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Distribution of betaine and choline under salt stress in lentil (*Lens esculenta Moench*)

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Abstract
The quantitative distributions of two major quaternary ammonium compounds betaine and choline were obtained by the use of both the specific but semi quantitative Thin Layer Chromatography method and the sensitive quantitative but less specific periodide assay. Lentil plants accumulated two organic metabolites betaine and choline not only control plants but also when exposed salt stress. The maximum betaine concentration (0.416 g/gm wt.), attained by mature plants of var.'PL-4' at 4mScm-1 EC, was apparently higher than of choline concentration (0.695 g/gm dry wt.) attained by mature plants of var. 'PL-406'. However, the rate of accumulation of both betaine and choline appeared to be the same in all varieties of lentil investigated.

Keywords: betaine and choline, salt stress and lentil

Introduction
Lentil, one of the main dry beans cultivated in India, serves as an excellent sources of readily digestible seed proteins and carbohydrates. Most stress plants species as lentil accumulate the quaternary ammonium compounds like choline and betaine in their cells in response to water, salt or cold (Storey *et al*., 1977; Wyne Jones, 1977; Hall *et al*., 1978; Hanson and Nelson, 1978) [28, 20, 10].These compounds may be either non-toxic of osmatica that effectively reduce the water potential, components generated due to pathological consequence of stress or products of ammonia detoxification (Hanson and Scott, 1980 [11]; Stewart and Hanson, 1980) [12] Glycine betaine(N,N,N-trimethyl glycine) has been found to accumulate in the leaves of several cereals and grasses in response to both rapidly imposed and long –term water deficits (e.g. Hanson and Nelson 1978; Hitz and Hanson 1980) [10]. In cereal and legumes crops like barley and lentil the accumulation results from de novo synthesis of betaine from 1-C and 2-C fragments (Hanson and Scott,1980) [11]. The betaine accumulated by barley leaves represent approximately 2% of the Kjeldahl nitrogen of the tissue Tully *et al*., 1979). Although certain plants, this species has been shown to contain higher levels (>100m mol.dry wt.-1) of B-alanine betaine and choline-o- sulfate when grown in saline conditions.
When subjected to salt stress, higher plants respond with a variety of integrated changes in, for example, the distribution of ions,their biochemical functions and their morphology (Cheesemen *et al*., 1988). Higher plants have been shown by Arakawa *et al*., 1992) that the level of betaine aldehyde dehydrogenase, increases several fold in whole leaves in response to salt stress, and that the expression of the gene for BADH is regulated both osmotic stress and by abscisic acid (Ishitani *et al*.,1995) [14]. It has been postulated that the differential distribution of salt and glycine-betaine between young and old leaves of salt stressed plants would allow the younger leaves to support sufficient metabolic and physiological activities for survival under high-salinity conditions. (Muthukumarasamy Panneerselvam, 1997 and Agarwal and Varshney, 1997) the adaptive value of betaine and choline distribution under field conditions.

There were following specific objectives
To determine whether organic metabolites like betaine and choline are actually responsible for tolerance of lentil crop plants to salts.
To assess the contribution of de novo synthesis to betaine accumulation leaves.
To compare the estimated metabolic cost of betaine accumulation under field condions with that of protein turn over was chosen a standard for comparision because it represents a large and critical item in the maintenance of respirestion of the leaf (Penning de vries 1975).
Lentil is an important and more useful proteinaceous pulse crop of India. It was thought worthwhile to study the exhibition of such organic metabolities as choline and betaine in order to have direct and indirect induction of physiological mechanism of salt tolerance in lentil (Lens esculenta Moench).

Materials and Methods
Seeds of two differentially salt tolerant varieties *viz*. PL-4 and Pl-406 of lentil (*Lens esculenta* Moench) were obtained from Genetic Resource Unit, International Crop Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru (A.P.) and G.B. Pant University of Agriculture and Technology, Pantnagar (Uttrakhand), India.

