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Combined Action of Copper with IAA on Individual Amino Acids and Microelement in Pods of Two Broad Bean Cultivars

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Abstract

It is worthy to mention that the two broad bean cultivars displayed a surprising situation during the fruiting stage of growth. The absolute amount of pods yield was higher in cv. Assiut 125 than in cv. Assiut 84. This situation was in contrast with that in the vegetative stage, where cv. Assiut 84 produced greater vegetative growth than cv. Assiut 125. Although the cv. Assiut 84 was less affected by the highest dose of copper, the cv. Assiut 125 was greatly affected by this dose. However, another surprising situation was recorded when the absolute values of fresh and dry matter of cv. Assiut 125 were much higher than cv. Assiut 84 at any used copper concentration. The fresh matters of the pods of cv. Assiut 84 and cv. Assiut 125 were 7.24 and 10.16 gm at the level of control and 6.69 and 7.59 gm at the level of 350 ppm Cu, respectively. Their dry matters were 1.99 and 3.52 gm at the level of control and 1.77 and 2.13 gm at the level of 350 ppm Cu^{2+} in cv. Assiut 84 and cv. Assiut 125, respectively. This contradiction was also extended when these copper-affected cultivars were sprayed by IAA. The cv. Assiut 84 responded slightly to IAA treatment. On the other hand, a considerable increase in the dry matter yield was recorded in the pods of cv. Assiut 125 at any used level of copper when compared to the non-sprayed plants treated by the same copper concentration. At the level of 350 ppm Cu^{2+}, the pod yield was 2-fold that of plants treated only by 350 ppm Cu^{2+}, without IAA spraying. Thus, even IAA behaved differently in its effect on the crop yield of the two tested broad bean cultivars. There are big and surprising variations in the accumulation of microelements and heavy metals in pods among the two broad bean genotypes, whatever the treatments used. These variations seemed to be complicated which might need further studies. The concentration of amino acids and their individuals also varied among the two cultivars; they increased in cv. Assiut 84 and decreased in cv. Assiut 125. The increased amino acids in cv. Assiut 84 are mostly used as precursors for phytochelatins. For the previous studies, thus the present work was conducted to explain the effect of Cu and interactive effect with IAA on two selected broad bean cultivars Assiut 84 and Assiut 125 during yielding production.

Keywords

Synergistic Effect, Amino Acids, Microelement, Broad Bean Cultivars

1. Introduction

Essential micronutrients are required in low concentrations, so that the plant can develop normally, but are toxic in high concentrations [1] [2]. Copper (Cu) has been considered to be an essential micronutrient for plants; it is an element of vital importance in seed production, disease resistance, and balance of water catchment [1] [3] and of other essential nutrients, that depend on copper solubility in the soil and the type of soil [4] [5] [6]. Scientists have been more interested in developing potential strategies to promote germination of seeds, growth of plants and higher crop production [7] [8] [9]. This goal is getting even more needed to alleviate the adverse effects of environmental pollutants, and the search for molecules mediating stress tolerance is a relevant step toward a better understanding of how lower plants respond to stress [10]. In particular IAA increased root and sometimes also shoot growth of plants that were stressed by salinity or heavy metals [11] [12] [13] [14] [15]. Diaz *et al.* (2017) [16] provide information about copper tolerance mechanisms. We evaluated the effect of copper (II) ions (control, 100 and 500 µM) on *C. quitens s*eedlings *in vitro*, determining morpho-physiological and biochemical variables. Copper showed a significantly negative effect on the development of new shoots (500 µM) and floral apex appearance (100 µM). The highest proline accumulation took place in seedlings subjected to 500 µM. Hamdia *et al.* (2018) [6]observed that when the copper stressed plants sprayed with IAA where a lot of these cations were transported in the same trend from the soil solution into the different parts of the two broad bean plants without any competition among these cations. The uptake, translocation and distribution of mineral ions are affected by various growth regulators among others by IAA. Massoud *et al.* (2018) [17] compare the efficiency of two types of treatments with the exogenous effectors (IAA, GA3, Ca and citric acid) against copper (Cu) toxicity: 1) the simultaneous application of “Cu^{++} effectors” at the beginning of germination (day 0) and 2) the abruption of Cu stress (exogenous effectors are added in the third day). Thus, this investigation was carried out to study the mechanisms of combined action of copper with IAA treatments on individual amino acids and microelements in pods of two broad bean plants.

2. Materials and Methods

2.1. Experimental Sites and Copper Treatments

Broad bean seeds cv. Assiut 84 and Assiut 125 were obtained from one of the active breading programs directed by Prof. Dr. Esmat Waly and Prof. Dr. Saeyd Abdellah, Faculty of Agriculture, Assiut University, Egypt. Broad bean plant is important economic crop plant and consider the first plant food for Egyptian people because it contains highly benefit protein and other essential elements for man healthy. Which has several common names (broad bean, fava bean, faba bean, horse bean, field bean, tick bean), is a species of bean (Fabaceae) native to North Africa and southwest Asia and is extensively cultivated elsewhere. In much of the world, the name broad bean is used for the large-seeded cultivars grown for human food. In Egypt, faba bean is the most common fast food item in the Egyptian diet, eaten by rich and poor alike. Egyptians eat faba beans in various ways; the most popular way of preparing faba beans is taking cooked beans, mashing them and adding oil, lemon, salt and cumin. The prepared beans, called fulmedames, are then eaten with bread. Faba bean is an excellent source of protein (20% - 25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and methionine-cystine (0.5%) in dry weight. It is also an excellent source of complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites (phenolics and levodihydroxy phenylalanine (L-DOPA), which is the precursor of the neurotransmitter dopamine and naturally found in seedlings, green pods and beans) [18] [19]. Broad bean seeds (cv Assiut 84 and Assiut 125) were obtained from one of the active breading programs directed by Prof. Dr. Esmat Waly and Prof. Dr. Saeyd Abdellah, Faculty of Agriculture, Assiut University, Egypt. From previous work Hamdia *et al.* (2018) [6] stated that at vegetative stage in cv. Assiut 84 the dry matter of stems and leaves increased as copper increased in the soil up to 200 ppm, then while a slight stimulation was obtained in leaves (about 4%), a slight reduction was recorded in stems (20%). The dry matter of roots remained more or less unchanged up to 200 ppm Cu, then about 24% reduction was recorded, which means that the three plant organs responded differently to copper treatments. In cv. Assiut 125 the dry matter of pods increased by copper treatment up to 200 ppm, which was more pronounced in leaves, then a highly significant reduction was recorded (about 30% in both). In roots, a gradual reduction was exhibited by increasing the copper concentration in the soil. This inhibitory effect was more obvious at the level of 350 ppm Cu (about 40% reduction). Broad bean seeds were surface sterilized by immersion in a mixture of ethanol 96% and H_2O_2 (1:1) for 3 minutes, followed by several washings with sterile distilled water. The concentrations of copper were chosen after preliminary experiments in which the seeds were subjected to different concentrations of copper. The chosen concentrations caused slight stimulation and moderate inhibition of seed germination from 100 ppm to 350 ppm copper treatment were recorded. Copper was added as copper sulphate ($CuSO_4$). Ten seeds were sown per pot, each pot contained 3.6 kg of garden clay soil. The clay soil comprise four components minerals and soil organic matter make up the solid fraction, whereas air and water comprise the pore space fraction. A typical agricultural soil is usually around 50% solid particles and 50% pores (Adapted from Brady and Weil, 2002 [20]). Soil particle of clay is <0.002

invisible to naked eye. Considerations of working in controlled environments were followed by Tibbitts & Langhans (1993) [21]. All pots were irrigated with tap water for four weeks until full germination. In preliminary experiments explained that low concentration is 100 ppm $CuSo_4$ and the high concentration is 350 ppm. The seedlings were then irrigated by different concentrations of $CuSO_4$ solutions (0, 100, 200, 350 ppm) and were classified into two groups.

