



*Journal*

*J. Biol. Chem.*  
*Environ. Sci.*, 2008,  
*Vol. 3(2): 45-57*  
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## EFFECT OF SOME GROWTH REGULATORS ON FLOWERS ABSCISSION AND YIELD OF FABA BEAN (*Vicia faba* L.) PLANT

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### ABSTRACT

Pot experiments were conducted in the green house, Faculty of Agriculture, Ain Shams University during the two successive seasons of 2005-2006 and 2006- 2007. Paclobutrazol (PP<sub>333</sub>) at 25, 50 ppm, naphthalene acetic acid (NAA) at 20, 40 ppm, CPPU (N-(2- chloro-4-pyridyl)-N- phenylurea) and salicylic acid (SA) at 10, 20 ppm each in addition to distilled water as control were applied as foliar spray at 60 and 75 days after sowing. Two samples were taken at 90 days after sowing and at harvest (180 days after sowing). Application of PP<sub>333</sub> at 50 ppm reduced plant height and at both concentrations gave an increase in branches and leaves number. Application of NAA showed the lowest number of branches. Application of PP<sub>333</sub> at 25 and 50 ppm gave marked increase in flowers number. Significant increase in flowers number was obtained by CPPU treatments and NAA at 40 ppm. CPPU and NAA treatments reduced flowers and immature pods abscission percentage. Significant increase in pods number/plant was obtained by PP<sub>333</sub>, CPPU and NAA treatments. The highest values of 100 seeds weight were obtained by NAA treatments. The best results of seeds weight/plant were recorded by PP<sub>333</sub> at 50 ppm and NAA at 40 ppm in both seasons. Applications of NAA resulted in high concentrations of endogenous auxin in leaves and total soluble carbohydrates in leaves and seeds. PP<sub>333</sub> treatments gave an increase in total soluble carbohydrates and proteins in leaves. CPPU treatments showed the highest values of total soluble proteins in leaves.

**Key words:** Faba bean, *Vicia faba*, Paclobutrazol, CPPU, Salicylic acid, Naphthalene acetic acid, Flowering, Abscission, Yield.

## INTRODUCTION

Faba bean (*Vicia faba*) is one of the most important winter crops of high nutritive value. Mature seeds of faba bean are a good source of protein (about 25% in dried seeds), starch, cellulose and minerals (Ibrahim, 2007).

Flowers and fruit abscission is a common phenomenon, especially under stress conditions, even under optimal conditions for growth and development, and in legumes it remains the main reason for low and varied seed yields (Prusiniski and Borowska, 2001). The percentage of abscission was found to be between 80-97% of total number of buds produced on faba bean plants (Kambal, 1969).

Bangerth (1989) developed two models supplementing each other to explain the abscission of generative organs. The competition model assumes that developing fruit and seeds assimilate acceptors, compete with vegetative organs for resources. The dominance model assumes that generative abscission is regulated by plant hormones. Seeds and fruit developed first are capable of inhibiting the development of those developed later. Auxin, indole-3-acetic acid (IAA) is a hormone which is directly responsible for abscission of generative organs. A high transport of IAA from the first developed, dominant, organ inhibits the withdrawal of IAA from fruit and seeds developed later.

Other workers have shown that auxin and cytokinins which slow the aging process, also retard fruit (Griggs *et al.*, 1970) and flower abscission (Roberts *et al.*, 1984). It was suggested that auxin block ethylene action.

Salicylic acid (SA) has shown to promote flowering in several plant species (Raskin 1992) in addition to fruit setting and yield of some legume plants such as dry bean (Zaghlool *et al.*, 2001) and mung bean (Zaghlool, 2002). Despite its mechanism of action is still unclear.

Plant growth retardants have shown to play role in abscission process. Rabie *et al.*, (1991) found that, chloromequat chloride (CCC) treatments as seed soaking of two faba bean varieties reduced abscission percentage of flowers and immature pods. Application of mepiquat chloride (Pix) at early flowering increased earliness and yield of cotton plants, Glover *et al.*, (1995).

