Exogenous 5-Aminolevulenic Acid Promotes Antioxidative Defence System, Photosynthesis and Growth in Soybean against Cold Stress

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Abstract
In the present study, the possibility of enhancing cold stress tolerance of young soybean plants (Glycine max [L.] Merr) by exogenous application of 5-aminolevulinic acid (ALA) was investigated. ALA was applied at various concentrations (0, 0.3, 0.6 and 0.9 mM) by seed priming and foliar application method. After ALA treatment, the plants were subjected to cold stress at 10 ± 0.5 °C for 72 h. Cold stress significantly decreased plant growth, relative water content, chlorophyll, photosynthesis and stomatal conductivity, while it increased electrolyte leakage and proline accumulation. ALA at low concentrations (0.3 mM) protected plants against cold stress, enhancing plant height, shoot fresh and dry weight, chlorophyll content, photosynthesis, stomatal conductivity as well as relative water content. Increase of electrolyte leakage was also prevented by 0.6 mM ALA. ALA also enhanced superoxide dismutase and catalase activities at 0.6 mM concentration especially under cold stress conditions. Proline increased with increasing in ALA concentration under both temperature conditions. In most cases, application of ALA by spraying method was better than seed priming method. Results showed that ALA, which is considered as an endogenous plant growth regulator, can be used effectively to protect soybean plants from the damaging effects of cold stress, by enhancing the activity of antioxidative enzymes, protecting cell membrane against reactive oxygen species and finally by promoting chlorophyll synthesis, leading to more intense photosynthesis and more carbon fixation, without any adverse effect on the plant growth.

Keywords: 5-aminolevulinic acid, cold stress, foliar application, priming, soybean

Introduction
About two thirds of the world’s landmass is annually subjected to temperatures below the freezing point and about half of it suffers from temperatures below -20 °C (Larcher, 2001). Low temperature may impose stress on a plant in a two-fold manner: by the effects of low temperature alone, and by dehydration of the cells and tissues when cellular water freezes (Beck et al., 2004). The plant response to low temperature stress can be divided into three distinct phases. The first is cold acclimation, which occurs at low, but above zero temperatures. The second stage, during which the full degree of tolerance is achieved, requires exposure to a period of sub-zero temperatures. The final phase is plant recovery after winter (Li et al., 2008). Low temperatures above the freezing point are deleterious to many crops of the tropics and subtropics which cannot acclimatize to cold. This kind of damage has been termed ‘chilling’ (Sakai and Larcher, 1987). Low temperature effects include damaged membranes (Xing and Rajashekar, 2001), reduced cellular respiration (Lee and Lur, 1997), increased abscisic acid levels (Nayyar et al., 2005), cryoprotectants and increased reactive oxygen species (Lee and Lur, 1997) which arises from imbalances of electron transport rate and the metabolic consumer activity of the reductive power (Beck et al., 2007). Cold acclimation is a process by which plants acquire freezing tolerance upon prior exposure to low non-freezing temperatures. Over wintering temperate plant species acclimatise during autumn, during which their metabolism is redirected towards synthesis of cryoprotectant molecules such as soluble sugars (saccharose, raffinose, stachyose, trehalose), sugar alcohols (sorbitol, ribitol, inositol) and low-molecular weight nitrogenous compounds (proline, glycine betaine) (Janská et al., 2010). Most temperate plants can cold-acclimate and acquire tolerance to extracellular ice formation in their vegetative tissues, while many important crops, such as rice, maize, soybean and cotton are chilling sensitive and incapable of cold acclimation. Given the fact that soybean plants are from subtropical regions, it is not surprising that they are particularly sensitive to cold. In addition, its cultivation is successful in climates with hot summers and temperatures below 20 °C and over 40 °C retard its growth significantly (Balestrasse et al., 2010). Following the expansion of soybean plants growing areas towards colder climates, acclimation to cold conditions has become a
major research target. Moreover, the combination of high light intensities and low temperatures, such as those experienced on cold but sunny mornings in spring, can cause irreversible damage to young soybean plants seedlings (Balestrasse et al., 2010). The sensitivity of soybean to night temperatures below 15 °C is reflected in the changes that occur in metabolism, growth, development and yield (Musser et al., 1983, 1984; Van Heerden et al., 2003). A single night of dark cold, with minimum temperatures of 8 °C, is sufficient to inhibit pod formation (Hume and Jackson, 1981). Therefore stress tolerance has become a major selection criterion in current soybean plants breeding programmers (Balestrasse et al., 2010).

