

Effect of Acetylsalicylic Acid, Indole-3- Bytric Acid and Gibberellic Acid on Plant Growth and Yield of Pea (*Pisum Sativum* L.)

¹Amal M. El-Shraiy and ¹Amira M. Hegazi

¹Agriculture Botany Department, Faculty of Agriculture, Ain Shams University. Cairo, Egypt

Abstract: Pots experiment were conducted during the two successive seasons of 2007 and 2008 to investigate the effect of acetylsalicylic acid (ASA) at 10 and 20 ppm, indole-3-bytric acid (IBA) at 50 and 100 PPM and gibberellic acid (GA₃) at 50 and 100 PPM on pea (*Pisum sativum* L.) cv Prgress mog. Growth regulators were applied as foliar spray at 25 and 35 days after sowing. Three samples were taken at 21, 45 and 70days after sowing coinciding with vegetative stage, flowering and harvest respectively. Application of ASA and IBA enhanced plant growth as indicated by plant height, number of leaves, fresh and dry weights in both seasons. Significant increase in 1000 seeds weight and pod parameters (length of pod, pods number / plant, seeds number / pod, pods fresh and dry weights) was recorded by ASA and IBA at harvest comparing with control and GA₃ treated plants. These positive effects of ASA and IBA were correlated with significant increase in total chlorophylls in leaves, total soluble proteins, praline, phenol, total soluble carbohydrates and sugars in seeds. Analysis of endogenous phytohormones in seeds at harvest revealed reduction in ABA concentration and an increase in zeatin riboside and GA₃ concentration in GA₃ treated plants. While plants treated with ASA and IBA showed an opposite trend.

Key words: peas (*Pisum sativum*), carbohydrate, proteins, yield, growth, chlorophyll, acetyl salicylic acid (ASA), indole-3-pytric acid (IBA), gibberellic acid (GA), Endogenous-phytohormones

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important leguminous vegetable crops grown during winter season in Egypt for local consumption and exportation. The pods of pea contain a great amount of protein and carbohydrates. So that pea is considered as one of the most important sources in human food nutrition throughout the world (Hussein *et al* 2006). Pea has high levels of amino acids, lysine and tryptophan, which are relatively low in cereal grains. Pea contains approximately 21-25 percent protein, high levels of carbohydrates, low contents of fiber and contain 86 percent of total digestible nutrients. These characters make pea plan an excellent livestock feed. Pea had 5 to 20 percent less of the trypsin inhibitors than soybean (Kent and Endres 2003). Plant growth regulators play an important role in producing high value horticultural crops and increasing the yield (Emongor, 1997). However, pea plants are highly sensitive to biotic and abiotic influences that affect the growth and yield. Several studies have been conducted to evaluate the response of crops, especially legumes (pea and lupin) and cereals (wheat), to different source–sink ratios during grain filling which affect grain yield and quality (Sandana. *et al*, 2009).

Acetylsalicylic acid (ASA) is a commercially available form of salicylic acid. It is known that in aqueous solutions, ASA is hydrolyzed almost entirely to SA, which is active ingredient (Mitchell and Broadhead, 1967). However, Salicylic acid is an endogenous growth regulator with phenolic nature, which participates in regulation of several physiological processes in plants, such as stomatal closure, ion uptake, inhibition of ethylene biosynthesis and transpiration (Khan *et al* 2003; Shakirova *etal*, 2003). The effect of salicylic acid on the physiological processes is variable depending on its concentration, plant species, developmental stages and environmental conditions El-Mergawi and Abdel-Wahed, 2004. Low SA dose enhanced growth of wheat and maize Khan *et al*,(2003); Shehata *et al*, (2001). Pierpoint, 1994; Pancheva *et al.*, 1996 reported that salicylic acid (SA) had periotic effects on the morphology and physiology of plants.

On the other hand, exogenous application of GA₃ increased cowpea plant height (James and Abraham, 1989), Emongor, 2007 Meanwhile, GA₃ reduced 100 seed weight and seed yield of common beans (Williams and de Mallorca, 1984).

Corresponding Author: Amal M. El-Shraiy, Agriculture Botany Department, Faculty of Agriculture, Ain Shams University. Cairo, Egypt

E-mail: amalelshraiy3@yahoo.com. Fax: 002 02 44444460

IBA is a synthetic auxin. Auxins are used commercially for enhancing crop production and regulation plant growth and development rapid growth such as shoot tissue, young leaves and developing seeds, elongation but do promote lateral root development (Nagel, 2001); increase seed weight and pod number (Cho, 2002). However, auxin application increase pod numbers, seed weight or seed yield but this based on varieties sensitivity and correct application timing (Cho, 2002; Leal-León, 2002.). Natural occurring auxin in plant (IAA) could also increase the grain-filling and mobilization significantly over control (Ray and Choudhuri, 1981).

The present study was undertaken to investigate the effect of growth regulators; ASA, IBA and GA₃ on pea plant growth, reproductively and some correlated biochemical constituents.

