EFFECT OF INDOLEACETIC ACID ON GROWTH AND ANTIOXIDANT PROPERTIES IN GLYCINE MAX SEEDLINGS EXPOSED TO ALUMINIUM TOXICITY

Mohammadi F.1*, R. A. Khavari-Nejad1,2

1Faculty of Biological Sciences, Kharazmi University, St. Shahid Mofateh, Tehran, Iran
2Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

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Summary: Two-week-old soybean (Glycine max) plants were subjected to factorial combination of five levels of aluminum chloride (0, 50, 100, 200 and 300 µM) and three levels of indoleacetic acid (IAA) (0, 50 and 100 µM). In the absence of IAA, treatment with increasing levels of Al progressively decreased net assimilation rate (NAR) and leaf water content per unit area (LWCA), but it increased the antioxidant enzyme activities, phenolic compounds and anthocyanin content. Aluminum concentration was increased in leaves and roots. Aluminum stress showed a substantial subtractive (p≤0.05) effect on plant growth. Exogenous IAA raised (p≤0.05) the activity of antioxidant enzymes and changed the content of anthocyanin and Al accumulation in roots and leaves depending on its concentration. Having been combined together, IAA and Al had additive effects on antioxidant enzyme activities, but their effects on NAR, LWCA, phenolic compounds, anthocyanin content and Al accumulation were moderate. Exogenous IAA counteracted the deleterious effects of Al stress and helped plants to grow successfully under these adverse conditions.


Keywords: aluminum toxicity; anthocyanins; ascorbate peroxidase; catalase; growth parameters; guaiacol peroxidase; indoleacetic acid; phenolic compounds; soybean.

Abbreviations: AlCl₃ – aluminum chloride; APX – ascorbate peroxidase; CAT – catalase; CHS – chalcon isomerase; DTZ – distal transition zone; EZ – elongation zone; GPX – guaiacol peroxidase; IAA – indoleacetic acid; LWCA – leaf water content per unit area; NAR – net assimilation rate; POD – peroxidase; PIN – pin-formed; UFGT – UDP-glucose: flavonoid 3-O-glucosyltransferase.

*Corresponding author: Farzanemohammadi90@yahoo.com
INTRODUCTION

Aluminum is a substantial abiotic factor limiting plant growth and productivity in many acidic soils throughout the world, and covers about 50% of earth’s surface (Yin et al., 2010). Aluminum contamination of soils due to intensive acid rain and industrial activities can cause environmental problems. In nature, Al is found in the form of aluminum silicate (Sun et al., 2010), potassium aluminum sulphate (KAl (SO$_4$)$_{12}$H$_2$O) and aluminum oxide (Al$_2$O$_3$) (Weast, 1979). Aluminum is hydrolyzed into Al$^{3+}$ cations in acidic soils and becomes a serious factor limiting plant growth and yield (Sun et al., 2010). Inhibition of root growth is a primary example of Al-induced injury in plants; hence, most researchers have focused their attention on plant root system (Chen, 2006). Roots are usually short and fragile having low output in absorption of food material and water (Sun et al., 2010). Preventive behavior of H$^+$-ATPase in the plasma membrane on top of the root cells (Mossor-Pietrazewska et al., 1997), modification of its structural properties and compounds of the lipid plasma membrane (Horst et al., 2010; Shen et al., 2005), increasing the oxidative stress, formation of callose and destruction of cellular skeleton ) Rengel and Zhang, 2003( are signs of Al toxicity in roots. In aerial plant parts, Al can infer cell changes and leaf structure, relative closure of stomatal apertures and decrease in photosynthetic rate resulting in chlorosis and necrosis in leaves (Williams et al., 2000).

Among plant hormones auxin plays a crucial role in modulating plant growth. It is also known to be involved in plant protection against heavy metal toxicity. Kollmeier et al. (2000) presented some evidence regarding the fact that distal transition zone (DTZ) is the first area of the root which is sensitive to Al in Zea maize. Regulation of root elongation inhibition in Arabidopsis by Al happens through restraining the transport of pin-ormed (PIN2) vesicles from the plasma membrane to endosomes. Besides, it has been reported that applying IAA into the elongation zone (EZ) would somehow dispel this inhibition (Sun et al., 2010).

