356.79*	1492.14**	432.98**	473.20**	4640.10*
ab	290600.49*	5836.34*	128.15	243.41*	35.14**	343.68
ac	31361.08	5983.77*	1346.15**	133.84*	5.24	242.65
bc	62609.21	30.89	110.91	57.58	8.82	722.98
abc	220688.21*	228.24	315.98*	47.97	11.07	166.46
E	62181.00	1887.68	83.75	41.03	4.64	581.65
CV (%)	10.26	11.95	9.89	13.01	6.21	10.54

**, * significant at 1% and 5% probability levels respectively.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to the method of Ramel *et al.* (2009) by monitoring the rate of ascorbate oxidation at 290 nm (2.8mm^{-1} cm^{-1}). The reaction composition (3 ml) included 1.5ml of 0.1M potassium phosphate buffer (pH 6.8) 0.5 ml of 6 mM ascorbate, 0.5 ml of 12mM H2O2 and 0.5 ml of enzyme extract (Ramel *et al.*, 2009). Proline was determined by following the method of Bates *et al*. (1973). Fresh leaves were extracted in sulphosalicylic acid, an equal volume of glacial acetic acid and ninhydrin solutions were added to the extract. The progenitor was heated at 100°C, then 5ml of toluene were added. The absorbance of the toluene layer was read on a spectrophotometer, at 528 nm. Proline (Sigma) was used for the standard curve (Bates *et al.,* 1973). Analysis of variance was applied using PROC ANOVA of SAS (version 9.1.3, 2004). Each treatment was analyzed in three transcripts. The comparison of the means was done at a probability level of 5 five percent by Duncan test.

RESULTS

Grain Yield

Investigation of analysis of grain yield variance showed that entire simple effects of experiment treatments on this trait in both experiment sites were significant at 1% of probability level (Table 1 and 2). Besides in Research Center field, effect of dual interaction of water deficit and ultrasonic waves as well as the three interaction of experiment treatments at 5% probability

Table 2. Analysis of variance (square mean) for effects of treatment on some qualitative criteria and yield of red bean in field of bean in Shahrood

Source of variance 2	Grain yield (kg/ha)	SOD (U/g Protein)	Peroxidase (U/g Protein)	Catalase (U/g Protein)	Proline (µmol g^{-1})	Ascorbate (µmol APX min^{-1} mg^{-1} protein)
r	151465.10**	9486.81**	1095.29**	268.79	315.50**	12187.45**
a	17128064.88**	25519.48**	1825.84**	3898.18**	653.01**	76353.99**
ra	26393.74	2103.36	509.86	217.05	9.41	1466.58
b	1422004.58**	4744.91*	805.51*	551.23*	1127.28**	3800.51*
c	2598237.73**	1558.93	624.75*	991.51**	310.87**	16096.41**
ab	135614.78**	120.73	368.86	23.49	49.36*	128.84
ac	207781.34**	1643.51	1100.59**	464.89*	11.10	1271.89
bc	377653.27**	2807.23	349.75	339.23*	12.78	59.77
abc	56132.58*	3415.33*	207.96	334.62*	11.34	1659.88
E	13322.47	1223.16	175.46	115.85	15.56	1252.62
CV (%)	8.68	10.21	13.42	10.18	8.00	11.47

**, * significant at 1% and 5% probability levels respectively

Table 3. Mean comparisons for effects of treatment on some qualitive criteria and yield of red bean in agriculture research center of Shahrood

1	Grain yield (kg/ha)	SOD (U/g Protein)	Peroxidase (U/g Protein)	Catalase (U/g Protein)	Proline (µmol g^{-1})	Ascorbate (µmol APX min^{-1} mg^{-1} protein)
A						
A1	2914.0[a]	306.35[c]	77.59[c]	36.45[c]	45.36[c]	299.62[a]
A2	2569.9[b]	359.93[b]	95.25[b]	47.40[b]	51.07[b]	239.07[b]
A3	1805.1[c]	424.42[a]	104.69[a]	63.84[a]	56.94[a]	147.64[c]
B						
B1	2253.82[b]	339.77[b]	85.87[b]	43.49[b]	44.58[b]	221.49[a]
B2	2605.56[a]	387.36[a]	99.15[a]	54.97[a]	57.66[a]	236.06[a]
C						
C1	2263.08[b]	346.61[b]	86.07[b]	45.76[b]	47.50[b]	217.42[b]
C2	2596.31[a]	380.53[a]	98.95[a]	52.70[a]	54.75[a]	240.13[a]

* Mean in each column followed by the same letter are not significantly different ($p<0.05$)

level showed significant effect on grain yield (Table 1). But in bean farm around Shahrood, entire dual effects at 1% significant level and three interactions of experiment treatments at 5% probability level showed significant effect on grain yield (Table 2).