MATERIALS AND METHODS

Plant Material and Treatments:

Pot experiments were conducted at the Experimental Farm of the Agricultural Botany Department, Faculty of Agriculture Ain Shams University, at Shoubra El-Kheima, Kalubia, Egypt during the two cultivation seasons of 2007 & 2008. Pea (*Pisum sativum* L. cv Progress mog), seeds were sown in plastic pots 40 cm diameter and 45 cm depth in October 15th and 30th in the two season respectively, each pot was filled with 10kg of clay loamy soil (40% clay, 35% silt, 25% sand) with 8 seeds/pot. Thinning was performed after 1 week later of germination leaving two plants per pot. Basal fertilization was performed as following; Phosphorous as calcium super phosphate (15.5% P₂O₅) was mixed with soil before sowing at the rate of 2 g/pot, Nitrogen as ammonium sulfate (33.5% N) at the rate of 2 g/pot and potassium as potassium sulfate (48% K₂O) at the rate of 1 g/pot and with 0.5 g/pot sulfur fertilizer were added twice at sowing and 20 days after sowing. Other recommended agriculture practices for pea cultivation was followed. Pots were arranged in complete randomized design with five replicates. Each replicate consisted of five pots with two plants per pot.

Pea plants were foliar sprayed twice at 25 and 35 days from sowing, with Acetyl salicylic acid as (ASA) 10 and 20 ppm, indole-3-butyric acid (IBA) at 50 and 100 ppm and Gibberellic acid (GA₃) at 50 and 100 ppm, against a control plants which treated with distilled water until complete covering of the plant foliage. Tween 20 at 0.05 ml/L was used as wetting agent.

Samples were taken at 21, 45 and 70 days after sowing for growth measurements; plant height, leaves number, shoots and roots fresh and dry weight, and chlorophyll analysis. Pods were gathered at 65, 70 days after sowing for yield measurements (length of pod per plant, pods number per plant, seeds number per pod, 1000 seeds weight, pods fresh and dry weights) and determination of biochemical constituents in seeds.

Biochemical Analyses:

Pea seed samples were taken for biochemical analyses including total soluble protein, total soluble carbohydrates, total soluble sugars, reduced and non-reduced sugars, phenols, proline and phytohormones.

Chlorophyll Determination:

Total chlorophyll (Chl) concentrations were determined in the fourth leaf (fully expanded leaves) from shoot tip. Samples of 0.1 g leaves was ground and extracted with 5 mL of 80% (v/v) acetone in the dark according to the methods described by Holder (1965). The mixture was filtered then the absorbance values at 645 and 663 nm were measured. Using Jenway 6105 UV/VIS Spectrophotometer.

Determination of Total Soluble Protein:

Total soluble protein was determined in seeds (one gram dry weight) using the method of Bradford (1976). The soluble protein concentration was calculated from the standard curve according to Read and Northcote (1981).

Determination of Total Soluble Carbohydrate:

Total soluble carbohydrates were extracted according to A.O.A.C. (1990), one gram of seeds sample was randomly taken and added to 30 ml HCL 2N. Tubes were placed in boiling water bath for 6 h. after cooling, samples transferred into calibrated flasks (100 ml). Total carbohydrates were estimated by the alkaline potassium ferricyanide method (Shales and Schales, 1945).

Determination of Total Soluble Sugars:

For extraction, one gram of seeds sample was taken and grounded in a mortar with ethanol 80% for 3 times. The extracts were combined and evaporated till dryness. The dried film was dissolved in 50 ml of 10 % aqueous isopropanol. Determination of total soluble sugars, reduced and non-reduced sugars were carried out according to the method by Shales and Schales (1945).

Determination of Phenols:

One gram seeds was taken and extracted with 80 % cold methanol (v/v) for three times at 0°C. The combined extracts were collected and filtered (Wt. No. 1). Then, the volume of sample were raised up to 25 ml with cold methanol. Phenol determination was carried out according to Daniel and George (1972).

Determination of Proline:

Proline determination was accomplished essentially as described by Bates *et al.* (1973).

Extraction and Determination of phytohormones:

Extraction of phytohormones (ABA, GA₃ and cytokinin (CKs)) was followed in accordance to method of Knecht and Brunisma (1973). C18 sep-pak cartridge was used to separate different groups of phytohormones by using different concentration of water and methanol as described by Lee *et al.*, (1989). Quantitative analysis of previous phytohormones was determined by HPLC as follows: water model µ6k, column: C18- 3.9 x 300 mm – Silica – based packing materials, mobilphase: Me-oH super purity 2% acetic acid, flow rate: 1.0 ml/min, filtration: filtrate through 0.45 µm filter, injector: waters 510, HPLC pump: waters 486 tunable, absorbance detector: model 680 automated. Determination method was followed as described by Bangerth, 1989.

Statistical Analysis:

Data were subjected to statistical analysis of variance and were calculated by using SPSS software according to SPSS Coakes and Steed (1999).

RESULTS AND DISCUSSION

Vegetative Growth Parameters:

As shown in Table (1), application of growth regulators significantly increased plant height comparing with control plants and the superiority was due to GA₃ at 100 ppm at the three sampling dates (21, 45 and 70 days after sowing) in both seasons. Application of ASA and IBA showed significant increases leaves number per plant, plant fresh and dry weight in the two seasons. The highest values were obtained by ASA at 20 ppm which recorded 18.43 leaves/plant, 19.68 gm fresh weight and 12.9 gm dry weight comparing with control plants which recorded 14.17 leaves/plant, 12.69 and 7.6 gm respectively, followed by IBA at 100 ppm in the three sampling dates in the two seasons.

The effect of GA₃ on plant height has been mentioned by several workers (Chauhan *et al* 2009; Leite *et al* 2003). The stimulation effect of ASA or SA on plant growth was confirmed by Abd El-Wahed. *et al* 2006 on yellow maize plants, Emogor, 2007 on cowpea plants and Martin-Mex *et al* (2005) on African violet plant. Maurya, 1975 found that IBA increased average number of leaves and fresh weight of onion bulbs.

