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A PROTEOMIC AND TARGETED METABOLOMIC APPROACH TO INVESTIGATE CHANGES IN *Stylosanthes scabra* VOGEL PLANTS IN RELATION TO NaCl SALINITY

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ABSTRACT

A pot culture was designed to examine the role of accumulated proline, choline and glycine-betaine in *Stylosanthes scabra* Vogel under saline conditions. The results indicated that proline was affected adversely by salinity stress, whereas glycine-betaine and choline contents were found to increase. Salinity caused decrease in proline contents might be due to disturbance in amino acid metabolism.

KEYWORDS: Proline, Choline, Glycine-Betaine, NaCl Salinity

Salinity is regarded as one of the important abiotic stresses that limits yield in various crops. It is estimated that about 2 billion ha of land that were once biologically productive are now degraded (Anon, 1992). The alarmingly rising human population has created an ever-expanding demand for food, fuel wood, fibre and fodder crops putting an intolerable pressure on land, water and other resources. Thus, species of the tropical pasture legume, *Stylosanthes* have shown promise as pasture and forage crops in the tropical and sub- tropical regions in Australia, Africa, South-east Asia and India.

During osmotic stress, plant induce processes that regulate the osmotic adjustment and maintain sufficient cell turgor for growth to proceed (Zimmermann, 1978). Since adjustment requires the control of intracellular inorganic ions in the cytoplasm through accumulation of organic compounds compartmented mainly in the cytoplasm (Jeschke *et al*., 1986) (Binzel *et al*., 1988) (Bohnert *et al*., 1995). These organic solutes termed as osmolytes, compatible solutes or osmoprotectants, are non- toxic molecules having relatively low molecular weight that raise osmotic pressure and protect some macromolecular structures against denaturation (Timasheff, 1992) (Bourot *et al*., 2000).

Decrease in proline contents due to salinity has been recorded earlier by Rodriguez *et al*., (1997) Samia El-Sayed, (2008) Sidari *et al*., (2008). However, most of the studies report a significant increase in proline contents. Salinity induced decrease in proline contents as observed in the present study, might be due to a disturbance in amino acid metabolism. But choline and glycine-betaine contents are significantly increased on exposure to salt stress. Our study hardly advocates for any role of proline in imparting tolerance to salinity in *Stylo* plants. This is because *Stylosanthes scabra* despite having the highest levels of choline in its tissues, is the most susceptible species to salinity treatment. For instance, Jacob *et al*., (1999) and Jackson and Seppelt (1995) did not find any accumulation of proline in *Prasiola* plants after different exposure to salt. Glycine-betaine is the predominant osmoprotectant and could be used as a reliable index of stress tolerance in *Stylos* plants.

MATERIALS AND METHODS

The certified seeds of *Stylosanthes* were procured from IGFRI, Jhansi (India). They were surface sterilized with 1% $HgCl_2$ for 15 minutes and thoroughly rinsed with distilled water. Seeds were placed in pots to germinate and were transplanted. Plants from each pot were harvested randomly at leafy stage (64 DAS) and at flowering stage (124 DAS). NaCl was added and mixed so as to fix the ECe of soil saturation extract at $4mScm^{-1}$. Ordinary garden soil was used as control ($1.2mScm^{-1}$).The treatments were applied at fortnightly intervals.

Proline contents were estimated by the method of Bates *et al*., (1973). 0.5g of sample was homogenized in a blender with 10ml of 3% aqueous sulphosalicyclic acid and centrifuged at 10,000g. 2ml of filtrate was reacted with 2ml of acid ninhydrin solution and 2ml of glacial acetic acid was boiled for 1 hour at 100^0C,in a water bath. The reaction was stopped in an ice bath and then 4 ml of toluene was added. The absorbance was read at 520nm on a UV visible spectrophotometer (Shimadzu-1601). The protein content was expressed as μmole g^{-1} fresh weight. The data were analysed by analysis of variance (ANOVA) method.

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Choline and glycine-betaine were determined by non-specific but per-iodide spectrophotometric method (Speed and Richardson, 1968) and by Direct reflectance Densitometry as described by Radecka *et al*. (1971) at CDRI, Lucknow (India). The proline and glycine-betaine contents were expressed as µg g^{-1} fresh weight.

RESULTS AND DISCUSSION

Under osmotic stress conditions various plants accumulate Proline and/or betaines (Wyn Jones and Storey, 1981). Glycine-betaine plays a major role in conferring resistance to drought, salinity and cold stress (Wyn Jones and Storey, 1981) (Zao *et al*., 1992) (Naidu *et al*., 1996). The present study reveals that salt stress brought about a marked depletion in protein content, whereas choline and glycine-betaine increased considerably.

The data tabulated in table revealed that under the influence of salinity, plants depicted 15.87% decline in proline content over control at leafy stage. Likewise, it declined 24.15% over control at flowering stage. The values were calculated to be 365.15µmole g^{-1} in control plants and 307.20 µmole g^{-1} in treated plants at leafy stage and 253.20 µmole g^{-1} in control plants and 192.05 µmole g^{-1} in treated plants at flowering stage.

