EFFECT OF SOME PLANT GROWTH REGULATORS ON SHEDDING PHYSIOLOGY, ANTIOXIDANT ENZYMES, GROWTH AND YIELD OF FABA BEAN

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ABSTRACT

In the greenhouse experiment *Vicia faba* L. cv. Giza 716 plant were treated with 25 & 50 ppm of paclobutrazol (PP_{333}), kinetin (Kin), naphthalene acetic acid (NAA) and 50 & 100 ppm of ethephon (Et) as foliar spray at the onset of flowering and fruiting. Plant height, branches and flowers number were recorded after 104 days from sowing in both seasons. Two samples were taken after 90 days from planting and at harvest. At the 1st sample date in the first season, biochemical analyses were conducted in leaves for determination of total soluble sugars, phenols, indoles, free amino acids, water soluble protein and isozymes. At harvesting time (2nd sample), number of pods, pods and seeds dry weights/plant were taken in both seasons. The obtained results revealed that PP_{333} at 50 ppm had significant and negative effect on plant height, flowers number, total phenols and indoles. The high concentration of Kin, Et and NAA treatments increased the number of pods, the dry weight of pods and seeds per plant whereas the opposite occurred in the same concentration of PP_{333} treatment. Seed yield has the same direction with the number of pods per plant and the dry weight of pods per plant. In general, NAA at 25 ppm has strongly stimulating effect on total soluble sugars, phenols, indoles and free amino acids in leaves, and seed index (100-seed weight). It seems that growth promoter NAA enhance the mobilization of photoassimilates to filling seeds. Proteins banding patterns and isozymes (superoxide dismutase, peroxidase, catalase, α, β-esterases

and acid phosphatase) data is related to the physiological state of the plant and its development.

Key Words: *Vicia faba*, NAA, PP_{333}, Kin, Ethephon, Shedding, Antioxidant enzymes.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important field crops in Egypt; it is cultivated throughout the country. It is an important member of *Fabaceae* and used as green and/or dry seeds, which considered an important source of plant protein for food and feed.

Abscission of buds, flowers and immature pods that fail to develop into fully mature pods in *V. faba* L. plants, is considered one of the most complicated problems in the crop production. The total shedding percentage was found to range from 80–97 % (Kambal, 1969). Competition for assimilates between vegetative and reproductive sinks during pod-filling could cause substantial flower drop and premature pod abscission (Peat, 1982) and consequent loss of yield.

Several authors reported the importance of plant growth regulators in resolving the problem of abscission or shedding (Ohkuma *et al.* 1965; Lewis and Varner, 1970; El-Antably, 1976 a &b; Rabie *et al.* 1991; Rademacher, 1995) and fruit set (Zhang, 1997; Djanaguiraman *et al.* 2004).

Protein and isozymes markers were used extensively to identify and study the genetic characters and relationships of many plants (Shawky *et al.* 2005). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are involved in scavenging of reactive oxygen species (Shanker *et al.* 2004). Orendi *et al.* (2001) reported that an increase in the peroxidase enzyme activity led to decrease of H_2O_2 content and lipid peroxidation leading to increased fruit set.

Therefore, this study designed to determine the effect of some growth regulators (Paclbutrazol (PP_{333}); ethephon (Et); kinetin (Kin); naphthalene acetic acid (NAA)) on shedding physiology, growth, fruit set and yield of faba bean plant under glasshouse conditions. In addition, the biochemical gene expression representing at quantitative

and/or qualitative changes in some biochemical components was also determined.

MATERIALS AND METHODS

Greenhouse experiment

This study was performed in the greenhouse at Faculty of Agric., Ain Shams Univ. during the seasons of 2007–2008. Five seeds of faba bean (*V. faba* L.) cv. Giza 716 were sown in 11th November on 35 cm diameter pots filled with sandy loam soil. Irrigation was applied as Hoagland nutrient solution (Gauch, 1972), one strength, (140 ml/Kg sand) twice a week. Thinning was conducted one week after sowing leaving 2 plants / pot.

Foliar spray of plant growth regulators

Plant growth regulators; ethephon "Break 48% ethephon" (Et) at 50 & 100 ppm, Naphthalene acetic acid (NAA), kinetin (Kin) and paclobutrazol (PP_{333}) at 25 & 50 ppm were used as foliar spray (till drip) at 48, 62 and 76 days after sowing corresponding with the onset of flowering and fruiting. Tween 20 at 0.05 ml/l was used as wetting agent.

Sampling dates and growth parameters

Ninety days post sowing, the 1st sample was taken for chemical analyses that conducted in leaves for determination of total soluble sugars, phenols, indoles, free amino acids, SDS-protein electrophoresis and isozymes in the first season.

Plant height and branch and flower numbers were recorded at 104 days after sowing in both seasons. Plant height was measured with a meter rule from the soil surface to the apex of the main stem. The number of branches on the main stem was recorded every two weeks from 48 days after sowing on two plants per pot. Flowers number was observed from flower initiation until 104 days post sowing. Flowers were counted on the main stem every three days.