Pod Yield and Yield Parameters:

Data in table (2) showed that application of growth regulators significantly increased yield parameters comparing with untreated plants and ASA at 20 ppm recorded the highest values for yield parameters in both seasons. Application of ASA and IBA showed significant increase in length of pod per plant, number of pod per plant, number of seed per pod, 1000 seeds weight and pods fresh and dry weights. The superiority of pod parameters observed with ASA at 20 ppm which recorded 13.7 cm length / pod, 14.3 pods number/ plant, 8.3 seeds number/pod, 175.1gm 1000 seeds weight, 16.2 and 6.2 gm fresh and dry weights respectively in both seasons. On the other hand, GA₃ treatment at 50 and 100 show a significant reduction in yield parameters.

The effect of ASA on yield parameters has been reported by with çanakç and Munzuroğlu (2000) who mentioned that acetyl salicylic acid (ASA) administration to the leaf caused an increase in fresh and dry weight gain of radish (*Raphanus sativus*,L). These results were in agreement with those of Singh and Kaur, 1980 on mung bean, Lang, 1986 on *Phaseolus vulgaris* and Singh, *et al* 2002 on onion plants. Similar findings were obtained by Resmi and Gopalakrishnan (2004) who mentioned that foliar application of cowpea plants with naphthaleneacetic acid (NAA) at 15, 30 and 45 days after sowing increased vegetative growth, fruit set, grain yield, seed yield, pod length, pod weight, pod number per unit area and pod number per plant of cowpea plants. However, Baraldiet *et al.* 1993 and Chhun *et al.*, 2004 indicated that foliar application with indole-3-butyric acid at the concentration 50 and 100 mg/l increased yield of rice and wheat.

The effect of GA₃ on pod parameters was agreement with Emogor (2002) who mentioned that GA₃ treatments had no significant effect on seed yield of bean plants and significantly reduced 1000 seed weight,

Table 1: Effect of ASA, IBA and GA₃ on some growth parameters of pea plants at various ages during the seasons of 2007 & 2008.

Treatments	Characters	1 st season						2 nd season		
		age (days after sowing)								
		21	45	70	21	45	70			
plant height	control	12.67	36.00	56.00	14.00	38.67	58.33			
	ASA 10 ppm	12.60	47.95	64.67	13.00	49.66	66.54			
	ASA 20 ppm	12.30	58.67	85.64	13.00	61.67	89.33			
	IBA 50 ppm	12.50	45.67	64.33	13.67	49.00	65.00			
	IBA 100 ppm	11.87	57.33	76.00	12.00	57.23	79.89			
	GA ₃ 50 ppm	11.97	93.33	143.00	13.00	98.86	146.67			
	GA ₃ 100 ppm	12.23	101.33	162.67	13.33	108.32	166.67			
No. of leaves/plant	control	5.00	10.67	14.17	6.00	9.00	13.63			
	ASA 10 ppm	5.33	12.54	16.34	5.67	12.87	15.67			
	ASA 20 ppm	5.33	15.67	18.43	6.00	16.67	19.67			
	IBA 50 ppm	5.33	12.41	16.98	5.67	13.67	15.52			
	IBA 100 ppm	5.67	13.98	17.77	5.67	14.65	16.98			
	GA ₃ 50 ppm	5.33	10.33	14.78	5.67	9.33	13.67			
	GA ₃ 100 ppm	5.00	10.67	13.89	5.67	8.67	12.33			
F. Wt /plant	control	4.91	9.85	12.64	4.87	10.9	12.87			
	ASA 10 ppm	5.12	11.93	13.78	4.98	12.3	17.89			
	ASA 20 ppm	4.89	14.00	19.68	5.74	14.4	21.34			
	IBA 50 ppm	4.96	10.73	13.35	5.88	11.8	16.99			
	IBA 100 ppm	5.12	12.87	15.64	4.96	12.5	19.87			
	GA ₃ 50 ppm	5.31	8.90	12.45	5.35	9.5	14.45			
	GA ₃ 100 ppm	4.93	7.80	10.97	5.77	7.9	11.64			
D. Wt /plant	control	2.31	5.1	7.6	2.54	4.2	8.0			
	ASA 10 ppm	3.45	7.7	9.8	2.87	5.3	11.9			
	ASA 20 ppm	2.59	9.8	12.9	3.12	6.3	14.3			
	IBA 50 ppm	3.65	6.0	8.8	3.25	5.0	11.2			
	IBA 100 ppm	3.87	7.1	10.3	2.98	5.1	13.2			
	GA ₃ 50 ppm	2.99	4.9	5.2	3.45	3.8	6.3			
	GA ₃ 100 ppm	3.45	4.0	4.9	3.63	2.5	5.1			
LSD for plant height	at 0.5 %	1.11	2.05	3.62	1.01	1.52	3.54			
LSD for no. of. leaves	at 0.5 %	0.01	1.63	1.85	0.01	1.45	1.74			
LSD for F. Wt/plant	at 0.5 %	0.84	1.87	2.64	0.77	1.81	2.12			