5-Aminolevulinic acid (ALA) with a molecular weight of 131 is the first compound in the porphyrin synthesis pathway, the pathway that leads to heme in mammals and chlorophyll in plants. In higher plants, ALA is synthesized from glutamate in a reaction involving a glutamyl tRNA intermediate and requiring ATP and NADPH as cofactors; its formation is the rate limiting step in chlorophyll biosynthesis (Castelfranco et al., 1974; Beale, 1990). The keto-amino acid ALA is an essential precursor in the biosynthesis of porphyrins such as chlorophyll, heme, vitamin B12 and other tetrapyrrole compounds (Stobart and Ameen-Bukhari, 1984). ALA is found in all plants and its concentration is regulated at low concentrations (Weinstein and Beale, 1985; Hotta et al., 1997b). Recently, it was found that ALA at low concentration has a promotive effect on growth and yield of several field crops (Hotta et al., 1997a, b; Watanabe et al., 2000). It has been reported that ALA at low concentrations can also increase cold resistance in rice seedlings (Hotta et al., 1998). Similarly, Hotta et al. (1998) and Zhang et al. (2006) showed that ALA increased cold and salt tolerance in rice and potato. It is also known that ALA biosynthesis in plants is inhibited by low and high temperatures (Hodgins and Öquist, 1989; Tewari and Tripathy, 1998). From previous studies, low concentrations of ALA could enhance the antioxidant level in spinach and pakchoi (Nishihara et al., 2003; Memon et al., 2009) and enhance plant’s tolerance to cold stress (Hotta et al., 1998; Wang et al., 2004). Exogenous application of ALA is a novel strategy and has been considered as an effective means of minimizing the cold-induced adverse effects. However, there are few reports about assessing the effects of exogenous application of ALA on soybean under cold stress conditions. Therefore, in the current study, the effects of exogenous ALA at different concentration and two methods of application, seed priming and foliar application, regarding the cold stress induced growth, physiological and biochemical responses in soybean were investigated.

Materials and Methods

Seeds of soybean (Glycine max L. cv ‘032’) were surface sterilized in a hydrogen peroxide/ethanol solution for 2 min (10 ml of 30% H2O2 and 75 ml of 96% ethanol filled up to 100 ml with sterile distilled water) and rinsed several times with sterile distilled water. Half of the seeds were
immersed in different concentration of 5-aminolevulinic acid comprised of 0, 0.3, 0.6 and 0.9 mM, another half of seeds were imbibed in distilled water. After 3 h incubation at 25 °C, four seeds of each group were planted out in 10 cm diameter plastic pots containing autoclaved peat and perlite (3:1 ratio) at depth of 1–3 cm (totally 48 pots). The pots were placed in a growth cabinet (L/D =16/8 h, T=28/25 °C and a light intensity of 175 µmol m⁻² s⁻¹) and watered with full strength of Broughton and Dilworth solution (B&D) (Broughton and Dilworth, 1971) containing 8 mM KNO₃ and 0.5 mM Ca(NO₃)₂. After two weeks growth, plants grown from untreated seeds were sprayed with 0, 0.3, 0.6 and 0.9 mM 5-aminolevulinic acid. Three days after foliar application, their electrical conductivity was also recorded (Cₑ). The experiment was carried out with three replicates and all treatments were arranged in a randomized complete blocks design arranged in factorial 3(2 × 2 × 4).

