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Impact of Salicylic Acid and Paclobutrazol Exogenous Application on the Growth, Yield and Nodule Formation of Common Bean

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Abstract: Paclobutrazol (PP_{333}) at 10, 20 ppm and salicylic acid (SA)at 10^{-2}, 10^{-3} M were applied as foliar spray to 30 and 45 day-old common bean *(Phaseolus vulgaris* L.) plants during March to June 2006 in the greenhouse of Agric. Botany Dept. in Fac. of Agriculture of Ain Shams Univ. at Cairo, Egypt. SA considerably increased plant height when applied at 10^{-2} M. In contrast, paclobutrazol significantly reduced plant height and shoot dry weight. The highest values of shoot dry weight were obtained by SA treatments. The maximum number of nodules per plant was achieved by the application of SA at 10^{-3} M. Whereas PP_{333} applications prevent nodule formation. The highest chlorophyll content was found with PP_{333} at 10 and 20 ppm. Significant increase in total protein in leaves was obtained with PP_{333} at 10 and 20 ppm followed by SA at 10^{-3} M. However the highest value of total protein in nodules was recorded with SA at 10^{-2} M then SA at 10^{-3} M. The present study is conducted to investigate the effect of exogenous application of SA and PP_{333} to find out whether both growth regulators had positively or negatively effect on growth, nodulation, chlorophyll, protein and yield of bean plant.

Key words: *Phaseolus vulgaris*, PP_{333}, SA, growth, nodule formation, chlorophyll, protein

INTRODUCTION

Common bean, *Phaseolus vulgaris*, is herbaceous annual plant, and now grown worldwide for its edible bean, pods are popular as a vegetable both dry and green bean. As a legume, beans provide the nitrogen-fixing bacteria which supply essential nutrient to other crops.

Rhizobium, is a bacteria that form nitrogen-fixing nodules on legume roots. Their association with legumes is a rather special plant-microbe interaction, in which the successful interaction does not induce a plant defense response (Abarca *et al*, 1998).

Rhizobium plays a very important role in agriculture by inducing nitrogen-fixing nodules on the roots of legumes such as peas, beans, clover and alfalfa (Downie and Brewin, 2007). Therefore, this symbiosis can relieve the requirements for added nitrogenous fertilizer during the growth of leguminous crops.

Salicylic acid (SA) is an endogenous plant growth regulator. It is involved in various physiological processes of plant growth and development (Klessig and Malamy, 1994) such as induction of flowering (Cleland and Ajami, 1974) and root growth stimulation (Gutiérrez-Coronado *et al*, 1998). SA also plays a major role during the early stages of *Rhizobium*-legume symbiosis (Rasmussen, *et al,* 1991). Nod factors produced by rhizobia, in response to legume produced flavonoids, affect SA content of the host plant during the early stages of nodulation. When Spronsen *et al* (2003) tested the effect of SA on both types of nodulation, they found that SA completely inhibits nodulation in indeterminate-type plants such as pea, vetch, alfalfa, and clover but not in determinate-type plants such as soybean, bean, and *Lotus japonicus*.

Triazole compounds including paclobutrazol (PP_{333}), were consider a gibberellin biosynthesis inhibitor. It blocks three separate steps in the biosynthetic pathway for the production of gibberellins (Zeevaart *et al*, 1993). However, when gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same number of leaves and internodes compressed into a shorter length (Quinlan, 1981).

Some morphological changes observed in triazole-treated oak plants include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio and elevated levels of epicuticular wax formation (Watson and Himelick, 2004).

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Paclobutrazol (pp_{333}) is a triazole compound used extensively in agriculture and horticulture as both plant growth regulator and fungicide. In ornamental crops, pp_{333} is used for reducing the size of plants, improving compactness, and increasing other functional aspects, such as the ability to resist both abiotic and biotic stresses.

