Physiological response of mung bean “Vigna radiata” plants to some bioregulators

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Summary
Pot experiments were conducted in the screen of the National Research Centre during two summer seasons 2006 and 2007 to study the physiological response of mung bean plants to some bioregulators (salicylic acid, glutathione or paclobutrazole). Vigna radiata plants were treated with different concentrations of salicylic acid (5, 10 and 15 mg/l), glutathione or paclobutrazole (PBZ) each at 50, 100 and 150 mg/l. The obtained results indicate that foliar application of glutathione or paclobutrazole each at a rate of 150 mg/l or salicylic acid at a rate of 15 mg/l significantly increased plant height, number of branches/plant, fresh and dry weights of leaves, fresh and dry weights of branches and yield (g/plant). On the other hand, an application of paclobutrazole at all the used treatments led to significant decrease in the plant height. Total carbohydrates and total proteins and mineral ions content of the produced seed were significantly increased as a result of foliar application of salicylic acid, glutathione or paclobutrazole at all the used levels. All the used treatments of bioregulators caused positive changes in the gels of protein electrophoresis and photosynthetic pigments of mung bean plants.

Introduction
Mung bean (Vigna radiata L. Wilczek) is a summer pulse crop with short duration (70-90 days) and high nutritive value. The seeds contain 22-28% protein, 60-65% carbohydrates, 1-1.5% fat, 3.5-4.5% fibers and 4.5-5.5% ash. They have many effective uses, green pods in cooking as peas, sprout rich in vitamins and amino acids. This crop can be used for both seeds and forage since it can produce a large amount of biomass and then recover after grazing to yield abundant seeds (MOHAMED and KARAMANY, 2005).

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. For example, it plays a role as natural inductor of thermogenesis in Arrum lilly, induces flowering in a range of plants, control ion uptake by roots and stomatal conductivity (RASKIN, 1992). There are experimental data indicating participation of SA in signal regulation of gene expression in the course of leaf senescence in Arabidopsis (MORRIS et al., 2000).

SARKHABUTDINOVA et al. (2003) reported that treatment of wheat plants with 0.05 mM salicylic acid increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Phytohormones are known to play a key role in plant growth regulation. It was found that salicylic acid treatment caused accumulation of both ABA and IAA in wheat seedlings. However, the SA treatment did not influence cytokinins content.

KAVEH et al. (2004) reported that very low dose (50 μM) of SA considerably enhanced the growth and carbohydrates metabolism of tea cuttings. Addition of 50 μM SA produced the most remarkable effects. There was a 2 fold significant increase in leaf area, leaf fresh weight and leaf dry weight. Leaf total soluble sugars (leaf TSS) was also doubled by this treatment. Invertase enzyme activity in SA treated cuttings was higher than in control with a significant increase for 50 μM SA. Optimal concentration of SA for leaf growth was 50 μM that caused a two fold significant increase in leaf area, fresh weight and dry weight. Salicylic acid application resulted in a significant increase in total soluble sugar content in leaves of camellia cuttings, thus maintaining the carbohydrate pool in the chloroplasts at a high level. This increase may be implicated in osmotic adjustment as it has been described in tomato SA-treated plants (TAHR et al., 2002).

The antioxidant thiol tripeptide glutathione (GSH) is regarded as one of the major determinates of cellular redox homeostasis KABB (2007) and homoglutathione (hGSH) are very abundant in legume root nodules. MORAN et al. (2000) emphasized the role of nodules plastids in antioxidant protection and in control of thiol synthesis, and suggest that plastids may be important in the stress response of nodules. Their results provide further evidence that thiol synthesis is critical for nodule functioning. YOUSSEFAN et al. (2001) reported that the biosynthesis of cysteine is regarded as the exclusive function of sulfur reduction in plants and a key limiting step in the production of glutathione (GSH), a thiol implicated in various cellular functions, including sulfur transport, gene expression, scavenging of reactive oxygen species (ROS), and resistance to biotic and abiotic stresses.

