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Improving Growth and Productivity of Faba Bean Plants by Foliar Application of Thiourea and Aspartic Acid

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Authors' contributions

This work was carried out in collaboration among the authors. All authors shared designing and writing the protocol. Authors designed the study, performed the statistical analysis. Authors AAA and HFA wrote the first draft of the manuscript. Authors EMR and AEFG managed the analyses of the study and managed the literature reviews. All authors read and approved the final manuscript.

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ABSTRACT

Two field experiments were carried out at the Research and Production Station of the National Research Centre, Noubaria, Egypt, during the winter season of 2010/2011 and 2011/2012. This study aimed to investigate the response of vegetative growth, yield and some metabolic constituents of field bean (*Vicia faba* L. var minor) cv. Giza402 to foliar application of bio-regulator Thiourea (TU; 250, 500 or 1000 mg L^{-1}), and amino acid Aspartic acid (AspA; 50, 100 or 150 mg L^{-1}) either alone or in combination. Foliar application of TU and Asp A either alone or in combination significantly increased the values of number of branches, leaves and pods/plant, total dry weight/plant, leaf area index, specific leaf weight and crop growth rate criteria. Total photosynthetic pigments content in leaves were increased by increasing TU (up to 1000 mg L^{-1}) and Asp A (upto150 mg L^{-1}) singly or in combination. Straw, seed and biological yields of faba bean plants were significantly increased by increasing TU and Asp A concentrations up to 1000 and 150 mgL^{-1}, respectively. Application of 1000 mgL^{-1} TU, singly and combined with Asp

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A at 150 mg L^{-1} caused significant increments in harvest index (2.9 and 25.5%) and seed yield ha^{-1} (46.4 and 101.9%), respectively, compared to their control plants. TU and Asp A, when applied alone, significantly improved the nutritional value and quality of faba bean seeds by increasing total carbohydrates, total crude protein, phosphorus, potassium, and calcium contents. In conclusion, foliar application of TU at 1000 mg L^{-1} and Asp A at 150 mg L^{-1}, singly or combined could be recommended to improve the yield, nutritional value and quality of faba bean seeds.

Keywords: Vicia faba; thiourea; aspartic acid; growth; photosynthetic pigments; yield; biochemical constituents.

1. INTRODUCTION

Faba bean (*Vicia faba* L.) Family (Fabaceae) is consumed world wide as an important source of plant protein for humans and animals [1]. Faba bean seeds contain 51-68% total carbohydrates [2], 28-30% protein [3] based on dry matter, which is considerable among vegetables. Protein quality is affected by essential amino acid composition, amino acid imbalance, digestibility and biological availability of the amino acids, and by the anti-nutritional activity of some components of the seeds [4]. In Saudi Arabia, Alghamdi [5] found that the seeds of faba bean (Giza-402 cultivar) contain 31.9% total crude protein consist of 18 amino acids, but deficient in sulphur containing amino acids (methionine and cystine). Thiourea (TU, CH_4N_2S) has been identified as an effective bioregulator involved in the phloem transport of sucrose and in the substrate binding site of the amino acid carrier [6]. TU enhances the formation of the ternary complex, sucrose H+ carrier, thus improving translocation of photosynthates while increasing the photosynthetically active leaf surface during grain filling in cereals and stimulating dark fixation of CO_2 in chickpea (*Cicerarietinum*) embryos [7]. Amino acids are not only building blocks of proteins but also participate in many metabolic networks that control growth and adaptation to the environment. They are important in many biological molecules, such as forming parts of coenzymes, or as precursors for the biosynthesis of molecules such as glutamine and Ornithine, which are precursors for nucleotides and polyamines, respectively [8]. Amino acids also serve as major transport molecules of nitrogen from vegetative to reproductive tissues. In young plants, amino acid biosynthesis is regulated by the metabolism of glutamine, glutamate, aspartate and as paragine which is then converted into all other amino acids by various biochemical processes [9]. Asparagines and glutamine are major amino acids transported from leaf canopy to developing seeds, then converted into all other amino acids including lysine and threonine and the free amino acids are subsequently incorporated into seed proteins [10]. Enriching crop plants in amino acids to improve their nutritional value has both economical and humanitarian interest [11]. In addition, amino acids play an important role in plant “stress” resistance; glutamate, cysteine, and glycine are involved in biosynthesis of glutathione [12].