The 2nd sample was taken at harvesting time (172 days post sowing) where pods number and pods and seeds weight/plant and 100 seeds weight were recorded in both seasons. The air dried pods and seeds samples were performed to determinate dry weight of pods and seeds.

Biochemical analyses

Total soluble sugars were estimated by the alkaline potassium ferricyanide method of Shales and Schales (1945). Phenols determination was carried out according to the method recorded by Daniel and George (1972). Indoles determination was carried out according to Larsen *et al.* (1962). Free amino acids were determined according to methods of Plummer (1978).

The statistical analysis of data was done by SAS 1996. Tukey test for separation between means was estimated using the following model $Y_{ijk} = \mu + trt_i + S_j + e_{ijk}$. The used design is completely randomized design.

SDS-PAGE analysis

Characterization of leaf water soluble protein at 90 days from sowing was carried out using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide according to Laemmli (1970) as modified by Studier (1973). Protein concentration was determined by the method of Bradford (1976), with bovine serum albumin as a standard. Banding patterns were photographed or schetched and the results were analyzed by the gel documentation system.

Isozymes Analyses

Electrophoretic separation of isozymes was achieved with 10% native-polyacrylamide gel electrophoresis (native PAGE) according to the method of Jonathan and Weaden (1990). Five isoenzymes: superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), esterases (α, β-EST) and acid phosphatase (ACPH) were electrophoretically analyzed. The methodology of Shaw and Prasad (1970); Beau-Champ and Fridovich (1971); Woodbury *et al.* (1971); Nadlony and Sequira (1980); Wendel and Weaden (1989); were followed for SOD, POD, CAT, α & β-EST and ACPH, respectively for visualizing the isoforms. The isoenzymatic fractions separated by electrofocusing were drawn and represented as zymograms. Isozyme phenotypes were interpreted on the basis of existing knowledge of isoenzyme structure and genetic control (Wendel and Weaden, 1989), as described by Jaaska (1997) for *Vicia* species. Various types of isozymes are specified following the nomenclature described by Jaaska (2001).

RESULTS AND DISCUSSION

Growth parameters

Data presented in Table (1) revealed the effect of foliar applications of PP_{333}, Kin, Et and NAA on plant height and branch and flower numbers of faba bean (104 days from sowing) as well as yield components (at harvest=2nd sample). In faba bean, the yield components are the number of pods per plant, the dry weight of pods per plant, the dry weight of seeds per plant and seed index (100-seed weight).

Plant treated with PP_{333} showed the lowest values of plant height. All treatments led to a significant decrease in plant height except NAA treatments as compared to the control. An insignificant decrease in plant height was obtained by NAA treatments. Applications of PP_{333} at 25 ppm and NAA at 50 ppm resulted in a significant increase in branches number while the rest treatments gave insignificant values when compared with the control. All treatments produced a significant decrease in flowers number except PP_{333} at 25 ppm. The latter treatment did not demonstrate significant value but it gave the highest value in this concern.

Yield components

The present data in Table (2) clearly demonstrate that the effects of applied plant growth regulators on yield components in the 2nd sample were greatly depended on the used concentration. The high concentration of applied plant growth regulators (PP_{333}, Kin, Et and NAA) has more effect than that of the low concentration. The high concentration of Kin, Et and NAA treatments increased the number of pods, the dry weight of pods and the seeds per plant whereas the opposite occurred in high concentration of PP_{333} treatment. PP_{333} treatment at 50 ppm induced the lowest pods and seeds yield than the rest treatments and control. These effects were mostly significant. However, the highest values of yield components were obtained by 50 ppm of NAA, followed by Kin at 50 ppm. Seed yield has the same direction with the number of pods per plant and the dry weight of pods. There was a negative significant effect relationship between 100-seed weight and applied plant growth regulators except NAA at 25 ppm.

Table 1. Effect of some plant growth regulators on growth parameters of faba bean after 104 days from sowing in both seasons.

Treatments	Plant Height (cm)			Branches Number/plant			Flowers Number/main stem		
	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	77.00	78.00	77.50	3.30	3.40	3.35	49.00	50.00	49.50
PP$_{333}$ 25	59.00	60.00	59.50	4.80	4.90	4.85	54.00	53.50	53.75
PP$_{333}$ 50	43.00	40.50	41.75	4.00	4.10	4.05	39.00	40.50	39.50
Kin 25	70.00	71.00	70.50	3.50	3.60	3.62	42.00	42.50	42.50
Kin 50	64.00	65.00	64.50	3.30	3.40	3.43	41.00	40.50	41.50
Et 50	67.00	68.00	67.50	3.80	3.90	3.85	41.00	41.50	41.50
Et 100	61.00	62.30	61.65	3.80	3.95	3.87	42.00	43.00	42.50
NAA25	72.00	73.00	72.50	3.80	3.90	3.85	43.00	43.50	43.50
NAA50	74.00	75.00	74.50	6.30	6.20	6.33	33.00	35.00	33.75
Mean	65.22	65.86		4.12	4.15		42.67	43.22	
MSD T		3.22			0.32			2.93	
MSD S					NS				
MSD T*S		5.69			0.55			5.22	

Table 2. Effect of some plant growth regulators on yield components of faba bean at harvest (2nd sample) in both seasons.