Glutathione plays a crucial role in controlling and maintaining the intracellular redox state NOCTOR and FOYER (1998). HELL and BERGMANN (1988, 1990) reported that little is known about the intracellular compartmentalization of GSH biosynthesis. They also reported that efficient regulation of the glutathione pool is thought to be particularly important in chloroplast metabolism, in which it provides the redox buffering capacity vital for efficient photosynthesis and is involved in processing the oxidizing species that are inevitably formed as a result of light capture and subsequent electron transport. In addition, MULLINEAUX and RAUSCH (2005) reported that glutathione plays an important role in the protection of the cells against oxidative stress. It is involved in the ascorbate/ glutathione cycle and the regulation of protein thiol-disulfide redox status of plants in response to abiotic and biotic stress.

Triazoles are a group of compounds that have been developed for use as either fungicides or plant growth regulators, although in varying degrees they possess both properties (FLETCHER et al., 1986).

Triazoles inhibit cytochrome P_150 mediated oxidative demethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenic acid in the gibberellin biosynthesis pathway (RADEMACHER, 1992); and it is likely that the former is responsible for fungicial properties whereas the latter conversion step is likely the primary basis for their plant growth regulatory properties. Triazoles can also protect plants from various environmental stress, including amoxia, air pollutants, drought, extreme temperatures, ultraviolet light and salinity (DAVIS et al., 1988; FLETCHER and HOFSTRA, 1988; BEKHATA et al., 2006; KANDIL and ELEWA, 2008).

Material and methods
Plant material and growth conditions
A pot experiment was carried out during two successive growth seasons of (2005/2006, 2006/2007) at the screen of National Research Centre, Dokki, Giza, Egypt. Seeds of Vigna radiata were
obtained from Field Crops Research Institute, Agricultural Research Centre, Ministry of Agriculture, A.R.E. The seeds were sown in clay pots, 30 cm in diameter, on February 21, 2006 and 2007, respectively. Each pot contained 8 kg loamy clay soil mixture. Fifteen days after sowing, the seedlings were thinned to the most three uniform plants in each pot and fertilized with two grams of potassium sulphate. Each pot received equal and adequate amounts of water. Phosphorous as calcium superphosphate was mixed with the soil before sowing at the rate of 2.0 g/pot. Three grams of nitrogen as ammonium sulphate in three applications (one g for each) with two weeks intervals started 30 days after planting, also, two grams of potassium sulphate were added as soil application. Other agricultural processes were performed according to normal practice. The plants were sprayed twice (after 30 and 45 days from sowing) with freshly prepared solutions of salicylic acid at a rate of 5, 10 or 15 mg/l or glutathione at a rate of 50, 100 or 150 mg/l or paclobutrazole at a rate of 50, 100 or 150 mg/l.

Treatments were distributed in complete randomized block design with five replications and each replicate consists of five pots.

**Sampling**

Three samples were taken during this investigation (each sample consists of five replicates) and divided as follows:

1st sample: was taken after two weeks from the second application of bioregulators and used for estimation of photosynthetic pigments.

2nd sample: was collected at flowering stage (i.e. after 85 days from sowing) and used for measuring and calculated vegetative growth parameters.

3rd sample “seed yield”: was taken at harvest time (120 days from planting). These samples were used for estimation of total carbohydrates, protein electrophoresis, total protein and mineral ion contents.

**Vegetative growth parameters**

In the second sample, plant height (cm), number of branches per plant, fresh and dry weights of leaves and stems (g/plant) were estimated. In the third sample, number of pods per plant, weight of pods (g/plant) and weight of seeds (g/plant) were measured. Plants were weighed and dried in an electric oven at 60°C till constant weight, ground to fine powder and stored in tubes with screw lids until needed.

