International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 2; Issue 6; November 2017; Page No. 229-231

Role of phosphotidyl choline and phosphotidyl ethanolamine in the biosynthesis of betaine in lentil (*Lens esculenta* Moench)

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Abstract
Present study examined the general biosynthetic route leading to the formation of glycine betaine probably involves the sequential methylations of ethanolamine to choline followed by a two steps oxidation of choline to glycine betaine. The role of phosphotidyl choline and phosphotidyl ethanolamine in the biosynthesis of betaine raise the significance of betaine accumulation during salt stress in lentil whether the accumulated turn over of methylations phospholipids be an a adaptive response to salinity or betaine accumulation turn over of phospholipid head groups during stress.

Keywords: betaine, choline, biosynthesis and *Lens esculenta* Moench

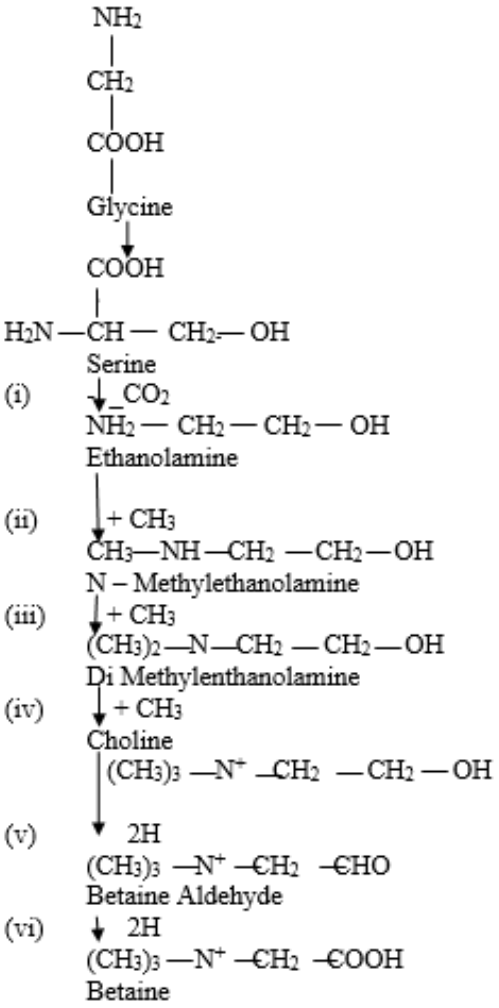
Introduction
Lens esculenta Moench belongs to the family-fabacae, it is an important pulse crop of India, mainly grow on low lying in undulated and marginal land. The biosynthetic pathway of glycine betaine has become a target for a genetic engineering approach to enhance the salinity and drought tolerance of crops (Mc cue and Hanson, 1990; Rathinasabhapathi *et al*., 1994; Holmstrom *et al*., 1994) [9, 8]. The accumulation of such Quaternary Ammonium compounds may represent an adaptive and osmoregulatory response to these environmental stresses. Extensive literature has shown that under environmental stress, plant cell synthesize various kinds of somatically active solute such as minerals, carbohydrates, glycine betaine, proline, serine, ethanolamine and betaine etc Delauney, A.J. *et al* 1993 [1], Premchandra, G. *et al* 1989 [10], Flowers and Colmer 2008 [5] reported that halophytes are remarkable plants which have the ability to complete their life cycle in a substrate rich in high saline that are normally toxic to other plants species.

In principle, the methylation of ethanol amine and the decarboxylation of serine could occur at the level of the free basis, or at the level of at least three types of derivatives; phosphoryl bases, nucleotide bases and phosphatides, in as much as these various potential methylation intermediates are inter convertible, the possible pathways between ethanolamine and choline can be viewed as a metabolic grid, comparising four parallel and interconnected methylation routes. Every pulse plants is itself a 'mini fertilizer factory' Lentil, one of the main dry beans cultivated in India, serve as an excellent sources of readily digestible seed proteins, carbohydrates, vitamins, fibre, potassium and iron, making it a favourite for people on meet free diets. Therefore, present study was aimed to assess the contribution of *denovo* synthesis phosphatidyl choline to betaine accumulation in lentil plants.

