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Effect of Indole-3-Acetic Acid and Benzyl Adenine on Growth Parameters and Yield of *Pisum sativum* L. Plants

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ABSTRACT

Pea (*Pisum sativum intisar2*) is one of the most important leguminous vegetable crops worldwide. This study was carried out to evaluate the effect of indole-3-acetic acid (IAA), benzyl adenine (BA) or their interaction on the growth vigor of both root and shoot, leaf area, pigment content as well as yield attributes and metabolism of pea plants. In the majority of cases, primily pea seeds in 10^{-9} µg/ml IAA, 10^{-6} µg/ml BA or their combination caused marked increase ($p \leq 0.05$) in root, shoot growth vigor, pigment content, total soluble sugars, total protein of pea leaves as well as yield attributes (i.e pods number/plant, no of seeds/ pod and 100 seed weight). The magnitude of increase of pea plants was more pronounced in the following order: IAA > (IAA+BA)> BA. In conclusion, the results cleared that IAA is the best treatment for improving the growth and productivity of pea plants.

Keywords: Benzyl Adenine, Indole-3-Acetic Acid, Pisum sativum.

INTRODUCTION

Pea (*Pisum sativum intisar2*) occupies the second rank after bean as primary dietary legumes (Graham and Vance, 2003). During the winter season in Egypt, the pea is cultivated for local consumption and exportation. Pea contains large amounts of amino acids, tryptophan and lysine, which are relatively low in cereal grains. Pea contains low contents of fiber, contain 86 percent of total digestible nutrients, 21-25 percent protein and high levels of carbohydrates. These features make the pea plant a perfect livestock feed (Kent and Endres, 2003). Pods of pea contain 58.6 g/100 g dietary fiber in addition to fructose, sucrose, glucose, and protein in addition to the most important fatty acid, oleic acid, in an observable amount (Mateos-Aparicio).

In order to face today's world increasing demand of agricultural products, chemical regulators of plant growth either natural or artificial in origin are currently used to produce high- value horticultural crops and to increase the yield (Emongor, 1997). Collectively, these chemical compounds affect all the aspects of plant growth, development as well as response to biotic "defense mechanisms" and abiotic stress (Santner and Estelle, 2009).

The main natural plant growth promotor is auxin that is responsible for cell enlargement, division, and differentiation (Teale *et al.*, 2006). The transportation of it from cell to cell ocurs via a unique transport system. Cytokinins affect seed germination, root and shoot differentiation, leaf senescence and plant-microbe interactions particularly the formation of nodules not only auxins (Santner and Estelle, 2009). Seed priming with indole-3-acetic acid (IAA) at 25 mg kg^{-1} stimulated the flag leaf growth, namely its fresh an dry masses, leaf area, pigment, saccharides and protein formation as well as its effect on $^{14}co_2$ fixation in wheat flag leaf (Aldesuquy, 2000).

This work was undertaken to investigate the efficacy of IAA, BA either alone or in combination to maximize pea plants biomass as well as their productivity.

MATERIALS AND METHODS

Pot experiments were carried out at the Experimental Farm of Faculty of Agriculture, Mansoura University, Egypt, during the cultivation seasons of October 2016/2017. A homogenous lot of pea seeds Intisar 2 (obtained from the Agricultural Research center, Giza, Egypt) were surface sterilized by soaking in 0.01M HgCl2 solution for one min, washed thoroughly with distilled water then, divided into four sets. Seeds of the 1st set were pre-soaked in distilled water to serve as a control, while seeds of the 2nd set were soaked in 10^{-9} µg/ml IAA, 3rd set was soaked 10^{-6} µg/ml BA, while the 4th set was soaked in a combination of the previous concentrations of IAA+BA; all seeds were left in the soaking solution for 3h (Aldesuquy, 2000). All treated seeds were planted in plastic pots (40 cm diameter and 45 cm depth); each pot was filled with about 3.5kg of sterilized peat moss soil containing 5 seeds. The fertilizers were mixed with soil before planting: 2gm/pot calcium superphosphate, 2gm/pot ammonium sulfate, and 1 g/pot potassium as potassium sulfate, including the control set.

Samples were taken after 75 days from planting at the flowering stage for the estimation of growth vigor of both root and shoot, pigment concentration(mg/g dwt), total soluble sugars(mg/g dwt) and total proteins(mg/g dwt). The samples of each treatment were five replicates for measuring growth vigor of root and shoot masses(gm), leaf area(m^2), yield analyses and triplicates for pigment content, total soluble sugars, and protein. Leaf area was measured by weighing the image of the 4th leaf and comparing that mass with the mass of a known area. Samples from the 3rd leaf of main shoot (numbered from the base) were taken for determination of pigment content. Chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+ chlorophyll b(chl a+b), chlorophyll a/chlorophyll b(chl a/b)and carotenoids contents were measured by the spectrophotometric method as recommended by (Metzner *et al.*, 1965).

