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Original Research

The role of gibberellic acid and zinc sulfate on biochemical performance relate to drought tolerance of white bean under water stress

Arefeh Abbasi[1], Abbas Maleki[2*], Farzad Babaei[3], Hooshmand Safari[4], Alireza Rangin[5]

[1] Department of Agronomy and Plant Breeding, College of Agriculture, Islamic Azad University of Ilam Branch, Ilam, Iran
[2] Department of Agronomy and Plant Breeding, College of Agriculture, Islamic Azad University of Ilam Branch, Ilam, Iran
[3] Department of Agronomy and Plant Breeding, College of Agriculture, Islamic Azad University of Ilam Branch, Ilam, Iran
[4] Research Department of Forests and Rangelands, Kermanshah Agricultural and Natural Resources Research and Education Center, Kermanshah, Iran
[5] Department of Biology, Islamic Azad University of Ilam Branch, Ilam, Iran

Correspondence to: maleki97@yahoo.com

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Abstract: In this study the effects of zinc sulfate and gibberellin on agro physiological of white bean under water deficiency were studied. Therefore, an experiment was conducted in a split-split plot design based on a randomized complete block with three replications in two places. The experimental factors included three irrigation levels, spraying of zinc sulfate in four levels and two levels of non-spraying and spraying of gibberellin. Analysis of measured data indicated that the water stress had a significant effect on all traits, except proline amount and 100 seeds weight. Spraying of zinc sulfate showed a significant effect on all traits except carotenoid value. Application of gibberellin had a significant effect on all traits except ion leakage, carotenoids, number of seeds per pod and grain yield. The interaction effect of stress×zincsulfate×gibberellin was significant on all traits except number of seeds per pod. In addition, comparison of means at 5% level, showed that application of 1.5 ml $L^{-1}$of zinc sulfate plus gibberellin improved bean biochemical properties. Under optimum water level, using of 4.5 ml $L^{-1}$of zinc sulfate and under severe water stress conditions, using of 4.5 ml L^{-1} of zinc sulfate plus gibberellin are recommended for obtaining the maximum crop performance.

Key words: Plant stress; PGR; Micronutrient; Irrigation regime.

Introduction

Malnutrition is one of the most important and worrying problems of human society. Protein deficiency in diet imposes the greatest harm to humans both physically and mentally (1). Bean (*Phaseolus vulgaris* L.) is one of the most valuable seeds and in many parts of the world is the main source of protein thus plays an important role in human diet. Beans contain about 2-4 times more protein than the cereals and 10- 20 times more than the tuberous plants (2). Bean plants with about 20 to 30 percent protein and 50 to 60 percent carbohydrates, are relatively good source of minerals and vitamins (3). Therefore, it is not only a vital nutrient for humans but also its stem and leaves can be used for animal feed. Another advantage of beans as a member of legume family is enriching the soil through nitrogen fixation (4). FAO data has shown that from 2011 to 2014 annually 337000 hectares were under beans cultivation with production of 445000 tons of beans (5).

Bean farming has always been confronted with many constraints including drought stress as an important factor which has a significant contribution to reducing the production of this crop. Recent studies have shown that only 7% from the global cultivation of beans received adequate water and 60% of its farming were under severe drought stress. Iran is located in arid and semi-arid regions with approximately 35% of lands have very dry climate, 29.9% dry, 20.1% semi-arid, 5% Mediterranean and 10% wet (cold-mountainous) climate conditions (6).

Low available water under drought stress conditions, reduces the photosynthesis and thus dry matter production (7). The main reason for reducing photosynthesis in drought stress conditions is the reduction of carbon entry due to the closure of the stomata. The stomata would be closed in response to water stress in order to reduce water loss. The non-stomatal limitations of photosynthesis are due to the increased mesophilic resistance to carbon dioxide transfer, the reduction of RuBisCO carboxylation and prevention of photosystem II activity. In addition, in the absence of water, the synthesis of chlorophylls and proteins would be reduced as well (8). Bean is susceptible to drought stress and its function is harmed even under short periods of stress. Drought stress reduces the water content, decreases the leaf water potential, the turgor pressure and cell growth. Plants respond to drought stress by creating a series of physiological changes. The accumulation of solutions materials in response to drought stress (osmotic regulation) is a way for turgor pressure maintaining (9). Severe water stress can cease the photosynthesis, impose metabolism disorder and eventually lead to plant death (10). One of the drought stress effects is disturbing the

Table 1. The physical and chemical characteristics of the experiment location.

K (Available) P.P.M	P (Available) P.P.M	Total N %	O.C %	C.E.C Meq/mg	T. N.V.%	pH	EC %	SP %	Sampling depth cm
640	9.2	13	1.26	30.8	15.2	7.6	0.73	54	0-20
380	2.8	6	0.63	30.2	17.5	7.7	0.49	55	60-20
240	2.4	3	0.29	28.0	24.5	7.8	0.44	52	95-60
220	2.8	4	0.38	28.0	26.0	7.8	0.48	51	125-95

Table 2. The meteorological and geographical characteristics of the Eslam Abad Ggarb and Khorram Abad.

