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INTERACTION EFFECTS OF BENZYLADENINE AND/OR PIX ON SOYBEAN PLANT:
1. SOME MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES

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ABSTRACT

Two pot experiments were carried out in the green house of Botany Department, National Research Centre, Dokki, Giza, Egypt, during two successive seasons (2000 and 2001). The aim of the present work was to study the effect of foliar application with benzyladenine, BA (25, 50 and 100 ppm), Pix (25, 50 and 100 ppm) and the interaction between BA and Pix on morphological and chemical analysis of soybean shoots (photosynthetic pigments, carbohydrate, protein and endogenous phytohormones).

The results revealed that foliar application with BA and/or Pix at all doses increased significantly the morphological criteria of shoot (Shoot length, stem diameter, fresh and dry weight, number of branches, number of leaves and leaves area/plant) and roots parameters (Root length and fresh & dry weights) at vegetative, flowering and fruiting stages. The most potent effect was observed due to the interaction treatment than the use of each growth regulator solely. The maximum promotive effect induced in all morphological growth was detected, in general, at 50 ppm BA + 50 ppm Pix at all stages of plant growth. The data also revealed that the photosynthetic pigments (chlorophylls and carotenoids) of soybean leaves significantly increased as affected by BA and/or Pix treatments. The most pronounced increase in photosynthetic pigments content was accompanied by the increase in total soluble sugar, polysaccharides and consequently total carbohydrates in soybean shoots in response to all treatments.

In the mean time, the amino-N, total soluble-N, protein-N and total-N increased in treated soybean plant's shoots in response to all concentrations of BA and/or Pix as compared to the untreated plants. The interaction treatments induced the maximum values of nitrogenous content as compared with the use of each growth regulator individually.

Benzyladenine treatments decreased ABA and increased IAA contents, while a reverse effect was observed in response to all Pix concentrations. The endogenous GA_3 and total cytokinins showed marked increases in response to all BA and/or Pix treatments.

Keywords: Soybean, benzyladenine (BA), pix, interaction, morphology, pigments, carbohydrate, protein and hormones.

INTRODUCTION

Benzyladenine is one of the naturally occurring cytokinins in plants (Van-Staden and Crouch, 1996 and Nair *et al.*, 2002). The mode of action of the cytokinins is promotion of cell division, delaying senescence, promotion of lateral buds development, gained growth and fruit set (Wilkins, 1984). Pix (1, 1 dimethyl piperidinium chloride) or mepiquot chloride, is a growth retardant, biologically active in modifying the growth habit of many cultivated crops (Oosterhius *et al.*, 1998).

The growth regulators induced a wide range effects on plant growth. In this concern, Ramadan (1992) on soybean plants found that BA foliar application increased the shoot length, stem diameter, number of nodes, number of leaves, leaves area and fresh and dry weights of shoots. Recently, several investigators found that the same effects on different plants when treated with BA (Vijay and Laxmi, 2001; Ramesh and Thirumurugany 2001; Patil *et al.,* 2002a and Sadak, 2005). Regarding the effect of Pix on morphological responses, Zaky *et al.* (1999) on *Vicia faba* and Youssef and Moussa (2001) on Bulgarian peppermint found that foliar application of Pix increased shoot length, number of internodes, circumference of stem, number of leaflets, average leaf area, shoot fresh and dry weights, root length and root fresh and dry weights. The effect of the plant growth regulators interaction on different criteria of plants growth was studied by many investigators. In this connection, Oosteruis and Coker (2000) found that GA_3+BA foliar application of cotton plants increased number of lateral roots per plant. Moreover, Abdel-Aziz (2002) found that the application of BA and/or Pix increased plant height, number of branches and fresh & dry weights of *Origanum majorana*.

Several authors reported that cytokinin and Pix increased the chlorophylls content and delaying senescence in different plants (Sivakumar *et al.*, 2000 on wheat; Shadi *et al.*, 2001 on maize; Abdel-Aziz, 2002 on *Origanum majorana* and Kassem and Namich, 2003 on cotton). Also, Patil *et al.* (2002b) showed that 6-benzyladenine foliar spraying increased significantly leaf chlorophyll content of soybean plants. The interaction effects of both BA + Pix on photosynthetic pigment was found by Abdel-Aziz (2002) who noticed that BA and/or Pix increased significantly these parameters than that resulted with using each one of them individually.

A vital role of benzyladenine or cytokinins in regulating the plant biochemical processes, cytokinins enhance the synthesis of different metabolites such as protein, RNA and carbohydrate (R^ese *et al.*, 1995), and also as protective effects against protein, RNA and DNA degradation (Ramanco *et al.*, 1980). In this respect, El-Shahaby *et al.* (1994) indicated that benzyladenine increased the nitrogen, protein (total soluble-N, protein-N, total protein and total-N) and carbohydrate contents (reducing sugar, sucrose and polysaccharides) in soybean plants. Pix induced major changes in the metabolism via controlling the carbohydrate and protein synthesis (Zhao and Oosterhuis, 2000). Yousef and Mousa (2001) on Bulgarian peppermint and Abdel-Aziz (2002) on *Origanum majorana* reported that the highest values of total carbohydrates were obtained due to foliar application of kinetin + Pix.