Total soluble sugars (TSS) was determined from dried pea shoot using the modified method described by (Yemm and Willis, 1954). A 0.1 ml of the alcoholic extract was added to 3.0 ml of freshly prepared anthrone in a boiling water bath for 10 minutes. After cooling, the absorbance was measured at 625 nm. Glucose was used as a standard sugar in this experiment.

Total soluble proteins were determined in dried pea shoot (0.05g) according to the method described by (Bradford, 1976). The soluble protein concentration was calculated from the standard curve of bovine serum albumin, BSA by (Read and Northcote, 1981).

Pods were collected 90 days after planting for yield measurements (number of pod per plant, seeds number per pod, 100 seeds weight, biological yield and straw yield).

Statistical Analysis

The SPSS software was used to analyze the data through the one-way analysis of variance (ANOVA) protocol. The Duncn[a] (Harmonic sample size of 3) option generates a set of letters to indicate significant differences between treatments at $\rho \leq 0.05$ by (Coakes and Steed, 1999).

RESULTS

Growth Vigor of Root and Shoot

A significant increase ($p \leq 0.05$) was noticeable after 75 days of growth of seed pretreated with IAA, BA or their combination (IAA+BA). This increase was reflected in the fresh and dry masses of both root and shoot, as well as in the lengths and leaf area of treated pea plants in comparison with control plants (Table 1). The increases can be arranged in a descending order as IAA> combination (IAA+BA) > BA, respectively. Comparing with control plants, the root fresh mass in all treatments 2-fold increase over untreated plants, shoot fresh mass of *Pea* plant significantly increased (1to 1,5-fold) in response to all the hormonal treatments; while, the leaf area showed ~ 1-fold increase.

Photosynthetic Pigment Concentration

The changes in photosynthetic pigment (chl a, chl b, chl a+b, chl a/b, carotenoids and total pigments) of *Pea* plants during the flowering stage in response to different hormonal treatments are presented in Table (2). Comparing with control plants, the content of chl a and chl b in all treatments 2-fold increase over untreated plants, chl a+b in pea leaves significantly increased (3 to 4-fold) in response to all the hormonal treatments; while, the ration of chla/chl b showed ~ 2-fold increase. In regard to carotenoids, the different hormonal treatments exerted a negative effect in the order of BA> IAA+BA> IAA.This negative trend observed in the carotenoids was reversed on the estimated overall values of the total pigments in pea plants, which doubled as shown in Table (2). The inter-relationship between chl a and b fraction can be clearly examined when the values of chlorophyll a/chlorophyll b ratio are taken into consideration.

Table 1. Effect of seed presoaking in IAA, BA and their combination on the growth vigor of the (root and shoot) of pea plants at flowering stage.

Parameters / Treatments	Growth vigor of the root			Growth vigor of the shoot			
	Root fresh mass(g)	Root dry mass(g)	Root length (cm)	Shoot fresh mass(g)	Shoot dry mass(g)	Shoot length(cm)	Leaf area (cm^2)
Control	0.55 ± 0.043 ᵃ	0.06 ± 0.003 ᵃ	20.00 ± 0.577 ᵃ	2.23 ± 0.136ᵃ	0.52 ± 0.035 ᵃ	37.33 ± 1.20 ᵃ	6.73 ± 0.176 ᵃ
IAA	1.34 ± 0.18 ᶜ	0.12 ± 0.01 ᶜ	29.00 ± 1.52 ᶜ	4.08 ± 0.26 ᶜ	0.91 ± 0.03 ᶜ	55.50 ± 1.04 ᶜ	10.60 ± 0.4 ᶜ
BA	0.93 ± 0.04 ᵇ	0.09 ± 0.01 ᵇ	23.67 ± 0.88 ᵇ	3.50 ± 0.13 ᵇ	0.66 ± 0.04 ᵇ	45.50 ± 0.28 ᵇ	8.53 ± 0.50 ᵇ
IAA+BA	1.03 ± 0.05 ᵇᶜ	0.08 ± 0.005 ᵃᵇ	23.00 ± 0.57 ᵃᵇ	3.54 ± 0.14 ᵇ	0.74 ± 0.02 ᵇ	48.83 ± 1.96 ᵇ	10.19 ± 0.79 ᵇᶜ

Values have the same letter are not significantly different at $\rho \leq 0.05$, the mean values of three replicates ±SE.

Table 2. Effect of seed presoaking in IAA, BA and their combination on pigment content (mg/g dwt) in the leaf of pea plants at flowering stage.