Treatments	Pods Number/Plant			Pods dry weight/Plant (g)			Seeds dry weight/Plant (g)			100-seed weight (g)		
	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	10.45	10.40	10.42	24.01	25.00	24.50	20.00	20.20	20.10	103.69	103.6	103.65
PP$_{333}$ 25	10.95	11.00	10.97	25.60	25.70	25.65	21.34	21.50	21.42	95.74	95.85	95.80
PP$_{333}$ 50	7.50	7.60	7.55	17.59	17.65	17.62	14.66	14.70	14.68	84.56	84.75	84.66
Kin 25	9.54	9.60	9.57	22.34	22.50	22.42	18.60	18.70	18.65	89.40	89.70	89.55
Kin 50	13.00	13.10	13.05	30.40	30.30	30.35	25.35	25.50	25.43	92.76	93.10	92.93
Et 50	9.99	10.00	9.99	23.38	23.50	23.44	19.48	19.60	19.54	96.96	97.00	96.98
Et 100	11.88	11.90	11.89	27.29	27.70	27.35	23.16	23.20	23.18	84.24	84.70	84.47
NAA25	9.75	9.80	9.77	22.82	22.90	22.86	19.01	19.15	19.08	104.18	104.3	104.24
NAA50	13.55	13.70	13.62	31.71	31.75	31.37	26.42	26.45	26.44	94.20	94.60	94.40
Mean	10.74	10.79		25.02	25.19		20.89	21.00		93.97	94.18	
MSD T		0.22			0.82			0.30			0.91	
MSD S						NS						
MSD T*S		0.39			1.38			0.52			1.74	

The obtained results indicated that PP$_{333}$ had significant negative effect on growth. In this respect, the results are in agreement with El-Shamey & Ibrahim (2004) and Wanas (2007); they found that application of PP$_{333}$ decreased plant growth and the plant have shorter internodes and more compact appearance. Kin and NAA effects are strongly dose-dependent (Kinet *et al.* 1993). Applications of kin and NAA promote photosynthetic rates, photoassimilates production, fruit set and growth. It has been suggested that the ability of cytokinins and NAA to mobilize assimilates to the area of application is responsible for increased fruit set and yield (Arteca, 1996). Ethylene is the key regulatory for fruit ripening and induced gene products (Theologis, 1992).

Chemical Analyses

As mentioned previously, chemical analyses in Table (3) were conducted in the leaves (total soluble sugars, phenols, indoles and free amino acids) at the 1st sample (90 days from sowing).

All treatments were ineffective on total soluble sugars concentration in leaves during reproductive stage except 25 ppm of NAA treatment. The latter treatment positively affected total soluble sugars concentration in leaves; this effect reached the level of significance. PP$_{333}$ at 25 & 50 ppm and Et and NAA at 50 ppm showed a significant decrease in total phenols concentration of leaves comparing with control, the lowest value referred to PP$_{333}$ at 50 ppm. The rest applications except NAA at 25 ppm were ineffective on total phenols. NAA at 25 ppm resulted in a significant increase in this concern. PP$_{333}$ treatments and Et treatment at 50 ppm produced a significant decrease in total indoles. Application of Et at 50 ppm gave the lowest concentration (89.21 µg / g f.wt.) comparing with control (167.42 µg / g f.wt.). An increase in indoles concentration was noticed by NAA treatments as compared to the control, the superiority was due to NAA at 50 ppm (256.5 µg / g f.wt.). There were no significant differences in free amino acids by applied plant growth regulators when compared to control. The highest and lowest values of free amino acids referred to NAA treatments at 25 & 50 ppm and PP$_{333}$ at 50 ppm, respectively. These results confirmed those of Zaghlool *et al.* (2001). They mentioned that NAA increased amino acid content in *Phaseolus vulgaris* and lentil.

Table 3. Effect of some plant growth regulators on biochemical components of faba bean leaves in the 1st sample (90 days from planting).

Treatments	Total soluble sugars (mg/g f.wt.)	Total phenols (µg/g f.wt.)	Total indoles (µg/g f.wt.)	Free amino acids (µg/g f.wt.)
Control	17.51	340.43	167.42	635.18
PP$_{333}$ 25	19.20	200.45	113.11	626.42
PP$_{333}$ 50	18.49	191.53	110.93	591.04
Kin 25	19.86	293.77	210.87	656.27
Kin 50	18.01	367.54	195.67	675.42
Et 50	19.38	212.12	89.21	645.56
Et 100	19.68	303.38	141.35	612.13
NAA25	22.37	403.91	249.98	690.03
NAA50	19.83	213.49	256.50	687.43
MSD T	2.83	55.94	27.97	55.94

In general, the present data demonstrated that NAA at 25 ppm has strongly stimulating effect on total soluble sugars, phenols, indoles and free amino acids (in leaves) and seed index (100-seed weight). It seems that growth promoter NAA enhance the mobilization of photoassimilates to filling seeds.

SDS-PAGE analysis

SDS-PAGE of leaf water-soluble was successfully used to assess the genetic variability of faba bean subjected to some plant growth regulators. The electrophoretic separation of these proteins extracted from leaves is shown in Figure (1).