The application of more than one of the plant growth regulators may act synergistically, antagonistically or additively depend upon the internal plant hormones levels (Audus, 1959). In turn the different physiological processes are directed. In this respect, Moussa and Sallam (1996) studying on maize and barley indicated that kinetin exerted promotive effects on IAA and GA_3 activities in presence of salinity. In the same ti.r e, kinetin treatments showed a slight increase in cytokinins activity in barley plants. Regarding the effect of Pix, Hardilik (1974) indicated that growth retardant might act through the production of IAA-oxidase and peroxidase which cause a decrease in IAA level. Pix act as a retardant which increase the endogenous growth inhibitory substances and induce a marked decline in auxins, GA_3 and cytokinins (El-Orabi, 1994 and El-Bahey *et al.*, 1999).

The aim of the present work was to study the effect of foliar application with benzyladenine (BA), Pix and the interaction between BA and Pix on some morphological and chemical analysis of soybean shoots (photosynthetic pigments, carbohydrate, protein and endogenous phytohormones).

MATERIALS AND METHODS

Two pot experiments were carried out in the green house of Botany Department, National Research Center (NRC), Dokki, Giza, Egypt, during two growing successive seasons (2000-2001) to study the effect of foliar application of Benzyladenine (BA) and/or Mepiquate chloride (Pix) on the yield and chemical composition of soybean plants.

A homogenous lot of soybean seeds (*Glycine max* L. Merr.) were selected and thoroughly washed with continuous tap water for a limited period (30 minutes) then dried and sown after inoculation with Okadin (*Rhizobium japonicum*) on the 15^{th} of May 2000 and 2001 in pots 30 cm. diameter. The pots contained equal amounts of soil (7 kg - from mixture of clay and sand 2 : 1 w/w respectively). A phosphorous fertilizer in the form of triple superphosphate (5 gm) was added to each pot. Ten seeds were sown in each pot and the irrigation was carried out according to the usual practice by adding equal amounts of water. After twelve days from sowing thinning was performed where five uniformed seedlings were left for experimentation in each pot. The plants were exposed to normal day length and natural illumination.

The pots were divided to Sixteen sets each of 8 pots. The experiment was performed as a complete randomized design with 8 replicates. BA and Pix were used as foliar application each in four concentrations (0, 25, 50 and 100 ppm) alone or in combination. The plants of each treatment were sprayed three times. The foliar spray was carried out after thirty days (Vegetative stage), forty five days (Flowering stage) and sixty days (Fruiting stage) from sowing throughout the two successive seasons (2000 and 2001).

After 15 days from spraying the growth regulators, the growth measurements (shoot & root lengths, stem diameters, number of branches, number of leaves, leaves area and fresh and dry weights of shoots and roots) were determined as well as photosynthetic pigments (chl. a, chl. b and carotenoids) and endogenous hormones (IAA, ABA, GA_3 and total cytokinins) at 45 days (vegetative stage), 60 days (flowering stage) and 75 days (fruiting stage) from sowing throughout the two successive seasons (2001 & 2002). Also, the chemical analyses of shoot were determined using the dried plant materials in the oven at 70°C for 48 hours till constant weight, then used for analysis of carbohydrate and protein content at the three growth stages.

Chemical analyses:

The plant pigments (Chlorophyll a, chlorophyll b and carotenoids) were determined according to the spectrophotometric method as recommended by Metzner ***et al.*** (1965).

Extraction procedure of carbohydrate fractions was carried out according to Younis (1963). Total soluble sugars were determined according to Bell (1955). Meanwhile, the polysaccharides were determined according to Younis (1963).

Total nitrogen was determined by using the modified micro-kjeldahl method of Peach and Tracey (1966) and calculated as percentage. Determination of amino nitrogen according to the method described by Muting and Kaiser (1963) and total soluble nitrogen fractions and total nitrogen according to Pirie (1955). The subtraction of the total soluble-N from total-N gave the value for protein-N, in which it multiplied by 6.25 (factor) to give the crude protein percentage.

Endogenous phytohormones were determined in fresh shoots after 60 days from sowing. The extraction was carried out according to Wasfy and Orrin (1975). The methylation as reported by Vogel (1975) was used for Gas Liquid Chromatography (GLC) for IAA, ABA and GA3. The GLC conditions for isothermal work were 2.8 m X4 mm glass column packed with acid, alkali and silanized diatomite C (100-120 mesh) and coated with 1% OV-17, temperatures; injector 250 C, column 230 C, and detector 300 oC flow rates; nitrogen 300 ml/min; hydrogen 33 ml/min, and air 330 ml/min range of 32X102 and chart speed of 3 cm/min. High Performance Liquid Chromatography (HPLC); isocratic UV analyzer ODS Hyparsil C18 column, 20 minutes gradient from 0.1 N acetic acid. pH 2.8 to 0.1 N acetic acid in 95% aqueous ethanol, pH 4. the flow rate: 1 ml/min., detection: UV 254 nm; was used for cytokinins measurements.

Statistical analysis:

Data were subjected to statistical analysis according to Snedecor and Cochran (1980). The means were compared by L.S.D. test at significant probability level of 0.05 and 0.01.

RESULTS AND DISCUSSION

Root growth:

The application of BA and/or Pix at all doses increased the length, fresh and dry weights of root/plant (Table 1). The most effective concentration of BA + Pix was 50 ppm at flowering and fruiting stages. In this respect, Abdel-Hamid (1997) showed similar effects on the fresh, dry weight and total root length of sweet pepper plants in response to BA treatment. In terms of Pix effect, similar results were obtained by Wasnik and Bagga (1996) on chickpea and Zaky *et al.* (1999) on soybean plant who recorded that the root length, root fresh weight as well as root dry weight/plant were increased significantly in response to Pix treatments.

