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Role of Indole-3-butyric Acid or/and Putrescine in Inproving Productivity of Chickpea (*Cicer arientinum* L.) Plants

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Abstract: The response of chickpea (*Cicer arientinum* L. cv. Giza 3) to treatment with two plant growth regulators putrescine (Put) and Indole-3-butyric acid (IBA) at 25, 50 and 100 mg L^{-1} applied either alone or in combinations was studied. Spraying of Put and IBA either individually or in combination significantly increased the plant height, number and dry weight of branches, leaves and pods/plant and leaf area/plant at the two growth stages. Total photosynthetic pigments in fresh leaves were significantly promoted as a result of application of Put or IBA. Generally, application of Put and/or IBA at 100 mg L^{-1} produced the highest numbers of pods which resulted in substantially the highest seed yield. Put and IBA increased the seed yield by 21.3 and 19.2%, respectively, while the combination of Put at 100 mgL^{-1} and IBA at 50 mgL^{-1} increased it by 27.4%. Greatest increases in straw and biological yield/fed (38.3 and 30.4%, respectively) were noted with the combination treatment of IBA 100 mg L^{-1} plus Put at 100 mg L^{-1}. Put and IBA significantly increased the nitrogen, phosphorus, potassium, total soluble sugars and total free amino acids in chickpea seeds over control, but the effects were less marked than those of their combination. This response was greater following treatment with IBA than with Put. It could be conclude that spraying Put or/and IBA on chickpea plants have promotion effects on the seeds yield criteria which have promising potential as sources of low-cost protein and minerals for possible use as food/feed supplements.

Key words: Chickpea, putrescine, Indole-3-butyric acid, growth, biochemical responses, photosynthetic pigments

INTRODUCTION

Grain legumes are important crop plants for their protein rich seeds that used as a major source of dietary protein for human and livestock consumption. In addition, legumes can be efficiently used for improving soil fertility (Manchanda and Garg, 2008).

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries (Jukanti *et al*., 2012). Chickpea is the cheapest and readily available source of protein (20.6%), (Gupta, 1987), carbohydrates (61.2%) (Gupta, 1987), rich in vitamins and minerals and is relatively free from anti-nutritional factors (Wang *et al*., 2010). Because of the great importance of chickpea as a cheapest and readily available source of protein food for large population groups, particularly in developing countries, many efforts have been made to improve chickpea productivity (Alvi *et al*., 2005; El-Kramany *et al*., 2009; Niari-Khamssi, 2011).

Plant growth regulators (PGRs) are representing one of the controlling factors that regulate growth, biosynthesis of chemical constituents, yield and may be improve adaptation of plants to environment. Polyamines (PAs) are polycationic compounds of low molecular weight that are present in all living organisms (Cohen, 1998). Some studies have shown the positive effects of PAs on cell division and its rate (Cvikrova *et al*., 1999), morphogenesis, floral initiation and development (Paschalidis and Roubelakis-Angelakis, 2005; Liu *et al*., 2006; Pang *et al*., 2007; Khan *et al*., 2012). PAs effectively retard senescence by retarding the loss of chlorophyll (Couee *et al*., 2004; Tang *et al*., 2004). Putrescine application (up to 5 mM) significantly increased the content of photosynthetic pigments of wheat (*Triticum aestivum*) plants (El-Bassiouny *et al*., 2008). PAs have been shown to be an integral part's response to stress (Walters, 2003; Alcazar *et al*., 2006; 2010). Exogenous putrescine and sermidine markedly modified the stress-induced effects in plants (Amooaghaie, 2011). Moreover, putrescine treatments significantly increased fresh and dry weights of bean plants (Nassar *et al*., 2003). Putrescine at 10^{-5} M increased grain and biological yield and grain index of wheat plant (Gupta *et al*., 2003).

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Exogenous application of putrescine and spermidine increased endogenous PGRs, particularly growth promoters (Indole-3- acetic acid, gibberellins and cytokinins) and decreased inhibitors (abscisic acid) (El-Bassiouny and Bakheta, 2005; Iqbal *et al*., 2006). Moreover, auxins are implicated in various metabolic processes as nucleic acid synthesis (Krishnamoorthy, 1981; Alvi *et al*., 2005).