In stressful conditions, higher activities of enzymatic antioxidants such as superoxide dismutase (SOD), peoxidases (POD) and catalase (CAT) and non-enzymatic constituents such as anthocyanins and phenolic compounds are necessary to create stress tolerance in plants. In general, peroxidases, catalase, anthocyanins and phenolic compounds are believed to increase the antioxidant response of plant in order to uphold a regular physiological and biochemical status in tissues having been affected by biotic and abiotic stressors (Kachout et al., 2009; Odjegba and Fasidi, 2007; Zhang et al., 2007).

In general, some environmental factors can affect plant growth. Moreover, it should be mentioned that Al and IAA affect the process of growth in different ways. So, to grasp a clear idea of this process, it was decided to do an experiment on the ameliorative role of IAA on Al-induced effects on selected processes to understand the biochemical and physiological mechanisms of Al toxicity in soybean.
MATERIALS AND METHODS

Plant materials and growth conditions
Soybean seeds (Glycine max (L.) Merr Var. williams) were provided from Karaj Seed and Seedling Institute. The seeds were sterilized in 4% sodium hypochlorite. After germination, the petri dishes were transferred into the light. Six-day-old seedlings were transferred into pots containing well moistened soil and suitable light conditions (16 h light and 8 h darkness). The seedlings in each pot were grown in Hoagland solution (Hoagland and Arnon, 1950) for 14 days. pH of the nutrient solution was adjusted to 5.5. Then pots were subjected to five different concentrations of AlCl₃ (0, 50, 100, 200 and 300 µM) and three different concentrations of IAA (0, 50 and 100 µM). After 40 days, plants were harvested to analyze the biochemical and physiological parameters.

Growth analysis
Four 14-day-old control plants and 40-day-old plants exposed to either Al or IAA alone or in combination were harvested. The wet weight of membrane of the plant such as root, stem, leaf and also the leaf surface was immediately calculated after harvest. They were then dried in an oven at 105°C for 24 h. The net assimilation rate (g m⁻² d⁻¹) and the leaf water content per unit area (g H₂O m⁻²) were calculated using the formula of Watson and Ivans (1991). NAR is the amount of the dried material produced by the plant in leaf level in unit of time which is obtained from the formula. In this equation $L_1$ and $L_2$ are equivalent to the first and final leaf level, respectively. LWCA shows the amount of water accumulated in leaves and it is calculated from the following formula:

$$NAR = \frac{1}{L} \times \frac{dW}{dt}$$

$$NAR = \frac{W_2 - W_1}{L_2 - L_1} \times \frac{LnL_2 - LnL_1}{t_2 - t_1}$$

$$LWCA = \frac{LFW - LDW}{L}$$

In this equation $LFW$ stands for the weight of wet leaves and $LDW$ shows leaf dry matter.

Enzyme activity assays
CAT activity was determined by the method of Dazy et al. (2008). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), 15 mM hydrogen peroxide (H₂O₂). 100 µl of enzyme extract was added to a final volume of 3 ml. The decrease of H₂O₂ was recorded at 240 nm per 30 s for 2 min. The activity of the enzyme was expressed as $\Delta OD_{240}$ min⁻¹ g⁻¹ FW.

POX activity was estimated according to the method of Dazy et al. (2008). The reaction mixture contained 25 mM potassium phosphate buffer, 20 mM guaiacol, 40 mM H₂O₂. 100 µl of enzyme extract was added to a final volume of 3 ml. The change in absorbance was monitored at 470 nm due to guaiacol oxidation. The activity of the enzyme was expressed as $\Delta OD_{470}$ min⁻¹ g⁻¹ FW.

Estimation of APX activity was performed according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer, 0.5 mM ascorbic acid, 0.1 mM H₂O₂. 200 µl of enzyme extract was added to a final volume of 3 ml. The
change in absorbance was recorded at 290 due to the reaction with ascorbate. The activity of the enzyme was expressed as $\Delta OD_{290}$ min$^{-1}$ g$^{-1}$ FW.

**Preparation of the enzyme extract**

Leaf sample (0.1 g FW) was homogenized in 2 ml of potassium phosphate buffer. After centrifugation of the samples at 15,000 x g for 12 min, the supernatant was used for enzyme assays.