Maximum increase in root parameters were recorded due to interaction effects (Table 1), the gained increase in root weight due to the interaction effect in this work might be due to the enhancement of shoot growth which transferred assimilates needed for root growth rather to direct effect on root growth such as affecting on cell division and/or elongation. This conclusion is in accordance with those obtained by Dawh (1989) and Shahin (1999).

Table (1): Mean values of root system parameters as affected by BA and/or Pix treatments in soybean during the two growing seasons.

Treatments (ppm)		Root length (cm)			Root fresh weight (g)			Root dry weight (g)		
		45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days
Control		13.7	21.0	36.1	3.0	8.3	11.8	1.2	3.7	4.8
BA	Pix									
25	0	17.1	23.1	40.6	3.8	8.8	12.6	1.4	4.0	5.8
50	0	18.2	24.3	41.1	4.1	9.4	13.2	1.6	4.7	6.3
100	0	17.5	23.6	41.6	4.2	9.1	12.7	1.6	4.3	6.3
0	25	17.5	24.7	43.9	4.7	11.1	13.3	1.3	4.7	6.4
0	50	19.0	26.6	45.0	5.0	12.5	14.2	1.8	5.1	6.8
0	100	20.6	26.6	45.2	4.8	13.2	13.4	1.7	4.8	6.8
25	25	18.0	25.7	44.1	4.9	11.0	13.9	1.4	5.2	6.7
25	50	19.6	26.5	46.0	5.5	12.5	14.6	1.8	5.8	7.7
25	100	20.1	27.2	45.3	5.2	12.7	14.2	1.7	5.7	6.6
50	25	19.0	27.0	45.2	5.1	11.7	14.2	1.6	5.5	7.4
50	50	20.5	27.8	47.3	5.5	13.4	15.3	1.8	6.1	8.1
50	100	20.4	27.8	45.3	5.0	12.7	14.1	1.8	5.7	6.5
100	25	18.9	27.4	43.7	5.0	11.2	13.7	1.5	5.4	7.0
100	50	19.7	27.8	46.9	5.3	12.6	14.6	1.6	5.8	7.2
100	100	19.1	27.1	45.4	4.5	12.4	13.0	1.6	5.5	6.3
L.S.D. for BA or Pix	(0.05)	0.5	2.0	1.7	0.6	0.8	0.8	0.13	0.45	0.93
	(0.01)	0.7	2.7	2.2	0.7	1.1	1.1	0.18	0.59	1.24
for BA x Pix	(0.05)	1.1	4.0	3.3	1.1	1.6	1.6	0.26	0.89	1.87
	(0.01)	1.4	5.4	4.4	1.5	2.2	2.2	0.34	1.19	2.48

Shoot growth:

Data presented in (Table 2) showed that there is an increase in the shoot length, stem diameter, number of leaves, leaf area and number of branches in BA-treated plants were followed by increases in fresh and dry weights of soybean shoots throughout the experimental stages in both seasons. The optimum increases were obtained by 50 ppm BA treatment. The increase in morphological criteria in BA-treated plants may be due to the role of BA on the regulation of cell division, differentiation and organogencsis in developing plants (Skoog and Armstrong, 1970 and Hall, 1973). These results are in agreement with the results obtained by Harb (1992) and Ibrahim *et al.* (2001). Also, Patil *et al.* (2002a) found that 25 or 50 ppm increased plant height, number of branches/plant, leaf area/plant, dry matter production and relative growth rate of soybean plants. The stimulatory effect of BA on shoot length could refer to the effect of growth regulators on cell division and cell enlargement (Rabie, 1996 and Cho *et al.*, 2002). Moreover, Montague (2000) indicated that the length, width, vein width and cell wall formation in cucumber cotyledons were promoted by BA treatments. He concluded that the increments in fresh weight may be due to the increase in the plant efficiency of water uptake, conservation and utilization under the effect of BA. Meanwhile, the increase in the dry weight of soybean treated, plant can be considered as reflection to the increase in photosynthetic activity (Table 3) and consequently carbohydrate (Table 4). Moreover, Vanderhoef and Dute (1981) stated that the dry matter accumulation of kinetin treated soybean plants was caused by the regulation of wall loosening and the precipitation of substances required for growth such as lipids, nucleic acids and protein.

The data (Table 2) revealed that all Pix concentrations (25, 50 and 100 ppm) increased significantly the previously mentioned growth parameters at vegetative, flowering and fruiting stages. The most pronounced increase was observed in response to 50 ppm. The promotive effect of Pix on morphological criteria was reported by several investigators (Channakesheva *et al.*, 1999 on maize; Sun *et al.,* 2000 on cotton; Youssef and Moussa, 2001 on *Bulgarian peppermint* and Abdel-Aziz, 2002 on *Origanum majorana*). The increment in shoot length may be attributed to the effect of Pix on endogenous gibberellin which in spite of it is a growth retardant, but when used in low concentrations could act as promoter (El-Orabi, 1994 on wheat; Nowak *et al.*, 1997 on *Vicia faba* and Lamas *et al.*, 2000 on cotton). While in increase in stem diameter could be attributed to the greatest increase in number of cortical layers, thickness of cortex, number and diameter of both vascular bundles and vessels per bundle (Louis, 1982).