The present study was undertaken to shed more lights on the role of cytokinins, auxin, salicylic acid and growth retardants at low concentrations on flowers and immature pods abscission in addition to seeds yield/plant of faba bean plants.

## MATERIAL AND METHODS

This study was conducted in the green house, Faculty of Agriculture, Ain Shams University during the two successive seasons of 2005-2006 and 2006- 2007. Five seeds of faba bean (*Vicia faba* L.) cv. Giza 716 were sown on November 2<sup>nd</sup> in each season in 35 cm. diameter pots filled with clay loamy soil (Table 1).

**Table (1): a) Mechanical and b) Chemical properties of the soil.**

### a) Mechanical analysis

Sand%	Silt%	Clay%
25.7	35.9	36.9

### b) Chemical analysis

pH	Organic matter %	Cations (meq/l)				Anions (meq/l)			
		Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Cl <sup>-</sup>	So <sub>4</sub> <sup>-2</sup>	Co <sub>3</sub> <sup>-2</sup>	HCO <sub>3</sub> <sup>-</sup>
7.25	0.92	5.4	0.50	4.00	1.10	1.50	5.80	---	3.70

Thinning was conducted at 10 days after sowing leaving 2 plants /pot. Pots were arranged in complete block design with 3 replicates (5 pots/replicate). Fertilization was applied with irrigation as Hogland nutrient solution (Gauch, 1972) one strength twice a week starting with thinning till 160 days after sowing.

### 1- Treatments

Paclobutrazol (PP<sub>333</sub>) at 25, 50 ppm, naphthalene acetic acid (NAA) at 20, 40 ppm, CPPU (N-(2- chloro-4-pyridyl)-N- phenylurea) and salicylic acid (SA) at 10, 20 ppm each in addition to distilled water as control were applied as foliar spray at 60 and 75 days after sowing. Tween 20 at 0.05 ml/l was added as a wetting agent.

### 2- Growth and yield measurements

Two samples were taken. The first sample was taken at 90 days after sowing where, plant height, number of branches and leaves were recorded. The second sample was taken at harvest (180 days after sowing) where, mature pods number, seeds weight/plant and 100seeds weight were recorded.

### 3- Abscission %

Starting with blooming, three plants were marked in each replicate and flowers number was recorded regularly each three days on the main stem and branches until the end of blooming.

Abscission of flowers and immature pods was recoded as following:

$$\text{Abscission \%} = \frac{\text{Flowers number} - \text{Mature pods number}}{\text{Flowers number}} \times 100$$

### 4. Chemical analysis

Chemical analysis was conducted for determination of total soluble carbohydrates, total soluble proteins and auxin (IAA) concentrations in the upper leaves of the main stem. Furthermore, total soluble carbohydrates were determined in seeds at harvest time.

#### 4.1. Determination of total soluble proteins.

One gram sample was mixed with 5 ml of extraction buffer (0.125 M tris borate, pH 8.9) then shaken for one hour and filtered. The supernatant were contained the soluble protein. A colorimetric determination of soluble proteins was carried out using the method of (Bradford, 1976).

#### 4.2. Determination of total soluble carbohydrates.

For extraction, 1.0g fresh weight of leaves or dry seeds were hydrolyzed with 30 ml HCl 2N. The tubes were placed in a boiling water bath for 6 hr. After cooling, resulting solution was filtered (wt.No.1). The sample was transferred into a calibrated flask (100 ml). Total carbohydrates were estimated by the alkine potassium ferricyanide method ( Shales and Schales, 1945).

#### 4.3. Determination of auxin

Endogenous phytohormone IAA was determined in leaves, two gram fresh weight of sample was extracted overnight in dark at 4°C with 80% redistilled methanol. The method for phytohormones extraction was followed as described by Knegt and Bruinsma (1973).

Sep-pak cartridge (C<sub>18</sub>) was used to separate auxin by aqueous solution of 40% MeOH containing 0.1 N acetic acid as described by Lee *et al* (1989).