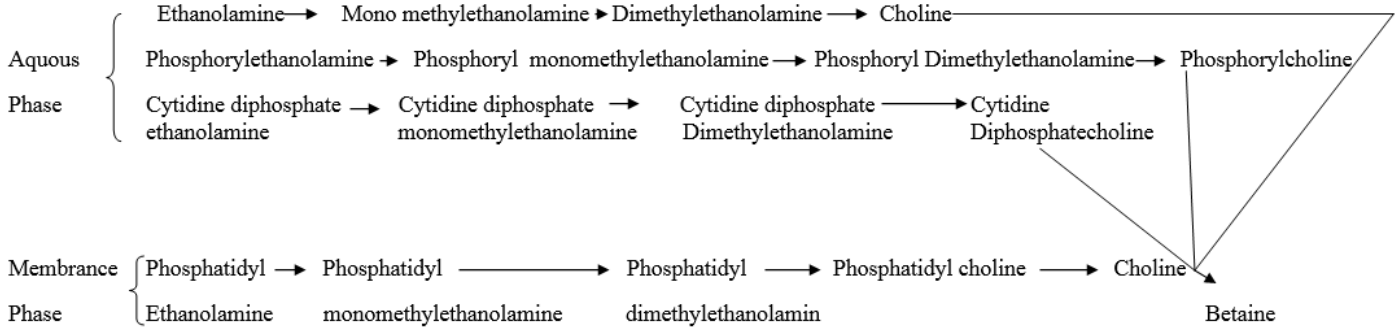
Material and Methods
Lentil seeds were procured from G.B. Pant university of Agriculture and Technology Pantnagar India, and surface sterilized by soaking is 1% $HgCl_2$ Plants were raised to leafy stage (30 days after sowing in 1m x 1m field beds in a controlled in a fields, the treatments with the salt solution of requiste 4-EC levels were given twice a week, alternating with watering of plant by equal amount of water to avoid excessive salt accumulation and loss of water due to evaporation from the soil. Plant grown in ordinary soil were used as control stressed as well as control plants were treated gradually. Biosynthesis of betaine and choline, the modified periodide method published by Speed and Richardson 1968 [14], Varshney *et al* 1988 [15] on the basis of by Wall *et al* 1968 [16]. The mixture was shaken and left for at least 90 minutes in an ice bath with intermittent shaking 2 ml of ice cooled H_2O was added rapidly to the mixture to reduce the absorbance of the blank and improve replication. This was quickly followed by 20 ml of 1,2 – Dichloro ethane at - 10^0C and the 2 layers mixed by a constant stream of our bubbles for 5 minutes, at 4^0C. The absorbance of the lower organic layer was measured at 365 n.m. 1,4 – Dioxan (6 : 9 : 5) purified extracts were applied to the Si gel G plates with the help of a thin layer chromatography multi – spotter. All TLC Plates were sprayed with Dragendorff's reagent, Betaine and choline were determined by direct reflectance densitometry in the visible range after TLC is described by Radecka *et al* 1971 [11]. The method was rapid as well as highly specific but lacked the precision of the periodide colorimetic assay.

Result and Discussion
A general biosynthetic pathway leading to the synthesis of glycine betaine may probably involve de carboxylation of ethanol amine to choline followed by a two steps oxidation of

choline to glycine betaine Scheme 1. Betaine is a metabolically inert accumulation of betaine and its precursors may be accelerated denovo synthesis from serine and 1 – C precursors (step 1 to VI in scheme I). This pathway as a whole might be energy – requiring and yet was accelerated in treated lentil plants, whose carbon income was severely cut. With in the pathway, it was specifically the synthesis and addition of methyl groups that demands the input of metabolic energy and so was especially likely to regulate traffic through the pathway. The methylations of ethanolamine and the decarboxylation of serine occurred at the level of free bases or at the level of at least three types of derivatives – phosphoryl bases, nucleotide bases and phosphtides. In as much as three various potential methylation intermediates are inter convertible, the possible pathway between ethanolamine and choline can be viewed as a 'metabolic grid.' Comparising of four parallel and inter connected methylation routes scheme – 2. It did not be made obvious from scheme – 1, whether this pathway in plants involves the free bases themselves or some bound forms of these. Accumulation of these compatible solutes reduces osmatic potential in the cytoplasm contributes to maintaining water homeostasis among several cellular compartments. Sairam, R.K., *et al* 2004 [13]. Betaine and choline contents accumulated finding corroborate the results of Weigel *et al* 1986 [17]. Hence, the biosynthesis of betaine and free choline in stressed and control plants of lentil depends upon the turn – over of the base mostly of larger, metabolically active fraction of total plant phosphotidyl choline Betaine accumulation by stressed legumin our plants may lentil of 2 to 3 fold increase in the turn over rate of this metabolically active phosphotidyl choline. Reduced level of protein is salt stressed plants may be due to reduced rate of protein synthesis (Dhir *et al*, 1975) [3]; accumulation of ABA (Dhingra and Cle land, 1975) [2] and increased protein hydrolysis (Dungey and Davis, 1982) [4]. Increased protein hydrolysis in tolerant genotypes may provide more amino acids for de novo synthesis of the required protein and to enhance availability of Quaternary ammonium compounds and phenols. Though, there are many organic molecules like proline, glycine betaine, glycerol, sugar and sugar alcohols whose accumulation is widely reported in a wide range of plant species (Greenway and Munns 1980) [7]. Proline may also serve as an intercellular osmotic solute for maintenance of osmotic balance between cytoplasm and vacuole (Flowers *et al*, 1977) [6].



Scheme I: A probable pathway of betaine synthesis via the generalized reaction sequence



Step II: A probable metabolic grid showing some inter relationship among four parallel methylation pathways leading from free ethanolamine to choline and betaine. The three successive methylations (horizontal arrows) are presented by physiologically irreversible steps

Conclusion

It may tentatively be concluded that the methylation steps in the biosynthesis of choline and betaine take place at the phosphoryl base level and at the phospholipid level. The role of phosphotidyl choline and perhaps also phosphotidyl ethanol amine in the biosynthesis of betaine in lentil plants need further investigations about the significance of betaine accumulation during salt stress, under salt stress conditions, the choline contents were found to be much lower than betaine. This may be due to the fact that betaine remained unmetabolised in the tissues, while choline being rapidly catabolised after having induction of salt stress.

Acknowledgement

The authors are immensely grateful to Dr. K. A. Varshney, Associate Professor in the Plant Physiology and Biochemistry Section, Department of Botany, Bareilly College Bareilly U.P. for the preparation of this manuscript and providing guidance during the work.

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