

EFFECT OF SOME PLANT GROWTH REGULATORS ON BIOCHEMICAL GENE EXPRESSION, GROWTH AND YIELD OF FABA BEAN

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ABSTRACT

In the greenhouse experiment *Vicia faba* L. cv. Giza 716 were treated with 25 and 50 ppm of paclobutrazol (PP₃₃₃), kinetin (Kin), naphthalene acetic acid (NAA) and 50 and 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. Two samples were taken after 90 days from planting and at harvest. At the 1st sample date, 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. Total seed proteins and soluble carbohydrates were evaluated in dry faba bean seeds. PP₃₃₃ 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 PP₃₃₃ treatment. Seed yield was correlated 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), soluble carbohydrates (in seeds) and seed index (100-seed weight). It seems that growth promoter NAA enhance the mobilization of photoassimilates to filling seeds. Protein banding patterns and isozymes of superoxide dismutase, peroxidase, catalase, α , β -esterases, acid phosphatase separation by electrophoresis were successfully used to faba bean subjected to PP₃₃₃, Kin, Et and NAA. The applied plant growth regulators affected the rate of gene expression in leaves more than in seeds. Their effects on growth, fruit set, yield, correlated components, proteins SDS-PAGE and isozymes were discussed below.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important field crops in Egypt; it is cultivated through out 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, 1976a & 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). Anti-oxidant 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 H₂O₂ content and lipid peroxidation leading to increased fruit set.

Therefore, this study designed to determine the effect of some growth regulators (Paclobutrazol (PP₃₃₃); ethephon (Et); kinetin (Kin); naphthalene acetic acid (NAA)) on 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.

MATERIAL AND METHODS

Greenhouse experiment: This study was performed in the greenhouse at Faculty of Agric., Ain Shams Univ. during the season of 2006–2007. Five seeds of faba bean (*V. faba* L.) cv. Giza 716 were sown on 11th November 2006 in 35 cm diameter pots filled with sandy loam soil. Irrigation was applied as Hoagland nutrient solution (Gauch, 1972), one strength (140ml/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 and 100 ppm, Naphthalene acetic acid (NAA), kinetin (Kin) and Paclobutrazol (PP₃₃₃) at 25 and 50 ppm for each 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 analysis that conducted in leaves for the determination of total soluble sugars, phenols, indoles, free amino acid, SDS-protein electrophoresis and isozymes.

Plant height, branches and flowers number were recorded at 104 days after sowing. 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, pods, seeds weight/plant and 100 seeds weight were recorded. The air-dried pods and seeds on plant were performed to determinate dry weight of pods and seeds. Chemical analysis was conducted in mature seeds for the determination of total carbohydrates and proteins electrophoresis.

Biochemical analysis: Total soluble sugars and carbohydrates 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). Total

amino nitrogen or 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 using the following model $Y_{ijk} = \mu + \tau_i + S_j + e_{ijk}$. The used design is completely randomized design.

SDS-PAGE analysis: Characterization of leaf water soluble protein (90 days from sowing) and total seed proteins (at harvest) 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). 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), acid phosphatase (ACPH) were electrophoretically analyzed. The methodology of Beau-Champ and Fridovich (1971); Wendel and Weaden (1989); Nadlony and Sequira (1980); Shaw and Prasad (1970) 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

Data presented in Table-1 revealed the effect of foliar applications of PP₃₃₃, Kin, Et and NAA on plant height, branches and flowers number 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).

Growth parameters: Plant treated with PP₃₃₃ showed the lowest values of plant height. All treatments led to a significant decrease in plant height except NAA treatments as compared to the controls. An insignificant decrease in plant height was obtained by NAA treatments. Applications of PP₃₃₃ 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 PP₃₃₃ 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-1** clearly demonstrate that the effects of applied plant growth regulators on yield components in the second sample were greatly depended on the used concentration. The high concentration of applied plant growth regulators (PP₃₃₃, Kin, Et and NAA) was more effect than the low concentration. The high concentration of Kin, Et and NAA treatments increased the number of pods per plant, the dry weight of pods per plant and the dry weight of seeds per plant whereas the opposite occurred in high concentration of PP₃₃₃ treatment. PP₃₃₃ 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 was correlated with the number of pods per plant and the dry weight of pods per plant. There was a negative significant effect relationship between 100-seed weight and applied plant growth regulators except NAA at 25 ppm. This effect was insignificant.

The obtained results indicated that PP₃₃₃ had significant and negative effect on growth. In this respect, the results are in agreement with El-Shamey and Ibrahim (2004), they found that application of PP₃₃₃

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).

