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Effect of Arginine and GA3 on growth, yield, mineral nutrient content and chemical constituents of Faba bean plants grown in sandy soil conditions

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Abstract: Two field experiments were carried out in successive winter seasons of 2012/2013 and 2013/2014 at the newly reclaimed sandy soil conditios Researches and Production Station of National Research Centre (NRC), Al Nubaria district, Al Behaira Governorate, Egypt. The objective was to study the effects of foliar application of arginine (200ppm or 300ppm) and Gibberellic Acid (GA3) (75ppm or150ppm)on vegetative growth, yield and yield components, as well as chemical constituents and mineral nutrients of faba bean shoots and seeds contents. Results of the combined analysis of the two seasons showed that foliar application of various concentrations of arginine or GA3 significantly improved vegetative growth of faba bean growth parameters, i.e. shoot length, root length, the fresh and dry weight of shoots and number of branches per plant as well as photosynthetic pigments contents at 75 days after sowing as compared with control treatment. Also, shoot chemical constituents i.e. total soluble sugars, total carbohydrates, polysaccharides, free amino acids, total phenol and IAA at 75 days after sowing significantly increased with foliar application treatments of arginine and GA3 as compared with control treatment. In addition the data revealed that, foliar spraying of either arginine or GA3 led to significant increases in faba bean yield and yield components as well as significant improvement of the nutritional status of faba bean plants and seeds nutrient, carbohydrates and protein content. The results showed that foliar application of arginine was more effective than GA3 in concern of growth, yield and yield components plant and seeds chemical constituents and nutritional status especially at the high concentration (300ppm arginin and 150ppm GA3).
Key words: Faba bean, Arginine, Gibberellic Acid (GA3), Sandy soil.

Introduction

Drought is one of the most serious world-wide problems for agriculture. Four-tenths of the world's agricultural land lies in arid or semi-arid regions. Other agricultural regions have consistently low rain-fall and rely on irrigation to maintain yields. In both circumstances, crop plants which can make the most efficient use of water and maintain acceptable yields will be at an advantage. Egyptian new reclaimed land is characterized as arid and semi-arid regions with poor soil nutrients and unfavorable environmental conditions (drought or high temperature). Water stress is one of the most important environmental stresses that can regulate plant growth and development, limit plant production, and alter the physiological and biochemical properties of plants. Water stress is known to increase the amount of secondary metabolites in plants. Accumulation of secondary metabolites is known as a defense mechanism of plants and plants can respond and adapt the water stress by altering their cellular metabolism to invoke various defense mechanisms[1, 2]. Drought stress often leads to the accumulation of reactive oxygen species (ROS). Excessive ROS production can cause oxidative stress to the photosynthetic apparatus seriously impair the normal function of cells[3].

Chloroplasts are the major organelles producing the reactive oxygen species (ROS) such as, the superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and singlet oxygen (O^{-1}) during photosynthesis[4]. The production of ROS can be particularly high, when plants are exposed to stress[5]. To scavenge ROS, plants posses specific mechanisms, which include activation of antioxidant enzymes[6] and non enzymatic antioxidants such as, carotenoids and ascorbic acid[7]. These compounds help the cells to maintain their dehydrated state and the structural integrity of the membranes so as to provide resistance against drought and cellular dehydration[8].

Recently great attention has been focused on natural and safety antioxidants substances, which have the ability, to free radicals and thereby form a protective screen around plant cells and hence increasing plant resistance to stress. The use of osmoprotectants as seed priming or as a foliar spray can be an economically viable strategy to enhance stress tolerance under adverse environmental conditions[9, 10]. Among these antioxidants amino acids like arginine and organic acids like pyruvic acid which considered as a source of nitrogen for plant. Amino acids are the fundamental ingredients for the process of protein synthesis. The important of amino acids came from their widely use for the biosynthesis of large variety on non protein nitrogenous materials i.e. pigments, vitamins, coenzymes, purine and pyrimidine bases[11].

Amino acids can directly or indirectly influence the physiological activities in plant growth and development such as exogenous application of amino acids have been reported to modulate the growth, yield and biochemical quality of squashe and garlic plants[12,13,14]. [15]on soybean found that treatments of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield. [16] Revealed that spraying strawberry plants with amino acids (peptone) at 0.5 and 1.0 g/L significantly increased total nitrogen, phosphorus and potassium in plant foliage as well as total yield, weight, TSS, vitamin C and total sugars content of fruits compared to control treatment.