As paragines and glutamine are highly efficient in promoting growth, yield and its components in faba bean [13] and snap bean (*Phaseolus vulgaris* L.) [14]. The potent impact of TU and aspartic acid (AspA) on various areas of plant structure and function has prompted many investigators to apply them to several crops aiming to control growth patterns and development coupled with enhanced systemic resistance to various harmful agents. TU significantly increased vegetative growth, protein content and yield of wheat [15] and maize [16]. Amino acids spray increased total carbohydrates, total proteins and mineral ions content of the mung bean (*Vigna radiate*) seed [17] and common bean (*Phaseolus* *vulgaris* L.) [18]. Considering the positive effects of TU and Asp A have had on productivity of several crop plants and to ascertain the possibility of increasing production efficiency and nutritive value of faba bean seeds, by spraying faba bean plants with different concentrations of TU and Asp A, individually or in combination. Agronomic traits, yield, quality of seeds and some metabolite constituents of faba bean (*Vicia faba* L.) cv. Giza-402 seeds were assessed.

2. MATERIALS AND METHOD

2.1 Experimental Proceures

Two field experiments were carried out at the Research and Production Station of the National Research Centre, Noubaria region (30 30.054' N - 30 19.421' E), Behira Governorate, Egypt during the winter sea sons of 2010/2011 and 2011/2012. Seeds of faba bean (*Vicia faba* L.; cv. Giza-402) were sown on 20th November in both seasons. The experimental design was a split plot with four replications. The TU treatment occupied the main plots and Asp A treatments were allocated at random in sub-plots. The plot area was 10.5m^2 (3.5m×3.0m) and consisted of five rows 70cm apart and the hills along rows were 20cm apart. Calcium super-phosphate (15.5%P_2O_5) was addedpre-sowing at 358 kg/ha to the soil; similarly, nitrogen in the form of ammonium nitrate (33.0%N) at the rate of 42kgN/ha as starter dose was added before first irrigation, Potassium sulphate (48%K_2O) was added at the rate of 119kg/ha to the soil in two equal doses at 21 and 35 days after sowing. Faba bean plants were irrigated and maintained during the whole growth season up to 100% of potential crop evapotranspiration (in 15 successive irrigations) using sprinkler irrigation system. The good agricultural practices, pest control, for growing faba bean in sandy soil were applied as recommended, except for bioregulators treatments. In both seasons, a foliar spray was applied twice to faba bean plants during elongation stage (at 45 and 60 days from sowing), with TU at 250,500or1000mgL^{-1}and/or Asp A at 50,100 or 150mg^{L-1}(i.e. singly and in combination, in all possible permutations). The interaction of different concentrations of both compounds was also assessed in addition to untreated plants (control).

2.2 Data Recorded

At 90 days after sowing (DAS) and 105 DAS (i.e., green yield) the plant growth characters measured were as follows: plant height, branch number, leaf number, pod number, dry weight/plant, and leaf area was measured. Leaf area per plant was worked out by leaf disc method [19] on dry weight basis. Thirty leaf discs having a known diameter (1cm^2) were collected randomly from fully expanded leaves throughout the plant canopy by avoiding midrib of the leaf. The discs thus collected and rest of the leaves was oven dried separately at 80°C for 72 hours. The dry weight of leaf discs and rest of the leaves was recorded and leaf area was computed and expressed in cm^2 per plant, Leaf area index was determined as the formula *LAI* = LA (cm^2)/P (cm^2), where LA=Leaf area and P=Land area according to [20],specific leaf weight(SLW) indicates the average leaf thickness and was determined by the method suggested by [21] and expressed as mg cm^{-2}) [SLW(g m^{-2})= Leaf dry weight per plant (mg)/ Leaf area per plant (cm^2) and crop growth rate. Crop growth rate (CGR) is the ratio of dry matter production per unit ground area per unit time, which was calculated by adopting the formula given by [22] and expressed as g m^{-2} day^{-1}.

CGR g m^{-2} day^{-1}) = $\frac{W2-W1}{T2-T1} \times \frac{1}{A}$. Where, W1= Dry matter of the plant (g) at time T_1; W2= Dry matter of the plant (g) at time T_2, A = Unit land area occupied by the plant (m^2).

The photosynthetic pigment [*chlorophylla (Chla), chlorophyllb (Chl b*), and carotenoids as well as total photosynthetic pigments (TPP)] concentration of fresh leaves were determined at 75 and 90 DAS. To determine total leaf chlorophyll and carotenoids concentrations, 2 g of fresh leaf tissue were used. The remainder of each plant, was then oven-driedfor 72 h at 70ºC, and its dry weight (DW) was recorded. Leaf total chlorophyll and carotenoids concentrations [in mg g^{-1} fresh weight (FW)] were estimated using 80%(v/v) acetone extracts and the spectrophotometric method [23].