MATERIALS AND METHODS

Pots Experiment:

Pots experiment was conducted in the greenhouse of Agric. Botany Dept. in Fac. of Agriculture of Ain Shams Univ. at Cairo, Egypt, during March to June 2006 with the common bean *Phaseolus vulgaris*, `cv. Bronco'. Plants were sown at 3rd March in 25 cm diameter plastic pots filled with a 2 kg of sandy soil. Fittings were inserted into the bottom of the pots to allow water drainage and to flow air into the pots. The pots were over seeded and thinned to one plant per pot after emergence. Soil was inoculated with a commercial preparation of *Rhizobium leguminosarum* bv. phaseoli strains after 10 days of germination. Plants were grown under well-watered conditions. Each pot was received fertilizer two times as follows 1.5 g nitrogen as ammonium nitrate (33.5% N) and 1 g potassium sulfate (48% K_2O). These fertilizers were added at 15 and 30 days from sowing. Phosphorous as calcium super phosphate (15.5% P_2O_5) was mixed with soil before sowing at the rate of 3 g/pot. Other recommended agriculture practices for bean cultivation was followed. The pots were arranged in complete randomized design with ten replicates. Each replicate consisted of four pots and one plant per pot. Plants were foliar sprayed two times (30 and 45 days from sowing) with paclobutrazol at 10 and 20 ppm, salicylic acid at 10^{-2}, 10^{-3} M and control plants were sprayed with distilled water until complete covering of the plant foliage. Tween 20 at 0.05 ml/L was used as wetting agent. Samples were taken at 70 days after sowing for growth measurements and chemical analysis from each treatment.

Growth Parameters:

Plant height, number of leaves, fresh and dry weights (g / plant) of shoots and roots were recorded (70 days after sowing). Shoots and roots were dried in an electrical oven at 75 °C till constant dry weight to determine fresh and dry weights.

Chemical Analysis:

Chlorophyll Determination:

Chlorophyll a, b and total chlorophyll (Ch) were determined in the second leaf from the plant tip of *Phaseolus vulgaris* after 70 days from sowing by extracting in 80% acetone according to the methods described by Holder (1965). The amount of pigments was determined spectrophotometrically and the content of chlorophyll was calculated according to Arnon (1949) as follows:

- $C_{a+b} = 20.2\ D_{645} + 8.02\ D_{663}$

Total Soluble Protein Determination:

Total soluble protein was determined (in a dry matter basis) in 3rd and 4th leaves from the tip using the method of Bradford (1976). The soluble protein concentration was calculated from the standard curve according to Read and Northcote (1981).

Statistical Analysis:

Data were subjected to statistical analysis of variance and were calculated according to SAS (1996).

RESULTS AND DISCUSSIONS

Plant Growth and Nodulation:

Data presented in Table (1) indicated that foliar application of salicylic acid generally had a positive effect on vegetative growth parameters (plant height, leaves number, shoots and roots fresh and dry weight) as compared to control or pp_{333} treatments except roots fresh and dry weight with pp_{333} at 20 ppm. These results confirming with Martín-Mex *et al* (2005) where SA at low concentrations affects plant size, number of leaves and flowers of African violet plant. Similar results were obtained by El-Shraiy, (2004) who reported that acetyl

Table 1: Growth parameters and nodules formation of bean plants as affected by pp_{333} and SA foliar application

Treatments	Plant height (cm)	Leaf number	Nodules number	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)
control	24.5	5.8	17.5	12.3	3.8	6.1	4.1
pp_{333} 10 ppm	23.3	6.3	0	14.5	4.6	6.9	5.0
pp_{333} 20 ppm	22.3	6.0	0	12.7	3.9	8.1	6.4
SA10^{-3} M	35.0	6.8	20.5	16.1	6.0	7.2	5.1
SA 10^{-2} M	38.5	7.8	12.6	16.8	6.1	7.9	5.2
LSD	9.204	1.222	5.505	2.711	1.526	1.091	1.117