In respect to the combined treatment effects on the morphological responses, they showed more or less similar way to either BA or Pix if applied individually (Table 2). It is clear that all concentrations of BA + Pix showed a clear additive or synergistic effect on most of the presented growth criteria in plants treated with either BA or Pix throughout all plant growth stages.

It is obvious from the results that the most improved shoot parameters due to interaction treatment was 50 ppm BA + 50 ppm Pix than the use of either BA or Pix alone. Similar effect can be discussed when Pix and other bioregulators were used such as Kinetin + ABA, GA + CCC, Pix + GA, Pix + IBA or promalin (GA + BA). In this respect, Mastalerz (1971) reported that the natural growth regulators in plant interact in various combinations to coordinate the plant growth and development. They exert their effects on the basis of the balance that prevail the tissues at particular stage of growth and development or under specific set of environmental conditions. The study of Ibrahim *et al.* (2001) on sun flower plant, found that all morphological criteria were improved due to the application of growth promotor (kinetin at 50 ppm) followed by the application of growth inhibitor, (ABA at 50 ppm) compared with the treatment of each alone. Moreover, Abdel-Aziz (2002) found that application of Pix (100 or 200 ppm) on *Majorana horten* + BA (20 or 40 ppm) and also Pix (the same concentration) plants plus IBA (indole butryic acid at 2 or 40 ppm) increased plant height, number of branches/plant, fresh weight/ plant, dry weight/ plant and air dry weight/plant in the first and second cultivar in both successive seasons compared with the used of BA alone or control plants. In addition, Kassem and Namich (2003) found that the spray of Pix + GA positively influenced the growth of cotton plats (plant height; nodes number, internode length, number of flower ad number of total fruiting sites/plant.

Table (2): Mean values of shoot system parameters as affected by BA and/or Pix treatments in soybean during the two growing seasons.

Treatments (ppm)		Shoot length (cm)			Shoot fresh weight (g)			Shoot dry weight (g)			Stem diameter (cm)			Leaves number/plant			Total leaves area/plant (cm^2)			Number of branches/plant		
		45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days
Control		26.0	32.6	49.2	11.5	20.4	25.6	3.4	5.7	9.9	0.45	1.07	1.29	7.3	13.7	26.7	365.8	490.0	1294.0	1.6	2.5	4.3
BA	Pix																					
25	0	26.7	36.4	59.5	12.1	21.9	27.4	3.8	6.1	11.0	0.53	1.26	1.54	8.9	15.2	30.5	454.8	681.9	1436.7	2.7	4.0	7.0
50	0	29.2	40.0	65.6	12.8	24.4	28.8	4.6	6.9	12.4	0.60	1.37	1.60	10.6	18.4	32.7	510.2	727.0	1542.6	3.7	5.3	7.9
100	0	29.4	41.2	67.3	12.6	23.2	29.6	3.9	7.1	12.0	0.65	1.43	1.70	10.6	17.7	30.9	465.7	710.4	1499.3	2.8	4.3	7.5
0	25	26.4	36.3	56.2	12.8	22.0	29.6	4.7	8.0	12.3	0.65	1.49	2.19	8.4	15.6	31.5	393.5	549.1	1490.1	3.5	4.9	7.5
0	50	27.1	39.0	58.7	13.1	24.5	31.0	5.1	8.3	13.7	0.81	1.61	2.33	10.6	19.0	34.3	422.9	654.3	1582.7	4.4	5.8	8.5
0	100	25.5	35.5	54.8	12.3	25.2	30.5	5.0	7.3	12.5	0.88	1.66	2.34	8.9	17.5	33.8	267.8	600.4	1343.6	2.6	4.5	5.9
25	25	27.9	41.0	64.5	12.7	24.3	29.5	4.7	7.0	13.5	0.69	1.55	1.96	9.8	19.1	32.6	475.3	704.0	1621.1	4.1	5.6	8.0
25	50	29.4	42.2	65.6	13.4	26.4	30.7	5.3	8.7	15.1	0.81	1.62	2.13	12.6	19.8	35.7	524.8	841.0	1657.2	4.8	6.6	9.2
25	100	27.7	40.6	61.9	13.7	26.0	30.4	4.9	7.9	13.7	0.87	1.66	2.19	10.3	18.8	34.5	349.2	732.2	1471.5	3.2	5.6	7.4
50	25	30.0	43.2	69.7	14.4	25.9	30.2	5.1	7.7	14.9	0.82	1.63	1.98	12.1	20.2	34.5	539.0	815.9	1754.9	4.6	6.2	8.5
50	50	31.3	46.0	72.3	15.7	27.7	34.0	5.4	9.0	16.0	0.85	1.70	2.04	13.7	22.3	38.9	557.4	881.1	1834.9	5.1	7.2	10.2
50	100	29.5	42.7	66.4	13.6	26.9	31.6	4.6	8.3	14.9	0.85	1.66	2.12	11.6	19.7	35.9	461.3	792.3	1742.8	3.1	5.3	8.0
100	25	30.0	44.4	72.0	14.3	23.9	30.8	4.5	8.0	13.9	0.79	1.56	2.00	11.9	19.7	34.0	477.1	775.7	1822.5	3.8	5.8	8.4
100	50	31.0	46.0	72.6	14.3	28.5	32.1	4.9	8.6	14.8	0.86	1.61	2.18	12.8	20.7	36.4	481.6	814.7	1891.6	4.5	6.4	8.9
100	100	28.0	42.0	64.5	13.4	26.3	29.1	4.6	7.9	14.9	0.81	1.52	1.96	10.0	18.6	33.6	381.2	835.4	1541.2	2.8	4.5	6.6
L.S.D. for BA or Pix	(0.05)	0.7	1.0	2.1	1.0	2.0	1.9	0.5	0.8	0.8	0.04	0.06	0.08	1.0	1.1	1.3	51.4	28.0	73.9	0.3	0.8	0.8
	(0.01)	1.0	1.4	2.8	1.4	2.7	2.5	0.6	1.1	1.1	0.05	0.08	0.11	1.4	1.5	1.7	68.4	37.2	98.3	0.4	1.0	1.0
for BA x Pix	(0.05)	1.5	2.0	4.3	1.2	4.05	3.8	0.9	1.7	1.7	0.08	0.13	0.16	2.0	2.2	2.5	102.9	56.0	147.9	0.6	1.6	1.6
	(0.01)	2.0	2.7	3.7	2.0	5.4	5.0	1.2	2.2	2.2	0.11	0.17	0.21	2.7	2.9	3.3	136.8	74.4	196.7	0.8	2.1	2.1