The results of SDS-PAGE protein profile of water-soluble protein fractions on faba bean leaves treated with some plant growth regulators revealed a total of 40 bands with different molecular weights (MW), ranging from about 80.00 to 10.00 kDa.

The soluble protein profiles of the leaves comprise seven common major bands and a number of minor bands. Electrophoretic analysis of protein patterns of the faba bean showed that the polypeptides with molecular weights of around 16, 25, 29, 38, 40, 42, 46, 60 and 80 kDa were the most prominent in the control (Fig. 1). The effect of the applied plant growth regulators resulted in the induction of new bands, i.e., band no. 38 with MW of around 73 kDa was noticed by 25 & 50 ppm of PP$_{333}$ & Kin treatments and enhanced

in 50 ppm of Et treatment. Band no. 39 with MW of about 75 kDa exhibited only in 100 ppm of Et treatment.

In control, band no. 34 with MW of around 60 kDa was disappeared in all treated plants; however, this band was replaced with two enhanced proteins of around 54 and 56 kDa in all treatments except in 50 ppm of NAA treatment which had a different profile. It was characterized by two bands with molecular weight of about 54 and 58 kDa, respectively.

There were similar electrophoretic profiles for bands no. 23, 24 with MW around 42 and 46 kDa, respectively by 25 & 50 ppm of each Kin and NAA and 50 & 100 ppm of Et treatments. An over accumulation for protein band no. 21 with MW of around 42 kDa was observed by 50 ppm of kin and Et, and 25 & 50 ppm of NAA treatments. These result indicated that the effect of these treatments led to increase of some protein band intensities compared to other treatments and untreated plants.

As compared with the control, the major polypeptide bands no.19 and 20 with MW of around 35 and 38 kDa respectively were not affected by applied treatments unless increase the intensity of most polypeptide except 50 ppm of NAA for band no. 19 & 20 and 25 ppm of NAA slightly decreased the intensity of band 19. On the other hand, all treatments resulted in the induction of three new bands no. 13, 15, 17 with MW of about 26, 28 and 30 kDa and increase the intensity of most polypeptide bands while minor bands no. 10, 11, 12 with MW of around 21, 23 and 24 kDa were induced by 50 ppm of PP_{333} and 25 & 50 ppm of Kin treatments.

There were two additional new bands no. 8 and 9 with MW about 19 and 20 kDa respectively were observed in the all treated plants but disappeared in 25 ppm of PP_{333} and 50 & 100 ppm of Et treatments. As for band no. 8 disappeared in 100 ppm of Et treatment. A strong activation of newly intensive band no.7 with MW about 17 kDa was observed under different treatments with applied growth regulators which was absent in 50 ppm of Kin and control.
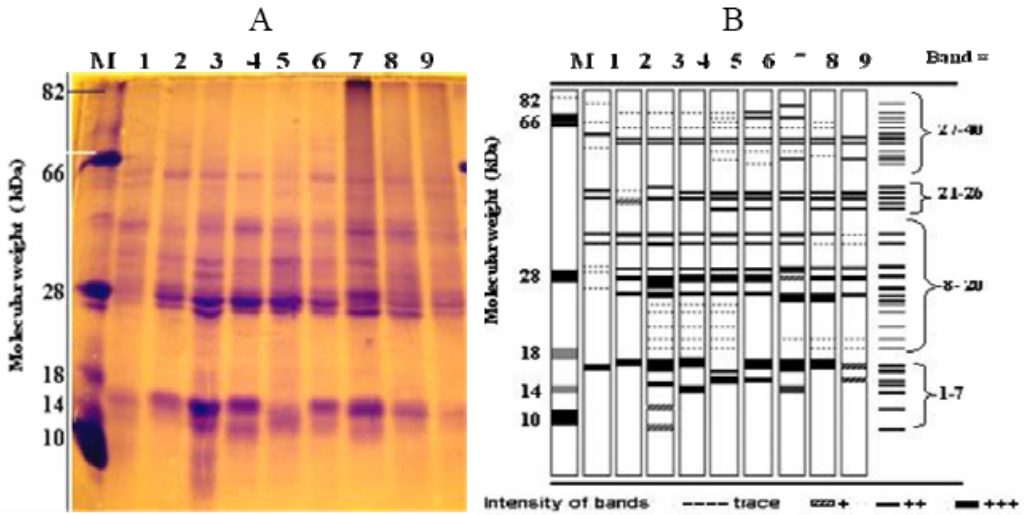
Fig 1. SDS-PAGE profile of water-soluble protein fractions of faba bean leaves treated with some plant growth regulators (A) illustrated in zymogram (B). (M = marker protein). Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

The overall results of leaf water-soluble protein patterns that obtained by SDS-PAGE were effective in studying genetic effect of each treatment for faba bean, whereas each treatment exhibited distinguishable bands. Using this technique was recommended by Kasarada *et al.* (1998) and Jaramillo *et al.* (1999) who mentioned that sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is widely used to separate cereal proteins which are directly related to genetic background of the protein.