At the time of harvest (105DAS), the mean values of yield and its related parameters, i.e., plant height, branch number, pod number, pod weight, seed index, seed and straw yields plant-1, seeds, straw and biological yields ha-1and harvest index were determined.

Seeds samples were taken from each plot and dried in an electric oven with adrift fan at 70°C for 48h until constant dry weight was achieved. Dry samples of seeds were used to determine total carbohydrate in using the colorimetric method described [24]. Crudeprote in percentage was extracted and determined by Micro-Kjeldahl method as described by AOAC [25].The value of total crude protein was calculated by multiplying total values of total-N by factor 6.25.
Seed mineral ions content: A known weight (0.5 g) of the dry ground plant seeds material was digested (using an acid mixture containing nitric acid: perchloric acid: sulfuric acid at the ratio of 8:1:1, respectively) according to the method described by [26], and extracted and the obtained extract was used for the estimation of mineral ions content. Calcium (Ca) and potassium (K) were determined photometrically by the flame photometer method as described [27], while phosphorus (P) was determined by atomic absorption spectrophotometer (using magnesium filter) according to [28]. Mineral contents were expressed in mg g^{-1} DW leaf tissue.

2.3 Statistical Analysis

A combined analysis of data for the two seasons was carried out according to the procedure outlined. All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split–plot design using means of “MSTAT-C” computer software package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability as described [29].

3. RESULTS

3.1 Growth Parameters

Data presented in Tables 1 and 2 show that exogenous application of TU either separately at 250,500 and 1000 mg l^{-1} and Asp A at 50,100 and 150 mg l-1 or any of their combinations, promoted almost all growth criteria compared to corresponding untreated control plants (i.e. neither treated with TU or Asp A). In most cases, the increments in growth parameters were often highly significant in comparison with untreated plants. The most effective treatments on most growth criteria were the combination of TU at 1000 mg l-1+ Asp A at 150 mg L-1 followed by TU alone at 1000mgL^{-1} for all growth parameters. Exogenous application of TU up to 1000 mg L-1 significantly increased the growth criteria studied, compared to corresponding untreated control plants. Furthermore, Asp A was less effective than TU in increasing vegetative growth of faba bean plants at the two growth stages Table 1. The increment in growth characters studied was maximum with

150 mg l-1 Asp A compared to control plants at 90 and 105DAS.

Table 1. Effect of thiourea and aspartic acid on growth characters of faba bean plants at 90 and 105 days from sowing (Combined analysis of two seasons)

Treatments (mgL^{-1})		Plant height (cm)		Number plant^{-1} branches		leaves		pods		Dry weight (g plant^{-1})	
		A	B	A	B	A	B	A	B	A	B
Thiourea	0.0	98.7	112.6	4.1	4.7	43.1	41.3	23.1	30.1	50.0	60.3
	250	101.3	119.5	4.6	4.9	48.3	45.6	26.3	33.4	52.1	63.2
	500	106.0	126.1	5.1	5.3	51.6	49.0	28.4	36.7	54.6	65.3
	1000	112.4	129.4	5.4	5.5	56.4	52.9	33.0	39.5	57.3	68.4
LSD at 5%		2.7	3.1	0.2	0.2	4.1	3.4	4.0	3.1	2.1	2.0
Asparttic acid	0.0	95.5	109.8	4.0	4.4	42.5	41.3	22.9	29.5	49.0	59.6
	50	97.6	112.7	4.3	4.6	46.6	43.7	25.2	32.3	51.4	62.7
	100	101.7	116.1	4.4	4.6	49.8	46.4	27.5	34.5	53.7	64.8
	150	106.9	120.9	5.0	5.1	50.7	49.3	31.5	37.6	55.8	66.9
LSD at 5%		2.1	2.8	0.2	0.2	3.3	2.7	2.3	2.1	2.1	2.0