Auxin determination was carried out according to Larsen *et al* (1962). One ml of the extraction was added to 4 ml PDAB 1% (Paradimethyl aminobenzaldehyde), shaken 3 min and allowed to stand for 60 min. The optical density was determined at 530 nm using spectrophotometer (Spectronic 21). Amounts were calculated according to standard curve of IAA.

### **5. Statistical analysis:**

Obtained data were subjected to analysis of variance using the method of SAS (1999).

## **RESULTS AND DISCUSSION**

### **1-RESULTS**

#### **1. Growth parameters**

Data presented in Table (2) clearly demonstrate significant reduction in plant height induced by PP<sub>333</sub> at 50 ppm comparing with other treatments including control plants in both seasons. Application of CPPU showed an increase in plant height, this effect reached the level of significance by the higher concentration (20 ppm). The rest of treatments showed insignificant response.

Despite the effects of PP<sub>333</sub> were insignificant comparing with untreated plants, they showed the highest values of branches and leaves number in the two seasons. The opposite trend was true by NAA which resulted in the lowest number of branches. SA gave approximately similar trend to control plants.

**Table (2): Effect of different concentrations of growth regulators on some growth parameters of faba bean plants at 90 days after sowing during the two successive seasons.**

Treatments	2005-2006			2006-2007		
	Plant height cm.	Branches No.	Leaves No.	Plant height cm.	Branches No.	Leaves No.
Control	66.67	2.34	37.47	68.33	2.56	41.63
PP <sub>333</sub> at 25 ppm	66.62	3.00	44.34	68.00	3.56	47.31
PP <sub>333</sub> at 50 ppm	52.55	2.77	43.00	54.44	3.42	45.66
CPPU at 10 ppm	70.35	2.00	40.00	72.00	3.00	43.00
CPPU at 20 ppm	76.00	2.00	40.87	77.58	2.97	43.00
SA at 10 ppm	72.00	2.32	37.33	74.74	2.00	40.00
SA at 20 ppm	68.65	2.30	36.00	75.00	2.68	38.67
NAA at 20 ppm	68.00	1.34	39.65	64.00	1.65	38.47
NAA at 40 ppm	69.64	1.32	36.09	68.00	1.67	37.45
M.S.D	6.67	1.10	8.04	7.16	1.35	8.77

## 2. Reproductive parameters

Application of PP<sub>333</sub> surpassed other bioregulators as well as the control in producing flowers/plant. The lower concentration was the most effective in both seasons, PP<sub>333</sub> at 25 ppm gave (95.17, 105.17 flowers/plant) and at 50 ppm gave (86.94, 94.06 flowers/plant) comparing with control plants (60.9, 62.5 flowers/plant) in the two seasons respectively (Table 3). CPPU at 10 ppm, SA at 20 ppm and NAA at 40 ppm showed significant increase in flowers number over the control in the first season. Similar effect was obtained in the second season by CPPU at 10 and 20 ppm and NAA at 40 ppm.

All treatments showed significant increase in pods number/plant in the two seasons with the exception of SA treatments (Table 3). The highest values were obtained by PP<sub>333</sub> at 25 ppm which recorded 19.76, 20.87 pods/plant comparing with control plants which recorded 11.39 and 12.72 pods/plant in the two successive seasons respectively.

Application of PP<sub>333</sub> at 25 and 50 ppm and NAA at 40 ppm gave significant increase in seeds yield/plant in the two seasons. CPPU treatments and NAA at 20 ppm showed insignificant increase.

Insignificant increase in 100 seeds weight was noticed by PP<sub>333</sub> at 25 ppm and SA at both concentrations in both seasons. Significant increase was gained by NAA treatments and the superiority was due to the higher concentration which recorded (100.45 and 104.27 g)

comparing with control plants (78 and 82g) in the two successive seasons.

### 3. Abscission percentage

Considering the two seasons, CPPU and NAA treatments showed significant reduction in flowers and immature pods abscission percentage than that of the control (Table 3). The lowest values were recorded by CPPU at 20 ppm (74.6%) in the first season and NAA at 20 ppm (74.02%) in the second season comparing with control plants (81.29, 79.64%) respectively.

