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PROLINE ACCUMULATION, WATER STATUS AND CHLOROPHYLL CONTENT IN LEAF IN RELATION TO SALT TOLERANCE IN SOYBEAN

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SUMMARY

An experiment was conducted to study the effect of salinity on proline accumulation, water status and chlorophyll content in leaf of a salt - tolerant (AGS 313) and a salt - susceptible (Shohag) soybean (*Glycine max* L) genotypes. The plants were grown in pots inside a vinylhouse with 50 and 100 mM NaCl solutions and tap water as a control. Leaf proline accumulation, water uptake capacity (WUC) and concentration of chlorophyll a, b and total chlorophyll of the two genotypes were compared at 15, 30, 45 and 60 days after treatment imposition. Marked variation in accumulation of proline was found between the two genotypes differing in salt tolerance. Proline accumulation was increased with the increasing salinity levels and with duration in both the genotypes. AGS 313 accumulated higher amount of proline under salt stress condition than that of Shohag at all the growth stages. Salinity also increased WUC in both AGS 313 and Shohag. The relative increase in WUC (% of the control) was greater in Shohag than that in AGS 313, which indicated that Shohag suffered more with water deficit than AGS 313. The chlorophyll a, b, and total chlorophyll in leaves of the two genotypes were decreased greatly with the increase in salt concentrations and with duration of exposure to salinity. The relative reduction (% of the control) of chlorophyll content was higher in Shohag than that in AGS 313. It was concluded that relatively higher amount of proline in AGS 313 had a greater osmoregulatory role in maintaining relatively lower WUC and higher leaf chlorophylls than those of Shohag.

Key words: **Chlorophylls, proline, salt tolerance, soybean, water uptake capacity.**

INTRODUCTION

Most of the leguminous species belong to the salt sensitive group of crops (Maas and Hoffman 1977). Soybean (*Glycine max* L. Merril), an important protein rich leguminous crop, suffers from a marked decrease in growth under salt stress conditions (Khan and Varshney 1986). Excessive salts in soil adversely affect the crop growth and yield (Munns and Tester 2008). However, magnitude of the effects of salinity varies with plant species, and types and levels of the salinity (Bishnoi *et al*. 1987). Salinity is also considered as the major abiotic stress that restricts the economic and efficient utilization of available land resources, by affecting adversely soil fertility, and crop productivity and quality (Viswanathan *et al*. 2005). Salts present in the ambient root environment generally alter a wide array of metabolic processes in plant culminating in stunted growth. Ashraf and Rasul (1988) reported that salinity reduced significantly leaf chlorophylls and also the protein and

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carbohydrate contents in different plant parts of mungbean. However, marked genotypic differences in salinity tolerance were also reported for different crops (Blum 1988, Raptan *et al*. 2001a, Aziz *et al* 2005, Sultana *et al*. 2007).

Salinity tolerance is a complex phenomenon that involves many physiological and biochemical processes (Greenway and Munns 1980). It is reported that the tolerant genotypes very often maintain better water relations for osmotic adjustment, lower amount of toxic ion Na^+, and higher photosynthetic activity than the susceptible ones (Lauchli and Wieneke 1979, Greenway and Munns 1980, Raptan *et al*. 2001b). However, the role of proline- a known compatible osmoticum found in plants under heat and drought stress (Blum 1988), on salinity tolerance of grain legume needs to be well clarified in the long term systematic studies. The present study was undertaken to analyze the magnitude of changes in proline accumulation, water status and chlorophyll content in leaf due to salinity in two soybean genotypes, differing in salinity tolerance, in order to understand the salt tolerance mechanisms of this crop.

MATERIALS AND METHODS

The seeds of soybean (*Glycine max* L.) genotypes AGS 313 and Shohag were sown in plastic pots of 24cm x 30cm in size, containing 12 kg air dried soil, inside a vinylhouse under natural light at the Environmental Stress Research Site of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh. In a preliminary experiment the genotype AGS 313 was found more salt tolerant in terms of growth than the genotype Shohag. When the 1st trifoliate appeared, 1000 ml of 0, 50 mM and 100 mM NaCl solutions were applied in each pot up to 60 days. Salt solutions were prepared by dissolving calculated amount of commercially available NaCl with tap water. Tap water was used as the control. The salt solution was applied stepwise with an increment of 25 mM in every alternate day till respective concentrations were attained. Treatment solutions were applied in excess so that the extra solution dripped out through the holes made at the bottom of the pots. Uppermost fully developed leaves were collected at 15, 30, 45, and 60 days after treatment imposition to measure proline, water uptake capacity and chlorophyll content. Four leaves from four separate plants in each treatment were considered for each measurement of the parameters. Leaf proline was estimated according to Bates *et al*. (1973). Water uptake capacity of leaf was measured using the following formula:

$$\text{Water uptake capacity (WUC)} = \frac{\text{Turgid weight} - \text{Fresh weight}}{\text{Dry weight}}$$