2.2. Copper Treatment and Combined with IAA

From two of previous groups which treated with different copper concentration (0, 100, 200, 350 ppm), one group was sprayed by 200 ppm IAA. In order to maintain the osmotic potential, the soil moisture content was kept near the field capacity using tap water. The seedlings were left to grow in natural conditions under these conditions for 150 days. At the end of the experimental period (5 months) yields of pods determined.

2.3. Laboratory Analysis for Metabolites

To determine the dry matter yields of pods were dried in an oven at 80˚C. Successive weighing was carried out until the constant dry weight of each sample was reached. The soluble carbohydrates were determined by the method of anthrone sulphoric acid which was stated by Fales (1951) [22]. The soluble proteins were determined according to the method adopted by Lowery *et al.* (1951) [23]. Calcium and magnesium determination by Schwarzenbach and Biedermann, (1948) [24] was employed. Potassium, Flamephotometer method using Carl Zeiss flamephotometer was used by Williams and Twine, (1960) [25].

3. Statistical Analysis

The triplicate sets of the experimental data for the different tested parameters were subjected to the one way analysis of variances (ANOVA) test in accordance with the experimental design using the SPSS program, version 13.0 and the means were compared using the least significant differences, L. S. D. at 0.05% levels [26].

4. Results

In cv. Assiut 84 the fresh and dry weights of pods increased progressively by increasing Cu concentration in the soil up to the level of 200 ppm. At this level, the percent of increase in fresh and dry matters was 40.6% and 33.7% over the control value, respectively. However, the fresh and dry matters of pods of cv. Assiut 84 reduced slightly at the concentration of 350 ppm Cu. The percent of reduction did not exceed 7.67% and 10.95% in fresh and dry matters, respectively. In cv. Asiut 125 the copper up to the level of 100 ppm induced an increase in fresh and dry matter yields (36.2% and 3.04%, respectively). Then a slight reduction was exhibited up to 200 ppm Cu^{2+} and a highly significant reduction was obtained only at the level of 350 ppm Cu^{2+}. This reduction of fresh and dry matters of pods was 25.3% and 39.4%, respectively, as compared to the control values. The various levels of Cu^{2+} considerably increased the soluble carbohydrates contents of pods of the broad bean cultivar Assiut 84. The highest accumulation was obtained at the level of 200 ppm Cu. At this level, the percent of increase was 42.78%. In cv. Assiut 125, the soluble carbohydrates in pods were stimulated up to the level of 200 ppm Cu^{2+}, and then a highly significant reduction in this fraction was recorded. In cv. Assiut 84 the soluble proteins remained more or less unchanged by the various concentrations of Cu^{2+}. The induction of Cu^{2+} on the accumulation of proteins in pods of cv. Assiut 125 went opposite to that in cv. Assiut 84, the soluble fraction decreased progressively. Potassium remained mostly unchanged at any concentration of Cu^{2+}. Some reduction was recorded at the higher dose of Cu^{2+} (15% reduction) in cv. Assiut 84 (**Tables 1-3**). A slight promotion in K^+ contents was revealed in pods of cv. Assiut 125 as a result of the various treatments by Cu^{2+} (**Table 3**). In Cv. Assiut 84 calcium content in the pods of cv. Assiut 84 decreased slightly up to 200 ppm Cu^{2+}. This reduction was constant at the two copper levels 100 and 200 ppm (14.29%). Then, a highly significant reduction was recorded only at the level of 250 ppm Cu^{2+} (42.86% of control). Pods of cv. Assiut 125 were able to maintain their Ca^{++} content at the level of control values at any used Cu level (the amount of Ca^{++} was 5 mg/gm dry weight at all levels of Cu). Cu treatments irregularly accumulated magnesium in pods of cv. Assiut 84 at any used level of Cu. It was 275% of control at the level of 100 and 350 ppm Cu and 225% at the level of 200 ppm Cu (**Table 3**). Irregular data of magnesium content in pods of cv. Assiut 125 were exhibited by the various levels of copper (**Table 3**). Magnesium content remained unchanged at the levels of 100 ppm and 350 ppm Cu and increased by 75% at the level of 200 ppm Cu.

4.1. Interaction with Phytohormone IAA

A marked and progressive accumulation of fresh and dry matter yields was exhibited as a result of IAA treatments whatever the concentration of Cu used in cv. Assiut 84. IAA increased the fresh and dry matter in pods by 28.59% and 15.27% at the level of 350 ppm Cu, the maximum value was recorded at 200 ppm Cu treatment. Phytohormonal treatments resulted in a considerable accumulation of the fresh and dry matters in pods that reached 54.34% and 22.31% over the control values, respectively in cv. Assiut 125, the maximum value was obtained at 100 ppm Cu treatment. In cv. Assiut 84 IAA treatments unchanged soluble cabohydrated content except at 350 ppm an increase was exhibited as compared with control plants. In cv. Assiut 125 an additional increase in the accumulation of carbohydrates was recorded as a result of IAA treatments. A marked and progressive accumulation of soluble proteins was recorded as a result of IAA treatments in cv. Assiut 84. IAA treatments induced a considerable accumulation in proteins of cv. Assiut 125 pods whatever the concentration of Cu^{2+} used. Some promotion in K content was exhibited by IAA treatments in pods of cv. Assiut 84. This promotion was higher at the lowest dose of Cu. In cv.

Table 1. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the fresh and dry matter yields in pods of the broad bean cultivars Assiut 84 and Assiut 125.

Treatment	$CuSO_4$ (ppm)	Cv. Assiut 84				Cv. Assiut 125			
		F. m.	%	D. m.	%	F. m.	%	D. m.	%
Absolute control	0	7.24	100	1.99	100	10.16	100	3.52	100
$CuSO_4$	100	8.04	111.09	2.48	124.46	13.84**	136.20	3.63	103.04
	200	10.18**	140.61	2.66*	133.70	9.24	90.96	2.99	84.91
	350	6.69	92.33	1.77	89.05	7.59**	74.71	2.13**	60.63
$CuSO_4$ + IAA	0	11.18**	154.41	2.33	117.23	16.21**	159.58	4.01	113.96
	100	12.05**	166.37	2.89**	144.95	19.01**	187.13	4.89**	138.94
	200	12.39**	171.13	2.82**	141.44	16.34**	160.90	4.71**	133.83
	350	9.31**	128.59	2.30	115.27	15.68**	154.34	4.30*	122.31
L. S. D. 0.05%		1.01		0.47		0.83		0.53	

*Significant differences and **highly significant differences as compared to the absolute control.

Table 2. The effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on soluble carbohydrates (mg·gm^{-1}·d·m.) and soluble proteins (mg·gm^{-1}·d·m.) in the pods of the broad bean cultivars Assiut 84 and Assiut 125.