Minimum Absolute Temperature(gradi)	Maximum Absolute Temperature(gradi)	Mean temperature (gradi)	Mean annual rainfall (mm)	Altitude (M)	Latitude	Longitude
-28.8	+41°C	+10.5°C	538	1346	34° 8′	47° 26′
-14.6oC	+47oC	+17.2oC	499 mm	1170 M	33o 30′	48o 18′

nutritional balance of the plant, however micro elements spraying, may be able to improve the situation of plant growth and somewhat increase the resistance of plant to stress conditions (11).

The Zn element is a necessary nutrient for optimum growth of plants and has a significant role in protecting and preventing the oxidation of vital cellular components such as chlorophyll (12). Zinc plays an important role in various metabolic and physiological processes of the plant, and regulates the activity of some enzymes, metabolism of carbohydrates and proteins, which is essential for different processes of the development and differentiation of plant cells (13), and also reducing the relative risk of reactive oxygen species (ROS) and protecting the cells against its attack (14). However, its excessive amount in plants affects the metabolism and process such as photosynthesis and as a result, limits root and leaf growth (15). It is well known that Zinc acts as a common agent (activator) for various enzymes and affects many biological processes (16). Zn also plays an important role in regulating the openness of stomata, as it contributes in maintaining the potassium in the stomata protective cells and increases relative water content of the leaf by reducing water loss of the leaves (17).

In the past decade, plant hormones such as gibberellic acid have been used as anti-oxidants against abiotic stress (10). The gibberellins increase the growth of plant cells. The longitudinal growth rate can be affected by the expansion of the cell wall and the amount of water absorption. It has been shown that gibberellin does not increase water absorption, but it increases the cell wall loosening and expandability. The use of gibberellic acid usually increases the growth and the expansion of root system, and also increases the tolerance to abiotic stresses (18). In general, various studies have shown that use of micro elements and growth stimulants under drought stress conditions increase plant tolerance to drought. The purpose of this study was to investigate the effect of zinc sulfate and gibberellin on the morphological and physiological characteristics of beans under different levels of water availability.

Materials and Methods

Experimental location

The present study was carried out to investigate the effect of zinc sulfate and gibberellin on biochemical and agro-morphological characteristics of white bean (*Phaseolus vulgaris* L.) under water stress conditions, in two place of 2016 and 2017 at Eslamabad-e Gharb Research Station, Kermanshah, Iran and Research Station KhorramAbad. The physical and chemical characteristics of the soil and the meteorological and geographical characteristics of the experiment location are presented in (Tables 1 and 2) respectively.

Experiment design

The experiment was conducted in a split-split plot design based on a randomized complete block with three replicates. The length of each main plot was 12m and its width was 3m and the length of the sub plots was 3m and its width was 3m and the length of the sub-sub plots was 3m and its width was 1.5m. The experimental factors included three irrigation levels (irrigation after 60, 90 and 120 mm evaporation from class A evaporation pan) as main plot, spraying of zinc sulfate in four levels of 0.0, 1.5, 3.5 and 4.5 ml L^{-1} as sub plot and two levels of non-spraying and spraying of gibberellin) mgl^{-1}) as sub-sub plot. Gibberellin and zinc sulfate were used at pre-flowering stage. Planting date was May 29, in both years of 2016 and 2017.

Measurement

Leaf electrolytes leakage (LEL) was measured according to Flinet et al. 1996 (19). Leaves were taken from each plant and then were cut into discs by puncher. The discs were washed using distilled water and then put into tubes containing 5 ml distilled water. After 24 h, the electrical conductivity of the samples was measured using EC meter (Jen way 4010). The samples were put in the freezer at -20 °C for 24 h and then EC was measured again. To break the cells and remove the contents of them into solution, the operation of freezing and melting was repeated several times. The electrical leakage was calculated using the following equation:

$$\frac{E_1}{E_2} \times 100$$

Chlorophyll a, b and total chlorophyll (CL_A, CL_B and CL_T) were measured according to Porra (2002) (20) method. Carotenoids were measured based on the methods described by Lichtenthaler and Wellburn (1983) (21). The leaf proline content (Proline) was determined using Bates (1973) method (22). The leaf sample (500 ml) was pulverized in 10 ml 3% sulfosalicylic acid and was filtered and then 2 ml of the sample were mixed with 2 mL ninhydrin acid (25.1 g ninhydrin plus 30 ml glacial acetic acid) and 2 ml glacial acetic acid. The sample were heated for 1 h at 100°C and then cooled down to 4° C for 30 min. In the next stage, 4 mL of toluene was added to the contents of each tube and mixed with vortex for 30 seconds. The tubes were kept at room temperature for 10 min. In this step two separate layers were created. The absorbance of the upper colored layer at 520 nm was measured using a toluene by a spectrophotometer and the amount of proline were calculated using the following equation.

$$\left(\frac{\mu g}{mL}\text{Proline}\times mLtoluene\right)\times\frac{\mu mol}{115/5\mu g}\times\frac{5}{sampleg}=\mu molprolinepergfreshsample$$

$$\left(\frac{\mu g}{mL}Proline\times mLtoluene\right)\times\frac{21}{sampleg}=\mu gprolinepergfreshsample$$

To measure the relative water content (RWC) was used of method that described by (23). So, for each plot, young leaves were selected on the same position on the plant and the fresh weight (Sample weight immediately after separating), saturation weight (Sample weight after 24 hours in distilled water) and dry weight (Sample weight after 24 hours at 70° C) were measured with 0.001 g accuracy and calculated using the formula:

RWC= ((Fw-Dw)/(Tw-Dw))×100

Where: Fw: fresh weight, Dw: dry weight and Tw saturation weight.

