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Impact of cysteine or proline on growth, some biochemical attributes and yield of faba bean

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Abstract : Two field experiments were carried out during two successive seasons (2013-2014, 2014-2015) to study the effect of two amino acids (L-cysteine or proline) as priming treatment on faba bean (*Vicia faba* cv. Sakha 4) plants at the rate of 20 and 40mgL^{-1}. The results indicated that all treatments of the applied amino acids induced significant increments regarding plant height, number of branches and leaves/plants as well as fresh and dry weight of stems and leaves. The used concentrations of L-cysteine or proline increased significantly all photosynthetic pigments (Ch1 a, b and carotenoids) content of leaves as well as N, P, K in the shoots as compared with untreated plants. Priming application with L-cysteine or proline showed significant increase of number of yielded pods/plant, weight of pods/ plant, weight of seeds/ plant as well as seeds yield/feddan. Treatment with 20 or 40 mg L^{-1}proline showed the highest level followed by L-cysteine at the rate of 20 mgL^{-1}. In addition, total carbohydrate, protein, P and K contents of dry seeds were significantly increased as compared to control plants. The ratio of essential amino acids (EAA)/non-essential amino acids (NEAA) was increased in yielded seeds of treated plants. Moreover, arginine showed the highest level of amino acids.

Key Word: Faba bean, seed priming, proline, cysteine.

Introduction:

Faba bean (*Vicia faba*) is one of the popular legume foods in Egypt with high yield capacity. The seeds have about 30% protein of their dry weight which contains most of the necessary amino acids for human nutrition.

Amino acids are fundamental ingredients in protein biosynthetic process and nearly twenty amino acids are involved in this biosynthetic process. Studies have been shown that amino acids can involve directly or indirectly in many plant's physiological activities such as protein biosynthesis.

Cysteine was documented that it is the primary organic compound contains reduced sulfur build by the plant[1]. Cysteine acts not only as amino acid in synthesis of protein but also as a precursor for some important biochemicals. It is an abundant and ubiquitous thiol group, this group of (Cys) furnishes disulfide bridges which are essential for structure and folding of protein and their function and satiability[2]. Another role of the thiol groups of cysteins, they often located to the active sites and act as a modulator to enzymatic reactions[3]. In addition, cysteine is the source molecule for important metabolites containing sulfur that are necessary for development such as the amino acid methionine, vitamins and cofactors[4]. The most important product of

cysteine is the glutathione (GSH) which has crucial function in cell defense and protection, principal antioxidant of cell, involved in active oxygen species (AOS) detoxification, protect protein against denaturation during stress[5].

Proline is an important amino acid plays different roles in plant. In addition, to its role in protein synthesis, it can protect plant from environmental stresses as well as singlet oxygen and free radical[6]. It acts as an intercellular structure[7] and conserves enzymes as well as membranes[8]. Moreover, it promotes the stability of some enzymes[9]. Accumulation of proline was also found in non-stressed condition as this amino acid provide energy for production need program of plant and plays role in flowering and development as a metabolite and also a signal molecule[10].

The present investigation was undertaken to study the effect of the two amino acids (L-cysteine or proline) as they are source of sulfur and /or nitrogen on growth yield and some biochemical constituents of faba bean grown in sandy soil.

Materials and Methods

Two field experiments were conducted at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt during the two growing seasons of 2013/2014 and 2014/2015 to study the physiological response of faba bean (*Vicia faba* cv. Skha 4) to priming application with different concentrations of L-cysteine (20, 40 mgL^{-1}) and proline (20, 40 mgL^{-1}). Seeds of faba bean were secured from Legume Research Department, Field Crop Institute, Agricultural Research Center, Giza, Egypt. The experiments were carried out under sandy soil conditions. The physical and chemical proprieties of the soil are presented in Table (1)

Table (1): Soil mechanical and chemical analysis characters.

