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Research Article

Modulation of Salinity Stress Using Phosphorus in Two Genotypes of Black Gram (*Vigna mungo*L.Hepper)

Dr. Indu Chaudhary

Assistant Professor, Department of Botany, New Standard College of Higher Education, Raebareli

Abstract

A soil culture experiment was carried out in wire house condition to investigate the response of two black gram (*Vigna mungo* L. Hepper) genotypes PU-31 and Azad-2 grown in saline water and amended with supplementary phosphorus (P). Plants were subjected to five different electrical conductivity levels of salinity viz. 0, 4, 6, 8 and 10 dS/m prepared by mixing NaCl, Na_2SO_4, $CaCl_2$ and $MgCl_2$ and two salinity levels with EC 8 and 10 dS/m were subjected with combination of 40 ppm P. Overall growth of plants of both varieties was found to be reduced when treated with saline irrigation water. Results showed that proline synthesis was more intense in case of PU-31 (154.0%) as comparison to Azad-2 (118.3%). Salt stress reduced the activity of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) but P amendment increased their activity. Thus, supplementary P reduced detrimental influence of salts and considerably alleviated the salt induced decrease in plant growth, dry matter yield as well as change in lipid per oxidation and electrolyte leakage percentage. The results indicated that supplementary P can mitigate the negative influence of irrigation water salinity on plant growth and physiological development.

Keywords: *Black gram, salinity, phosphorus, anti-oxidative enzymes.*

Introduction

Salinity is one of the most prominent abiotic factor affecting crop yield in arid and semiarid regions (Subramanyam, *et al*., 2010). It is estimated that of the 230 million ha of arable land, 45 million are affected by salinity (FAO, 2008). Salt contaminated soils (ECe >4 dSm^{-1} or 40 mM NaCl or osmotic potential<0.117MPa) are defined as saline lands which directly affect plant growth and development particularly the vegetative growth prior to reproductive stage, (Ashraf, 2010). Most of crop species i.e. beans, eggplant, onion, pepper, corn, sugarcane, potato and cabbage are sensitive to salinity (ECe 1.0-1.8 dSm^{-1}), which reduce crop productivity about 6-19 % (Chaum and Kirdmanee, 2009). Salinity stress caused generation of excessive reactive oxygen species (ROS), which leads to cell toxicity, membrane damage and cell death (Chookhampaeng, 2011). To control the level of ROS and to protect the cells, plants possess low molecular weight antioxidants compounds and antioxidant enzymes such as CAT, POX and SOD which scavenge the ROS (Mishra, *et al*., 2009). P is an important element of key molecules such as nucleic acids, phospholipids, and ATP; consequently, plants cannot grow without a reliable supply of this nutrient (Schachtman, *et al*., 1998). In addition to the importance of P in plant, the agronomic literature is full of examples of grain, fibre and forage yield increases due to proper maintenance of P fertility. Therefore, Supplementary P has a role in alleviation of the adverse effects of salinity on plant biomass for a variety of crop plants (Kaya, *et al*., 2003). A positive effect of P under saline conditions also has been reported in wheat (Abrol, 1968) and sorghum (Indulkar & More,

***Corresponding Author:**
Dr. Indu Chaudhary;

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1985). Based on these studies, the present investigation was focussed to understand the influence of P on salt stressed black gram plants and to compare the degree of tolerance in two genotypes.

Materials and Methods

The seeds of two black gram genotypes (Azad-2 and PU-31) were obtained from Indian Institute of Pulse Research (IIPR), Kanpur. The pot experiment was conducted in wire house. Seeds were sown in 310 cm^2 area earthen pots lined with polyethene bags and filled with a mixture of garden soil and compost in the ratio of 3:1. In each pot, 10 seeds were sown. The saline solution was made using a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ in the ratio of equimolar basis of various EC levels viz. 0, 4, 6, 8 and 10 dSm^{-1} and two salinity levels with EC 8 dS/m and 10 dS/m were subjected with combination of 40 ppm phosphorus. Salt treatment was begun 15 days after sowing. After 30 days of salt treatment, plants were harvested and leaves were collected for various physiological studies. Chlorophyll was estimated by the method of Arnon (1949), amended by Lichtenthaler (1987). CAT (EC 1.11.1.6) activity was assayed by the modified method of Euler and Josephson (1927).

Statistical Analysis

The experimental data were tested for significance by using least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test was performed with analysis tool from Microsoft office excel 2016.

