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Amelioration of Chilling Injuries in Mung Bean (*Vigna radiata* L.) Seedlings by Paclobutrazol, Abscisic Acid and Hydrogen Peroxide

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Abstract: The present study was carried out to study the effect of chilling stress on some metabolic products and antioxidative system in mung bean (*Vigna radiata* L.) plant and try to alleviate chilling injuries by using paclobutrazol, abscisic acid and H_2O_2. Twenty-day-old seedlings were randomly separated into equal three groups, the first group was left in green house at 35°C, the second one was chilled at 5°C for 5 or 10 h and the last one was treated with 0.0, 25 and 50 mg L^{-1} paclobutrazol, 0.5 and 1 mM abscisic acid or 0.0, 0.1 and 0.5 mM H_2O_2 before transforming to 5°C. The results showed that, chilling stress induced significant increased lipid peroxidation, membrane leakage and hydrogen peroxide level, while activities of catalase, peroxidase and ascorbate peroxidase were decreased. In addition, total chlorophyll, total carbohydrates, protein content and proline level decreased following exposure to 5°C. Paclobutrazol, abscisic acid and H_2O_2 treatments ameliorated the chilling injuries by lowering lipid peroxidation, membrane leakage and H_2O_2 level and increasing in total chlorophyll, carbohydrates, protein content, proline level and antioxidant enzymes activities. Based on these results, it was presumed that the stress protection caused by paclobutrazol, abscisic acid and H_2O_2 probably contributes to some extent to the enhanced activity of the free-radical scavenging systems.

Key words: Chilling stress, paclobutrazol, abscisic acid, H_2O_2, antioxidant enzymes, mung bean (*Vigna radiata* L.) plant

INTRODUCTION

Cultivated plants are often subjected to different types of environmental stress during their growth in the field, which could result in reduction of their yield. Low temperature stress (chilling) induces considerable changes in biochemistry and physiology of plants (Katterman, 1990). Exposure of watermelon seedlings to low temperatures retards growth, delays flowering, reduces total yields and quality and even kills the plants (Korkmaz and Dufault, 2001).

Electrolyte leakage indicator as chilling injuries increased in *Stylosanthes guianesis* under chilling conditions due to damage of the membrane system (Zhou *et al*., 2005). In addition, prolonged exposure to low temperatures increased the leakage of solutes in mung bean seedlings, such as soluble sugars and free amino acids (Chang *et al*., 2001). Chilling-enhanced lipid peroxidation, as indexed by malondialdehyde (MDA) content, in maize cells (Chen and Li, 2002) and wheat seedlings (Berova *et al*., 2002). These deteriorative symptoms in wheat seedlings were ameliorated by the paclobutrazol (PBZ) treatment (Berova *et al*., 2002) due to enhancing the activity of the free-radical scavenging systems.

Chilling induces oxidative stress (Prasad *et al*., 1994) during which Reactive Oxygen Species (ROS), including hydrogen peroxide (H_2O_2), are accumulated in concentrations higher than necessary for normal metabolism. High levels of H_2O_2 could promote lipid peroxidation in the membranes (Senaratna *et al*., 1987) in the absence of increased antioxidant mechanism. The H_2O_2 excess can be removed by catalase and the ascorbate-glutathione pathway (Apel and Hirt, 2004). The higher

activities of defense enzymes and higher content of antioxidant under stress were associated with tolerance to chilling (Huang and Guo, 2005). Activities of catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase were decreased in chilling sensitive cultivar of rice (Huang and Guo, 2005), cucumber (Heelee and Bumlee, 2000; Saropulos and Drennan, 2002) and *Stylosanthes guianesis* (Zhou *et al*., 2005) under chilling stress.

Many plant species accumulate free proline in response to salinity, drought and cold (Delauney and Verma, 1993; Hare and Cress, 1997; Yoshiba *et al*., 1997). Positive correlations between the accumulation of endogenous proline and improved cold tolerance have been found in maize (Zhou *et al*., 2002), rye (Koster and Lynch, 1992), wheat (Dörffling *et al*., 1997), grapevine (Ait-Barka and Audran, 1997), potato (Swaaij *et al*., 1986) and *Arabidopsis thaliana* (Nanjo *et al*., 1999). Proline has been suggested to play multiple roles in plant stress tolerance. It acts as a mediator of osmotic adjustment (Yoshiba *et al*., 1997), a stabilizer of proteins and membranes (Rudolph *et al*., 1986), an inducer of osmotic stress-related genes (Iyer and Caplan, 1998) and a scavenger of Reactive Oxygen Species (ROS) (Saradhi *et al*., 1995).

