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Studies of Enzyme Glutamine Synthetase (GS) in *Sesuvium Portulacastrum* (L.), an Associate Halophyte

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ABSTRACT

Sesuvium portulacastrum L. (Aizoaceae) is a pioneer, psammophytic associate halophyte of subtropical, Mediterranean regions. It dominates in coastal and warmer zones of the world. Apart from being utilized as a vegetable by local people and forage for domestic animals in the coastal area, environmentally too it is utilized for the bio-reclamation of saline soil in the arid and semiarid regions. Coastal soils as well as sea water, which permeate the soil characteristically, have a poor content of available nitrogen. In contrast, halophytes which inhabit these areas have high protein content. This is because halophytes have the ability to conserve nitrogen and recycle it through their body metabolism. Efficient enzyme mechanism for Nitrogen metabolism in halophytes has been thoroughly studied and communicated. In present investigation, S*esuvium portulacastrum* (L.) is used as a model system representing an associate halophyte with efficacy in Nitrogen utilization in saline conditions. To begin with kinetics of enzyme Glutamine synthetase (GS) (EC: 6.3.1.2) is studied in terms of effect of varying temperature, pH and concentration of enzyme and substrate. The same study would be extended to other important enzymes of Nitrogen metabolism to get an insight in efficacy of such halophytes to conserve available Nitrogen from saline soils and help in phytoremediation of saline soils.

1. Introduction

Associate halophytes grow in the fringe area of mangrove swamps, get inundated 1-5 times per fortnight during spring tide and are also found growing in mesophytic habitat. *Sesuvium portulacastrum* L. (Seapurslane) is one such fast growing, herbaceous, dichotomous, perennial, pioneer, psammophytic halophyte naturally growing in the subtropical, mediterranean, coastal and warmer zones of the world. *Sesuvium portulacastrum* is found occurring on the coastlines of five continents and widely distributed as a pioneer strand species on tropical and subtropical shores (Lonard and Judd, 1997). It grows naturally in the subtropical, mediterranean, coastal and warmer areas around the world (Balasubramanian et al., 2006). *Sesuvium* frequently grows in the backshore topographic zone on sandy beaches as the initial pioneer species just above the high tide line on barrier islands. It is also a common species on the margins of hurricane washover channels, disturbed roadsides, and tidal flats.

In the tropics, the species occurs on estuarine mudflats adjacent to mangrove swamps (Joshi and Bhosale, 1982), in salt marshes and on calcareous shorelines, on the margins of lagoons, on coral sand and rubble shorelines. It is also found along coasts and river mouths and in lower mountains (Hammer, 2001). In India, it grows among the eastern and western coastal regions as inland or seashore species including areas where mangrove plants are found. This includes coastal regions of Gujarat, Maharashtra, Goa, Kerala, Tamilnadu, Andhra Pradesh and Orissa. The present study on *Sesuvium portulacastrum* L. was done keeping following objectives in mind *S. portulacastrum* (L.), as a model system representing an associate halophyte with efficacy in nitrogen utilization in saline conditions.

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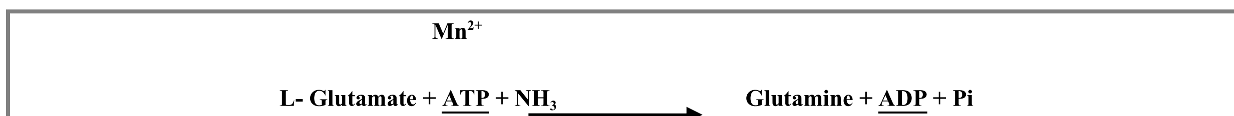
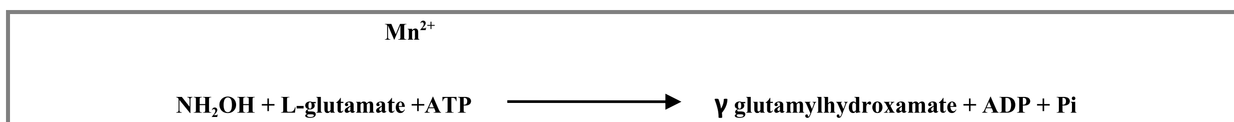
To study kinetics of enzyme Glutamine synthetase (GS) (EC: 6.3.1.2) in terms of effect of varying temperature, pH and concentration of enzyme and substrate. The same study would be extended to other important enzymes of Nitrogen metabolism to get an insight in efficacy of such halophytes to conserve available Nitrogen from saline soils and help in phytoremediation of saline soils. To get an insight in the world of associate halophyte helping in natural conservation of mangrove locations. Glutamine synthetase, a key enzyme of ammonia assimilation, catalyses the ATP dependent production of glutamine from glutamate and ammonia. The native molecular weight of GS from different plant tissues is in the range of 320,000 to 400,000 (Stewart et al, 1980; McCully and Hirel, 1983). The enzyme consists of 8 identical subunits of 39,000 to 45,000 (McCormack et al., 1982; Cullimore et al., 1983).