Chlorophylls were estimated and expressed as mg g^{-1} fw using the following equation (Witham *et al*. 1986):

$$\text{Chlorophyll a} = [12.7\ (D_{663}) - 2.69\ (D_{645})] \times [V/1000 \times W]$$

$$\text{Chlorophyll b} = [22.9\ (D_{645}) - 4.68\ (D_{663})] \times [V/1000 \times W]$$

$$\text{Chlorophyll (total)} = [20.2\ (D_{663}) - 8.02\ (D_{645})] \times [V/1000 \times W]$$

RESULTS AND DISCUSSION

Proline accumulation: Proline accumulation in the NaCl treated plants of the two soybean genotypes was increased with the increasing in plant age till 60 days (Fig. 1). Under control condition, no significant difference in proline accumulation between Shohag and AGS 313 was observed, though Shohag accumulated a slightly higher amount of proline than that of AGS 313. Proline accumulation increased with the increase in salinity levels and with the duration in both the genotypes. However, the rate of increase was higher in the salt tolerant AGS 313 than that of salt sensitive Shohag. Accumulation of proline in response to salinity was also observed by Weimberg *et al.* (1982) in sorghum and Reddy and Vora (1983) in bajra. The accumulation of proline during salt stress was probably due to the consequence of reduction in cell osmotic potential for the maintenance of osmotic balance between cytoplasm and vacuole (Flowers *et al.* 1977). The higher amount of proline accumulation in AGS 313 was associated with the better salt tolerance mechanisms of this genotype than that of Shohag.

Water uptake capacity: Water uptake capacity (WUC) of a plant indicates the amount of water required to reach turgid weight (Sangakkara *et al.* 1996). A higher

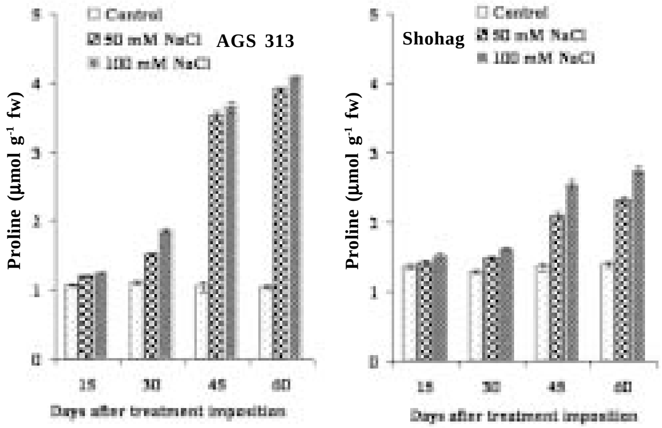

Fig. 1. Effect of salinity on proline content (µ mol g^{-1} fw) of leaf in salt tolerant (AGS 313) and salt sensitive (Shohag) soybean genotypes at different days after treatment imposition. Bars represent standard error (± SE)

value of WUC of a plant means the plant suffers to a greater degree from moisture stress than the plant with a lower WUC. Salinity increased the WUC in both the genotypes as compared to the plants under control, and the WUC increased with the increase in duration of plant exposure to salinity. Between the two genotypes, the susceptible Shohag had higher value of WUC under saline conditions than that of salt tolerant genotype, AGS 313 (Fig. 2). The higher WUC in Shohag indicated that this genotype suffered more from water stress aroused from salinity effect than the genotype AGS 313.

Chlorophyll content: At all the levels of salinity, AGS 313 had greater amount of chlorophyll a and total chlorophyll than those in Shohag; though, chlorophyll b was higher in Shohag than that in AGS 313 (Fig. 3, 4 & 5). In general, chlorophyll contents were reduced by the salinity and the reduction was conspicuous at the higher level of salinity in both the genotypes, though variation in the extent of reduction between the genotypes was evident. Reddy and Vora (1983) reported that a decrease in chlorophyll content in salt affected plants might be attributed due to the increased activity of the chlorophyll-

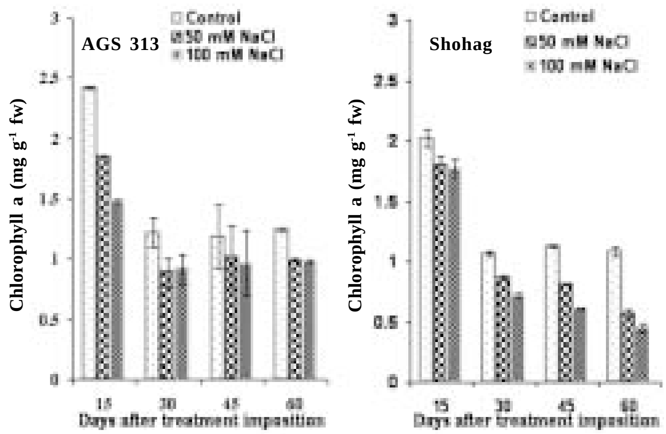

Fig. 3. Effect of salinity on chlorophyll a (mg g^{-1} fw) of salt tolerant (AGS 313) and salt sensitive (Shohag) soybean genotypes at different days after treatment imposition. Bars represent standard error (± SE)