Indole-3-butyric acid significantly increased the vegetative growth criteria, seed yield and protein content in rice (Chhun *et al*., 2004), maize and onion plants (Amin *et al*., 2006; 2007). Soaking chickpea seeds with 10^{-8} M IAA improved nitrate reductase activity, total protein, calcium and potassium contents (Alvi *et al*., 2005). Moreover, improvement in nitrogen metabolism, photosynthesis and yield of chickpea were noted with the application of 10^{-8} M of IAA, IBA, or 4-Cl-IAA (4-chloroindole-3-acetic acid) (Hayat *et al*., 2009).

Considering the positive benefit that Put and IBA have had on productivity of several crop plants and to ascertain whether these two PGRs would also be able to improve morphological parameters and seed nutritional value of a cereal-based diet.

Therefore, effect of spraying chickpea plants with different concentrations of putrescine and Indole-3-butyric acid, individually or in combination on some morphological criteria, yield, as well as some metabolic constituents of the seeds were assessed in this work.

MATERIALS AND METHODS

Two field experiments were carried out at the Agricultural Experimental Station, National Research Centre, Nubaria, Behira Governorate, Egypt, during the two successive seasons of 2010/2011 and 2011/2012 to study the effect of foliar application of different concentrations of Put and IBA, individually or in combination on some growth criteria, photosynthetic pigments and yield as well as some metabolic constituents of chickpea seeds.

The experimental design was split-plot design with four replications. The IBA treatment occupied the main plots and Put treatments were allocated at random in sub-plots. The plot area was 10.5 m^2 (3.5×3.0 m) and consisted of five rows 70 cm apart and the distance between hills along the row 25 cm apart.

Seeds of chickpea (*Cicer arietinum* L.) cv. Giza 3, were obtained from Agricultural Research Centre, Giza, Egypt and sown on 23 November in both seasons. The normal cultural treatments of growing chickpea in the location were followed. Pre-sowing, 30 kg/fed. calcium super-phosphate (15.5% P_2O_5) was applied to the soil. While, nitrogen fertilizer (35 kg N/fed) as ammonium nitrate (33.5% N) were applied in two equal doses before the first and second irrigation, respectively.

In both seasons, a foliar spray was applied twice to chickpea plants during vegetative growth at 45 and 60 days after sowing (DAS) with putrecine (Put) as polyamine (Polycarbonic and aliphatic nitrogenous compounds $[(H_3N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH_3])$(approx 97%) obtained from Sigma Company, USA and Indole-3-butyric acid (IBA, Aldrich). The two bioregulators were applied at 25, 50 and 100 mgL^{-1} either individually or in combination, in all possible permutations. Solutions were sprayed over the entire surface of plant including the adaxial and abaxial surfaces of leaves. No additives or surfactants such as Tween were added. In addition, untreated plants (control) were sprayed only with tap water.

The plant growth characters were measured at 75 and 90 days after sowing i.e., green yield as plant height, number of branches, leaves and pods/plant, dry weight of branches, leaves and pods/plant and leaf area (cm^2/plant) following the suggestions of Bremner and Taha (1966). Photosynthetic pigment (chlorophylls a, b, carotenoids as well as total pigments) in the fresh leaves was determined at two growth stages (75 and 90 DAS) according to Saric *et al*. (1967).

At harvest time, ten guarded plants were taken out randomly from the middle two ridges of each plot to determine the mean values of yield and its related parameters, i.e. plant height, number of pods and seeds/plant, dry weight of pods/plant, seed and straw yield per plant and harvest index [(grain yield/ biological yield) ×100]. Seed, straw and biological yields per feddan (fed = 4200 m^2) were determined by harvesting the whole plot area.

Plant samples were taken from each plot and dried at 70°C for 48 h until constant dry weight. Representative dry samples of seeds were taken from each plot to determine the total soluble sugars using the phenol sulphuric method (Dubois *et al*., 1956). Crude protein percentage was calculated by multiplying the values of total nitrogen by 6.25 (AOAC, 1988). Phosphorus was determined according to Watanab and Olsen (1965) and potassium by Jackson (1965).The method of Rosen (1957) was used to estimate the total free amino acids.

