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SYNTHESIS OF CHOLINE AND GLYCINE BETAINE AND ALLEVIATION OF SODIUM CHLORIDE TOXICITY

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ABSTRACT

The experiments were conducted to examine the effect of choline and glycine-betaine, synthesized and accumulated in alfalfa plants (*Medicago sativa* L.) on alleviation of sodium chloride stress. Soil salinity (1.2 to 12.0 $mScm^{-1}$ECe) brought about an accumulation of these osmolytes. However, they presented their net destruction upon relief of stress.Choline was found to accumulate in the range of 24.88[-1]6.32 µ mol g^{-1} fresh wt. and varied to rising extent under increasing ECe levels of sodium chloride. Glycine betaine levels were high (>64.90 µ mol g^{-1} fresh wt.) under 1.2 $mScm^{-1}$ECe and went on increasing gradually with rising salinity levels (4-12 $mScm^{-1}$ECe). The synthesis and accumulation of both the osmolytes in shoot always exceeded roots. Discrete spots of glycine and serine as observed on thin layer chromatograms revealed that the synthesis of choline and glycine betaine could take place *de novo* from carbon precursor ethanol amine-a product of amino acid serine. It may be suggested that the estimated metabolic cost of glycine betaine synthesis via choline approached the cost of protein turn over. Since both these osmoprotectants might mitigate the toxicity of sodium chloride salt, it may presumably be consistent with their adaptive value. A search for their synthesizing potential variability is warranted.

KEYWORDS : Choline, Glycine Betaine, Sodium Chloride Toxicity, Alfalfa

Quaternary ammonium compounds, also called quaternary amines are salts of quaternary ammonium cations with an anion. Choline and glycine-betaine, the best characterized quaternary ammonium compounds, have been reported to synthesize and accumulate in large amounts (10-30% of the total cellular nitrogen) in most plants (Hanson and Nelson, 1978; Hitz and Hanson, 1980; Wyn Jones and Storey, 1978; Rhodes and Hanson, 1993). These osmoprotectants accumulate in plants as an adaptive mechanism to environmental stress such as salinity (Thomas et al., 1992). Choline is important as a precursor of lecithin in plasma membranes and together with its acid derivative glycine-betaine, it acts as a methyl donor (Toyosawa and Nishimoto, 1967). In addition to its role as osmoprotectant, glycine-betaine has been reported to stabilize photosynthetic reactions, the structure of extrinsic proteins of the pigment system II complex and ATP synthesis (Mamedovet al., 1991) as well as cell membranes (Jolivetet al., 1982) and activation of various enzymes (Gorham, 1995).

Both of the aforesaid compounds may be either nontoxic osmotica that effectively reduce the water potential, components generated due to a pathological consequence of stress or products of ammonia detoxification (Cavalieri, 1979; Greenway and Munns, 1980; Steward and Hanson, 1980; Storey et al., 1977; WynJones et al., 1977).

In most organisms, glycine-betaine is biosynthesized by oxidation of choline in two steps. The intermediate betaine aldehyde is generated by the action of the enzyme mitochondrial choline oxidase (choline dehydrogenase). Betaine aldehyde is further oxidised to betaine by the enzyme betaine aldehyde dehydrogenase.

In the present paper, we report the results of work on the synthesis and accumulation of choline and glycine-betaine in alfalfa plants (*Medicago sativa* L.). This work evaluates the potential usefulness of both these osmolytes as cumulative index of sodium chloride toxicity.

