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Enhancing the Defensive Mechanism of Lead Affected Barley (*Hordeum vulgare* L.) Genotypes by Exogenously Applied Salicylic Acid

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Abstract

Lead is a non-essential element reduced plant growth and development that may cause multifarious disturbance in physiological, biochemical and structural integrity of plants. SA is an efficient signal molecule induces systemic resistance responses that control local defense reactions in plants. To evaluate the effect of SA on photosynthetic activity of Pb affected plants therefore a pot experiment was conducted on barley genotype Juo-93 and Juo- 87 in the Old Botanical Garden, University Of Agriculture, Faisalabad, Pakistan. Three treatment levels (0, 100 and 200 µM) of lead sulphate ($PbSO_4$) were applied in thrice replication with or without SA (0.5 mM) along half strength of Hoagland's solution till the termination of experiment. The experiment was arranged in a completely randomized design. During course of study growth and pigments modulations were recorded. The result indicated that growth parameters such as root and shoot length, leaf area, fresh weight of shoot and root, dry weight of shoot and root were reduced under lead toxicity. Pb stress damaged the photosynthetic pigments such as Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids but chlorophyll a/b was increased under Pb stress whereas exogenous application of SA alleviated the negative effect of lead toxicity. Juo- 93 showed more tolerance to Pb toxicity as compared to Juo-87.

Keywords: carotenoid, lead toxicity, barley, chlorophyll, salicylic acid

1. Introduction

Heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades (Gisbert et al., 2003; Castro et al., 2011). These metals unlike other organic pollutants are not degraded and converted into harmless compounds via biological processes. Elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants (Hall, 2002; Li et al., 2010). Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element (Capuana, 2011). Heavy metals effects chlorophyll content in plants by interfering with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient (Meers et al., 2010). Lead is very toxic environmental pollutant which is widely distributed in the soil and contaminated water. The key enzyme for chlorophyll biosynthesis i.e. α-amino laevulinate dehydrogenase is strongly inhibited by Pb ions (D. D. K. Prasad & A. R. K. Prasad, 1987). Photosynthesis is especially affected by lead exposure (Bazzaz et al., 1975); chlorophyll and carotenoids contents, photosynthetic rate and CO_2 assimilation are strongly decreased (Eun et al., 2002).

Seed treatment or foliar application of chemicals like glycinebetaine, kinetin, salicylic acid (Gunes, 2007; Karlidag, 2009) may increase yield of different crops due to reduction in stress induced inhibition of plant growth (Khan et al., 2003; Elwana & El-Hamahmyb, 2009). Salicylic acid is an endogenous growth regulator of phenolic nature and acts as potential non-enzymatic antioxidant which participates in the regulation of many physiological processes in plants (Simaei et al., 2012; Horvath et al., 2007), such as stomatal closure, photosynthesis, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan et al., 2003; Arfan et al., 2007). Chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA under lead stress (Bosch et al., 2007; Najafian et al., 2009). Barley (*Hordeum vulgare* L.) is a highly adaptable cereal grain and considered to be the most drought and salinity tolerant among cereals (Ceccarelli et al., 1987; Belaid & Morris, 1991).

The aim of this project is to explore the response of barley under Pb contamination and ameliorating potential of SA towards metal toxicity.

2. Materials and Method

Seeds of two barley genotypes Juo-93 and Juo-87 were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Ten seeds of good vigor of both genotypes were graded and sown in sand filled (5 kg) plastic pots (30 × 20 cm size). The pots were kept in a net house under bright sunlight. The climatic conditions were 30±2 °C with 50% relative humidity at the time of experiment. Lead sulphate ($PbSO_4$) of 100 and 200 µM along with 0.5 mM SA was applied by mixing with Hoagland's solution till the termination of experiment. Control was without treatment of SA and Pb for both barley genotypes for comparison. After 58 days of germination, prior to uprooting of plants, the intact plants were measured for shoot length and leaf area. Leaves of Juo-93 and Juo-87 varieties for each treatment was sampled and stored in an ice bath, immediately brought in to the Lab and grinded in 80% acetone for the estimation of photosynthetic pigments following spectrophotometric method (Hitachi-U-2001, Japan) (Yoshida et al., 1976; Davies, 1976). Plants were uprooted from three replicates for the measurement of root length, shoot and root fresh weight. For dry weight determination, the shoot and root parts were properly washed with water, blotted dry, wrapped in labelled paper bags and kept in an oven at 70 ^{o}C for one week.

The three factors factorial experiment was arranged in a completely randomized way. The collected data was statistically analyzed using LSD Test and their means were compared applying DMRT comparison Test.