Table 2: Effect of ASA, IBA and GA₃ on yield components of pea plants during the seasons of 2007 & 2008

treatment	1 st season						2 nd season					
	length of pod (cm)	No of pods / plant	No of seed / pod	1000 seeds weight (gm)	pod F.Wt. (gm)	pod D.Wt. (gm)	length of pod (cm)	No of pods / plant	No of seed / pod	1000 seeds weight (gm)	pod F.Wt. (gm)	pod D.Wt. (gm)
control	9.7	6.0	6.0	111.7	8.3	3.3	8.6	6.7	5.7	140.00	9.5	2.4
ASA 10 ppm	12.3	12.9	7.7	140.7	12.4	5.6	11.7	13.3	7.7	150.43	13.1	3.4
ASA 20 ppm	13.7	14.3	8.3	175.1	16.2	6.8	13.3	16.7	8.7	174.62	16.7	4.8
IBA 50 ppm	10.3	8.7	7.7	120.7	10.9	4.4	10.7	9.3	8.0	111.25	10.4	3.0
IBA 100 ppm	11.0	12.3	8.0	140.7	12.9	5.4	11.3	13.7	8.3	136.80	13.0	4.1
GA ₃ 50 ppm	3.8	1.9	2.3	94.3	3.8	1.9	5.3	2.0	2.0	80.00	3.2	1.6
GA ₃ 100 ppm	2.3	1.0	1.3	52.5	2.3	1.1	3.0	1.0	1.0	30.00	1.9	1.1
LSD	2.54	1.64	1.44	5.61	2.78	1.98	1.88	2.41	1.24	6.11	2.45	1.12

seed yield, seed number/ pod and number of pods/plant of common beans (*Phaseolus vulgaris*). However, the role of GA₃ in the development of parthenocarpic fruits in pea was confirmed by Bonde (2008). He found that gibberellic acid caused the formation of seedless pods from a number of weeks application. These results may explain the reduction in seeds yield in GA₃ treated plants.

Effect of Plant Growth Regulators on Biochemical Parameters:

Chlorophyll Concentration:

As seen in table (3) significant difference was observed in total chlorophyll concentration of leaves to application of ASA, IBA and GA₃. Application of ASA and IBA recorded higher significant level in chlorophyll concentration at the age of samples 21 and 45 day after sowing than in control and GA₃ treatments.

The superiority of chlorophyll concentration observed with ASA 20 ppm and IBA 100 ppm. at the age of sample 45 days after sowing. Meanwhile, GA₃ treatment give a significant decreased at the same age. These results were in agreement with Türkyılmaz *et al.*, (2005) who suggested that, foliar spray with salicylic acid increased Chl a, Chl b and other photosynthetic pigments in bean plants under normal field conditions. The stimulation effect of ASA on chlorophyll concentration was confirmed by Zhao *et al.*, 1995 on soybean and Sinha *et al.* (1993) on maize plant. While, the role of SA in the chlorophyll levels was mentioned by Abreu

and Munne-Bosch (2009) who revealed that SA deficiency is associated with reduced damage to the photosynthetic apparatus as well as chlorophyll levels. The effect of IBA on chlorophyll concentration has been mentioned by several workers (Ludwig-Muller, 2000; Abd El-Wahed. *et al* 2006). Otherwise, GA₃ treatments show the opposite trend for chlorophyll concentration. However, the role of GA₃ in decreasing chlorophyll content was confirmed by Leite *et al.*, (2003) who found that applying gibberelline increased leaf area in soybean, consequently decreased chlorophyll concentration per leaf unit area.

Table 3: Effect of ASA, IBA and GA₃ on total chlorophyll concentration (mg/ml) in bean leaves at various ages during the seasons of 2007 & 2008.

	Total chlorophyll (mg/gm)					
	1 st season			2 nd season		
	age (days after sowing)					
	21	45	70	21	45	70
control	1.09	1.33	0.90	1.18	1.66	0.81
ASA 10 ppm	1.21	2.44	0.88	2.02	2.13	1.21
ASA 20 ppm	1.24	3.63	0.79	3.09	3.74	1.99
IBA 50 ppm	1.04	2.12	0.98	2.07	2.12	0.96
IBA 100 ppm	1.05	2.98	0.89	2.10	2.55	0.87
GA ₃ 50 ppm	0.94	1.16	0.76	1.14	1.74	0.56
GA ₃ 100 ppm	1.10	1.31	0.49	1.07	1.96	0.35
LSD	0.07	0.12	0.03	0.13	0.24	0.09

Total Soluble Protein Concentration:

Table (4) showed that treatment of ASA and IBA significant increased in total soluble protein. However, opposite trend was noticed with GA₃ treatment which give significant reduction as compared with control plant. On the other side, ASA at 20 ppm reach the highest value in total soluble protein concentration as compared to control plant. Similar findings were obtained by Abd El-Wahed. *etal*, (2006), working in yellow maize, salicylic acid (SA) application led to significant effect on biochemical constituents such crude protein, oil and total carotenoids content also. Whereas Vardhini and seeta (1998) found that exogenous Table (3) application of SA enhanced levels of DNA, RNA, Soluble protein and fat content of peanut seed. However, changes in vegetative and reproductive growth parameters of pea plants by the application of gibberellic acid gave a lowest trend in this respect and resulted in reduction of biochemical component as represented in protein and amino acid. However, Hariharan and Unnikrishnan (2005) mentioned that the effect of GA₃ on free amino acid and protein contents vary according to the concentrations of the hormone used, but does not follow a uniform pattern, there is a marked reduction in the total amount of free amino acid as the fruit matures, and this decline is steeper in the treated category, GA₃ treatment leads to qualitative and quantitative changes in the free amino acid as compare to control plants.

