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Response of Broad Bean and Lupin Plants to Foliar Treatment with Boron and Zinc

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Abstract: This study investigate the growth, yield and certain metabolic activities of both broad bean and lupin plants in response to foliar treatment with either Boron (75ppm) or Zinc (100ppm). Plants were grown in natural loamy soil conditions and treated twice with each of the applied micronutrients. The treated plants showed significant stimulation in most of the growth and yield characteristics (lengths of shoots and roots, number of leaves/plant, plant biomass, number of pods/plant, number of seeds/plant and weight of 100-seed).Also, treatment with either B or Zn caused significant increases in the contents of photosynthetic pigments, soluble carbohydrates and soluble proteins in the two tested plants. Treatment with the micronutrients greatly affected the activities of amylases, proteases, catalases and peroxidases of the two tested plants. Levels of endogenous gibbrellic acid (GA_3) and indole acetic acid (IAA) were markedly increased, while levels of abscisic acid (ABA) were decreased in both the two tested plants due to the treatment with either B or Zn.

Key words: broad bean, lupin, foliar treatment, boron and zinc.

INTRODUCTION

Agricultural production and productivity are directly linked with nutrient availability and uptake. To sustain high crop yields, the application of nutrients is required. Mineral nutrients are essential for plant growth and development (Marchner, 1995). Boron is an essential micronutrient for plants, and plant requirements for this nutrient are lower than the requirements many other nutrients. It is the only nonmetal among the micronutrients and also the only micronutrient present over a wide pH range as a neutral molecule rather than an ion (Epstein and Bloom, 2005). Although B uptake of crop plants is not higher than the uptake of other nutrients, its deficiency has been reported in many parts of the world (Fageria *et al*., 2002 and Ross *et al*, 2006). Zinc is a micronutrient needed in small amounts by crop plants, but its important in crop production has increased in recent years (Fageria, 2009). It is considered to be the most yield-limiting micronutrient in crop production in various parts of the world (Fageria and Baligar, 2005 and Duffy, 2007). So, the present investigation was undertaken to study the growth, yield and certain metabolic responses of both broad bean and lupin plants to foliar treatment with either B or Zn.

MATERIALS AND METHODS

Seeds of broad bean "*Vicia faba*" (Giza-716) & lupin "*Lupinus albus*" (Giza-1) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Uniform broad bean and lupin seeds were planted in natural loamy soil conditions in Botanical garden, Botany and Microbiology Dept., Fac. of Sci., Al-Azhar Univ., in two plots containing 6 ridges (3 for each plot). The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated when ever required. Each of the two experimented plants was divided into 3 groups representing the foliar treatments with, distilled water (as controls), 75 ppm B as boric acid, and the third group sprayed with 100 ppm Zn as zinc sulphate. Concentrations of the used micronutrients were chosen according to a preliminary experiment in which they caused a maximum germination percentage. The plants were sprayed twice with the above mentioned treatments. The first treatment was made when the age of plants was 33 days, while the second treatment was made when the age of plants was 65 days. The plant samples were collected for analysis when the plants were 40 (Stage I) and 72 (Stage II) days old. At the end of the growth season, analysis of the seeds yielded from the different treatments and the control were done. Contents of chlorophylls were estimated using the method of Vernon and Selly (1966). Contents of soluble carbohydrates were measured according to the method of

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Umbriet *et al* (1969). Contents of soluble proteins were estimated according to the methods of lowery *et al* (1951). Activities of amylases were determined using the method of Afifi *et al* (1986). Proteases activities were estimated using the method of Ong and Guacher (1972). Catalases activity were measured according to the method of Chen *et al* (2000). Peroxidase activity was assayed according to the method of Bergmeyer (1974). Contents of endogenous GA_3, IAA and ABA were estimated using HPLC according to the method of Knegt and Brunima (1973). Statistical analysis of the obtained results was done using (T.test &L.S.D.) according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Growth and Yield Responses:

The obtained results (Tables 1, 2 & 3) revealed that most of investigated growth characteristics (length of shoots and roots, number of leaves/plant, fresh and dry weight of shoots, and roots, number of pods/plant, number of seeds/plant and the weight of 100-seeds) of both broad bean and lupin plants were markedly increased in response to the application of either B or Zn. These effects were stronger at the second stage of growth. The stimulative effects of either B or Zn on plant growth were also obtained by many workers (Tobbal, 1999; Hemantaranjan *et al*., 2000; Rizk and Abdo, 2001; Wanas, 2002 and Gomaa, 2003). Madny (2004) reported that, in broad bean, plant height, leaf area/plant, total dry weight/plant, number of pods/plant, number of seeds/pods, seeds weight/plant and the 100-seed weight were significantly increased in response to the treatment with B (50 ppm). Khudsar *et al*. (2004) studied the effect of Zn (50, 100, 200, 300 and 400 µg/g soil dry mass) on *Artemisia annua* plants. They found that, total leaf area, length and dry mass of shoots and roots were increased with the age of the plant. El-Sallami and Gad (2005) found that, Zn sprays at 100 ppm increased the vegetative growth measurements plant height, number of leaves as well as the fresh and dry weights of aster plants. Mahmoud *et al*. (2006) found that, treating broad bean plants with B (25 -50 ppm) significantly increased plant height, total dry weight, number of pods/plant, number of seeds/pod and the seed yield.

Photosynthetic Pigment:

Results of the present study (Table 4) revealed that, contents of chlorophyll a, b and total chlorophyll (a + b) of broad bean and lupin plants were, mostly, significantly increased in response to the application of either B or Zn. These results are in agreement with the results obtained by Rashed and Ahmed (1997) on broad bean plants. They found that, chlorophyll contents were increased by using foliar application with Zn (50 ppm). Hassanein *et al*. (2000) found that, spraying cow pea (*Vigna sinensis*) plants with 10, 50 and 250 mg/L^{-1} of B or Zn caused high significant increases in the contents of chlorophyll (a) and chlorophyll b. Also, Tobbal (2006) showed that, spraying *Celosia* plants with Zn (400 mg/L^{-1}) increased contents of chlorophyll a, b and total (a + b). The same author reported that, the increase in chlorophyll a, b and total chlorophyll (a + b) could be ascribed to the effect of this element on increasing the biosynthesis of photosynthetic pigments and/or retarding their degradation.

Soluble Carbohydrates:

Results of the present work (Table 5) revealed that, contents of total soluble carbohydrates were significantly increased in shoots, roots and yielded seeds of broad bean and lupin plants in response to the treatment with either B or Zn. This was the case throughout the two stages of growth. The stimulatory effects of the micronutrients (B and Zn) as regards contents of total soluble carbohydrates in different plants were recorded by other investigators. Zaky *et al*. (1999) found that, spraying cow pea plants with B or Zn at 10 and 50 m/L increased the reducing sugars, sucrose, polysaccharides and total carbohydrates contents. Tobbal (2006) revealed that, contents of total soluble carbohydrates of both *Celosia* and *Zinnia* shoots were significantly increased in response to the treatment with Zn.

Soluble Proteins:

In the present study, it was found (Table 6) that protein contents in shoots, roots as well as in the yielded seeds of broad bean and lupin plants were significantly increased in response to the treatments with either B or Zn. In accordance with the obtained results, Rizk and Abdo (2001) studied the effect of B as foliar spraying on mung bean plants; they found that, the contents of crude proteins were increased**.** Gamal El-Din (2005) reported that, Zn treatments (100 and 200 mg/L) increased protein contents of fenugreek seeds. Also, Tobbal (1999) found that, the contents of soluble proteins in shoots, roots and yielded seeds of fenugreek and chickpea plants were increased in response to the treatment with Zn (100 ppm) as foliar spraying.