Character	Value	Character	Value
Sand %	88	K	10.18
Silt %	4	Ca mg/100g	92.0
Clay %	7.2	Mg	18.4
Texture	Sandy	Na	12.36
pH (1: 2.5 water)	8.83	Fe	8.92
E.C(mmhos/cm)(1:2.5)	0.12	Mn mg/kg	8.34
CaCO3 %	4.8	Zn	0.13
O.M %	0.24	Cu	0.10
P	0.22		

Experimental Procedure:

Faba bean seeds were primed with L cysteine and proline for 12 hrs. While, the untreated seeds were soaked with water. Faba bean seeds were sown on 3rd December in the two seasons. The experiment was made in randomized complete block design with three replicates. The experimental land is divided into five plots, each contained one treatment. The plot was ridged, four meters long, 50 cm apart, and hills were spaced at 20 cm distance, and three seeds were sown in each hill. The plants were thinned to one plant per hill at 21 DAS. Soil preparation, fertilizer application and cultural operations followed the normal practices of faba bean cultivation in the vicinity.

Data recorded:

A random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment to study the morphological characters at 75 days after sowing.

1. *I - Morphological Characters:*
2. Shoot height (cm).
3. Number of leaves and branches / plant.

4. Stem fresh and dry weight / plant (g).
5. Leaves fresh and dry weight / plant (g).

Moreover, photosynthetic pigments (chlorophyll a, b and caortenoids) were determined in fresh leaves. Whereas, mineral contents (nitrogen, phosphorus and potassium) were determined in dried leaves.

II -Yield Characters:

A random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment at harvest time to determine; number of pods per plant, weight of pods and seeds per plant (g) and Seed yield/fed (Kg).

Moreover, carbohydrate, protein, P and K were determined in the yielded seeds.

III-Biochemical Studies:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the fresh leaves at 75 days from sowing were determined as the method described by Moran [11]. Nitrogen, phosphorus and potassium were determined in the leaves on the basis of dry weight according to Chapmanand Pratt [12]. Nitrogen content of the yielded seeds was determined and multiplied by 6.25 to calculate the crude protein content [13]. Total carbohydrates were determined using the colorimetric method described by Dubois *et al.*[14].

Statistical Analysis:

Data on morphological and yield characters as well as on seed quality were subjected to conventional methods of analysis of variance according to Snedecor and Cochran [15].The least significant difference (L.S.D.) for each was calculated at 0.05 level of probability.

Results and Discussion:

Vegetative growth:

Data presented in (Table 2) showed that, priming seeds with the used concentration of L-cysteine or proline results in significant increase of growth criteria represented by shoot height, number of leaves and branches/plant as well as fresh weight and dry weight of stems and leaves/plant compared to the corresponding controls. Maximum increase was obtained in response to 20 mg L^{-1} cysteine or 20 and 40mgL^{-1}proline. The results coincide with other investigators who revealed that amino acid treatments induce increase of vegetative criteria for different crops as cucumber, wheat, onion, snap beans and sunflower[16-20]. Similar finding were reported in response to L cysteine application on other plants ([21] on *Lupinus termis* L. and [22] on *Alluim cepa*). With respect to proline application, [23] showed that foliar spray of this amino acid enhanced some criteria of vegetative growth as plant height, number of leaves and shoot dry weight of *Vicia faba*.

Table (2): Effect of cysteine or proline on growth characters of faba bean after75 days form sowing (combined analysis of two seasons)

Treatments		Shoot height(cm)	No. of leaves /plant	No. of branches/plant	Fresh weight of stem /plant (g)	Fresh weight of leaves/plant(g)	Dry weight of stem /plant(g)	Dry weight of leaves /plant(g)
Control	0	29.83	14.67	1.00	24.22	31.12	5.16	7.56
L-Cysteine	20	40.50	27.17	3.00	61.78	62.50	8.64	10.50
	40	38.17	19.67	2.00	45.46	49.27	9.79	8.77
Proline	20	40.40	22.67	2.17	55.23	53.37	10.32	10.98
	40	42.50	17.00	1.00	44.12	54.70	10.22	11.79
L.S.D at 5%		5.14	0.246	2.150	4.690	3.996	1.23	2.952

Table (3): Effect of cysteine or proline on photosynthetic pigments in fresh leaves and macroelement (NPK) in dried leaves of faba bean after75 days form sowing (combined analysis of two seasons)