The occurrence of newly synthesized bands and the absence of others, in treated plants under different treatments apparently is indicated by the occurrence of either enhancement or repression of gene expression in these plants. This might alter the produced proteins in response to plant growth regulators either at the transcription or post-transcription levels of gene expression.

In the present study, applied growth regulators induced considerable variations in the protein patterns. These variations have been manifested as the novel expression of some polypeptides; the

absence of others and over expression of a third class of polypeptides. A wide range of variations was found either in band density or intensities. Several of new proteins which are synthesized in response to plant growth regulators have been reported as stress-proteins in plants (Hoyos & Zhang, 2000; Patharkar & Cushman, 2000). These modifications in gene expression can be illustrated as plant response for plant growth regulators.

In general, it can be concluded that the application of applied plant growth regulators affected the expression of different plant genes, where some of them were suppressed, while some novel proteins were induced. These effects were correlated with the type of plant growth regulators involved in the treatment and the dose, which reach to organ.

From these results we can conclude that there is a remarkable change in polypeptides in response to different plant growth regulator treatments. It appears that a number of polypeptides were increased or decreased in intensity while others were disappeared, or new polypeptides were expressed. Most of these modifications appear to be a part of metabolic changes in response to developmental stages (Ibrahim *et al.* 2005).

Isozymes analyses
  1- Superoxide dismutases
Superoxide dismutases (SOD) are ubiquitous metalloenzymes that catalyze the dismutation of superoxide radicals and thus prevent oxidative damage in all organisms. Three classes of SODs, differing in the metals at their catalytic active site, are known in plants. The CuZnSODs are localized in the cytosol, chloroplasts, nucleus, and apoplast, the MnSODs present in the mitochondria and peroxisomes, and the FeSODs occur in the chloroplasts (Ogawa et al. 1996).

Several SOD isoforms were identified. Four of them being CuZnSOD, zone and one of higher mobility FeSOD zone (Fig. 3). However, it was not possible to identify MnSOD in faba bean leaves under the experiment condition. The activity gel of SOD revealed that 50 ppm of Kin, 100 ppm of Et and 25 & 50 ppm of NAA treatments produced a high level of FeSOD isoform. In the CuZnSOD zone four SOD isoenzymes were found, three of them being most prominent (No.1, 3 and 4), while the remaining one was very faint band; also only one of them (isoenzyme No. 2) responded to the stress applied by

25 & 50 ppm of Kin, 50 &100 ppm of Et and 25 ppm of NAA treatments when compared with control. Similar results were reported by Srivalli *et al.* (2003).These authors observed five SOD isoforms, four of them being Cu/Zn SODs. The activity of SODII increased during water stress in all cases. SOD III and IV did not show any differences among treatments. The enhancement of total SOD activity in stressed plants was due to the SODII isoenzyme. Unique new isoforms in all the analyzed samples in CuZnSOD zone (No. 4) was detected than in the control, and this may be a result of gene dosage (Aragoncillo *et al.* 1978). SOD catalyses the disproportion of superoxide radicals and converts them to molecular oxygen and H_2O_2 (Srivalli and Khanna-Chopra, 2001). In the growth regulators treated plants, more activity isoforms of SOD were observed compared to control plants. SOD plays an important role in protecting cells against the toxic effects of superoxide radicals produced during oxidative burst (Halliwell and Gutteridge, 2000). This indicates the possible role of plant growth regulators in the retention of reproductive parts through subdued accumulation of ROS.

Variable responses of SOD to dehydration stress have been reported in literature depending on plant species, tissue and stage of development including decreased activity (Quatracci *et al.* 1994); lack of effect (Bartoli *et al.* 1999) and increased activity (Srivalli *et al.* 2003).

Concerning scavenging enzymes of reactive oxygen species (ROS), Superoxide dismutase activity (SOD) in faba bean was increased with growth regulators spray. Electrophoretical analyses of isozymes are the most popular methods for the detection of gene products (Kalinowski *et al.* 2001).
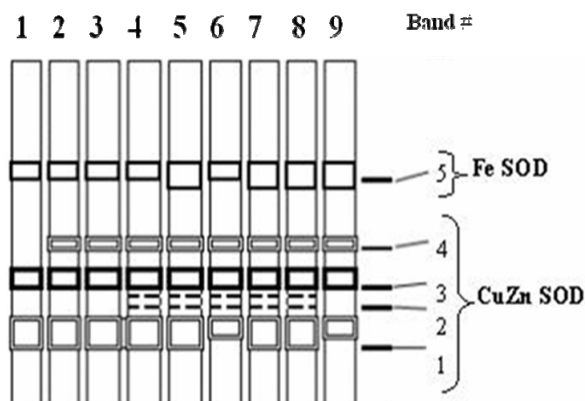
Fig. 3. Effect of plant growth regulators on isozyme banding patterns of superoxide dismutases (SOD) in faba bean leaves illustrated in zymogram. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP_{333} 25, PP_{333} 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