**Relative water content**

Relative water content (RWC), expressed as a percentage was determined in soybean leaves according to the formula:

\[
\text{RWC} = \left( \frac{\text{FW} - \text{DW}}{\text{SW} - \text{DW}} \right) \times 100
\]

where FW, DW and SW mean fresh weight, dry weight and saturated weight, respectively. FW was measured just after collection of the leaves at the end of the cold stress and DW was measured after drying the leaves at 70 °C for 48 h.

**Electrolyte leakage**

Electrolyte leakage was determined by the procedure described by Dionisio-Sese and Tobita (1998). Leaf discs (100 mg) were thoroughly washed in distilled water thereafter the discs were heated in 10 ml of double distilled water at 25 °C for 2 h. Then electrical conductivity was recorded by EC meter (Cₑ). Subsequently the same samples were autoclaved at 121 °C for 20 min and then their electrical conductivity was also recorded (Cₑ). The electrolyte leakage was calculated as:

\[
\text{Electrolyte leakage} = \left( \frac{C₀}{Cₑ} \right) \times 100
\]

**Chlorophyll**

Chlorophyll was extracted in 80% acetone from the leaf samples according to the method of Arnon (1949). Extracts were filtrated and content of total chlorophyll was determined by spectrophotometry at 645 and 663 nm, respectively. The content of chlorophyll was expressed as mg g⁻¹ fresh weight according to the formula:

\[
\text{Total chlorophyll} = \left( 20.2 (D_{645}) + 8.02 (D_{663}) \right) \times V/1000 \text{ W}
\]
Superoxide dismutase (SOD)
Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium according to the method of Giannopolitis and Ries (1977). The reaction mixture contained 100 µl crude extract, 500 µl 10 mM H₂O₂ and 1.4 ml 25 mM potassium phosphate buffer. The decrease in absorbance was recorded at 240 nm for 1 min using a spectrophotometer (Cintra GBC, Dandenong, Victoria, Australia). Catalase activity of the extract was expressed as Abs mg⁻¹ protein min⁻¹.

Catalase
Catalase activity was estimated by the method of Cakmak and Horst (2006). The reaction mixture contained 100 µl crude extract, 500 µl 10 mM H₂O₂ and 1.4 ml 25 mM potassium phosphate buffer. The decrease in absorbance was recorded at 240 nm for 1 min using a spectrophotometer (Cintra GBC, Dandenong, Victoria, Australia). Catalase activity of the extract was expressed as Abs mg⁻¹ protein min⁻¹.

Proline
Proline content of leaves and roots was determined according to method of Bates et al. (1973). Samples (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v) and then centrifuged at 12,000 rpm for 15 min. Two ml of the supernatant were added to a test tube and then two ml glacial acetic acid and two ml freshly prepared acid ninhydrin solution were added. The test tubes were incubated in a water bath for one h at 100 °C and then allowed to cool to room temperature. Four ml of toluene was added to the tubes and mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min, to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve, and was expressed as mg g⁻¹ fresh weight.

Statistical analysis
For all variables, analysis of variance (ANOVA) was performed using the GLM procedure in SAS version 9.1. Main and interaction effects of experimental factors were determined. Where interactions between two factors were significant, it has been presented the results in the form of a combination of treatments and not separately or individually. The significance of differences among treatment means was compared by LSD at the 5% probability level.