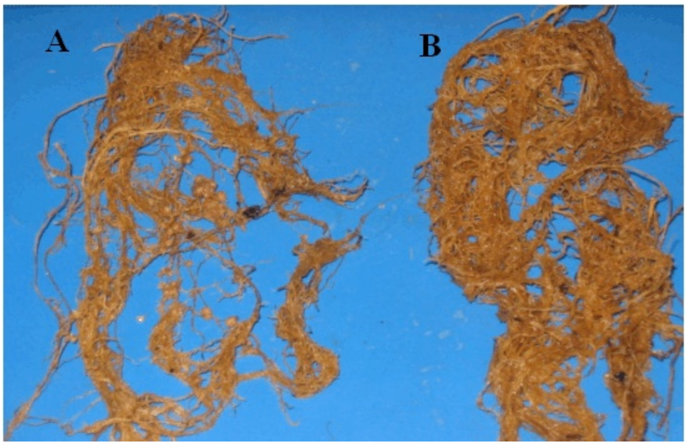

Photo 1: Root of bean plants illustrated nodules formation: A. roots in plants received SA (10^{-3}M). B. roots in plants received pp333 (10 and 20ppm).

salicylic acid treatments on potato plants promoted plant growth, plan height and number of leaves per plant. However, SA at 10^{-2} M had the same trend except with nodule number. The highest nodule number was obtained from plants treated with SA at 10^{-3} M (Photo, 1). Such results may be due to that late application of SA promoted and increased nodulation. Since Takashi *et al* (2002) indicated that salicylic acid inhibited only early nodule formation and did not affect the growth of formed nodules of soybean.

Spronsen *et al* (2003) reported that, addition of SA may interfere with nodule formation in indeterminate-type nodulating plants such as pea, vetch, alfalfa, and clover. Whereas in determinate-type nodulating plants such as soybean, bean, and *Lotus japonicus* no effect of SA would be expected. When testing the effect of SA on both types of nodulation, they found that SA indeed completely inhibits indeterminate-type nodulation but not determinate-type nodulation in plants.

Concerning paclobutrazol effects, pp_{333} at 20 ppm increased root fresh and dry weight as compared to pp_{333} at 10 ppm and control treatments.

Total root length increased significantly after triazole treatment. Among the triazoles, Triadimefon showed the strongest effect, followed by pp_{333}. Triazole treatment was found to increase the root growth in cucumber, and this was associated with increased levels of endogenous cytokinins. The stimulatory effect of triazole compounds in rooting may be due to an inhibition of GA synthesis (Fletcher and Arnold, (1986), Vettakkorumakankav *et al*, 1999, Sankhla and Davis, 1999). Gopi *et al* (2005) also showed that, triazole treatment was found to increase the root growth in cucumber and this was associated with increased levels of endogenous cytokinins. The increased cytokinin levels might increase cell division and thereby lead to increased dry weight in the triazole-treated plants.

However, pp_{333} application significantly decreases vegetative growth which may be explained as paclobutrazol blocks the biosynthesis of the active gibberellins GA_s. These results confirmed by Zeevaart *et al* (1993). The morphological response to paclobutrazol is the reduction in internode length and this effect has been observed in herbaceous (Quinlan, 1981; Setia *et al*, 1995). Our results are in agreement with (Sankhla *et al*, 1985) who reported that paclobutrazol is an experimental growth retardant which considerably reduced plant height, leaf area of soybean. Also, Mahgoub (2006) indicated that foliar application of all paclobutrazol treatments on *Calendula officinalis* L. plants significantly decreased plant height, however, fresh and dry weight of leaves per plant was increased by paclobutrazol application compared with control treatments. Same results were also obtained by Singh (2004) who reported that the shortest plants on marigold, fresh leaf biomass and dry leaf biomass were obtained with 10 and 15 mg paclobutrazol per plant.

Concerning the inhibitory effect of pp_{333} on nodule formation (Photo, 1), it could also be suggested that GA_s have a role in early stages of nodule formation and GAs are essential for induction of cortical cell division and differentiation of the nodule primordium. Lievens *et al* (2005) showed that, GAs are needed to initiate intercellular invasion, therefore, addition of inhibitors of GA synthesis prior to bacterial inoculation completely blocked nodulation.

Chlorophyll and Protein Content:

Data in Table (3) and figures (1 and 2) illustrated that paclobutrazol application significantly increased chlorophyll b and total chlorophyll compared with other treatments. The highest values were obtained with pp_{333} at 10 ppm followed by 20 ppm and SA at 10^{-3} M respectively compared with control meanwhile no differences were detected in chlorophyll a concentration.