In general, chilling injury is characterized by reduction of chlorophyll levels in wheat plant (Berova *et al*., 2002) and inhibition of the photosynthetic process (Hodgson and Raison, 1989). Maize leaves grown at low temperature are characterized by a very low photosynthetic performance (Haldimann *et al*., 1996), changes in the content and the composition of the pigments (Haldimann *et al*., 1996; Haldimann, 1998), changes in the activities of several enzymes of photosynthetic carbon assimilation (Stamp, 1987) and reduced activities of photosystem I and photosystem II (Robertson *et al*., 1993).

Paclobutrazol, which is a member of the triazole family of plant growth regulators, has been found to protect severa1 crops from various environmental stresses, including drought, chilling, heat and UV-B radiation (280-320 nm) (Davis and Curry, 1991; Lurie *et al*., 1994; Pinhero and Fletcher, 1994; Kraus *et al*., 1995; Paliyath and Fletcher, 1995). Even though paclobutrazol-induced stress tolerance is reported to be due to increased antioxidant enzymes (Paliyath and Fletcher, 1995; Kraus *et al*., 1995), increased levels of proline (Mackay *et al*., 1990) and chlorophyll content (Berova *et al*., 2002). Foliar applications of uniconazole reduced electrolyte leakage and malondialdehyde accumulation caused by freezing stress (Berova *et al*., 2002). Enhanced chilling tolerance in triazole-treated cucumber (Upadhyaya *et al*., 1989) and tomato (Senaratna *et al*., 1988) was associated with increased antioxidant enzyme concentrations. Triazole-induced tolerance to low temperature stress has been associated with increased levels of endogenous abscisic acid (Fletcher *et al*., 2000), which has increased free proline (Chen and Li, 2002) and scavenging systems (Fath *et al*., 2001; Zhou *et al*., 2002).

High levels of endogenous abscisic acid (ABA) are related to increase chilling tolerance and an increase in ABA content before low temperature exposure might be an essential step in activating a protection mechanism against chilling (Zhang *et al*., 1986). ABA-decreased chilling injuries in tomatoes (King *et al*., 1982), cucumbers (Yamazaki *et al*., 1995) and *Stylosanthes guianesis* (Zhou *et al*., 2002) is partially associated with enhanced scavenging systems and increases in endogenous free proline content in rice (Chou *et al*., 1991), maize (Chen and Li, 2002) and cucumber plants (Flores *et al*., 1988). However, ABA-treated maize cells displayed less lipid peroxidation and accumulated proline in response to chilling and enough to improve chilling tolerance (Chen and Li, 2002).

H_2O_2 can play dual roles in plants exposed to chilling stress, inducing not only oxidative damage, but also acting as a signal for induction of defenses, resulting in chilling tolerance (Prasad *et al*., 1994). Exogenously supplied of H_2O_2 induced chilling tolerance in maize plant exposed to 4°C (Prasad *et al*., 1994) and mung bean seedlings (Chih-Wen *et al*., 2003) by increasing antioxidant enzymes such as catalase and peroxidase and lowering electrolyte leakage.

Thus, the objective of this research was to carried out to examine the effect of low temperature on some metabolic activities and antioxidant enzymes of mung bean (*Vigna radiata* L.) plant and trying to alleviate the injuries caused by low temperature by using paclobutrazol (PBZ), Abscisic acid (ABA) and hydrogen peroxide (H_2O_2).

MATERIALS AND METHODS

Plant Material and Growth Conditions

Mung bean (*Vigna radiata* L.) seeds were obtained from the Agricultural Research Center (ARC), Giza, Egypt. Seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed with distilled water. Seeds were immersed in running water overnight and then grown in plastic pots (25 cm in height and 20 cm in diameter) which equally filled with beat-moss. All pots were kept in a green house under natural photoperiod and a temperatures regime of 35/22°C (day/night) and irrigated regularly every two days until chilling treatment. Thinning was carried out after 10 days from germination so that six seedlings of symmetrical growth rates were left per pot. This study was carried out at Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