Arginine is one of essential amino acids considered the main precursor of polyamines which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine[17]. Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and developmental processes in higher plants[18].

The application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean [19] and in wheat[20, 21]. Moreover, [22,23]recorded the positive role of arginine in alleviating the inhibition occurs as in result of exposing plants to stress.

Faba bean *(Vicia faba L.)* is one of the most important winter crops of high nutritive value in the world as well as in Egypt. Mature seeds of faba bean are good sources of protein (about 25% in dried seeds), starch, cellulose, vitamin C and minerals[24]. Cultivation of faba bean leads to increase of soil nitrogenous compounds[25] so it can be consumed for humans consumption. Faba bean are consumed as fresh faba bean pods, seeds, conservative faba bean and as a dried seeds. At the same time they are used for animal food, broken seeds are mixed into animal diet and the vegetative parts of the plants are used as the animal fodder[26]. Thus, many efforts have been consistly made to increase its productivity.

The objective of this study was to examine the effects of foliar application of arginine and GA3 on growth, some physiological responses, and yield and yield components, nutritional status and chemical constituents of faba bean plants grown under newly reclaimed sandy soil conditions.

Materials and Methods

A field experiment was carried out at the experimental Station of National Research Centre, Nubria district El-Behrea Governorate – Egypt, during the two successive winter seasons of 2012/2013 and 2013/2014. The soil of both experiments sites were Newly Reclaimed sandy soil where mechanical and chemical analysis is reported in Table (1) according to[27] seasons. The aim of this work was to investigate drought stress mitigation by foliar application of arginine and Gibberellic acid (GA3) on faba bean plant grown under newly reclaimed sandy soil. The experimental design was randomized complete block design with four replicates. Seeds of faba bean c.v Giza-3 were sown on the 15th November in both seasons in (5 ridges, each 3m in length and 0.70m in width) in hills 20 cm apart with 2 seeds per hill. The recommended agricultural practices of growing faba bean were applied and the pre-sowing, 150 kg/fed. of calcium super-phosphate (15.5 % P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 75 Kg/fed. was applied at five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52 % K_2SO_4) was added at two equal doses of 50 kg/fed. before the first and third irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Faba bean plants were foliar sprayed with arginine and GA3 at the rate of 200 and 300 ppm and75 and 150 ppm for each, respectively. In both seasons, foliar application of arginine and GA3 was carried out twice; after 45 and 60 days from sowing. Skipping the irrigation at 45 and 60 days after sowing (DAS) after sprayed (arginine and GA3). Plant samples were taken after 75 days from sowing for measurements growth characters were measured in terms of, plant height shoots fresh and dry weight, roots length, root fresh and dry weight. Plant samples were dried in an electric oven with drift fan at 70°C for 48 hr. till constant dry weight. Plant samples were taken for chemical analysis after 75 days from sowing for measurements of total soluble sugars, total carbohydrates, free amino acids, total IAA, total phenol content.

Table (1): Mechanical and chemical analyses of the experimental soil.

Properties / Year	2012/2013		2013/2014	
	30 cm depth	**60 cm depth**	**30 cm depth**	**60 cm depth**
Mechanical analysis				
Sand (%)	91.20	93.70	92.33	93.12
Silt (%)	3.70	3.90	2.95	3.12
Clay (%)	5.10	3.40	4.78	3.76
Soil texture	Sandy	Sandy	Sandy	Sandy
Chemical analysis				
PH	7.40	7.80	7.50	7.69
E.C. m mohs/cm2	0.30	0.50	0.50	0.70
CaCO3 (%)	1.40	1.00	1.65	0.95
Organic matter %	0.30	0.21	0.24	0.22
Soluble N ppm	8.10	9.20	7.45	8.25
Available P ppm	3.20	3.60	4.12	4.65
Exchangable K ppm	20.00	23.50	26.00	24.55

Chemical analysis:

Photosynthetic pigments:

Total chlorophyll a and b and carotenoids contents in fresh leaves were estimated using the method of[28]. The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophyll a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/100g FW.