Treatments		Chl. a (mg/gFw)	Chl. b (mg/gFw)	Car. (mg/gFw)	Chla+b (mg/gFw)	Chl. a+b/c (mg/gFw)	Total pigments (mg/gFw)	Microelements		
								N%	P%	K%
Control	0	0.665	0.309	0.190	0.974	5.126	1.164	2.880	0.263	2.047
L-Cysteine	20	0.817	0.352	0.248	1.169	4.714	1.417	3.007	0.300	2.367
	40	0.740	0.348	0.226	1.088	4.814	1.314	3.210	0.417	2.490
Proline	20	0.946	0.376	0.235	1.322	5.626	1.557	3.107	0.288	2.230
	40	0.866	0.327	0.290	1.193	4.114	1.483	3.500	0.320	2.310
L.S.D at 5%		0.103	0.060	0.001				0.060	0.002	0.060

Photosynthetic pigments:

Priming treatment of L-cysteine or proline on bean plants induced significant increase in chlorophyll (a), and chlorophyll (b) (Table 3). The results obtained by proline matched the findings of [23] on faba bean and [20]on sunflower. The high level of chlorophylls by proline application may be due to the role of proline in protection chloroplast from oxidation damage [6].This amino acid has an important role in protecting enzymes as well as membranes [8] or elevating the level of some ions asMg^{+2} which needed for chlorophyll synthesis[24].

Similar results were recorded by using L-cysteine [21]on *Lupinus termis* L. and [22]on *Allium cepa*). Regarding the role of L-cysteine in increasing photosynthetic pigments could be arise from the fact that the chloroplast is the main source of sulfide *via* sulfate reduction in the sulfur assimilation pathway[1].

Carotenoids content showed significant increases due to faba bean seed priming with L-cysteine or proline (Table2). It is well recorded that carotenoids have vital importance as they act as photoreceptors pigments [25] and as modulator for eliminating singlet oxygen [26].

Minerals content:

Application of L-cysteine or proline induced significant increase of N, P, and K contents of faba leaves (Table 3). with respect to N content, the magnitude of increase was more pronounced by applying proline at the rate of 40 mgL^{-1} followed by 40 mgL^{-1} L-cysteine whereas, seed priming with 40 mgL^{-1} L-cysteine produced the highest significant content of P or K of faba bean leaves. [22] reported significant increases in total nitrogen, and amino acids in onion plants treated with L-cysteine or methionine. L-cysteine is a source of nitrogen and sulfur major macronutrients essential for plant growth and development, moreover, L-cysteine is a reduced sulfur donor molecule involved in the synthetic of essential biomolecules and defense compound such as glutathione[27]. The positive effect of proline might be due to its action as an important source of nitrogen and their role in protecting metabolites from oxidation[8,28]. Similar enhancement of N, P, K content was reported in response to proline application on faba beans[23].

Yield and yield components:

Seed soaking with L-cysteine or proline significantly increased yield components per plant as well as yield of seeds per feddan of faba bean as compared with untreated plants (Table 4). Treatment with 20 or 40 mgL^{-1} proline produced the highest significant values of weight of pods per plant, weight of seeds per plant and yield of seeds per feddan followed by 20 mgL^{-1} L-cysteine. Whereas, application of the higher concentration of L-cysteine (40 mgL^{-1}) showed the lowest significant increase of all yield parameters.

The improvement of faba bean yield by applying L-cysteine or proline could be a reflection of vegetative growth, photosynthetic pigment and minerals which in turn lead to an increase of photosynthetic assimilates production that translocated to the reproduction organs during maturation and ripening stages of the pods and seeds. This effect might be due to the role of L-cysteine as a source of nitrogen and sulfur and it has shown to be the main factor limiting glutathione production which has essential roles in the storage and transport of reduced sulfur as a powerful cellular reactant[5,29]. With respect to proline, it has been established that this amino acid can feed energy to support plant metabolism of production process in plants [30]. Moreover,

this amino acid can regulate some needs of cell plant such as acceleration of organ growth, meristem stimulation and cell division[10].

Seed quality:

Carbohydrate content of dry produced seeds was increased significantly by application of all used concentrations of L-cysteine or proline (Table4).The highest increments were induced by either40 mgL^{-1}cysteine or 40 mgL^{-1}proline followed by 20 mgL^{-1}proline. Protein content showed also significant increase with regarding to all treatments of L-cysteine or proline. Maximum increase was induced by 40 mgL^{-1} cysteine or 40mgL^{-1}proline followed by 20mgL^{-1} cysteine. In this connection several reports indicated that application of amino acids increased carbohydrate and protein content of other plants [21]on *lupinns termis* L. and [17]on wheat.