Table-1: Effect of some growth regulators on growth parameters and yield components of faba bean

Treatments	Plant height (cm) ²	Branches No./plant ²	Flowers No./main stem ²	Pods No./Plant ³	Pods dry wt./Plant (g) ³	Seeds dry wt./Plant (g) ³	100-seed wt. (g) ³
Control	77a	3.30c	49a	10.30cd	24.01cd	20.00d	103.69a
PP ₃₃₃ 25	59e	4.80b	54a	10.94cb	25.60cb	21.34dc	95.74b
PP ₃₃₃ 50	43f	4.00cb	39b	7.51e	17.59e	14.66e	84.56d
Kin 25	70bc	3.50c	42b	9.54d	22.34d	18.60d	89.40cd
Kin 50	64ed	3.30c	41b	13.00a	30.40a	25.35ba	92.76cb
Et 50	67dc	3.80cb	41b	9.99cd	23.38cd	19.48d	96.96b
Et 100	61e	3.80cb	42b	11.88b	27.29b	23.16bc	84.24d
NAA25	72bac	3.80cb	43b	9.75d	22.82cd	19.01d	104.18a
NAA50	74ba	6.30a	33c	13.55a	31.71a	26.42a	94.20cb
MSD T	5.59	1.12	5.59	1.12	5.59	2.78	5.59

2 = after 104 day from sowing.

3 = at harvest (2nd sample).

wt. = weight.

Chemical Analysis: As mentioned previously, chemical analyses shown in Table-2 were conducted in the leaves (total soluble sugars, phenols, indoles and free amino acids) at the 1st sample (90 days from sowing) and in seeds (total soluble carbohydrates) at harvest (2nd sample).

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 and this effect reached the level of significance.

PP₃₃₃ at 25 and 50 ppm, Et and NAA at 50 ppm showed a significant decrease in total phenol concentration of leaves comparing with control, the lowest value referred to PP₃₃₃ 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.

PP₃₃₃ 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 $\mu\text{g} / \text{g}$ f.wt.) comparing with control (167.42 $\mu\text{g} / \text{g}$ 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 $\mu\text{g} / \text{g}$ f.wt.).

There were no significant differences in free amino acids by applied plant growth regulators observed when compared to control. The highest and lowest values of free amino acids referred to NAA treatments at 25 and 50 ppm and PP₃₃₃ at 50 ppm, respectively. These results confirmed with Zaghlool et al., (2001). They mentioned that NAA increased amino acid content in *Phaseolus vulgaris* and lentil.

No significant differences in total soluble carbohydrates were noticed between all treatments and control except Et treatment at 100 ppm, which showed significant reduction. NAA treatment at 25 ppm gave highest value in this concern.

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

Table-2: Effect of some plant growth regulators on biochemical components of faba bean leaves and seeds.

Treatments	Total soluble sugars ¹ (mg/g f.wt.)	Total phenols ¹ ($\mu\text{g}/\text{g}$ f.wt.)	Total indoles ¹ ($\mu\text{g}/\text{g}$ f.wt.)	Free amino acids ¹ ($\mu\text{g}/\text{g}$ f.wt.)	Total soluble carbohydrates ³ (mg/g f.wt.)
Control	17.51b	340.43bc	167.42c	635.18bdac	327.78ba
PP ₃₃₃ 25	19.20b	200.45d	113.11d	626.42bdc	316.00bac
PP ₃₃₃ 50	18.49b	191.53d	110.93d	591.04d	324.12ba
Kin 25	19.86ba	293.77c	210.87b	656.27bac	298.95bc
Kin 50	18.01b	367.54ba	195.67b	675.42ba	297.57bc
Et 50	19.38b	212.12d	89.21d	645.56bdac	288.84bc
Et 100	19.68ba	303.38c	141.35c	612.13dc	264.27c
NAA25	22.37a	403.91a	249.98a	690.03a	361.35a
NAA50	19.83ba	213.49d	256.50a	687.43a	321.55ba
MSD T	2.83	55.94	27.97	55.94	55.94

1 = after 90 day from sowing in leaves (1st sample). 3 = at harvest in seeds (2nd sample).

SDS-PAGE analysis

SDS-PAGE of leaf water-soluble and total seed proteins 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 and seeds are shown in Figures-1 and 2 respectively, and their densitometry analysis is illustrated 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 growth regulators resulted in the induction of new bands, i.e., band no. 38 with MW of around 73 kDa was noticed by 25 and 50 ppm of PP₃₃₃ & 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 kDa and 56 kDa in all treatments except in 50 ppm of NAA treatment had a different profile. It showed two bands with molecular weight of about 54 and 58 kDa, respectively.