Data for both growing seasons was carried out according to Snedecor and Cochran (1990). Data was combined since CV% was <5%. Treatments means were compared using Fisher's least significant differences (LSD) at p = 0.05.

RESULTS

Growth parameters: Data presented in Table 1 and 2 show that spraying Indole-3-butyric acid (IBA) and putrecine (Put) at 25, 50 and 100 mg L^{-1} individually or in combination promoted almost growth criteria compared to corresponding untreated plants. In all cases, the

Table 1: Effect of Indole-3-butyric acid (IBA) and Putrescine (Put) on growth characters of chickpea plants at (A) 75 and (B) 90 days after sowing. (Combined analysis of two seasons)

	Growth parameters							
			Number plant^{-1}					
	Plant height (cm)		Branches		Leaves		Pods	
Treatments (mgL^{-1})	A	B	A	B	A	B	A	B
IBA								
0	67.8	69.0	12.9	14.9	135.10	160.1	50.3	60.0
25	70.9	74.5	14.8	16.9	164.20	181.7	63.9	68.1
50	78.4	88.1	16.7	19.4	169.20	189.8	66.6	72.2
100	89.7	92.3	20.7	23.5	196.19	210.9	68.4	74.3
LSD at 5%	3.0	5.1	1.9	2.0	24.50	19.0	3.4	5.9
Put								
0	66.3	70.3	12.3	14.4	134.5	159.6	49.7	59.9
50	73.4	79.2	14.5	16.6	162.9	179.8	60.7	66.6
75	82.3	87.4	17.5	19.7	178.8	190.8	62.9	69.0
100	87.8	91.8	18.6	21.5	192.4	199.4	65.3	71.2
LSD at 5%	7.2	8.8	2.1	2.1	26.0	20.1	5.8	6.9

	Dry weight (g plant^{-1})							
	Branches		Leaves		Pods		Leaf area (cm^2 plant^{-1})	
Treatments (mgL^{-1})	A	B	A	B	A	B	A	B
IBA								
0	4.94	5.90	3.43	3.97	5.89	7.57	1091	1080
25	6.19	4.64	4.19	4.86	7.49	8.66	1170	1099
50	6.71	8.90	4.49	5.42	8.13	8.71	1253	1147
100	8.14	9.26	5.66	5.59	8.59	9.11	1267	1157
LSD at 5%	1.22	1.56	0.76	0.70	1.53	0.87	12	11
Put								
0	4.84	5.76	3.13	3.80	5.57	7.33	1068	1043
25	5.89	6.89	4.06	4.59	7.59	8.41	1107	1060
50	6.90	7.79	4.32	5.12	7.89	8.67	1178	1104
100	7.87	8.78	4.91	5.30	8.26	8.97	1240	1123
LSD at 5%	1.03	1.12	0.89	0.78	2.00	0.96	12	95

increments in growth parameters were often highly significant in comparison with untreated control plants. The most effective treatments on most growth parameters were the combination between Put at 100 mg L^{-1} and IBA at 100 mg L^{-1} followed by IBA alone at 100 mg L^{-1} treatment.

When considered Put as a single factor, The results in Table 1 illustrate that application of Put up to 100 mg L^{-1} significantly increased the plant height, number of branches, leaves and pods/plant, dry weight of branches, leaves and pods/plant and leaf area compared to untreated plants at 75 and 90 days after sowing (DAS). Furthermore, when considering IBA as a single factor, it was noted that IBA treatments were more effective than Put treatments in increasing vegetative growth of chickpea plants at the two different growth stages (Table 1).

The increment in growth characters studied was more pronounced with spraying IBA at 100 mg L^{-1}. An increase of 57, 42 and 20% over control occurred in dry weight of branches, leaves and pods at 90 DAS with the application of (100 mg L^{-1}) IBA on chickpea plants, respectively. While the Put showed corresponding increments 52, 40 and 22%, respectively (Table 1).