From each plot, five bush beans were randomly selected and number of pods per plant (NPP) and number of seeds per pods (NSPP) were measured. Then the root length (RL) for five plants and weight of 100 seeds (W100S) were measured in each plots. Finally seed yield for each plot was recorded as well.

Results

Analysis of variance

Considering that the effect of place and the interaction effects of the place with other studied factors were not significant (the results were not shown), therefore, in order to better interpret the results, the average data obtained from two places was used for analysis. This study results showed that the water stress had a significant effect on all measured traits, except proline value and weight of 100 seeds (Table 3). The Zinc sulfate spray showed a significant effect on all traits except carotenoid value.

The use of gibberellin had a significant effect on all traits except ion leakage, carotenoids value, number of seeds per pod and grain yield (Table 3). The interaction effect of stress×zinc sulfate, showed a significant difference for all traits. The interaction effect of stress×gibberellin also showed a significant effect on all traits except total chlorophyll, number of seeds per pod, number of pods per plant and weight of 100 seeds. The interaction effect of gibberellin×zinc sulfate did not show a significant effect on number of seeds per pod. Finally, the three-way interaction effect of stress×zinc sulfate×gibberellin was significant on all traits except the number of seeds per pod.

The results of means comparison by Duncan's methods at 5% level for main effects of water stress, zinc sulfate and gibberellin factors are presented at (Table 4) and means comparison of some traits for inte-

Table 3. Variance analysis of mean square of studied traits.

S.O.V	df	Mean square of traits					
		LEL	Cl_A	Cl_B	Cl_T	Carotenoid	Proline
Rep	2	12.13^{ns}	0.02^{ns}	0.10^{ns}	0.25^{ns}	13.72*	278.6^{ns}
(S)	2	936.03**	28.41**	12.91**	68.64**	268.38**	2629.6^{ns}
Erorr1	4	4.81	0.3	0.23	0.82	1.67	1736.7
(Zn)	3	98.12**	12.90**	6.84**	36.38**	6.44^{ns}	8645.5**
S×Zn	6	57.47*	7.56**	1.16**	8.25**	82.58**	2951.2**
Erorr2	18	17.59	0.44	0.08	0.38	2.94	468.65
Gibberellin	1	15.40^{ns}	11.99**	4.59**	34.13**	1.31^{ns}	1319.0*
S×GA	2	32.27*	1.48**	0.52*	0.50^{ns}	26.41**	1697.7**
Zn×GA	3	75.53**	5.09**	0.45*	5.54**	48.46**	1152.5**
S×Zn×GA	6	16.19*	15.60**	1.43**	19.05**	61.46**	1621.9**
Erorr3	24	5.84	0.11	0.12	0.21	2.83	243.3
S.O.V	**df**	**Mean square of traits**					
		RWC	**RL**	**NSPP**	**NPP**	**W100S**	**Yield**
REP	2	41.25^{ns}	123.11^{ns}	0.84^{ns}	3.40^{ns}	0.59^{ns}	1856^{ns}
Stress	2	1037.61**	23.03^{ns}	5.91**	142.64*	20.21^{ns}	35627**
Erorr1	4	23.67	144.49	0.21	14.48	3.15	572
Zinc sulfate	3	1457.44**	79.49*	0.33*	46.16**	14.37**	26331**
S×Zn	6	151.1**	99.69**	0.66**	237.57**	12.01**	25542**
Erorr2	18	10.31	22.77	0.09	8.51	2.16	2401
Gibberellin	1	134.45**	0.54^{ns}	0.45^{ns}	37.56*	81.03**	1402^{ns}
S×GA	2	193.01**	28.28**	0.01^{ns}	7.26^{ns}	5.33^{ns}	12809**
Zn×GA	3	15.80**	6.22^{ns}	0.06^{ns}	82.07**	12.37*	3683^{ns}
S×Zn×GA	6	167.38**	16.15*	0.15^{ns}	19.67*	11.00**	10068**
Error4	24	RWC	4.59	0.15	5.74	2.79	1273

df*, ** and ns significant at5%, 1%   Duncan's test and no significant, respectively.

Table 4. Mean comparison for main effects of studied factors by Duncan's method at 5% level.