There were similar electrophoretic profiles for bands no. 23 and 24 with MW of around 42 and 46 kDa, respectively by 25 and 50 ppm of each Kin and NAA and 50 and 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, 50 ppm of Et, 25 and 50 ppm of NAA treatments. These results 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 were not affected by applied treatments unless increase the intensity of most polypeptide except 50 ppm of NAA for band no. 19 and 20 of 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 and 17 with MW of about 26, 28 and 30 kDa increases the intensity of most polypeptide bands while minor bands no. 10, 11 and 12 with MW of around 21, 23 and 24 kDa were induced by 50 ppm of PP₃₃₃ and 25 and 50 ppm of Kin treatments.

There were two additional new bands no. 8 and 9 with MW of about 19 and 20 kDa were observed in the all treated plants but disappeared in 25 ppm of PP₃₃₃ and 50 and 100 ppm of Et treatments. The band no. 8 disappeared in 100 ppm of Et treatment. A strong activation of newly intensive band no.7 with MW of about 17 kDa was observed under different treatments with applied growth regulators which was absent in 50 ppm of Kin and control.

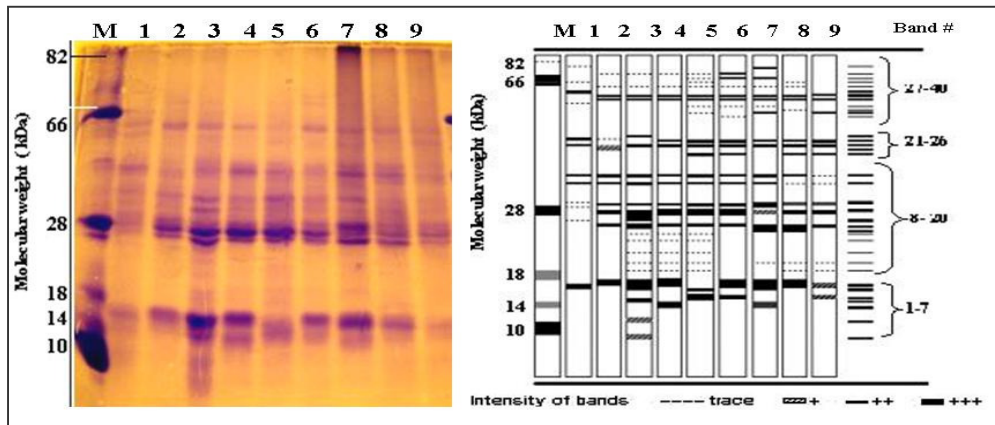


Fig-1: SDS-PAGE profile of water-soluble protein fractions of faba bean leaves treated with some plant growth regulators (A) illustrated in zymogram (B). (M = marker protein). Lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9 represent control, PP₃₃₃25, PP₃₃₃50, Kin 25, Kin50, Et 50, Et 100, NAA 25 and NAA50, respectively.

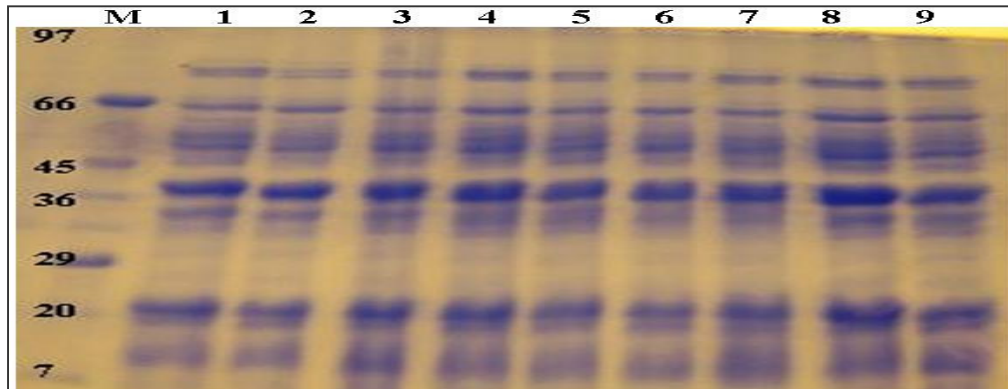


Fig-2: SDS-PAGE profile of total seed storage protein fractions of faba bean leaves treated with some plant growth regulators. (M=marker protein). Lanes from 1 to 9, refer to Figure 1.

The overall results of leaf water-soluble and total seed storage 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. This technique has 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 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. Hassanien *et al.*, (2004) noted these changes as the effect of some herbicides on faba bean. Where some genes were promoted (up regulated) and gave intensive or normal band, while some others were suppressed (down regulated) and gave less intensive or absent bands.