Data in Table 2 show significant increases in some growth parameters under the effect of combination of different concentrations of Put and IBA when compared to control plants. The combination treatment of IBA at 100 mg L^{-1} + Put at 100 mg L^{-1} enhanced the most growth parameters-to great extent- at the two physiological growth stages. This superior combination treatment caused a significant increase (17%) in the leaf area/plant at 75 DAS compared to untreated plants.

Photosynthetic pigments: Data presented in Table 3 show that Chl a, b, carotenoids and total photosynthetic pigments in chickpea leaves reached a maximum value at 90 DAS. Generally, foliar application with either Put or IBA at any concentration significantly increased the Chl a, b and carotenoids and consequently the total pigments more than controls at 75 and 90 DAS. The most effective concentration was 100 mg L^{-1} of either IBA or Put at the two growth stages.

It is worthy to mention that IBA treatments were more effective in increasing the Photosynthetic pigments than Put treatments. IBA treatments had much higher photosynthetic pigments than Put by 5 and 4% at 75 and 90 days after sowing, respectively and it increased by 28 and 27%, respectively, as compared to controls (Table 3).

Table 2: Effect of interaction between Indole-3-butyric acid (IBA) and Putrescine (Put) on growth characters of chickpea plants at (A) 75 and (B) 90 days after sowing. (Combined analysis of two seasons)

Treatments (mgL^{-1})		Growth parameters							
				No. $Plant^{-1}$					
		Plant height (cm)		Branches		Leaves		Pods	
IBA	Put	A	B	A	B	A	B	A	B
0	0	65.2	70.1	12.3	14.3	135.1	158.9	44.3	52.50
	25	74.2	77.9	13.6	16.4	159.2	176.8	53.4	60.30
	50	81.3	85.3	15.7	19.6	163.6	182.7	59.7	66.20
	100	86.6	91.7	17.8	21.7	174.4	189.8	62.9	71.10
25	0	67.6	72.7	13.0	15.5	137.6	160.1	47.6	54.20
	25	76.4	79.5	14.6	17.8	164.7	179.3	59.0	65.70
	50	84.7	89.4	16.5	20.8	167.8	187.4	62.6	69.40
	100	89.8	95.5	19.7	22.1	178.9	196.6	67.3	74.50
50	0	69.9	75.2	14.9	15.2	139.0	165.8	49.4	57.50
	25	79.4	82.2	16.3	18.3	169.1	186.9	62.5	69.70
	50	86.1	90.7	17.7	20.3	170.3	190.0	64.5	71.80
	100	92.3	96.8	19.7	23.4	179.3	198.1	67.6	76.80
100	0	70.4	77.7	14.8	16.5	142.3	170.3	51.7	60.06
	25	80.5	86.6	16.7	18.7	174.4	189.4	63.9	70.10
	50	88.5	92.8	18.8	22.0	176.5	196.5	66.9	73.60
	100	94.7	98.8	20.8	24.1	180.6	206.6	69.1	79.20
	LSD at 5%	5.1	6.0	1.3	1.8	6.5	7.0	1.6	3.10

Treatments (mgL^{-1})		Dry weight (g $plant^{-1}$)							
		Branches		Leaves		Pods		Leaf area (cm^2 $plant^{-1}$)	
IBA	Put	A	B	A	B	A	B	A	B
0	0	4.60	5.98	4.05	4.23	6.39	7.62	1076	1070
	25	5.09	6.89	4.63	4.69	8.34	8.78	1088	1084
	50	5.89	7.38	4.88	4.87	8.96	9.67	1109	1097
	100	6.66	8.06	5.03	4.96	9.31	10.31	1124	1111
25	0	4.89	6.38	4.33	4.39	6.88	7.89	1090	1087
	25	5.55	7.44	4.76	4.81	8.72	9.57	1098	1093
	50	6.19	8.69	4.90	4.93	9.20	10.18	1147	1106
	100	7.46	8.97	5.13	5.03	9.53	11.02	1209	1156
50	0	5.09	6.77	4.49	4.44	7.30	8.36	1101	1095
	25	5.86	7.89	4.81	4.96	9.09	10.12	1143	1130
	50	6.58	8.76	5.02	4.98	9.51	11.43	1170	1156
	100	7.80	9.49	5.39	5.32	9.64	12.16	1229	1209
100	0	5.29	6.96	4.52	4.56	7.65	9.71	1137	1106
	25	6.06	7.92	4.9	4.99	9.29	11.19	1156	1124
	50	7.37	9.19	5.33	5.12	9.74	12.21	1237	1195
	100	8.86	9.86	5.6	5.36	9.87	12.69	1257	1234
	L.S.D. at 5%	0.33	ns	ns	ns	ns	0.50	14	12