Enzymes Activities:

In the present investigation, it was found that(Table 7), treatment with B resulted in, broad bean plants, significant increases in the activity of catalases, significant decreases in the activities proteases & peroxidases and insignificant changes as regards the activity of amylases. The same treatment caused, in lupin plants, significant increases in the activities of amylases & catalases, significant decrease of proteases activity, while activities of peroxidases were insignificantly affected. According to these results, it seems likely to state that the effect of B as regards the activities of certain enzymes may depend largely on several factors such as the level applied the age and the type of plant species. In this respect, Zaky *et al.* (1999) found that, the high level of B (250 ppm) decreased the protease activity in shoot of cowpea plants, while the lower level (10 & 50 ppm) significantly increased activities of amylases and proteases.

The obtained results revealed also that, application of Zn caused variable responses as regards the activities of the detected enzymes, in broad bean plants, activities of amylases were markedly increased, activities of proteases were significantly decreased, and insignificant changes were recorded as regards the activities of catalases and peroxidases. In lupin plants, treatment with Zn caused significant increases in the activities of amylases and peroxidases, activities of proteases were significantly decreased while activities of catalases were insignificantly affected. The potent effects of Zn on stimulating the activities of certain enzymes were recorded by other investigators. In this respect, Tobbal (2006) summarized that, treating *Celosia* plants with Zn resulted in insignificant increases in the activities of amylases, proteases, catalases, peroxidases and polyphenoloxidases. El-Mashad (1998) reported that, application of Zn caused highly significant increases in peroxidases activities of shoots and roots of *Vigna sinensis* plants. Contrary to the stimulatory effects of Zn as regards enzymes activities, Tobbal (1999) revealed that, activities of proteases in fenugreek and chickpea plants were significantly decreased in response to treating the plants with Zn 0.1%. El-Gebaly *et al.* (2003) pointed out that, treatment with Zn at 0.06% to flax plants insignificantly affected the activities of peroxidases and polyphenoloxidases enzymes.

Endogenous Hormones:

In both broad bean and lupin plants, the obtained results (Table 8) revealed that, contents of GA_3 and IAA were significantly increased in response to the treatment with either B or Zn. The same treatments caused significant decreases in the contents of ABA of the two studied plants. The obtained results are in agreement with those of other investigators. Zaky *et al.* (1999) found that, both B and Zn at (10 & 50 ppm) increased the activities of endogenous gibberellins of cowpea plants. Tobbal (2006) found that, Zn treatments greatly increased contents of IAA and GA_3 in both *Celosia* and *Zinnia* plants while contents of abscisic acid (ABA) were decreased.

Table 1: Effect of B and Zn on shoot length (Cm/plant) , root length (Cm./plant)and the number of leaves/plant of broad bean and lupin .Each value is mean of 8 replicates ± standard error of means.

Broad bean plants	Treatment	Shoot length(Cm. /plant)		Root length(Cm. /plant)		Number of leaves	
		stage 1	stage II	stage 1	stage II	stage 1	stage II
	control	33.37± 0.61	72.51± 0.22	13.72±0.07	14.50±0.54	8.74±0.16	46.17±0.28
	B	36.28±0.36 **	75.88±0.94 **	13.84±0.04**	16.13±0.19 *	11.84±0.91**	50.38±1.07**
	Zn	38.35±0.18 **	76.51±0.37 **	13.87±0.20**	17.25±0.10**	10.55±0.22**	48.01±0.39**
Lupin plants	control	23.16 ± 0.37	44.21±0.56	10.88±0.39	16.75±0.13	13.01±0.12	18.31±0.24
	B	25.33±0.27**	47.12±0.61**	8.56±0.13**	17.02±0.13	13.13±0.19	18.88±0.55
	Zn	23.38 ± 0.49	47.21±0.36*	9.24±0.03 *	18.75±0.1*	14.38±0.1*	20.36±0.8*

*significant at 5% confidence level; ** significant at 1% confidence level.

Table 2: Effect of B and Zn on fresh and dry weights of shoots and roots (g/plant) of broad bean and lupin plants .Each value is mean of 8 replicates ± standard error of means.