The major seed storage proteins in faba bean like soybean are glycinin (11S globulin) and b-conglycinin (7S globulin), accounting for approximately 70% of the total storage proteins (Derbyshire *et al.*, 1976). The glycinin protein is composed of acidic subunits, with approximate molecular weight of 45 and 38 kDa, and

basic subunit with 22 kDa; the β - conglycinin is composed of major subunits of α - α and β -subunits with 76, 72, and 48 kDa, respectively (Shuttuck-Edidens and Beachy, 1985).

The data suggest that accumulation of all protein bands were insensitive to the applied growth regulators. These results suggest that no difference between the treatments in the accumulation of proteins might be due to time and treatment and their effect on flowering and fruit set. From these results, it is concluded that there is a remarkable change in polypeptides in response to different plant growth regulators treatments. It appears that a number of polypeptides increased or decreased in intensity others disappeared or new polypeptides expressed. Most of these modifications appear to be a part of metabolic changes in response to developmental stages (Ibrahim *et al.*, 2005).

In general, plant growth regulators treatments PP₃₃₃, Kin, Et and NAA affected the rate of gene expression in leaves more than in seeds. These effects were correlated with the type of plant growth regulators involved in the treatment and the dose, which reach to organ. As for quantity of protein, it was observed that the proteins in leaves source less than in fruits sink (Zaghlool *et al.*, 2001).

Isozymes analyses: 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 in the mitochondria and peroxisomes and the FeSODs in the chloroplasts (Ogawa *et al.*, 1996).

Several SOD isoforms were identified. Four of them being CuZnSOD zone while one is 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 50ppm 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 (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 have 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 of SOD isoforms 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 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).

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).

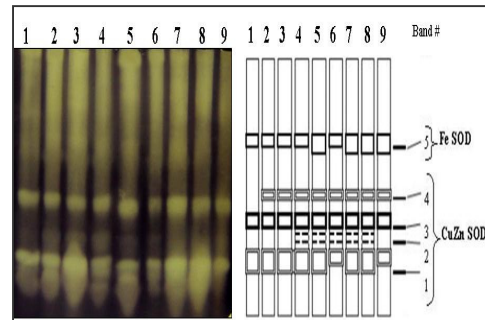


Fig.-3: Effect of plant growth regulators on isozyme banding patterns of Superoxide dismutases (SOD) in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure-1.

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 PP₃₃₃ (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).

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 H₂O₂ as the enzyme has a high affinity to H₂O₂. Orendi *et al.* (2001) reported that an increase in the peroxidase enzyme activity led to decrease of H₂O₂ 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 and 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 deterioration during abscission leading to increased fruit set (Djanaguiraman *et al.*, 2004)

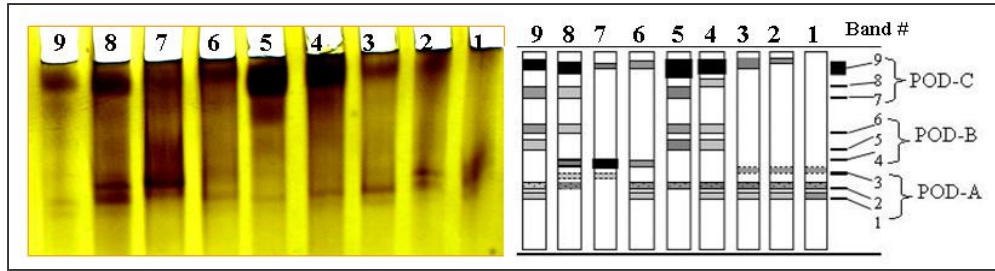


Fig-4: Effect of plant growth regulators on isozyme banding patterns of Peroxidase (POD) in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure 1.

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. The expression of specific catalase isoenzymes is important and critical against oxidative stress induced by a given environmental stress (Scandalios et al., 1984).

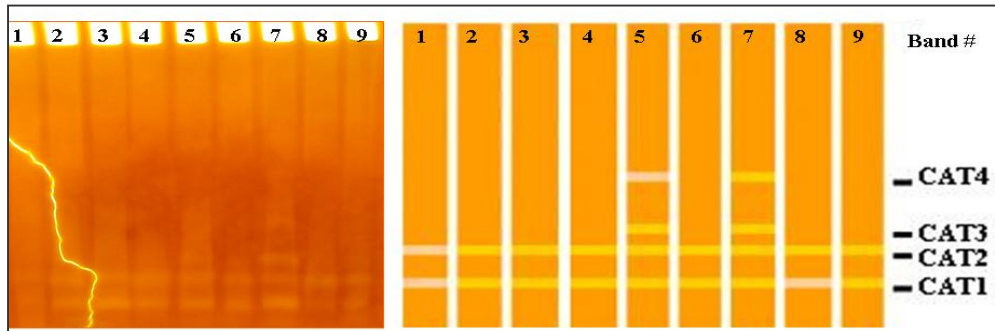


Fig-5: Effect of plant growth regulators on isozyme banding patterns of Catalase (CAT) in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure 1.