Parameters	Photosynthetic pigment Content (mg/g dwt)					
Treatments	Chl a	Chl b	Chl a+b	Chl a/b	Carotenoids	Total pigments
Control	1.06 ± 0.13 ᵃ	0.85 ± 0.01 ᵃ	1.91 ± 0.12 ᵃ	1.25 ± 0.16ᵃ	0.90 ± 0.05 ᵃ	2.80 ± 0.08 ᵃ
IAA	2.52 ± 0.14 ᶜ	1.55 ± 0.21 ᵇ	4.08 ± 0.35 ᵇ	1.66 ± 0.15 ᵃ	1.33 ± 0.15 ᵇ	5.41 ± 0.20 ᶜ
BA	2.00 ± 0.05 ᵇ	1.43 ± 0.10 ᵃᵇ	3.42 ± 0.05 ᵇ	1.41 ± 0.13 ᵃ	1.09 ± 0.00 ᵃᵇ	4.51 ± 0.05 ᵇ
IAA+BA	2.17 ± 0.13 ᵇ	1.57 ± 0.34 ᵇ	3.75 ± 0.21 ᵇ	1.53 ± 0.34 ᵃ	1.22 ± 0.01 ᵇ	4.97 ± 0.22 ᵇᶜ

Values have the same letter are not significantly different at$\rho \leq 0.05$, the mean values of three replicates ±SE.

Total soluble sugars and total proteins concentration

The total soluble sugars of pea plants shoot showed a significant increase under all the hormonal treatments used and the most significant effect was recorded to IAA alone followed by the combined treatment and BA treatment was the least significant treatment (Table 3). BA treatment led to a significant increase in total protein concentration of the shoot of pea plants compared with untreated plants, however; hormonal mixture treatment showed higher protein concentration. The highest significant increase in protein contents was due to IAA as shown in (Table 3).

Pod yield and yield Attributes:

Data in Table (4) showed that the application of growth regulators (hormones) either alone or in combinations significantly increased yield parameters compared with untreated control plants. The treatment with IAA recorded the highest values for yield parameters at the sampling date of 90 days. BA also significantly increased the number of pod per plant, seeds number per pod, 100 seed weight, biological yield, and straw yield, however, hormonal mixture treatment showed higher yield values compared with BA treatment.

Table 3. Effect of seed presoaking in IAA, BA and their combination on the total soluble sugars and total protein (mg g^{-1} dwt) in the shoot of pea plants at flowering stage.

Parameters / Treatments	Total soluble sugars (mg g^{-1} dwt)	Total Protein (mg g^{-1} dwt)
Control	0.99 ± 0.026 ᵃ	0.22 ± 0.003 ᵃ
IAA	3.86 ± 0.04 ᶜ	0.43 ± 0.01 ᵈ
BA	3.00 ± 0.04 ᵇ	0.31 ± 0.003 ᵇ
IAA+BA	3.75 ± 0.03 ᶜ	0.34 ± 0.003 ᶜ

Values have the same letter are not significantly different at $\rho \leq 0.05$, the mean values of three replicates ±SE .

Table 4. Effect of seed presoaking in IAA, BA and their combination on yield and yield attributes of pea plants at yield stage.

Parameters / Treatments	Number of pod /plant	Number of seeds /pod	Fresh weight of 100 seeds (g)	Dry weight of 100 seeds (g)	Fresh weight of biological yield (g)	Dry weight of biological yield (g)	Fresh weight Straw yield (g)	Dry weight of Straw yield (g)
Control	1.00 ± 0.00 a	1.00 ± 0.00 a	28.00 ± 0.28 a	9.13 ± 0.06 a	1.39 ± 0.26 a	0.11 ± 0.01 a	1.95 ± 0.02 a	0.55 ± 0.02 a
IAA	2.00 ± 0.00 b	2.67 ± 0.66 b	35.69 ± 0.15 d	12.17 ± 0.16 c	2.26 ± 0.09 b	0.68 ± 0.02 d	3.57 ± 0.18 c	1.03 ± 0.02 d
BA	1.33 ± 0.33 ab	1.33 ± 0.33 ab	31.33 ± 0.13 b	9.71 ± 0.05 b	1.73 ± 0.005 a	0.31 ± 0.01 b	2.27 ± 0.02 a	0.64 ± 0.01 b
IAA+BA	1.67 ± 0.33 ab	2.00 ± 0.00 a	33.45 ± 0.11 c	9.95 ± 0.02 b	1.87 ± 0.04 ab	0.44 ± 0.02 c	2.70 ± 0.14 b	0.77 ± 0.03 c

Values have the same letter are not significantly different at $\rho \leq 0.05$, the mean values of five replicates ±SE.