**Chemical analysis**

**Photosynthetic pigments:**

An accurate weight (0.5 g) of fresh young leaves of mung bean were homogenized in 85% acetone and used for determination of photosynthetic pigments (chl. a, chl. b and carotenoids ) using spectrophotometric method developed by LORENZEN et al., 1965. The samples were measured at 663, 664 and 452.5 nm respectively.

**Total carbohydrates in mung bean seeds:**

These were determined in fine dry powder of the mung bean produced seeds by using the colorimetric method described by HERBERT et al., 1971.

**Gel electrophoresis of mung bean seeds:**

Some plant seeds were stored in deep freezer at -20°C until used for estimation of electrophoretic protein using continuous polyacrylamide gel electrophoresis in presence of sodium dodecyl sulfate (cont. SDS-Page) according to WEBER and OSBORN (1969).

**Total protein in mung bean seeds:**

Total nitrogen of mung bean produced seeds was extracted and determined by Micro-Kjeldahl method as described by A.O.A.C. (1984). The value of total crude protein was calculated by multiplying total values of total-N by factor 6.25.

**Seed mineral ions content:**

A known weight (0.5 g) of the dry ground plant seeds material was digested (using an acid mixture containing nitric acid: perchloric acid: sulfuric acid at the ratio of 8:1:1, respectively) according to the method described by CHAPMAN and PRATT (1976) extracted and the obtained extract was used for the estimation of mineral ions content. Calcium, potassium and sodium were determined photometrically by the flame photometer method as described by Brown and LILLIAN (1946) while magnesium and phosphorus were determined by atomic absorption spectrophotometer (using magnesium filter) according to CARPINA et al. (1990).

**Statistical analysis**

Data obtained (means of the two growing seasons) were subjected to standard analysis of variance procedure appropriate to the randomized complete block design applied in this study. The values of LSD were obtained whenever F values were significant at 5% level as reported by SNEDECOR and COCHRAN (1980).

**Results and discussion**

**Vegetative growth parameters**

Data illustrated in Fig. 1 indicate that foliar application of salicylic acid (SA) significantly increased plant height, number of branches, fresh and dry weights of leaves and branches, especially in plants treated with 20 mg/l SA.

GUTIÉRREZ-CORONADO et al. (1998) reported that spraying the shoots of soybean plants with salicylic acid significantly increased the growth of shoots and roots measured after seven days of treatment. Meanwhile, SARKHABUTIDINNOVA et al. (2003) reported that treatment of wheat plants with 0.05 mM SA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Phytohormones are known to play a key role in plant growth regulation. It was found that SA treatment caused accumulation of both ABA and IAA in wheat seedlings. However, SA treatment did not influence cytokinin content.

KAVEH et al. (2004) reported that application of SA at very low dose (50 μM) enhanced the growth and carbohydrate metabolism of tea cuttings. Addition of 50 μM SA produced the most remarkable effects. There was a 2 fold significant increase in leaf area, leaf fresh weight and leaf dry weight. Optimal concentration of SA for leaf growth was 50 μM that caused a two fold significant increase in leaf area, fresh weight and dry weight. TALAAT (2005) reported that foliar application of salicylic acid and/or tryptophan influenced the vegetative growth of geranium plants, especially at a rate of 100 μM/L. Data illustrated in Figs. 1 and 2 also show that foliar application of glutathione to mung bean plants significantly promoted growth parameters such as plant height, number of branches, fresh and dry weight of leaves, number and weight of pods and seeds/plant. The highest recorded values were obtained from the application of GSH at a rate of 150 mg/l.

