Electrophoretic studies of isoenzymes of Glutamate dehydrogenase (EC 1.4.1.3) (GDH) in *Arthocnemum indicum* Moq

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Abstract

Arthocnemum indicum Moq. is a succulent and halophyte which generally grow in marshy land which is frequently washed by marine water and the soil is generally deficient in nitrogen content. The plant has adapted to nitrogen stress by evolving efficient nitrogen assimilating isozymes. In present investigation 4 isoenzymes of Glutamate dehydrogenase (EC 1.4.1.3)(GDH) are separated electrophoretically from the shoot of *Arthocnemum indicum* Moq.

Key words: *Arthocnemum indicum* Moq.,isozyme, halophyte, nitrogen metabolism

Markert and Moller (1959) described the term 'isozyme' for the first time for the multiple forms of an enzyme. These enzymes are with similar or identical substrate specificity, occur in the same organism but differ in certain physico-chemical properties. According to Vessel and Bearn (1962), the duplication of gene with subsequent mutations at both the parent and the daughter loci could be the biological mechanism for producing multiple molecular forms of an enzyme. However, Rider and Taylor (1980) suggested that the multiplicity of the enzyme might be due to genetic and primary causes, where organism carries multiple genes, each one coding a different type of enzyme subunit. It may also be due to posttranslational causes, as homogenous enzyme subunits are modified differently resulting in a range of subunits from a single gene. Multiple forms of an enzyme may also result due to the influence of environment on the molecules of proteins. Lumry and Erying (1954) termed these forms as 'conformational forms'.

Isozymes commonly occur in microorganisms (Brown *et al.,* 1975) plants (Liu, 1975) and animals (Georgiev, 1975) and have been extensively studied by several workers (Vezina *et al.,* 1987, Chen and Cullimore, 1989 ; Cai and Wong, 1989). Isozymes are known to function in the biochemical modulation of intracellular reactions (Ting *et al.,* 1975). Even though isozymes exhibit tissue specificity, which implies a significant physiological role for them, they are essentially alike in enzyme activity (Markert and Apella, 1961). According to Kay *et al.* (1967), isozymes differ from one another in several catalytic properties, as affinity for the substrate, behaviour towards coenzyme, pH optima, thermal stability and sensitivity to inhibitors.

Environmental conditions are known to have a great influence on the isozyme pattern of different enzymes. Pahlich (1972) observed changes in the isozyme pattern and electrophoretic mobility of GDH due to environmental changes. Similarly, Srivastava and Singh (1987) also reported variation in the number of GDH isozymes due to variation in nutritional and environmental conditions. Modified activity of certain enzymes under saline environment are either due to conformational changes (Kalir

and Poljakoff-Mayber, 1975) of the enzyme molecule or due to the changes in isozyme pattern (Sanglikar, 1982). Hasson-Porath and Poljakoff-Mayber (1969) based on their isozyme pattern of MDH reported that Na_2SO_4 did not affect the isozyme pattern. However, NaCl caused the appearance of new isoenzymes in pea root tips. According to Somero (1975), isoenzymes serve an important mechanism for broadening the environmental tolerance range of the organisms.

MATERIAL AND METHODS

The plant material of *Arthocnemum indicum* Moq.were collected from the natural habitat and brought to the laboratory in polythene bags and used for the study of protein profile and isozymes of glutamate dehydrogenase (GDH).

Enzyme Extraction:

Plants were collected from natural habitats and brought to the laboratory in polythene bags. The plants were washed with deionized water and blotted dry. One g fresh leaf material was ground vigorously in 10 ml chilled extraction buffer (0.2M Tris-HCl pH 8, 3.5 mM $MgCl_2$ and 2.5% Polyethylene glycol) using a mortar and pestle. The homogenate was then passed through 4 layers of muslin and the filtrate was centrifuged at 10,000 rpm for 20 minutes. The debris was removed and the supernatant was used as the enzyme source. Throughout the extraction, procedure the temperature was maintained around $0^{\circ}C \pm 2^{\circ}C$. Minimum quantity of buffer was used to get concentrated extract.

