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Response of antioxidant enzyme activity and pigment content in common bean (*Phaseolus vulgaris* L.) seedlings under salt stress

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Abstract. In order to evaluate the effects of osmotic stress on fresh weight, pigment content and responses of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), a factorial experiment was carried out under laboratory conditions with five common bean genotypes and two salt stress levels induced by NaCl (control and 400 mM). Electrophoretic analyses were performed for three antioxidant enzymes SOD, POX and CAT in leafs of common bean genotype seedlings using 8% slab polyacrylamide gels. For SOD, POX and CAT, three, three and one isozymes were observed, respectively. Salinity was decreased fresh weight and chlorophyll content. Anthocyanins and carotenoids concentration was increased in common bean genotypes. Statistical analysis showed that salinity stress has a significant effect on fresh weight, pigment content and enzymatic activities in common bean seedlings. SOD, POX and CAT activities were increased significantly in the salt stress compared with control condition about 40, 21 and 30.5 percent, respectively. These results seem to indicate that 41218 genotype of common bean tolerance to salt stress is associated with enhance activity of antioxidant enzymes.

Keyword: Antioxidant enzyme, Common bean, Pigment content, Salinity.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops of the legume family and one of the major sources of calories and proteins in human nutrition. Plants growing on land experience a multitude of environmental stresses. These stresses include drought, salinity, waterlogging, extremes of temperature, radiation, mineral deficiency or excess etc. Oxidative stress is one of the various influences caused by salt stress (Ashraf 2009). When molecular O2 undergoes reduction, it gives rise ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$). Singlet oxygen ($^1O_2$), which may arise due to reaction of O_2 with excited chlorophyll, is also considered as one of the potential ROS. ROS are extremely reactive in nature because they can interact with a number of other molecules and metabolites such as DNA, pigments, proteins, lipids, and other essential cellular molecules which lead to a series of destructive processes (Mittler 2002). Stomatal closure resulting from osmotic stress limits CO2 availability for photosynthetic carbon assimilation thereby causing high accumulation of superoxide in chloroplast which cause photoinhibition and hotooxidation can damage therein. ROS in a plant during stress are generated through pathways such as photorespiration, mitochondrial respiration, and from the photosynthetic apparatus (Ashraf 2009). Plants have the ability to scavenge/detoxify ROS by producing different types of antioxidants. Antioxidants can be generally categorized into two different types, i.e., enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase, monodehydroascorbatereductase, dehydroascorbatereductase and glutathione reductase. The commonly known non-enzymatic antioxidants are glutathione, ascorbate, anthocyanin, carotenoids and tocopherols (Gupta et al. 2005).

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The present study was to evaluate the effect of salinity on fresh weight, pigment content and changes in activity profile of some antioxidant enzymes properties of common beans.

2. MATERIAL AND METHODS

2.1. Plant material and salt stress treatment

The seeds of five common bean genotypes were surface sterilized in sodium hypochlorite (2.5% for 30 S) and were washed immediately with large volume of sterile distilled water. Five days old seedlings were then transferred into specially designed dishes containing 1/2 strength sterile Hoagland's nutrient solution with added micronutrients and 0.04 mM ferrous ion as Fe–EDTA, (pH 5.6) as described. The seedlings were grown at 26 °C under 16 h light: 8 h dark photoperiod. Salt stress on white beans was induced by incubating plants in half-strength Hoagland's nutrient solution containing NaCl at a final concentrations of 400 mM for 48 h. Plants grown on half-strength Hoagland's medium without NaClserved as control (NageshBabu andDevaraj 2008).

2.2. Pigment content

Chlorophyll-a, chlorophyll-b, carotenoids and anthocyanins were determined in leaves. About 200 mg weight of fresh leaf was placed in a mortar half full with liquid nitrogen and it was ground to powder. Then, pigments were extracted from the powdered sample by adding 2.0 ml of the extraction solvent 85% acetone and 15% Tris stock buffer (1% w/v Tris final concentration; adjusted to pH 8 with HCl) previously cooled in ice. The extract was centrifuged at 12,000 g for 3 min. A defined quantity of supernatant (1 ml) was removed and diluted to 3.0 ml. Its absorbance was measured at 537, 663, 647 and 470 nm in a 1-cm path length cell (Yaryura et al. 2009).