Total soluble sugars (TSS):

Total soluble carbohydrates (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates[29]. TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol SpectrocololourimeterVEB Carl Zeiss[30].

Total carbohydrate:

Determination of total carbohydrates was carried out according to [31]. A known mass (0.2-0.5 g) of dried tissue was placed in a test tube, and then 10 ml of sulphuric acid (1N) was added. The tube was sealed and placed overnight in an oven at 100ºC. The solution was then filtered into a measuring flask (100ml) and completed to the mark with distilled water. The total sugars were determined Colorimeterically according to the method of[32] as follows: An aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5% aqueous phenol solution followed by 5 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed in a water bath at 23-30ºC for 20 minutes. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Indole acetic acid

A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0OC. The combined extracts were collected and made up to a known volume with cold methanol. Then take 1ml of the methanolic extract and 4ml of PDAB reagent (para-dimethylamino benzoic acid 1g dissolve in 50 ml HCl, 50 ml of ethanol 95%) and left for 60 min in 30-400C. The developing colour was spectophotometrically measured at wave length of 530 nm. As described by Larsen P et al [33].

Total phenol contents:

The same extract of IAA were used for phenol content, 0.5 ml of the extraction was added to 0.5 ml Folin, shaked and allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by[34].

Free amino acids:

Free amino acid content was extracted according to the method described by[35]. Free amino acid was determined with the ninhydrin reagent method[36]. 1 ml acetate buffer (pH 5.4) and 1 ml chromogenic agent were added to 1 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocolololourimeter VEB Carl Zeiss.

Results and Discussion

Vegetative growth and photosynthetic pigments:

As shown in table (2) the results of average of two seasons revealed that the foliar application of various concentrations of arginine (200 and300ppm) or GA3 (75and 150ppm) induced significant increases of faba bean studied parameters, i.e. shoot length, root length, the fresh and dry weight of shoots and number of branches per plant at 75 days after sowing as compared with control treatment as an average of the growing seasons. It is clear from the present results that increasing concentration of either arginine or GA3 led to increases in growth characteristics and the highest values of growth parameters were attained from arginine foliar spray treatment at 300ppm as well as arginine was more effective than GA3. These results are in harmony with the finding and illustrated by [19, 37] on bean and [38]on wheat, they reported that the application of arginine significantly promoted the growth and increased the fresh and dry weight. Also, [39,19]concluded that exogenous application of polyamine (end product of arginine) promote cell division, cell differentiation and general growth promotion and also help to stabilize membrane and wall properties[40] and protect plant against environmental stress[41]. Moreover, the significant increase in growth parameters of sorghum plants due to foliar application of arginine at the rate of 200 or 300 ppm were recorded by [42].

The results in same table also showed that, spraying faba bean plants with arginine (200 and300ppm) or GA3 (75 and 150 ppm) increased foliar photosynthetic pigments contents as compared with control plants and there were gradually increase with increasing arginine or GA3 concentration. Similar promoting effects of arginine and GA3 on plant photosynthetic peigments content had been observed by [19] on bean and [38]on wheat.

Table (2): Effect of foliar application with Arginine and GA3 on growth and photosynthetic pigments content of faba bean plants at 75 days after sowing (Average of two seasons).

Treatments	Shoot length (Cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Number of branches/ plant	Chlor ophyll (a)	Chlor ophyll (b)	Carote noids	Total pigment s
						mg /g Fresh weight			
Control	72	7.30	19.80	2.70	4.0	1.253	0.503	0.202	1.991
Arginine (200ppm)	85	8.00	28.90	4.60	5.0	1.499	0.603	0.329	2.431
Arginine (300ppm)	97	10.50	32.00	6.60	8.0	1.980	0.753	0.367	3.100
GA3 (75 ppm)	80	9.00	25.80	3.80	5.0	1.286	0.544	0.305	2.102
GA3 (150 ppm)	84	9.80	30.90	5.20	7.0	1.456	0.574	0.312	2.342
LSD5%	3.62	0.62	1.03	0.21	1.86	0.476	0.010	0.020	0.10

Shoot chemical constituents at 75 days after sowing:

Data in Table (3) clear that arginine or GA3 foliar spray at both concentrations increased significantly shoot chemical constituents i.e. total soluble sugars, total carbohydrates, polysaccharides , free amino acids, total phenol and IAA at 75 days after sowing as compared with control treatment. The obtained results indicate superiority and preference of arginine in increasing the above mentioned chemical compounds compared with GA3 treatment under the conditions of the experiment. The highest values of different chemical constitute of shoot plant at 75 days after sowing were obtained from arginine foliar spray at 300ppm. The results in the same table clear that the above mentioned chemical compounds determined of faba bean shoot increased gradually with increasing arginine or GA3 levels as compared with those unsprayed plants. The increment in the IAA as an endogenous growth promoting hormone in response to arginine and GA3 treatments might be attributed to their effects on enhacing the biosynthesis of the endogenous growth promoters and / or decreasing their inactivation. These results could be supported by the results obtained by[20,38,22]who indicated that arginine was the most effective compound in increasing soluble carbohydrate, polysaccharides, total carbohydrates, total amino acid and protein contents of wheat plants and grains under normal or stressed condition.

Table (3): Effect of Arginine and GA3 on shoot chemical constituents at 75 days after sowing (average of two seasons)

Treatment	Total Soluble Sugars	Total Carbohydrates	polysaccharides	Free amino acids	Total phenol	IAA (µg /100g fw)
	%			(mg / 100gdw)		
Control	3.88	21.49	17.61	62.40	111.30	18.30
Arginine (200ppm)	5.64	33.50	27.86	205.10	159.00	45.30
Arginine (300ppm)	7.00	35.48	28.48	230.30	180.30	66.50
GA3 (75 ppm)	4.38	25.07	20.69	167.80	148.50	40.20
GA3 (150 ppm)	5.01	27.81	22.80	186.40	177.60	55.70
LSD5%	0.35	0.65	0.69	1.12	7.09	0.38

The increase in the different growth promoters could be occurred through retarding the biosynthesis of hormone degradative enzymes and/or repressing their activities or through preventing the transformation of these active substances into inactive forms. These results agreed, in part, with those obtained by [43] who demonstrated that, putrasein (arginine forming substance) increased the auxin content in treated plants due to retarding the destruction of endogenous auxins through decreasing the activity of IAA–oxidase of wheat and mung bean plants respectively.

Yield and its components

Data in (Table 4) showed the Plant height at harvest, mean pod weight, mean seed Weigh/ pod,100-seed weight, seeds, straw and biological yield per /feddan as a mean average of were significantly increased by foliar spraying plants with arginine or GA3 concentrations while, Pod length and number of seeds/pod were not significantly affected. On the other hand arginine or GA3 at low concentrations had no significant effect on plant height, Mean seed weigh /pod and 100-seed weight, respectively. The highest values of seeds and straw yields and the parameters of yield components were attained from arginine foliar spray at 300 ppm. The positive action of these substances foliar application (arginine and GA3) on faba bean yields and yield attributes studied mainly attributed to these substances play an important role in the multi-biological processes in plant through enhancement of foliar chlorophyll contents and plant vegetative growth (Table 2). These increments of seed yield and yield components could be due to antisenescence effect of arginine.[21] proved that, putrescine (arginine forming substance) is intimately involved in salt treated wheat plant thereby regulating growth, development and grain yield. Moreover, [19]concluded that, arginine induce early flowering and fruiting of bean plants.

Table (4): Effect of Arginine and GA3 on seed yield and yield components (average of two seasons)

Treatment	Plant height (cm)	Pod length (cm)	Mean pod weigh (g)	Mean no. of seeds/pod	Mean seed Weigh /pod (g)	100-seed weight (g)	Seed yield (ton/fad.)	Straw yield (ton/ fed.)	Biological yield (ton/ fed.)
Control	87.33	8.33	3.21	3.33	3.11	67.01	1.21	1.80	3.16
Arginine (200ppm)	94.33	9.00	3.85	3.67	3.86	69.70	1.73	2.04	3.96
Arginine (300ppm)	103.67	9.67	4.10	3.67	4.13	71.63	2.22	2.95	4.92
GA3 (75 ppm)	90.67	8.67	3.60	3.33	3.55	67.67	1.40	1.99	3.61
GA3 (150 ppm)	96.67	9.33	4.03	3.67	3.97	70.03	1.90	2.70	4.21
LSD5%	8.18	NS	0.25	NS	0.55	1.64	0.11	0.12	0.37

