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Progressive changes in oxidative enzymes and some biochemical constituent of chickpea genotype under salinity stress

RESHMA C. WADEKAR, S.S. KARANDE, NEELISHA HADWALE AND R.M. JADHAV

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SUMMARY

A pot culture experiment was conducted to study the progressive stress responses and mechanism of salinity stress tolerance in chickpea genotype. Two chickpea genotype (one tolerant and one susceptible) CSG-8962 and Vijay of different adaptation were taken for the study in control and saline stress (150mM Nacl) condition at interval of 7,14 and 21 days . The sample was analyzed for the levels of peroxidase and polyphenol oxidase enzymes and some of the key biomolecules like reducing sugars, soluble protein, proline, polyphenol and free amino acids to find out the biochemical markers involved in identifying the salt tolerance in chickpea cultivar. The result reveled that the activity of oxidative enzyme peroxidase and polyphenol oxidase, proline, free amino acids and polyphenol were found to be increased comparatively higher in salt tolerance cultivar than the susceptible cultivar under the salinity stress situation. Where the soluble protein content in salt susceptible cultivar decreased with salinity stress.

Key words : Chickpea, Peroxidase, Polyphenol oxidase, Proline, Soluble protein, Polyphenol reducing sugar, Free amino acids and salinity stress

Chickpea (*Cicer arietinum* L.) is the world's second most important pulse crop next only pigeonpea and cultivated in more than 41 countries occupying approximately 15 present of the total pulse area in the world (Datta, 2002). Among the various stress condition salinity causing losses quantitatively in chickpea is one of the major. Chickpea is cool-season legumes grown extensively through out world (12.03M ha), particularly in the India subcontinent, countries of North Africa, North America, West Asia and Mediterranean region (Anonymous, 1999). In India, it is grown on 8.40 Mha areas. Legumes have been found to be highly susceptible to words saline environment, which ultimately manifest in reduced growth and metabolism. Abiotic stress is known to disturb the intracellular water balance of the biological organisms. To counteract such condition, plant accumulates various low molecular weight compounds such as sugar, sugar alcohols amino acids and quaternary ammonia compounds. These metabolites, widely known as compatible solutes or osmolytes are neutral, non-toxic and do not interfere with normal metabolic reaction even in high concentration (Pujni *et al*., 2007). Salt tolerance mechanisms include array of characters, alterations and progressive adaptation in tolerance type and any flow that reflect on the metabolic failures resulting in sensitive behavior (Vasantha and Rajalakshmi, 2009). With these objective of studying progressive changes in chickpea and experiment was designed with tolerant and susceptible variety.

Correspondence to:
RESHMA C. WADEKAR, Department of Biochemistry and Molecular Biology, College of Agricultural Biotechnology, LONI (M.S.) INDIA
Authors' affiliations:
S.S. KARANDE AND R.M. JADHAV, Department of Plant Biotechnology, College of Agricultural Biotechnology, LONI (M.S.) INDIA
NEELISHA HADWALE, Department of Animal Biotechnology, College of Agricultural Biotechnology, LONI (M.S.) INDIA

MATERIALS AND METHODS

The chickpea cultivar CSG-8962 and Vijay are available at all India Co-ordinated Pulses Improvement Project MPKV Rahuri, were used for the present investigation. The seeds were surface sterilized with aqueous solution of 0.1% mercuric chloride and then thoroughly washed with distilled water. The seeds were soaked in cold water and placed in germinating paper for even germination for 2 days. Uniformly germinated seed were sowed in pots filled with soil. Two replication were maintained for each cultivar and treatment. When the seedling reaches 3-4 leaf stage salinity stress of 150mM NaCl was imposed. The shoot of both cultivar were analyzed at 7, 14 and 21 days after imposition of salinity treatment. During the growing of plant the water level was maintained with distilled and saline water respectively. The fresh shoot samples were collected for analysis of biochemical analysis. The proline contains in the sample were determine by the Bates *et al*. (1975). The soluble protein was determination by the procedure of Lowry *et* *al*. (1951). The free amino acids were estimated by method of Rosen (1957). Reducing sugar was determine by Nelson Somagy (1944). Peroxidase and polyphenol oxidase enzyme activity was measured spectrophotometricaly by monitoring the increase in absorbance at 420 nm and polyphenol by Swain and Hills, (1959).

RESULTS AND DISCUSSION

The data presented in fingers on change in the oxidative enzymes and biochemical constituent under salinity stress in chickpea genotypes. The oxidative enzymes *viz*. peroxidase and polyphenol oxidase were increased under the influence of salinity. Peroxidase activity was increase from 7 to 21 days (Fig.1) and increased was more in tolerant cultivar than susceptible. A significant increase in peroxidase activity influence of salinity was recorded for wheat and rice (Sgherri *et al*.,2000 and Srivalli ., 2003), Polyphenol oxidase activity increased under influence of salinity. The varietal behavior different under salinity. In CSG-8962 , polyphenol oxidase activity increased gradually from 7 to 21 days (Fig. 2) while in Vijay an increase was slow . The result is similar with Hernandez *et al.* (2000).