Results and Discussion
Effect of cold stress and 5-Aminolevulinic acid on plant height
Analysis of variance showed that there was a significant effect of the cold stress and ALA treatments as well as the interaction of them on plant height. There was no significant effect of the ALA application method (Table 1). Plant height significantly decreased by cold stress treatment and increased by ALA application (Table 2). The highest plants were observed when 0.3 and 0.6 mM ALA was applied either in stressed plants or unstressed plants (Table 2). On the other hand, the shortest plants were observed in the control treatment (no stress and no ALA application). It is notable that further increase in
ALA concentration (0.9 mM) showed no increase in plant height (Table 2). Temperature within the chilling range can limit the soybean growth at all phenological stages. The present results support the claim of the former reports that low temperature had been identified as being powerful inhibitors for plant growth (Ercoli et al., 2004; Rodríguez et al., 2005; Xia et al., 2009). Earlier studies have shown that temperature alters the plant's auxin response by altering its biosynthesis (Gray et al., 1998), whereas cold temperature affects the polar and lateral transport of auxin (Wyatt et al., 2002; Nadella et al., 2006) a decrease in plant height is expected to bring modifications to the internode length or number. Although these studies demonstrate a link between auxin and temperature stress, the molecular and cellular mechanisms that regulate the auxin response under temperature stress, particularly for cold, remain elusive (Shibasaki et al., 2009). It has been found that low concentrations of ALA had a promotive effect on soybean plant height. It was reported that a low concentration of ALA had a promotion effect on cold resistance in rice (Hotta et al., 1998). Such type of ALA induced growth improvement has already been studied in date palm, potato, oilseed rape, pakchoi etc. (Youssef and Awad, 2008; Naeem et al., 2010).

Effect of cold stress and 5-Aminolevulinic acid on plant weight

As can be seen from Table 1, interaction of temperature × ALA concentration was significant on shoot fresh weight while shoot dry weight was only affected by individual effect of temperature and ALA concentration. Exposure to cold stress dramatically reduced the shoot fresh weight in untreated plants with ALA (Table 2). It is interesting to remark that there was no significant difference between the two temperature regimes when ALA was applied. Shoot fresh weight reached its maximum value on account of 0.3 mM ALA and then reduced with increase in ALA concentration (Table 2). Shoot dry weight was inhibited significantly under cold stress (Fig. 2). By contrast, it increased because of 0.3 mM ALA application; however the rate of increase decreased consistently with increased ALA concentration (Fig. 3). Similar results were obtained in the case of root dry weight, whereas cold stress decreased root dry weight whiles ALA increased (Table 2). Root fresh weight was affected neither by cold stress nor by ALA application. These results indicate that ALA treatment not only counteracted cold stress damage but also stimulated the growth and dry matter accumulation through increased chlorophyll content and photosynthetic CO₂ absorption. It also has been found that plants treated with 0.3 mM ALA produced the highest shoot fresh weights, whereas a higher concentration of ALA decreased the growth. The increase in fresh or dry weight may be attributed to the promotive effects of ALA on phloem transport (Bindu Roy and Vivekanandan, 1998). The present findings are in agreement with Hotta et al. (1997a) and Watanabe et al. (2006) who demonstrated that low concentrations of ALA improve growth rates and photosynthesis in barley, potato, radish, garlic, kidney bean and grapevines.

Effect of cold stress and 5-Aminolevulinic acid on relative water content

Table 2 shows that RWC decreased under cold stress respect to controls (25°C). Treatment with ALA protected against this effect. Treatment with 0.9 mM ALA under cold stress conditions showed a major RWC loss respect to controls. Conversely, the highest RWC value was related to 0.6 mM ALA treatment under none cold stress conditions (Table 2). Measurement of RWC showed that RWC decreased in leaves at the end of cold stress, whereas a higher concentration of ALA decreased consistently with increased ALA concentration (Table 2). Shoot dry weight was inhibited significantly under cold stress (Fig. 2). By contrast, it increased because of 0.3 mM ALA application; however the rate of increase decreased consistently with increased ALA concentration (Fig. 3). Similar results were obtained in the case of root dry weight, whereas cold stress decreased root dry weight whiles ALA increased (Table 2). Root fresh weight was affected neither by cold stress nor by ALA application. These results indicate that ALA treatment not only counteracted cold stress damage but also stimulated the growth and dry matter accumulation through increased chlorophyll content and photosynthetic CO₂ absorption. It also has been found that plants treated with 0.3 mM ALA produced the highest shoot fresh weights, whereas a higher concentration of ALA decreased the growth. The increase in fresh or dry weight may be attributed to the promotive effects of ALA on phloem transport (Bindu Roy and Vivekanandan, 1998). The present findings are in agreement with Hotta et al. (1997a) and Watanabe et al. (2006) who demonstrated that low concentrations of ALA improve growth rates and photosynthesis in barley, potato, radish, garlic, kidney bean and grapevines.

