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Research Article

EFFECT OF NaCl INDUCED SALT STRESS ON BIOCHEMICAL PARAMETERS OF *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.

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ABSTRACT
Salinity is one of the major abiotic stresses which limits growth and productivity of plants. The effects of NaCl induced salt stress on the activities of various key enzymes (guaiacol peroxidase, catalase, α-amylase) and total protein content was analyzed *in vitro* in two genotypes (RGC- 1002 and RGC-197) of *Cyamopsis tetragonoloba*. The biochemical results have shown that both the varieties of *C. tetragonoloba* respond well under *in vitro* salt stress by modifying the various biochemical pathways to prevent salt induced oxidative damage.

Keywords: Salinity stress, NaCl, *Cyamopsis tetragonoloba*, Guaiacol peroxidase, Catalase, α-amylase, Soluble proteins

INTRODUCTION
Salinity is a major limiting factor to crop productivity in the world especially in semiarid regions. Salinity adversely affects plant growth and development, with nearly 20% of the world cultivated area and about half of the world irrigated lands being affected by salt stress (Sairam and Tyagi, 2004). This problem is more acute in arid and semiarid regions with low rainfall, which adversely contribute to increase soil salinity (Viégas *et al.*, 2001). In these areas, the problem of soil salinity is aggravated by the use of low quality water in irrigation associated with inadequate methods of soil management (Ferreira-Silva *et al.*, 2009). Increased concentration of salt ions in plants causes osmotic stress, ionic toxicity, nutritional deficiencies and inhibition of various biochemical and physiological processes (Munns, 2002). High salt concentration disrupts homeostasis in water relations and changes the ion distribution at cellular level (Bastías *et al.*, 2004). Altered water homeostasis ultimately leads to plant death.
Cyamopsis tetragonoloba (L.) Taub (Leguminosae), popularly known as cluster bean in English and Guar in Hindi, is a multipurpose crop grown in India and Pakistan for feeding, green fodder, vegetable, green manure and grain purposes. It is one of the important crops for the climatic conditions of Rajasthan because of its drought-tolerant nature (Kherawat *et al.*, 2013). The crop is mainly cultivated under water limiting conditions in arid and semi-arid regions of Rajasthan. Understanding the biochemical mechanisms for plant salinity stress tolerance is still a major challenge in biology and agriculture to identify suitable traits at early stage that would support plant breeders in development of salt tolerant crop plants. Adaptation to salinity stress is associated with metabolic adjustments that lead to the modulation of different enzymes and metabolites. A complete understanding on how plants response to salt stress at biochemical, physiological and molecular level is essential for the development of salt-tolerant varieties of crop plants. Therefore, the present study was carried out to identify the effects of NaCl-induced salt stress on various key enzymes and soluble protein in two varities (RGC-1002 and RGC-197) of *C. tetragonoloba*.

MATERIALS AND METHODS
Plant materials and Salt treatment: Seeds of two genotypes RGC- 1002 and RGC-197 of cluster-bean were obtained from Agriculture Research Institute, (Durgapura, Jaipur). These seeds were then surface sterilized with 0.1% $HgCl_2$ for 3-5 min. followed by a dip in 90% ethanol for 30 seconds. The explants were inoculated vertically on plain MS medium (Murashige and Skoog, 1962). Salt stress was provided

by adding different concentration (0.3%, 0.5 %, 0.7 %, 0.9% and 1.0%) of NaCl into MS medium. MS medium without NaCl was used as control. Leaves from 12 days plantlets were harvested for biochemical assay.

Sample preparation: Leaf samples were macerated in cold pestle mortar by adding 50 mM potassium-phosphate buffer (pH 7.4) to fine slurry and then centrifuged at 10,000 g for 20 minutes at 40 ^{o}C. The supernatant thus collected was used for enzyme assay and protein estimation. Results are the averages of three replicates.

Biochemical assay: Guaiacol H_2O_2 method was used for assaying the activity of guaiacol peroxidase (Racusen and Foote, 1965). Catalase activity was determined by the method of Teranishi *et al.* (1974). Bernfeld's method (1995) was used for assaying the activity of α- amylase. Bradford's method (1976) was used for the estimation of soluble proteins.

RESULTS AND DISCUSSION

Higher amount of salt in soil inhibits plant growth and development. Salt stress causes disruption of ionic equilibrium, inhibition of enzymatic activity, osmotic imbalance, membrane disorganization, inhibition of cell division and expansion, reduction in photosynthesis and production of reactive oxygen species (ROS). Reactive oxygen species (ROS) production is a normal biochemical event that occurs in plants. The ROS are generated during normal cellular metabolism, but there is evidence that ROS production is increased when plants are exposeded to biotic or abiotic stresses. ROS cause oxidative damage to nucleic acids (Imlay, 2003). In the present study, activity of two ROS scavenging enzymes guaiacol peroxidase and catalase were analyzed in RGC- 1002 and RGC-197 of cluster-bean growing under *in vitro* salt stress (Fig.1 A-B). Peroxidase activity increased continuously in the leaves of *C. tetragonoloba* plants subjected to salt stress conditions under *in vitro* condition (Fig. C, D). The variety RGC-1002 showed the high expression of peroxidase enzyme as compare to the RGC-197. Highest activity of peroxidase was observed in *C. tetragonoloba* RGC-1002 cultured on MS medium containing 0.9 % NaCl. Increased peroxidase activity caused by salt-induced oxidative stress is well reported (Lin and Kao, 2002), and appears to be caused either by overexpression of genes responsible for peroxidase expression (Mittal and Dubey, 1991). .