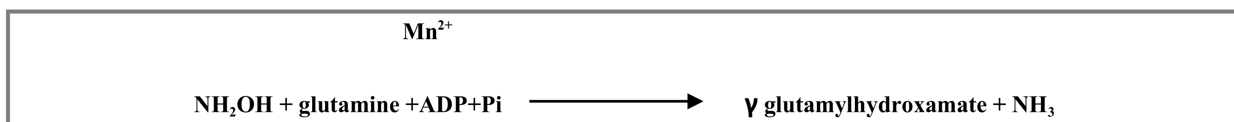
The reaction mechanism of GS involves the binding of substrate in an ordered sequence (Meister, 1974). First, the complex of ATP and divalent cation (Mg^{++}, Mn^{++} or Co^{++}) binds to the enzyme followed by glutamate which reacts to form an enzyme bound γ glutamyl-phosphate. Ammonia, thus binds to the enzyme, attacks the phosphoryl group, resulting in the formation of tetrahedral intermediate before the products are released. GS is important as it is involved in the assimilation, storage and translocation of ammonia in higher plants (Yemm and Folkes, 1958; Lingnowski et al., 1971). The end product glutamine is an important metabolite as it serves a building block of protein and as nitrogen donor in various biosynthetic pathways (Kanamori and Matsumoto, 1972).



In addition to catalysing the synthesis of glutamine, GS catalyses the formation of glutamylhydroxamate when ammonia is substituted by hydroxylamine



GS also catalyses the transferases reaction



Materials and methods

GS activity was studied by the method of Elliot (1955) with some modifications.

Reagents:

Extraction buffer-0.1M Tris-HCl containing 1mM EDTA, 1mM Cysteine and 0.1% v/v Mercaptoethanol (pH 7.0)

Assay buffer-0.1M Tris-HCl, (pH 7.0) $MgSO_4.7H_2O$ 0.1M (pH adjusted to 7.0 With NaOH)

The reaction was initiated by the addition of sodium glutamate, which was replaced in the blank by buffer. After incubation at 30ºC for 30 minutes, 1.0 ml of ferric chloride reagent was added to each tube and the absorbance was read at 540 nm in EQIP-Tronics digital spectrophotometer (EQ 820).The protein content of the enzyme was estimated by the method of Lowry *et al.* (1951). The specific activity of the enzyme is expressed as ΔOD/mg protein/30 minutes.

Hydroxylamine-0.1M (pH adjusted to 7.0 with NaOH) ATP-0.06M, Na Glutamate- 0.6M. Ferric Chloride Reagent-Prepared from equal volumes of 10% $FeCl_3.6H_2O$ in 0.2 N HCl, 24% Trichloroacetic acid and 50% HCl.

Principle

GS catalyses the formation of glutamine from glutamate with the simultaneous cleavage of ATP to ADP.Glutamate serves as the primary substrate and hydroxylamine supplies the amino group. The product γ- glutamylhydroxymate develops yellowish brown colour with ferric chloride reagent, which is estimated Spectrophotometrically.

Enzyme Extraction

One g fresh leaves were ground using 10ml chilled extraction buffer. The homogenate was filtered through 4 layers of muslin and the filtrate was centrifuged at 10,000 rpm for 20 minutes. The supernatant thus obtained was used as the source of enzyme. Throughout the procedure, the temperature was maintained near $0^0C\pm2^0C$.