Proline is a compatible osmolyte that accumulate in greater proportion under abiotic stress. Proline contain increases in all days under salinity in both varieties (Fig. 3) but increase was higher in salt tolerant cultivar CSG 8962 as compare to Vijay. Same trend was observed in sugarcane cultivar under salinity stress (Vasantha and Rajalakshmi, 2009) in the sugarcane cultivar. Salt tolerant cultivar of barley accumulated over 80% proline than control and the levels increased only 40% in a sensitive cultivar (Kumar *et al*., 1981). Damame *et al*. (2008) also proposed that the higher increase in proline content was stimulated by salt in the tolerant cultivar than susceptible in chickpea. Proline accumulation remained higher under stress condition and accumulation helps to maintain turger and promotes continued growth under moisture stress condition (Pankaj kumar *et al*., 2006).In the present study increased trend of proline content with the stress suggests it protective and stabilized role under stress.

The soluble protein content was significantly decreases in salinity condition and decrease was more in Vijay cultivar (Fig.4). On other hand, soluble protein content in salt susceptible cultivar decrease in salinity condition, these result are in agree with those of Garg, (2002) and Agarwal and Pandey (2003). The polyphenol content was increase in both cultivar in both control and saline condition but the increase was higher in tolerant cultivar in saline situation (Fig.5). Damame *et al*. (2008) reported induced salinity increases polyphenol content in chickpea seedling.

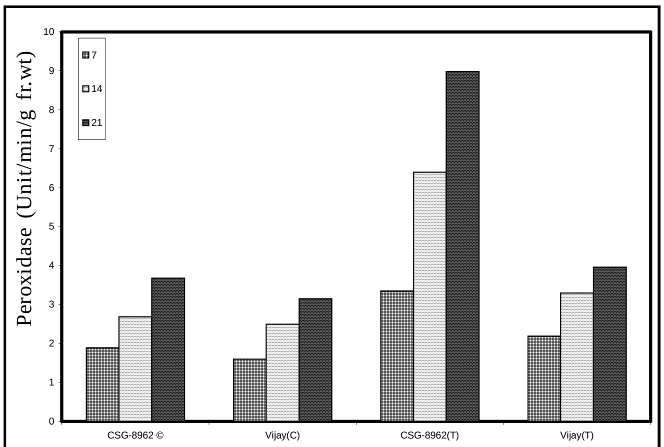

Fig.1 : Prograssive change in peroxidase activity in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

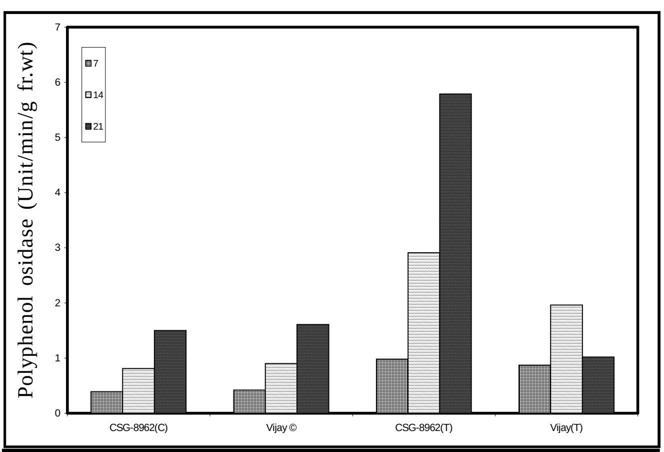

Fig.2 : Polyphenol oxidase activity in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

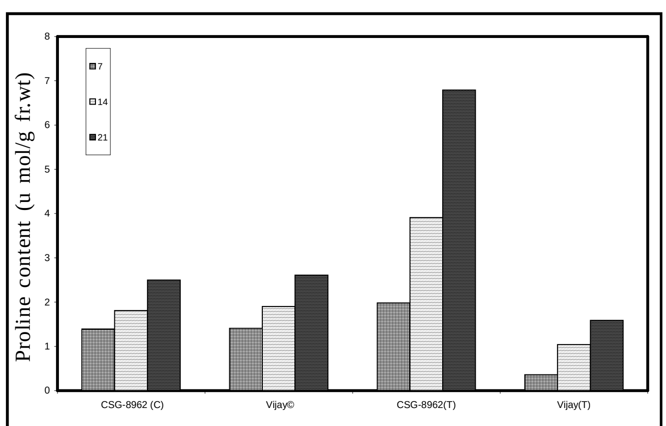

Fig.3 : Proline contein in chickpea as influence by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