Broad bean plants	Treatment	F. wt of shoot (g./plant)		F. wt of root (g./plant)		D. wt of shoot (g./plant)		D. wt of root (g./plant)	
		stage 1	stage II	stage 1	stage II	stage 1	stage II	stage 1	stage II
	control	19.29±0.92	109.34±0.94	2.24 ± 0.11	8.31±0.17	1.58±0.17	10.49±0.62	0.19±0.03	1.94±0.06
	B	30.21±1.51**	182.39±0.12**	2.58±0.07	11.83±0.23**	2.69±0.22*	16.13±1.62*	0.22±0.02	1.63±0.01**
	Zn	22.83±1.34 *	155.53±1.52**	3.16±0.14**	10.94±0.15**	2.16±0.17	12.64±0.54*	0.26±0.03	1.89±0.07
Lupin plants	control	7.24±0.18	47.43±0.17	0.53±0.03	3.51±0.06	0.87±0.04	6.35±0.03	0.08±0.01	0.79±0.02
	B	7.06±0.19	62.43±0.8**	0.26±0.01**	4.13±0.05**	0.89±0.01	7.91±0.05**	0.06±0.02	0.91±0.04
	Zn	9.51±0.87*	68.26±0.5**	0.47±0.01	4.67±0.06**	0.88±0.07	8.94±0.16**	0.07±0.01	0.84±0.02

*Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 3: Effect of B and Zn on Yield characters of lupin plants .Each value is meaning of 8 replicates ± standard error of means.

Treatments		No. of pods/plant	No. of seeds/plant	100-seed weight (g./plant)
Broad bean plants	Control	21.34 ± 0.31	60.87 ± 0.32	109.36 ± 0.94
	B	29.37 ± 0.21**	89.81 ± 0.15**	124.31 ± 1.13**
	Zn	31.72 ± 0.72**	105.39 ± 0.21**	135.27 ± 0.96**
lupin plants	Control	14.37 ± 0.07	36.14 ± 0.21	89.95 ± 0.17
	B	19.83 ± 0.21**	47.95 ± 0.17**	98.36 ± 0.14**
	Zn	23.57 ± 0.08**	58.31 ± 0.23 **	101.24 ±0.15**

*Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 4: Effect of B and Zn on the chlorophyll contents (mg/g fresh weight) of broad bean and lupin plants .Each value is mean of 3 replicates ± standard error of means.

Broad bean plants	Treatment	Chlorophyll (a)		Chlorophyll (b)		Total (a+b)	
		stage I	Stage II	Stage I	Stage II	Stage I	Stage II
	control	6.72 ± 0.04	6.34 ± 0.05	4.13 ± 0.05	3.95 ± 0.02	10.85 ± 0.03	10.89 ± 0.04
	B	8.14±0.03**	8.45±0.13**	5.04 ±0.01**	4.21±0.02**	13.18±0.01**	12.66±0.10**
	Zn	8.42±0.01**	9.62±0.04**	5.77 ±0.01**	5.61±0.01**	14.19±0.13**	15.23±0.12**
Lupin plants	control	7.55 ± 0.13	8.76 ± 0.15	3.16 ± 0.01	3.59 ±0.30	10.71 ± 0.14	12.35 ± 0.15
	B	8.96 ± 0.13**	9.45 ± 0.03*	5.03 ± 0.21**	4.46 ±0.02	13.99±0.13**	13.91±0.11**
	Zn	9.11 ± 0.07**	11.63±0.03**	3.91 ± 0.02**	5.34±0.14*	13.02±0.07**	16.97±0.16**

*Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 5: Effect of B and Zn on the total- soluble carbohydrate contents (mg/g dry weight) of broad bean and lupin plants .Each value is mean of 3 replicates ± standard error of means.