Catalase is involved in the degradation of hydrogen peroxide into water and oxygen, is the most effective antioxidant enzymes in preventing oxidative damage. In the present study, catalase activity was significantly increased in both the varieties of cluster bean (Fig. E, F). Highest catalase activity was observed in RGC- 1002 growing on 0.5% NaCl. RGC-1002 had higher activity of catalase enzyme than RGC-197. High salt stress significantly declined the catalase activity in both the genotypes of cluster bean. The increase activity of catalase is an adaptive trait to overcome salt damage by reducing toxic levels of H_2O_2 and provide protection against oxidative stress (Chawla *et al.*, 2013).

Activity of α-amylase declined slightly till 0.7% NaCl treatment and declined sharply thereafter in variety RGC-1002 plants subjected to salt stress (Fig. G). In *C. tetragonoloba* RGC-197 α-amylase activity was continuously decreased under *in vitro* salt stress conditions (Fig. H). Similarly, under salinity, decreased α-amylase activity has been noted in various crop plants such as *Hordeum distichum* (Tipirdamaz *et al.*, 1995), *Triticum aestivum* (Siddiqui *et al.*, 2006), *Triticum durum* (Almansouri *et al.*, 2001), *Vigna radiata* (Promila and Kumar, 1998) and *Cicer arietinum* (Kaur *et al.*, 2000).

The soluble protein contents initially increased in variety RGC-1002 of *C. tetragonoloba*. A drastic reduction in soluble protein contents was observed when RGC-1002 varieties were cultured on medium containing more than 0.5 % NaCl (Fig. I). On the other hand, no significant effect on total protein content was observed in variety RGC-197 when cultured up to 0.5% NaCl (Fig. J). A slight increment in total protein content was observed in RGC- 197 variety of cluster bean growing on nutrient medium containing 0.7% NaCl. Higher concentration of NaCl reduced the concentration of total protein content in variety RGC-197. Decrease in protein content under salt stress might be due to the effect on the protein biosynthesis machinery.

The present work suggests that both the varieties (RGC-1002 and RGC-197) of *C. tetragonoloba* respond under salt stress by modifying the various biochemical pathways. Rapid accumulation of protective antioxidative enzymes (peroxidase and catalase) suggests that both varieties response well to minimize the adverse effects of salt induced oxidative stress.

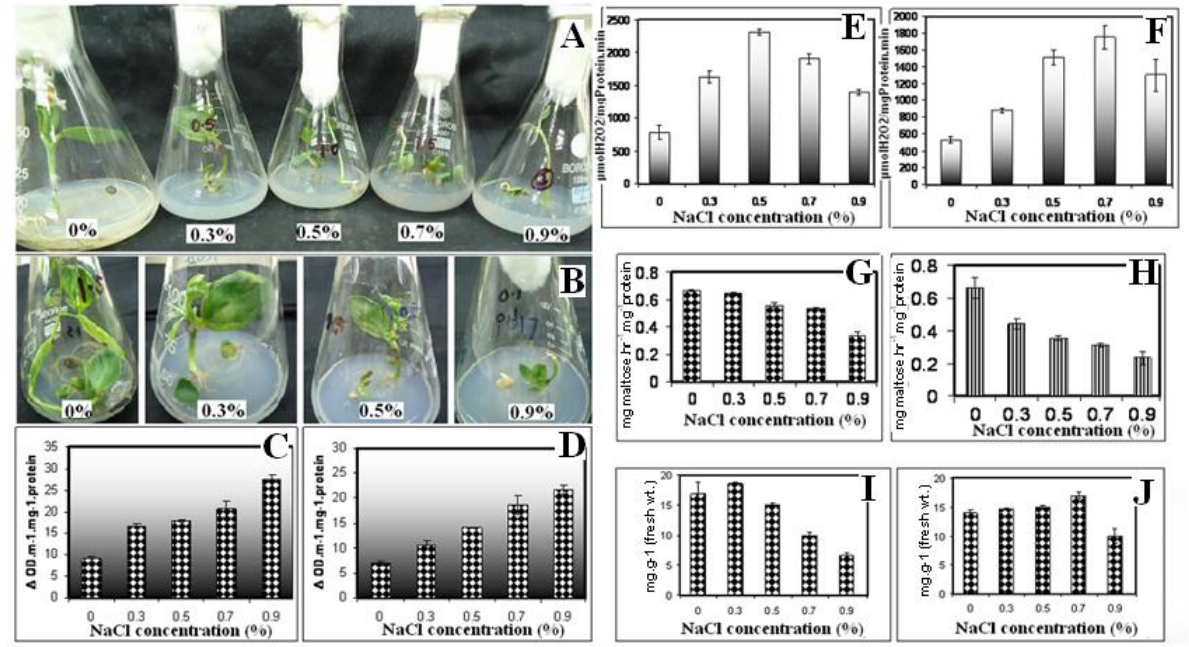

Figure 1: (A) *C. tetragonoloba* variety RGC-1002 (A) and RGC-197 (B) growing under *in vitro* salt stress; Salinity induced changes in Guaiacol peroxidase in RGC-1002 (C) and RGC-197 (D); Catalase in RGC-1002 (E) and RGC-197 (F); α-amylase in RGC-1002 (G) and RGC-197 (H); and Soluble protein in RGC-1002 (I) and RGC-197 (J)

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