Treatment	$CuSO_4$ (ppm)	Sol. Carb.	%	Sol. Pro.	%
cv. Assiut 84	0	10.74	100	59.70	100
$CuSO_4$	100	12.10*	112.66	55.97	93.75
	200	15.33**	142.78	53.73*	90.00
	350	13.71**	127.72	47.39**	79.37
$CuSO_4$ + IAA	0	9.83	91.57	139.42**	233.54
	100	10.74	100	184.04**	308.27
	200	11.64	108.44	166.35**	278.63
	350	14.68**	136.75	162.89**	272.83
L. S. D. 0.05%		0.84		0.39	
cv. Assiut 125	0	6.68	100	46.27	100
$CuSO_4$	100	7.12	106.53	41.42**	89.52
	200	7.89**	118.15	40.30**	87.10
	350	5.11**	76.51	39.54**	85.45
$CuSO_4$ + IAA	0	11.26**	168.51	139.42**	301.33
	100	13.39**	200.48	113.08**	244.39
	200	15.85**	237.27	118.85**	256.86
	350	9.51**	142.36	93.65**	202.41
L. S. D. 0.05%	L. S. D. 0.05%	0.83		0.28	

*Significant differences and **highly significant differences as compared to the absolute control.

Table 3. Effect of $CuSO_4$ and $CuSO_4$ plus IAA treatments on ($mg \cdot gm^{-1} \cdot d \cdot m.$) in pods of the broad bean cultivars Assiut 84 and Assiut 125.

Treatment	$CuSO_4$ (ppm)	K^+	%	Ca^{2+}	%	Mg^{2+}	%
cv. Assiut 84	**0**	12	100	5.25	100	1.8	100
$CuSO_4$	**100**	11.5	95.83	4.5*	85.71	4.95**	275
	200	10.6	88.33	4.5*	85.71	4.95**	275
	350	10.2	85.00	3.0**	57.14	4.05**	225
$CuSO_4$ + IAA	**0**	11.9	99.17	5.5	104.76	8.1**	450
	100	13.6	113.33	5.25	100	10.3**	575
	200	12.7	105.83	4.9	93.33	9**	500
	350	11.6	96.67	4.9	93.33	9.0**	500
L. S. D. 0.05%		**1.50**		**0.54**		**0.71**	
cv. Assiut 125	**0**	10.1	100	1.5	100	3.6	100
$CuSO_4$	**100**	11.8*	116.83	1.5	100	3.6	100
	200	10.5	103.96	1.5	100	6.3	175
	350	10.5	103.96	1.5	100	3.6	100
$CuSO_4$ + IAA	**0**	10.2	100.99	3.25**	216.67	10.8**	300
	100	12.0*	118.81	3.00**	200	9.9**	275
	200	13.1**	129.70	3.00**	200	8.55**	237.5
	350	12.6**	124.75	3.25**	216.67	4.95*	137.5
L. S. D. 0.05%		**1.16**		**0.18**		**0.99**	

*Significant differences and **highly significant differences as compared to the absolute control.

Assiut 125 a highly significant increase in K contents was recorded when the Cu-affected plants were sprayed by IAA (Table 3). The pods of cv. Assiut 125 seemed to be more potassium-accumulators than the pods of cv. Assiut 84, whatever the used type of treatments. IAA treatments ameliorated the drastic effects of Cu on Ca^{+2} content in pods of cv. Assiut 84 (Table 3). This promotion was more pronounced at the highest dose of Cu^{2+}. A marked and progressive increase in Ca^{2+} content in pods of cv. Assiut 125 was obtained by IAA treatments. Ca^{2+} content was 2.5-fold that of control as a result of 350 ppm Cu^{2+} plus IAA. In cv. Assiut 84 a huge accumulation of magnesium was recorded by IAA treatments (Table 3). The percent of this increase was 350%, 475%, 400% and 325% at the level of 0, 100 200 and 350 ppm Cu, respectively. IAA resulted in a marked and progressive, but irregular, increase in magnesium content in pods of cv. Assiut 125 (Table 3). The percent increase in pods treated by 0 Cu^{2+} plus IAA, 100 ppm Cu^{2+} plus IAA, 200 ppm Cu^{2+} plus IAA and 350 ppm Cu^{2+} plus IAA was 200%, 175%, 137% and only 37.5%, respectively (Table 3).

4.2. The Concentration of Some Microelements and Interaction with IAA

The most striking feature in this work is that while the pods of cv. Assiut 84 accumulated a huge amount of Al at the level of control, the concentration of 350 ppm Cu^{2+} slowed down this accumulation of Al to about 50%, which may be one of the defense mechanisms of this cultivar against copper toxicity (**Tables 4-6**). This was recommended in plants treated by IAA when the concentration of Al was highly significantly retarded at the level of control as well as the level of 350 ppm Cu^{2+}, it was reduced to about 70% in both (**Figure 1**). Thus, the mechanism of IAA in alleviating the toxic copper effect in the pods of this cultivar may be associated with the retardation of Al content (**Table 4**). In this context a surprising situation was recorded in cv. Assiut 125. The pods of this cultivar accumulated small amounts of this toxic element (Al) at the level of control. This small amount of Al was reduced more in pods treated by 350 ppm Cu^{2+} (**Figure 2**). This was associated with the converting situation of the two cultivars when transported into the fruiting stage. The amount of Cd seemed to be very small in the pods of the two tested broad bean cultivars grown under different treatments, which indicates that the used soil is very rare in Cd ion (**Table 4**). However, the highest amount of this heavy metal was found in cv. Assiut 84 only at the level of controls (control and control plus IAA). In general, pods treated by IAA, either with or without copper, accumulated higher amounts of Co than those untreated by IAA (**Table 4**). This again confirmed the alleviating role of IAA (cobalt is considered an essential microelement when present at suitable concentrations). There is a considerable variation in the Cu contents in pods among the two tested cultivars. In cv. Assiut 84, the pods treated by 350 ppm Cu accumulated a high amount of Cu in relation to its control (1.6 to 2.8 mg/100 gm dry matter) (**Table 4**; **Figure 1**). On the other hand, the pods of cv. Assiut 125 succeeded to keep the Cu content around its control value, which again might confirm the opposing situation of the two cultivars from the vegetative into the fruiting stages of growth (**Table 4**; **Figure 1**). Another important situation was also located in the variations in the accumulation of Fe in the pods of the two broad bean cultivars grown under different copper treatments (**Table 4** and **Figure 1**). In cv. Assiut 84 a huge accumulation of Fe was recorded in pods treated by 350 ppm Cu^{2+}, in relation to the control (16.04 to 44.00 mg/100 gm dry matter). Interestingly, the opposite occurred in cv. Assiut 125 where the accumulation of Fe was retarded by 350 ppm Cu^{2+}, treatment (**Table 4**; **Figure 1**). Phytohormonal treatments seemed to play different roles in the accumulation of Fe (**Table 4**; **Figure 1**). While IAA induced insignificant changes in the Fe content of the pods of cv. Assiut 84, it, on the other hand, induced a marked increase in this element in the pods of cv. Assiut 125, particularly in pods treated by IAA without copper treatment (**Table 4**; **Figure 1**). Zn content in pods of cv. Assiut 84 reduced to about 50% as a result of 350 ppm Cu^{2+} treatment (**Table 4**; **Figure 1**). In cv. Assiut 125, as in the case of Fe, there is no obvious difference in

Table 4. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the contents of some microelements (mg·100 g^{-1}·d·m.) in the broad bean cultivars Assiut 84 a and Assiut 125 (b).