Polyacryamide Gel Electrophoresis:

PAGE was carried out at low temperature ($4^{\circ}C \pm 1^{\circ}C$) according to the method of Zweig and Whitaker (1967). The gels used for the separation of anionic samples in the present experiment were 7.5% running gels; stacking pH 8.3. The gels were cast in neutral glass plates (7 cm long, 8 cm wide and 0.75 mm thickness with 10 wells) of vertical electrophoresis unit (BIO-RAD, MINI PROTEAN-II). The gel was polymerized with polymerizing catalysts like ammonium persulphate and TEMED (accelerator of polymerization of gel). For the electrophoresis, the buffer was prepared using 6g Tris-HCl, 28.8 g glycine and volume was made to one liter. The buffer was diluted 10 times and pH was adjusted to 8.3 before use.

The enzyme extract was mixed with bromophenol blue as a front marker and loaded in a polymerized gel wells. The entire gel plate was fitted in the electrophoretic unit, it was flooded with running buffer and the anodal, and cathodal ends were connected to the power system. The electrophoretic run was carried out at a current of about 2 mA /well, at a constant voltage of 150 volts. The run was carried out for about 90 minutes until the bromophenol marker front migrated to the other end of the plate. Subsequently, the gel was loosened with a jet of water and removed from the glass plate.

Detection of GDH:

Isoenzymes of GDH were detected on the gels by the method of Brewer and Singh (1970).

The staining mixture was prepared by dissolving 0.25 M L-glutamic acid, 1.5 mM NAD, 0.163 mM phenazine methosulphate (PMS) and 0.43 mM nitroblue tetrazolium (NBT) in 0.125M phosphate buffer (pH 9.0). The staining mixture was prepared shortly before use as the reactants are less stable in solution.

For detecting GDH isoenzymes, the gels were incubated in the staining mixture at 37˚C. GDH activity generates NADH, which reduces phenazine, which in turn reduces NBT. The reduced NBT is an insoluble formazan, which is coloured.

RESULTS AND DISCUSSION

Three bands G1, G2, G3, and G4 of Rf values of 0.05, 0.21, 0.43 and 0.67were observed representing GDH activity in the leaves of *Arthocnemum indicum* Moq. (Figure 1). Isoenzymic nature of GDH in higher plants is well established (Thurman *et al.,* 1965; Yue, 1969). Number of GDH isoenzymes varied amongst different plants studied and upto 7 isozymes have been reported in *Pisum sativum* (Hartman, 1973), *Medicago sativa* (Hartman *et al.,* 1973), *Arabidopsis thaliana* (Cammaerts and Jacob, 1985) and *Vitis vinifera* (Loulakakis and Angelakis, 1990). Ratajczak *et al.* (1986) observed 8 isozymes of GDH in lupin root nodules. According to Srivastava and Singh (1987), isozymic number of GDH enzyme varies with plant species as well as with nutritional and environmental conditions.

Kanamori *et al*. (1972) detected new isozymes of GDH on the zymogram of PAGE due to ammonia treatment. Similar observation of synthesis of new isozymes of GDH under high levels of ammonia in the cellular environment was made by several workers (Ratajczak *et al.,* 1977, Givan, 1979). According to Loulakakis and Angelakis (1991), ammonia induces expression of isozyme. Plants growing under high salt concentration are known to accumulate high concentration of ammonia (Strogonov, 1964), which may lead to synthesis of new isozyme of GDH. Isozymic studies of GDH in leguminous plants have suggested that the GDH isozymic pattern is the result of an adaptation of the cell to nitrogen metabolism (Mazurowa *et al.,* 1980, Avhad 2012) suggesting a physiological role of GDH isoenzymes in the regulation of nitrogen metabolism. However, in the present study three isozymes of GDH were detected in the leaves. This may be in response to halophytic nature of the plant.