Table 2: Yield of bean plants as affected by pp_{333} and SA foliar application

Treatments	Pods number/plant	Pods FW (g)	Pods DW (g)
control	4.8	5.0	3.9
pp_{333} 10 ppm	6.3	6.4	5.5
pp_{333} 20ppm	3.5	3.9	2.9
SA10^{-3} M	7.0	8.7	6.2
SA 10^{-2} M	5.8	5.5	4.1
LSD	2.041	2.457	1.812

Table 3: Chlorophyll a, b, total chlorophyll and total soluble protein concentration in leaves and nodules of bean plants as affected by pp_{333} and SA foliar application

Treatments	Ch a mg/l	Ch b mg/l	Total Ch mg/g	Total soluble protein	
				Leaves	Nodules
control	0.007	0.010	1.67	0.39	0.29
pp_{333} 10ppm	0.008	0.011	1.84	0.85	-
pp_{333} 20ppm	0.008	0.010	1.74	0.59	-
SA 10^{-3} M	0.008	0.009	1.71	0.50	0.53
SA 10^{-2} M	0.004	0.005	0.92	0.45	0.68
LSD	0.0021	0.0035	0.5016	0.242	0.422

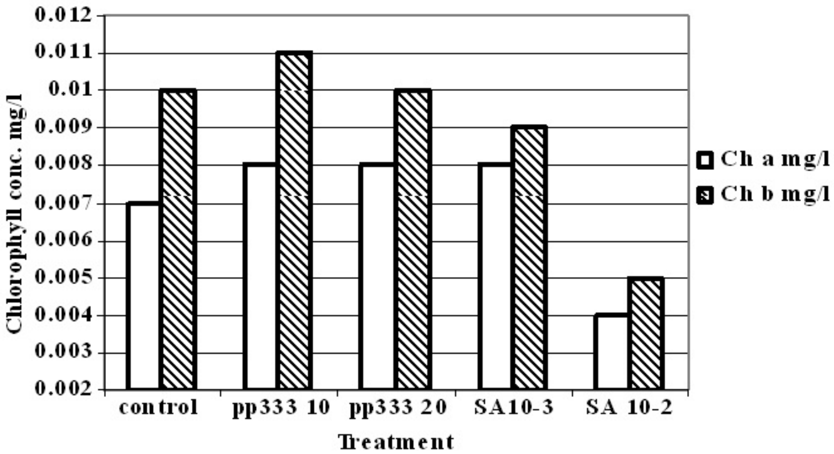

Fig. 1: Ch a and b concentration in leaves of bean plants as affected by pp333 and SA foliar application

Paclobutrazol increased chlorophyll content, this may be partly due to the observed increase in mass of the root system which is the major site of cytokinin biosynthesis (Sopher *et al*., 1999). The increase in cytokinin levels was associated with stimulated chlorophyll biosynthesis (Fletcher *et al*., 2000). Also Grossmann (1992) reported that, triazoles were found to increase the cytokinin content in many plants like pumpkin, oil seed and rape seedlings.

However, SA at 10^{-2} M. significantly reduced chlorophyll a, b and total chlorophyll as compared with other treatments and control. Similar results were obtained by Mahgoub *et al*, (2006) who found that foliar application of paclobutrazol significantly increased chlorophyll a and chlorophyll b in *Calendula officinalis* L. plant. Also Setia *et al*, (1995) noticed that leaves in paclobutrazol-treated *Brassica carinata* plants exhibited higher chlorophyll content, and they remained intact on plants for longer than the controls.