Alfa esterase (α -EST) zymograms (Fig. - 6) showed 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 and 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 PP₃₃₃ treatment. Isoesterases 4 was performed only by 50 ppm of PP₃₃₃. 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 PPP₃₃₃ treatment.

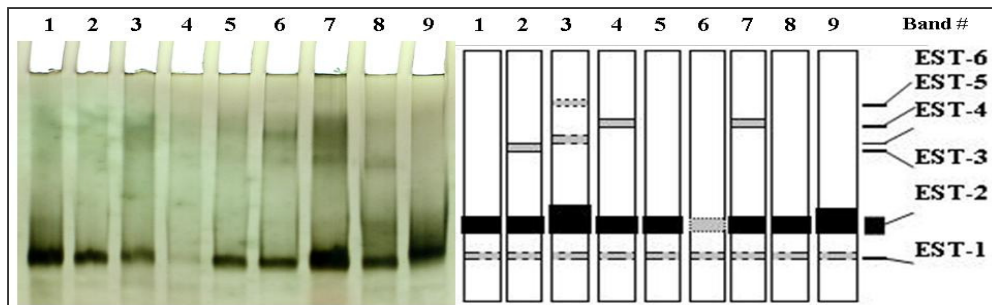


Fig-6: Effect of plant growth regulators on isozyme banding patterns of α -Esterase (EST) in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure 1.

Nine isozymes of β -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 PP₃₃₃. The 50 ppm of PP₃₃₃ 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.

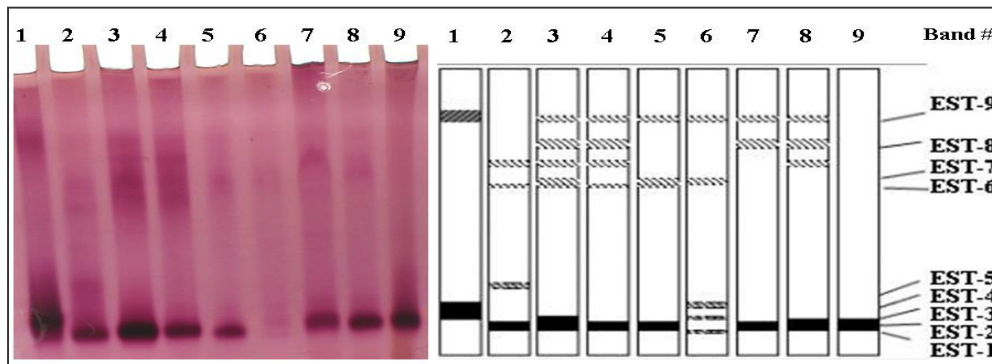


Fig-7: Effect of plant growth regulators on isozyme banding patterns of β -Esterase in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure 1.

Four isozymes of 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. Isoform 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 PP₃₃₃ 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).

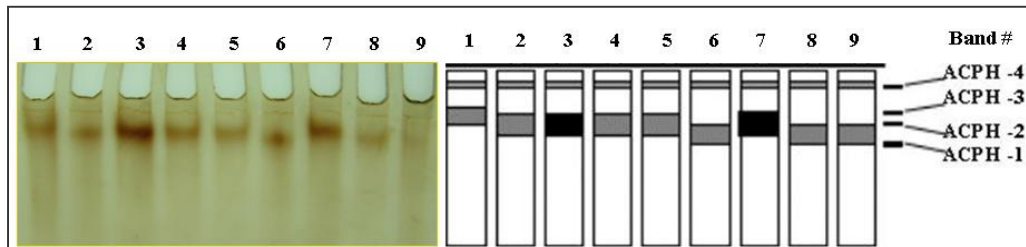


Fig-8: Effect of plant growth regulators on isozyme banding patterns of Acid phosphatase (ACP) in faba bean leaves (A) illustrated in zymogram (B). Lanes from 1 to 9, refer to Figure 1.

Acid phosphatase is believed to be important for many physiological processes, including regulation of soluble phosphorous (Yan et al. 2001). Free soluble phosphate plays vital role in energy transfer, metabolic regulation, 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, irradiance, temperature, and type of ions present (Bohnert et al., 1995).

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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