These results are in agreement with those obtained by many investigators (HELL and BERGMANN, 1988, 1990; BERGMANN and RENNENBERG, 1993) all reported that little is known about the intracellular compartmentalization of GSH biosynthesis. They also reported that efficient regulation of the glutathione pool is
Fig. 1: Effect of salicylic acid, glutathione and paclobutrazole on growth characters of Vigna radiata L. plants (vertical bars represent LSD).
thought to be particularly important in chloroplast metabolism, in which it provides the redox buffering capacity vital for efficient photosynthesis and is involved in processing the oxidizing species that are inevitably formed as a result of light capture and subsequent electron transport. Rennanberg (1996) suggested that glutathione functions as a storage pool for excess cysteine and as the principal form in which organic sulfur is transported in many plants. Buwalda et al. (1990) also emphasized that the promotive effect of glutathione might be due to its effect as a reservoir of reduced sulfur as the amino acid cysteine which is a component of the antioxidant glutathione and is the principal form in which organic sulfur is transported in many plants. Noctor and Foyer (1998) reported that glutathione plays a crucial role in controlling and maintaining the intracellular redox state. In addition, Youssefian et al. (2001) and Chaparzadeh et al. (2004) reported that biosynthesis of cysteine is regarded as the exclusive function of sulfur reduction in plants, and a key limiting step in the production of glutathione (GSH), a thiol implicated in various cellular functions, including sulfur transport, gene expression, scavenging of reactive oxygen species (ROS), and resistance to biotic and abiotic stresses. In this connection, Talaat and Aziz (2005) reported that foliar application of 100 mg/l glutathione, 100 mg/l nicotinic acid or 200 mg/l ascorbic acid significantly increased plant height, number of branches/plant, fresh and dry weights of herb, fresh and dry weights of flower-heads yield (g/plant).

Data illustrated in (Figs. 1 and 2) indicate also that foliar application of paclobutrazole to mung bean plants at all the used concentrations caused significantly decreased in plant height. On the other hand, application of the same treatments increased the number of branches, number of leaves and number of pods per plant, especially in plants treated with 150 mg/l PBZ. Similar results were obtained for fresh and dry weights of leaves and branches. Weight of pods and seeds followed the same trend. The results recorded in this work are in agreement with those obtained by many investigators using different triazole compounds at various concentrations (Fletcher and Hofstra, 1988; Batts et al., 1991; Bekhteta, 1992; Hathout, 1995; Wu et al., 1996; El-Kady, 2002). In addition, Bekhteta, et al. (2006) and Kandil and Elewa, (2008) reported that using uniconazole at different levels on various plants caused significant increase in the number of branches or tillers/plant, respectively.

**Chemical constituents**

Data illustrated in Fig. 3 indicate that foliar application of salicylic acid to mung bean plants significantly increased chlorophyll a and chlorophyll b contents in the leaves, especially in plants treated with the concentration of 150 mg/l. Total carotenoids followed the same trend. Data in the same figure also indicate that salicylic acid
treatments promoted total carbohydrates and total protein contents in the produced seeds of mung bean plants.

Obtained data show that application of salicylic acid at all the used treatments caused considerable increases in total carbohydrates and total proteins of the produced seeds as compared with the amounts obtained from the seeds produced from the control plants. In this connection, Talaat (2005) reported that application of SA at the same treatment significantly increased total sugar percentage, total protein percentage, essential oil percentage and essential oil yield of Pelargonium graveolens.

It is also evident that mineral ions contents of Ca, P, K, Na, Mg followed the same trend (Fig. 4). The highest values for all the studied
minerals were obtained from the application of paclobutrazole at 150 mg/l as compared with other treatments. In this concern, Raskin (1992) reported that salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes of plants. For example it plays a role as natural inductor of thermogenesis in *Arun* lily, induces flowering in a range of plants and controls ion uptake by roots and stomatal conductivity. There are experimental data indicating that SA
participation in signal regulation of gene expression in the course of leaf senescence in Arabidopsis (MORRIS et al., 2000). Poliar application of glutathione to mung bean plants significantly increased photosynthetic pigments (Fig. 3). Data illustrated in the same figure indicate that spraying mung bean plants with different concentrations of glutathione at the rate of 50, 100 or 150 mg/l significantly increased total carbohydrates and total protein contents of the produced seeds. Meanwhile, mineral ion contents (P, K, Mg, Ca and Na) were also increased as a result of glutathione treatments, especially at the concentration equal 150 mg/l (Fig. 4).