**Estimation of anthocyanin and total phenolics content**

Anthocyanin and phenolic compounds were determined using the method of Dai et al. (2006). Samples (0.1 g FW) were homogenized in 1% HCl-methanol (5 ml). After 24 h, extracts were filtered, and each filtrate was diluted with 1% HCl-methanol to 10 mL.

The absorption at 530 and 600 nm was calculated and then subducted from each other so as to obtain the amount of anthocyanin. Also, absorption at a wavelength of 280 nm was measured spectrophotometrically for phenolic compounds content. The content of anthocyanins and phenolic compounds was expressed as mg g$^{-1}$ FW and µg g$^{-1}$ FW respectively.

**Estimation of Al concentration**

After being dried at 80°C, roots and leaves were ashed for 6 h at 500°C. Each sample was dissolved in 3.5 % (v/v) HCl, and the samples were used for Al determination using an atomic absorption spectrophotometer (Shimadzu, Model AA-360-O$_2$). Al concentration was expressed as µg g$^{-1}$ DW.

**Statistical analysis**

The data were subjected to two-factor analysis of variance (ANOVA) using SPSS version 17 and SAS version 9 software at level of $P<0.05$.

**RESULTS**

**Growth and biomass yield**

The results from the growth analysis after application of AlCl$_3$ and/or IAA are presented in Table 1. The deleterious effect of Al on growth was significantly ($p \leq 0.05$) alleviated after the combined application of Al and IAA. The increase in Al level caused a decrease in both NAR and LWCA. After treatment with 300 mM Al the plants showed the greatest decrease in NAR and LWCA (1.27 gm$^{-2}$d$^{-1}$ and 15.52 gH$_2$Om$^{-2}$, respectively). Results showed also that the exogenous application of IAA countered the deleterious effects of Al. For instance, in the presence of 300 mM Al together with 50 and 100 mM IAA, compared to a medium lacking auxin (300 µl Al), the amounts of NAR and LWCA were changed from 1.27 to 1.53 and 1.38 and from 15.52 to 19.61 and 17.87, respectively.

**Antioxidant enzyme activities**

In plants, GPX, APX and CAT activities (Fig. 1A, B and C) increased progressively with increasing Al stress (300 µM). These contents were significantly higher in plants treated with Al and IAA. The activities of the three enzymes, GPX, PXA and CAT increased and the highest increase was observed in plants treated with 300 mM Al (0.065, 0.252 and 0.057 $\Delta OD_{290}$ min$^{-1}$ g$^{-1}$ FW, respectively) (Fig. 1). The addition of IAA to the nutrient solution in the presence of Al caused an
Table 1. Effect of different concentrations of AlCl₃ and IAA on NAR [g m⁻² d⁻¹] and LWCA [g (H₂O) m⁻²] in Glycine max. Values are significantly different at P<0.05 from control. Values are Average ± SE.

<table>
<thead>
<tr>
<th>AlCl₃ [µM]</th>
<th>IAA [µM]</th>
<th>NAR [g m⁻² d⁻¹]</th>
<th>LWCA [g (H₂O) m⁻²]</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>3.25±0.136a</td>
<td>39.35±1.97a</td>
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<tr>
<td>0</td>
<td>50</td>
<td>3.17±0.159a</td>
<td>37.39±2.07ba</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>3.10±0.110ba</td>
<td>35.32±2.19bc</td>
</tr>
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<td>50</td>
<td>0</td>
<td>2.64±0.078c</td>
<td>32.92±1.50c</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>2.75±0.193bc</td>
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</tr>
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</tr>
<tr>
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<td>100</td>
<td>2.10±0.054c</td>
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<tr>
<td>300</td>
<td>100</td>
<td>1.38±0.089f</td>
<td>17.87±2.14hg</td>
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</tbody>
</table>

increase in the activities of these enzymes. The results showed that in the presence of 300 µl Al + 100 µl IAA the activities of GPX, PXA and CAT were 1.18, 1.23 and 1.40-fold higher, respectively than in plants grown in solution lacking IAA. In general, the effects of Al and IAA on each of the three enzymes were additive.