Table : Proline, choline and glycine-betaine contents of Stylos plants under artificial salinization

Sl. No.	Parameters	Growth stages	Untreated (1.2 $mScm^{-1}$)	Treated (4.0 $mScm^{-1}$)
1.	Proline (µmol g^{-1})	I*	365.15	307.20
		II*	253.20	192.05
2.	Choline (µg g^{-1})	I*	22.20	30.60
		II*	24.43	31.93
3.	Glycine-betaine (µg g^{-1})	I*	34.87	39.07
		II*	36.20	41.17

*Leafy stage; **Flowering stage

For proline			
SEm±	7.853	7.853	11.106
CD at 5% P	23.669	23.669	33.474
For choline			
SEm±	0.3800	0.3800	0.537
CD at 5% P	1.146	1.146	1.619
For glycine-betaine			
SEm±	0.408	0.408	0.577
CD at 5% P	1.230	1.230	1.740

The data portrayed in table indicated that choline content is significantly greater in treated plants (30.60 µg g^{-1}) over control plants (22.20 µg g^{-1}) at leafy stage. It was increased by 37.84% over control. Similarly, at flowering stage, it increased by 31.93 µg g^{-1} in treated plants over control plants showing 24.43 µg g^{-1}. The percentage increment was calculated to be 30.69%.

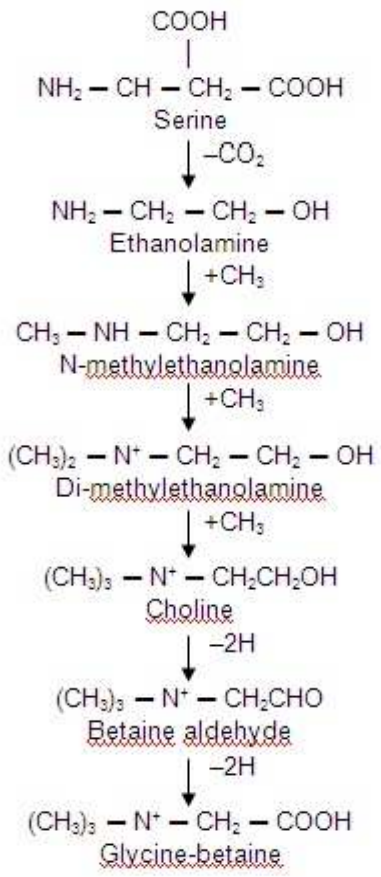
The glycine-betaine content also showed marked increase under salinization. At leafy stage, it increased by 39.07 µg g^{-1} over control i.e. 34.87 µg g^{-1}.Likewise,at flowering stage, it increased by 41.17 µg g^{-1} over control i.e. 36.20 µg g^{-1}. The percentage increment was calculated to be 12.04% and 13.73% at leafy and flowering stage respectively.

Similar findings have been recorded earlier by Rodriguez *et al*. (1997) Samia El-Sayed Saffan (2008) Sidari *et al*., (2008) in respect of proline content. Salinity induced decrease in proline contents as observed might be due to a disturbances in amino acid metabolism. Role of proline in *Stylosanthes scabra* may be investigated further. There are reports that accumulation of proline in response to salinity is not mandatory.

The glycine-betaine was found to accumulate more than choline. This may be due to the fact that accumulate choline is catabolized rapidly after induction of salt stress whereas the accumulated glycine-betaine remains unmetabolised in the tissues. These findings are similar to

those of Ahmad and Wyn Jones, 1979; (Browman and Rohringer, 1970) (Hanson *et al*. 1978).

A general biosynthetic solute leading to the formation of glycine-betaine is a two-step oxidation of choline catalysed by choline mono-oxygenase and betaine aldehyde dehydrogenase (BADH) in chloroplasts (Brouquisse *et al*., 1989) (Papageorgiov *et al*., 1991) (Rhodes and Hanson, 1993). Our studies on both treated and control plants revealed accumulation of serine besides choline and glycine-betaine which reflects that the ultimate precursor of betaine might be an amino acid serine.



Pathway of glycine-betaine synthesis

Therefore, synthesis of glycine-betaine probably involves the decarboxylation of serine and sequential methylation of ethanol amine to choline followed by a two step oxidation of choline to glycine-betaine. The activity of betaine aldehyde dehydrogenase (BADH), a terminal enzyme of the glycine-betaine biosynthetic pathway exhibited significant inclination in a stylos plants subjected to stress of NaCl salt.

This modest increase in the activity of betaine aldehyde dehydrogenase can be attributed to increased levels of protein and transcripts for this enzyme (Weretilnyk and Hanson, 1989, 1990) (McCue and Hanson, 1992).

Hence proline, choline and glycine-betaine contents can be considered as one of the most physiological criteria for assessing combatment of osmotic stress in stylos plants.

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