Fed.* = $4200m^2$

Shoot mineral nutrients content, at 75 days after sowing

N, P, K, Mg, Fe, Zn and Mn contents in faba bean shoot at 75 days after sowing as an average of the two growing seasons (Table 5) were significantly increased under different concentration of both arginine and GA3 compared with control plants, except only Mn content at 75 ppm GA3 treatment. Data also in the same Table clearly indicated that, macro and micronutrients concentration were gradually increased with increasing arginine and GA3 levels and the highest N, P, K, Mg, Fe, Zn and Mn contents in faba bean shoot were gained from arginine foliar spray at 300 ppm. These results are in good harmony with those obtained by[44] they reported that foliar application of putrescine (one product of arginine) enhance the uptake of K, Ca and Mg by chick pea plants. Also, [45] reported that external application of arginine increased the uptake of N, P K in leaves of mung bean plants.

Table (5): Effect of Arginine and GA3 on shoot mineral nutrients content, at 75 days after sowing (average of two seasons).

Treatment	N	P	K	Mg	Fe	Zn	Mn
	%				mg/kg		
Control	0.89	0.20	0.63	0.42	75.33	17.73	9.53
Arginine (200ppm)	0.97	0.26	1.55	0.87	105.33	32.80	11.47
Arginine (300ppm)	1.00	0.31	1.85	0.96	191.33	44.10	12.40
GA3 (75 ppm)	0.94	0.24	0.83	0.63	87.00	20.43	9.90
GA3 (150 ppm)	0.96	0.26	0.93	0.73	167.33	38.70	11.60
LSD5%	0.03	0.01	0.06	0.04	8.30	1.99	0.73

Chemical analysis of yielded seeds:

Data in (Table 6) clear that, the foliar spray of faba bean plants with either arginine or GA3 at different concentrations increased significantly N, P, K, Mg, Fe, Zn, Mn, Carbohydrates and total crude protein contents in faba bean seeds as an average of the two growing seasons as compared with the control treatment. Data also in the same Table show that GA3 foliar spray at 75ppm had no significant effect on seed Fe, Zn, Mn and Carbohydrates contents. The highest values of seeds mineral nutrients, Carbohydrates and total crude protein contents were gained by foliar spray with arginine at 300 ppm. These results could be supported by the results obtained by[20,38,22] who indicated that arginine was the most effective compound in increasing soluble carbohydrate, polysaccharides, total carbohydrates, proline, total amino acid and protein contents of wheat plants and grains under normal or stressed condition. Moreover,[45] reported that external application of arginine increased the uptake of N, K, Ca, Mg and P in mung bean seeds.

Table (6): Effect of Arginine and GA3 on seed nutrients, carbohydrates and protein contents of the yielded seed (average of the two seasons).

Treatment	N	P	K	Mg	Fe	Zn	Mn	Seed T. Carbohydrates	Seed T. crude protein
	%				mg / kg			%	
Control	3.31	0.42	0.93	0.28	66.33	56.23	15.07	54.70	22.66
Arginine (200ppml)	3.70	0.49	1.29	0.42	143.33	66.40	16.67	58.90	24.27
Arginine (300ppm)	4.15	0.51	1.39	0.56	189.67	81.70	32.03	63.00	26.84
GA3 (75 ppm)	3.41	0.46	1.03	0.30	72.67	58.60	16.17	56.40	23.60
GA3 (150 ppm)	3.47	0.47	1.26	0.37	168.33	70.43	17.20	61.30	25.00
LSD5%	0.02	0.02	0.06	0.02	6.62	3.79	1.58	2.42	0.93

Conclusion

As compared with control treatment the obtained results indicated that foliar spraying of Arginine (200 and 300 ppm) or GA3 (75 and 150 ppm) had positive significant effects on growth, yield and yield components of faba bean plants grown under newly reclaimed sandy soil conditions. Also, foliar treatments significantly improved nutritional status of faba bean plants and seeds carbohydrates, protein and nutrients content, as well as photosynthetic pigments and chemical constituents contents of faba bean plants. Moreover, the obtained results indicated to superiority and preference of arginine than GA3 foliar spraying.

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