Results

Figures show the changes in chlorophyll content in both varieties i.e PU-31 and Azad - 2. As it is evident from the data that the response of black gram at different EC levels of saline irrigation water depicted remarkable variation caused by salinity. The content of Chl T, Chl a, Chl b and carotenoids were dropped in PU-31 at all EC levels except 4EC in Azad-2 genotype, while the synthesis of pigment decrease at all EC level. Phosphorus application significantly ($P<0.01$) increased the content of Chl T up to 72.5 and 66.7% treated with 8 and 10EC in PU-31 while in Azad-2 it was 12.2% at 10EC. Similarly, P application raised Chl a significantly of about 27.1% at 10EC in PU-31 and in Azad-2, 25.9% at 8EC. In PU-31, chl b was significantly increased of about 100% and 78.6% at 8 as well as 10EC respectively but in case of Azad-2 it was 20% at 10EC. Carotenoids revealed significant data of about 72.2% at 8EC in PU-31 while it was 12.5% in Azad-2 at 10EC using P. The present study showed that application of P enhanced the content of Chl a, Chl b, Chl T and carotenoids. It was observed that the expression of CAT activity depends on EC level, genotype and P supplementation. CAT activity was significantly ($P< 0.05$) increased in the leaves of PU-31 genotypes treated with 4EC level while decreased at higher levels. However, in the leaves of Azad-2 genotype, CAT activity was gradually decreased as the salinity level increased. But the application of P at 8 and 10EC of both *Vigna* genotypes a significant recovery was observed.