**Anthocyanin and phenol contents**

In control plants, the content of anthocyanin and phenolic compounds (Fig. 2A and B) progressively increased with increasing Al stress (300 µL). The addition of IAA reduced the content of anthocyanin and phenolic compounds compared to plants without IAA treatment. For example, anthocyanin and phenolic compounds contents in plants treated with 300 µM Al + 100 µM IAA in combination were about 1.26 and 2.90-fold lower, respectively than in the plants treated with 300 µM Al.

**Uptake of aluminum**

Leaf and root Al content (Fig. 3A and B) were largely affected by adding Al (0, 50, 100, 200 and 300 µM). Al-treated plants accumulated high amounts of Al in their leaf and root tissues compared to control plants. The addition of IAA reduced leaf and root Al content compared to plants without IAA treatment.

**DISCUSSION**

The present study showed that a decrease in growth of Glycine max was recorded under Al stress conditions. Similar reports have been made for different plant species during heavy metal stress conditions (Vassilev et al., 2002;
Figure 1. Effect of different concentrations of AlCl3 and IAA on CAT (A), GPX (B) and APX (C) in *Glycine max*. Values are significantly different at $P<0.05$ from control. Values are Average ± SE.

Figure 2. Effect of different concentrations AlCl3 and IAA on Anthocyanin (A) and Phenol (B) contents in *Glycine max*. Values are significantly different $P<0.05$ from control. Values are Average ± SE.
Yamamoto et al., 2003). A decrease in growth could be due to binding of Al to the cell wall. Linking of Al to the wall causes changes in the layer potential which leads to activating some calcium channels and the result of increasing cytoplasmic Ca$^{2+}$ would be two destructive actions in the cell: 1. Formation of callose 2. Destruction of the cellular skeleton (Rengel and Zhang, 2003). The formation of depleted callose by Al in top of the roots has a direct relationship with inhibition of root growth (Bhuja et al, 2004). That is due to the formation of callose which causes the absorption of water to get little and hence blocks the plasmodesmata and in this way inhibits the transfer from cell to cell (Ishikawa and Evans, 1995). It has also been reported that Al increases the amounts of ferulic and deferulic acids in the cellular wall to a great degree which leads to thickness of the wall, since these two acids take part in the lignification process which consequently leads to root growth inhibition (Haluskova et al., 2010) (Table 1). Al causes various adverse effects, such as prevention of cell division and ion fluxes, disorganization of the cytoskeleton dynamics and plasma membrane function and stability as well as disruption of signal transduction pathways. This heavy metal also decreases biomass production via inhibition of phosphorus absorption into cells (Shamsi et al., 2007). Al increases IAA-oxidase activity (Abdalla, 2008), decreases auxin biosynthesis and prevents its translocation from root meristem to the elongation zone (Barcelo and Poshenrieder, 2002; Massot et al., 2002). Our results showed that the combined IAA+Al treatment ameliorated the deleterious effect of Al stress and enhanced growth (NAR and LWCA) (Table 1).

Production of reactive oxygen species (ROS) has been recognized among many effects of heavy metals in plants, and they have shown to have destructive effects.
on proteins and membrane lipids, nucleic acids and photosynthetic pigments. Al induces the oxidation of phenolic compounds which results in production of $O_2^-$ (Pereira et al., 1999), probably the heavy metal affects NADPH oxidation activity thus consequently increasing $O_2^-$ (Romero-Puertas et al., 2004). Autoxidation of transition metals such as Cu or Fe leads to the production of $O_2^-$ and subsequently $H_2O_2$ and OH$^-$ formation via Fenton reactions. Missing one electron from $O_2$ in the electron transport chain is the typical source of $O_2^-$ (Sigaud-Kutner et al., 2003).