2.3. Enzymes extraction and electrophoresis

The crude extract of fresh and healthy leaves from adult plants were prepared with separate mortar and pestle in a Tris-HCl extraction buffer pH 7.5 (Tris 50 mM, sucrose 5%, ascorbic acid 50 mM, sodium metabisulfite 20 mM, PEG 2% and 2-Mercaptoethanol 0.1% before use) with a ratio of 1 mg µl-1 and centrifuged at 4ºC and 10,000 rpm for 10 minutes using small Eppendof tubes. Enzyme extracts were immediately absorbed onto 3×5 mm wicks cut from Whatman 3 mm filter paper and loaded onto 8% horizontal slab polyacrylamide gel (0.6×15×12 cm) using TBE (Tris-Borate-EDTA) electrod buffer (pH= 8.8). Electrophoresis was carried out at 4 0C for 3 h (constant current of 30 mA, and voltage of 180 V) (Valizadehet al. 2013).

2.4. Statistical analysis

An image analysis program (MCID software) was used to measure D×A (optical density×area) for each enzyme activity. Data was analyzed by using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA), and is presented as mean ± SE. For treatment showing a main effect, means have compared by Duncan test. $P \leq 0.01$ was considered as significant differences between treatments.

3. RESULT AND DISCUSSION

Analysis of variances for fresh weight, pigment and 7 above mentioned isozymes activities showed that the salinity stress has a significant effect on fresh weight, pigment content and enzymatic activities in common bean seedlings. The differences between genotypes of common bean were significant fresh weight, pigment content and enzymatic activities. Salinity × genotype interactions were significant for fresh weight and all enzymes except one POX (POX2) isozymes (variance analysis not shown).

3.1. Fresh weight

Appearance of common bean genotype seedlings after salt-treated is shown in Fig. 1. Salinity was decreased fresh weight of all common bean genotypes. 41218 genotype of common bean was the higher fresh weight than other bean genotypes (Fig. 2).Salinity is a major environmental factor that limits plant growth and crop productivity, and different crops may have varying salt-tolerant mechanisms (Asishet al. 2004).

3.2. Pigment content

Pigment content of leaves from both stressed and control common bean plants are presented in Table 1. Although salt stress decreased chlorophyll-*a*, chlorophyll-*b* and total chlorophyll but, salinity increased anthocyanin and carotenoids concentration in common bean genotypes. As a general rule, anthocyanins are considered light attenuators and antioxidants. In this context, it is believed that under stress situations, their main function is the quenching of the reactive oxygen species generated by stress (Neill and Gould 2003). The efficacy of light captured to drive photosynthesis is strongly related to the chlorophyll concentration in the leaf. The change in chlorophyll contents was used to evaluate the influence of environmental stress on plant growth and yield. Many studies indicated that high chlorophyll concentrations are associated with improved yield under oxidative stress conditions (Verma et al. 2004).

3.3. Superoxide dismutase

The specific SOD activity was increased in the leaf of common bean seedlings under stress conditions. Increase in enzyme activity coincided with a variable increment in the individual isoform expression. Three isozymes (SOD_1, SOD_2 and SOD_3) were detected (Fig. 3). The most common isoforms of SOD known in the literature are copper–zinc containing superoxide dismutase (Cu/Zn-SOD), manganese containing (Mn-SOD) and iron containing (Fe-SOD) (Gaber, 2010).

The ability of plants to overcome oxidative stress partly relies on the induction of SOD activity and subsequently on the up regulation of other downstream antioxidant enzymes (Alscher et al. 2002). Increment in the SOD activity may be attributed to the increased production of the superoxide ($O_2^{\bullet-}$) as substrate that lead to induced expression of genes encoding SOD. In red common bean seedlings an increase in SOD activity was detected under salinity stress (Moharramnejad and Valizadeh, 2014). Higher SOD activity in 41218 compared with other common bean genotypes can also be explained by less efficiency susceptible group in scavenging of $O_2^{\bullet-}$ under oxidative stress conditions (Fig. 4).