Table 4: Effect of ASA, IBA and GA₃ on total soluble protein , Phenols and Proline concentrations (mg / g) in pea seeds at harvest during the seasons of 2007 & 2008.

Treatment	protein (mg/g)		Phenol (mg/g)		Proline (mg/g)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
control	56.35	59.59	2.33	4.76	0.98	1.11
ASA 10 ppm	77.30	79.57	4.88	6.77	6.87	8.97
ASA 20 ppm	88.50	95.25	7.94	8.74	9.77	11.74
IBA 50 ppm	60.76	62.84	3.44	5.43	7.96	8.45
IBA 100 ppm	64.40	77.33	5.41	7.02	8.78	9.78
GA ₃ 50 ppm	49.98	41.77	2.45	3.66	2.55	3.31
GA ₃ 100 ppm	40.15	38.41	3.21	4.89	3.64	4.79
LSD	3.69	4.12	1.34	1.77	1.87	2.01

Proline and Phenol Concentrations:

Data reported in Table (4) show that ASA has a significant effect in total phenols and proline. However, foliar application of acetylsalicylic acid and indole-3-butyric acid at both concentrations gave the highest values for total phenols and proline. Concerning, Sing *et al.*,1995 on kharif onion and Amin *et al.* 2006 and 2007 on maize, indicated that foliar application of indole-3-butyric acid at the concentration 50 and 100 mg/l to maize plants were the most effective treatments for increasing chemical constituents as compared with control. IBA treated plants showed significant increases in most mentioned constituent. Comparing the values of the total phenols and proline, it was noticed that ASA at 20 ppm and IBA at 100 ppm gave the highest significant

increase of phenol and proline concentrations whereas GA₃ in both concentrations gave the lowest values. Henrique, 2006 suggested that the only significant difference observed was between NAA treatments, mixed or not with PBZ, where NAA alone showed the highest phenol content.

Total Soluble Sugars and Carbohydrates Concentrations:

Total soluble carbohydrates and sugars (reducing and non-reducing) concentrations in seeds are demonstrated in Tables (5). Foliar application of ASA and IBA significantly increased carbohydrates and sugars contents as compare to control. These results were found to be in agreement with Amin et al, 2007; Henrique, 2006 they explained that SA treatment regulated sugar contents (translocation from source to sink) and spraying salicylic acid caused significant increase in total soluble sugars. Also, they observed that an application of NAA or Table (5)

Table 5: Effect of ASA, IBA and GA₃ on total soluble carbohydrates, total soluble sugar, reduced sugar and non-reduced sugar concentrations (mg/g F.Wt.) In pea seeds at harvest during the season 2007 & 2008.

Treatment	Total soluble carbohydrates (mg/g)		Total soluble sugar (mg/g)		Reduced sugar (mg/g)		Non-reduced sugar (mg/g)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
control	18.87	19.54	5.47	6.77	2.54	3.01	2.93	3.76
ASA 10 ppm	23.74	26.54	8.94	9.08	3.66	4.12	5.28	4.96
ASA 20 ppm	36.98	37.41	11.45	11.88	5.74	4.94	5.71	6.94
IBA 50 ppm	21.66	22.13	8.65	9.23	3.87	3.77	4.78	5.46
IBA 100 ppm	26.25	25.02	9.88	10.01	4.97	4.63	4.91	5.38
GA ₃ 50 ppm	10.02	11.33	3.87	3.21	1.54	1.89	2.33	1.32
GA ₃ 100 ppm	7.51	6.77	2.25	2.56	1.21	1.55	1.04	1.01
LSD	4.66	3.87	1.02	1.65	0.87	0.76	0.98	0.81

IBA increased total sugars content showed the highest content, while IBA at 2000 mg L⁻¹ gave the highest reducing sugars contents.

Several works, Habba, 2003 on onion, Sing *et al.*, 1995 on kharif onion and Amin *et al.*, 2006 on maize, indicate that foliar application of indole-3-butyric acid at the concentration 50 and 100 mg/l had the same stimulation effects in carbohydrate and sugars. On the other hand, application of GA₃ showed opposite significant decrease for carbohydrate and sugar in seeds in both season.

Endogenous Hormones (Analyze Phytohormones):

Endogenous phytohormones (GA₃, CKs(zeatin-riboside) and ABA) in pea seeds at harvest were greatly affected by ASA, IBA and GA₃ treatments presented in Figure (1,2 and 3). Application of ASA and IBA increased endogenous GA₃ and CKs levels more than ABA levels comparing to control plants and GA₃ treatments. However, the high concentrations of GA₃ treatments increase the level of endogenous growth promoters (GA₃ and CKs) and decreased the level of endogenous growth inhibitors (ABA) as compared to control plants. The physiological role of GA₃ as active growth promoting hormone was documented by several reports Mahgoub,1992 and Magda *et al*,2007 reported that spraying rose plants with GA₃ at the concentration 50,100,200 ppm increased the level of IAA, IAN and decreased the level of ABA. The effect of ASA and IBA on endogenous phytohormones levels has been mentioned by Tompsett and Schwabe, 1974. Exogenous auxin application reduced gibberellin-like substance levels and caused devernization Jarvis and Shaheed (1986). and IAA-oxidase activity was controlled by phenolic acids (diphenols inhibit the IAA-oxidase). The effect of salicylic acid has been known to be present in some plant tissues for quite some time, but has only recently been recognized as a potential plant growth regulator (PGR) which play an important role in regulating a member of plant physiological processes (Arfan *et al* 2007).