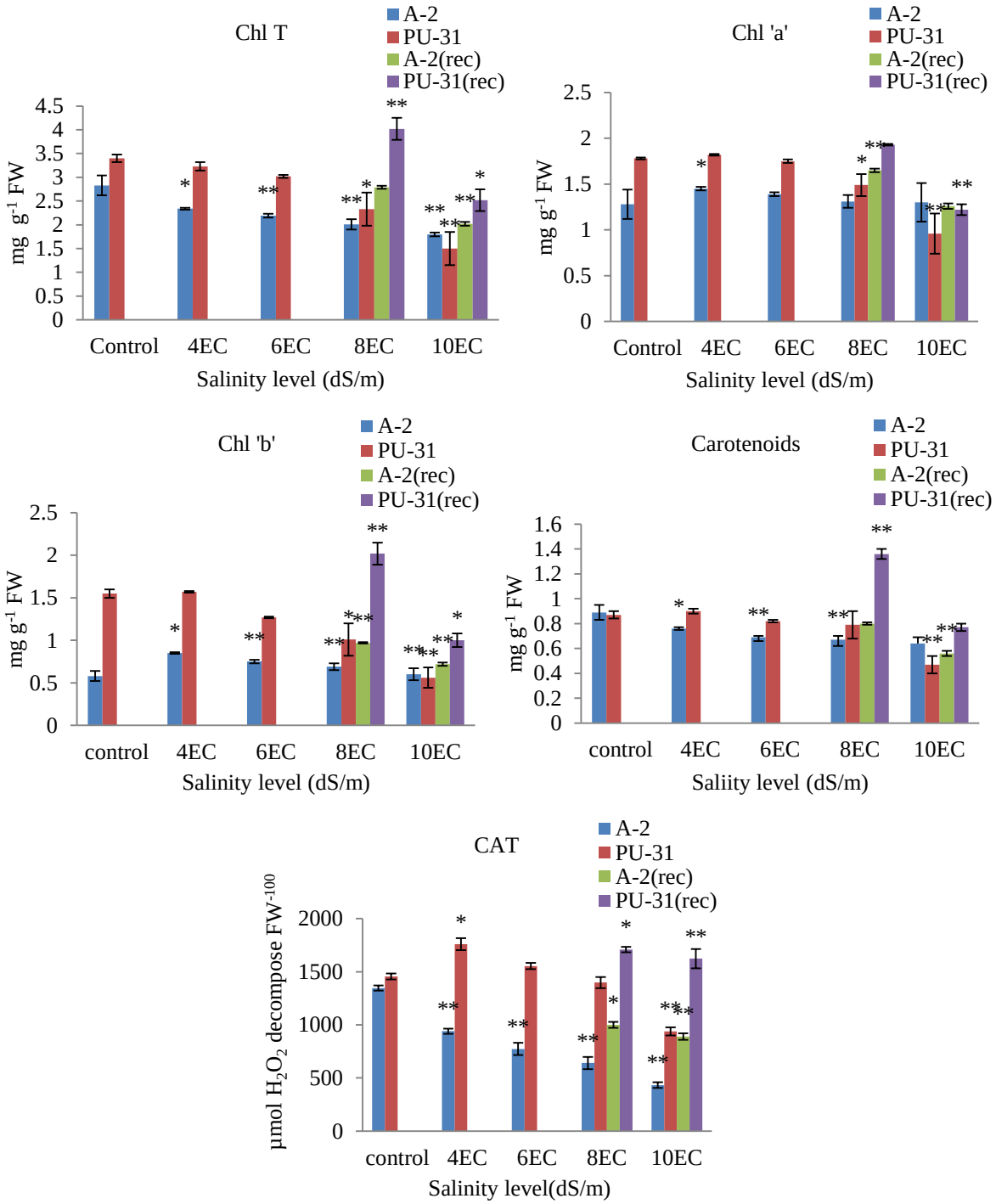

Figure 1: Effect of salinity on Chl T, Chl a, Chl b, Carotenoids and Catalase activity and their remediation with P in *Vigna mungo* (PU-31 and Azad-2) observed at 30 days old plants.

Bars represent ± S.E. Statistically significant (*) at P<0.05 and (**) at P<0.01. rec (recovery with 40ppm P).

Discussion

Salinity stress may induce damage to the photosynthetic apparatus (Yasseen,1983). Reduction in photosynthetic pigments such as Chl a and Chl b has been reported in some earlier studies on different crops,eg., sunflower, *Helianthus annuus* (Akram and Ashraf, 2011). This work was supported by Cha-um and Kirdmanee, (2009) in salt-stressed seedlings of two maize cultivars viz. Saccharata and Ceratina. However, researchers summarized the results by showing that reduction in chlorophyll may be

due to variation in its synthesis between the plant species or variation in specific enzymes under saline conditions (Keutgen and Pawelzik, 2007). Carotenoids, being antioxidants, have the potential to detoxify the plants from the effects of reactive oxygen species (Verma and Mishra, 2005). Salinity induced decline in carotenoid contents in both genotypes was observed. These results concur with those found by Gadallah (1999) on P. vulgaris L. and Singh, *et al*., (2008) in maize and wheat genotypes. Carotenoids are considered as shielding pigment because it protects chlorophyll a molecule from solarisation and function as collectors of light energy for photosynthesis. Plants use a number of enzymatic and non-enzymatic antioxidants to prevent oxidative damage and keep reactive oxygen species concentrations within a narrow functional range (Ozgur, *et al*., 2013). The decrease in chlorophyll levels in salt stressed plants has been considered as a typical symptom of oxidative stress. (Smirnoff, 1996). Plants detoxify the ROS species by maintaining the high activities of antioxidant enzymes i.e. SOD, POD, CAT (Sekmen, *et al*., 2012). The activity of antioxidant enzymes has been reported to increase under saline conditions in case of salt tolerant cotton (Meloni, *et al*., 2003). Catalase activity in maize (Azevedo, *et al*., 2006) and *Sesamum indicum* (Koca, *et al*., 2007) was found to be differing under the influence of salt. Accordingly, the above findings confirmed that phosphorus remediates deleterious impact of salinity and averting plants from oxidative damage. CAT scavenges H_2O_2 by breaking it into less reactive gaseous oxygen and water, and increase in its activity is related to an increase in stress tolerance (Agarwal, S. and Shaheen, R. 2007). Phosphorus has a significant effect to reduce the adverse effect of salinity on growth. As phosphorus increased, it ameliorated the effect of salinity on growth of different parts of plants (Knight, *et al*., 1992). These results indicated that deleterious effects of salinity on chlorophyll content can be largely mitigated by supplementary P. These findings were in agreement with Kaya, *et al*., 2001a and 2001b who showed similar effects in cucumber, pepper and tomato.

Conclusion

Plants grown under salt stress exhibited reduced pigment contents and increased CAT activity as compared to control plants. Our findings highlight the profound role of P in salt stress mitigation in crop plant like black gram by improving plant growth and support the efficacy of its use in crop cultivation. The results of this experiment also depict the differences of salt stress tolerance of both *Vigna* genotypes and confirm that PU-31 genotype is relatively more tolerant as comparison to Azad-2 under salinity stress.

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