Superoxide Dismutase (SOD)

The results of variance analysis of superoxide dismutase showed that the simple effect of water deficit stress in both sites at 1% probability level affected superoxide dismutase content (Table 1 and 2). The effect of ultrasonic waves in the Research Center field at 1% probability level and the effect of 24-epi-brassinolid foliar application as well as the interaction of water deficit and ultrasonic waves and the interaction of water deficit and 24-epi-brassinolid foliar application in Research Center field at 5% probability level also affected the content of superoxide dismutase enzyme (Table 1). However in bean farm, the effect of ultrasonic wave radiation and the three interactions of the experiment treatments at 5% probability level affected the content of superoxide dismutase enzyme (Table 2).

The results of comparison between the averages of dual interaction of water deficit stress and ultrasonic

Table 4. Mean comparisons for effects of treatment on some qualitative criteria and yield of red bean in a field of bean in Shahrood

2	Grain yield (kg/ ha)	Superoxide Dismutase (U/g Protein)	Peroxidase (U/g Protein)	Catalase (U/g Protein)	Proline (µmol g^{-1})	Ascorbate (µmol APX min^{-1} mg^{-1} protein)
A						
A1	3611.36[a]	293.40[c]	86.08[c]	37.66[c]	41.71[c]	300.90[a]
A2	2555.47[b]	348.23[b]	99.18[b]	49.26[b]	49.62[b]	242.32[b]
A3	1227.11[c]	385.04[a]	110.74[a]	73.01[a]	56.45[a]	143.10[c]
B						
B1	2265.90[a]	330.74[a]	93.93[b]	49.40[b]	54.86[a]	218.50[a]
B2	2663.39[b]	353.71[a]	103.39[a]	57.22[a]	43.67[b]	239.05[a]
C						
C1	2196.00[b]	335.64[a]	94.50[a]	48.06[b]	46.32[b]	207.63[b]
C2	2733.30[a]	348.81[a]	102.83[a]	58.56[a]	52.20[a]	249.92[a]

* Mean in each column followed by the same letter are not significantly different ($p<0.05$)