2-Peroxidase

Peroxidase (POD) zymogram (Fig. 4) revealed three zones of independent variation pattern among various treatments, indicating the existence of three heterozymes. However, only the most intensely stained POD-C could be recorded for all treatments compared with control. A strong activation of newly isoform 9 in zone POD-C was observed under different treatments with growth regulators compared with control. On the other hand, the intensity of this isoform was 4-fold higher in the leaves under 25, 50 ppm of Kin and NAA treatments. In the spectrum of PP_{333} (25, 50 ppm) treatment only unique high active isoenzyme was visible (No. 9) whereas the moderate moving isoenzymes had disappeared. Similar trends could be seen for Et (50, 100 ppm) treatments but in this case an addition of isoenzymes remained in the spectrum – isoenzymes No. 4 and some fast moving isoenzymes had disappeared No. 3 & No. 1, 2 upon 50, 100 ppm of Et treatments respectively. The activity of the fast moving isoenzyme (No. 4) enhanced significantly under the experimental conditions 100 ppm of Et and 25 ppm of NAA treatments. The activity of the moderate No. 6 and slow No. 7 migrating isoenzymes increased

by 50 ppm of Kin and NAA treatments whereas heterozygous slow (No. 8) migrating isoenzymes was recorded only for Kin 25 ppm.

The induction of peroxidase (POD) activity in plants occurs in response to numerous biotic and abiotic stimuli, including exposure to pathogens or elicitor preparations, chemical oxidizing agents, red light, and mechanical stimuli (Casal *et al.* 1994). The generality of this response is probably related to the multiple forms and overlapping functions of POD in normal plant development and following induction by abiotic and biotic elicitors (Lagrimini and Rothstein, 1987). POD is believed to play roles in auxin catabolism, the oxidation of phenolics to form lignin, the cross-linking of hydroxyproline-rich glycoproteins in plant cell walls, and the production and breakdown of hydrogen peroxide and other reactive oxygen species (Klotz and Lagrimini, 1996).

Different isoforms of peroxidases are found in chloroplasts, mitochondria, peroxisomes and cytosol. The different isoforms are also regulated differentially in response to stress and development (Ye *et al.* 2000). From this investigation, it was clearly established that peroxidase isoforms were increased compared to control plants. The increase in peroxidase isoforms was to scavenge even low concentrations of H_2O_2 as the enzyme has a high affinity to H_2O_2. Orendi *et al.* (2001) reported that an increase in the peroxidase enzyme activity led to decrease of H_2O_2 content and lipid peroxidation leading to increased fruit set.

The above mentioned findings may confirm the interpretation of Booij *et al.* (1993a, b) who reported that peroxidase activity considered an early marker for root ability and/or shoot formation.

Peroxidase has been shown a first edge in a process of hardening and lignification of cell walls in plantlet stage. On contrary, the reduction of peroxidase contributes to the maintenance of the tissues in a more meristematic state by reducing the lignification of the walls, and allowing their transformation into buds or shoots (Thorpe & Gaspar, 1978). Peroxidase is inversely proportion with auxin level and auxin-cytokinin ratio (Booij *et al.* 1993 a, b).

Superoxide radicals are known to inhibit peroxidase activity (Matysik *et al.* 2002) and thereby efficient scavenging of superoxide is a must for enhanced peroxidase activity. Such increased peroxidase activity as observed in growth regulators treated plant confirms the role of SOD in protecting POD enzyme from superoxide radicals

(Rabinowich and Fridovich , 1983). To a great extent, the differences in SOD activity were shown to be related to subcellular localization of SOD isoforms and to the cellular decompartmentalization that results from membrane deterioration during oxidative burst (Droillard and Paulin, 1990). In growth regulators treated plants, enhanced expression and forms of SOD indicate the possible participation of growth regulators in delaying the membrane detoriation during abscission leading to increased fruit set (Djanaguiraman *et al.* 2004).
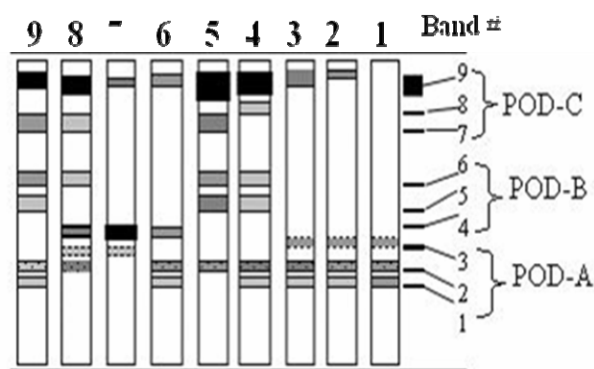
Fig. 4. Effect of plant growth regulators on isozyme banding patterns of Peroxidase (POD) in faba bean leaves. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

3- Catalase

In the present study (Fig 5), four major bands with catalase activity were detected but only two of them (CAT1 and CAT2) were the most prominent in the control and other treatments. The activity of the CAT 1 and CAT 2 isoenzymes increased significantly in response to plant growth regulators with high intensity than control. A strong activation of newly isoenzymes (CAT3 and CAT4) was observed under high concentration of Kin (50 ppm) and Et (100 ppm) treatments.