(a)

Element	Cont.	350 ppm Cu^{2+}	Cont. + IAA	350 ppm Cu^{2+} + IAA
Aluminum	28.40	15.20**	9.70	10.90
Iron	16.04	44.30**	15.80	17.00*
Zinc	6.93	3.90**	4.40	5.50
Boron	2.80	2.90	2.60	2.50
Manganese	2.60	2.60	2.90	2.90
Cadmium	0.02	0.01	0.02	0.01
Cobalt	0.02	0.01	0.05	0.03
Lead	0.40	0.20	0.20	0.20
Chromium	0.21	0.41	0.20	0.20
Copper	1.60	2.80	2.50	2.90

(b)

Element	Cont.	350 ppm Cu	Cont. + IAA	350 ppm Cu + IAA
Aluminum	8.70	7.40	12.40	11.30
Iron	13.60	10.40**	24.20	14.20**
Zinc	2.70	2.50	4.10	3.60
Boron	2.30	1.90	2.70	2.50
Manganese	1.70	2.02	2.30	2.50
Cadmium	0.01	0.01	0.01	0.01
Cobalt	0.02	0.02	0.02	0.02
Lead	0.2	0.01	0.2	0.1
Chromium	0.2	0.20	0.22	0.23
Copper	1.7	1.9	2.20	2.30

*Significant differences and **highly significant differences as compared to the absolute control.

the accumulation of Zn between the control pods and the 350 ppm Cu^{2+}-treated pods (Table 4; Figure 1). Hormonal treatments stimulated the accumulation of this element in the pods of the two tested cultivars treated with or without copper (Table 4; Figure 1). However, as in the case of Al, the highest amount of Zn was obtained in the control pods of cv. Assiut 84 (Table 4; Figure 1). A small amount of Pb was recorded in pods of the two tested cultivars under the various treatments (Table 4; Figure 1). The dominant concentration of Pb was 0.2 mg/gm dry matter, except for the pods of cv. Assiut 125 treated by 350 ppm Cu^{2+} or 350 ppm Cu^{2+} plus IAA (0.1 mg/100 gm dry matter) and in pods of cv. Assiut 84 treated by H_2O (0.4 mg/100 gm dry matter) (Table 4; Figure 1). The amount of Mn in the differently treated pods was more or less similar in the two cultivars (Table 4; Figure 1). It fluctuated between 2.3 and 2.9 mg/100 gm dry matter,

Table 5. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the individual amino acids in the broad bean cultivar Assiut 84 (mg/100 gm protein).

Amino acids	Cont.	%	350 ppm Cu^{2+}	%	Cont. + IAA	%	350 ppm Cu^{2+} + IAA	%
Aspartic acid	6.60	100	7.19	108.94	7.54	114.24	8.76	132.73
Threonine	2.13	100	2.18	102.35	2.30	107.98	2.11	99.06
Serine	3.62	100	3.97	109.67	4.03	111.33	3.68	101.66
Glutamine	7.29	100	8.72	119.62	9.00	123.46	7.94	108.92
Proline	0.44	100	0.46	104.55	0.44	100	0.43	97.73
Glycine	5.21	100	5.85	112.28	5.80	111.32	5.70	109.40
Alanine	3.96	100	4.21	106.31	4.37	110.35	4.97	125.51
Valine	2.65	100	2.76	104.15	2.86	107.92	2.60	98.11
Methionine								
Isoleucine	2.11	100	2.23	105.69	2.27	107.58	1.13	53.55
Leucine	4.61	100	4.95	107.38	5.13	111.28	5.88	127.55
Tyrosine	1.55	100	1.56	100.65	1.69	109.03	1.22	78.71
Tyrosine							1.00	
Phenylalanine	1.63	100	1.65	101.23	1.77	108.59	1.60	98.16
Histdine	1.14	100	1.33	116.67	1.74	152.63	1.53	134.21
Lysine	3.07	100	3.59	116.94	3.80	123.78	3.41	111.07
NH_3	6.34	100	7.54	118.93	7.98	125.87	10.34	163.09
Argenine	2.71	100	3.36	123.98	5.29	195.20	4.11	151.66
Total	**55.07**	**100**	**61.55**	**111.77**	**65.99**	**119.83**	**66.40**	**120.57**

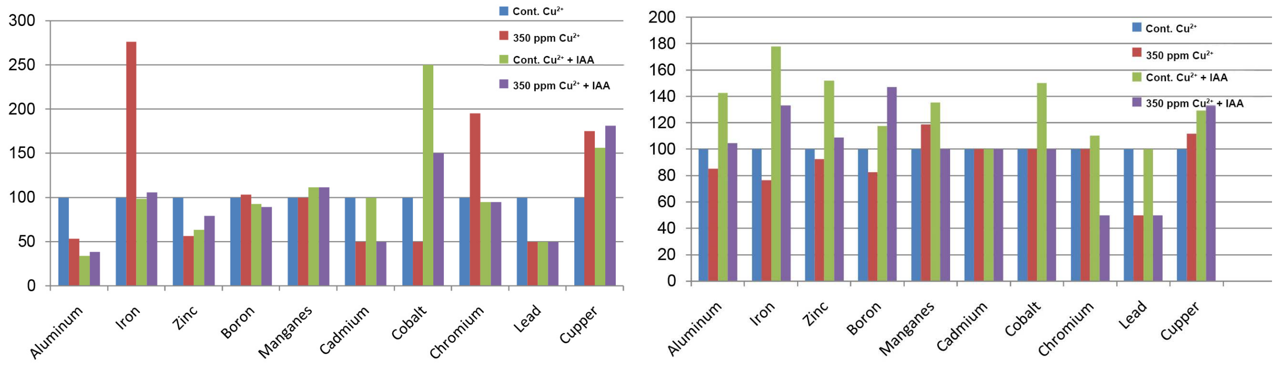
except for pods of cv. Assiut 125 under H_2O irrigation (1.7 mg/100 gm dry matter). Also there is a general promotion in the contents of this element as a result of IAA treatment, whatever the cultivar or the treatment (Table 4; Figure 1). The amount of B was more or less similar under the different treatments in the pods of the two tested cultivars (2.5 to 2.9 mg/100 gm dry matter), except for the pods of cv. Assiut 125 treated by 350 ppm Cu (1.9 mg/100 gm dry matter) (Table 4; Figure 1). Also there is some promotion in the accumulation of this element in the pods of cv. Assiut 125 either treated or untreated by copper in relation to the control values when sprayed by IAA (Table 4; Figure 1).

4.3. Contents of Total and Individual Amino Acids

Amino acids contents and their individuals varied considerably among the two tested cultivars. Copper treatment enhanced the accumulation of amino acids in the pods of cv. Assiut 84 in relation to its control. Additional enhancement in these contents was observed when the copper-treated plants were sprayed by IAA. On the other hand, in cv. Assiut 125 the total amino acids contents dropped highly significantly by copper treatment. However, they were considerably

Table 6. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the individual amino acids in the broad bean cultivar Assiut 125 (gm/100 gm protein).