3. Results

Genotypes Juo-93 and Juo-87 showed significant ($P < 0.05$) differences for growth attributes under SA and Pb treatments. Juo-93 performed better than Juo-87 under high concentration of Pb. Salicylic acid (SA) application was found beneficial for the growth of both barley genotypes. Length as well as fresh and dry weights of shoot and root was enhanced by SA as compared to control. Juo-93 showed great shoot and root length, their fresh weights as compared to Juo-87 in control. Rooting medium application of SA enhanced length and fresh weights of both upper and lower parts of both genotypes. Pb level of 100 µM was less toxic for shoot length and fresh weights whereas 200 µM Pb was more damaging for root length and fresh weights. Although SA application in combination with Pb (100 µM and 200 µM) treated plants improved the growth of both genotypes. Similar response in shoot and root dry weights were observed in the presence of SA and Pb treatments as shown by other growth parameters (Figure 1).

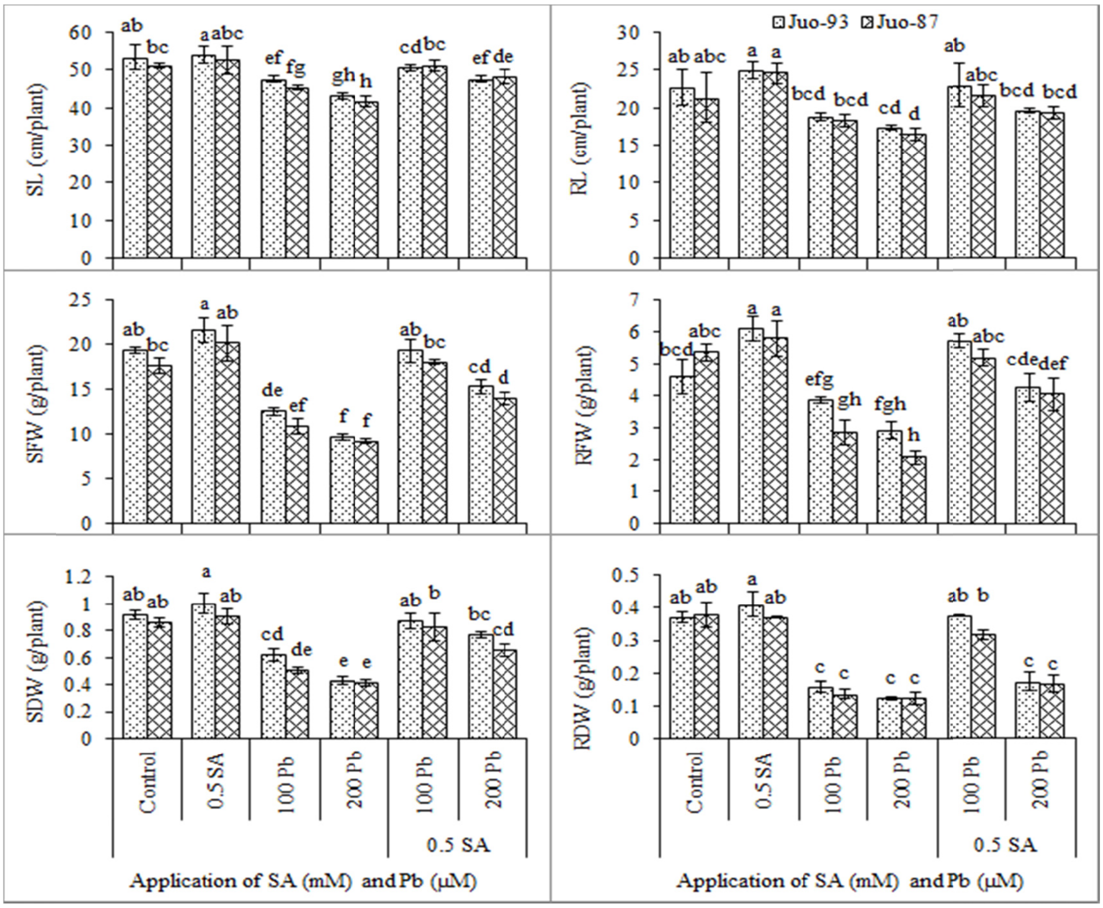

Figure 1. Graphical presentation of growth attributes of two barley genotypes (Juo-97 and Juo-87) treated with SA (0.5 mM) and Pb (100 and 200 μM)

Note. SL = shoot length, RL = root length, SFW, SDW = shoot fresh and dry weights, RFW, RDW = root fresh and dry weights. Histogram and Error bars represent Means±SD, while labels represent significance levels of treatments and genotypes ($P < 0.05$).

