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CHANGES IN CRUDE PROTEINS, TOTAL AMINO ACIDS AND CHLOROPHYLL CONTENTS OF STYLOS (*Stylosanthes scabra* VOGEL) UNDER SALINE CONDITIONS

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ABSTRACT

NaCl salinity is a major abiotic stress limiting the productivity and the geographical distribution of many plant species. Roots are the primary site of salinity perception. An attempt was made to examine the effect of NaCl salinity on the concentration of crude proteins, total amino acids and chlorophyll contents in Stylos plants. Significant antagonistic changes in crude proteins, total amino acids and chlorophyll contents were recorded under salinity exposures. The percentage of crude proteins content decreased by 52.78% over control at leafy stage and 26.45% at flowering stage, whereas, total amino acids declined by 22.22% over control at leafy stage and 37.14% at flowering stage. The reduction was recorded 7.91% in Chl'a', 5.26% in Chl'b' and 7.08% in total chlorophyll contents in leaves exhibiting the Chl a/b ratio 1.00:0.97. In the plants of flowering stage ,the corresponding values were found 10.40% in Chl'a', 21.57% in Chl'b' and 21.17% in total chlorophyll contents revealing Chl a/b ratio 1.00:1.14. These biochemical parameters may be suggested to act as cumulative index of salt tolerance in Stylos plants.

KEYWORDS : Crude proteins, total amino acids, Chlorophyll contents, *Stylosanthes scabra*, salinity.

Among the range legumes, Stylos (*Stylosanthes scabra* Vogel) has been found to provide good quality of fodder. It has attracted many studies in different countries on its biology and utilization. This pasture legume is currently used as cut-and-carry feed and for grazing cattles. The Indian Grassland and Fodder Research Institute (IGFRI) at Jhansi began evaluation of *Stylosanthes* germplasms in 1974. CSIR, New Delhi, has recognized the potential of *Stylosanthes* in the reclamation of wastelands.

Decreasing protein level in this legume due to salinity has been reported (Sharma et al.,1996; Salem et al.,2002 and Mohamed,2003). Stewart and Larher (1980) have found an accumulation of amino acids in the presence of salinity, leading to a dynamic adjustment of N-metabolism. Varshney (2006) suggested that the increase in free amino acids could contribute to the tolerance of the plants to salt stress through an increase in osmotic potential, or as a reserve of N, principally for the synthesis of specific enzymes.

A number of studies have reported the salinity effects on chlorophyll contents of Stylos plants (Brougham,1960; Billore and Mall, 1976; Misra and Misra,1981).The present investigation therefore, attempts to analyze the biochemical basis of NaCl salt tolerance in Stylos plants. This study is envisaged to be useful in future breeding programmes for developing salt tolerant Stylos plants.

MATERIALS AND METHODS

The certified seeds of *Stylosanthes* were procured from IGFRI, Jhansi. They were then surface sterilized by using 1% $HgCl_2$ for 15 minutes with constant shaking. These were then thoroughly rinsed 3 to 4 times with distilled water. The experiment was laid out in randomized plots (1x1m) in two groups of two each. The seed beds were floored by a polythene sheet at a depth of 40cm to maintain constancy of ions. Seeds of *Stylos germplasm* were sown in all four beds equidistantly. In two beds, the treatment of $4mScm^{-1}$ was given to study the effect of salinity on crude proteins, total amino acids and chlorophyll contents in *Stylosanthes scabra* plants.

Crude proteins were determined by using Micro-Kjedahl's method of Snell and Snell, (1955). Total amino acids composition was determined by HPLC method as described by Cocking and Yemm (1954). To separate the amino acids a Superpac (Pharmacia) ODS-2 column coupled to an LKB dual pump HPLC system (Model 2150), controlled by a gradient generator, model 2152 was used. Data were recorded and processed with an LKB integrator (Model 2221) and expressed in mmol/kg dry matter and mol%. Chlorophyll contents were determined by the method of Brougham (1960) standardized by Varshney and Baijal (1977). Further, Arnon (1949) technique was followed to determine the amount of Chl'a' and Chl'b' by measuring the optical density on a Spectrophotometer Type 127 at 663 nm and 645 nm. The check readings at 652 nm

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Table 1: Crude Proteins and Total Amino Acid Contents of Stylos Plants Under Artificial Salinization

(Values are Averages of 12 Replicates)

Sl. No.	Parameters	Growth stages	Untreated (1.2 $mScm^{-1}$)	Treated (4.0 $mScm^{-1}$)
1.	Crude proteins (mg g^{-1})	I*	11.50	5.43
		II**	7.90	5.81
2.	Total amino acids (mg g^{-1})	I*	0.63	0.49
		II**	0.70	0.44

*Leafy stage; **Flowering stage

For crude protein			
SEm±	0.274	0.274	0.387
CD at 5% P	0.825	0.825	1.167
For total amino acids			
SEm±	0.007	0.007	0.009
CD at 5% P	0.0210	0.0210	0.029

were also carried out and results were expressed as average values. Chla/b ratio was then calculated. The data were analyzed by analysis of variance (ANOVA) method.

RESULTS AND DISCUSSIONS

NaCl salt stress brought about a significant decrease in crude proteins, total amino acids and chlorophyll contents of *Stylos* plants investigated. These parameters are an important feature in determining the nutritional value of *Stylos* plants.