The contents of P or K of faba bean seeds significantly increased by L-cysteine or proline applications (Table 4). Treatment with 40mgL^{-1}cysteine or proline showed the pronounced increase. This can be attributed to the high levels of P and K in treated faba bean leaves which may lead to the translocation of these elements from leaves to the storage organ (seeds).

Table (4): Effect of cysteine or proline on yield components and carbohydrate, protein, P, K content of yielded seeds of faba bean (combined analysis of two seasons)

Treatments		No. of pods /plant	Wt of pods / plants (g)	Wt of seeds / plant(g)	Seed yield/ fadan (kg)	Carbohydrate %	Protein %	P%	K%
Control	0	6.40	16.71	12.96	362.9	40.62	28.28	0.130	3.566
L-Cysteine	20	11.47	29.02	21.81	610.80	42.57	31.22	0.166	3.818
	40	8.13	21.01	17.22	482.10	43.63	33.38	0.191	3.977
Proline	20	10.o7	31.30	24.09	674.4	43.29	31.83	0.152	3.671
	40	11.40	32.51	25.11	703.0	44.21	33.99	0.173	3.878
L.S.D at 5%		1.05	1.41	1.93	54.4	0.619	0.253	0.02	0158

Table (5): Effect of cysteine or proline on amino acids contents of faba bean yield seeds

Treatments	Control	Cysteine		Proline	
	0mg/L^{-1}	20 mg/L^{-1}	40 mg/L^{-1}	20 mg/L^{-1}	40 mg/L^{-1}
Amino acids Mg/g dw	Essential amino acids				
Threonine	2.78	5.87	7.85	5.79	7.65
Valine	2.98	7.68	9.85	8.49	9.68
Methionine	0.47	1.02	1.41	0.98	0.75
Leucine	4.98	6.98	8.01	6.35	8.35
Isoleucine	3.78	5.85	6.25	4.68	6.25
Phenyalanine	4.97	4.36	5.68	5.78	4.68
Histidine	3.78	4.98	6.78	5.75	6.68
Lysine	4.98	6.24	6.98	6.75	5/64
Total	37.97	59.33	72.39	60.92	67.93
	Non-Essential amino acids				
Agrginine	9.25	16.35	19.58	16.35	18.35
Proline	5.64	10.35	12.75	13.36	12.65
Aspartic	8.41	10.35	13.68	10.87	16.35
Serine	4.52	5.68	7,25	6.58	8,75
Glutamic Acid	16.87	17.35	19.25	19.25	18.35
Glycine	5.68	5.97	6.87	6.98	7.58
Alanine	5.67	7.35	8.45	7.25	8.34
Tyrosine	3.24	6.02	7.68	6.35	7.68
Cysteine	0.98	3.58	2.41	1.57	1.12
Total	64.26	77.97	88.99	83.56	91.5
Total Essential and non-essential	102.23	137.3	161.38	144.48	159.43
Ess. AA/Non. ESSAA	0.590	0.761	0.813	0.729	0.742
NH_4	13.25	11.32	10.65	11.35	10.68

Amino acid composition of seeds:

Amino acids composition is an important feature in determining the nutritional value of faba bean seeds. Eighteen amino acids were detected including essential amino acids (EAA) i.e threonine, valine, methionine, leucine, isoleucine, phenyl alanine, histidine, lysine, arginine and non-essential amino acids (NEAA), i.e Aspartic acid, serine, glutamic, proline glycine, cysteine, alanine, tyrosine (Table 5). Arginine showed the highest level of (NEAA) followed by glutamic acid, while, valine followed by leucine were the highest level as NEAA. These results were previously recorded by [31]. Lysine is very important national attribute in faba bean seeds[32]. Arginine and lysine amino acids showed the highest level of amino acids in faba bean [33]. These increased contents of different amino acids in response to amino acid treatment are in accordance with those of [34] and [35], they found that arginine treatment increases different amino acid constituents of wheat grains and hot pepper fruits respectively. Also, [36] treated wheat plant with glutamic acid and found that different amino acids of wheat grains increased.

In conclusion, this study can show the promotive effect of the two amino acids (L-cysteine, proline) on growth, photosynthetic pigments, minerals content as well as quantity and quality of the yield of fababean. Proline at the rate of 40mgL^{-1} showed the superior effect.

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