Treatments (mgL^{-1})		Fourth leaf area (cm^2)		Leaf area index		Specific leaf weight ($mg\ cm^2$)		CGR ($mg\ cm^2\ day^{-1}$)
		A	B	A	B	A - B	B - C	A-B
Thiourea	0.0	1332	1691	3.01	2.97	4.80	3.87	1276
	250	1540	1989	3.27	3.08	4.97	4.28	1336
	500	1719	2164	3.32	3.14	5.39	4.46	1567
	1000	1940	2337	3.44	3.21	6.02	5.29	1708
LSD at 5%		181	170	0.11	0.06	0.16	0.19	120
Asparttic acid	0.0	1322	1544	2.96	2.87	4.59	3.68	1245
	50	1479	1878	2.99	2.92	4.74	3.94	1309
	100	1680	1970	3.11	3.05	4.89	4.19	1391
	150	1791	2080	3.17	3.09	5. 51	4.68	1457
LSD at 5%		110	97	0.05	0.03	0.12	0.18	76

A: 90 days from sowing, B: 105 days from sowing, CGR: crop growth rate

Regarding the combined effect of TU and Asp A on vegetative growth, similar significant increases were obtained in all growth parameters with using different concentrations of TU and AspA except for number of branches plant^{-1}, leaf area index and specific leaf weight showed insignificant response. TU at 1000mgL^{-1} + Asp A at 150mgL^{-1} enhanced growth parameters the most at the two stages of growth (Table 2).

3.2 Photosynthetic Pigments

Data presented in Tables 3 and 4 show that Chl. a and b, carotenoids and total photosynthetic pigments (TPP) in the leaves reached a maximum value at 90DAS. Generally, foliar application of either TU or AspA at any concentration or their combination significantly increased the Chl*a* and *b*, carotenoids and consequently the TPP more than controls at 75 and 90 DAS. The most effective concentrations were 1000 and 150 mg l-1 of either TU or AspA, respectively and their combination at the two growth stages. Foliar spray of bean plants with TU at 250, 500 and 1000 mg l-1 significantly increased photosynthetic pigments compared to untreated plants (Table 3). The highest recorded value of Chl. a and b, carotenoids and TPP in the bean leaves was recorded with 1000 mg l-1 TU at the two growth stages, while Asp A up to 150mgL^{-1} also significantly increased these pigments Table 3. Moreover, the interaction between TU and Asp A significantly increased Chl. a and b, carotenoids and TPP content in the leaves of bean plants more than controls at both growth stages Table 4, the most effective treatment being TU at 1000 mg l-1 + Asp A at 150 mg l-1.

Table 2. Effect of interaction between thiourea (TU) and aspartic acid (AspA) on growth characters of faba bean plants at 90 and 105 days from sowing (Combined analysis of two seasons)

Treatments (mgL^{-1})		Plant height (cm)		Number plant^{-1}						Dry weight (g plant^{-1})	
				branches		leaves		pods			
TU	AspA	A	B	A	B	A	B	A	B	A	B
0.0	0.0	90.4	101.2	3.1	3.6	39.2	41.3	20.9	22.8	41.3	51.3
	50	97.3	106.3	3.3	3.8	42.3	44.5	22.8	24.6	46.4	54.4
	100	101.4	109.4	3.6	4.3	44.3	46.6	23.7	25.4	48.5	56.5
	150	108.8	112.4	4.1	4.4	47.4	48.1	23.9	27.0	50.6	59.6
250	0.0	98.7	108.5	3.3	3.8	41.7	42.9	22.6	24.6	44.6	54.5
	50	109.6	113.6	3.4	3.9	43.8	45.8	24.9	27.6	48.7	59.4
	100	112.8	116.8	3.8	4.4	46.9	48.7	26.4	29.7	50.4	62.3
	150	114.7	119.9	4.3	4.6	50.0	50.7	27.5	31.8	52.8	65.7
500	0.0	100.7	112.0	3.6	3.8	42.1	44.6	23.8	25.9	46.8	56.5
	50	111.5	119.7	3.7	4.5	44.1	46.5	25.8	29.1	49.9	60.8
	100	113.6	121.3	3.8	4.7	47.2	49.4	28.4	31.6	52.1	63.1
	150	116.4	123.3	4.5	5.0	49.6	52.2	29.6	33.3	53.1	66.5
1000	0.0	105.2	116.4	3.7	4.2	43.4	46.1	24.9	27.3	48.2	59.5
	50	116.2	120.6	4.4	4.7	46.5	49.0	27.7	30.5	51.4	63.7
	100	118.2	126.8	4.45	5.1	50.3	52.9	29.8	34.6	53.4	67.6
	150	120.6	130.7	4.6	5.2	51.8	54.6	32.0	39.7	56.6	69.5
LSD at 5%		2.4	3.2	n.s.	n.s.	2.1	1.5	1.12	1.53	2.8	3.1