Paclobutrazol, Abscisic Acid or Hydrogen Peroxide Treatments

Twenty-day-old, seedlings were used for chilled treatments are separated into three groups. The first group was left in green house at 35°C (negative control). Seedlings of the second group were transferred to 5°C and 45 μmol m^{-2} sec^{-1} in an light incubator for various lengths of time (5 and 10 h) (positive control). The root portions of the whole seedlings of the third group were immersed in 0.0, 25 and 50 mg L^{-1} paclobutrazol or 0.5 and 1 mM ABA for 24 h or in 0.0, 0.1 and 0.5 mM H_2O_2 for 4 h at 27°C in darkness. The treated seedlings were washed with distilled water, transplanted in peat moss and further incubated at 27°C for an additional 12 h before transforming to 5°C (5 and 10 h). At the end of the chilling period, the treated plants were returned to the pre-experimental conditions in the greenhouse, where they have recovered for 5 days. Treatments were replicated using 4 pots. 25-day-old plants were harvested, weighed, frozen and stored in deep-freezer for further analysis.

Measurement of Chilling Injury

The conductivity of electrolyte leakage in the cell sap from leaf discs was used as a measure parameter of chilling injury (Sukumaran and Weiser, 1972).

Determination of Malondialdehyde Content

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was assayed spectrophotometrically using TBA-MDA assay (Minotti and Aust, 1987).

Assay of H_2O_2

H_2O_2 concentration in the incubation medium of treated leaf explants was measured by the FOX I method (Jiang *et al*., 1990; Wolf, 1994) based on the peroxide-mediated oxidation of Fe^{2+} with xylenol orange.

Antioxidant Enzymes

Enzymes extracts were prepared in 20 mL chilled extraction buffer (pH 7.5). Extracts were then centrifuged at 6000 rpm for 20 min at 5°C. Enzyme assays were conducted immediately following extraction.

Determination of Catalase Activity

Catalase activity was assayed in a method following Aebi (1983). Activity was determined by following the decomposition of H_2O_2 at 240 nm.

Determination of Peroxidase Activity

Peroxidase activity was determined by following the dehydrogenation of guaicol at 436 nm (Malik and Singh, 1980).

Determination of Ascorbate Peroxidase (ASPX) Activity

ASPX activity was determined using the method of Nakano and Asada (1987). Activity was determined by following the H_2O_2 dependent decomposition of ascorbate at 290 nm.

Estimation of Proline

Proline was assayed colorimetrically using the method of Bates *et al*. (1973) at wave length 520 nm.

Determination of Total Protein

The colorimetric method for the determination of protein according to Lawry *et al*. (1951) at wave length of 500 nm was used.

Estimation of Chlorophyll Content

Total chlorophylls were estimated in the fresh plant leaves according to the procedure of Lichtenthaler (1987).

Determination of Total Carbohydrates

The total available carbohydrate content were extracted according to Smith *et al*. (1964) and estimated colorimetrically by the Phenol-Sulphuric Acid Method as described by Dubois *et al*. (1951).

Statistical Analyses

Analysis of variance (ANOVA) and student t-test was performed on all data using SPSS program (Version 11.0).

RESULTS

Electrolyte Leakage

Chilling stress induced a significant increase in electrolyte leakage percentage of mung bean seedlings when compared to the negative control (Fig. 1). It was also found that increasing time of chilling stress increased electrolyte leakage. In addition, electrolyte leakage of paclobutrazol, abscisic acid or hydrogen peroxide-treated plants, which chilled for 5 h, decreased to the negative control level after the plants were recovered for 5 days.

Lipid Peroxidation (LP) Level

Lipid peroxidation, expressed as MDA levels, in mung bean seedlings was significantly increased with increasing chilling time. The percentage of increasing was 88.83 and 131.7% in the seedlings exposed to 5°C for 5 or 10 h, respectively, relative to the negative control (Fig. 2). Lipid peroxidation level was significantly decreased after treating the mung bean seedlings with paclobutrazol, abscisic acid or hydrogen peroxide. The decrease was more pronounced at 1 mM ABA and 0.5 mM H_2O_2 in the seedlings exposed to 5°C for 5 h.

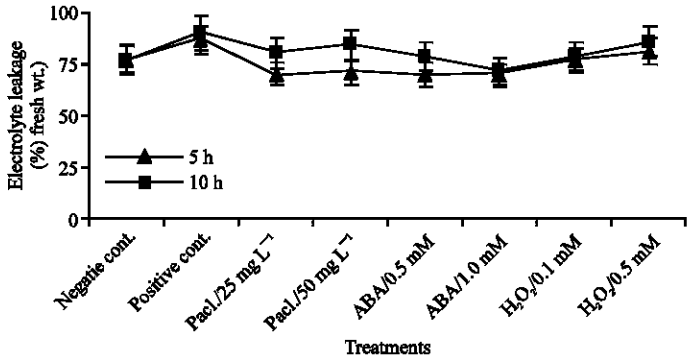

Fig. 1: Electrolyte leakage expressed as (%) fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} Pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