**Table (3): Effect of different concentrations of growth regulators on flowers and mature pods number, seeds yield/plant and abscission% of faba bean plants during the two successive seasons.**

Treatments	2005 - 2006					2006 - 2007				
	Flowers No.	Mature pods No.	Seeds Wt/plant g	100 Seeds Wt g	Abscission %	Flowers No.	Mature pods No.	Seeds Wt/plant g	100 Seeds Wt g	Abscission %
Control	60.90	11.39	29.67	78.00	81.29	62.5	12.72	33.00	82.00	79.64
PP <sub>333</sub> at 25 ppm	95.17	19.76	42.03	85.27	79.22	105.17	20.87	45.44	89.64	80.14
PP <sub>333</sub> at 50 ppm	86.94	17.06	38.67	74.13	80.38	94.06	16.93	40.67	77.54	82.00
CPPU at 10ppm	68.83	16.99	35.13	79.90	75.31	71.72	18.55	39.00	81.53	74.13
CPPU at 20 ppm	63.06	16.01	34.17	77.90	74.60	73.22	17.04	37.15	82.69	76.74
SA at 10 ppm	61.43	10.67	29.00	84.33	82.63	61.21	12.81	32.48	89.18	79.08
SA at 20 ppm	66.17	11.33	34.00	85.32	82.87	66.47	12.56	33.27	89.53	81.11
NAA at 20 ppm	63.78	15.04	34.40	90.67	76.42	65.62	17.05	37.35	95.33	74.02
NAA at 40 ppm	66.63	16.38	37.13	100.45	75.40	70.24	17.20	39.67	104.27	75.52
M.S.D	3.88	1.19	6.24	7.43	1.67	4.12	2.17	6.23	7.22	2.65

## 4. Chemical analysis

### 4.1. Total soluble carbohydrates

As shown in Table (4), significant increases (over the control) in total soluble carbohydrates in leaves were obtained by PP<sub>333</sub>, SA and NAA treatments. Meanwhile, application of CPPU at both concentrations showed insignificant differences. Naphthalene acetic acid recorded the highest values in the first season by 40 ppm (12.85mg/g) and in the second season by 20 ppm (13.19mg/g) comparing with control plants which recorded 7.24 and 7.66 mg/g in the two successive seasons.

Significant increase in total soluble carbohydrates in seeds was recorded by PP<sub>333</sub> at 25 ppm and CPPU and NAA at both concentrations in the two seasons. Salicylic acid treatments showed insignificant effect. The superiority was also due to NAA treatments especially the higher concentration which recorded 499.35 and 510.72 mg/g comparing with control plants (378.21 and 385.44 mg/g) in the two successive seasons. Application of PP<sub>333</sub> at 50 ppm gave insignificant increase in total soluble carbohydrates in seeds.

#### 4.2. Total soluble proteins

All treatments showed significant increase in total soluble proteins in the first season with the exception of NAA at 20 ppm which showed significant reduction as compared with control. In the second season, the significant increase was obtained by CPPU at both concentrations and SA at 20 ppm. However, the highest values were recorded by CPPU at 10 and 20 ppm which gave (14.58, 15.37 mg/g) and (14.81, 14.58mg/g) comparing with control plants (8.75, 8.83 mg/g) in the two seasons respectively.

**Table (4): Effect of different concentrations of growth regulators on total soluble carbohydrates, total soluble proteins and auxin (IAA) concentrations in leaves of faba bean plants at 90 days after sowing and total soluble carbohydrates in seeds at harvest during the two successive seasons.**