### Table 3. Interaction between temperature, ALA concentration and method of application

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Method of application</th>
<th>Concentration</th>
<th>Electrolyte leakage (%)</th>
<th>Chlorophyll (mg g⁻¹ FW)</th>
<th>Stomatal conductivity (mol m⁻² s⁻¹)</th>
<th>Catalase (ΔAbs mg protein⁻¹ min⁻¹)</th>
<th>Proline (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold stress (10°C)</td>
<td>Seed priming</td>
<td>0 mM</td>
<td>85.27a</td>
<td>1.42j</td>
<td>0.01g</td>
<td>1.37def</td>
<td>9.02h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 mM</td>
<td>69.85b</td>
<td>2.72a</td>
<td>0.08ab</td>
<td>1.72c</td>
<td>18.28e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 mM</td>
<td>23.67c</td>
<td>1.94gh</td>
<td>0.04cde</td>
<td>2.44b</td>
<td>22.87c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 mM</td>
<td>88.76a</td>
<td>1.75fg</td>
<td>0.02de</td>
<td>1.26fg</td>
<td>9.25h</td>
</tr>
<tr>
<td></td>
<td>Foliar application</td>
<td>0 mM</td>
<td>88.83a</td>
<td>1.77ja</td>
<td>0.01g</td>
<td>1.26fg</td>
<td>9.25h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 mM</td>
<td>65.74b</td>
<td>2.40bcd</td>
<td>0.05abc</td>
<td>1.64cd</td>
<td>17.13f</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 mM</td>
<td>15.82f</td>
<td>2.05fg</td>
<td>0.04de</td>
<td>3.47a</td>
<td>18.59e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 mM</td>
<td>36.56d</td>
<td>2.23cdef</td>
<td>0.02f</td>
<td>1.47cde</td>
<td>24.41f</td>
</tr>
<tr>
<td>None-stress (25°C)</td>
<td>Seed priming</td>
<td>0 mM</td>
<td>13.86f</td>
<td>2.37cde</td>
<td>0.01e</td>
<td>1.15gh</td>
<td>4.25j</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 mM</td>
<td>12.07f</td>
<td>2.61ab</td>
<td>0.06a</td>
<td>0.93hi</td>
<td>6.77e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 mM</td>
<td>12.09f</td>
<td>2.21def</td>
<td>0.05ab</td>
<td>0.71j</td>
<td>18.3w</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 mM</td>
<td>13.89f</td>
<td>2.17ef</td>
<td>0.05bde</td>
<td>1.20ij</td>
<td>23.15f</td>
</tr>
<tr>
<td></td>
<td>Foliar application</td>
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<td>15.30f</td>
<td>2.45cde</td>
<td>0.04e</td>
<td>0.62j</td>
<td>3.9j</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 mM</td>
<td>11.66f</td>
<td>2.20dfe</td>
<td>0.05abc</td>
<td>1.276g</td>
<td>5.81i</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 mM</td>
<td>12.06f</td>
<td>1.69f</td>
<td>0.05bde</td>
<td>2.06f</td>
<td>6.11i</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 mM</td>
<td>14.66f</td>
<td>2.06f</td>
<td>0.03f</td>
<td>1.01gh</td>
<td>14.32g</td>
</tr>
<tr>
<td>LSD (%)</td>
<td></td>
<td>5.66</td>
<td>0.21</td>
<td>0.00</td>
<td>0.28</td>
<td>1.00</td>
<td></td>
</tr>
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</table>

Values are mean (n = 3) and differences between means were compared by least significant difference test.
agreement with the current results. The ALA treatment significantly increased RWC over the control and also overcome the adverse effect caused by cold. This increase in RWC might be due to increase in plant growth attributes.