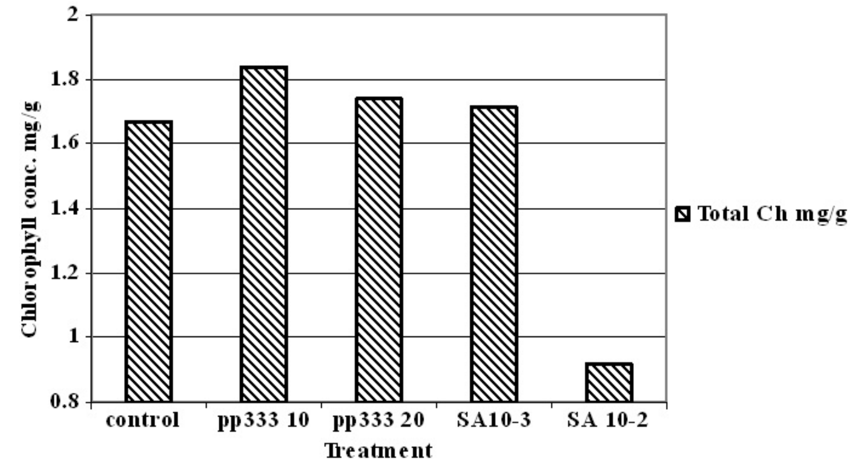

Fig. 2: Total chlorophyll concentration in leaves of bean plants as affected by pp333 and SA foliar application

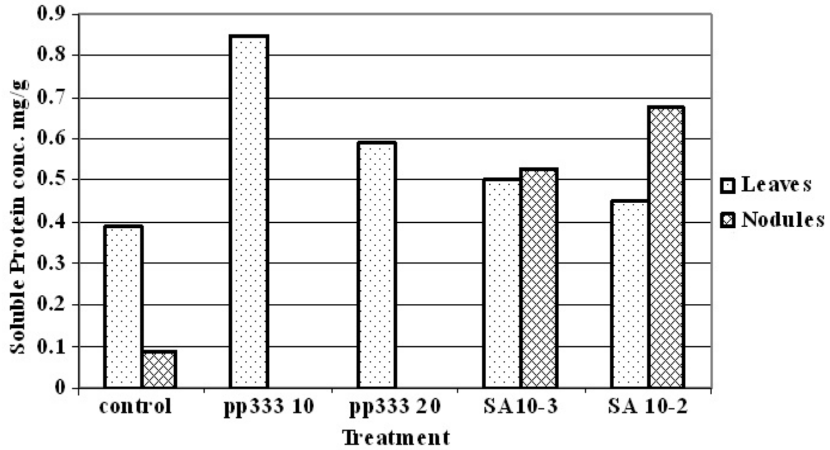

Fig. 3: Total soluble protein concentration in leaves and nodules of bean plants as affected by pp_{333} and SA foliar application

Regarding protein content as shown in Table, 3 and figure,3, the highest significant treatment for increasing total soluble protein content in leaves was recorded by pp_{333} at 10 ppm followed by pp_{333} at 20 ppm, SA at 10^{-3} M and SA at 10^{-2} M respectively.

In concern to nodule total soluble protein, SA at 10^{-2} M gave the highest value followed by SA at 10^{-3} M comparing with control. It is obvious that low salicylic acid concentration (10^{-3}M) favored high protein concentration in leaves than that in nodules and vice versa was true by high SA concentration (10^{-2}M).

Our results could be explained by the findings obtained by (Sankhla *et al*, 1985) who reported that paclobutrazol-treated soybean plants had increased soluble protein contents compared to controls. Also Setia *et al*, (1995) noticed that the seeds from paclobutrazol-treated *Brassica carinata* plants had higher levels of proteins.

Pod Yield:

Application of SA at 10^{-3} M was the best treatment for all yield parameters (pod number, pod fresh and dry weight) followed by pp_{333} at 10 ppm compared with other treatments (Table, 2). Same trends were obtained by Cleland and Ajami, (1974), that salicylic acid (SA) when applied to herbaceous plants affects many physiological processes such as induction of flowering.

The obtained results were in agreements with many investigators. Mahgoub *et al* (2006) reported that foliar application of paclobutrazol on *Calendula officinalis* L. plants significantly increased number of flowers per plant, flowers fresh and dry weight (g/plant) compared with control treatments.

In support of these results, significant influence of paclobutrazol in increasing number of flowers has been experimentally substantiated by de Baerdemaeker *et al* (1994) and Singh *et al* (1999). Also, Singh (2004) reported that Paclobutrazol at 10 ppm could be applied to enhance flowering and increase the yield of large seeds of pot marigold. However, pp_{333} at 20 ppm decreased the whole yield parameters compared with other treatments and control.

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