Treatments	2005-2006				2006-2007			
	Total soluble carbohydrates mg/g		Total soluble proteins mg/g	Auxin µg/g	Total soluble carbohydrates mg/g		Total soluble proteins mg/g	Auxin µg/g
	leaves	seeds			leaves	seeds		
Control	7.24	378.21	8.75	55.35	7.66	385.44	8.83	59.21
PP <sub>333</sub> at 25 ppm	9.85	407.07	9.25	53.46	10.04	425.37	10.11	58.16
PP <sub>333</sub> at 50 ppm	8.72	392.46	9.92	57.59	8.94	398.64	9.83	57.95
CPPU at 10 ppm	7.78	420.78	14.58	57.82	7.94	439.59	14.81	67.44
CPPU at 20 ppm	7.33	429.81	15.37	65.88	7.58	432.87	14.58	59.57
SA at 10 ppm	8.57	363.72	9.60	54.66	9.62	380.01	9.72	63.66
SA at 20 ppm	9.95	376.89	10.48	68.83	10.14	388.59	10.63	73.08
NAA at 20 ppm	10.96	456.30	7.92	73.39	13.19	471.21	8.09	77.48
NAA at 40 ppm	12.85	499.35	9.33	95.55	11.11	510.72	9.57	99.42
M.S.D	1.13	6.46	0.1	14.8	0.79	14.67	1.76	9.98



### 4.3. Endogenous auxin

Data presented in Table (4) reveals significant increases (over the control) in auxin (IAA) concentration obtained by NAA at both concentrations in both seasons as well as SA at 20 ppm in the second season. The rest of treatments showed insignificant effect. Application of NAA at 40 ppm recoded the maximum values (99.55 and 99.42  $\mu\text{g/g}$ ) comparing with control plants (55.35 and 59.21  $\mu\text{g/g}$ ) in the two seasons respectively.

## 2- DISCUSSION

Application of PP<sub>333</sub> in the present study induced significant reduction in plant height by the high concentration (50 ppm). This effect refers to the role of PP<sub>333</sub> as a growth retardant. Growth retardants are sometimes called as antigibberellins because they inhibit gibberellin biosynthesis which reduces gibberellin levels and causes a decrease in shoot elongation (Arteca 1996). At the same time an increase in branches and leaves number was noticed by PP<sub>333</sub>. However, PP<sub>333</sub> belongs to a highly active group of plant growth retardants, the "triazole". In addition to blocking gibberellin biosynthesis, triazole compounds have been shown to inhibit IAA (Law and Hamilton 1989) and increase cytokinin concentration (Izumi et al., 1988) thereby, promoting lateral bud break (Klee et al., 1991) and increase branches number. The increase in branches number implied an increase leaves number. In this regard, reduction in branches number was induced by NAA (synthetic auxin). The effect of NAA was concomitant with high concentration of endogenous auxin.

Marked increase in flowers number was obtained by PP<sub>333</sub> followed by CPPU treatments. PP<sub>333</sub> treatments in the present study resulted in an increase total soluble carbohydrates and proteins in leaves in addition to branches and leaves number as previously mentioned. These effects resulted in an improvement flower production.

As for the stimulating effect of CPPU upon flowering, cytokinins have been shown to increase the flower count in *Schumbergera truncate* (Ho et al., 1985). The promotive effects of cytokinins on flowering may be indirect since it has been shown in *Pharbitis nil* that cytokinins can increase the translocation of flower stimulus and assimilates from induced leaves (Ogawa and King 1979).

The increase in pods number and the subsequent increase in seeds yield/plant by PP<sub>333</sub> were expected to be correlated with the high production of flowers. While the increase in pods number induced by NAA and CPPU resulted from the stimulation of fruit setting and development. These results of NAA and CPPU on fruit setting were confirmed by other workers on legume plants (Prusiniski and Borowska, 2001). It has been suggested that the ability of cytokinins to mobilize assimilates to the area of application is responsible for increase fruit set (Arteca 1996). Furthermore, the increase in 100 seeds weight and seeds weight/plant by NAA is correlated with the enhancement in total soluble carbohydrates in leaves and seeds. CPPU treatments showed the highest values of total soluble proteins in leaves.

With regard to abscission percentage, the best results were due to NAA and CPPU treatments. Arteca (1996) mentioned that conditions which promote high carbohydrates and stimulates photosynthesis retard abscission. It is thought that high carbohydrates in the plant contribute to the vigor of fruits and leaves. This increased vigor will enable these organs to more readily synthesize plant growth substances required for growth, development and inhibition of abscission.