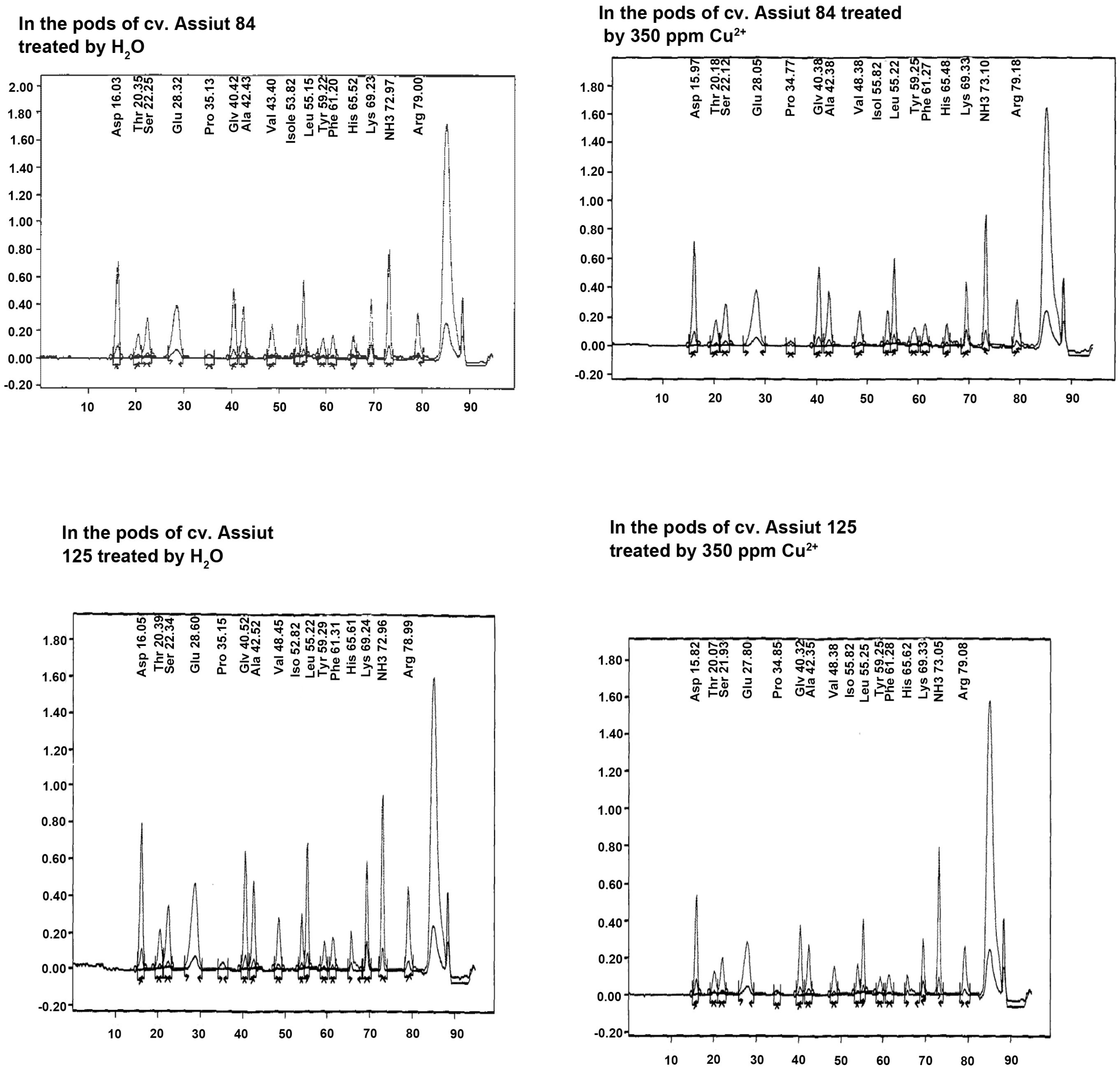
Amino acids	Cont.	%	350 ppm Cu	%	Cont. + IAA	%	350 ppm Cu + IAA	%
Aspartic acid	7.53	100	4.42	58.70	5.50	73.04	10.17	135.06
Threonine	2.58	100	1.34	51.94	1.86	72.09	3.54	137.21
Serine	4.18	100	2.30	55.02	3.31	79.19	5.92	141.63
Glutamine	9.61	100	5.14	53.49	7.00	72.84	13.54	140.89
Proline	0.55	100	0.21	38.18	0.41	74.55	0.76	138.18
Glycine	6.13	100	3.47	56.61	4.75	77.49	8.49	138.50
Alanine	4.61	100	2.56	55.53	3.74	81.13	6.15	133.41
Valine	2.96	100	1.59	53.72	2.31	78.04	3.47	117.23
Methionine							3.12	
Isoleucine	2.34	100	1.29	55.13	1.90	81.20	7.22	308.55
Leucine	5.32	100	2.92	54.89	4.01	75.38	0.31	5.83
Tyrosine	1.36	100	0.88	64.71	0.41	30.15	1.48	108.82
Phenylalanine	1.77	100	0.96	54.24	0.75	42.37	2.17	122.60
Phenylalanine					1.25			
Histdine	1.87	100	1.01	54.01	1.31	70.05	2.37	126.74
Lysine	4.31	100	1.73	40.14	2.73	63.34	4.53	105.10
NH_3	7.26	100	5.24	72.18	4.73	65.16	10.76	148.21
Argenine	4.34	100	2.67	61.52	3.14	72.35	6.63	152.77
Total	**66.72**	**100**	**37.73**	**56.55**	**49.12**	**73.62**	**90.61**	**135.81**



(a) (b)

Figure 1. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on percent contents of some microelements ($mg \cdot 100 \cdot g^{-1}$ d·m.) in the broad bean cultivars Assiut 84 (a) and Assiut 125 (b).

enhanced by Cu^{2+} plus IAA treatment. In cv. Assiut 84 all of the individual amino acids increased by Cu^{2+}, H_2O plus IAA or Cu^{2+} plus IAA treatments, except for isoleucine and tyrosine which dropped as a result of 350 ppm Cu^{2+} plus IAA treatment. On the other hand, the contents of these amino acids dropped highly


In the pods of cv. Assiut 84 treated by H_2O

In the pods of cv. Assiut 84 treated by 350 ppm Cu^{2+}

In the pods of cv. Assiut 125 treated by H_2O

In the pods of cv. Assiut 125 treated by 350 ppm Cu^{2+}

Figure 2. Effect of $CuSO_4$ and $CuSO_4$ plus IAA on the individual amino acids (gm/100 gm protein) in the pods of broad bean cultivars Asiut 84 and Assiut 125.

significantly in the pods of the copper-treated cv. Assiut 125. IAA treatment of the copper-affected pods of this cultivar succeeded in increasing the accumulation of all of these amino acids (except for leucine) over the values of the control plants. The uptake of nutrients was greatly impacted in response to copper stress.