The application of promalin (GA + BA) has a pronounced effect on increasing the vegetative growth of many plants (Jacyna, 1995; Rabie, 1996 and Suh Jeung and Suh, 1997). The promotive effects of interaction on vegetative growth could be attributed to its effect on several factors such as: element uptake (Bist, 1990), auxin content (Rabie, 1996) and the chlorophyll content (Ranwala *et al.*, 2000).

Photosynthetic pigments content:

Data in Table (3) showed, in general, that all concentrations of BA and/or Pix induced significant increase in photosynthetic pigments of soybean plants. The most pronounced increment was profound in response to 50ppm BA+50ppm Pix in all pigment contents at all plant growth stages.

Table (3): Mean values of chlorophyll contents (mg/100g fresh weight) of soybean leaves at three stages during as affected by BA and/or Pix treatments in soybean during the two growing seasons.

Treatment (ppm)		Chlorophyll a			Chlorophyll b			Carotenoids		
BA	Pix	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days
Control		123.5	142.8	163.4	62.4	69.8	72.6	30.4	42.3	43.1
25	0	124.4	160.1	165.6	64.7	72.1	73.8	31.5	44.9	46.5
50	0	125.9	139.3	168.6	65.8	77.1	78.3	34.4	48.5	54.0
100	0	124.7	141.9	164.7	64.8	76.1	73.9	34.6	48.3	49.3
0	25	124.2	154.1	164.5	63.9	71.3	75.8	31.6	41.6	43.7
0	50	127.7	156.6	166.4	68.6	77.0	86.7	33.6	42.7	66.5
0	100	124.9	143.2	162.8	67.2	72.8	77.1	32.3	43.4	65.5
25	25	124.8	160.1	167.1	65.5	77.3	78.3	31.8	46.3	54.4
25	50	129.6	157.0	168.0	66.3	78.2	89.3	36.1	46.9	68.7
25	100	126.7	153.8	166.8	66.7	76.7	81.4	32.9	49.2	65.8
50	25	129.3	167.5	168.9	67.2	78.1	85.9	35.9	49.1	58.8
50	50	139.2	160.6	175.2	71.5	94.3	98.9	39.6	52.3	79.6
50	100	126.8	143.3	168.4	68.9	78.0	88.1	35.2	48.0	67.2
100	25	128.3	144.6	166.2	68.3	76.9	85.8	35.7	41.6	58.1
100	50	138.1	157.1	175.3	68.3	78.3	88.2	37.4	51.0	68.1
100	100	123.3	142.8	168.0	65.5	67.0	87.0	32.5	41.2	56.0
LSD for BA or Pix (0.05)		0.3	1.7	1.2	0.6	0.5	1.2	0.5	0.7	0.6
LSD for BA or Pix (0.01)		0.5	2.3	1.6	0.8	0.7	1.7	0.7	1.0	0.8
for BA x Pix (0.05)		0.7	3.4	2.3	1.2	0.9	2.4	0.9	1.5	1.2
for BA x Pix (0.01)		0.9	4.6	3.2	1.6	1.3	3.3	1.3	2.1	1.6

The general increases in chlorophylls and total pigments in response to BA treatments is coincide with the results obtained by the recent researches of Sivakumar *et al.* (2000) on wheat, Ramesh *et al.* (2001) and Patil *et al.* (2002) both on soybean and El-Abagy *et al.* (2003) on faba bean plants. The increment of pigments content due to BA application could be attributed to inhibition of chlorophyll degradation and/or the promotion of chlorophyll synthesis. In this respect, the findings of Sudria *et al.* (2001) supported the presented results who found that cytokinins application accelerated the development and modification of the chloroplast and other components of photosynthesis such as CO_2 assimilation and activity of photosynthetic enzymes and consequently promoted the photosynthetic activity.

The increments in pigments content due to pix application were confirmed by Zaky *et al.* (1999) who showed that Pix increased chlorophyll a, chlorophyll b, chlorophyll a + b and carotenoid contents in *Vicia faba* plants. Similar effects were observed due to Pix application on pigments contents on different plants (Wasnik and Bagga, 1996 on chickpea and Abdel-Aziz 2002; on *Origanum majorana*). It was found that there were close correlation between the changes of the total chlorophylls and carotenoids in response to Pix foliar application. Similar results were obtained by Zavirove and Saloheva (1979) who reported that the growth retardant CCC application on bean plants enhanced carotenoids content which act as protective agents against photo-destruction of chlorophyll and in turn cylic photo-phosphorelation.