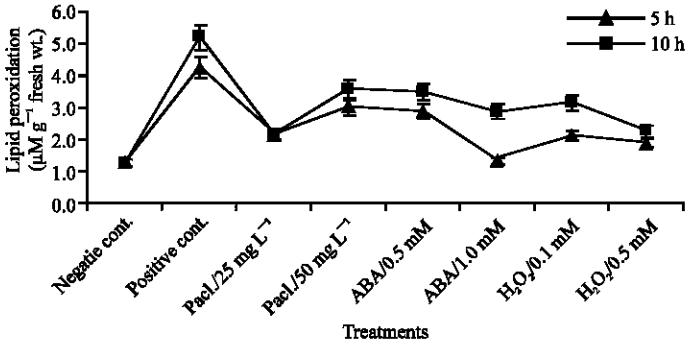

Fig. 2: Lipid peroxidation as malondialdehyde content (MDA) expressed as μmole g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

H_2O_2 Level

It was noticed that chilling stress at 5°C induced a highly significant increase in H_2O_2 content comparing with unchilled plants as shown in Fig. 3. In addition, increasing time of chilling stress increased H_2O_2 content when compared to the negative control. The percentage of increasing was 86.76 and 237.86% in the seedlings exposed to 5°C for 5 or 10 h respectively, compared to the negative control. Pretreatment with paclobutrazol, abscisic acid and H_2O_2 lowered H_2O_2 level of mung bean seedlings. The highest reduction in H_2O_2 level was observed at 25 mg L^{-1} paclobutrazol (70.1%) and 1.0 mM ABA (66.85%) in seedlings chilled for 5 h, in comparison with the positive control.

Catalase Activity

The results in Fig. 4 revealed that low temperature stress induced a significant decrease in catalase activity when compared to the negative control. This reduction increased with increasing time of stress.

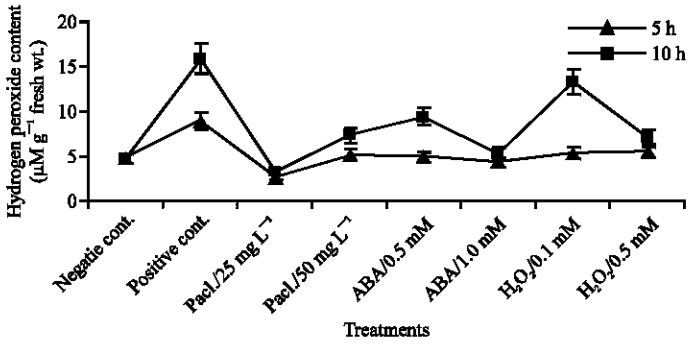

Fig. 3: H_2O_2 content expressed as μM g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5, 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

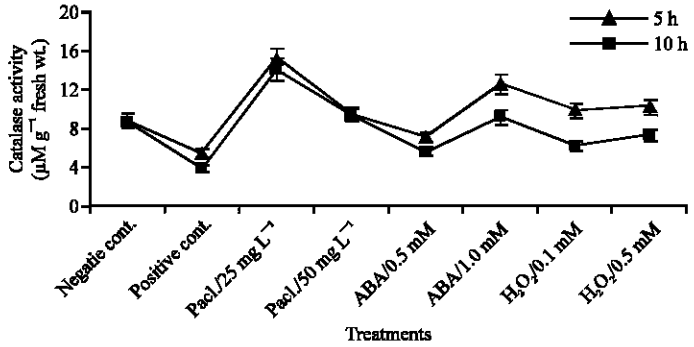

Fig. 4: Catalase activity expressed as unit g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

Paclobutrazol, abscisic acid or hydrogen peroxide treated seedlings showed an enhancement catalase activity at all concentrations used in both seedlings stressed for 5 or 10 h over that of the positive control. The highest increasing in catalase activity was observed at 25 mg L^{-1} paclobutrazol (70.90 and 178.88%), 1.0 mM ABA (43.99 and 134.95%) and 0.5 mM H_2O_2 (15.58 and 88.60%) in seedlings chilled for 5 h, in comparison with the negative and the positive control, respectively.