		Fourth leaf area (cm^2)		Leaf area index		Specific leaf weight ($mg\ cm^2$)	
		A	B	A	B	A-B	B-C
	0.0	1034	1123	2.86	2.80	3.43	2.90
	50	1057	1167	2.93	2.90	3.67	3.52
	100	1087	1249	2.96	2.93	4.49	3.69
	150	1108	1370	3.27	3.09	4.65	3.82
250	0.0	1140	1245	2.94	2.90	3.56	2.96
	50	1278	1351	2.96	2.94	4.70	3.90
	100	1289	1370	2.98	2.97	4.78	4.59
	150	1353	1479	3.33	3.21	4.82	4.77
500	0.0	1267	1540	2.97	2.99	3.66	2.98
	50	1360	1662	2.99	3.01	4.79	3.87
	100	1468	1743	3.38	3.30	4.83	4.74
	150	1528	1810	3.34	3.29	5.46	4.87
1000	0.0	1409	1665	2.99	3.01	4.76	3.78
	50	1469	1777	3.29	3.18	4.88	3.69
	100	1580	1856	3.42	3.39	5.69	4.89
	150	1597	1949	3.49	3.41	6.42	5.95
LSD at 5%		67	86	NS	NS	NS	NS

A: after 90 days from sowing, B: after 105 days from sowing.

Table 3. Effect of thiourea and aspartic acid on photosynthetic pigments contents (mg g^{-1} fresh weight) in the leaves of faba bean plants at 75 and 90 days from sowing (Combined analysis of two seasons)

Treatments (mgL^{-1})		75 days after sowing				90 days after sowing			
		Chl a	Chl b	Carotenoids	TPP	Chl a	Chl b	Carotenoids	TPP
Thiourea	0.0	1.72	0.92	0.86	3.50	2.31	1.41	1.46	5.18
	250	1.89	1.08	0.98	3.95	3.20	2.59	1.69	7.48
	500	2.55	1.29	1.58	5.42	3.67	2.70	2.56	8.93
	1000	2.91	1.58	1.77	6.26	3.94	2.89	2.72	9.55
L.S.D. at 5%		0.16	0.14	0.10	0.40	0.23	0.19	0.21	0.63
Asparttic acid	0.0	1.64	0.80	0.79	3.23	2.06	1.33	1.25	4.64
	50	1.78	0.95	0.96	3.69	2.34	1.54	1.46	5.34
	100	2.09	1.23	1.39	4.71	2.92	1.84	1.59	6.35
	150	2.25	1.33	1.49	5.07	3.64	2.76	2.66	9.06
LSD at 5%		0.13	0.11	0.08	0.32	0.20	0.16	0.18	0.54

TPP: total photosynthetic pigments

Table 4. Effect of interaction between thiourea (TU) and aspartic acid (AspA) on photosynthetic pigments contents (mg g^{-1} fresh weight) in the leaves of faba bean plants at 75 and 90 days from sowing (Combined analysis of two seasons).

Treatments (mgL^{-1})		75 days after sowing				90 days after sowing			
TU	AspA	Chl a	Chl b	Carotenoids	TPP	Chl a	Chl b	Carotenoids	TPP
0.0	0.0	1.56	0.54	0.86	2.96	1.64	0.69	1.49	3.82
	50	1.67	0.63	0.97	3.27	1.76	1.44	1.59	4.79
	100	1.76	0.79	1.50	4.05	2.34	1.58	1.68	5.60
	150	1.83	1.39	1.66	4.88	2.52	1.76	1.77	6.05
250	0.0	1.62	0.60	0.96	3.18	1.77	1.48	1.54	4.79
	50	1.74	0.73	1.49	3.96	2.49	1.67	1.66	5.82
	100	2.23	1.49	1.69	5.41	2.62	2.28	2.39	7.29
	150	2.48	1.59	1.79	5.86	3.01	2.47	2.47	7.95
500	0.0	1.69	0.69	0.98	3.36	2.50	1.56	1.60	5.66
	50	2.33	1.59	1.60	5.52	2.72	2.48	2.49	7.69
	100	2.42	1.67	1.74	5.83	3.26	2.60	2.59	8.45
	150	2.51	1.77	1.79	6.07	3.39	2.67	2.63	8.69
1000	0.0	1.76	0.74	1.55	4.05	2.66	2.44	1.68	6.78
	50	2.49	1.66	1.69	5.84	3.34	2.56	2.54	8.44
	100	2.53	1.78	1.82	6.13	3.46	2. 71	2.64	8.81
	150	2.60	1.80	1.88	6.28	3.59	2.79	2.70	9.08
LSD at 5%		0.04	0.06	0.08	0.18	0.11	0.10	0.09	0.30