3.4. Peroxidase and Catalase

Three and one isozymes were detected for POX and CAT in leaf of common bean genotypes, respectively (Fig. 3). POX, APX and CAT are three important H2O2 scavenging antioxidant enzymes functioning in different sub cellular compartments (Noctor and Foyer 1998). Increase in POX and CAT activity in leaves of common bean genotypes (Fig. 4). The result indicates that in salt stress, mean activities of SOD, POX and CAT isozymes are significantly higher than normal conditions (Fig. 4). Similar increases in the activities of SOD, POX and CAT have been reported in French bean (NageshBabu and Devaraj 2008) and alfalfa (Wang et al. 2009). Significant roles of POX have been suggested in plant development processes, which was involved in scavenging of H_2O_2 produced in chloroplast (Gaber 2010). Increases in the activities of CAT have been reported in soybean (Combaet al. 1998).

4. CONCLUSION

Evaluation of effect of relatively high salinity (400 mMNaCl) on common bean genotypes showed that salt stress decreased fresh weight and chlorophyll content but, increased anthocyanins, carotenoids concentration and antioxidant enzymes activity. These results seem to indicate that 41218 genotype of common bean tolerance to salt stress is associated with enhance activity of antioxidant enzymes.

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Figure 1. Effect of salt stress (48 h) on growth of common bean seedlings.

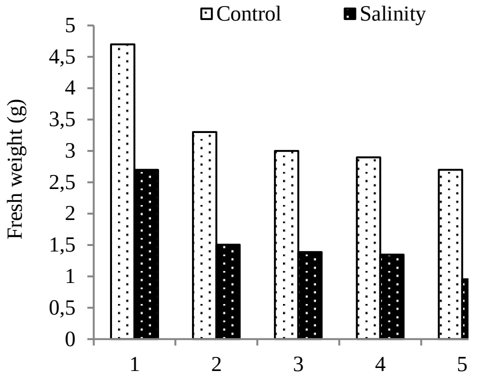


Figure 2. Fresh weight of common bean seedlings under salt-treated.

Table 1. Mean comparison pigment contents in common bean seedlings under control and salt stress (400 mM) conditions

Pigment (μmol g^{-1} fresh weight)	Control	Salinity
Carotenoid	1.110±0.106[b]	1.166±0.184[a]
Anthocyanins	0.097±0.001[b]	0.102±0.005[a]
Chlorophyll-a	1.331±0.115[a]	1.229±0.146[b]
Chlorophyll-b	0.634±0.052[a]	0.600±0.112[b]
Chlorophyll a/b	2.116±0.014[a]	1.099±0.080[b]
Total chlorophyll	1.966±0.171[a]	1.900±0.254[b]