The data shown in Table 1 revealed that under artificial salinity, plants depicted 52.78% decline in crude protein content over control at leafy stage. Similarly, 26.45% reduction was recorded at flowering stage. The values were calculated to be 11.5 mg g^{-1} in control plants whereas 5.43 mg g^{-1} in treated plants at leafy stage and 7.9 mg g^{-1} in control plants whereas 5.81 mg g^{-1} in treated plants at flowering stage.

The total amino acid content also declined with the induction of salinity. At leafy stage, it was found to be 0.63 mg g^{-1} and 0.49 mg g^{-1} at control and treated plants, respectively. The percentage was determined 22.22% over control. At flowering stage, the values were examined 0.70 mg g^{-1} and 0.44 mg g^{-1} at control and treated plants, respectively. The percentage was noted to be 37.14% over control. Our results are in agreement with those of Sharma et al., (1996); Luttus et al., (1996); Huan-Wen et al., (1999); Silveira et al., (2001); Salem et al., (2002) and Mohamed (2003).

It is well known that growth of plants under salinity stress is less than unstressed plants and this of course is due to the inhibition of protein synthesis in the cell. Many reports indicated that the biosynthesis of some amino acids was dramatically decreased by increasing the level of salinity and consequently concentration of protein recorded significant decrease in comparison with the control plants. Shen et al., (2007) indicated the decrease in protein content in wheat grains with increasing soil salinity. Similarly, Saffan, (2008) has also reported reduction of amino acids content in wheat and barley plants under salt stress. Levine et al., (1990) reported that salt stress causes inhibition of protein synthesis and disturbs nucleic acid metabolism.

Reduction in crude protein content was reported in rice leaf (Luttus et al. 1996) and in *Coleus blume* tissues (Gilbert et al. 1998) in response to salinity. Silveira et al. (2001) correlated the lowered crude protein contents in salt-stressed cowpea with low nitrate reductase activity and could account for a decline in protein growth. The impaired protein synthesis by other green cultivars such as red kidney

Table 2: Chlorophyll Contents of *Stylos* Plants Under Artificial Salinization

(Values are Averages of 12 Replicates)

Sl. No.	Parameters	Growth stages		Untreated (1.2 $mScm^{-1}$)	Treated (4.0 $mScm^{-1}$)
1.	Chlorophyll content (mg g^{-1}fr wt)	I*	Chl a	0.177	0.163
			Chl b	0.323	0.306
			a:b ratio	0.548	0.531
			Total Chl	0.522	0.485
		II**	Chl a	0.202	0.181
			Chl b	0.394	0.309
			a:b ratio	0.513	0.585
			Total Chl	0.633	0.499

*Leafy stage; **Flowering stage

For Chl a			
SEm±	0.004	0.004	0.005
CD at 5% P	0.011	0.011	0.016
For Chl b			
SEm±	0.003	0.003	0.005
CD at 5% P	0.009	0.009	0.014
For Total Chl			
SEm±	0.009	0.009	0.136
CD at 5% P	0.029	0.029	0.041

beans (Frota and Tucker ,1978), alfalfa (*Medicago sativ*a L.) (Pessarakli and Huber,1991), pea (Kahane and Poljakoff,1968), wheat (Abdul Kadir and Paulen,1982) have been reported previously by many investigators. Thus, it can be concluded from the present study that decreased amino acid incorporation into protein was reported as the reason for the depressed protein synthesis in Stylos plants.

Total amino acids also showed a declining trend. Our findings are in consonance with the earlier reports (Levitt,1972). Suggested that decreased availability of amino acids might also be due to denaturation of enzymes involved in the amino acid formation.

Data in Table 2 depicted that the concentration of chlorophylls decreased with an increase in soil salinity. However, the Chla/b ratio at flowering stage showed contradiction.

At leafy stage, the Chl'a' content was amounted 0.177 mg g^{-1} and 0.163 mg g^{-1} in control and treated plants, respectively. The calculated percentage decline was 7.91% over control . Chl'b' values were recorded 0.323 mg g^{-1} and 0.306 mg g^{-1} in control and treated plants, respectively. The calculated percentage was recorded 5.26% over control. The Chla/b was depicted 1.00 : 0.97. The total chlorophyll values were calculated to be 0.522 mg g^{-1} and 0.485 mg g^{-1} in control and treated plants, respectively. The calculated percentage was examined 7.08% over control.

At flowering stage, the Chl'a' amounted 0.202 mg g^{-1} and 0.181 mg g^{-1} in control and treated plants, respectively. The calculated percentage reduction was 10.40% over control. Chl'b' remained 0.394 mg g^{-1} and 0.309 mg g^{-1} in control and treated plants, respectively. The percentage reduction was calculated to be 21.57% over control. The Chla/b ratio was found to be 1.00 : 1.14. Total

chlorophyll was calculated to be 0.633 mg g^{-1} and 0.499 mg g^{-1} in both control and treated plants and percentage reduction was 21.17% over control.

Our results agree with those of Chandra et al.(1993) in barley, in bajra (Reddy and Vora,1985), in mungbean (Singh et al.1994), black gram (Ashraf,1989) and in chickpea (Singh and Singh,1999).According to Reddy and Vora (1983) the declined green pigments might be attributed to the increased activity of the chlorophyll degrading enzyme chlorophyllase. Thus, more or less adverse effects of salinity supports the view that exhibition of green pigments is indicately associated with nitrogen economy of Stylos plants.

Hence, chlorophyll contents and crude proteins along with total amino acids can be considered as one of the physiological criteria for assessing combatment of osmotic stress in Stylos plants.

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