Stress mm evaporation from class A	LEL %		Cl_A mg/g Fw		Cl_B mg/g Fw		Cl_T mg/g.Fw		Carotenoid mg g FW^{-1}		Proline mg g FW^{-1}	
60%	25.37	c	10.45	a	6.04	b	16.49	a	18.18	c	147.23	b
90%	34.31	b	9.91	b	6.55	a	16.46	a	21.49	b	164.11	a
120%	37.39	a	8.36	c	5.10	c	13.55	b	24.86	a	144.95	b
Stress mm evaporation from class A	**RWC %**		**RL cm**		**NSPOD Seed no pod**		**NPODP Pod no plant**		**W100S g**		**Yield kg ha^{-1}**	
60%	63.68	a	26.63	b	5.83	a	29.30	a	28.78	a	209.74	a
90%	52.49	b	28.39	a	5.25	b	26.21	b	28.01	a	199.56	a
120%	52.11	b	28.25	a	4.85	c	24.49	c	26.95	b	138.51	b
Zinc sulfaml L-1	**LEL %**		**Cl_A mg/g.Fw**		**Cl_B mg/g.Fw**		**Cl_T mg/g.Fw**		**Carotenoid mg g FW^{-1}**		**Proline mg g FW^{-1}**	
Control	35.38	a	9.02	b	5.28	b	14.30	c	21.62	ab	141.78	bc
1.5	32.28	b	8.68	c	5.47	b	14.25	c	22.30	a	183.85	a
3.5	32.09	b	10.40	a	6.33	a	16.92	a	20.95	b	148.33	b
4.5	29.68	c	10.19	a	6.52	a	16.52	b	21.17	ab	134.44	c
Zinc sulfa ml L-1	**RWC %**		**RL cm**		**NSPOD Seed no pod**		**NPODP Pod no plant**		**W100S g**		**Yield kg ha^{-1}**	
Control	45.89	d	28.47	a	5.20	a	28.24	a	29.08	a	147.60	b
1.5	51.58	c	24.65	b	5.19	a	27.51	ab	28.00	ab	204.22	a
3.5	61.25	b	29.32	a	5.39	a	24.57	c	26.93	b	153.20	b
4.5	65.67	a	28.57	a	5.46	a	26.33	b	27.65	b	225.40	a
Gibberellin	**LEL %**		**Cl_A mg/g.Fw**		**Cl_B mg/g.Fw**		**Cl_T mg/g.Fw**		**Carotenoid mg g FW^{-1}**		**Proline mg g FW^{-1}**	
Yes	31.89	a	9.16	b	5.64	b	14.81	b	21.65	a	147.82	b
No	32.82	a	9.98	a	6.15	a	16.19	a	21.37	a	156.38	a
Gibberellin	**RWC %**		**RL cm**		**NSP Seed no pod**		**NPP Pod no plant**		**W100S g**		**Yield kg ha^{-1}**	
Yes	54.73	b	27.84	a	5.23	a	27.39	a	28.98	a	187.02	a
No	57.46	a	27.67	a	5.39	a	25.94	b	26.85	b	178.19	a

Means with the same letter are not significantly different from each other (P>0.05 ANOVA followed by DMRT).

ractive effects of stress×zinc sulfate×gibberellins are presented at (Table 5).

Leaf electrolytes leakage

Among the stress levels, the highest LEL was due to irrigation after 120% evaporation from the evaporation pan with an average of 37.4% and showed significant difference with other irrigation levels. Lowest LEL was for St60% with an average of 25.4%. The lowest amount of LEL was associated with applying 4.5 ml L^{-1} zinc sulfate with average of 29.7% and had a significant difference with other zinc sulfate levels. The highest LEL was related to control level (no application of zinc sulfate) with average of 35.4% that showed a significant difference with other levels. The spraying of gibberellin did not show any significant effect on LEL compared to non-spraying of gibberellin.

Mean comparison of studying treatments for LEL by Duncan's method at 5% level showed that the highest LEL was for treatment of St90 (43.2%) that did not have significant difference with other treatments of St120GA, St120Zn3.5GA, St120, St120Zn4.5, St90Zn1.5 and St120Zn4.5GA. The lowest percentage of LEL belonged to St60Zn4.5 with amount of 21.2% that did not have a significant difference with other treatments of St60Zn3.5, St60Zn1.5GA, St60Zn4.5GA, St90Zn4.5GA, St60GA and St60Zn1.5 (Table 5). The lowest leaf electrolytes leakage was observed under normal irrigation conditions.

Photosynthetic pigments

According to this study, the highest amount of Chlorophyll A in the stress levels was for irrigation after 60% evaporation from the evaporation pan with an average of 10.45 mg/g.Fw that has a significant difference with other levels of irrigation regimes. The lowest amount of ClA was for St120% with average of 8.36 mg/g. Fw. On the other hand, the highest amount of Chlorophyll B in the stress levels was for St90% with an average of 6.55 mg ml-1 that has a significant difference with other levels and the lowest amount of ClB was for St120% (5.10 mg/g.Fw). In addition, the highest amount of-Chlorophyll T in the stress levels was for St60% with an average of 16.49 mg/g.Fwand did not show significant difference with levels of St90%. The lowest amount of ClT was for St120% (13.55 mg/g.Fw). The highest amount of carotenoid in the stress levels was for St120% with an average of 24.86 mg/g.Fw and was significantly different than other levels of stress and the lowest amount of carotenoid was for St60% with average of 18.18 mg/g.Fw.