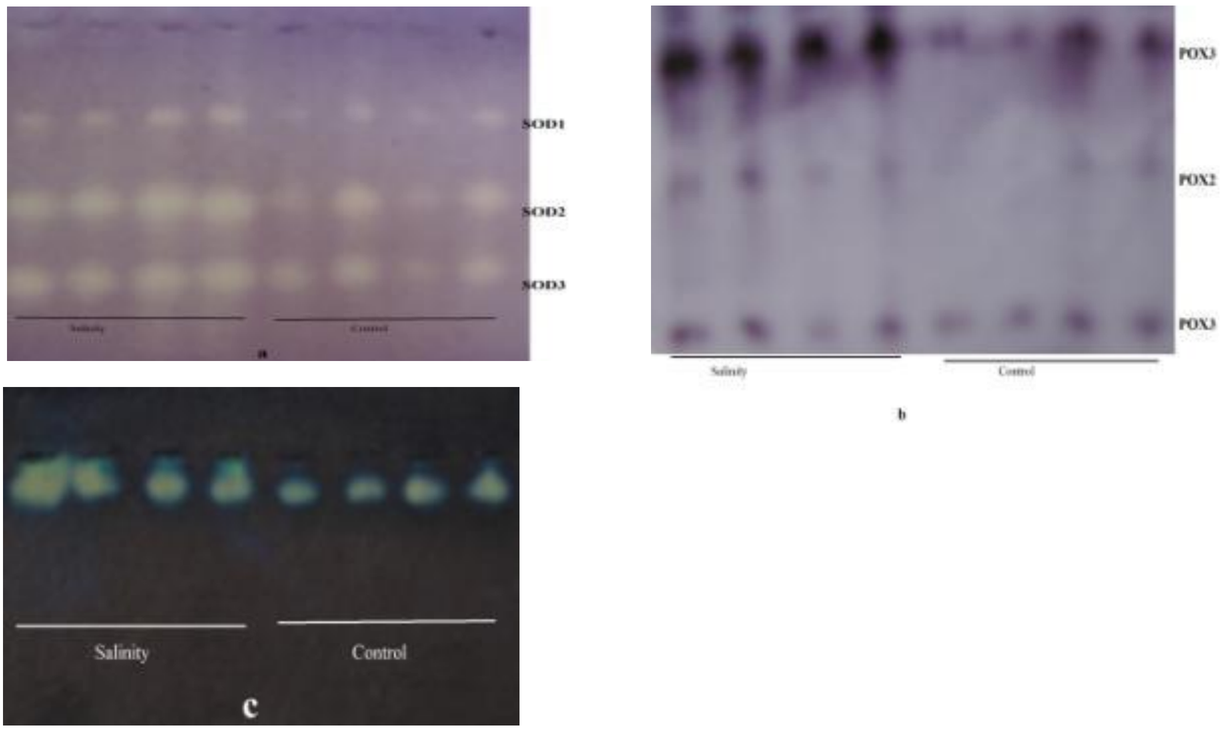
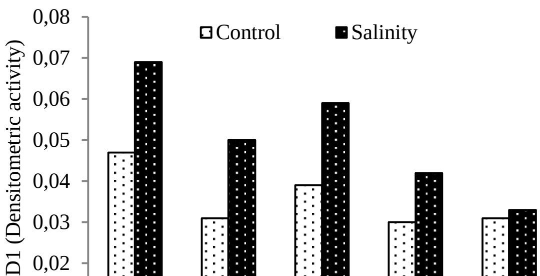
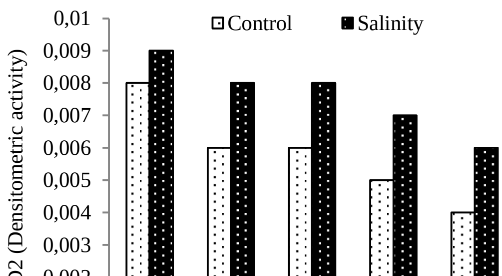


Figure 3. Example of isozyme pattern and relative activity of SOD (a), POX (b) and CAT (c) in the leafs of common bean seedlings for two stress conditions





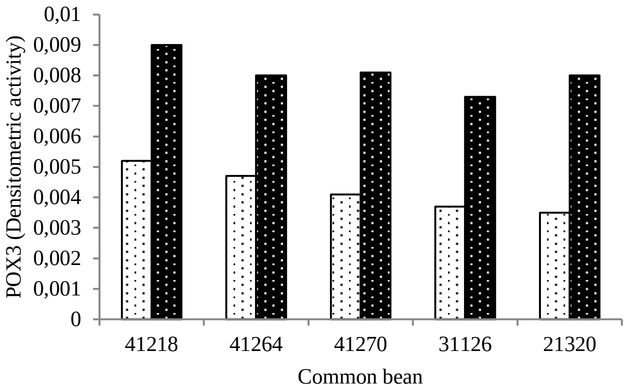


Figure 4. SOD_1, SOD_2, SOD_3, POX_1, POX_3 and CAT of common bean seedlings under control and salt stress conditions