TPP: total photosynthetic pigments

3.3 Yield

Data presented in Tables 5 and 6 show that the application of TU significantly increased the values of yield and its related criteria more than control plants, except the harvest index criteria that not significantly affected by TU application. The most promising results were for pod number plant L^{--1}, straw and seed yields ha-1 were obtained with application of 1000mgL^{-1} TU. The increments of these criteria were 25.3, 40.5 and 46.4% more than the control, respectively, and increased the seed quality by increasing the seed index (increased 19.0% more than the control). Exogenous application of AspA alone significantly increased bean yield at any concentration used. The maximum increase in yield criteria was obtained with spraying AspA at 150mgL^{-1}, where the increase in the straw yield haL^{-1}, seed yield haL^{-1} and harvest index were 37.4,75.8, and 16.8% more than the control) while pod weight $plantL^{-1}$ increased 20.8% more than the control with $150mgL^{-1}$ Asp A Table 5. In all cases, the increments in bean yield were often highly significant in comparison with untreated controls. It's worthy to mention that the increments in faba bean yield and yield components were pronounced under application of TU than that of Asp A. Furthermore, bean yield was far more sensitive to the interaction between TU and Asp A. The highest increase in bean yield (seed yield ha-1, straw yield haL^{-1} and harvest index increased 101.9, 40.0, and 25.5%, respectively, relative to their controls) were obtained by foliar application of $1000mgL^{-1}$ TU+$150mgL^{-1}$ Asp A followed by 1000 mg l-1 TU for all previous parameters Table 6.

Table 5. Effect of thiourea and aspartic acid on yield and its components of faba bean at 105 days from sowing (Combined analysis of two seasons).

Treatments (mgL^{-1})		Plant height (cm)	Number $plant^{-1}$		Yield (g $plant^{-1}$)			Yield (t fed^{-1})		Seed Index (g)	Harvest Index %
			branches	pods	Pods	Straw	Seed	Seed	Straw		
Thiourea	0.0	110.3	4.0	31.6	85.6	104.8	73.2	2.20	3.14	69.4	41.1
	250	121.7	4.6	33.3	91.5	116.2	79.7	2.39	3.49	73.3	40.7
	500	125.7	4.9	38.3	105.4	132.9	91.3	2.74	3.99	76.3	40.7
	1000	130.5	5.0	39.6	108.3	147.2	107.3	3.22	4.41	82.6	42.2
LSD at 5%		5.8	0.2	1.2	2.8	11.5	6.7	0.34	0.31	3.4	NS
Aspartic acid	0.0	109.4	3.9	30.5	82.1	101.6	52.4	1.57	3.05	68.9	34.0
	50	112.3	4.4	32.6	88.3	113.3	73.7	2.21	3.40	71.3	39.4
	100	119.2	4.6	35.2	97.1	128.0	83.0	2.49	3.84	75.4	39.3
	150	126.0	4.7	36.8	99.2	139.6	92.1	2.76	4.19	78.5	39.7
LSD at 5%		3.5	0.3	1.1	2.3	10.8	5.3	0.26	0.29	3.0	2.3

Table 6. Effect of interaction between thiourea (TU) and aspartic acid (AspA) on yield and its components of faba bean plants at 105 days from sowing (Combined analysis of two seasons).