The most amount of ClA for main effect of zinc sulfate belonged to the level of Zn3.5 with average of 10.40 mg/g.Fw and did not show significant difference with level of Zn4.5. The lowest amount of ClA was for applying 1.5 ml L-1 of zinc sulfate which was also significantly different with other levels. On the other hand, the highest amount of ClB for main effect of zinc sulfate belonged to the level of 4.5 ml L-1 of zinc sul-

Table 5. Mean comparison for studied treatments.

Irrigation regime	zinc sulfate levels	Application of gibberellin	LEL (%)		Chlorophyll total mg/g.Fw		Carotenoid mg g FW^{-1}		Proline mg g FW^{-1}		Root length cm		Yield mg g FW^{-1}	
Irrigation after 60% evaporation from evaporation pan (normal condition)	0.0 ml L^{-1}	+	25.50	fg	11.74	j	14.63	ij	119.38	fgh	33.75	a	219.25	bcd
		-	33.27	cde	19.70	a	14.22	j	159.51	c-f	27.50	a-e	212.46	bcd
	1.5 ml L^{-1}	+	22.68	g	15.00	gh	19.20	fgh	133.46	d-h	22.33	de	132.38	efg
		-	25.78	fg	16.23	ef	27.20	b	172.98	bcd	23.58	cde	184.75	b-f
	3.5 ml L^{-1}	+	29.92	ef	17.91	bc	17.34	ghi	168.09	cde	28.42	a-e	200.58	b-e
		-	21.39	g	18.32	b	16.32	hij	181.69	abc	23.83	b-e	140.13	d-g
	4.5 ml L^{-1}	+	23.25	g	18.28	b	18.15	fgh	116.07	gh	26.08	a-e	218.33	bcd
		-	21.17	g	14.74	gh	18.35	fgh	126.66	e-h	27.50	a-e	370.08	a
Irrigation after 90% evaporation from evaporation pan (moderate stress)	0.0 ml L^{-1}	+	34.61	b-e	17.11	cde	23.54	cd	159.73	c-f	33.42	ab	111.38	fg
		-	43.16	a	14.61	h	24.23	c	146.52	c-h	31.17	a-d	111.00	fg
	1.5 ml L^{-1}	+	35.15	b-e	12.45	j	27.71	b	207.40	ab	23.67	b-e	364.63	a
		-	38.76	abc	15.16	gh	18.86	fgh	213.09	a	25.42	a-e	205.46	b-e
	3.5 ml L^{-1}	+	35.06	b-e	16.63	def	21.01	def	112.25	h	31.92	a-d	237.96	b
		-	32.41	de	18.05	bc	17.58	ghi	156.11	c-g	29.83	a-d	210.50	b-e
	4.5 ml L^{-1}	+	25.32	fg	17.57	bcd	18.65	fgh	162.41	cde	25.92	a-e	199.58	b-e
		-	30.02	ef	20.11	a	20.32	efg	155.41	c-g	25.75	a-e	156.00	c-g
Irrigation after 120% evaporation from evaporation pan (severe stress)	0.0 ml L^{-1}	+	37.41	a-d	10.20	k	22.38	cde	155.83	c-g	19.67	e	116.92	fg
		-	38.31	a-d	12.43	j	30.74	a	109.68	h	25.33	a-e	114.58	fg
	1.5 ml L^{-1}	+	36.43	bcd	12.37	j	17.30	ghi	163.86	cde	26.25	a-e	187.08	b-f
		-	34.88	b-e	14.31	hi	23.53	cd	212.33	a	26.67	a-e	151.04	c-g
	3.5 ml L^{-1}	+	37.60	a-d	14.86	gh	31.06	a	156.71	c-g	29.67	a-d	29.88	h
		-	36.16	bcd	15.77	fg	22.37	cde	115.12	gh	32.25	a-c	100.17	g
	4.5 ml L^{-1}	+	39.81	ab	13.61	i	28.77	ab	118.64	fgh	33.00	a-c	226.25	bc
		-	38.53	a-d	14.81	gh	22.77	cde	127.46	e-h	33.17	a-c	182.13	b-f

df*, ** and ns significant at5%, 1%   Duncan's test and no significant, respectively.

fate with average of 6.5 mg/g.Fw and no significant difference with level of Zn3.5. The lowest amount of ClB was for control level of zinc sulfate, with no significant difference with level of Zn1.5. In addition, the highest amount of ClT for main effect of zinc sulfate were obtained at the level of Zn3.5 with average of 16.9 mg/g.Fw that has a significant difference with other levels. The lowest amount of ClT was for level of Zn1.5 with no significant difference with control level. The comparison of means for carotenoid showed that the highest amount of carotenoid was due to the level of Zn1.5 with average of 22.3 mg/g.Fw that did not show significant difference with control level. The lowest amount of carotenoid was for the level of Zn3.5 and has significant different only with the level of Zn1.5.