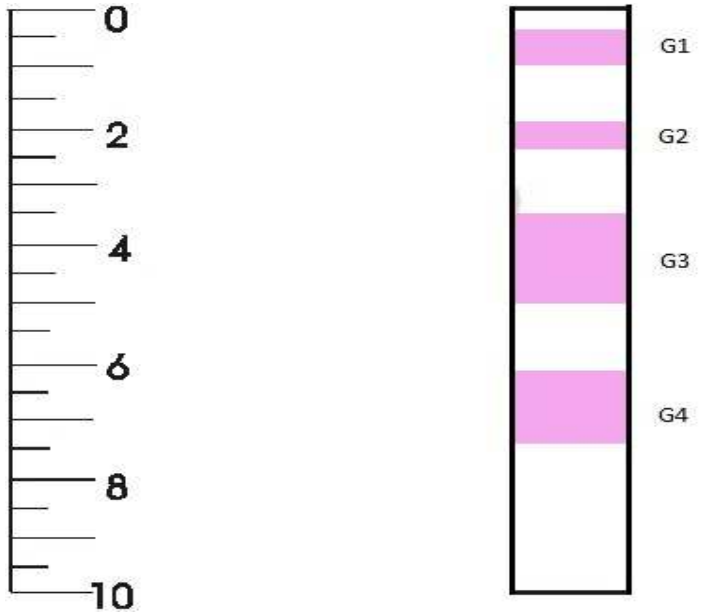

Figure 1. Zymogram depicting isoenzymes of Glutamate dehydrogenase (GDH) from the leaves of *Arthocnemum indicum* Moq.

CONCLUSION

Electrophoretic studies of Glutamate dehydrogenase (GDH) enzyme extracted from the shoot of *Arthocnemum indicum* Moq., revealed 4 isoenzymes of Rf values of 0.05, 0.21, 0.43 and 0.67.

From the present investigation it can be concluded that even though the plants of *Arthocnemum indicum* Moq. succulent and halophyte generally grow in soil which is poor in nitrogen content, it has a high nitrogen content in the plant which is due to an efficient enzyme machinery responsible for assimilating the nitrate and ammonia available to the plant.

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BIBLIOGRAPHY

Avhad Anil (2011) Studies on mechanisms of Nitrate uptake and assimilation in *Sesuvium portulacastrum* L. Ph.D. Thesis of University of Mumbai

Brewer GJ and Singh CF (1970) An introduction to isozyme technique. Academic Press, New York, London.

Brown CS, Kline EL and Umbarger HE (1975) Single reaction with multiple function. Multiple enzymes as one of three patterns in microorganisms. In: Isoenzyme. II. Physiological function Ed.CL Markert, pp. 249-274. Academic Press, New York, San Francisco, London.

Cai X and Wong PP (1989) Sub unit composition of glutamine synthetase isozymes from root nodules of bean (*Phaseolus vulgaris* L.). *Plant Physiol*. 91: 1056-1062.

Cammaerts D and Jacobs M (1985) A study of the role of glutamate dehydrogenase in the nitrogen metabolism of *Arabdiopsis thaliana. Planta.* 163: 517-526.

Chen FL and Cullimore JV (1989) Location of two isoenzymes of NADH-dependent glutamate synthase in root nodules of *Phaseolus vulgaris* L. *Planta.* 179: 441-447.

Georgiev P (1975) Studies on the activity of selected enzymes and isoenzymes in experimental heart infarct in dogs. In:Isozymes : II.Physiological function Ed. CL Markert, pp 181-192, Academic Press, New York, San Francisco, London

Givan CV (1979) Metabolic detoxification of ammonia in tissues of higher plants. *Phytochem.* 18: 375-382.

Hartman T (1973) Endogen and exogen ausgeloste Anderung-des Isoenzym spectrums der NAD - spezifischen glutamate dehydrogenase in Spro B *Von Pisum sativum. Planta.* 111: 129-136.

Hartman T, Nagel M and Ilert HI (1973) Organ spezifische multiple formen der glutamate dehydrogenase in *Medicago sativa. Planta.* 111: 119-128.

Hasson-Porath E and Poljakoff-Mayber A (1969) The effect of salinity on the malic dehydrogenase of pea roots. *Plant Physiol.* 45: 1031-1035.