Treatments (mgL^{-1})		Plant height cm	No. pods $plant^{-1}$	Yield (g $plant^{-1}$)			Yield (t fed^{-1})		Seed index g	Harvest index %
TU	AspA			Pods	Straw	Seed	Seed	Straw		
0.0	0.0	110.6	28.7	73.0	104.9	53.4	1.60	3.15	64.1	33.7
	50	115.3	29.3	75.3	114.4	63.3	1.90	3.43	66.5	35.6
	100	117.5	30.4	79.0	119.3	69.4	2.09	3.58	69.1	36.8
	150	120.4	31.9	81.6	123.4	85.3	2.56	3.70	73.3	40.9
250	0.0	115.5	29.5	76.3	108.0	54.5	1.64	3.24	67.4	33.5
	50	118.6	30.6	80.8	116.9	79.7	2.39	3.51	71.2	40.5
	100	121.8	31.7	82.2	127.0	83.3	2.50	3.81	74.6	39.6
	150	123.9	33.0	84.9	132.5	87.0	2.61	3.98	79.3	39.6
500	0.0	120.9	30.3	82.0	112.1	57.5	1.73	3.36	72.4	33.9
	50	123.7	32.4	86.3	118.3	85.3	2.56	3.55	78.5	41.9
	100	125.2	34.2	92.5	133.5	88.7	2.66	4.01	80.5	39.9
	150	128.3	35.9	96.1	136.6	89.7	2.69	4.10	84.7	39.6
1000	0.0	122.4	32.3	87.1	116.6	83.3	2.50	3.50	79.7	41.7
	50	125.6	34.4	94.3	124.1	87.0	2.61	3.72	81.8	41.2
	100	129.7	36.5	98.6	137.8	100.3	3.01	4.13	84.9	42.1
	150	131.7	39.7	105.4	147.1	107.7	3.23	4.41	89.0	42.3
LSD at 5%		2.1	1.3	2.6	4.3	3.2	0.11	0.13	3.7	2.2

3.4 Chemical Constituents

Data presented in Table 7 shows that foliar application of either TU or AspA at any concentration significantly increased the total carbohydrates, crude protein %, nitrogen, phosphorus, potassium and calcium in the seeds of bean compared with controls at 105 DAS (1^{st} harvest date). The most effective treatment was $1000mgL^{-1}$ and 150mgL-1 of either TU or Asp A, respectively. Furthermore, the results obtained indicate that the highest level of total carbohydrates (59.93,57.09%), crude protein (25.12,24.29%), nitrogen (4.02, 3.89 mg g-1DW), phosphorus (3.97, 3.49 mg g-1 DW), potassium (6.55,5.80mgg-1DW), and calcium (1.85,1.64mgg-1DW) in bean seeds were obtained for either 1000 mg L^{-1}TU or 150 mgl^{-1} AspA, respectively compared with their controls. Its worthy to minion that, the combination between TU and AspA at any concentration used had insignificant effects on the total carbohydrates, protein, N, P, K and Ca concentrations in faba bean seeds. Therefore the data were excluded.

Table 7. Effect of thiourea and aspartic acid on chemical constituents of faba bean seeds at 105 days from sowing (Combined analysis of two seasons).

Treatments (mgL^{-1})		Total carbohydrate	Crude protein %	N %	P	K	Ca
					($mg\ 100\ g^{-1}$ DW)		
Thiourea	0.0	53.2	22.0	3.52	1.89	4.39	0.49
	250	55.1	23.3	3.72	2.74	5.77	0.97
	500	57.6	24.1	3.85	3.34	5.89	1.59
	1000	59.9	25.1	4.02	3.97	6.55	1.85
L.S.D. at 5%		2.3	1.0	0.16	0.59	0.39	0.18
Aspartic acid	0.0	52.3	21.9	3.50	1.76	3.87	0.37
	50	53.9	22.8	3.64	2.56	4.56	0.80
	100	55.9	23.3	3.73	2.76	5.49	0.89
	150	57.1	24.3	3.89	3.49	5.80	1.64
LSD at 5%		1.3	0.4	0.07	0.19	0.30	0.16

4. DISCUSSION

The present study indicates that application of TU up to 1000 mg l-1and AspA up to150 mg l-1, individually or in combination; greatly promote the vegetative growth and dry matter production of faba bean plants, possibly through increased translocation of sucrose metabolites from source to sink [30], improved the plant growth potential and photosynthetic efficiency [31], participation in the synthesis of auxin and/or cytokine in, enhancement of cell division and chlorophyll accumulation. TU (1000 mg l-1) has shown a much better ability than AspA (150 mg l-1) on promoting growth and yield of faba bean. In other studies, TU promoted growth and photosynthetic pigments in *Brassica juncea* [31], wheat [15], horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] [32], two sunflower cultivars [33] and cluster bean (*Cyamopsis tetragonoloba* L.) [34]. The growth inhibitor hormone (abscisic acid) was decreased in level in pretreated wheat with TU [15]. Thiourea also increase nitrogen uptake, metabolic processed and hence increased the growth and dry matter accumulation in maize [16]. On the other hand, the amino acid Trp increased the content and activity of endogenous PGRs which promote linear growth of plant organs [35,36] due to its conversion in to IAA [37]. It has not been distinguished that increased total nitrogen came of nitrate or amino acids [38].