Table 3: Effect of Indole-3-butyric acid (IBA) and Putrescine (Put) on the photosynthetic pigments content (mg/g fresh weight) in the leaves of chickpea plants at 75 and 90 days after sowing. (Combined analysis of two seasons)

Treatments (mgL^{-1})	At 75 days after sowing				At 90 days after sowing			
	Chl. a	Chl. b	Carotenoids	Total pigments	Chl. a	Chl. b	Carotenoids	Total pigments
IBA								
00	0.65	0.17	0.27	1.09	0.68	0.19	0.30	1.17
25	0.70	0.19	0.30	1.19	0.74	0.21	0.31	1.26
50	0.79	0.21	0.33	1.33	0.81	0.23	0.35	1.39
100	0.82	0.23	0.35	1.40	0.87	0.24	0.37	1.48
LSD at 5%	0.03	0.02	0.02	0.03	0.05	0.02	0.02	0.03
Put								
00	0.64	0.16	0.27	1.07	0.68	0.19	0.29	1.16
25	0.69	0.18	0.29	1.16	0.73	0.21	0.31	1.25
50	0.76	0.19	0.31	1.26	0.79	0.22	0.34	1.35
100	0.80	0.21	0.33	1.34	0.84	0.23	0.36	1.43
LSD at 5%	0.04	0.01	0.02	0.02	0.03	0.02	0.03	0.03

On the other hand, the interactions between IBA and Put show insignificant effects on Chl. a, b, carotenoids and total photosynthetic pigments content in chickpea leaves at the two stages of growth, therefore the data were excluded.

Table 4: Effect of Indole-3-butyric acid (IBA), Putrescine (Put) and their interaction on yield characters of chickpea plants. (Combined analysis of two seasons)

		Yield characters								
		Number plant^{-1}			Yield (g plant^{-1})			Yield (kg feddan^{-1})		
Treatments (mgL^{-1})	Plant height (cm)	Pods	Seeds	Pods	Seeds	Straw	Seeds	Straw	Biological	Harvest index
IBA										
0	78.1	50.30	74.90	22.1	16.6	19.4	743	991	1734	0.42
25	88.3	57.50	80.53	26.7	18.6	20.5	769	1090	1859	0.43
50	95.8	67.80	89.00	30.1	20.3	23.0	859	1160	2019	0.42
100	99.2	76.90	93.10	33.3	22.4	23.8	886	1210	2096	0.42
LSD at 5%	4.0	2.96	3.01	2.9	1.3	0.8	23	120	92	ns
Put										
0	76.7	49.2	74.30	21.3	16.2	19.0	723	989	1712	0.42
50	87.3	54.6	79.30	25.6	18.0	21.3	809	1030	1839	0.43
75	92.4	65.3	84.40	29.1	19.4	22.4	839	1120	1959	0.43
100	97.6	72.4	89.40	33.3	21.1	23.4	877	1160	2037	0.43
LSD at 5%	5.1	3.1	4.10	3.8	1.0	1.0	34	20	110	ns