Enzyme Assay

Initial 3ml of assay mixture consisted of:

Assay buffer	0.1M
$MgSO_4.7H_2O$	7.5 mM
Hydroxylamine	2.5 mM
ATP	7.5 mM
Na Glutamate	75 mM
Enzyme source	as per the requirement

GS extracted from the leaves of *Sesuvium portulacastrum* was assayed at different pH ranging from 6 to 8.5. Effect of variation of substrate concentrations were also studied, wherein ATP concentration was varied between 1.5 mM and 10.5 mM, Na-glutamate was varied between 0.625 mM and 5 mM and hydroxylamine concentration was varied between 75 mM and 375 mM in the assay mixture. Enzyme concentration in terms of protein was varied between 0.081mg and 0.486mg in the assay mixture to understand GS activity in terms of active enzyme protein participation.

3. Results

Effect of hydrogen ion variation on glutamine synthetase activity:

The response of Glutamine synthetase (GS) enzyme extracted from the leaves of *Sesuvium portulacastrum* to different hydrogen ion concentration in the range of pH 6 to 8.5 is recorded in Figure 1.

The enzyme activity was observed to be maximum at pH 7. At the extremes of pH range selected, the enzyme activity remained low.

Effect of ATP variation on glutamine synthetase activity

Figure 3.11 represent the rate of activity of the GS extracted from the leaves of *Sesuvium portulacastrum* as a function of ATP concentration in the assay medium. The enzyme activity had a linear relationshipwith ATP till a concentration of 7.5 mM, beyond which, the enzyme activity recorded low value than its maximum at 7.5 mM. The V_{max} obtained from the graph for 10.3 mM ATP concentration in the assay mixture was 0.12 ΔOD/mg protein/30 minutes with a corresponding K_m of 4.2mM ATP.

Effect of Na-glutamate variation

The response of enzyme GS obtained from leaves of *Sesuvium portulacastrum* to varying concentration of substrate, Na glutamate is depicted in Figure 2.The plot of Na-glutamate variation showed a linear relationship upto 3.5 mM Na-glutamate concentration, beyond which there is slowing of enzyme activity. Beyond 4.5 mM concentration of substrate there was a sharp decline in enzyme activity. The V_{max} for 3.75 mM Na-glutamate concentration in the assay mixture is 0.17ΔOD with a corresponding K_m1.8 mM Na-glutamate

Effect of hydroxylamine variation

The results of GS enzyme extracted from the leaves of *Sesuvium portulacastrum* when studied as a function of hydroxylamine concentration are represented in Figure 3. The enzyme recorded a rapid increase in the activity in the range of 75 mM to 300 mM hydroxylamine concentration. At concentrations higher than 300mM, the enzyme activity recorded a sharp decline. The V_{max} obtained at complete saturation of enzyme by substrate Hydroxylamine was 0.15ΔOD with a corresponding K_m of 170 mM Hydroxylamine.

Effect of enzyme variation

The concentration of enzyme protein was varied in the assay mixture to study the response of ratios of enzyme and substrate available in the assay mixture. Figure 3.14 depicts the correlation between the rates of enzyme activity as a function of enzyme protein concentrations in the assay medium. From the figure 5, it can be observed that the enzyme extracted from the leaves of *Sesuvium portulacastrum* exhibits a direct proportionality to the changes in protein concentration from 0.089mg protein to 0.712 mg of protein in the assay mixture.