**Effect of cold stress and 5-Aminolevulinic acid on electrolyte leakage**

In this study, electrolyte leakage was affected by different temperature regimes and ALA concentrations, but not by method of ALA treatment (Table 1). The electrolyte leakage of soybean plants presented in this work decreased with increasing ALA concentration up to 0.6 mM and then increased slightly due to 0.9 mM ALA (Table 3). Under conditions of cold stress 0.6 mM ALA indicated a drastic reduction in electrolyte leakage (Table 3). For comparison purpose, foliar application of ALA was more effective for maintenance of membrane integrity than seed priming. It worth mentioning that there was no significant difference between 0.6 mM ALA with all other concentrations and methods of application at 25 °C, indicating this treatment overcome the adverse effect caused by cold. Increment in electrolyte leakage is a good reflection of oxidative damage to membrane lipids and other vital molecules such as proteins, DNA and RNA.

In the current study, cold stress increased electrolyte leakage compared to controls, in agreement with results of other studies (Schwanz and Polle, 2001). Cold stress causes overproduction of ROS, which ultimately imposes a secondary oxidative stress in plant cells (Foyer et al., 1997). Peroxidation of membrane lipids due to ROS may result in enhanced membrane fluidity, which may lead to enhanced electrolyte leakage and support the hypothesis that cold stress can induce membrane lipid peroxidation. Reduced membrane integrity resulting in electrolyte leakage has been reported with freezing in wheat (Pukacki et al., 1991). Foliar application of ALA particularly 0.6 mM extremely prevented the enhancement of electrolyte leakage. Thus, it is assumed that plants treated with ALA might be more efficient in scavenging of ROS. The probable reason behind the decreased electrolyte leakage is the application of ALA, as it is an essential precursor in the biosynthesis of haeme, so its exogenous application boasts up the activity of haeme-based biomolecules (APX, POD and CAT) and helped to scavenge the ROS to protect against the potentially harmful effects caused by reactive oxygen under cold stress condition (Liu et al., 2011).

**Electrophotograph of different ALA concentrations on chlorophyll**

Chlorophyll content was affected by individual effect of different temperature regimes and ALA concentrations (Table 1). Generally chlorophyll content significantly decreased by cold stress treatment and increased by ALA application (Table 3). The highest chlorophyll content was observed when soybean seeds were primed in 0.3 mM ALA solution and grown either under cold stress conditions or no stress conditions. It means that seed priming with low concentration of ALA overcomes cold stress in soybean. However, chlorophyll content decreased due to further increase in ALA concentration (Table 3). These ups and downs can be explained on one hand from the fact that ALA at low concentrations, acts as a regulator of chlorophyll and heme biosynthesis and, while on the other hand, oxidative stress might have occurred as a result of ROS generated by higher ALA concentrations. Furthermore, it is clearly known that ALA is an essential precursor in the biosynthesis of porphyrins such as chlorophyll and heme (Zhang et al., 2006). So it is easy to understand why exogenous supply of ALA would result in increased chlorophyll content. Low temperature is one factor that may limit photosynthetic activity via chlorophyll degradation. Musser et al. (1984) reported that low temperatures appear to damage the structure of chloroplasts and reduce the content of chlorophyll pigments. Previous studies have shown that chlorophyll can be bleached under cold stress due to generated ROS and oxidative stress (Noriega et al., 2007).
Effect of cold stress and 5-Aminolevulinic acid on photosynthesis and stomatal conductivity