			Yield characters								
			Number plant^{-1}			Yield (g plant^{-1})			Yield (kg feddan^{-1})		
Treatments (mgL^{-1})		Plant height (cm)	Pods	Seeds	Pods	Seeds	Straw	Seeds	Straw	Biological	Harvest index
0	0	70.9	50.6	76.5	22.8	17.9	18.4	690	839	1529	0.45
	25	76.8	59.4	84.8	25.6	19.4	20.0	738	927	1665	0.44
	50	81.8	64.3	89.2	28.5	21.5	22.1	770	964	1734	0.44
	100	87.6	69.8	93.9	31.1	22.8	22.7	809	1060	1869	0.43
25	0	74.5	54.1	79.3	23.9	18.3	19.2	739	860	1599	0.45
	25	79.6	62.6	86.4	27.5	20.7	21.6	742	998	1740	0.44
	50	84.0	69.7	92.4	31.8	22.3	23.5	813	1020	1833	0.44
	100	89.7	74.8	96.5	33.4	23.9	24.0	844	1100	1944	0.42
50	0	76.0	59.5	82.6	26.5	19.7	21.5	744	969	1713	0.43
	25	87.3	66.6	87.7	29.9	21.0	23.9	770	1020	1790	0.43
	50	92.4	73.2	94.8	34.9	22.7	24.1	826	1120	1946	0.42
	100	96.9	79.6	97.8	36.0	23.9	24.8	879	1140	1969	0.42
100	0	78.4	62.3	84.5	28.1	19.9	22.7	760	976	1736	0.43
	25	89.7	69.5	89.0	32.9	21.9	23.9	813	1060	1873	0.43
	50	94.6	79.6	96.1	35.6	23.7	24.5	827	1140	1967	0.42
	100	99.5	84.9	99.2	38.0	24.0	25.0	833	1160	1993	0.42
	LSD at 5%	4.3	5.1	4.6	2.1	1.2	0.5	21	26	100	ns

Yield and its components: Data presented in Table 4 show that application of Put alone up to 100 mg L^{-1} significantly increased the chickpea yield criteria (number of branches, pods and seeds/plant, seed and straw yield per plant and per fed and biological yield/fed, while harvest index were not differ than control plants. This response was greater following treatment with IBA than with Put.

Application of IBA and Put increased the seed yield by 21.3, 18.3%, respectively, while the combination treatment of IBA at 50 mgL^{-1} and Put at 100 mgL^{-1} increased it by 27.4%, compared to untreated plants (Table 4).

Application of IBA exhibited more chickpea seed yield if compared to the untreated plants (Table 4). The most promising results (0.88, 1.21 and 2.10 t/fed) were obtained with 100 mg L^{-1} IBA for seed, straw and biological yield, respectively.

Furthermore, chickpea yield was more sensitive to the interaction between Put and IBA (Table 4). The highest increase in number of pods and seeds/plant, dry weight of pods/plant, seeds and straw yield/plant, as well as seed, straw and biological yield/fed were obtained by foliar application with 100 mg L^{-1} Put+100 mg L^{-1} IBA followed by 100 mg L^{-1} IBA (Table 4). On the other hand, the interaction between Put and IBA show insignificant changes in harvest index compared to the control plants.

Chemical constituents: Data presented in Table 5 show that application either Put or IBA at any concentration as well as their combination increased nitrogen, phosphorus, potassium, crude protein, total sugar and total free amino acids in the dry seed of chickpea compared with their control. The most effective treatment in this concern was 100 mg L^{-1} of either Put or IBA and their combination. Increasing the concentration of IBA from 25 to 50 and to 100 mg L^{-1} cause a significant increase in the values of P % only, while the rest of chemical constituents studied were not significantly affected by increasing the concentration of IBA and Put (Table 5). However the three concentration of the two bioregulators caused a significant increase in the values of chemical constituents compared to untreated plants.

Table 5: Effect of Indole-3-butyric acid (IBA), Putrescine (Put) and their interaction on chemical constituents of chickpea seeds (Combined analysis of two seasons)

Treatments (mgL^{-1})	N %	P %	K %	Crude protein %	Total soluble sugar %	Free amino acids (mg g^{-1} DW)
IBA						
00	3.2	0.34	1.98	20	4.96	61.01
25	3.5	0.35	2.19	21.9	5.99	69.3
50	3.8	0.39	2.26	23.8	6.39	73.26
100	4.0	0.42	2.32	25.2	6.43	76.18
LSD at 5%	0.3	0.03	0.16	2.1	0.86	5.01
Put						
00	3.1	0.33	1.96	19.4	4.89	60.71
25	3.4	0.38	2.1	21.3	5.94	67.6
50	3.6	0.39	2.16	22.6	6.18	70.01
100	3.7	0.4	2.2	23.1	6.29	73.44
LSD at 5%	0.3	0.05	0.12	1.8	0.62	6.33