As regard to the interaction effect of both BA + Pix, it is worthy to mention that the maximum values of pigments contents were recorded in response to the effect of BA + Pix than that obtained for each one alone. The present results agreed with those of Abdel-Aziz (2002) on *Origanum majorana* who found that the interaction effect of BA with Pix induced a significant increments in all photosynthetic pigments (Chl. a, chl. b and carotenoids) compared with the treatment with BA and Pix solely. Also, Kassem and Namich (2003) showed the interaction effect of both GA_3 + Pix at early flowering stage induced a highly significant increase in chl. a, chl. b, chl. a+b and carotenoids of cotton treated leaves. It is concluded that the interaction effects of both BA and Pix induced an additive effects.

Carbohydrate content:

The different concentrations of BA and/or Pix at (25, 50 and 100 ppm) induced a highly significant increase in total soluble sugars, polysaccharides and consequently total carbohydrates in soybean shoots at vegetative, flowering and fruiting stages of plant development (Table 4). The magnitude of response was more pronounced with the plants sprayed with 50 ppm BA + 50 ppm Pix in total soluble sugar, polysaccharide and total carbohydrate in all stages of plant growth. This increase in total carbohydrate pool in soybean shoots in response to BA applications is supported by stimulation in photosynthetic pigments (Table 3) and the accumulation of the dry matter in the shoots of BA treated plants (Table 2). In agreement with these results Talaat and Youssef (1998) and Balbaa (2002) who found that cytokinins treatments increased CO_2 fixation leading to more sugar synthesis.

Concerning the effect of Pix, Nepomuceno *et al.* (1997) reported that Pix-treated plants had higher sucrose and starch due to a decrease in the activity of ribulose diphosphatc carboxylase enzyme in cotton treated plants. Zhao and Oosterhuis (2000) showed that Pix plus (Pix + *Bacillus cereus*) and mepiquat chloride improved leaf CO_2 exchange rate and increased leaf starch content in cotton plants due to starch accumulation in chloroplasts as a primarily mechanism for storing carbon when the role of photosynthesis exceeded the leaf capacity to export saccharides (Stitt, 1984).

Table (4): Effect of BA and/or Pix on carbohydrate contents (mg/100 g dry weight) in shoots of soybean plants at three stages during 2001 season.

Treatment (ppm)		Total Soluble Sugars			Polysaccharides			Total Carbohydrates		
BA	Pix	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days
Control		4.4	8.9	11.9	197.8	243.7	344.2	202.2	252.6	356.1
25	0	6.3	10.3	12.9	216.5	261.9	364.3	222.8	272.2	377.2
50	0	7.7	12.4	14.2	227.5	311.2	381.0	235.2	323.6	395.2
100	0	7.4	12.0	13.2	204.8	292.4	367.2	212.2	304.4	380.4
0	25	6.0	10.9	12.9	240.6	271.9	360.3	246.6	282.8	373.2
0	50	8.2	13.5	14.4	291.0	321.3	382.2	299.2	334.8	396.6
0	100	7.5	12.4	13.6	269.3	290.4	375.2	276.8	302.8	388.8
25	25	7.4	12.4	14.7	234.2	294.8	390.3	241.6	307.2	405.0
25	50	8.5	15.2	16.5	288.1	329.6	432.1	296.6	344.8	448.6
25	100	7.8	13.0	14.6	270.4	324.4	410.0	278.2	337.4	424.6
50	25	8.0	14.1	15.8	256.2	332.7	424.2	264.2	346.8	440.0
50	50	10.1	16.5	18.3	308.3	370.3	473.7	318.4	386.8	492.0
50	100	8.7	15.6	16.7	230.5	351.2	444.1	289.2	366.8	460.8
100	25	8.0	13.4	15.6	248.8	320.8	409.4	256.8	334.2	425.0
100	50	9.2	15.7	17.7	302.6	351.1	445.3	311.8	366.8	463.0
100	100	7.7	14.5	15.2	300.3	332.5	413.2	308.0	347.0	428.4
LSD for BA and or Pix	(0.05)	0.3	0.6	0.1	3.1	1.3	4.9	1.6	2.4	4.9
	(0.01)	0.4	0.8	0.2	4.3	1.7	6.8	2.2	3.4	6.8
BA X Pix	(0.05)	0.6	1.2	0.2	6.2	2.5	9.9	3.2	4.9	9.9
	(0.01)	0.8	1.7	0.4	8.6	3.5	13.6	4.4	6.7	13.6

The additive effect of both growth regulators BA+Pix on carbohydrate content of soybean shoot compared to their solely usage was confirmed by Abdel-Aziz (2002) using the interaction between BA + Pix; Kassem and Namich (2003) using GA + Pix and Yousef and Mousa (2001) using kinetin + Pix, since they all found that the carbohydrate fractions increased significantly in response to the interaction treatments.