In maize catalase is present as four isoenzymes (Scandalios *et al.* 1984). Srivalli *et al.* (2003) established an induction of a new catalase isoform in water stressed rice seedlings that reached a maximum at severe water stress. Catalases and superoxide dismutases are the most efficient antioxidant enzymes (Scandalios *et al.* 1984). The expression of specific catalase isoenzymes is important and critical against

oxidative stress induced by a given environmental stress (Scandalios *et al.* 1984).
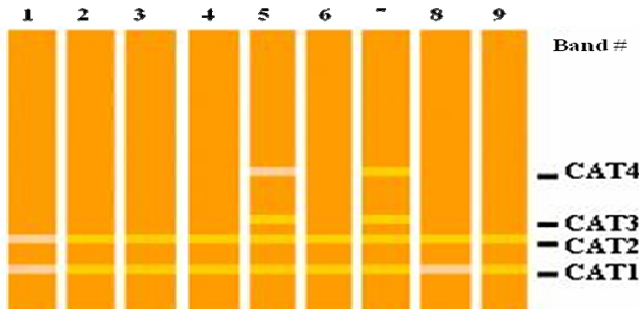

Fig. 5. Effect of plant growth regulators on isozyme banding patterns of Catalase (CAT) in faba bean leaves. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

4- α esterase

Alfa esterase (α-EST) zymograms (Fig. 6) showed, as a rule, one major band together with 1–5 fainter bands, evenly spaced bands of slower mobility. The latter are considered to be modified isoforms of the basic isozyme. Two fastest moving isoesterases (EST) 1& 2 were detected with high frequency in all the analyzed samples, as opposed to the remaining four bands observed with lower frequencies. Band 3 was detected only by 25 ppm of PP$_{333}$ treatment. Isoesterases 4 was performed only by 50 ppm of PP$_{333}$. Isoesterases 5 was present upon 25 ppm of Kin and 100 ppm of Et treatments. Specific variants, recorded by the mobility of the slowest major band No. 6 by 50 ppm of PPP$_{333}$ treatment.
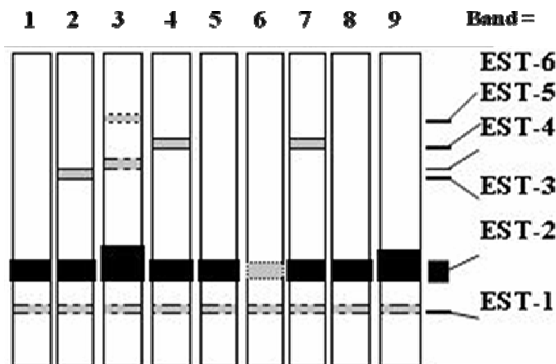
Fig. 6. Effect of plant growth regulators on isozyme banding patterns of α-Esterase (EST) in faba bean leaves. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

5- ß-esterase

Nine isozymes of ß-esterase were detected as slow and fast migrating zones, each consisting of five and four EST morphs, which were visualized on the gels in the analyzed samples (Fig. 7). Isozyme 1 and 4 were present only in 50 ppm of Et. Band 2 was present in all treated plants, except for the control and 50 ppm of Et. Band 3 was present only in the control. Isoform 4 was detected only in 25 ppm of PP$_{333}$. The 50 ppm of PP$_{333}$ and Kin treatments showed four slow moving (6, 7, 8, 9) bands, while the 50 ppm of Et and Kin treatments showed two slow moving (6,9) bands**.**
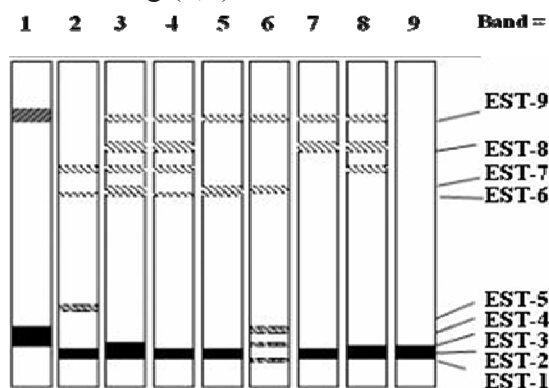

Fig. 7. Effect of plant growth regulators on isozyme banding patterns of β-Esterase in faba bean leaves. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

6- Acid phosphatase

Four isozymes of acid phosphatase ACPH (Fig. 8) were detected after separation in polyacrylamide gels. Isozyme 4 with high mobility was present in all the analyzed samples. The relative intensity and the number of ACPH bands on the zymograms varied depending on different treatments. Isophorm 1 was detected by 50 ppm of Et and 25, 50 ppm of NAA treatments. This band was absent in the phenotypes of the control and other treatments. The activity of the slow moving

isoenzyme (No. 2) enhanced significantly by 50 ppm of PP$_{333}$ and 100 ppm of Et treatments under the experimental conditions. The activity of the slow (No. 3) migrating isoenzyme was observed in the control plants. The appearance of ACPH isoform 2 in 55% of treatments and isoform 1 in 33% may be connected to the expression of new genes. The detection of new isozymes in the treatments, not expressed in the control, may be a result of the recombination between different alleles. It has been shown that unique alleles could be formed also by intragenic recombination (Golding and Strobeck, 1983).