DISCUSSION

The plant growth regulators hormones are structurally unrelated small molecules derived from various metabolic pathways. These compounds are important regulators of plant growth and control responses to both living and nonliving treatments (Santner and Estelle, 2009). They influence all aspects of plant growth and metabolism. In this study, the application of these regulators significantly increased shoots and roots and leaf growth parameters in addition to its significant positive effect on the yield attributes of pea plants comparing with control plants. The superiority was observed in IAA treated plants over BA or the combined treatments. These results were agreed with other studies that were carried out on *Phaseolus vulgaris* (Lang, 1986). IAA bosses many different effects, such as inducing cell elongation and cell division with all subsequent results for plant growth and development (Teale *et al.*, 2006). On a larger scale, IAA serves as a signaling molecule necessary for the coordination of growth and development of plant organs (Aldesuquy, 2000). BA is a synthetic cytokinin that stimulates cell division in plants. Among other actions, it spurs plant growth, improves fruit quality, and sets blossoms (Polisetty *et al.*, 1997). BA is regarded as a synergetic stimulator of Auxin-induced ethylene production that would express the observable decrease in growth parameters of pea plants in response to a combined treatment (Yoshii and Imaseki, 1981).

The changes in photosynthetic pigments (chl a, chl b, chl a+b, chl a/b, carotenoids, and total pigments) of treated plants compared with the control plants, significantly increased in response to all hormonal treatments. The same pattern was recorded for carotenoids, total chla+b and total pigments, as these hormones increase plant leaf area leading to increases in plastids numbers so occasionally pigment content increased. These results were agreed with those obtained in a previous study in which BA-treated leaves had higher specific and dry weights, and the chlorophyll and carotenoid contents were greater than water controls (Adedipe *et al.*, 1971). The increase in photosynthetic pigments has been observed in the mustard plant due to exogenous IAA treatment, that led to an increase in plant growth parameters as well as yield due to increase in photosynthetic rate (Hayat *et al.*, 2001).

Application of BA and hormonal mixture had slightly increased the sugar content as compared with the control plants due to the significant increased in photosynthetic rate but, the superiority was due to IAA. BA is a synergistic stimulator for auxin-induced ethylene production, it increases the sugar content parallel to the rate of ethylene production in the presence of IAA, but fails to increase the sugar content in the absence of IAA while ethylene production was significantly stimulated by BA (Yoshii and Imaseki, 1981).

Total soluble proteins concentration in pea tissue treated with BA increased compared with untreated plants(SCHROEDER, 1984) also, showed that application of BA increases total proteins content per cotyledon of *Pisum sativum* plants due to increased concentration of amino acids that results from nitrogen fixation process by nodules which activated by the addition of chemical fertilizers but; hormonal mixture showed higher protein concentration than BA and the superiority was due to IAA; similar findings were obtained (ABDEL and Amin, 2006).

In conclusion, the use of IAA as seed priming before planting improves growth masses and productivity of plants, in general, and pea plants in specifics.

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تاثير الاندول حمض الخليك والبنزيل ادنين على النموومحصول لنباتات البسله

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البسله انتصار٢ من اهم محاصيل البقوليات فى العالم.هذه الدراسه اجريت لتقييم تاثير هرمون الاندول حمض الخليك والبنزيل ادنين كل على حده او معا (الاندول حمض الخليك +البنزيل ادنين) على كل من صفات النمو لكل من المجموع الجزرى والمجموع الخضرى ومحتوى صبغات البناء الضوئى والمحصول والايض لنبات البسله. فى اغلب الحالات وجد ان تهيئه بذور البسله فى الاندول بتركيز ١٠-٩ ميكروجرام بالملى اوالبنزيل ادنين بتركيز ١٠-٦ ميكروجرام بالملى او معا بيسبب زياده ملحوظه عند بى فاليو اقل من او يساوى ٠.٠٥ فى صفات النمو النمو لكل من المجموع الجذرى والمجموع الخضرى ومحتوى الاصباغ وتركيزالسكريات الذائبه والبروتين الكلى فى الاوراق وكذلك المحصول بمعني(عدد القرون فى النبات وعدد البذور داخل كل قرن ووزن ١٠٠ بذره). مقدار الزياده فى نباتات البسله رتب بالترتيب الاتى الاندولحمض الخليك اكبر من(الاندول +البنزيل) اكبر من البنزيل ادنين. فى النهايه هذه النتائج وضحت ان الاندول حمض الخليك افضل معامله لتحسين النمو والمحصول لنباتات البقوليات.

الكلمات المفتاحيه:البنزيل ادنين والاندول اسيتك اسد والبسله.