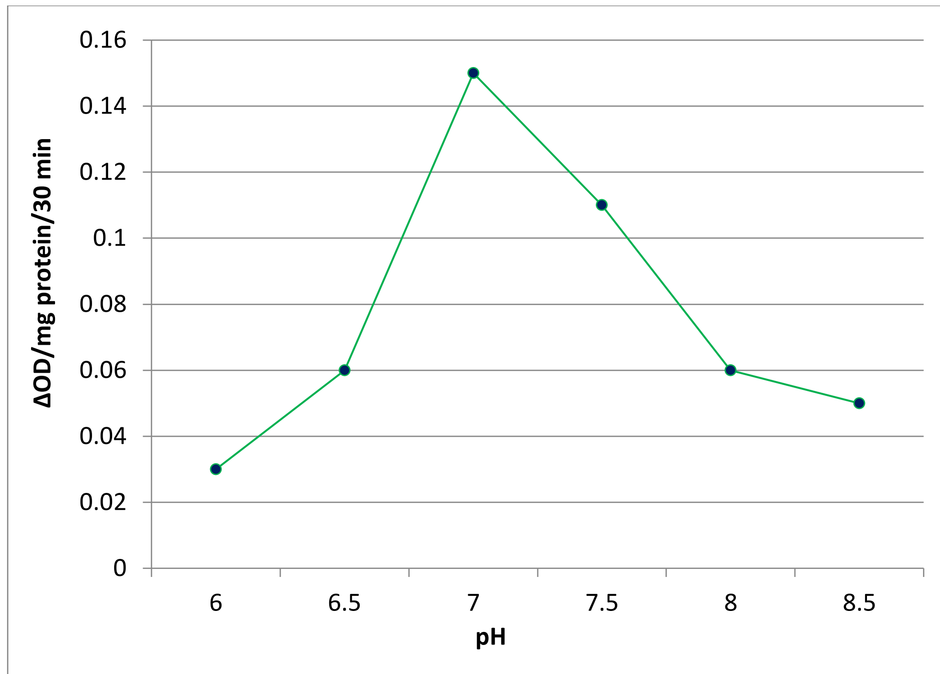


Figure 1. Effect of pH variation on the *in vitro* activity of GS from the leaves of *Sesuvium portulacastrum*

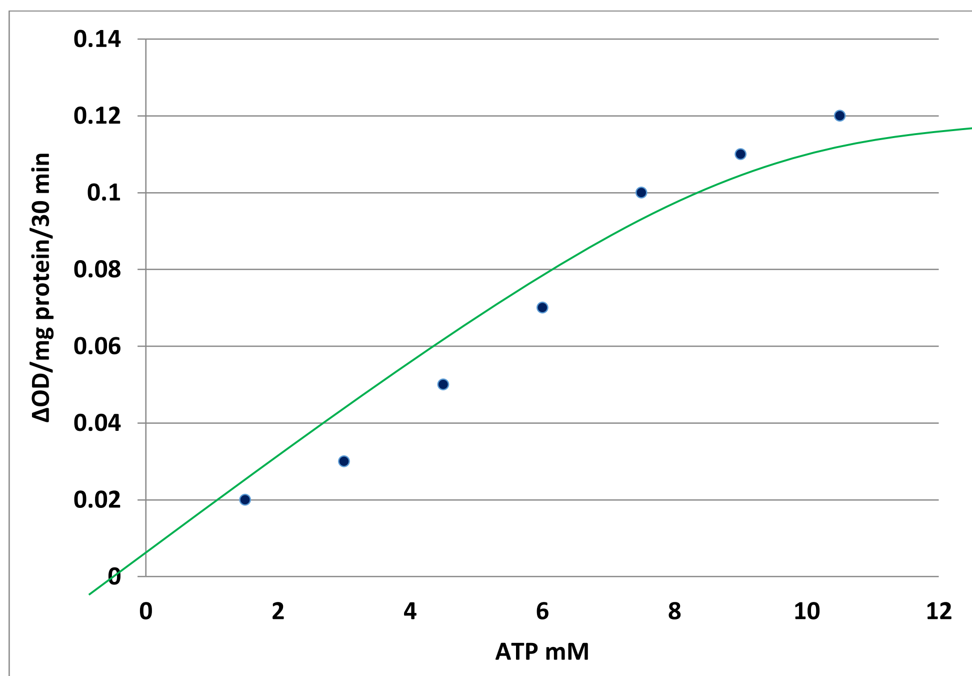


Figure 2. Effect of ATP variation on the *in vitro* activity of GS from the leaves of *Sesuvium portulacastrum*