Peroxidase Activity

After exposure of mung bean seedlings to chilling stress, the peroxidase activity decreased as shown in Fig. 5. In addition, prolonged exposure to chilling stress decreased peroxidase activity. Treatment with different concentrations of paclobutrazol, abscisic acid or hydrogen peroxide significantly increased peroxidase activity over that of the negative and positive control. Paclobutrazol and abscisic acid, however, introduced the highest peroxidase activity.

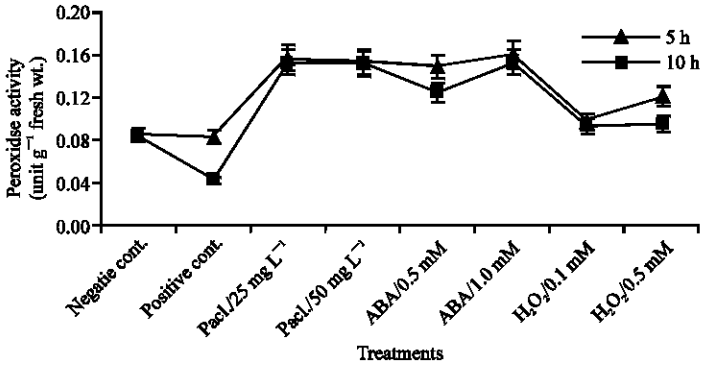

Fig. 5: Peroxidase activity expressed as unit g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

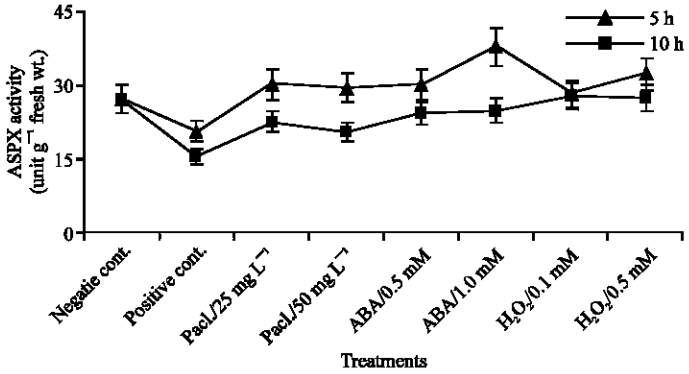

Fig. 6: ASPX activity expressed as unit g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

Ascorbate Peroxidase (ASPX) Activity

The ASPX activity was determined in leaves of mung bean seedlings chilled at 5°C for 5 or 10 h as shown in Fig. 6. The data showed that chilling stress induced a significant decline in ASPX activity when compared to the negative control. Treating of mung bean seedlings with paclobutrazol, abscisic acid or hydrogen peroxide significantly increased ASPX activity. Paclobutrazol and abscisic acid, however, introduced the highest ASPX activity in seedlings chilled for 5 h, in comparison with the positive control.

Proline Content

Control mung bean seedlings had low proline content after chilling for 5 or 10 h at 5°C in comparison with unchilled control (Fig. 7). Under chilling conditions, paclobutrazol, abscisic acid and H_2O_2-treated plants had higher proline content than positive and negative control but abscisic acid introduced the highest proline content.

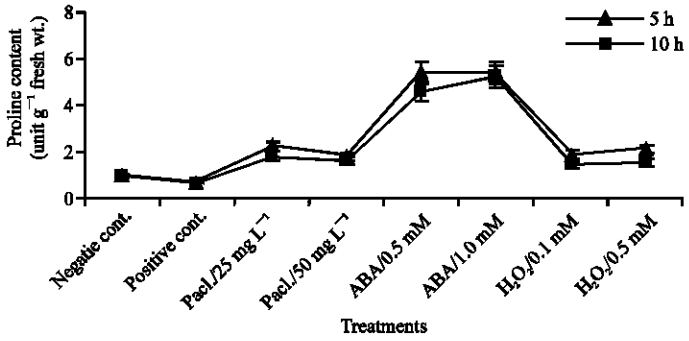

Fig. 7: Proline content expressed as μM g^{-1} fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

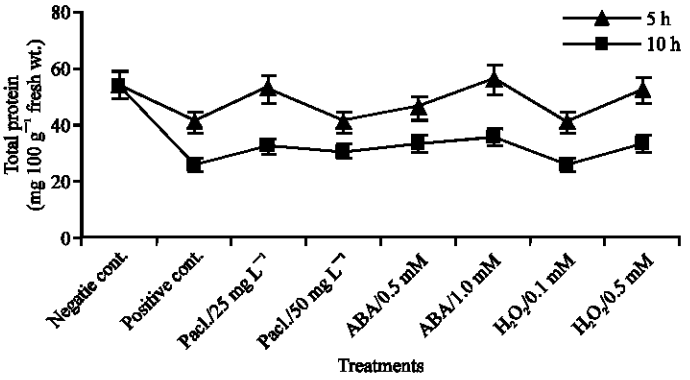

Fig. 8: Total protein expressed as mg/100 g fresh wt. in the leaf extract of mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