Plant cells induce ROS scavenging enzymes, such as superoxide dismutase, peroxidase and catalase which is vital for plants to tolerate the oxidative stress (Kachout et al., 2009). Increased accumulation of GPX, APX and CAT in soybean plants in response to Al treatment is shown in Fig. 1. Similar reports have been made for many plant species under heavy metal stress conditions (Cakmak and Horst, 1991; Haluskova et al. 2010; Odjegba and Fasidi, 2007; Szolosi et al. 2011; Zhang et al. 2007). This could be due to the effects of the heavy metal on producing various reactive oxygen species (ROS) and expression of antioxidant enzymes genes (Neill et al. 2002; Vranova et al. 2002). Al-induced increase in POD activity is associated with root lignification and decreased $H_2O_2$ content. Furthermore, lignification and suberization of roots are interpreted as defense reactions limiting the entry of extra heavy metal into the root cells (Haluskova et al., 2010). Fig. 1 indicates an increase in the contents of GPX, APX and CAT in soybean plants, being exposed to IAA. Such an increase could be due to increased expression of genes of these enzymes in response to heavy metal stress (Balestrasse et al., 2001; Barket et al., 2007).

Enhancement of anthocyanins and phenolic compounds in Al-treated plants correlated positively ($p\leq0.05$) with Al concentration of internal tissue (Fig. 2). However, the increase of anthocyanins and phenolic compounds under heavy metal stress has been recently studied (Dai et al. 2006). Such an increase in anthocyanins and phenolic compounds could be due to an increase in the PAL gene expression in response to heavy metal stress (Basak et al., 2001; Michalak, 2006; Santiago et al., 2000; Winkel-Shirey, 2002). Metal ions decompose lipid hydroperoxide (LOOH) by cleavage of O-O bonds and produce radicals of lipid alkoxyl which initiate free radicals oxidation. Phenolic antioxidants prevent peroxidation of lipids by trapping the lipid alkoxyl radical. This activity depends on the number and position of the hydroxyl groups in the molecules (Millic et al., 1998). The results on IAA (Fig. 2) were in agreement with the achievements of Jeong et al. (2004). Such a decrease in the content of anthocyanins and phenolic compounds could be due to preventing the chalcon synthase (CHS) and UDP-glucose: flavonoid 3-O-glucosyltransferase (Ufgt) genes expression.

The increased activity of GPX, APX, CAT and content of anthocyanin and phenolic compounds in soybean plants in response to Al treatment could be due to enhanced accumulation of Al (Figs. 1, 2, 3) through the creation of peroxidases that may have a role in heavy metal accumulation through polymerization of phenols (Fig. 1A, B, 3). The combined IAA+Al treatment ameliorated the deleterious effect of Al stress and enhanced...
growth (NAR and LWCA) (Table 1).

The concentration of heavy metals in roots is higher than in leaves and this may be due to the fact that roots are the first organs to be in contact with the heavy metals. In leaves, the concentration of heavy metals depends on the age, e.g., the highest concentration of AI has been found in older leaves of a plant (Shen and Ma, 2001; Singh and Agrawal, 2010). Plants may store and accumulate the elements in nontoxic forms in roots and leaves (Fig. 3A and B). Binding of heavy metals to the cell wall of the roots and leaves, their storage in vacuole and their chelating with organic compounds like citrate are the main mechanisms for accumulation of heavy metals (Ma, 2000; Memon et al., 2001). Phenolic compounds increase in response to environmental stress and one of the roles of these compounds may be to bind specifically to unnecessary elements such as Al. Peroxidases may have a role in heavy metal accumulation by polymerization of polyphenols. Another way in Fabaceae family is loading that causes accumulation of heavy metal in vacuoles of epidermal cells. This method appears to cause the least damage to the photosynthetic apparatus (Chen et al., 2005; Milner and Kochian, 2008; Watanabe and Osaki, 2002). Our results presented in Fig. 3 demonstrated that when soybean plants exposed to Al stress were treated with IAA, the accumulation of Al decreased. This result was in agreement with the decrease in the levels of anthocyanins and phenolic compounds in these plants (Jeons et al., 2004) (Fig. 2A and B). Also, our results clearly showed that treatment with IAA led to reduced accumulation of Al and induced the re-growth of the plants (Fig. 3, Table 1).

REFERENCES


Physiology, 45: 543–549.


Ma J F, 2000. Role of organic acids in detoxification of Aluminum in higher
Effect of IAA on *Glycine max* exposed to aluminium toxicity


Shen H, L F He, T Sasaki, Y Yamamoto, S J Zheng, A Ligaba, X L Yan, S J