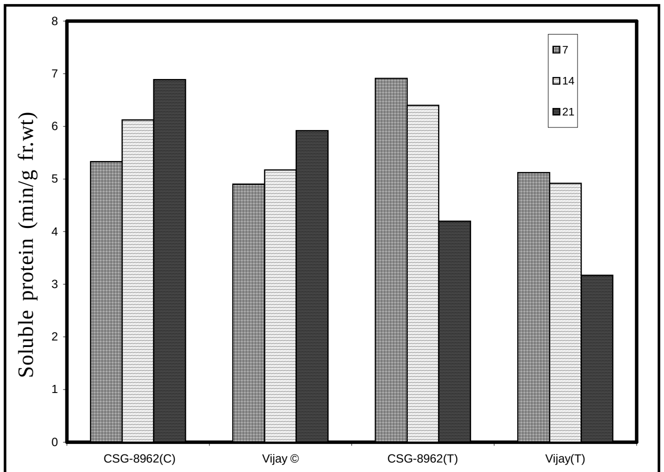

Fig.4 : Soluble protein in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

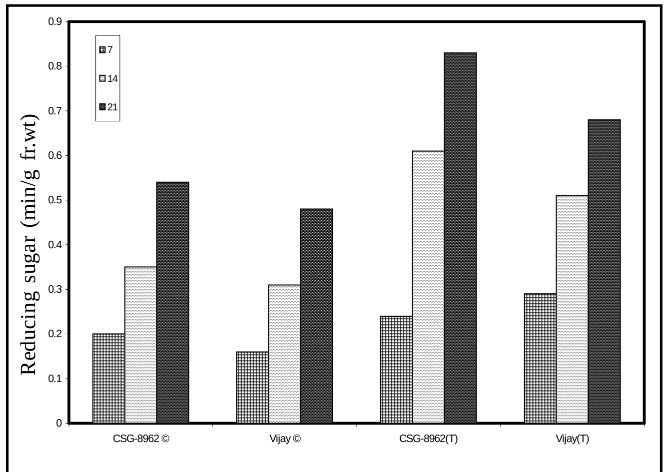

Fig.6 : Reducing sugar conten in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

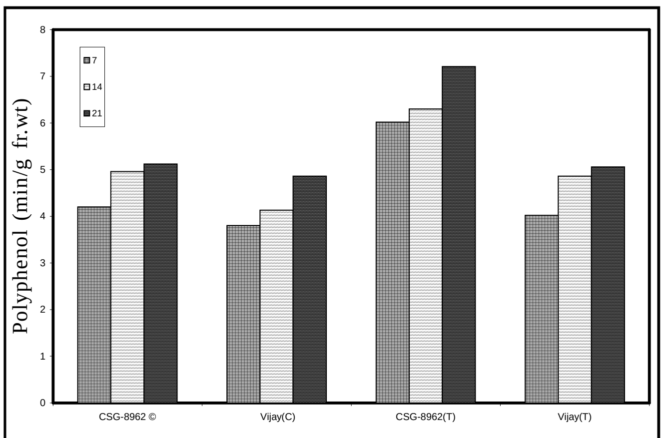

Fig.5 : Polyphenol activity in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

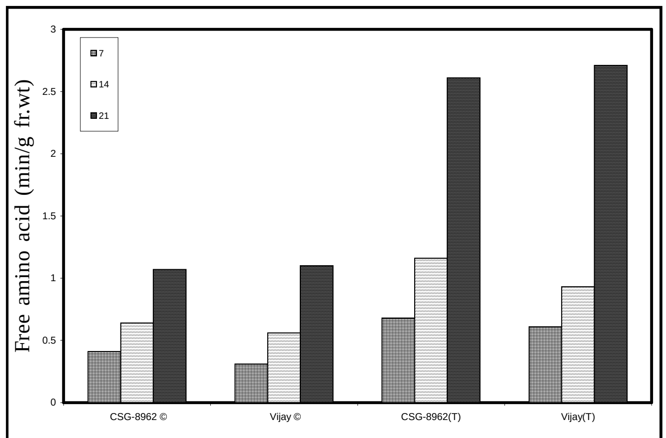

Fig.7 : Free amino acid activity in chickpea as influences by salinity at 7, 14 and 21 days, C and T indicate control and salinity treatment

The reducing sugar was higher in tolerant cultivar CSG-8962 in salinity situation, but the increase was observed in both cultivars in both situations(Fig.6). Cram, (1976) was view that of the major osmotic adjustment in glycophytes subject to salinity environment. Osmotic adjustment in plant exposed to salinity stress depends largely on soluble sugar. The accumulation of sucrose in plants has been widely reported as a response to salinity or drought (Ashraf, and McNeilly, 2004). Free amino acid content was also increase in both cultivar in both situation but it was higher in susceptible cultivar in both situation but was higher in susceptible cultivar(Fig.7). Free amino acid play vital role in salinity stress and free amino acid are known to be increases significantly in 8 canola line with increase in salt concentration of the growth medium is reported by Qasim, (2000) is similar with present study.

It can be concluded from the present study that the oxidative enzymes peroxidase and polyphenol oxidase and biochemical constituent like proline, soluble protein, reducing sugar, polyphenol and free amino acid appears to be a predominant biochemical indicator in chickpea genotype to study the tolerance mechanisms.

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