Induction of salt stress
The salt stress was induced by addition of NaCl, Na_2CO_3 and $NaHCO_3$ salt to distilled water calculated as per the conversion formula of Richards (1954) for 2 and 4 $mScm^{-1}$ electrical conductivities at 25 ^{0}C. Meq/1=10xECx10^3 For field culture experiments, ordinary soil, having ECe=1.2$mScm^{-1}$, was used as control. A cross verification of EC maintenance was made by using direct reading conductivity meter (Systronics make). Nevertheless, the salt solutions of different EC values were examined for their pH values. The corresponding pH values for 4 ECe eas noted 8.1. Sampling: At three stages of growth i.e. leafy stage (30 days after sowing), flowering stage (45 days after sowing) and maturity stage (150 days after sowing, Six plants from each pot were harvested at random selection. The roots were

washed in tap water. All the samples blotted immediately and the fresh weight of each part was taken separately. The data for complete plant, were computed by the addition of values of shoot and root.

Colorimetric assays

Betaine and choline both were determined by the non-specific periodide method in which choline and betaine are selectively precipitated at different pHs, So that the following expression is established. Total Quaternary ammonium compounds (pHs 2.0) – Choline = betaine modified periodide method, published by speed and Richardson (1968) for the determination of Quaternary ammonium compounds was developed further by Varshney *et al*., (1989) [27] on the basis of findings Wall *et al*., (1960) [28]. Precisely 0.2 ml of either the acid or alkaline for choline potassium tri iodide reagent was added to sample containing between 10-15 mg of quaternary ammonium compounds in water. The mixture was shaken and left for at least 90 minutes in an ice bath with intermittemt shaking 2 ml of ice cooled wter was added rapidly to the mixture to reduce the absorbance of the blank and improve replication. The choline and betaine both were determined by referring the readings previously prepared standard curves. Thin Layer Chromatography (TLC) also provided a means of assessing the specificity of the periodide assay for both choline and betaine. These were determined by direct reflectance densitometry in the visible range after TLC as described by Radecka *et al* (1971) [17]. The method was rapid as well as highly specific but lack the precision of the periodide colorimetric assay. By employing the sensitive but non-specific periodide assay with the highly specific but semiquantitative T.L. photo densitometry, betaine and choline levels in plants extracts were determined by a relatively routine and rapid procedure at CDRI, Lucknow (India).

Results

In general betaine and choline accumulated due to salt stress In plants of both lentil varieties PL-4 and PL-406 and the distribution was noted increased markedly with an increase in both NaCl, Na_2CO_3 and $NaHCO_3$ regimes. Glycine betaine (N, N, N, Trimethyl glycine): -NaCl salt brought about a considerable distribution of betaine in plants of lentil var. PL-4 at leafy stage, flowering stage and maturity stage. However, the magnitude of accumulation was recorded relatively more at leafy stage. Both carbonate and bicarbonate salts of sodium also depicted accumulation of betaine in leafy stage, flowering stage and maturity maintaining the trend same (Table-2). It is interesting to note that accumulate betanie went up considerably with age of plants.

Table 2: Betaine and choline in two varieties of lentil under salt stress (TLC Denistometry).

Var.				Betaine				Choline
PL-4	**Control**	**NaCl**	**$NaHCO_3$**	**Na_2HCO_3**	**Control**	**NaCl**	**$NaHCO_3$**	**Na_2CO_3**
L	27.5	26.9	27.2	23.2	9.5	11.3	11.2	12.7
F	28.9	29.5	27.6	25.6	8.**9**	10.7	14.5 1	8.3
M	30.2	30.9	32.1	26.9	9.1	10.9	14.7	18.5

Var.				Betaine				Choline
PL-406	**Control**	**NaCl**	**$NaHCO_3$**	**Na_2HCO_3**	**Control**	**NaCl**	**$NaHCO_3$**	**Na_2CO_3**
L	28.9	28.4	29.3	24.3	14.3	15.2	18.3	16.4
F	32.1	32.0	29.8	29.8	26.5	14.9	15.6	17.6
M	33.7	34.7	35.6	28.2	15.0	16.8	17.9	19.7

L = Leafy stage, F= Flowering stage, M= Maturity stage.