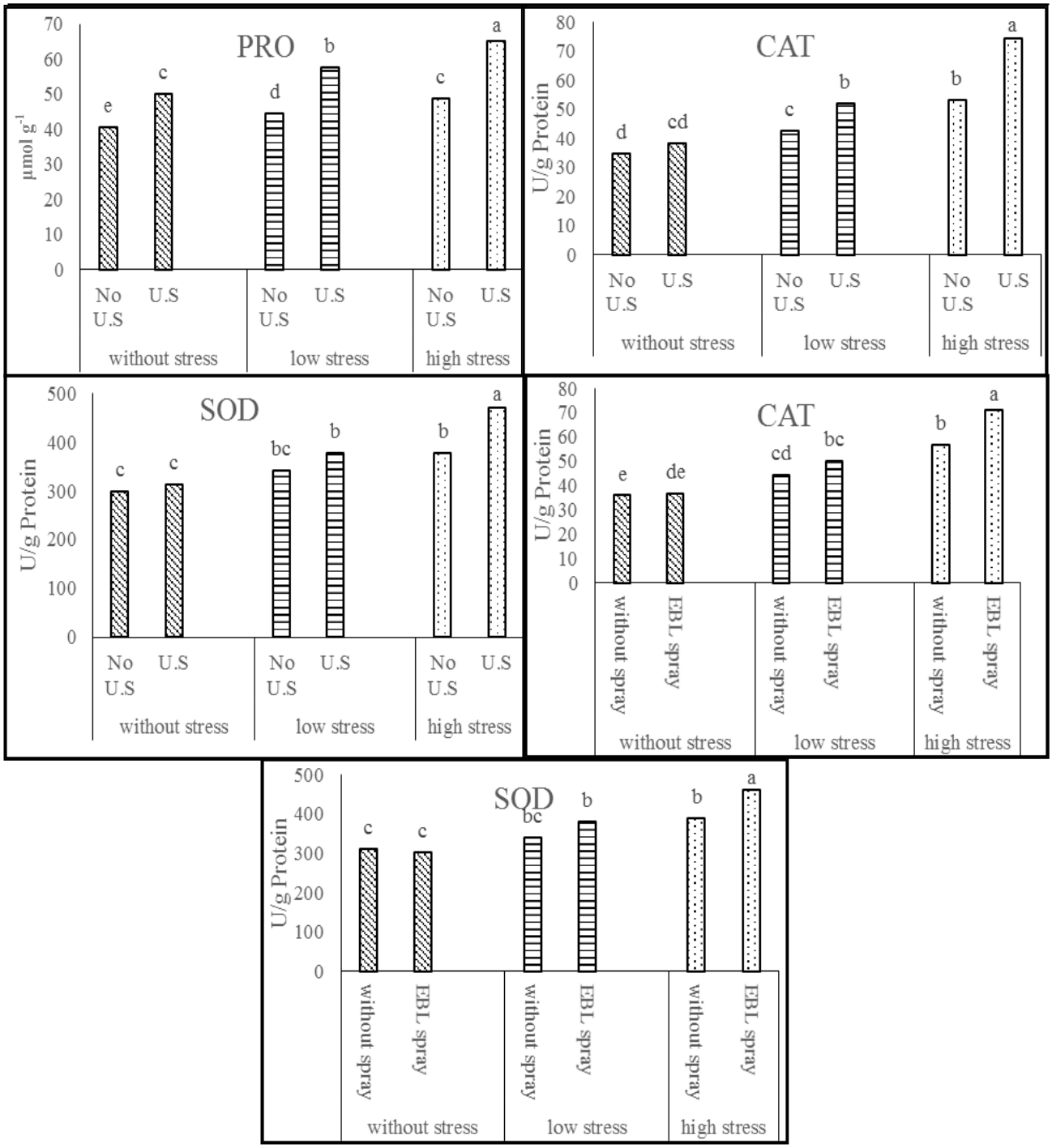

Figure 1. Double interaction of water stress - EBL and water stress - Ultra Sonic on CAT, SOD and PRO content in *Phaseolus vulgaris* in agriculture research center of Shahrood

waves in Research Center field showed that the maximum amount of superoxide dismutase was obtained from severe stress treatment and ultrasonic wave radiation (470.35) and the minimum amount of superoxide dismutase enzyme was obtained from non-use of stress treatment and non-radiation of ultrasonic waves (299.60). The minimum content of superoxide dismutase treatment with two treatments of lack of stress and wave radiation and mild stress and non-radiation of waves were categorized in one statistical group. But in general there was 37% difference in the content of superoxide dis-