MATERIALS AND METHODS

The seeds of alfalfa plants (*Medicago sativa* L.) var. Pant Ragini, obtained from Indian Grassland and Fodder Research Institute, Jhansi (India), were surface sterilized and sown in plots of equal size (1 M^2) during winter season 2014. Salt stress was induced by adding solution of NaCl and $CaCl_2$ in 1:1 ratio separately to the soil in order to obtain the EC of soil saturation extract at 4, 8 and 12 $mScm^{-1}$ at $25^0C \pm 1^0C$ following the method of Varshney et al., 1988. The soil of control plot had 1.2 $mScm^{-1}$ECe. The amount of salts were determined as per the formula of Richards (1954) [meq/l = 10 EC 10^3]. The ECe of different salinity levels were cross checked on a direct reading

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conductivity meter. The plants were exposed to natural light daily from morning to evening. The average day and night temperature varied from 24^0C to 31^0C and 9^0C to 15^0C respectively. The experiment was conducted in three replications and 12 seedlings were maintained in each replication. Fifteen day old plants were subjected to the analysis of osmolytes under study.

For glycine-betaine and choline determination, blades of the youngest and fully expanded leaves were removed, transported on ice to the laboratory, frozen in liquid N and freeze dried. The dried leaves were weighed and ground in a Micro-wiley Mill to pass a 40-mesh screen. An accurately weighed subsample of milled material (about 20mgs) from each sample was extracted with 5ml of H_2O at 100^0C for 1hr and centrifuged to clear. Choline and betaine in the cleared extract were determined by Pyrolysis assay after ion exchange purification/TLC and by periodide colorimetric method (Hitz and Hanson, 1980).

RESULTS AND DISCUSSION

The results obtained by per iodide assay given in Table 1 indicate that both choline and glycine betaine accumulated in alfalfa plants under NaCl salinity stress. Choline accumulated 24.8 μ mol g^{-1} in shoot of control plants while 16.32 μ mol g^{-1} in root portion of the same. This solute increased upto 8 $mScm^{-1}$ and slightly decreased under 12 $mScm^{-1}$ECe i.e. the values recorded were 25.8; 29.9 and 27.2 μ mol g^{-1} in shoot. In roots the corresponding values were 16.2; 16.4 and 10.9 μ mol g^{-1} under 4, 8 and 12 $mScm^{-1}$ ECe respectively.

As regards the glycine betaine, it accumulated 64.9 μ mol g^{-1} in shoot of control plants while only 8.2μ mol g^{-1} in root portion of the same. Under 4, 8 and 12 $mScm^{-1}$ECe, the values recorded were 66.3, 69.51 and 61.9 μ mol g^{-1} respectively.

The observations of both per iodide assay and TLC densitometry were found more or less same (Table 1). Therefore, for the sake of brevity the results of per iodide assay were expressed. Our findings are in agreement with those of Arakawa et al., 1990; Rhodes and Hanson 1993; Varshney et al., 1988.

Thin layer chromatograms of amino acids were run as discrete spots (figure 1). Glycine and serine amino acids appeared besides betaine and choline in solvent butanol-acetic acid-water in the samples of alfalfa plants. Thus, it may be suggested from these results that choline

Table 1: Choline, Glycine Betaine and Quaternary Ammonium Compounds in Alfalfa Plants Grown Under Varying NaCl Salinity Regimes
(Values are Mean of Three Determinations)

	Soil Salinity Regimes ($mScm^{-1}$)							
	Control (1.2)		4		8		12	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Choline (μ mol g^{-1}frwt)								
Per iodide Assay	24.88	16.32	25.89	16.29	29.91	16.45	27.20	10.95
TLC Method	26.31	14.32	29.34	15.11	36.32	14.31	31.15	10.51
Glycine betaine (μ mol g^{-1}frwt)								
Per iodide Assay	64.90	08.20	66.30	16.85	69.51	14.06	61.92	14.32
TLC Method	64.85	08.34	65.26	09.95	71.62	16.16	62.87	11.45
Quaternary ammonium compounds(μ mol g^{-1}frwt)								
Per iodide Assay	98.80	18.69	98.97	19.27	102.36	19.64	84.94	13.44
TLC Method	98.10	17.45	97.31	18.99	102.91	18.32	83.95	14.33