It could be concluded from the present study that both ASA and IBA improved pea plants growth and productivity throughout enhancing and up regulating some chemical contents such as total soluble proteins, total phenols, proline, total soluble carbohydrates and sugars in seeds at harvest. From commercial point of view, not only low concentration of ASA treatment could be recommended to enhance plant growth and productivity but to induce plant resistance against biotic and abiotic stresses as well (Raskin *et al*, 1990). Furthermore, plants treated with ASA showed high level of ABA in seeds at harvest. The high level of ABA induce seeds dormancy and subsequently, prevents seeds sprouting in pods.

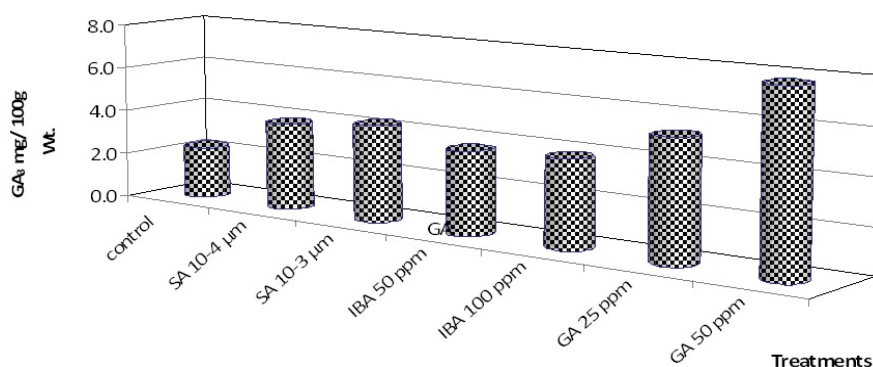


Fig. 1: Effect of different concentrations of ASA, IBA and GA3 on endogenous hormones (GA3) during two season 2007 & 2008.

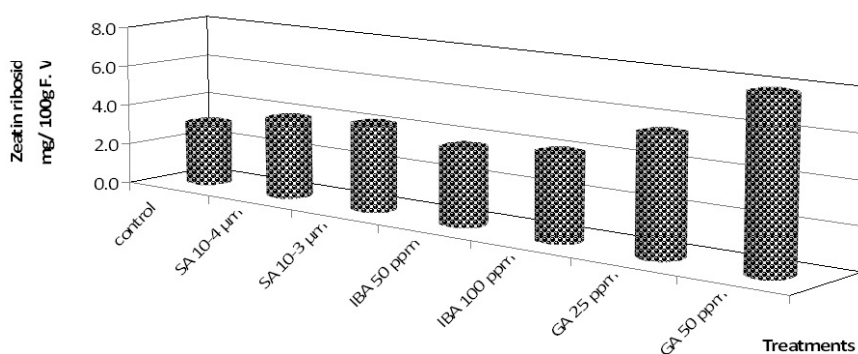


Fig. 2: Effect of different concentrations of ASA, IBA and GA3 on endogenous hormones (Zeatinriboside) during two season 2007 & 2008.

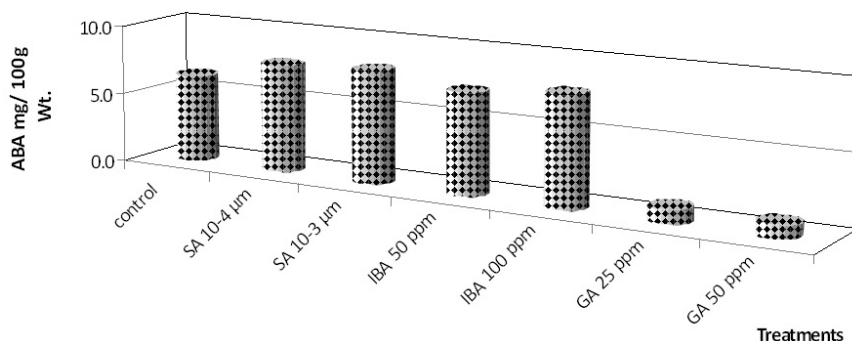


Fig. 3: Effect of different concentrations of ASA, IBA and GA3 on endogenous hormones (ABA) during two season 2007 & 2008.

REFERENCES

- A.O.A.C., 1990. Official Methods of Analysis. 15 th ed. Association of official agricultural chemists, Washington, DC, USA, pp: 59-60.
- Abd El-Wahed, M.S.A., A.A. Amin and El-Sh. M. Rashad, 2006. Physiological effect of some bioregulators on vegetative growth, yield and chemical constituents of yellow Maize plants. *World J. of Agric. Sci.*, 2(2): 149-155.
- Abreu, M.E. and S. Munne'-Bosch, 2009. Salicylic acid deficiency in NahG transgenic lines and sid2 mutants increases seed yield in the annual plant *Arabidopsis thaliana*. *Journal of Experimental Botany*, 2: 1-11.
- Amin, A.A., EL-Sh. M. Rashad and H.M.H. EL-Abagy, 2007. Physiological Effect of Indole - 3 - Butyric Acid and Salicylic Acid on Growth, Yield and Chemical Constituents of Onion Plants. *Journal of Applied Sciences Research*, 3(11): 1554-1563.