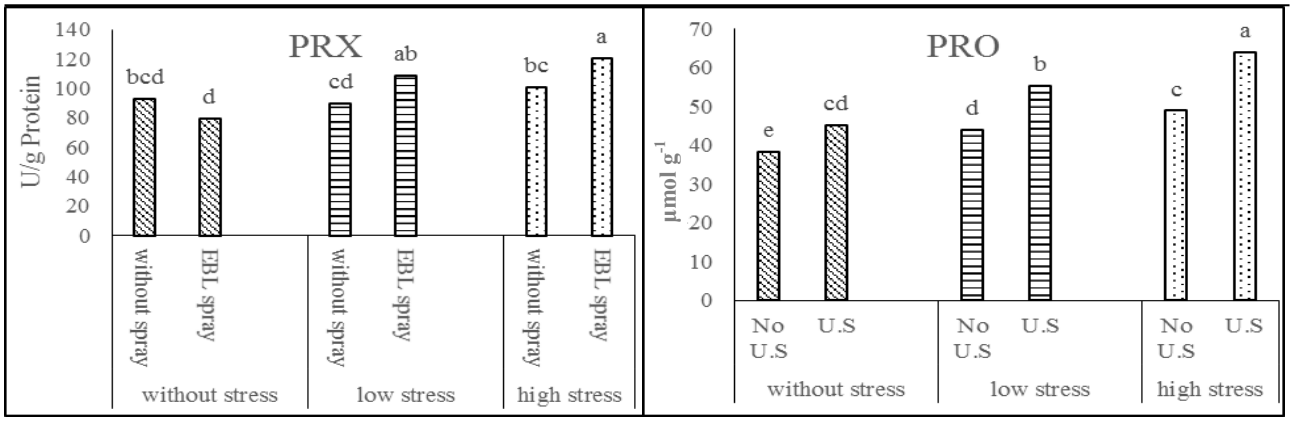

Figure 2. Double interaction of water stress - EBL and water stress - ultra sonic on PRO and PRX content in *Phaseolus vulgaris* in a field of bean in Shahrood

mutase enzyme between maximum and minimum treatments (Figure 1).

Peroxidase

Investigation of variance analysis of peroxidase enzyme showed that in Research Center field the entire simple effects and dual interactions of water deficit and 24-epi-brassinolid foliar application affected the peroxidase enzyme content at 1% probability level. The three interactions of experiment treatments at 5% probability level also affected the peroxidase enzyme content (Table 1). But in bean farm around Shahrood, the simple effects of water deficit and dual interaction of water deficit and 24-epi-brassinolid foliar application at 1% probability level and simple effect of ultrasonic radiation and simple effect of 24-epi-brassinolid foliar application at 5% probability level affected the peroxidase enzyme content (Figure 2).

The results of comparison between the averages of three effects of peroxidase enzyme content Research Center field showed that the maximum amount of peroxidase enzyme was obtained from severe stress treatment, radiation of ultrasonic waves and 24-epi-brassinolid foliar application (121.79). But mild water deficit stress treatment, radiation of ultrasonic waves and 24-epi-brassinolid foliar application with 109.39 peroxidase enzyme with maximum treatment were categorized in one statistical group and the least amount of peroxidase enzyme was obtained from control treatment, nonuse of stress, non-radiation of ultrasonic waves and lack of 24-epi-brassinolid foliar application (71.32) (Table 3).

The results of comparison between the averages of peroxidase enzyme content showed that radiation of ultrasonic waves increased the peroxidase content to 10% and changed from 93.93U/g Protein in non-radiation of ultrasonic waves treatment to 103.39 U/g Protein in radiation of ultrasonic waves treatment (Table 3). But examination of dual interactions (water deficit and 24-epi -brassinolid foliar application) in bean field around Shahrood showed that the maximum amount of peroxidase enzyme was obtained from severe stress treatment and 24-epi-brassinolid foliar application (120.67) and that the minimum amount of peroxidase enzyme was obtained from nonuse of severe stress treatment and 24-epi-brassinolid foliar application (79.19), So, there was 35% difference between the comparison of two treatments of minimum and maximum content of peroxidase enzyme (Figure 3).

Catalase

Investigation of variance analysis of peroxidase enzyme showed that in Research Center field, the entire simple effects at 1% probability level and dual interactions of water deficit and 24-epi-brassinolid foliar application as well as water deficit and 24-epi-brassinolid foliar application affected the peroxidase enzyme content at 5% probability level (Table 1). But in bean farm around Shahrood, the simple effects of water deficit and