Total Protein

The results of Fig. 8 show that chilling stress induced a significant decrease in total protein content when compared to the negative control. This reduction increased with increasing time of stress. The percentage reduction being 24.38 and 52.59% in the plants chilled at 5 and 10 h, respectively, compared to the negative control. Paclobutrazol, abscisic acid and H_2O_2 treatments enhanced protein contents over that of positive control, but still lower than the negative control.

Total Chlorophyll

The data revealed that low temperature stress for 5 and 10 h induced a significant decrease in total chlorophyll content in mung bean seedlings when compared to negative control (Table 1). Treating mung bean seedlings with paclobutrazol, abscisic acid and H_2O_2 induced a significant increase in total chlorophyll content when compared to the positive control level but still decreased than the negative control.

Table 1: Effect of chilling time on total chlorophyll and carbohydrate contents of mung bean

	Chilling time (h)			
	5 h		10 h	
Treatments	Total chlorophyll Mean±SE	Total carbohydrates Mean±SE	Total chlorophyll Mean±SE	Total carbohydrates Mean±SE
Negative control	12.60±0.462	28.42±0.142	12.60±0.462	28.42±0.142
Positive control	9.19±0.115	19.11±0.306	7.20±0.058	14.86±0.326
Paclobutrazol treatment (mg L^{-1})				
25	11.89±0.064	25.46±0.184	9.57±0.133	22.45±0.223
50	11.29±0.121	22.50±0.289	9.16±0.092	22.22±0.459
F-ratio	5.559	191.194	44.223	131.805
p-value	0.043	0.000	0.000	0.000
Abscisic acid treatment (mM)				
0.5	10.33±0.173	19.13±0.105	9.48±0.069	15.47±0.184
1.0	10.86±0.081	19.84±0.171	9.86±0.115	19.71±0.519
F-ratio	16.932	1325.093	37.557	404.450
p-value	0.003	0.000	0.000	0.000
Hydrogen peroxide treatment (mM)				
0.1	11.26±0.081	25.88±0.090	9.14±0.092	22.41±0.168
0.5	11.09±0.052	23.97±0.248	9.06±0.035	18.30±0.179
F-ratio	9.221	167.00	54.938	967.00
p-value	0.015	0.00	0.000	0.00

Total chlorophyll content expressed as μg g^{-1} dry weight and total carbohydrates expressed as mg glucose/100 g dry wt. in mung bean seedlings pretreated with 0.0, 25 and 50 mg L^{-1} pacloputrazol, 0.0, 0.5 and 1 mM ABA or 0.0, 0.1 and 0.5 mM H_2O_2. The seedlings then subjected to chilling stress at 5°C for 5 or 10 h. Then, the treated plants were transferred to the pre-experimental conditions in the green house, where they have recovered for 5 days. Data represented are mean of 3 replica±SE

Total Carbohydrates

The data in Table 1 showed that increasing time of chilling led to decreasing in total carbohydrates when compared to the negative control. Increasing in total carbohydrates content after the chilling stress was higher in paclobutrazol, abscisic acid and $H_2O_2^-$ treated seedlings in comparison with the positive control, but still lower than the negative control. The increase was much more pronounced at paclobutrazol and H_2O_2 treatments.

DISCUSSION

When the plants are exposed to unfavorable conditions, Activated Oxygen Species (AOS) can react with pigments, membranes, enzymes and nucleic acids, thereby modifying their functions (Sun and Leopold, 1995). Autocatalytic peroxidation of membrane lipids can be triggered by AOS, resulting in loss of membrane semipermeability (Basaga, 1989), one of the primary mechanisms of stress injury. Electrolyte leakage and lipid peroxidation level in mung bean plant increased under chilling conditions (Fig. 1, 2). This result is in agreement with Zhou *et al*. (2002), who reported the membrane system of *Stylosanthes guianensis* was damaged under chilling stress due to the induction of oxidative damage is related to the imbalance of ROS production (Bowler *et al*., 1992). ROS are highly reactive and have the potential to damage membrane lipids, proteins, chlorophyll and nucleic acids, thus disrupting the homeostasis of the organism (Scandalios, 1993). Lyons (1973) proposed that membranes of the plant under low temperature become less fluid, their protein components can no longer function normally, causing water and soluble materials to leak out into the intercellular spaces. Paclobutrazol, abscisic acid or H_2O_2 treatments decreased membrane leakage and lipid peroxidation in mung bean plant (Fig. 1, 2). This result is in agreement with Berova *et al*. (2002) and Chen and Li (2002), they found that the lipid peroxidation content in paclobutrazol-treated wheat and maize seedlings was reduced. The ability of ABA-treated plants to protect cellular membranes during chilling