The spraying of gibberellin significantly decreased ClA, ClB and ClT content, but the spraying of gibberellin did not show significant effect on carotenoid amount (Table 4). The comparison of means of studying treatments for ClT by Duncan's method at 5% level showed that the highest ClT obtained for treatment of St90%Zn4.5 (20.11 mg/g.Fw) and did not have a significant difference with treatment of St60%. The lowest amounts of ClT belonged to St120%GA with amount of 10.20 mg/g.Fw that has a significant difference with the other treatments.

The comparison of means of studying treatments for amount of carotenoid showed that the highest amount was for treatment of St120%Zn3.5GA with average of 31.06 mg/g.Fw and no significant difference with St120%, and St120%Zn4.5GA. The lowest amount of carotenoid belonged to St60% with amount of 14.2 mg/g.Fw and did not have a significant difference with St60%Zn3.5 and St60%GA (Table 5).

Proline

The highest amount of proline under water stress was for irrigation after 90% evaporation from the pan evaporation, with an average of 164.1 µg ml^{-1} and had a significant difference with other levels of water stress. The lowest amount of proline was for St120% with average of 144.9 µg ml^{-1} and did not show a significant difference with the level of St60%.

The highest amount of proline for levels of zinc sulfate as main effect belonged to the level of Zn1.5 with average of 183.8 µg ml^{-1} with significant difference with other levels of Zn. The lowest amount of proline was for the level of Zn4.5 that only showed significant difference with the control level.

The comparison of means amounts of proline showed that the highest amount was for treatment of St90%Zn1.5 with average of 213.09 µg ml^{-1} and did not have a significant difference with St120%Zn1.5, St90%Zn1.5GA andSt60%Zn3.5. The lowest amount of proline belonged to St120% with amount of 109.68 µg ml-1and did not have a significant difference with St90%Zn3.5GA, St90%Zn3.5, St60%Zn4.5GA, St120%Zn4.5GA, St60%GA, St60%Zn4.5, St120%Zn4.5, St60%Zn1.5GA and St90% too. In normal conditions, a moderate amount of proline content was observed as only applying of 3.5 ml L-1 of zinc sulfate caused a significant increase in proline. In moderate and severe water stress conditions, the use of 1.5 ml L^{-1} of zinc resulted in a significant increase in proline content.

Relative water content

Among the water stress levels, the highest RWC was for level of St60% with an average of 63.68% that showed significant difference with other levels and the lowest RWC was for St120% with an average of 52.11% that did not have significant difference with level of St90%.

Reviewing zinc sulfate level effect on RWC showed that the highest amount with average of 65.67% was obtained when 4.5 ml L^{-1} of zinc sulfate was used and had a significant difference with other levels. The lowest RWC was for control level (not using zinc sulfate) with average of 45.9%.

Root length

The highest length of root under water stress levels was for St90% with an average of 28.4 cm and did not show a significant difference with level of St120%. The lowest root length was for St60% with average of 26.6 cm and had a significant difference with other levels of irrigation. Therefore, root length increased at first step of increasing irrigation duration (moderate water stress) and its growth was ceased at the second step of increasing irrigation duration (severe water stress). The comparison of means of root length showed that the highest root length was for treatment of St60%GA with average of 33.7 cm and had a significant difference with St120%GA, St60%Zn1.5GA, St60%Zn1.5, St90%Zn1.5GA andSt60%Zn3.5. The lowest root length belonged to St120%GA with average of 16.9 cm.

Yield and yield components

The highest number of seeds per pod in water stress levels was for irrigation after 60% evaporation from the evaporation pan with an average of 5.8 and has a significant difference with other levels of irrigation and the lowest NSP was for St120% with average of 4.85 that has a significant difference with other levels. The highest number of pod per plant in the stress levels was for St60% with an average of 29.3 that has a significant difference with other levels of irrigation and the lowest NPP was for St120% with average of 24.5 and had a significant difference with other levels. The highest weight of 100 seeds obtained under water stress level of St60% with an average of 28.8 g and did not show a significant difference with the level of St90%. The lowest W100S was for St120% with average of 26.9 g and has a significant difference with other levels of irrigation. The highest yield in the stress levels obtained at St60% with an average of 209.7 $kg.m^2$ and did not show a significant difference with the levels of St90%. The lowest yield for seed was for St120% with average of 138.51 $kg.m^2$ and had a significant difference with other levels of irrigation.

Analysis of variance results showed that zinc sulfate, did not have a significant effect on the NSP at any applied level. The highest NPP in response to zinc sulfate obtained at control level with average of 28.2 and did not have a significant difference with the level of Zn1.5 and the lowest NPP was for the level of Zn3.5, and had a significant difference with other levels. The highest weight for 100 seeds for levels of zinc sulfate obtained at control level of zinc sulfate with average of 29.1 g and did not show significant difference with the level of Zn1.5 and the lowest W100S was for the level of Zn3.5 that did not show significant difference with the levels of Zn4.5. The highest yield in response to zinc sulfate main effect obtained at the level of Zn4.5 with average of 225.4 kg.m^2 and did not have a significant difference only with level of Zn1.5. The lowest yield was for the control level and did not show significant difference with Zn3.5.