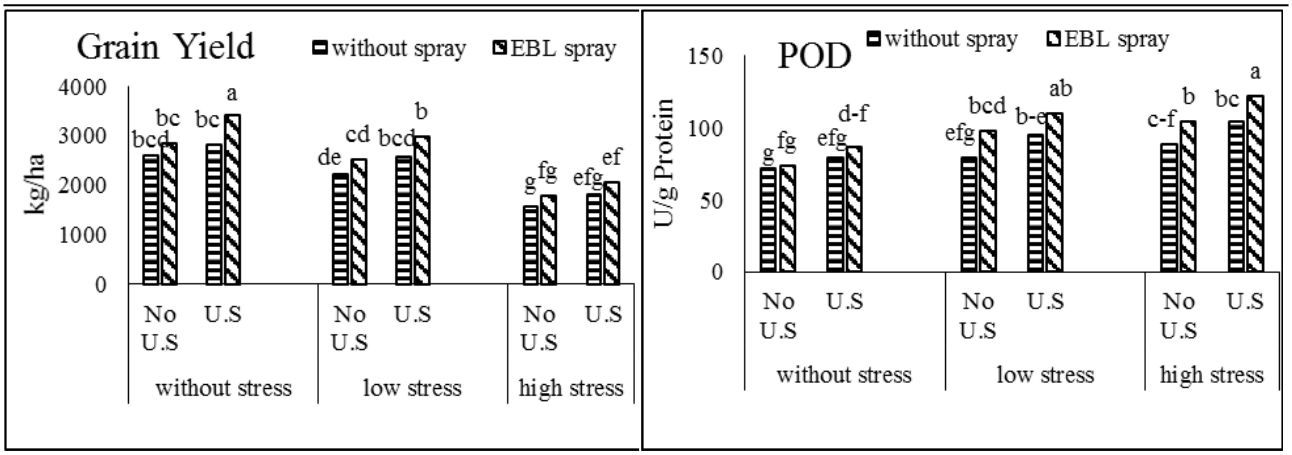

Figure 3. Interaction of drought stress, ultrasound irradiation and 24-epibrassinolide spray on yield and peroxidase content of *Phaseolus vulgaris* in agriculture research center of Shahrood

24-epi-brassinolid foliar application at 1% probability level and simple effect of ultrasonic radiation and effects of water deficit interaction and 24-epi-brassinolid foliar application, radiation of ultrasonic wave and 24-epi-brassinolid foliar application and the effect of three interactions of treatments affected catalase enzyme at 5% probability level (Table 2).

The results of comparison between the averages of dual effects in Research Center field showed that in dual interactions of water deficit and ultrasonic waves, the maximum amount of catalase enzyme was obtained from severe stress treatment and radiation of ultrasonic waves (74.50) and minimum amount of catalase enzyme was obtained from lack of severe stress and non-radiation of ultrasonic waves (34.63) while the content of catalase enzyme more than 53% was lower than maximum treatment. But examination of the effect of dual interactions of water deficit stress and 24-epi-brassinolid foliar application showed that the maximum amount of catalase enzyme was obtained from severe stress treatment and 24 -epi-brassinolid foliar application (70.89) which was 50% lower than minimum content of catalase enzyme treatment. The minimum amount of catalase enzyme in this interaction was also obtained from dual interactions of nonuse of stress and lack of 24-epi-brassinolid foliar application (36.00) (Table 3).

The results of comparison between the averages of three effects in bean farm around Shahrood showed that the maximum amount of catalase enzyme was obtained from severe stress treatment, non-radiation of ultrasonic waves and 24-epi-brassinolid foliar application (89.4). But this treatment with two treatments of severe water deficit treatment, radiation of ultrasonic waves and 24-epi-brassinolid foliar application were categorized in one statistical group. The minimum amount of catalase enzyme content was obtained from nonuse of stress, non-radiation of ultrasonic waves and lack of 24-epi-brassinolid foliar application (35.23). The content of catalase enzyme more than 53% was lower than maximum treatment (Figure 4).

Proline

Investigation of results of variance analysis showed that proline amino acid content was affected by the entire simple effects at 1% probability level in both fields under study (Table 1 and 2) but dual interactions of water deficit and ultrasonic wave radiation in Research Center field at 1% probability level in bean farm around Shahrood affected the proline content at 5% probability level (Table 1).

The results of comparison between the averages of proline amino acid showed that 24-epi-brassinolid foliar application (47.50 micromoles per gram) increased the content of this amino acid to 13% in Research Center

field, However in 24-epi-brassinolid foliar application treatment, proline amino acid content was 54.75 micromoles per gram (Table 3). Besides, in bean farm around Shahrood, 24-epi-brassinolid foliar application increased the content of this amino acid to 11.5% and reached to 52.20 micromoles per gram (Table 4).

Investigation of dual interactions of water deficit and ultrasonic waves in Research Center field showed that the maximum amount of proline amino acid was obtained from severe stress treatment, radiation of ultrasonic waves (65.21) and the minimum amount of aforesaid amino acid was obtained from nonuse of stress treatment and non-radiation of ultrasonic waves (40.51) which reduced to 39% compared to maximum proline content treatment (Table 3).