may be attributed, in part, to higher activities of reactive oxygen scavenging enzymes such as catalase, ascorbate peroxidase and superoxide dismutase, which are known to be up-regulated by ABA (Fath *et al*., 2001). The data of antioxidant enzymes support this suggestion as showed in Fig. 4-6.

One of the reactive oxygen species that accumulates in plant tissues during cold stress is hydrogen peroxide (H_2O_2) (Kang *et al*., 2003). The results of the present study revealed that chilling stress induced a significant increase in hydrogen peroxide (H_2O_2) level of mung bean seedlings (Fig. 3). These data are in according of these obtained by Aroca *et al*. (2005) who stated that Chilling can cause H_2O_2 accumulation in *Zea mays* plant could be attribute to decrease activity of key antioxidant enzymes (catalase and superoxide dismutase) activity (Scandalios *et al*., 1997; Polle, 1997). The results of the present study showed that, H_2O_2 decreased after treatment of mung bean seedlings with paclobutrazol, abscisic acid or H_2O_2 (Fig. 3). This result is in agreement with Parasad *et al*. (1994), who reported that maize seedlings pretreated with H_2O_2 or ABA and exposed to 4°C induced chilling tolerance could attribute to increase the antioxidant enzymes. Paclobutrazol, H_2O_2 and ABA treatments protect wheat from oxidative stress by enhancing antioxidant enzyme activity (Kraus *et al*., 1995) or other factors involved in membrane stability (Prasad *et al*., 1994).

Higher plants possess several enzymatic and non-enzymatic scavenging systems to minimize deleterious effects of Reactive Oxygen Species (ROS) including H_2O_2. Hydrogen peroxide is scavenged by catalases, peroxidase and ascorbate peroxidase (Kim *et al*., 2005). The results of the present work showed that chilling stress induced a significant decrease in catalase, peroxidase and ascorbate peroxidase activities in mung bean seedlings (Fig. 4-6). A large body of evidence has shown that the antioxidant enzyme systems are altered under abiotic stresses, including chilling (Kim *et al*., 2005). The chilling treatment decreased significantly both the catalase and the ascorbate peroxidase activities in rice (Huang and Guo, 2005) and *Stylosanthes guianesis* (Zhou *et al*., 2002). Mung bean seedlings treated with paclobutrazol, abscisic acid or H_2O_2 had higher activity of catalase, peroxidase and ascorbate peroxidase (Fig. 4-6). Yong *et al*. (2003) suggests a higher CAT activity in rice plant more efficient scavenging of H_2O_2, which would result in better protection against this toxic molecule during chilling and thus protect rice leaves from chilling injury. ABA pretreated increased activities of antioxidant enzymes in maize seedlings and reduces the degrees of oxidative damage under stressful conditions (Zhou *et al*., 2002). In addition, paclobutrazol and ABA-induced chilling tolerance due to increased antioxidant enzymes and protected the membranes from oxidative stress (Prasad *et al*., 1994; Paliyath and Fletcher, 1995).

In the present study, the results in Fig. 7 revealed that low temperature stress induced significant decreased in proline content in mung bean seedlings, while pretreatment with paclobutrazol, ABA or H_2O_2 induced a significant increase in its content. However, proline content in the present study decreased with increasing time of chilling stress. The present results are supported by that of Chen and Li (2002), who reported most of the accumulated proline in maize cells, was lost to the medium during a prolonged chilling treatment. The leakage might be attributed to membrane damage (Chen and Li, 2002), as evidenced by the chilling-enhanced lipid peroxidation (Fig. 2). It has been shown that plant membrane damage during chilling is related to the peroxidation of membrane lipid due to the stress-induced accumulation of free radicals (Wise and Naylor, 1987). Whether proline prevents membrane lipid peroxidation in plant tissue by acting as an antioxidant to counteract the chilling-induced free radicals (Xin and Li, 1993). ABA induces chilling tolerance in maize plant by increasing free proline content (Chen and Li, 2002). Heber *et al*. (1973) showed that proline is capable of preventing freezing-induced inactivation of membrane activities. Whether proline prevents membrane lipid peroxidation in plant tissue by acting as an antioxidant to counteract the chilling-induced free radicals is under investigation. These results suggest that increased chilling tolerance by overexpression of reactive oxygen scavenging enzymes in plants (Mckerise *et al*., 2000) might be due to the prevention of proline