Acid phosphatase is believed to be important for many physiological processes, including regulation of soluble phosphorous (Yan *et al.* 2001). Free soluble phosphate reserves plays vital role in energy transfer, metabolic regulation, important structural constituent of biomolecules like phytin bodies in the ungerminated seeds, protein and nucleotide phosphorylation (Ehsanpour and Amini, 2003). The exact role of phosphatases in the germinated seeds is still not clear, because metabolism of these compounds can be affected by a number of environmental factors such as stress type, irridance, temperature, and type of ions present (Bohnert *et al.* 1995).
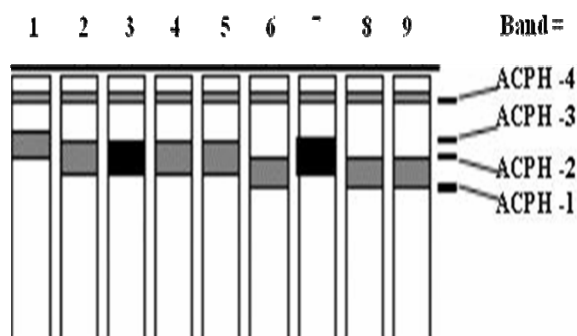

Fig. 8. Effect of plant growth regulators on isozyme banding patterns of Acid phosphatase (ACPH) in faba bean leaves. Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP$_{333}$ 25, PP$_{333}$ 50, Kin 25, Kin 50, Et 50, Et 100, NAA 25 and NAA 50, respectively.

These all observations may also be a result of plant growth regulators controlling the expression of structural genes (Zivy *et al.* 1992) and or gene suppression (Galili and Feldman 1984) and gene dosage (Aragoncillo *et al.* 1978). New isozymes patterns may also possible that control possess enzymatic silent genes activated in the

new combinations of different treatments as a result of the neutralization of suppression.

The present study clearly indicates that application of plant growth regulators significantly increased the antioxidant enzymes and other enzymes resulting increased fruit set and yield.

Generally, isozymes can be identified on zymographs. Either the loss or induction of isozymes can have a profound effect on metabolism. Therefore, total soluble proteins and isozymes (superoxide dismutase, peroxidase, catalase, α, β-esterases and acid phosphatase) data is related to the physiological states of the plant and its development. Data show differences in band numbers and intensities. These variations were due to genetic differences among developmental stages and applications of plant growth regulators. These results are in harmony with Baaziz *et al.* (1994); Booij *et al.* (1995).

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تأثير بعض منظمات النمو النباتية على فسيولوجيا التساقط وانزيمات مضادات الأكسدة والنمو والمحصول للفول البلدى

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تم زراعة بذور الفول البلدى صنف جيزة 716 فى الصوبة ورشت فى بداية الإزهار والعقد بالبكلوبيوترازول والكينتين ونفثالين حمض الخليك بتركيزي 25 ، 50 جزء فى المليون والايثيفون بتركيزي 50 ، 100 جزء فى المليون. سجل طول النبات وعدد الأفرع والأزهار بعد 104 يوم من الزراعة. تم أخذ عينتان عند عمر 90 يوم وفى الحصاد. فى العينة الأولى فى الموسم الأول أجريت التحليلات البيوكيميائية فى الأوراق لتقدير السكريات الكلية الذائبة والفينولات والاندولات والأحماض الامينية الحرة والبروتين الذائب فى الماء ومشابهات الإنزيمات. عند الحصاد (العينة الثانية) أخذت القياسات التالية عدد القرون والوزن الجاف للقرون و البذور للنبات فى كلا الموسمين. تم تقييم البروتينات والكربوهيدرات الكلية لبذور الفول الجافة.

أدت معاملة البكلوبيوترازول بتركيز 50 جزء فى المليون لتاثير سلبى ومعنوي على ارتفاع النبات وعدد الأزهار والفينولات الكلية والاندولات. أدى التركيز العالى لمعاملات الكينتين والايثيفون ونفثالين حمض الخليك لزيادة عدد القرون والوزن الجاف لكل من القرون والبذور للنبات فى حين حدث العكس مع معاملة البكلوبيوترازول بالتركيز العالى. ارتبط محصول البذرة بعدد القرون ووزن القرون لكل نبات. على وجه العموم معاملة نفثالين حمض الخليك بتركيز 25 جزء فى المليون ذات تأثير مشجع قوى على مستوى السكريات الكلية الذائبة والفينولات والاندولات والأحماض الامينية الحرة (فى الأوراق) ومرجع البذرة (وزن 100 بذرة). حيث أن منبه النمو نفثالين حمض الخليك يشجع حركة نواتج التمثيل الضوئى لامتلاء البذور. يمكن استخدام التفريد الكهربى للبروتينات ومشابهات الإنزيمات "سوبر اكسيد ديسميوتيز والبيروكسيديز والكتاليز والفا وبيتا استيريز والفوسفاتيز الحامضى" للتعبيرعن فسيولوجيا التساقط. وبناء علية تكون بيانات البروتينات الكلية الذائبة و مشابهات الأنزيمات مرتبطة بالحالة الفسيولوجية للنبات وتطوره.