Ascorbate

Investigation of variance analysis of ascorbate enzyme showed that in Research Center field, the entire simple effects at 1% probability level and simple effects of ultrasonic wave radiation and 24-epi-brassinolid foliar application affected ascorbate enzyme content at 5% probability level (Table 1), but in bean farm around Shahrood, the simple effect of water deficit and 24-epi-brassinolid foliar application was at 1% probability level and simple effect of ultrasonic radiation affected the ascorbate content at 5% probability level (Table 2).

The results of comparison between the averages of water deficit showed that in both planting sites (Table 3 and 4), ascorbate enzyme content in red bean reduced with increment of water deficit as though the amount of ascorbate enzyme in Research Center field (147.64 µmol min^{-1} mg^{-1} protein) (Table 3), and bean farm around Shahrood (143.10µmol min^{-1} mg^{-1} protein) were obtained after severe stress treatment (irrigation after 120 mm evaporation from evaporation pan) which decreased the content of ascorbate enzyme (Table 4), compared to the

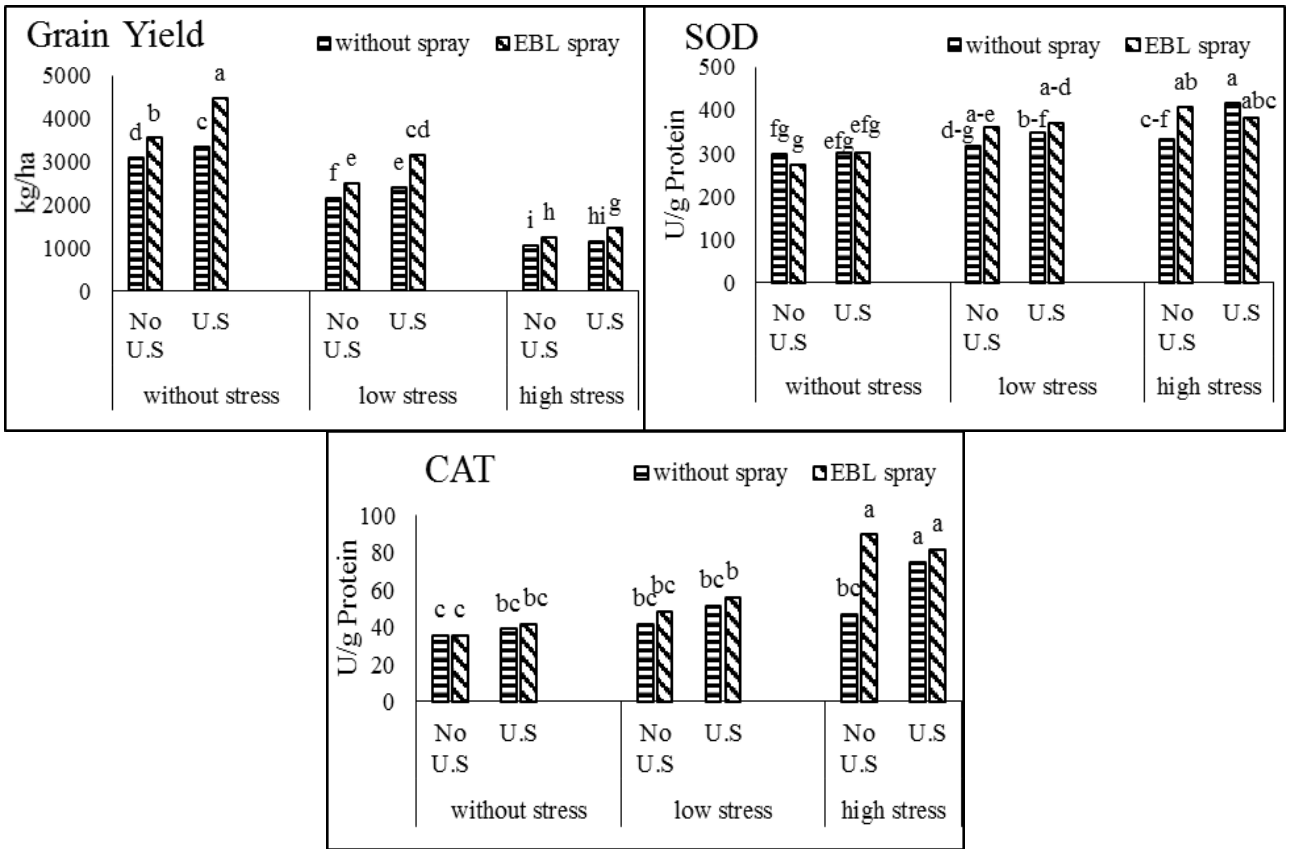

Figure 4. Interaction of drought stress, ultrasound irradiation and 24-epibrassinolide spray on yield, SOD and catalase content of *Phaseolus vulgaris* in a field of bean in Shahrood

lack of water deficit stress treatment, to 51 and 53% in Research Center farm (299.62 μmol min^{-1} mg^{-1}protein) and bean farm around Shahrood (300.90 μmol min^{-1} mg^{-1}protein) respectively. But ultrasonic radiation on bean seeds in Research Center field increased the content of ascorbate enzyme to 7% and changed it to 236.06 μmol min^{-1} mg^{-1} protein (Table 3).

In bean farm around Shahrood, the content of ascorbate enzyme was increased under the influence of ultrasonic radiations to 9% and reached to 239.05 μmol min^{-1} mg^{-1}protein (Table 4). Moreover, 24-epi-brassinolid foliar increased the content of ascorbate enzyme respectively to 10 and 17% in Research Center farm and bean farm around Shahrood as though maximum content of ascorbate enzyme was 240.13 μmol min^{-1} mg^{-1} protein and bean farm around Shahrood was 249.92 μmol min^{-1} mg^{-1} protein (Table 3 and 4).

DISCUSSION

Grain Yield