- Amin, A.A., Rashad, M. El - Sh. and F.A.E. Gharib, 2006. Physiological response of maize plants (*Zea mays* L.) to foliar application of morphactin CF and indole-3-butyric acid. *J. of 125 Biol. Sci.*, 6(3): 547-554.
- Arfan, M., H.R. Athar and M. Ashraf, 2007. Does exogenous application of salicylic acid through the root in g medium mod ul e growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. *J. Plant Physiol.*, 164: 685-694.
- Bangerth, F., 1989. Dominance among fruits/ sinks and the search for a correlative signal. *Physio. Plant*, 76: 1-7.
- Baraldi, R., G. Bertazza, S. Predieri, A.M. Bregolo and J.D. Cohen, 1993. Uptake and metabolism of indole-3-butyric acid during the in vitro rooting phase in pear cultivars (*Pyrus communis*). *Acta Horti.*, 329: 289-291.
- Bates, L.S., R.P. Waldren and I.D. Treare, 1973. Rapid determination of free proline for water-stress studied. *Plant and Soil*, 39: 205-207.
- Bonde, E.K., 2008. Effects of gibberellic acid on growth and parthenocarpy in the dwarf telephone pea. *Physiologia Plantarum*, 19(2): 356-364.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein – Dye binding. *Anal. Biochem*, 72: 248-254.
- Çanakçı, S. and Ö. Munzuroğlu, 2000. Effects of sprayed acetylsalicylic acid application to the leaf of bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) seedlings on transpiration rate and weight changes. *Yüzüncü Yıl University. J. Inst. Sci.*, 7(1): 83-92.
- Chauhan, J.S., Y.K. Tomar, A. Badoni, N.I. Singh, S. Ali and Debarati, 2009. Morphology, Germination and early Seedling Growth in *Phaseolus mungo* L. with Reference to the Influence of Various Plant Growth Substances. *Journal of American Science*, 5(7): 34-41.
- Chhun, T., S. Taketa, S. Tsurumi and I. Masahiko, 2004. Different behaviour of indole-3-acetic acid and indole-3-butyric acid in stimulating lateral root development in rice (*Oryza sativa* L.). *Plant Growth Regul.*, 43: 135-143.
- Cho, *et al.*, 2002. *Plant Growth Regulation*, 36: 215-221.
- Coakes, S.J. and L.G. Steed, 1999. *SPSS Analysis without Anguish*, John Wiley Sons, New York.
- Daniel, H.D. and C.M. George, 1972. Peach seed dormancy in relation to indigenous inhibitors and applied growth substances. *J. Amer. Soc. Hort. Sic.*, 97: 651-654.
- El-Mergawi, R.A. and M.S.A. Abdel-Wahed, 2004. Diversity in Salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize. *Egypt. J. Agron.*, 26: 49-61.
- Emongor, V.E., 1997. The prospective of plant growth regulators in Kenyan Agriculture. In: proceedings of the National Horticulture Conference, Agong, S. G., L. S. Wamoyo and F. K. Obwara (Eds). Progress and prospects in Kenya's Horticulture Development Towards the year 2000 and beyond, pp: 227-229.
- Emongor, V.E., 2002. Effect of benzyladenine and gibberellins on growth, yield and yield components of common bean (*Phaseolus vulgaris*). *Uniswa Res. J. Agric. Sci. Technol.*, 6: 65-72.
- Emongor, V. 2007. Gibberellic acid (GA₃) influence on vegetative growth, nodulation and yield of cowpea (*Vigna unguiculata* (L.) Walp, *J. of Agronomy*, 6(4): 509-517.
- Habba, E.E., 2003. Physiological studies of some growth regulators on the growth, yield and chemical constituents of onion plants (*Allium cepa* L.). *J. Agric. Sci. Mansoura Univ.*, 28(3): 1645-1653.
- Hariharan, M. and K. Unnikrishnan, 2005. Effect of gibberellic acid on variations in free amino acid and total protein contents in developing kernel of cashew. *International Society for Horticultural Science. Ann. Rev. Plant Physiol.*, 24: 571-598.
- Holder, M., 1965. Chlorophylls. In: T.W. Goodwin, ed, *Chemistry and Biochemistry of Plant Pigments*. Academic Press, New York, pp: 461-488.
- Hussein, M.M., N.H.M. El-Geready and M. El-Desuki, 2006. Role of putrescine in resistance to salinity of pea plants (*Pisum Sativum* L.). *J. of Appl. Sci. Res.*, 2(9): 598-604.
- James, C.O. and P.G. Abraham, 1989. Effect of seed-pretreatment with some plant growth regulators on germination, growth and yield of cowpea (*Vigna sinensis* Endl.). *Japan. J. Crop Sci.*, 58(4): 641-647.
- Jarvis, B.C. and A.I. Shaheed, 1986. Adventitious root formation in relation to the uptake and distribution of supplied auxin. *New Phytologist*, 103: 23-31.
- Kent, M.B.S. and G. Endres, 2003. *Field pea production*. North Dakota State University, Fargo, North Dakota.
- Khan, W., B. Prithviraj and D.L. Smith, 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. *J. Plant Physiol.*, 160: 485.