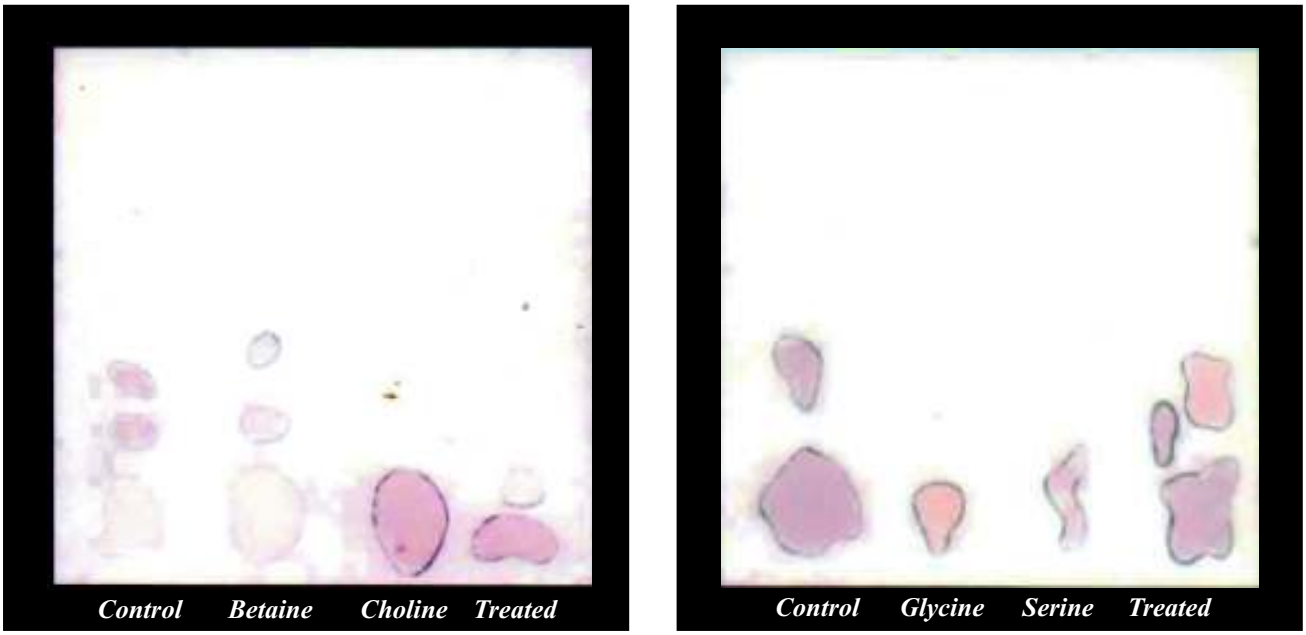
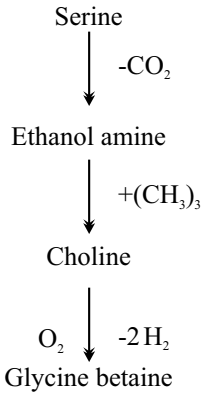


Figure 1: Thin Layer Chromatography of Amino Acids Run as Discrete Spots in Strong Acid Solvent Butanol-Acetic Acid-Water (BuA) in the Plant Samples of Alphalpha (*Medicago sativa* L.) Control and Treated (NaCl), Co-Chromatograph of the Standards Betaine, Choline, Glycine and Serine

might synthesize by sequential three step methylation of ethanolamine and choline could subsequently convert into glycine betaine by two oxidation steps.



Our results revealed that the catabolic system of glycine betaine was perhaps blocked by increasing salinity levels. In this way a substantial amount of glycine betaine could be maintained in stressed alfalfa plants. Similar results have been obtained in bacterial studies of Bernard et al. (1986). Thus, glycine betaine behaves as an inert end product during stress (Ahmad and Wyn Jones, 1979; Ladyman et al. 1980; Hanson and Wyse, 1982).

It must also be emphasized here that choline, the precursor of glycine betaine, is present in alfalfa plants and become available for production glycine betaine (Wyn Jones and Storey, 1981). The presence as well as role of choline oxidase and betaine aldehyde dehydrogenase enzymes need further studies.

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