Treatments (mgL^{-1})		N %	P %	K %	Crude protein %	Total soluble sugar %	Free amino acids (mg g^{-1} DW)
0.0	0	3.0	0.33	1.94	18.9	4.91	59.61
	25	3.1	0.35	2.09	19.6	5.87	62.29
	50	3.2	0.37	2.11	20.1	6.19	65.38
	100	3.3	0.39	2.14	20.9	6.26	69.44
25.0							
	0	3.1	0.34	1.96	19.2	5.88	60.52
	25	3.2	0.36	2.10	19.7	6.14	64.64
	50	3.5	0.38	2.15	21.6	6.22	66.76
	100	3.5	0.39	2.19	22.1	6.31	70.8
50.0							
	0	3.1	0.35	2.12	19.6	6.01	62.91
	25	3.5	0.37	2.16	21.8	6.21	66.02
	50	3.6	0.39	2.20	22.2	6.28	72.41
	100	3.6	0.40	2.22	22.6	6.39	75.29
100.0							
	0	3.2	0.36	2.19	19.8	6.19	64.30
	25	3.7	0.38	2.23	22.9	6.29	67.46
	50	3.9	0.40	2.29	24.3	6.33	73.50
	100	4.3	0.43	2.34	25.5	6.44	77.69
	L.S.D. at 5%	0.1	ns	ns	0.5	ns	1.76

Furthermore, the results in Table 5 indicate that among the treatments Put (100 mg L^{-1}) followed by IBA (100 mg L^{-1}) and the combination between the two bioregulators at 100 mg L^{-1} recorded the highest level of N, P,K, crude protein, total soluble sugars and total free amino acids.

DISCUSSION

The present study indicated that foliar application with Put and IBA up to 100 mg L^{-1}, single or in combination greatly promoted the vegetative growth and dry matter production of branches, leaves and pods by enhancing cell division and chlorophyll accumulation. In other crops, Put stimulated growth by increasing the amount of endogenous promoters (auxin, gibberellins and cytokinins) accompanied by a decrease in the content and activity of inhibitors (ABA) (El-Bassiouny and Bakheta, 2005; El-Bassiouny *et al.*, 2008). Abbas and El-Saeid (2012) reported that IBA treatment increased endogenous GA concentrations in lemon grass plants by 10% and 23% compared with the control for plants treated with 25 and 100 ppm, respectively. In this study, Indole-3-butyric acid has shown more pronounced effect than putrescine (100 mg L^{-1}) in promoting growth and yield of chickpea plants may be due to increase the nitrogen uptake and regulation of different metabolic processes. Similarly, promoting effects of IBA on growth and photosynthetic pigments was obtained on maize and onion (Amin *et al.*, 2006, 2007). Intuitively the combination of Put and IBA increased vegetative growth and dry matter production of chickpea more effective than individual treatments.

Photosynthetic pigments of chickpea leaves-at 75 and 90 DAS- were significantly enhanced by application of Put or IBA. Since, Put or IBA treatments might retard Chl destruction and/or increase their biosynthesis or stabilize the thylakoid membrane. PAs may retard senescence and chlorophyll loss by altering the stability and permeability of membranes and protecting chloroplast from senescing (Gonzalez-Aguilar *et al.*, 1997). PAs have been shown to affect Chl stabilization and antisensecence (Da Silva, 2006; Ioannidis *et al.*, 2009).