As regards Var.PL-406, the distribution of betaine was also found obvious. However, plants of flowering stage demonstrated tremendous increase as compare to mature plants. Both soil salinity and sodicity proved favourable to betaine accumulation. However, mature plants nurtured by 4mScm^{-1} solution of $NaHCO_3$ presented a significant accumulation of betaine (Fig. 1 -2). Betaine can be a very useful cytoplasmic osmatic, especially since, at high high concentrations it is relatively non-toxic to the cells.

Thus by and large, salt stress induced an accumulated of betaine in the leaves of control plants as well as the plants under exposure of salts.

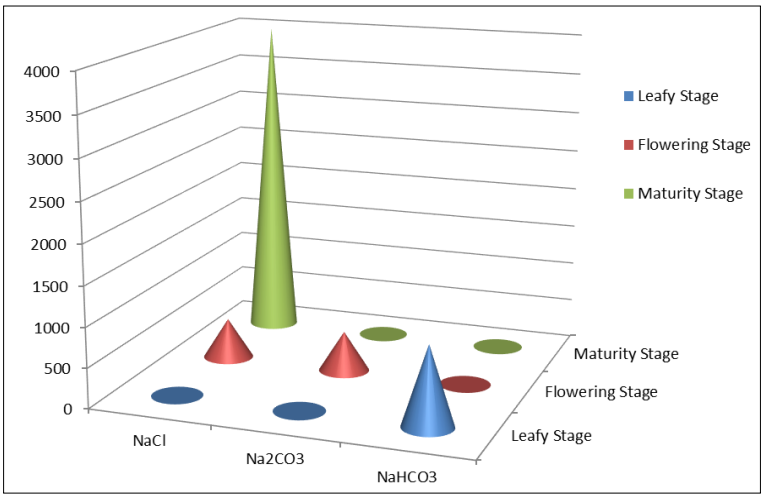

Fig 1: Betaine in variety PL-4 of Lentil under salt stress (% increase over control)

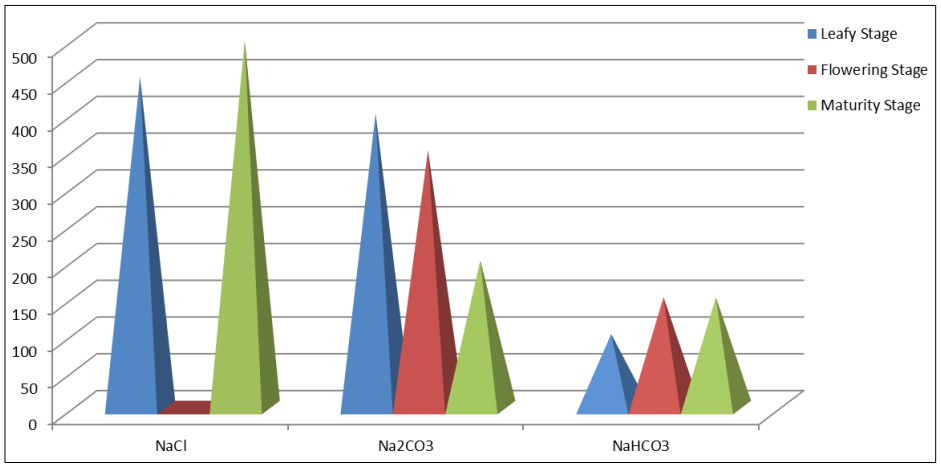

Fig 2: Betaine in variety PL-406 of lentil (*Lens esculenta* Moench) under salt stress (% increase over control).

Choline

Like betaine, choline another organic metabolites was also found accumulated in plants of lentil. The magnitude accumulation increased not only with age of plants but also with a treatment of chloride, carbonate and bicarbonate salt of sodium in Var. PL- 4. While comparing the stress of three different salts Na_2CO_3 proved relatively more favourable than NaCl and $NaHCO_3$ (Table-1).