Nitrogenous constituents:

The interaction of BA and Pix caused, in general, highly significant increases in nitrogenous fractions (Amino-N, total soluble-N and total-N) of soybean shoot compared with the increase due to BA or Pix treatments alone (Table 5). The concentration of 50 ppm BA + all Pix levels induced the maximum values of nitrogen constituents at flowering and fruiting stages. The increase in total soluble-N of shoots was accompanied by a marked increase in total protein-N parallel to growth rate stimulation of shoot (Table 2). These results may be attributed to the incorporation of amino acids into protein and/or to the translocation of the soluble-N to the young leaves and developing fruits. Wilkins (1984) suggested that BA not only promote photosynthetic activity, but also increased RNA and protein synthesis. Also, Khalil and Mandurah (1990) emphasized that kinetin delayed the loss of total soluble protein in cowpea plants via preventing a rapid decline in protein content and nucleic acids due to the depressive effect of kinetin on the proteolytic enzymes.

Table (5): Effect of BA and/or Pix on nitrogenous content (mg/100 g dry weight) in shoots of soybean plants at three stages during 2001 season.

Treatment (ppm)		Amino-N			Total soluble-N			Protein -N			Total-N		
BA	Pix	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days	45 days	60 days	75 days
Control		5.6	6.4	3.3	11.7	8.7	6.8	29.1	39.3	32.5	40.8	48.0	39.3
25	0	6.1	6.7	3.6	11.6	9.5	8.0	32.2	41.6	33.0	43.8	51.1	41.0
50	0	7.6	7.8	4.7	12.7	9.8	9.1	32.9	44.3	33.1	45.6	54.1	42.2
100	0	6.4	7.5	4.6	12.1	9.2	6.2	32.8	43.0	35.1	44.9	52.2	41.3
0	25	6.7	7.7	3.5	18.5	13.5	5.6	23.9	38.8	35.6	42.4	52.3	41.2
0	50	7.9	9.9	4.6	17.0	11.2	6.6	26.2	44.2	35.6	43.2	55.4	42.2
0	100	6.8	9.0	4.6	19.8	13.8	9.6	24.6	39.3	30.8	44.4	53.1	40.4
25	25	7.1	8.9	4.8	12.2	9.9	9.1	32.3	45.4	33.6	44.5	55.3	42.7
25	50	7.9	10.0	6.0	13.1	11.1	9.7	32.2	48.7	34.4	45.3	59.8	44.1
25	100	6.6	9.9	5.8	14.1	10.1	9.2	31.5	47.8	33.8	45.6	57.9	43.0
50	25	8.9	9.9	5.7	11.7	10.1	9.6	36.8	47.0	34.5	48.5	57.1	44.1
50	50	7.0	11.7	6.8	16.6	14.3	9.7	30.5	50.0	37.5	47.1	64.3	47.2
50	100	6.8	10.5	5.4	13.0	12.4	6.8	33.2	50.0	36.6	46.2	62.4	43.4
100	25	7.0	8.7	5.2	14.3	9.3	6.9	32.1	46.0	35.8	46.4	55.3	42.7
100	50	8.9	10.7	6.1	18.1	12.1	9.3	30.5	49.8	34.9	48.6	61.9	44.2
100	100	6.9	8.8	4.2	13.6	10.0	7.9	31.8	48.3	32.8	45.4	58.3	40.7
LSD for BA and or Pix (0.05)		0.3	0.2	0.3	0.1	0.1	0.03	0.5	0.4	0.4	0.4	0.6	0.4
(0.01)		0.4	0.3	0.4	0.2	0.2	0.05	0.6	0.6	0.5	0.5	0.8	0.5
BA X Pix (0.05)		0.5	0.5	0.5	0.3	0.2	0.07	0.9	0.9	0.8	0.7	1.2	0.7
(0.01)		0.8	0.7	0.7	0.4	0.4	1.00	1.3	1.2	1.0	1.0	1.7	1.0

The increment in nitrogenous constituents were supported by Zayed *et al.* (1985) on Okra plants and Zaky *et al.* (1999) on *Vicia faba* plants who found that the lower concentration of Pix induced a remarkable increase in amino-N, total soluble-N, protein-N as well as total-N in the shoots and roots during vegetative, flowering and fruiting stages. Also, marked increments were induced that in DNA, RNA and reduction in protease enzyme in both shoots and roots.

Our results concerning the increase in nitrogen fractions in soybean shoot in response to the interaction treatments (BA + Pix) are in agreement with those obtained by Abdel-Aziz (2002) who found that combining Pix (100 or 200 ppm) with BA (20 or 40 ppm) caused a marked increase in nitrogen content compared to the used individual growth regulators in the shoot of *Origanum majorana*. This was attributed to the increase in absorption, translocation of nitrate and their assimilation in addition to the increase in nitrogen fixation.

Endogenous phytohormones:

The presented data in Table (6) revealed that BA alone or in combination with Pix increased the content of IAA, GA_3 and total cytokinins, while ABA decreased due to all treatments. The increase in endogenous IAA and GA_3 contents in response to treatment with BA or in combination with Pix might be attributed to the effect of these applied hormones on increasing the biosynthesis of IAA auxins and gibberellins and/or decreasing their degradation through decreasing the activity of IAA-oxidase.

Table (6): Effect of BA and/or Pix on endogenous phytohormones (IAA, ABA, GA and cytokinins), ug/g fresh wt. of soybean plants during 2001 season.