Results of analysis of variance for yield showed that the highest seed yield was for treatment of St60%Zn4.5 with average of (370.1 kg.m^{-2}) and did not show significant difference with St90%Zn1.5GA. The lowest seed yield obtained at St120%Zn3.5GA with average of (29.88 kg.m^{-2}). Therefore, under normal conditions, using 4.5 ml L^{-1} of zinc sulfate, under moderate water stress conditions, use of 1.5 ml L^{-1} of zinc sulfate plus gibberellin and under severe water stress conditions, use of 4.5 ml L^{-1} of zinc sulfate with gibberellin, have resulted in 74.2, 71.6 and 6.5 percentage higher yield than yield obtained under normal condition, respectively.

Discussion

The significant effect of irrigation regime on the studied traits revealed the important role of water in plant growth processes, which has also been reported in several other studies (24). Zinc is an essential nutrient for normal growth in crops and plays an important role in protecting cellular components such as chlorophyll by preventing their oxidation (12). However, excessive use of zinc on plants can disturb metabolic processes such as photosynthesis and transpiration and thus decrease the plant growth due to reduced root growth and leaf chlorosis (15). Gibberellins are plant hormones with a wide range of activities including seed germination and cell elongation (25). The foliar use of gibberellins improved plant morphology and physiology (26).

It is realized that the rate of leaf electrolytes leakage increased step by step with reducing the irrigation level. The cell membrane stability under water stress is one of the most important factors in drought tolerance. Accordingly, increasing electrolytes leakage decreases the membrane stability and thus decreases drought tolerance (27). The significant increase in electrolytes leakage was observed under water stress conditions in common bean (28) which were consistent with this study results. It seems that using of zinc and gibberellin was not effective in reducing LEL under this condition and only, when were used at 4.5 ml L^{-1} zinc sulfate, the LEL increased significantly. On the other hand, in moderate stress conditions, by using of gibberellins, the amount of LEL decreased and there was no significant difference with normal conditions. In severe stress conditions, LEL was highly increased and using zinc sulfate and gibberellin did not have a reducing effect on the value of LEL. In stress condition, increasing of the reactive oxygen species is the reason of oxidative damage in many cellular components and cause of the cell membrane peroxidation and increasing electrolyte leakage (29). Using zinc sulfate results in reduction of ion leakage in common bean (30). In this study also, it was observed that with increasing use of zinc, there was a significant decrease in electrolyte leakage.

Chlorophyll content as an indicator of leaf photosynthesis is one of the criteria to assess drought tolerance of plants (31). Our results showed that chlorophyll a content decreased step by step with increasing irrigation duration, while chlorophyll B content increased in moderate stress and decreased in severe stress. In addition, the total chlorophyll content in severe stress significantly decreased compared to normal and moderate stress conditions. (32) reported that drought stress significantly decreased chlorophyll content which resulted in lower plant growth and productivity. (33) reported that high levels of chlorophyll in stress conditions increased the photosynthesis rate and, thus increased drought tolerance. Carotenoids play an important role in utilization of light and increasing plants production and enhance plants drought tolerance and the oxidative damage caused by drought stress (10). It is found in this study that with increasing stress severity, the carotenoid content significantly increased, while other studies for various plants have indicated that with increasing the severity of stress, the level of carotenoid decreases (34).

In this study it is found that with enhancing dosage of zinc sulfate, the photosynthetic pigments, except carotenoid content, significantly increased. This increasing of photosynthetic pigments content is related to functional role of zinc in activation of enzymes in the complex pathway of pigments biosynthesis and some antioxidant enzymes, e.g. glutathione reductase and ascorbate peroxidase, which prevent the reduction of pigments synthesis by active oxygen radicals (2). Therefore, we observed that the total chlorophyll content was high under normal irrigation conditions. However, it showed that different levels of gibberellin and zinc sulfate in normal irrigation conditions had a different effect on chlorophyll content, in addition, in this condition, the use of gibberellin and zinc sulfate reduced the total chlorophyll content. Conversely, under moderate stress condition, application of 4.5 ml L^{-1} zinc sulfate significantly increased the total chlorophyll content, but under severe stress, the use of zinc sulfate and gibberellin did not show a significant effect on chlorophyll content.

As the intensity of water stress increased, carotenoid levels also increased. In other words, under normal irrigation conditions, the least amount of carotenoid was observed in bean leaves and it seems that in normal conditions, the use of gibberellin and zinc sulfate were the cause of carotenoid increase. In this condition, using the low level of zinc sulfate (1.5 ml L^{-1}), caused the highest increase in carotenoid level. Under moderate water stress condition, the use of gibberellin and zinc sulfate, only when used at low level of zinc sulfate (1.5 ml L^{-1}) plus gibberellin, resulted in carotenoid increase. However, under severe stress conditions, the use of 3.5 ml L^{-1} of zinc sulfate plus gibberellin resulted in highest rate of carotenoid.