Broad bean plants	Treatment	Shoot		Root		Seeds
		stage 1	stage II	stage 1	stage II	
	control	67.21 ± 0.06	64.13 ± 0.37	50.16 ± 0.21	51.21 ± 0.82	80.36 ± 0.27
	B	73.07 ±0.94**	76.05 ±0.27**	73.23 ±0.19**	72.07 ±0.81**	109.27±0.26**
	Zn	81.27 ±0.27**	83.51 ±0.91**	69.05 ±0.27**	71.28 ±0.83**	114.13±0.19**
Lupin plants	control	72.21 ± 0.36	74.24 ± 0.72	56.27 ± 0.19	54.01 ± 0.37	79.36 ± 0.13
	B	81.65 ±0.28**	84.36 ±0.09**	63.81 ±0.17**	66.35 ±0.16**	98.45 ±0.16**
	Zn	83.42 ±0.27**	91.27 ±0.18**	72.07 ±0.26**	69.41 ±0.94**	98.17 ±0.21**

*Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 6: Effect of B and Zn on the total- soluble protein contents (mg/g dry weight) of broad bean and lupin plants .Each value is mean of 3 replicates ± standard error of means.

Broad bean plants	Treatment	Shoot		Root		Seed
		stage 1	stage II	stage 1	stage II	
	control	96.36 ± 0.17	101.12 ± 0.28	74.23 ± 0.28	70.26 ± 0.22	126.21 ± 0.20
	B	117.29±1.02**	121.54±1.01**	81.09±0.91**	81.21±0.19**	161.34 ± 0.21**
	Zn	123.91±1.11**	139.41±0.24**	90.08±0.27**	92.86±0.82**	157.27 ± 0.30**
Lupin plants	control	83.23 ± 0.36	86.11 ± 0.29	76.26 ± 0.11	72.21 ± 0.07	138.21 ± 0.21
	B	80.36 ± 0.91	94.29 ± 1.02**	80.20±0.18**	87.08±0.91**	157.17 ± 0.5**
	Zn	87.17 ±0.72*	107.22±1.03**	89.36 ±0.86**	91.51 ±0.75**	164.24±0.13**

*Significant at 5% confidence level; ** Significant at 1% confidence level.

Table 7: Effect of B and Zn on the activity of amylase (s), protease, catalase and peroxidase of broad bean and lupin plants. Each value is mean of 3 replicates ± standard error of means.

Treatments		Amylase (mg/g fresh weight)		Protease (mg/g fresh weight)		Catalase (μg/g fresh weight)		Peroxidase (mg/g fresh weight)	
		Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Broad bean plants	Control	1.02 ± 0.20	0.12 ± 0.01	1.02 ± 0.05	2.20 ± 0.33	0.05 ± 0.02	0.17 ± 0.03	1.56 ± 0.20	0.65 ±0.03
	B	1.23 ± 0.07	0.16 ± 0.03	1.02 ± 0.06	0.74±0.03**	0.05 ± 0.03	0.29 ± 0.02*	0.73 ±0.14*	0.37±0.0*
	Zn	1.38 ± 0.03	0.67±0.03**	1.03 ± 0.40	1.03 ±0.02*	0.07 ± 0.01	0.16 ± 0.02	1.04 ± 0.24	0.75 ±0.04
Lupin plants	Control	0.17 ± 0.03	0.07 ± 0.02	0.54 ± 0.02	0.75 ± 0.02	0.12 ± 0.02	0.15 ± 0.04	0.45 ± 0.07	1.44 ±0.43
	B	0.42 ± 0.17	0.35±0.02**	0.70±0.02**	0.65±0.02*	0.26±0.03*	0.37 ± 0.03*	2.38±0.13**	3.58 ±0.89
	Zn	0.40 ± 0.19	0.24±0.01**	0.56 ± 0.14	0.62 ±0.03*	0.0.08 ±0.01	0.20 ± 0.02	3.41±0.58**	3.58±0.68*

*Significant at 5% confidence level; **Significant at 1% confidence level.

Table 8: Effect of B and Zn on contents of endogenous GA_3, IAA and ABA (mg/100g f.wt) of broad bean plants at stage II of plant growth.

Broad bean plants	Treatments	Levels of endogenous hormones		
		GA_3	IAA	ABA
	control	3.69	4.01	0.41
	B	4.51	5.01	0.29
	Zn	5.28	6.17	0.23
Lupin plants	control	2.97	3.18	0.28
	B	3.83	4.01	0.22
	Zn	4.21	5.11	0.22
LSD at 0.05		0.035	0.03	0.02

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