leakage. From these results, it appears that paclobutrazol, ABA and H_2O_2 may influence chilling tolerance in mung bean by increasing free proline content (Xin and Li, 1993).

As showed in the present study (Fig. 8), chilling stress induced a significant decline in total protein content in mung bean seedlings. Paclobutrazol, abscisic acid and hydrogen peroxide significantly increased protein content in the plant under investigation. The decline in protein content may be due to extensive damage of protein synthesizing system in various crop (Hallman *et al*., 1973) and tree (Espindola *et al*., 1994) or synthesis or activation of large quantities of proteolytic enzymes (Bewley and Black, 1982) as protease (Krishna *et al*., 2000). Adva and Waisel (1975) reported that at temperatures of 0 to 10°C, hydrophobic bonding in proteins decreases, causing conformation changes in protein structure and irreversible injury to plants. Plants synthesize a spectrum of new proteins on exposure to different environmental stresses including cold stress (Sutton *et al*., 1992). Proteins induced by stress fall into three categories: (i) those inducible by stress and ABA; (ii) specifically induced by stress but not by ABA and (iii) inducible by ABA. Therefore, it is quite evident that many of these stress-responsive proteins.

AOS generated under environmental stresses are highly reactive and have the potential to damage membrane lipids, proteins, chlorophyll and nucleic acids, thus disrupting the homeostasis of the organism (Rao *et al*., 1996). Low temperatures generally cause a decrease in the entire metabolism and in the photochemical steps of photosynthesis (Oliveira *et al*., 2002). In mung bean plant, chilling stress induced a significant decline in total chlorophyll content, while paclobutrazol, abscisic acid and hydrogen peroxide treatments induced significantly increased total chlorophyll content when compared to the positive control (Table 1). Leaf chlorophyll content was reduced significantly after plants were subjected to freezing stress and foliar sprays of paclobutrazol retarded the degradation of chlorophyll (Zhou and Leul, 1998). It was established that paclobutrazol prevent the decline in total chlorophyll content in corn plants after exposure to chilling temperature (Pinhero and Fletcher, 1994). Paclobutrazol-induced freezing tolerance was accompanied by increased activities of various antioxidant enzymes, including superoxide dismutase, catalase and peroxidase (Zhou and Leul, 1998). In addition, the protection of photosynthesis can be realized by reducing either superoxide or H_2O_2 levels, thereby reducing the possibility of hydroxyl radical formation (Payton *et al*., 2001).

As seen in results (Table 1), mung bean showed a significant decline in carbohydrates content when faced a chilling stress, but treating mung bean seedlings with paclobutrazol, abscisic acid and hydrogen peroxide significantly increased carbohydrates content when compared to the positive control. The stress effect on photosynthetic activity is manifested in many different ways, leading to efficiency loss in the photosynthetic apparatus, such as decreased use of photons for NADPH, ATP and carbohydrate production (Oliveira *et al*., 2002). Haldimann (1999) observed in maize leaves grown at low temperature is likely to be related to the fact that such leaves display reduced proportions of reaction centre core complexes to light harvesting antenna complexes in photosystem I and photosystem II (Nie and Baker, 1991). This feature is associated with reduced electron transport activities in both photosystems (Nie *et al*., 1995). Low temperatures generally cause a decrease in the entire metabolism and in the photochemical steps of photosynthesis, which are interdependent on the biochemical phase and, as expected, more directly influenced by low temperatures (Öquist, 1983). In addition, cold stress can affect photosynthesis rates by inhibiting the light and dark reactions of photosynthesis (Katterman, 1990) and change in the activities of several enzymes of photosynthetic carbon assimilation (Stamp, 1987).

In conclusion, mung bean plant is sensitive to low temperature. The result of the present investigation suggested that paclobutrazol, abscisic acid and H_2O_2 improved chilling injuries by decreasing lipid peroxidation, electrolyte leakage and H_2O_2 level and increasing catalase, peroxidase, ascorbate peroxidase activities and free proline content.

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