Juo-93 was found to be more efficient photosynthetically as compared to Juo-87 in control as well as treatment applications. SA broaden the leaf area in barley genotypes directly provide more surface area for photosynthetic activity. The chlorophyll and carotenoids contents increased in both by the increment of SA. Pb was proved to be toxic and directly affect the concentration of photosynthetic pigments in barley. Plants treated with 200 μM Pb had less chl-a, chl-b, chl-T in Juo-93 and Juo-87 as compared to 100 μM Pb. Application of SA in combination with both the treatments of Pb (100, 200 μM) enhanced the amount of chl-a, chl-b, chl-T and carotenoids, more in Juo-97 in comparison to Juo-87. Chl a/b ratio depicted that chl-a was more in both barley genotypes as compared to chl-b which shows that chl-b is more sensitive to Pb as compared chl-a (Figure 2).

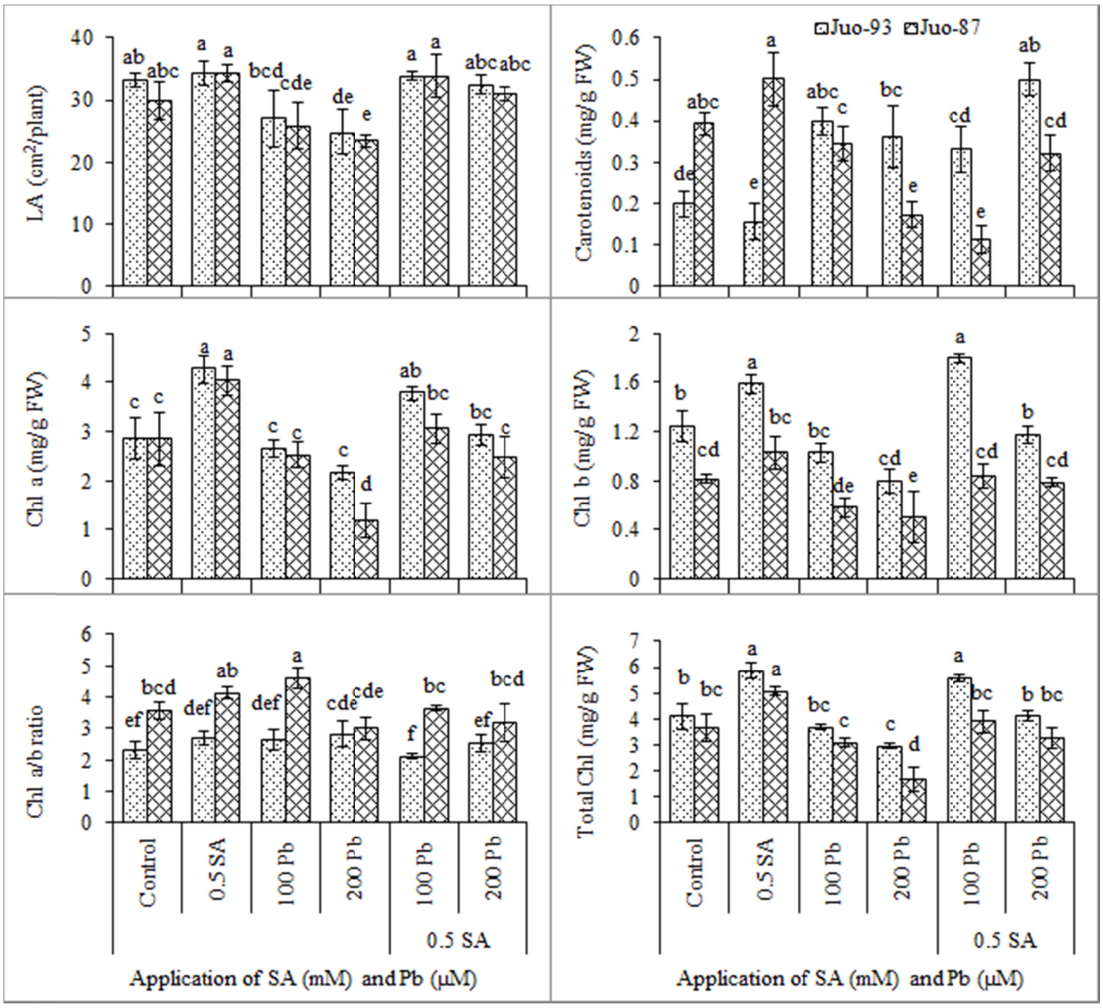

Figure 2. Graphical presentation of photosynthetic pigments of two barley genotypes (Juo-97 and Juo-87) treated with SA (0.5 mM) and Pb (100 and 200 μM)

Note. LA = leaf area, Chl a = Chlorophyll a, Chl b = Chlorophyll b. Histogram and Error bars represent Means±SD, while labels represent significance levels of treatments and genotypes ($P < 0.05$).