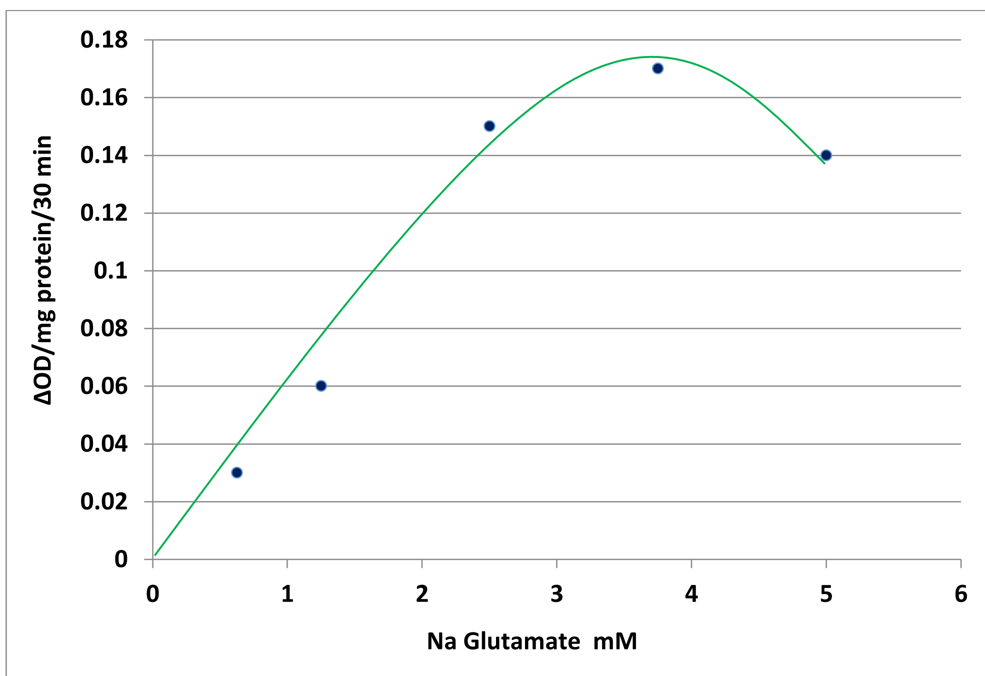


Figure 3. Effect of Na-Glutamate variation on the *in vitro* activity of GS from the leaves of *Sesuvium portulacastrum*

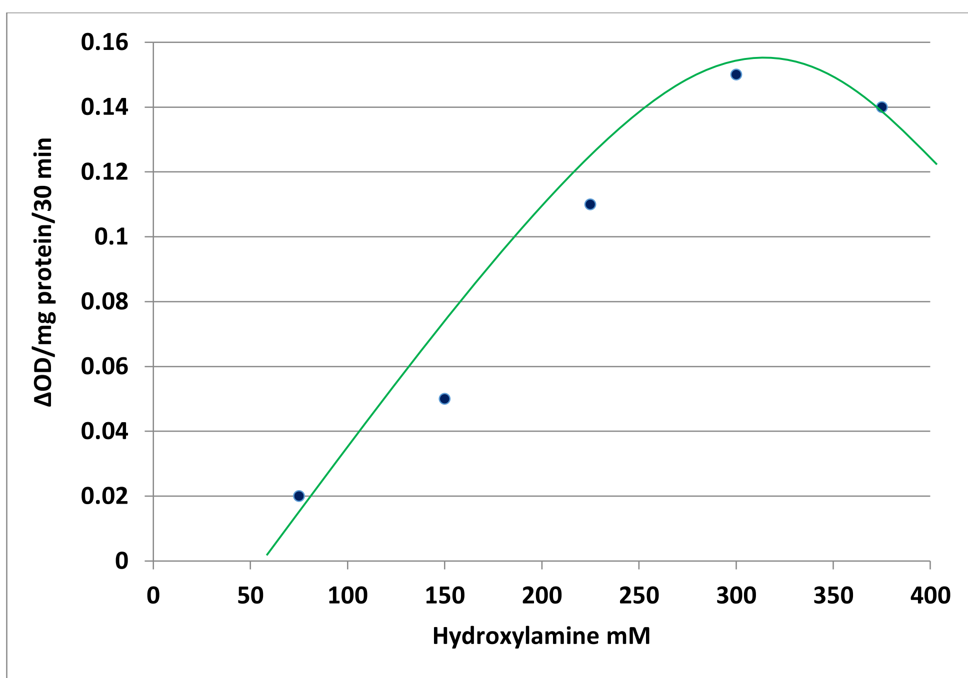


Figure 4. Effect of Hydroxylamine variation on the activity of GS from the leaves of *Sesuvium portulacastrum*