5. Discussion

It is worthy to mention that the two broad bean cultivars displayed a surprising situation during the fruiting stage of growth to copper treatments. The absolute amount of pods yield was higher in cv. Assiut 125 than in cv. Assiut 84, this might be interpreted by the effect of the environmental stresses which is different with the different species, cultivars intensity, rate and duration of exposure and the stage of crop growth [27] [28] [29]. Hamdia *et al.* (2018) [30] stated that there are major differences in the response of the two broad bean cultivars Assiut 84 and Assiut 125 to copper treatments during the vegetative stage from one hand and at the crop yield from the other. We preferred to use the growth criteria of roots, stems and leaves during the vegetative stage as a suitable selection criterion for the copper responses of the two cultivars. The alterations in anatomical parameters and grain yield of different crop plants as a result of heavy metal stress have been previously reported [31] [32] [33] [34]. The data in fresh and dry matter yields of pods revealed interesting results, although the cv. Assiut 84 was less affected by the highest dose of copper, the cv. Assiut 125 was greatly affected by this dose (around 37% reduction in fresh and dry matter of pods compared to insignificant changes in these values in cv. Assiut 84). However, another surprising situation was recorded when the absolute values in fresh and dry matter of cv. Assiut 125 were much higher than cv. Assiut 84. The fresh matter in cv. Assiut 84 and cv. Assiut 125 was 7.24 gm and 10.16 gm at the level of control and 6.69 gm and 7.59 gm at the level of 350 ppm Cu^{2+}, respectively. Their dry matter was 1.99 and 3.52 gm at the level of control and 1.77 and 2.13 gm at the level of 350 ppm Cu^{2+} in cv. Assiut 84 and cv. Assiut 125, respectively. This contradiction was also extended when these copper-affected cultivars were sprayed by IAA, the cv. Assiut 84 responded slightly to IAA treatment. On the other hand, a considerable increase in the dry matter yield was recorded in the pods of cv. Assiut 125 at any used level of copper when compared to the non-sprayed plants treated by the same copper concentration. At the level of 350 ppm Cu^{2+}, the pod yield was 2-fold that of plants treated only by 350 ppm Cu^{2+}, without IAA spraying. Thus, even IAA behaved differently in its effect on the crop yield of the two tested broad bean cultivars. Thus, the different effects of IAA (the complexity and diversity in the behavior of IAA) were not a concentration effect. These different effects might be due to the synergistic effect between IAA and the different growth hormones during the different growth stages, particularly flowering which are accompanied by the appearance of florigen and phytochromes (the flowering and fruiting hormones) [35] [36] [37]. The data of carbohydrates contents in pods reveal that although the carbohydrates content increased considerably by copper in the cv. Assiut 84, especially at the level of 200 ppm Cu^{2+} addition, another side there is a slightly increase in cv. Assiut 125 up to 200 ppm only then, a reduction was recorded at 350 ppm Cu^{2+} treatment was recorded. Another situation was recorded a reduction in proteins values, was exhibited in cv. Assiut 84 and cv. Assiut 125. Thus, is there a correlation between increase in carbohydrate and a reduction protein metabolism in the pods of cv. Assiut 84 and cv. Assiut 125? The answer seemed to be complicated as these variable responses may be attributed to differences in genes expression and carbohydrates and proteins functions in different plant tissues. It has been shown that heavy metals also reduce biomass accumulation [5] [38] [39] [40] as a result of adverse effects upon key metabolic processes such as photosynthesis [41] (Rodriguez *et al.* 2012), mineral nutrition [42] and interactions with water [30] [33] [43]. The phytohormones, especially GAs, alleviate heavy metal stress before influencing other important developmental processes, such as flowering initiation, seed germination, and increasing plant height in crop plants [44] [45]. Similarly, in auxin groups, indole-3-acetic acid (IAA) is responsible for cell division, elongation, differentiation, root initiation, regulation of gene expression, and ROS homeostasis under abiotic stress conditions to enhance the growth and development process of the plant [46]. It is presumed that these findings might contribute to better understanding the response mechanisms of the two broad bean cultivars to copper stress and to further insights into copper-soil interaction in natural environments. There is a big variation in the accumulation of microelements and heavy metals in pods among the two broad bean genotypes. First of all, the water-treated pods of cv. Assiut 84 accumulated huge amount of aluminum. Interestingly, this amount of Al reduced by about 50% in pods of cv. Assiut 84 when treated by 350 ppm Cu^{2+} and decreased by more than 66% and 67% as a result of control plus IAA and 350 ppm Cu^{2+} plus IAA treatments respectively. The following situation was recorded in cv. Assiut 125, a) there are no significant changes of Al content in cv. Assiut 125 copper-treated pods. b) these values were considerably less than those of cv. Assiut 84. c) IAA enhanced the accumulation of this element in pods of cv. Assiut 125. A contrasting effect of IAA was exhibited IAA considerably reduced the accumulation of Al in cv. Assiut 84, it stimulated the accumulation of this element in the pods of cv. Assiut 125. These problematical behaviors (the correlation between Cu and Al) evoked us to point out that: Firstly there are two contrasting situations in the effect of Cu^{2+} on the accumulation of Al. In cv. Assiut 84 the amount of Al was reduced to about 50% by copper treatment, which means that the presence of copper in soil prevented the uptake of aluminum. On the other hand, in cv. Assiut 125 the concentration of Al in copper-treated pods seemed to be more or less similar to those of control. Accordingly, the differences in the uptake of Al could be attributed to cultivar variations rather than the effect of copper. Secondly this was recommended in hormonal-treated plants, that while IAA treatment dropped Al content in cv. Assiut 84, it stimulated the Al content in cv. Assiut 125, which again confirmed the complicated correlations of these genotypes and the role played by copper as well as IAA and the concentration effect of this dilemma might be ruled out. Al interference with the uptake, transport and utilization efficiency of most of the mineral elements have been well documented [47]. Tani and Barrington (2005) [48] showed that plant Cu and Zn uptake increased with two transpiration rates, with higher levels of Cu, Zn uptake by buckwheat was significantly reduced, while Zn had a slight but non-significant impact on Cu uptake. Previously and in a study exposing wheat plants to the same conditions, Cu significantly increased Zn uptake, while Zn had a slight but insignificant negative effect on Cu uptake. The buckwheat roots contained the greatest levels of Cu and Zn, indicating their role in moderating heavy metal uptake. The accumulation of iron was also interesting, in cv. Assiut 84, Fe considerably accumulated in copper-treated pods (it reached 276.14% of control). The opposite cut held in cv. Assiut 125 where iron was reduced by 23.6% in copper-treated pods in comparison with those of control. Hormonal treatments behaved differently among the two cultivars treated or not treated by copper. In cv. Assiut 84, IAA induced insignificant changes in iron content at the level of controls, but it considerably dropped the amount of iron in copper-treated plants, it retained the amount of Fe in copper-treated pods around their control. In cv. Assiut 125, while IAA nearly doubled the Fe content at the level of controls, it also, as in the case of cv. Assiut 84, retained the amount of Fe in copper-treated pods around their control. Why copper induced this considerable accumulation of iron only in cv. Assiut 84, is still a complicated question. Again, like aluminum, iron is copper independent, it is cultivar-dependent. Pätsikkä *et al.* (2002) [49] observed that excess Cu in hydroponic medium induces a Fe-deficiency in bean plants. Chen *et al.* (2004) [50] observed that Fe-deficiency induces Cu accumulation in *Commelina communis* L. plants. Yruela (2005) [51] reported that Cu and Fe compete in ion-uptake. Furthermore, Rombolà *et al.* (2005) [52] found that Fe deficiency increases the Cu content and decreases the Zn content in leaf blades of sugar beet grown hydroponically. Cu and Fe antagonism often occurs in plants grown under Cu toxicity [53]. These reports are not in agreement with our results. However, the opposite scenario has been also observed in oregano [54], rice seedlings [55] and wheat (*Triticum aestivum* L. cv. Vergina) [56] plants exposed to Cu toxicity in soil. An increasing concentration of soil Cu^{2+} resulted in a parallel increase in leaf Cu content with no reduction in the leaf Fe and Mg. Lara and Luca (2004) [57] reported that as the medium Cu concentration increased from 0.1 to 50 µM, iron uptake was strongly stimulated as indicated by tissue Fe concentration. Increasing the media $CuSO_4$ concentration to 100 µM had no effect stimulating Fe uptake. These apparently contradictory results may be explained by different tolerance strategies adopted by different plants [58] [59] [60] [61]. Bernal *et al.* (2007) [62] demonstrated that Cu interacts differently with Fe and Zn depending on the pathway through excess Cu is supplied. In the present work, cultivars variations as well as copper effect were also recorded in the absorption of zinc. While the Zn content in the pods of cv. Assiut 84 was more than double of that in cv. Assiut 125 at natural conditions, under copper treatments, while Cu caused a drop in the Zn content to about 50% in cv. Assiut 84, it, on the other hand, induced insignificant changes in this cation in cv. Assiut 125. In agreement with our data, Panou-Filotheou and Bosabalidis (2004) [63] reported that soil Cu^{2+} affected negatively the accumulation of Zn in roots of oregano. Additionally, an antagonist interaction between Cu and Zn was observed in *Chlamydomonas reinhardtii* [58]. Zinc plays an important role as an essential trace element in all living systems from bacteria to humans [64]. Interestingly, the opposite event was recorded in boron contents. While B remained around the control values in the copper-treated pods of cv. Assiut 84, it decreased significantly in pods of cv. Assiut 125 as a result of copper treatment. Results from Laboratory and Screen house experiments have been made by Azeez *et al.