These results are in agreement with those obtained by TALAA and AZZ (2005) who reported that total sugar percentage and total nitrogen percentage of *Matricaria chamomilla* L. plants were significantly increased as a result of foliar spray of glutathione. In this concern BUWALDA et al. (1996) and NOCTOR et al. (1997) all reported that the amino acid cysteine is a component of the antioxidant glutathione. The influence of cysteine availability on glutathione levels reflects the importance of glutathione as a reservoir of reduced sulphur (BUWALDA et al., 1990). Recently, KATTAB (2007) found that foliar application of glutathione on canola plants grown under salt stress increased photosynthetic pigments content especially carotenoids which play a role to protect the photosystems by reacting with lipid peroxidation products.

Data illustrated in Fig. 4 also show that application of paclobutrazole at all used levels led to significant increase in the mineral ion (P, K, Mg, Ca and Na) content of the produced *Vicia faba* seeds. These results are in agreement with that obtained by BEKHETA (2000) and KANEHL and ELEWA (2008) who used growth retardant uniconazole on *Vicia faba* and *Aswan majus* plants, respectively. Data illustrated in Fig. 2 show that application of paclobutrazole at the rate of 150 mg/l led to noticeable increase in the content of photosynthetic pigments, total carbohydrates and total proteins as compared to the untreated plants. Meanwhile, mineral ions contents were also increased as a result of paclobutrazole treatments, especially at 150 mg/l (Fig. 4).

In this concern, many investigators reported that Paclobutrazole caused positive changes in the amounts of naturally occurring substances, e.g., carbohydrates, proteins, amino acids and mineral ion content (BEKHETA, 2004; GOMATHINAYAGAM et al., 2007; BORA et al., 2007).

**Protein electrophoresis:**
Tab. 1 reveals the changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the newly formed leaves of mung bean plants treated with different concentrations of SA, GSH or PBZ. The molecular weights of the proteins ranged between 8.1-2460 kDa and exhibited a maximum number of 21 bands. The scanning profile of such detected protein bands revealed that the band number 20 having the molecular weight of 8.3 kDa produced the highest intensity of protein which recorded 42.1% in plants treated with 100 mg/l GSH.

### Tab. 1: Comparative analysis of relative area (%) of each band of the coomassie blue-stained gels of mung bean plants treated with different concentrations of salicylic acid (SA), glutathione (GSH) and paclobutrazole (PBZ).

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<th>Control</th>
<th>SA (5mg/l)</th>
<th>SA (10 mg/l)</th>
<th>SA (15 mg/l)</th>
<th>GSH (50 mg/l)</th>
<th>GSH (100 mg/l)</th>
<th>GSH (150 mg/l)</th>
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Treatment of mung bean plants with 5 mg/l salicylic acid led to the appearance of 14 protein bands ranging between 8.1-2460 kDa. Comparing with the features of protein banding pattern obtained from the control plants, it is evident that such treatment induced the appearance of 5 newly protein bands having the molecular weights of 2460, 1810, 841, 562, 375, respectively.

The electrophoretic pattern of the plants treated with 10 mg/l SA showed the presence of 11 protein bands ranging between 2460-22.4 kDa. It is evident that treatment with 10 mg/l SA induced the appearance of 4 new protein bands. The former newly protein bands having molecular weights of 2460, 1260, 841 and 562 kDa. This was accompanied by the disappearance of two bands of those detected in the control plants. These two bands have the molecular weights of 16.4 and 15.0 kDa.

Application of salicylic acid at 15 mg/l showed the existence of 12 protein bands with the molecular weights ranging between 1810-8.1 kDa as compared to the protein bands obtained from the control plants. The data show that this treatment induced the appearance of 3 new protein bands having the molecular weights of 1810, 1010 and 562 kDa.