As far as the plants of Var.PL-406 are concerned the trend of choline accumulation was maintained the same except NaCl treated matured plants where choline accumulated more, however not to that level at which it should have been. Thus, choline accumulation was found to take place almost in the same way as betaine (Fig 3-4).

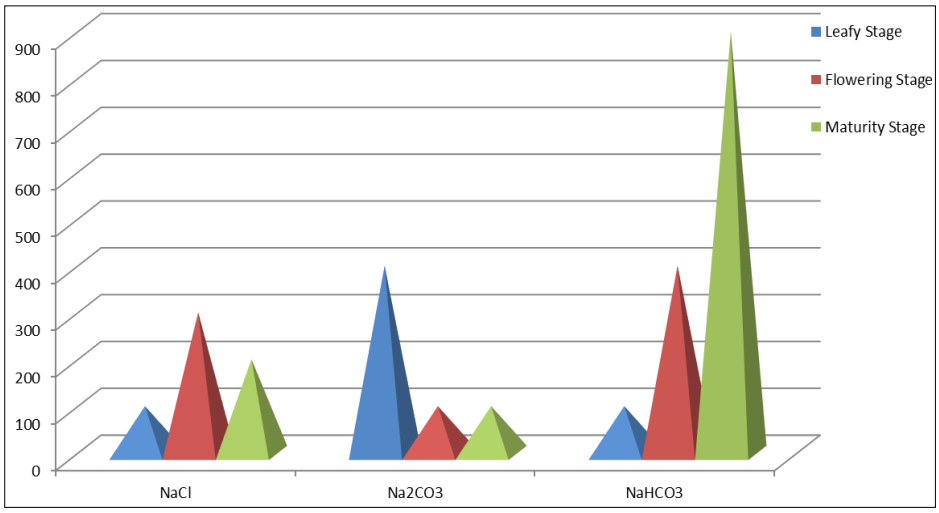

Fig 3: Choline in variety PL-4 of lentil (*Lens esculenta* Moench) under salt stress (% increase over control).

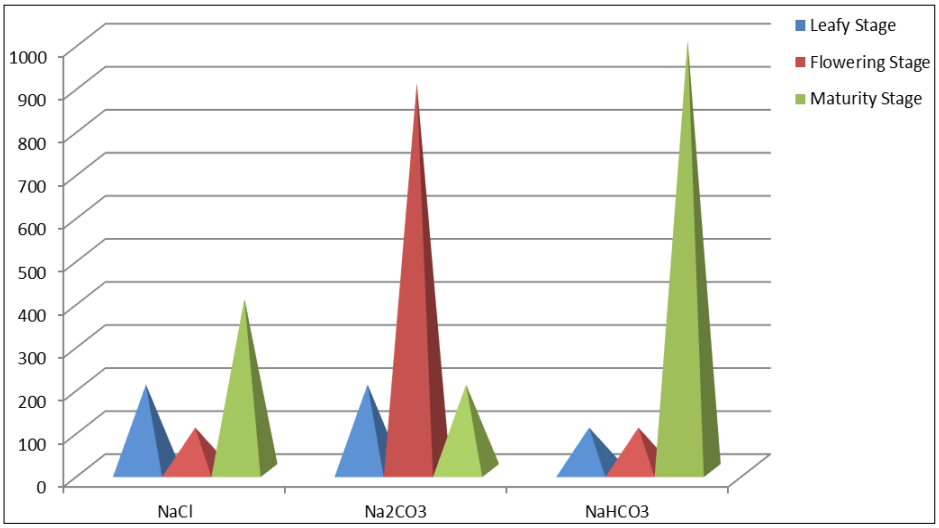

Fig 4: Choline in variety PL-406 of lentil (*Lens esculenta* Moench) under salt stress (% increase over control).

TLC Densitometrys

Experiment related to TLC densitometry was conducted to understand the quantitative determination of choline and betaine plants of lentil exposed to salt stress (Table 1). The observations were not only found statically significant but also the trend almost parallel to those of less specific method "Periodide Assay". Therefore, the results of latter have been discussed for the sake of brevity presuming those of former as confirmatory.

Table 1: Bataine, choline and total QACs in two varieties of lentil under salt stress (Periodide Assay).