Treatment (ppm) BA	Pix	IAA	ABA	GA	Total cytokinins
Control		18.40	4.61	92.58	76.32
25	0	22.71	2.10	114.59	88.16
50	0	34.03	1.94	160.45	97.83
100	0	33.28	1.85	130.46	108.29
0	25	16.59	4.97	98.28	83.65
0	50	17.26	5.07	103.20	94.11
0	100	13.71	5.99	100.95	73.07
25	25	23.69	3.53	120.06	107.89
25	50	20.17	2.69	106.94	113.07
25	100	19.99	2.42	95.71	89.11
50	25	26.25	4.23	112.24	86.22
50	50	24.86	4.07	103.84	104.34
50	100	23.21	3.97	101.66	109.96
100	25	20.92	4.27	93.85	82.29
100	50	21.86	4.68	115.70	89.01
100	100	19.71	5.93	100.25	72.74

In this respect, Lau and Yang (1973) found that kinetin increased the free auxin content of mungbean hypocotyls segments. Also, Goodwin (1978) stated that BA treatment not only increased the extractable active gibberellins, but also the rate of synthesis of GA_{20} from GA_9. Moreover, Blagoeva *et al.* (2004) found that exogenous application of cytokinin and BA stimulated dihydrozeatin 7-glucoside up to 40% and endogenous zeatin up to 35% in radish seedlings. The decrease in ABA content due to hormonal treatments could be attributed to the shift of the common precursor isopentenyl pyrophosphate into the biosynthesis of cytokinins and/or gibberellins instead of ABA (Bouza *et al.*, 1993 and Hopkins and Hüner, 2004).

Concerning the effect of Pix on endogenous phytohormones, it is clear from Table (6) that the gradual reduction in IAA followed by increment in ABA contents in response to all concentration. The reduction in IAA content due to Pix application may be attributed to the production of IAA oxidase which controls the IAA level in plants. Similar conclusion was reported by Omar *et al.* (1985) and Zaky *et al.* (1999). The observed increase in ABA, GA_3 and cytokinin level in response to Pix treatments were also confirmed by Due *et al.* (1979) who reported that Pix induced a significant increase in the endogenous levels of each of zeatin, zeatin riboside and zeatin glucosides in soybean treated plants. Also, El-Bahey *et al.* (1999) found a clear increment in gibberellins activity and ABA during the different growth stages of *Vicia faba* plants with using low concentration of Pix.

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تأثيرات تداخل البنزيل أدنين و/أو البكس على نبات فول الصويا:
١- بعض الاستجابات المورفولوجية والفسيولوجية

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أجريت تجربتي أصص في صوبة قسم النبات بالمركز القومي للبحوث بالدقي ، الجيزة ، مصر خلال الموسمين المتتالين (٢٠٠٠ ، ٢٠٠١). وكان الهدف من هذا العمل هو دراسة تـأثير الرش بالبنزيل أدنين والبكس (٢٥ ، ٥٠ ، ١٠٠ جزء في المليون) لكل منهما ، والتفاعل بينهمـا على الشكل الظاهري والتحليل الكيماوي لنبات فول الصويا (الصبغات الضوئية ، الكربوهيدرات ، البروتين ، والهرمونات الداخلية).

أوضحت النتائج أن المعاملة بجميع التركيزات المستخدمة من البنزيل أدنين أو الـبكس أدت إلى زيادة معنوية في القياسات المورفولوجية للمجموع الخضري (طول النبات ، قطر الساق ، الوزن الطري والوزن الجاف ، عدد الأفرع ، عدد الأوراق ، المساحة الورقية لكل نبات) وقياسات المجموع الجذري (طول الجذر ، والوزن الطري والوزن الجاف) خلال مراحل النمو الخضـري والإزهار والإثمار. كان التأثير الأكثر حدوثاً قد لوحظ نتيجة معاملة التفاعل بينهما مقارنة باستخدام كل منظم نمو على حدة. وبصفة عامة فإن التأثير الإيجابي الملحوظ على خصائص النمو الظاهري كان عند المعاملة بـ ٥٠ جزء في المليون بنزيل أدنين + ٥٠ جزء في المليون بكس في جميـع مراحل نمو النبات.

كما أظهرت البيانات أيضاً زيادة الصبغات الضوئية (الكلوروفيلات والكاروتينات) فـي أوراق فول الصويا بدرجة معنوية نتيجة المعاملات التجريبية. وكانت الزيادة الملحوظة في هـذه الصبغات مصحوبة بزيادة في السكريات الذائبة والسكريات العديدة وبالتالي الكربوهيدرات الكليـة لنبات فول الصويا استجابة لكافة المعاملات التجريبية. في نفس الوقت ، ارتفع محتوى نبات فول الصويا من النتروجين الأميني ، النتروجين الذائب الكلي ، نتروجين البروتين ، والنتروجين الكلي نتيجة المعاملة بجميع تركيزات البنزيل أدنين و/أو البكس مقارنة بالنباتات غير المعاملة. كما عظم التفاعل بين المركبين قيم المكونات النتروجينية مقارنة بتلك المتحصل عليها عند استخدام كل منهما على حده.

أدت معاملات البنزيل أدنين إلى تقليل محتوى النبات من حمـض الأبسيسـيك وزيـادة محتواه من إندول حمض الخليك ، بينما لوحظ وجود التأثير العكسي مع كـل تركيـزات الـبكس المستخدمة. بينما أظهرت تركيزات هرمون الجبريللين الـداخلي والسـيتوكينينات الكليـة زيـادة ملحوظة مع كافة المعاملات التجريبية من البنزيل أدنين و/أو البكس.