* (2015) [65] showed significant decrease in soil available P, Zn and Fe as rates of Cu increase over control experiment. The effect was more pronounced at application rate above 20 $mg \cdot kg^{-1}$ Cu. Gradual decrease in maize plant height, stem girth, leaf areas index, P, Zn and Fe uptake were observed as rate of Cu application increased. Hormonal treatment in most cases stimulated the accumulation of Zn in the two cultivars. Thus, the effect of IAA on Zn content was more or less similar in the two cultivars. This was opposite to its effect on Al and Fe, IAA induced almost insignificant changes in B contents. The amount of B was nearly the same in the two cultivars, whatever the treatments used, except for cv. Assiut 125 where there is some reduction in this element as a result of copper treatment. There are no significant changes in manganese content in pods of the two broad bean cultivars, whatever the treatments used. Yang and You (2009) [66] reported that Al can also reduce Mn toxicity and Mn accumulation in the plant. Thus, it is speculated that Al exerts an antagonistic effect on Mn uptake, and thus leads to alleviated Mn toxicity. Aluminum interference with P uptake might result in P deficiency in plants grown on acid soils or in nutrient solutions [67]. The pods of the two cultivars received very minute amounts of cadmium and cobalt, whatever the treatments used. Consequently, there are no differences in the accumulation of the two elements, which indicated that the used natural soil was very poor in Cd and Co. Lead (Pb) belongs among nonessential metals for plants and has no known biological function, and probably is one of the most frequently encountered heavy metals in polluted environment [68] [69]. Lead content is reduced by 50% as a result of copper treatment in the two tested cultivars. IAA mostly induced insignificant changes in Pb content except for cv. Assiut 84 plants irrigated by H_2O, where this element was reduced by 50% in relation to the control. The data of chromium indicated that while copper increased Cr content by about 100% in cv. Assiut 84, it, on the other hand, induced insignificant changes in pods of cv. Assiut 125 in relation to the control values. The results of copper content in pods of cv. Assiut 84 and cv. Assiut 125 reveal that while exogenous application of Cu^{2+} nearly doubled the Cu^{2+} content in pods of cv. Assiut 84, it induced a slight increase in Cu^{2+} content in pods of cv. Assiut 125. The percent increase in Cu contents in pods of cv. Assiut 84 and cv. Assiut 125 were 75% and only 11% respectively. Unexpectedly, the amount of copper increased highly significantly when the copper-treated plants were sprayed by IAA. This stimulatory effect in the uptake of Cu by IAA was more pronounced in cv. Assiut 84 than in cv. Assiut 125. It is worthy to mention that the amount of Cu in pods of cv. Assiut 84 subjected to 350 ppm Cu^{2+} was similar to that in pods treated by 350 ppm Cu^{2+} plus IAA, 2.8 and 2.9 mg/100 gm dry matter, respectively. The corresponding values in cv. Assiut 125 were 1.9 and 2.3 mg/100 gm dry matter in pods of 350 ppm Cu^{2+} and 350 ppm Cu^{2+} plus IAA, respectively, which also recommended the different responses to IAA according to the cultivars variations and the different treatments. Interactions of Zn, Mn, and Cu with macro and micronutrients are either synergistic, antagonistic or have no effects, depending on crop species and nutrients under investigation [70]. No consistent correlation was found between the Cu levels in the nutrient solution and either Fe or Mn levels in roots, stems, or leaves [59]. However, copper had significant synergistic effect on the uptake of P, K, and Mn in the upland rice plants. However, concentration of Ca, Mg and Fe were significantly decreased in these plants with the application of copper. Copper did not influence significantly uptake of Zn in rice plants. In bean plants, Cu application significantly increased uptake of Zn and had no significant effect on uptake of P, K, Ca, Mg, Mn andiron [71]. Ahmed *et al.* (2012) [72] reveal that treatments with different levels of Zn (0, 37.5 and 75 ppm), tryptophan (25, 50 and 100 ppm) and IAA (100, 200 and 300 ppm) foliar applications improved growth characters (shoot length, shoot thickness, leaves number, and leaves area), yield and fruit quality through their favorable effects on leaves chemical composition (plant pigments, total sugars, total soluble phenols, total free amino acids, tryptophan and endogenous plant hormones balance of leaves) as well as nutritional status (N, P, K, Mg, Zn, Cu, Fe and Mn concentrations) of leaves of *Valencia orange* trees. Chuan *et al.* (2016) [73] study the effect of various growth parameters on heavy metal uptake of vetiver grass and PGP traits enhanced the heavy metal remediation in the sterile Laboratory scale. Fassler *et al.* (2010) [74] he use of plants for phytoextraction of heavy metals from contaminated soil is limited by the ability plants to grow on these soils and take up the target metals, as well as by the availability of the metals for plant uptake in the soil solution. The hypotheses of this study were that the growth-promoting phytohormoneauxin (indole-3-acetic acid, IAA) can alleviate toxic effects of metals on plants and increase metal phytoextraction in combination with the biodegradable chelating agent ethylene diamine disuccinic acid (EDDS). Neto *et al.* (2017) [33] stated that heavy metals are natural non-biodegradable constituents of the Earth's crust that accumulate and persist indefinitely in the ecosystem as a result of human activities. Since the industrial revolution, the concentration of cadmium, arsenic, lead, mercury and zinc, amongst others, have increasingly contaminated soil and water resources, leading to significant yield losses in plants. These issues have become an important concern of scientific interest. Understanding the molecular and physiological responses of plants to heavy metal stress is critical in order to maximize their productivity. Recent research has extended our view of how plant hormones can regulate and integrate growth responses to various environmental cues in order to sustain life. In the present review we discuss current knowledge about the role of the plant growth hormones abscisic acid, auxin, brassinosteroid and ethylene in signaling pathways, defense mechanisms and alleviation of heavy metal toxicity. The data of individual amino acids reveal that there is a big difference in the concentration of the total amino acids among the two broad bean cultivars as a result of the different treatments (Cu^{2+} or Cu^{2+} plus IAA). In cv. Assiut 84, there is some activation in the total amino acids content in the pods as a result of Cu^{2+} or Cu^{2+} plus IAA treatments in relation to the absolute control value. This enhancement was more pronounced under the interactive effect of Cu^{2+} and IAA. This increase in the amino acids concentrations means that this cultivar used these amino acids to phytochelatin the excess copper and consequently to reduce its toxicity. This may be one of the defense strategies adopted by this cultivar and led to the maintenance of the values of the crop yield mostly around the control values, even at the severe dose of copper (the dry matter of the 350 ppm Cu^{2+}-treated pods is about 90% that of control pods). These increased amino acids could be used as free radical scavenger agents. The scavenging of the reactive oxygen species in heavy metal-treated plants is known to be a very important defense mechanism against the heavy metals toxicity. Interestingly, another situation was recorded in cv. Assiut 125, where the total amino acids in the pods dropped markedly by Cu^{2+} treatment. They were reduced by about 43% as a result of copper treatment. This situation confirms the role of amino acids as a defense mechanism against copper toxicity when we take into consideration the highly significant drop in the crop yield of this cultivar as a result of 350 ppm Cu^{2+} treatment (the pods dry matter was about 60% that of control as a result of 350 ppm Cu^{2+} treatment) (Sharma and Dietz (2006) [75]. Generally, heavy metal stress leads to a decrease in endogenous levels of auxins. For example, arsenic (As) is able to alter levels of three auxins (IAA, NAA, and indole-3-butyric acid, IBA) in *Brassica juncea* [76]. In another case, short-term cadmium treatment disturbed IAA homeostasis in barley root tips [77]. Previous work also indicates that Cadmium (Cd) suppresses primary root elongation in *Arabidopsis* [78]. Despite the detrimental effect of heavy metal in auxin metabolism, it has been reported that exogenous application of these phytohormones can rescue the endogenous levels of the auxins. An increase in the biomass of roots and stems of sunflower (*Helianthus annuus*) plants grown in soil moderately contaminated with lead (Pb) was observed after the addition of the phytohormone IAA [79]. Exogenous supply of IAA also improved the growth of *Brassica juncea* exposed to As [76]. In the same way, the application of different levels of L-TRP (a precursor of auxin) to the roots of rice seedlings growing in contaminated soil enhanced plant growth and yield under Cd stress, when compared to untreated seedlings in Cd-contaminated pots without this auxin precursor [80]. Some recent approaches showed that this synergistic or additive interaction between heavy metal and auxin can be used as a protective mechanism against toxicity in crop plants or as a useful tool in phytoremediation programs for detoxification of polluted areas. Tandon *et al.* (2015) [81] evaluate the application of six concentrations of two representative natural auxins (IAA and IBA), and a synthetic auxin (1-Naphthalene acetic acid), in wetland and non-wetland plant species in a water environment. The authors showed that exogenous auxin supply increased phytoremediation efficiency in wastewater treatment [33].