Water deficit causes oxidative stress which interferes physiological functions of cells. Because of the generation of reactive oxygen species in cell culture, this stress leads to oxidative damages similar to superoxide anion, hydrogen peroxide, hydroxyl radicals. To vanish these toxic species, there is need to very effective antioxidant system (enzymatic and non-enzymatic system) in plant cells (Mozaffari, 2004). It has been reported that the ultrasonic beam affects the biomolecules through structural changes, oxidation and free radical formation, such as superoxide anion, hydrogen peroxide and hydroxyl radicals and so provides the ground for changes in developmental characteristics (Hamed *et al.,* 2008).

Rawling *et al.* (2001) conducted a trial on soybean under heat radiation and 'x' neutrons treatments. They found that significant genetic variety will be generated under the influence of ray in grain yield, plant height, maturity time and seed size. Besides, certain mutations have been successfully applied by breeders to change the genetic structure of canola and mustard and mutations were identified with favorable some economic characteristics (Javed *et al.,* 2003).

Superoxide Dismutase

Ionizing beams enter into tissue and cells react with different atoms and molecules and produce free radicals in cells. Depending on the intensity of the beam, positive or negative changes in morphological, physiological and biochemical processes occur in plants (Kiong *et al.,* 2008).

Examination of dual interactions (water deficit and 24-epi-brassinolid foliar application) in Research Center field showed that the maximum amount of superoxide dismutase enzyme was obtained from severe stress treatment and 24-epi-brassinolid foliar application (460.49) and the minimum amount of aforesaid enzyme was obtained from lack of water deficit treatment and 24-epi-brassinolid foliar application (301.52). The minimum content of superoxide dismutase treatment with two treatments of lack of stress, non-radiation of waves as well as mild water deficit and non-radiation of waves were categorized in one statistical group. But in general there was 35% difference in the content of superoxide dismutase enzyme between maximum and minimum treatments.

The results of comparison between the averages of three treatments in bean farm around Shahrood for superoxide dismutase enzyme content showed that the maximum amount of superoxide dismutase enzyme was obtained from severe water deficit stress treatment, radiation of ultrasonic waves and 24-epi-brassinolid foliar application (417.8) and the minimum amount of superoxide dismutase enzyme was obtained from lack of water deficit treatment, non- radiation of ultrasonic waves and 24-epi-brassinolid foliar application (272.3). 35% of severe stress increased the superoxide dismutase content in ultrasonic wave radiation condition.