Photosynthesis rate was affected by different temperature regimes and ALA concentrations (Table 1). Typically, photosynthesis rate decreased due to cold stress (Fig. 4) and increased on account of ALA application (Fig. 5). Photosynthesis rate was significantly reduced with increasing ALA concentration (Fig. 5). Similarly, stomatal conductivity was reduced because of cold stress and improved by 0.3 mM ALA under cold stress conditions and 0.3 or 0.6 mM ALA at 25 °C (Table 3). The hereby observations support this assumption and show that application of low levels of ALA increased stomatal conductivity and photosynthetic rate. Cold stress induces various biochemical and physiological responses in plants and affects almost all plant functions including photosynthesis. Decrease of photosynthesis induced by low temperatures is a well-known response of chilling-sensitive plants (Yadegari et al., 2008). The obtained data are in agreement with the findings of Hotta et al. (1997a, b) who suggested that growth and yield of several crops were improved by ALA application due to increased chlorophyll content and increased photosynthetic activity. Accumulated evidence suggested that exogenous ALA at low concentrations can promote photosynthesis in some plant species. ALA treatment could improve the biosynthesis of phycocyanin and chlorophyll in algae and eventually improve cell growth (Sasaki et al., 1995) and could significantly restore the photosynthetic ability under low light condition in melon (Wang et al., 2004) and watermelon (Sun et al., 2009).

Effect of cold stress and 5-Aminolevulinic acid on superoxide dismutase and catalase activity

Cold treatment resulted in higher enzyme activities of superoxide dismutase (Table 2). ALA application did not change superoxide dismutase activity at 25 °C. Conversely, under conditions of cold stress, superoxide dismutase activity increased with increasing ALA concentration (Table 2). The highest catalase activity was observed when plants were treated with 0.6 mM ALA at 10 °C. Generally, the antioxidant enzymes enhanced their activities remarkably with ALA treatment under cold stress. The effects of antioxidant enzymes in low temperature stress have been demonstrated in many experiments (Li et al., 2000; Wu et al., 2004). Superoxide dismutase converts O₂ radicals into H₂O₂ and O₂; and then catalase dismutases H₂O₂ into water and O₂. The probable reason behind the enhanced activities of catalase activity due to ALA is that it is an essential precursor in the biosynthesis of haeme, so its exogenous application boosts up the activity of haeme-based biomolecules such as superoxide dismutase and catalase (Lu et al., 2011). In addition, ALA undergoes enolization and further metal-catalyzed aerobic oxidation at physiological pH to yield superoxide radical, hydrogen peroxide and hydroxyl radical. Therefore, accumulation of ALA enhances reactive oxygen species levels leading to oxidative stress (Balestrasse et al., 2010).

Effect of cold stress and 5-Aminolevulinic acid on proline accumulation

Proline, soluble sugars and free amino acids have been mentioned as important compatible solutes in osmoregulation and protect plants from stress through different mechanisms, including cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity and stabilization of proteins/enzymes (Sairan and Tyagi, 2004; Ashraf and Foolad, 2007). An elevation of proline accumulation in the cold stressed plants was also observed in present study. Moreover, proline accumulation increased with increasing ALA concentration under both temperature conditions (Table 3). However, the highest accumulation was observed under cold stress conditions. It is notable that when seeds were primed with ALA, proline accumulation was higher. It is generally known that plants subjected to stress synthesize organic solutes such as glycine betaine, proline and sugar alcohols for turgor maintenance (McCue and Hanson, 1990). Contradictory results have been previously reported about whether proline plays protective role in stressed plants or just a symptom of injury under stress conditions (Misra and Gupta, 2005; Tatar et al., 2010). Also, it has been reported that ALA increases fructan (polyfructosylsucroses) content as nonstructural carbohydrate in spinach (Yoshida et al., 1995). The main question is how ALA leads to increased proline? The answer to this is not known yet; nonetheless it seems that ALA might be involved in proline synthesis and accumulation.

Conclusions

The present study provides evidence for the protective role of ALA against cold stress in young soybean plants. ALA application was effective in improving soybean growth such that 0.3 mM concentration was relatively more effective than the other concentrations used in the current study. Moreover, foliar-applied of ALA was more helpful than seed priming method. It has been also present data showing that ALA activated the enzymatic defence system and enhanced chlorophyll content, stomatal conductivity and photosynthesis. In addition, results suggest that application of ALA decreases electrolyte leakage and increases proline accumulation. Overall, ALA application can be recommend mitigating serious problems occurring under cold stress, to prevent crop losses in soybean due to low temperatures.

References