Varieties					**PL-4**				**PL-406**
Stage	**Control**	**NaCl**	**Na_2CO_3**	**$NaHCO_3$Control**	**NaCl**	**Na_2CO_3**	**$NaHCO_3$**	**Stage**	**Control**
Betaine	A	0.001	0.005	0.012	0.034	0.002	0.045	0.002	0.049
(ugg^{-1}	B	0.165	0.176	0.2	0.023	0.039	0.048	0.065	0.61
Drywt.)	C	0.327	0.416	0.010	0.011	0.10	0.088	0.086	0.246
Choline	A	0.370	0.472	0.657	0.634	0.557	0.541	0.682	0.517
(ugg^{-1}	B	0.471	0.515	0.577	0.588	0.605	0.582	0.695	0.607
Drywt.)	C	0.582	0.515	0.578	0.672	0.567	0.411	0.555	0.662
TQACs	A	0.583	0.477	0.669	0.668	0.579	0.586	0.684	0.566
(ugg^{-1}	B	0.636	0.657	0.777	0.611	0.644	0.630	0.760	0.617
Drywt.)	C	0.909	0.699	0.588	0.683	0.577	0.449	0.641	0.908
D. 5%	**Varities**	**Ece**	**eplicates**	**Var.**	**VXR**	**TxR**	**VxTxRP**	**level x ECe**	
P				Lavel			x ECe		
	0.0160.	020	0.023	0.029	0.033	0.041	0.058		

Where
A= Leafy stage
B= Flowering stage
C= Maturity stage

Discussion

Higher plants particularly cereals and grass accumulate large amount of organic solutes betaine and choline inside their cells in response to water, salt and cold stress (Wyn Jones and Storey,1978; Hanson and Nelson, 1978; Stewart and Hanson 1980; Hanson and Scott,1980 [11]; Greenway and Munns,1980; Cavalieri Haung,1981; Varshney*et al* 1988) [10, 20]. An effort was made to know, what happens for such organic solutes in leguminous crop lentil (*Lens esculenta* Moench). Under salt stress, the betaine and choline contents were observed several fold higher than controlled conditions. However, the magnitude of distribution of choline and betaine appeared to be approximately the same both lentil varieties This seems becaus the distributed betaine remains unsaltstress, whereas, the distributed choline is catabolised rapidely after the induction of sait stress, whereas, the distributed betaine remains unmetabolised in the tissues (Cavalieri and Hung, 1979; Hanson and Nelson, 1978) [5, 10]. It is possible that lentil plants distributed choline to combat the current stress only and betaine to combat the current stress as well as accumulate in the tissue to the future salt stress. Ahmid and Wyn Jones 1979 [2], have, however, reported the distributed choline and betaine contents in shoots due to their import from roots. Present study reveal high concentration of betaine than found in the tissue analysed here. It is possible that betaine might still play a protective role for protein or membranes even when present ay concentrations too low for an osmotic role. Obviously, more work is warranted in this context and also wipe off the drawback that the large proportion of the valuable cellular nitrogen is locked up into choline and betaine leading to crucial nitrogen economy of lentil plants.

Conclusion

The present studies concluded that Lentil plants distributed two organic metabolites choline and betaine not only in control plants but also when exposed to salt stress. Under stress, the betaine and choline contents were observed several fold higher than controlled conditions. However, the magnitudes of distribution of choline and betaine appeared to be approximatel the same in both lentil varieties. The exact relationship between the concentrations of glycinebetaine and choline and osmotic quantities is not known as yet nor the distribution of such organic solutes has been emphasised in vacuole and cytoplasm of cell separately. The maximum betaine concentration, attained by mature plants of var. PL-4 at $4mScm^{-1}$ ECe, was apparently higher than choline concentration attained by mature plants of var. PL-406. Lentil is an important pulse crop of India. It was thought worthwhile to study the exhibition of such organic metabolites as choline and betaine in order to have direct and indirect induction of physiological mechanism of salt tolerance in lentil (*Lens esculenta* Moench).

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