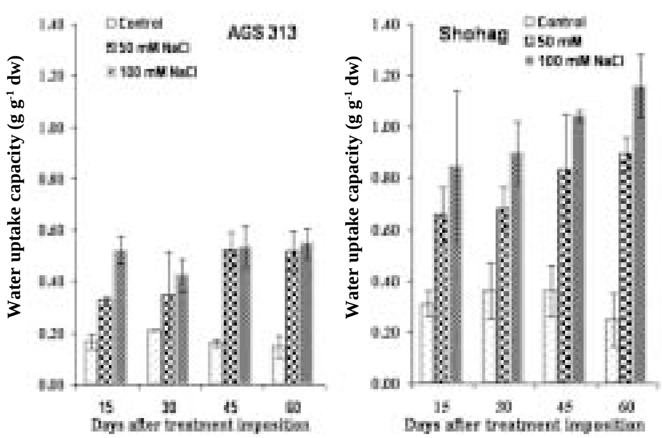

Fig. 2. Effect of salinity on water uptake capacity of salt tolerant (AGS 313) and salt sensitive (Shohag) soybean genotypes at different days after treatment imposition. Bars represent standard error (± SE)

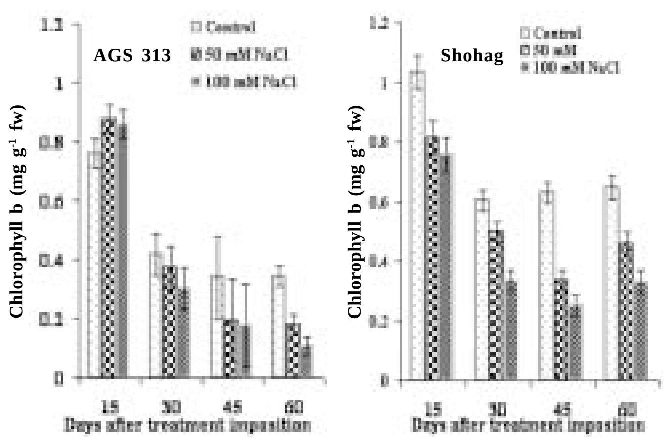

Fig. 4. Effect of salinity on chlorophyll b (mg g^{-1} tissue) of salt tolerant (AGS 313) and salt sensitive (Shohag) soybean genotypes at different days after treatment imposition. Bars represent standard error (± SE)

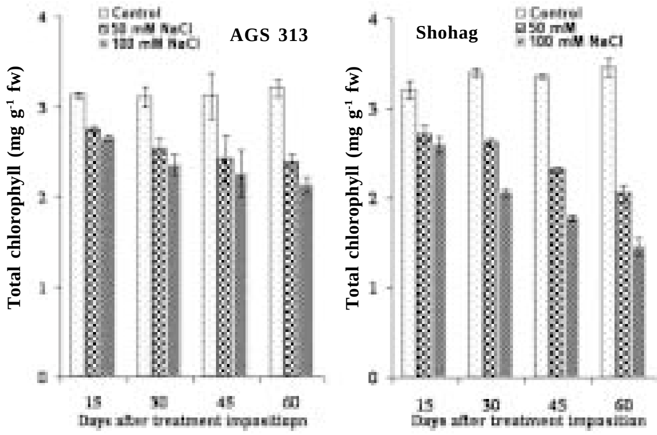

Fig. 5. Effect of salinity on total chlorophyll (mg g^{-1} tissue) of salt tolerant (AGS 313) and salt sensitive (Shohag) soybean genotypes at different days after treatment imposition. Bars represent standard error (± SE)

degrading enzyme chlorophyllase. Salinity induced reduction in chl. a, chl. b and total chl. was also reported in mungbean (Singh *et al*. 1994) and black gram (Ashraf 1989). It has been suggested by Strogonov *et al.,* (1970) that the specific enzyme which is responsible for the synthesis of green pigments was suppressed by the effect of salinity. It has also been suggested by the same workers that the synthesis of total chlorophyll and proportion of its components depended on the biology and developmental stage of the plant, and also on the nature and concentration of salts.

Maintenance of osmoregulation is an important mechanism of salinity tolerance, and tolerant plant usually show better osmoregulation than the susceptible one (Greenway 1973, Greenway and Munns 1980). Higher level of proline accumulation due to salinity plays an important role in maintaining better osmoregulation in AGS 313 under condition of comparatively lower WUC and higher chlorophyll content than the genotype Shohag. Thus, AGS 313 suffered less from the salinity stress than that of Shohag.

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