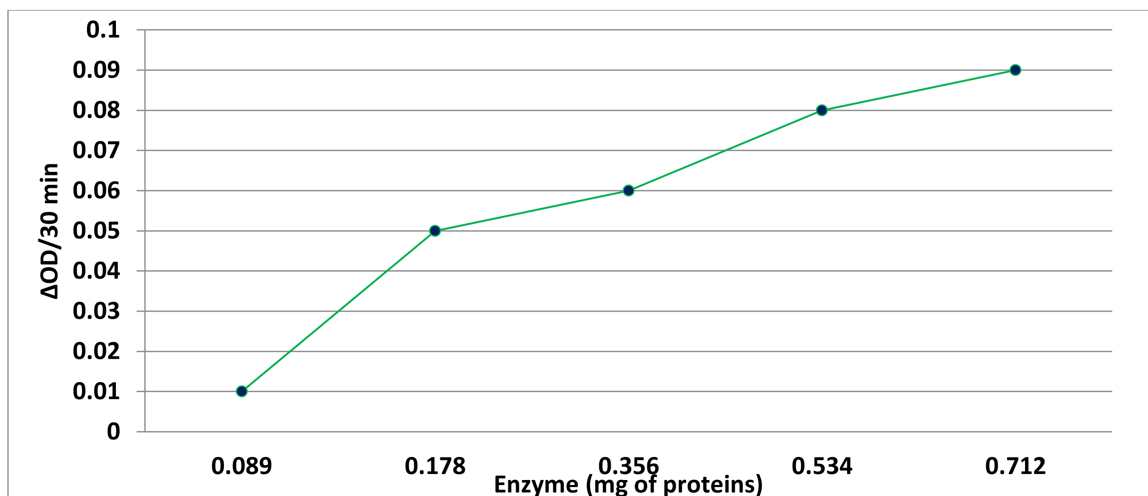


Figure 5 Effect of enzyme variation on the *in vitro* activity of GS in leaves of *Sesuvium portulacastrum*

4. Discussion

In higher plants, GS has been reported to occur in seeds (Elliot, 1953), seedlings (Webster, 1964), roots (Kanamori and Matsumoto, 1972), root nodules (Dunn and Klucas, 1973) and shoots (O'Neal and Joy, 1973). In leaves apart from its primary function of assimilation of ammonia, GS is also responsible for reassimilation and detoxification of large amounts of ammonia released during photorespiration (Tingey and Coruzii, 1987).

In halophytes, high GS activity has been reported earlier. According to Steward and Rhodes (1978) and Boucard and Billard (1979), GS in shoot play a greater role in nitrogen assimilation under saline conditions than roots. In opinion of Bottasin et al., (1985), salt resistance depends on the capacity of halophytic plants to withstand the inhibition of GS by NaCl, as salt adaptation in halophyte is a shift of nitrogen metabolism towards glutamate route. High affinity of GS for ammonia (Km 100-200 mM) (Steward and Rhodes, 1977) as against low affinity of GDH for ammonia (Stewart et al., 1980), further supports functioning of glutamate route to GDH system for nitrogen metabolism in halophytes.The pH optima and the kinetic properties of the enzyme GS depend upon the cation present (O'Neal and Joy, 1974). With Mg^{+2} as the cofactor the pH optimum is in the range of pH 8.0 and with Mn^{+2} as the cofactor, it is in the range of pH 5.0.The Km values for glutamate (1-13 mM) and ATP (0.1 - 1.5 mM) for GS greatly vary and are proportional to the concentration of each other. The results obtained in present investigation are in accordance with the findings of these scientists.