4. Discussion

Lead (Pb) concentration increases in cultivated soils due to irrigation with sewage effluents. The other major cause is the use of lead arsenate in pesticides (Paivoke, 2002). The exhausting of Pb from automobiles and industrial emissions pollutes atmosphere for plants as well as humans (Awofolu, 2004). Pb is non-essential heavy metal that sharply decreases crop productivity (Sengar et al., 2008). Pb is considered as protoplasmic poison which is taken up by plants from soil and compartmentalized in vacuolar sequestration by phytochelatins (Anjum et al., 2015; Sharma et al., 2016). The entry of Pb from leaves is dependent on leaf morphology and their ability to absorb Pb from the aerial sources (Sharma & Dubey, 2005) however, bulk of the Pb is taken up by plants from the soil through roots (Fahr et al., 2013). The translocation of Pb is highly restricted to upper parts of the plants through roots (Aziz et al., 2007; Tangahu et al., 2011). Calcareous or soil rich in phosphate precipitates Pb and make it non-toxic to plants (Li et al., 2013). Application of Pb decrease the shoot and root length of two barley genotypes as studied in Brassica (Pallavi & Rama, 2005; Ghani et al., 2016). Growth data result showed that 100 μM and 200 μM level of lead reduced the shoot and root length, their fresh and dry weight and leaf area per plant (Hussain et al., 2013). Reduction in the root biomass has also been reported in turnip and lettuce when treated with lead (Hassanein et al., 2013; Gupta & Sinha, 2007). Dry mass production of plant was dependant completely on light harvesting that was required for the generation of reducing powers (ATP and NADPH) during the photosynthesis light reaction and reducing powers use in dark reaction for photo-assimilate production (Taiz & Zeiger, 2010).

In present study, 200 μM level of lead was found toxic for both barley genotypes while applied level of SA (0.5 mM) in the rooting medium was effective in improving all the above mentioned growth parameters that were slightly affected by Pb-stress. Important indication of stress tolerance in plants was prolific system of roots by increasing cell division of root apical meristems (Yang et al., 2000; Shakirova et al., 2003; Vicente & Plasencia, 2011). SA application under Pb-stress enhanced the dry weight of root although relatively better in barley genotype Juo-93.

Pb application was toxic for photosynthetic attributes such as chlorophyll a and b, total chlorophyll, carotenoids, but application of SA enhanced the contents of these pigments. Juo-93 showed less reduction in chlorophyll a, b, total chlorophyll and carotenoids contents under Pb-stressed conditions and reduced ratio of Chlorophyll a/b was also observed in this genotype than Juo-87. Key determinant of final productivity was maintenance of photosynthetic pigments under stressful circumstances (Wahid et al., 2009). Carotenoids not only harvest light on photosystems but acts as antioxidant mainly protecting photosystems from the action of ROS generated in chloroplast (de Passcale et al., 2001; Huchzermeyer & Koyro, 2005). Chlorophyll ratio that is used as stress indicator enhanced with increase in heavy metal treatment (Monni et al., 2001). Improvement in chlorophylls ratio under environmental stress has been reported in leaves of spinach (Delfine et al., 1993). Under Pb stress rooting medium application of SA improved the contents of chlorophyll pigments, especially Chl-a, b and total chlorophylls (Khodary, 2004). Exogenous SA of 0.5 mM concentration is beneficial in improving growth and protecting photosynthetic pigments from salts toxicity as in barley (Metwally et al., 2003; Ananieva et al., 2004).

SA application increased the antioxidation capacity and protection of photosynthetic apparatus against Pb stress. Pb translocation through roots mainly follow apoplastic pathway. Roots tend to hinder the movement of Pb in apoplast by binding it with carboxyl groups of carbohydrates galacturonic acid and glucuronic acid in the cell wall (Sharma & Dubey, 2006). Pb transport from the soil to the root cells possibly through voltage gated Ca-channels of plasma membrane isolated from roots of wheat and corn plants (Marshall et al., 1994; Huang et al., 1994). This voltage gated Pb transport was blocked by nifidipine (a Ca-channel blocker) (Tomsig & Suszkiw, 1991). Exogenous SA might be involves in activation of nifedipine, blocking the Ca-channels for Pb translocation into the roots. SA resulted in improved growth of two barley genotypes by restricting the Pb at root levels.

5. Conclusion

In conclusion, SA (0.5 mM) is found to be beneficial in enhancing Pb tolerance in two barley genotypes. Juo-93 showed better growth than Juo-87 under lead and salicylic acid treatments. Anatomical, molecular and genomic studies of SA and Pb treated roots should be done in order to understand the tolerance mechanism and selected level of SA (0.5 mM) should be tested in field trails so that it can be recommended for metal toxic soils.

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