Peroxidase

Destructive processes of membrane become activated in stress condition and leads to peroxidation of membrane lipids. Brassinosteroids affects the composition of fatty acids and membrane permeability and has positive effect on the concentration of solutes.

Some researchers have shown that the activity of antioxidant enzymes, especially peroxidase will increase after irradiation to cope with the damage caused by oxidative stress (Hamed *et al.,* 2008). Thus it can be stated that use of radiation in increasing competitiveness strength of plant is effective in the event of stress.

Catalase

Wani and Anis (2008) examined the seeds of one variety of chickpea under 750, 1000 and 1250 Gray of gamma rays treatments and selection for desirable agronomic traits was implemented in several stages. They found that bushes under the 750 gray level had higher thousand kernel weight and yield compared to parent. Rice seedlings exposed to saline stress and treated with BR showed a significant increase in the activities of CAT, SOD and Glutathione Reductase (GR) and a slight increase in APX (Nunez *et al.,* 2003).

Proline

However the investigation of dual interactions (water deficit and ultrasonic waves) in bean farm around Shahrood showed that the maximum amount of proline enzyme was obtained from severe stress treatment and radiation of ultrasonic waves (64.04) and the minimum amount of aforesaid enzyme was obtained from non-use of severe stress treatment and non-radiation of ultrasonic waves (38.18) which decreased to 41% compared to maximum proline content treatment.

Plants can absorb water until they have lower water potential than environment. Usually the major part of osmotic regulation may continue through increment of increased concentration of different dissolved substances such common sugars, organic acids, ions especially potassium. Cytosol enzymes in high concentrations of ions are strongly prevented and so ions mostly accumulate within vacuoles, where they are not in contact with cytosol enzymes or intercellular organelles. Because of this type of allocation of compatible solutions which do not interfere with enzyme functions, they accumulate in the cytoplasm for balancing water potential in cells. These types of materials such as glycine betaine, proline and poly L, create a compatible environment for macromolecules, especially proteins (Kafi, 1996). Proline accumulation has positive and direct relation with increased resistance to water drought and salinity stress induced in plants (Saneoka, 2004) which was consistent with the results of our study and we observed significant increase in amount of proline in water deficit stress.

Similar report is available about water deficit stress on wheat, corn and rice which indicated that proline accumulation in cytoplasm acts like a smotricom in macromolecular structural protection in an environment where ionic equilibrium has been disturbed (Nayyar, 2003). It seems that 24-epi-brassinolid foliar application in present paper has increased osmotic adjustment during stress. Proline increase results in inflation maintenance and reduction of membrane damage in plants and so tolerance to water deficit stress will increase by osmotic adjustment method (Pandey and Agarwal, 1998). Accumulation of soluble sugar into the cells plays important role in osmotic adjustment and helps to decrease water potential of cell and keep much amount of water into the cells and maintain turgor under water stress conditions.

The foliar spray of either with 24-epiBL or 28-homoBL significantly enhanced the growth, photosynthesis, antioxidant enzymes and proline content in aluminium stressed mung bean plants. 24-epiBL enhanced the level of antioxidant system (SOD, CAT, peroxidase and glutathione reductase and proline), both under stress and stress-free conditions. The influence of 24-epiBL on the antioxidant system was more pronounced under stress situation, suggesting that the elevated level of antioxidant system, at least in part, increased the tolerance of mus-

tard plants to saline and/or nickel stress, thus protected the photosynthetic machinery and the plant growth (Ali *et al.,* 2008).

Ascorbate

It is now well known that salinity exerts oxidative stress due to the production of variety of Active Oxygen Species (AOS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH^-) radicals, which cause oxidative damage in plants (McCord, 2000). To scavenge these toxic species, plants develop antioxidant enzymes, such as SOD, Peroxidase (POX), Ascorbate Peroxidase (APOX), CAT and GR. Since their activities and transcripts are altered when plants are subjected to stress, changes in the levels of antioxidant enzymes have been used to assess the effect of different stressors including salinity (Filiz *et al.,* 2004).

CONCLUSION

The results of this survey showed that using ultrasonic waves and 24-epi-brassinolid foliar application significantly can be effective for reduction of water deficit stress during growth and improves quantitative and qualitative properties of plant.

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