The mechanism for drought tolerance might be associated with accumulation of proline as an osmotic pressure regulator (35). In fact, increasing the rate of proline in plants, by improving the osmotic pressure regulation, can quickly increase their tolerance to drought. Therefore, it is found that under moderate water stress, the amount of proline significantly increased compared to optimum irrigation, but on the other hand, the amount of proline decreased under severe water stress. (35) showed that increase of the proline content of fababeans under water stress condition depends on the genetic potential of the studied cultivars and the proline content were higher in tolerant cultivars with increasing tension level, while in the sensitive cultivars proline content did not change as tension level increased.

Our results showed that the applying of zinc in beans at low doses increased the proline and at higher doses, proline decreased significantly. (36) reported a decrease in the amount of soybean leaf proline under different levels of irrigation by using the Zn and reported that receiving Zn via leaf in plant, reduced a large portion of glutamate that was involved in chlorophyll biosynthesis. This compound plays a role in the proline biosynthesis pathway. The spraying of gibberellin significantly decreased proline amount. Therefore, it was concluded that under different irrigation regimes, the effect of gibberellin and different levels of zinc sulfate on the proline amount of common bean was varied. These differences are due to the different interaction effects of these factors in different moisture conditions.

In conclusion, it can be stated that, under moderate and severe water stress conditions, applying of 1.5 ml L^{-1} of zinc sulfate is the cause of proline increase. The plants grown under water stress conditions, due to increasing of osmotic compounds, and decrease of water in the intracellular begin to absorb more water from the soil (37). In this study a direct correlation between soil moisture content and relative water content of leaf was monitored. By decreasing soil moisture and increasing the duration of irrigation period, the relative water content of leaves decreased and resulted in a significant reduction in amount of morphological traits, especially yield and yield components.

The zinc element plays a major role in regulation of stomatal opening, because of its important role in maintaining potassium in the stomata guard cells, and increasing the relative water content under water stress conditions (17). In this study, it was observed that by increasing application of Zn, the relative water content of the leaf showed a significant increase, which was consistent with the report by (30). Gibberellin spray (54.7%) significantly reduced RWC in comparison with non-gibberellin spraying (57.5%).

The influence of water stress is varied on physiological trait expression and this difference could be attributed to differences between the nature of the traits, as some characters are more sensitive to water stress effects than others (38).

The highest length of root for levels of zinc sulfate as main effect belonged to the level of Zn3.5 with average of 29.3 cm and has a significant difference only with level of Zn1.5. The lowest root length was for the level of Zn1.5. (39) reported that using of Zn increased root and shoot dry weight. The spraying of gibberellin did not have a significant effect on root length. In other words, the root length increased with increasing Zn, but this increase was not statistically significant. (40) reported that gibberellin did not have a significant effect on plant height and significantly decreased root length.

Based on these results, the root length does not have a highlighted change under experimental conditions. However, in normal and moderate water stress conditions gibberellin resulted in highest root length. Under severe water stress, gibberellin plus 4.5 ml L^{-1} of zinc sulfate had an important role on increasing of root length. So gibberellin is effective in increasing root length of beans, when the water stress is low or absent, however under severe water stress conditions, using zinc sulfate (4.5 ml L^{-1}) plus gibberellin would increase root length.

It is concluded that morphological traits of yield and yield components with increasing duration period of irrigation and reduction of plant available water were significantly decreased. The quality of Zn levels effect on morphological traits was varied. Zn deficiency results in biochemical and physiological changes in beans, and its application can significantly improve bean growth (39). The seed yield showed a significant increase with increasing Zn dose. The seed number per pod increased with increasing Zn value, but this increase was not statistically significant (41) stated that zinc has important role in auxin biosynthesis as a plant growth regulator (42). Increasing grain yield mostly were due to increasing of number of seeds per pod, because the number of pods per plant and the weight of 100 seeds were decreased significantly with increasing dose of Zn. (43) reported that combined application of Mn and Zn caused an increase in primary yield components through number of grains per pod, number of pods per plant, and productivity itself.

The spraying of gibberellin did not have a significant effect on the NSP and seed yield, but significantly increased NPP and W100S. Our results showed a significant increase only for 100 seeds weight and number of pods per plant by using gibberellin. On the other hand, application of gibberellin did not show a significant effect on seed number per pod and yield. (17) studied the effect of growth regulators under different moisture and salinity conditions on snap beans, and found that the effect of growth regulators was significant only for number of pods per plant and did not indicate significant effect on biomass and pod weight per plant.

In our experiment, it was observed that the agro-morphological and biochemical traits were affected by various irrigation regimes and application of gibberellin and zinc sulfate in different irrigation conditions showed a different effect on the study traits. In addition, it was found that application of 1.5 ml $L^{-1}$of zinc sulfate plus gibberellin improved bean biochemical properties. Under normal conditions, application of 4.5 ml $L^{-1}$of zinc sulfate and under severe water stress conditions, using of 4.5 ml L^{-1} of zinc sulfate plus gibberellin is recommended for obtaining the maximum performance of yield. However, in moderate stress conditions, use of 1.5 ml $L^{-1}$of zinc sulfate is recommended.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AA and AM conceived the project. FB and HS did all the experiments and analysis, overall research was supervised by AR. The manuscript was written by AA. All authors read and approved the final manuscript.

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