However, application of NAA in the present study resulted in high levels of endogenous auxins in leaves. It has been shown that when endogenous levels of auxin in the leaves or other plant organs are reduced, abscission typically occurs (abeles *et al.*, 1992).

The role of SA in the present study was unclear. Application of SA at 20 ppm showed significant increase in flowers number but this effect was restricted to the first season. Application of SA positively affected the determined biochemical constituents in leaves especially the high concentration (20 ppm) but this did not reflect on reduction in abscission or promotion in seeds yield/plant.

Finally, in respect to flower production and seeds yield, the best results in this study were due to PP<sub>333</sub> treatments. As for reducing abscission of generative organs, NAA and CPPU worked better than other treatments in this regard and NAA was responsible for the highest values of 100 seeds weight.

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## تأثير بعض منظمات النمو على تساقط الأزهار ومحصول نبات الفول (*Vicia faba* L.)

سناء عبد الرحمن مصطفى زغول

قسم النبات الزراعى - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر.

تمت هذه الدراسة فى الصوبة الخاصة بقسم النبات الزراعى ، كلية الزراعة ، جامعة عين شمس خلال موسمى 2005-2006 و 2006-2007 وقد شملت المعاملات الرش الورقى لنباتات الفول عند عمرى 60 و 75 يوم من الزراعة بكلا من الباكلوبترازول (PP<sub>333</sub>) بتركيزى 25 و 50 جزئى فى المليون و حامض النفتالين أسيتيك (NAA) بتركيزى 20 و 40 جزئى فى المليون و حامض الساليسيليك (SA) و CPPU بتركيزى 10 و 20 جزئى فى المليون لكل منهما ، كما تم رش نباتات المقارنة بالماء المقطر. وقد تم أخذ عينتين عند عمر 90 يوم من الزراعة وعند الحصاد (180 يوم).

### وتتلخص أهم النتائج فيما يلى:

- أدت معاملة PP<sub>333</sub> بتركيز 50 جزئى فى المليون إلى نقص ملحوظ فى طول النبات.
- أدت معاملات PP<sub>333</sub> بتركيزى 25 و 50 جزئى فى المليون إلى زيادة عدد الأفرع والأوراق فى حين أدت معاملات NAA إلى نقص فى عدد الأفرع.
- أدت معاملات PP<sub>333</sub> بتركيزى 25 و 50 جزئى فى المليون إلى حدوث زيادة كبيرة فى عدد الأزهار كما تسببت معاملات CPPU و NAA بتركيز 40 جزئى فى المليون إلى حدوث زيادة معنوية فى عدد الأزهار.
- أظهرت معاملات CPPU و NAA نقص معنوى فى نسبة تساقط الأزهار والقرون غير الناضجة.
- أدت معاملات PP<sub>333</sub> و CPPU و NAA إلى حدوث زيادة معنوية فى عدد القرون للنبات.
- سجلت أعلى قيم لوزن المائة بذرة من خلال معاملات NAA .
- أعطت معاملات PP<sub>333</sub> بكلا التركيزين و NAA بالتركيز الأعلى أفضل نتائج وزن البذور للنبات فى كلا الموسمين.
- أدت معاملات NAA إلى زيادة تركيز الأكسين فى الأوراق وزيادة تركيز الكربوهيدرات الكلية الذائبة فى الأوراق والبذور.
- أدت معاملات PP<sub>333</sub> إلى زيادة تركيز الكربوهيدرات الكلية الذائبة فى أوراق و البذور والبروتينات فى الأوراق وأعطت معاملات CPPU أعلى تركيز للبروتينات الكلية الذائبة فى الأوراق.
- لم تعطى معاملات SA تأثيرا ملحوظا على محصول البذرة/النبات أو خفض نسبة التساقط.

وتتلخص أهم النتائج فى أن معاملات PP<sub>333</sub> هى الأفضل بالنسبة لإنتاج الأزهار ومحصول البذرة للنبات. وبالنسبة لتقليل نسبة تساقط الأعضاء النكاثرية كانت معاملات NAA و CPPU هى الأفضل وللحصول على أعلى قيم لوزن المائة بذرة فإن معاملات NAA هى الأفضل.