- Lang, O.F.P., 1986. Regulators del crecimiento VIII: effects del acido acetyl salicilico ylo dimetil sulfoxido en el rendimiento agronomico de phaseolus vulgaris I. testis de maestri Jen Cienia C.P., Montecillo.
- Leal-León *et al.*, 2002. *Plant Cell, Tissue and Organ Culture*, 71: 133-139.
- Lee, B., P. Martin and F. Bangerth, 1989. The effect of sucrose on the levels of abscisic acid, indole acetic acid and zeatin /zeatin riboside in wheatears growing in liquid culture. *Physiol.Plant*, 77: 73-80.
- Leite, M.V., C.A. Rosolem, D. Rodrigues, 2003. Gibberelin and cytokinin effects on soybean growth, *Sci. Agric.*, 60(3): 537-541.
- Ludwing-Muller, J., 2000. Indole-3-butyric acid in plant growth and development. *Plant growth regul.*, 32: 219-230.
- Magda, M.K., M.A. Shalaby and M.H. Mahgoub, 2007. Effect of some growth regulators om the levels of endogenous hormones and chemical constituents of rose plant. *J. Agric. & Environ. Sci.*, 2(6):720-730.
- Mahgoub, M.H., 1992. Physiological studies on the flowering of roses (*Rosa Sp.*). M. Sc. Faculty of Agric., Cairo University.
- Maurya, A.N. and S. lai, 1975. Effect of plant regulators on the growth and development of onion (*Allium cepa L.*) seeds with low temperature and IAA on the growth, chemical composition and yield of bulb 1 Arab weed control Conf.st., pp: 209-228.
- Mitchell, A.G. and J.F. Broadhead, 1967. Hydrolysis of solubilized aspirin. *J. Pharm. Sci.*, 56(10): 1261-1266.
- Nagel, *et al.*, 2001. *Annals of Botany*, 88: 27-31.
- Pancheva, T.V., L.P. Popova and A.N. Uzunova, 1996. Effect of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol.*, 149: 57-63.
- Pandey, D. and R.K. Pathak, 1981. Effect of Rootstocks, IBA and Phenolic Compounds on the Rooting of Apple Cuttings. *Propagation of Horticulture*, 13: 105-110.
- Pierpoint, W.S., 1994. Salicylic acid and its derivatives in plants: medicines, metabolites and messenger molecules. *Bot. Res.*, 20: 163-235.
- Raskin, I., 1995. Salicylic acid plant hormones physiology. *Biochem. Mol. Biol. New york USA*, pp: 188-205.
- Ray, S. and M.A. Choudhuri, 1981. Effects of Plant Growth Regulators on Grain-filling and Yield of Rice *Annals of Botany*, 47: 755-758.
- Read, S.M. and D.H. Northcote, 1981. Minimization of variation in the response to different proteins of the coomassie blue G.dye – binding assay for protein. *Anal. Biochem.*, 116: 53-64.
- Resmi, R. and T.R. Gopalakrishnan, 2004. Effect of plant growth regulators on the performance of yard long bean (*Vinga unguiculata var. sesquipedalis(L) vercourt*). *J. Trop. Agric.*, 42: 55-57.
- Sandaña, P.A., C.I. Harcha and D.F. Calderini, 2009. Sensitivity of yield and grain nitrogen concentration of wheat, lupin and pea to source reduction during grain filling. A comparative survey under high yielding conditions. *Field crop research*, 114(2): 233-243.
- Shakirova, F.M., A.R. Sakabutdinova, M.V. Berzukova, R.A. Fathutdinova and D.R. Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinith. *Plant Sci.*, 164: 317.
- Shales, O. and S.S. Schales, 1945. A sample method for determination of glucose in blood. *Arch. Biochem.*, 8: 285.
- Shehata, S.A.M., S.I. Ibrahim and S.A.M. Zaghlood, 2001. Physiological response of flag leaf and ears of maize plant induced by foliar application of kinetin (kin) and acetyl salicylic acid (ASA). *Ann. Agric. Sci. Ain Shams Univ. Cairo*, 46: 435-449.
- Sing, S., K. Sing and S.P. Singh, 1995. Effect of hormones on growth and yield characters of seed crop of kharif onion (*Allium cepa L.*). *Indian. J. plant physiol.*, 38(3): 193.
- Singh, G. and M. Kaur, 1980. Effect of growth regulators on padding and yield of mung bean (*Vigna radiate L.*) Wilezek. *Indian J. Plant Physiol.*, 23: 366-370.
- Singh, P., N. Tewari and P.K. Katiyar, 2002. Pretransplant seedling treatment with growthregulators and their effect on the growth and bulb production of onion (*Allium cepa L.*) *Progressive, Agric.*, 2(2): 181-182.
- Tompsett, P.B. and W.W. Schwabe, 1974. Growth Hormone Changes in *Chrysanthemum morifolium* *Annals of Botany*, 38: 269-285.
- Türkyılmaz, B., L.Y. Aktaş and A. Güven, 2005. Salicylic acid induced some biochemical and physiological changes in *Phaseolus vulgaris L.* *Science and Engineering Journal of Firat Univ*, 17(2): 319-326.
- Vardhini, V.B. and R.R.S. Seeta, 1998. Effect of brassinosteroids on nodulation and nitrigenase activity in groundnut (*Arachis Hypogaea L.*). *Plant Growth Regul.*, 28: 165-167.

Williams, P.M. and M.S. de Mallorca, 1984. Effect of gibberellins and the growth retardant ccc on the nodulation of soya. *Plant Soil*, 77: 53-60.

Zhao, H.J., X.W. Lin, H.Z. Shi and S.M. Chang, 1995. The regulating effects of phenolic compounds on the physiological characteristics and yield of soybean. *Acta Agron. Sin.*, 21: 351-355.