Put increased endogenous cytokinins, which stimulate Chl biosynthesis and chloroplast differentiation in wheat (*T. vulgare*) (Xie *et al.*, 2004; El-Bassiouny *et al.*, 2008). On the other hand, IBA increased photosynthetic pigments content due to the stimulatory effect on the amount of metabolites synthesized through enhancement of cell division and chlorophyll accumulation which leads to higher rate of photosynthesis (Ludwig-Muller, 2000). El-Tohamy *et al.* (2012) reported that the higher levels of putrescine significantly increased all vegetative growth parameters, yield, T.S.S. of fruits and total chlorophyll content of leaves of Cape gooseberry (*Physalis peruviana* L.).

Promoting effects of PAs and IBA on photosynthetic pigments was observed in cucumber (*Cucumber sativus* L.) (He *et al.*, 2002); common bean (*Phaseolus vulgare*) (Nassar *et al.*, 2003); maize (*Zea mays* L.) and onion (*Allium cepa* L.) (Amin *et al.*, 2011; Ghodrat *et al.*, 2012). Put treatments concomitantly with increasing Mg levels (a structural component of Chl) retarded senescence (Kao, 1994; El-Bassiouny *et al.*, 2008). Although, combination of Put and IBA caused insignificant increases in photosynthetic pigments of chickpea leaves (Data not shown).

Chickpea yield and quality of seeds were significantly enhanced by application of Put and IBA, especially at the highest concentration. Rice grain filling and 1000-seed weight were positively correlated with PA content (Yang *et al.*, 1996). On the other hand, auxins (IBA) are involved in many aspects of growth and development of plants (Ludwig-Muller, 2000).

IBA promotes growth, increases building metabolites, retards senescence, enhances cell division, chlorophyll accumulation and stimulates dry matter production as a result of higher photosynthetic activity and consequently increased translocation and accumulation of microelements in plant organs (Shafey *et al.*, 1994; Chhun *et al.*, 2004). IBA increased vegetative growth, seed yield per plant and per feddan, seed protein content in rice (Chhun *et al.*, 2004), maize and onion (Amin *et al.*, 2006, 2007). The combination between Put and IBA was more effective than Put or IBA individually for increasing the productivity of chickpea plants by enhancing photosynthetic activity, accumulating dry matter and retarding senescence. Consequently, these treatments increased translocation and accumulation of certain metabolites in plant organs which affected yield quantity and quality.

Foliar application with either Put or IBA and their combination increased nitrogen, phosphorus, potassium, crude protein, total soluble sugars percent and total free amino acids in the dry seeds of chickpea notably due to their bioregulatory effect on enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites. PAs linked with particular proteins (Serafini-Fracassini and Del Duca, 2008) and this covalently bound PA-protein complexes have been reported in artichoke tubers (*Helianthus tuberosus*) (Dinnella *et al.*, 1992) and tobacco (*Nicotiana* spp.) (Sawhney and Applewhite, 1993). PAs are able to bind with pectin and polysaccharides (D'oraci and Bagni, 1987) and increased α-amylase activity (Tipirdamaz *et al.*, 1995). Spm and Put significantly increased the total sugar content but decreased total free amino acids in chamomile (*Matricaria chamomilla*) leaves (Abd El-Wahed and Krima, 2004; Abd El-Wahed and Gamal El Din, 2005). On the other hand, IBA promotes growth, increases building metabolites and consequently increases translocation and accumulation of building metabolites to seeds. The highest values of total sugar and crude protein content in soybean, wheat, rice and maize (Dybing and Lay, 1982; Ludwig-Muller, 2000; Chhun *et al.*, 2004) and highest total free amino acids, total phenols and total Indoles in onion (Amin *et al.*, 2007) were obtained by foliar application of IBA at 100 mg L^{-1}.

The combination treatments of Put and IBA at 100 mg L^{-1} was very effective in enhancing nitrogen, phosphorus, potassium, crude protein, total sugar content and total free amino acids and consequently increased nutritional value of chickpea seeds as a cereal-based diet more than Put and IBA used individually.

In conclusion, application of Put and IBA (up to 100 mg L^{-1}) individually or in combination, resulted in a significant increase in morphological attribute, including number of pods, seed, straw and biological yield per fed, the main consumer-related traits. The use of Put and IBA improved the biological yield and nutritional value of chickpea seeds, the highest prior in selecting high yielding chickpea plants.

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