Auxins promote growth, increase building metabolites, retard senescence, enhance cell division, chlorophyll accumulation and stimulate dry matter production as a result of higher photosynthetic activity and consequently increased translocation and accumulation of micro elements in plant organs [39,40]. Previously, amino acids application has been reported to increase growth, flowering parameters and yield of faba bean [41,42], mung bean [17], common bean [18] and, tomato plants [43]. Both aspartic acid and glutathione increased plant growth, anthocyanin, α-tocopherol, ascorbic acid and enzymatic activities [43]. Intuitively, combination of TU and AspA was more effective for increasing vegetative growth and dry matter production of faba be an plants as compared to single treatments. Faba bean yield and quality of pods was significantly enhanced by TU, especially at high concentration (1000 mg it L^{-1}). TU increased yield (seed, straw and biological yield/ha) more effectively than AspA by enhancing photosynthetic activity, accumulating dry matter and consequently, increased translocation and accumulation of certain metabolites in plant organs which affected their yield and its components (Tables 3,4). On the other hand, amino acids application has been reported to increase yield of faba bean [41,42], mung bean [17] and tea [44].

The combined treatments of TU and AspA were more effective as compared to TU used alone for increasing yield by enhancing photosynthetic activity and accumulation of dry matter which affected their yield and quality. Photosynthetic pigments of bean leaves (Tables 5,6) were significantly enhanced by the application of TU or AspA and/or their combination. TU and AspA might concomitantly retard the chlorophylls destruction and/or increase Nlevels (structural component of Chl) and enhance their biosynthesis or stabilize the thylakoid membrane which would lead to a higher rate of photo synthesis. Amino acids provide plant cells with an immediately available source of nitrogen, which generally can be taken by the cells more rapidly than inorganic nitrogen [45]. The promotive effects of TU on photosynthetic pigments were observed for cluster bean [34], wheat [15] and sunflower [33]. Amino acids and putrescine treatments concomitantly within creasing Mglevels (structural component of chlorophyll) and retarding senescence [46]. Glutathione, Tryptophan and phenylalanine up to 100 ppm significantly increased photosynthetic pigments content in mung bean [17], broad bean (*Vicia faba* L.) [42] and *Antirrhinum majus* plants due to their important role in the biosynthesis of chlorophyll molecules which in turn affected total soluble sugars content [47].

Raeisi et al. [42] reported that higher mRNA transcription up to 2.5 fold, activation of reproductive growth- involved hormones, production of carbohydrates, higher absorption and element transition, higher protein in plants caused improvement of yield and related parameters in plants in environmental stresses [44,48]. Similarly, in this study, the combination of TU at 1000 mg l-1 and AspA at 150 mg l-1 increased the photosynthetic pigments of bean leaves. A foliar application of either TU or AspA increased not ably total soluble sugars percent, crudeprotein, nitrogen, phosphorus, potassium and calcium in the dry seeds of faba bean Table 7, possibly due to the bioregulatory effect on enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites [31]. Pandey et al. [31] reported that the enhanced photosynthetic efficiency was found to be coupled with increased expression of TPT and higher enzyme activity of FBPase that indicated the availability of sufficient building blocks for sucrose biosynthesis in TU treated leaves. They added that TU modulating different plant processes such as source strength, translocation of photo assimilates, sink strength and pod photosynthesis rate. Similarly, amino acids serve as major transport molecules of nitrogen from vegetative to reproductive tissues [10]. Seeds of faba bean have high protein content (30% of their dry weight) which contain most of the necessary amino acids for human and animal nutrition and low sulphur amino acids concentrations [49]. TU significantly increased the content of total sugar, protein, and total phenolic compounds [31,33]. On the other hand, glutathione stimulate total carbohydrates and total proteins and mineral ions content of mung bean plants [17]. Spraying chamomile plant with amino acids (ornithine, proline or phenylalanine) increased total nitrogen% and crude protein [17]. Amino acids increased total soluble sugars and total free amino acids in mung bean [17] and snap bean [15].

5. CONCLUSION

In conclusion, thiourea (TU) and aspartic acid (AspA) can be easily applied as a foliar application to faba bean plants in the field. Application of TU at 1000 mg l-1 and AspA at 150 mg l-1, singly or combined, resulted in a significant increase in all morphological attributes, scored highest number of pods which resulted in substantially higher seed yield and quality. The use of TU and AspA as bioregulatory compounds thus open up a new avenue for increasing faba bean yield, improving quality and nutritional value around the world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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