The electrophoretic banding pattern of proteins resulted from the application of glutathione at 50 mg/l on mung bean plants showed the appearance of 10 protein bands with molecular weights ranging from 1810 to 8.1 kDa. Three new bands were shown to be induced as a result of this treatment having molecular weights of 1810, 1260 and 667 kDa. Meanwhile, two protein bands disappeared having molecular weights of 16.4 and 15.0 kDa.

One new protein band having the molecular weight of 8.7 kDa was induced as a result of treating plants with 100 mg/l GSH. On the other hand, three bands disappeared as a result of this treatment. These protein bands have the molecular weights of 16.4, 15.0 and 8.1 kDa.

Treatments of mung bean plants with 150 mg/l GSH resulted in the induction of two new protein bands having molecular weights of 667 and 375 kDa. This treatment also resulted in the disappearance of two protein bands of molecular weights of 16.4 and 15.0 kDa.

Treatment of mung bean plants with 50 mg/l PBZ led to the appearance of 14 protein bands ranging between 8.1-1810 kDa and induced the appearance of 5 newly protein bands having the molecular weights of 1810, 1010, 562, 422, 375 kDa, respectively.

Treatment of mung bean plants with 100 mg/l PBZ resulted in the appearance of 12 protein bands. Five of these bands are new and having the molecular weights of 1810, 1010, 562, 17.4 and 8.7 kDa.

The treatment led to the disappearance of two protein bands having the molecular weights of 9.9 and 8.1 kDa.

Treatment of mung bean plants with 150 mg/l PBZ led to the appearance of 13 protein bands. Six of these bands are new and having the molecular weights of 1810, 1260, 667, 562, 18.5 and 17.4. This treatment led also to the disappearance of two protein bands having molecular weights of 16.400 and 8.1 kDa.

The outcome of the obtained results clearly indicate that spraying mung bean plants with different concentrations of SA, GSH or PBZ led to the appearance of new protein bands which varied according to the applied concentration. The existence of such newly formed protein bands in treated mung bean plants might be explained basing on the potentiality of SA, GSH or PBZ to trigger the expression of specific genes along DNA molecule in the target cells, a process which appears to play a key role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns and yield of the produced plants. This accompanied by a persistent effect carrying over to the progeny via alteration of DNA-binding protein receptors mechanism which might amplify the signal-transduction pathway, this suggestion is reinforced by the findings of Jacobsen and Beach (1985) and Abdel-Hamid (2002). Paclobutrazole (PBZ) is a triazole compound of antigibberellin nature and is involved in reducing abscisic acid, ethylene and indole-3-acetic acid while increasing cytokinin levels. Though each plant hormone evokes many different specific biochemical, physiological and/or morphological responses (Chaney, 2004). Recently, many investigators reported that paclobutrazole caused changes in the amounts of naturally occurring substances, e.g., carbohydrates, proteins and amino acids (Bekheta, 2004; Gomathinayagam et al., 2007; Bora et al., 2007).

In this respect, Raskin (1992) reported that salicylic acid plays a role as natural inducer of thermogenesis in Arabidopsis, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity. There are experimental data indicating participation of SA in signal regulation of gene expression in the course of leaf senescence in Arabidopsis (Morris et al., 2000).

Talaat (2005) reported that treatment of Petargonium graveolens plants with glutathione significantly increased protein content. Talaat and Aziz (2005) also reported that total nitrogen percentage of Matricaria chamomilla L. plants were significantly increased as a result of foliar spray of glutathione.

Bekheta (2004) showed that application of PBZ combined with gibberellic acid (GA3) on wheat plants changed the electrophoretic profile of protein patterns.

In conclusion, by comparing the effects of foliar treatments of Vigna radiata plants with salicylic acid, glutathione or paclobutrazole it is apparent from the previous results that foliar treatments had beneficial influences for improving plant growth, seed yield quality and quantity.

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