References

Balasubramanian R, Thilo R, Ahmed D, Ralf S, Bernhard H, Ahlert S and P Jutta (2006) *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survivein saline habitats. *Plant Physiol Biochem*44:395–408

Bottasin A, Cacco G and M Saccomani (1985) Nitrogen absorption and NaCl resistant and NaCl susceptible millet genotypes (*Pennisetum americanum). Can. J. Bot.*63: 517-520.

Boucaud J and JP Billard (1979) Etude comparee des activities glutamate dehydrogenasique et de glutamine synthetasique dans les recines et les parties aerinnes d'un halophyte, obligatory la *Suaeda maritima* var. macrocarpa. *Physiol. Plant.*44: 31-37.

Cullimore JV and M J Bennet (1988) The molecular biology and biochemistry of plant glutamine synthetase from root nodules of *Phaseolus vulgaris* L. and other legumes. *J. Plant Physiol*. 132: 387-393.

Dunn SD and RV Klucas (1973) Studies on possible routes of ammonium assimilation in Soybean root nodule baltereoids. Can. *J. Microbiol*., 19: 1493-1499.

Stewart GR, Mann AF and PA Fentem (1980) Enzymes of glutamate formation: GDH, GS and GOGAT In: The biochemistry of plants, Vol. 5, Ed. B.J. Miflin, pp. 271-327, Academic Press, New York, London, Toranto, Sydney, San Francisco.

Tingey SV and GM Coruzzi (1987) Glutamine synthetase in *Nicotiana plumbaginifolia* cloning and *in vivo* expression. *Plant Physiol*., 84: 366-373.

Elliot WH (1953) Isolation of glutamine synthetase and glutamotransferase from green peas. J. *Biol. Chem*., 201: 661-672.

Elliot WH (1955) Methods in enzymology7 vol. II.Eds. SPColowick and NO Kaplan, pp.337-342, Academic Press Inc. Pub. New York.

Hammer K (2001) Aizoaceae. In: Hanelt P, Institute of Plant Genetics and Crop Plant Research (eds) Mansfeld's encyclopedia on agricultural and horticultural crops, vol1. Springer Verlag, Berlin, Heidelberg, New York, 1986,pp 223–227

Joshi GV and LJ Bhosale (1982) Estuarine ecology system of India. In: "Contribution to the ecology of Halophytes". Tasks for vegetation science, vol. 2 ed. By DN sen and KS Rajpurohit. Pp. 21-31. Dr. Junk Publishers, The Hague, Boston, London, 1982.

Kanamori T and H Matsumoto (1972) Glutamine synthetase from rice plant roots. Arch. *Biochem. Biophys*., 152: 404-412.

Lingnowski EM, Splittstoesser WE and K Chou (1971) Glutamine Synthesis in germinating seeds of *Cucurbita mosch ata*. *Plant Cell Physiol*., 12: 733-738.

Lonard RI and FW Judd (1997) The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L.) *J Coast Res* 13(1):96–104

Lowry OH, Rosenbrough NJ, Farr AL and RJ Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 49: 64-71.

McCormack DK, Farnden KJF and MJ Boland (1982) Purification and properties of glutamine synthetase from the plant cytosol fraction of lupin nodules. Arch. *Biochem. Biophys.* 218: 561-571.

Meister A (1974) Glutamine synthetase of mammals. In: *The Enzymes*, Ed. P. D. Boyer, Vol 10: pp. 699-754. Academic Press, New York.

O'Neal D and KW Joy (1973) Glutamine synthetase of pea leaves. 1. Purification, Stabilization and pH optima. Arch. *Biochem. Biophys*. 159: 113-122.

O'Neal D and KW Joy (1974) Glutamine synthetase of pea leaves-divalent cation effects, substrate specificity and other properties. *Plant Physiol*., 54: 773-779.

Stewart CR and D hodes (1978) Nitrogen metabolism of halophytes III Enzymes of ammonia assimilation.*New Phytol.* 80: 307-316.

Webster G (1964) In: Modern methods of plant analysis. Eds. H. F. Linskens, B. D. Sanwal and M. W. Tracey, Vol. 7, pp. 392-420, Springer-Verlag, Berlin.

Yemm E W and BF Folkes (1958) The metabolism of amino acids and proteins in plants. Ann. Rev. *Plant Physiol*., 9: 245-280.