The role of IAA application in alleviating the adverse effects of stress factors including heavy metals was also suggested [82]; such as, the decrease in the level of ROS and the increase in seed germination rate, growth and nitrogen metabolism [83]. Exogenous GA3 is also able to overcome the inhibitory effects of different environmental stresses on seed germination and other physiological parameters; such as dry matter contents, chlorophyll, relative water, proline and mineral nutrients, activities of superoxide dismutase, peroxidase and polyphenol oxidase, as well as extent of electrolyte leakage [84]. Another situation was exhibited by this cultivar when its plants were sprayed by IAA. While the amino acids content decreased by 26% as a result of H_2O plus IAA treatment, they, on the other hand, increased by about 36% as a result of Cu^{2+} plus IAA treatment. The data also revealed that while the concentration of most of the individual amino acids increased as a result of Cu^{2+} or Cu^{2+} plus IAA treatments in cv. Assiut 84, the opposite cut held in cv. Assiut 125, where most of the individual amino acids decreased by Cu^{2+} or H_2O plus IAA treatments. However, the concentration of most of these individual amino acids increased surprisingly as a result of the interactive effect of Cu^{2+} and IAA. Amino acids and carboxylic acids such as citrate, malate and histidine are potential ligands for heavy metals and could play a role in tolerance and detoxification. Citrate, malate and oxalate have been involved in transport of metal ions through the xylem and vacuolar sequestering [85]. The significant and proportional change in amino acid or organic acid concentration elicited by a change in metal exposure was shown by histidine response in plants that accumulate nickel [3]. If we take into consideration the concentration of proteins in the pods of the two cultivars at the level of 350 ppm Cu^{2+}, we can notice that: a. In cv. Assiut 84, the high concentration of proteins was corresponding to high concentration of amino acids, compared to the control values, which again recommended the ability of this cultivar to regulate nitrogenous compounds. b. In cv. Assiut 125, it seemed that this cultivar failed to regulate these nitrogenous compounds at the severe dose of copper. Which again recommended the differences in copper tolerance between the two cultivars? The ability to regulate the nitrogenous compounds were considerably recommended when the two broad bean cultivars were treated by IAA at the level of 350 ppm Cu^{2+} (the great accumulation of both proteins and amino acids). Therefore, it can be concluded that the copper tolerance was closely associated with the up-regulation of nitrogen metabolism. Cultivars variations were also revealed in proline content. The pods of cv. Assiut 84 maintained the proline content mainly around the control value, whatever the treatment used is (Cu^{2+}, IAA or Cu^{2+} plus IAA). On the other hand, and surprisingly, while copper treatment reduced the proline content by about 62% in relation to the control, the Cu^{2+} plus IAA treatment elevated the proline content by about 38%. Accordingly, copper played two contrasting effects in the accumulation of proline in the pods of the two tested cultivars, an insignificant effect in cv. Assiut 84 and an inhibitory effect in cv. Assiut 125. Copper played two contrasting effects in the accumulation of proline in the pods of the two tested cultivars, an insignificant effect in cv. Assiut 84 and an inhibitory effect in cv. Assiut 125. Interestingly, the same pattern was recorded by IAA, where it induced insignificant changes in cv. Assiut 84 and stimulated the production of proline in cv. Assiut 125. This means that the biosynthesis of proline may be dependent on the cultivars variations rather than the effect of copper or IAA. However, previous results recorded the accumulation of proline in copper-treated crop plants [86]. Backor *et al.* (2004) [86] reported that proline inhibited metal-induced loss of potassium ion. Our results are in contrast with this observation in cv. Assiut 125, that while copper stimulated the absorption of potassium, it reduced the proline content by more than 60%. Additionally and surprisingly, another situation was observed in cv. Assiut 84, that this cultivar maintained both of them (proline and potassium) mainly around the control. Additionally, there is no correlation in the absorption and accumulation of calcium and magnesium and the criteria of proline in the two selected broad bean cultivars. Accordingly, these confusing results weakened the physiological significance of proline. Zhang *et al.* (2008) [87] indicated that Cu-responsive proline synthesis is closely related to NO generation in *C. reinhardtii*, suggesting the regulatory function of NO in proline metabolism under heavy metal stress. Azooz *et al.*, (2012) [88] support the biphasic effect of copper on Hassawi wheat growth. The stimulatory effect of Cu^{2+} on the biosynthesis of free amino acids, proline and antioxidant enzyme activities could serve as important components of antioxidative defense mechanism against Cu^{2+} toxicity. Noreen *et al.* (2018) [34] showed that application of 400 µM copper caused accelerated quantities of proline, protein and calcium.

Therefore, it can be concluded that there are differences in copper tolerance between the two cultivars and their interactions with IAA, it was closely associated with the regulation of nitrogen metabolism, microelement and proline content.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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