Kalir A and Pljakoff-Mayber A (1975) Malic dehydrogenase from *Tamarix* root. *Plant Physiol*. 55: 155-162.

Kanamori T, Konishi S and Takahashi E (1972) Inducible formation of glutamate dehydrogenase in rice plant roots by addition of ammonia to the media. *Physiol. Plant.* 26: 1-6.

Kay E, Shannon LM and Lew JY (1967) Peroxidase isoenzyme from horse radish roots. II. Catalytic properties. *J. Biol. Chem.* 242: 2470-2473.

Liu, EH (1975) Substrate specificities of plant peroxidase isoenzymes: Physiological function Ed. CL Markert, pp.837-850. Academic Press, NewYork, San Francisco, London.

Loukakis KA and Angelakis KAR (1991) Plant NAD(H) glutamate dehydrogenase consists of two subunit polypeptides and their participation in the seven isoenzymes occurs in an ordered ratio. *Plant Physiol*. 97: 104-111.

Loukakis KA and Angelakis KAR (1991) Plant NAD(H) glutamate dehydrogenase consists of two subunit polypeptides and their participation in the seven isoenzymes occurs in an ordered ratio. *Plant Physiol*. 97: 104-111.

Lumry R and Erying H (1954) Conformation changes of proteins. *J. Phys. Chem*. 58:110.

Markert CL and Apella E (1961) Physico-chemical nature of isoenzymes. *Ann. N.Y.Acad. Sci*. 94: 678-690.

Markert CL and Moller F (1959) Multiple forms of enzymes: tissue, ontogenetic and species specific patterns. *Proc. Natl. Acad. Sci*. 45: 753-763.

Mazurowa H, Ratajczak W and Ratajczak L (1980) Glutamate dehydrogenase characteristics in the organs and root nodules of *Lupinus luteus* L**.** *Acta Physiol. Plant*. 2: 167-177.

Pahlich E (1972) Evidence that multiple molecular forms of glutamic dehydrogenase from pea seedlings are conformers. *Planta*. 104: 78-88.

Ratajczak L, Kozoniak D, Mazurawa H, Ratajczak W and Pris-Glowacki W (1986) Glutamate dehydrogenase isoforms in lupin root and root nodules. *Physiol. Plant.* 67: 685-689.

Ratajczak L, Ratajczak W and Mazurowa H (1977) Isoenzyme pattern of GDH- a reflection of nitrogen metabolism in *Lupinus albus*. *Acta. Soc. Bot. Pol*. 46: 347-357.

Rider CC and Taylor CB (1980) Structural aspects of isoenzymes In: "Isoenzymes" Chapman and Hall pp. 8-10, London, New York.

Sanglikar RN (1982) Physiology of marine plants. Studies in *Acanthus ilicifolius*, Linn. Ph.D. Thesis, Univ. of Bombay.

Somero GN (1975) Isoenzyme II. Physiological function. Ed. CL Markert (1975), Academic Press, New York, San Francisco, London.

Srivastava HS and Singh RP (1987) Role and regulation of L-glutamate dehydrogenase activity in higher plants. *Phytochem.* 26(3): 597-610.

Srivastava HS and Singh RP (1987) Role and regulation of L-glutamate dehydrogenase activity in higher plants. *Phytochem.* 26(3): 597-610.

Strogonov BP (1964) "Physiological Basis of Salt Tolerance of Plants as affected by varius types of salinity" Akad. Nauk. USSR, from Russian Israel Progr. Sci.Transl. Jerusalem.

Thurman DA, Palin C and Laycook MV (1965) Isoenzymic nature of L-GDH of higher plants. *Nature* (London). 207: 193-194.

Ting IP, Fuhr I, Curry R (1975) Malate dehydrogenase isozymes in plant, preparation, properties and biological significance. In: Isozymes II. Physiological function Ed. CL Markert, pp 369-389. Academic Press, NewYork, San Francisco, London.

Vessel ES and Bearn AG (1962) Variation in the lactic acid dehydrogenase of vertebrate erythrocytes. *J. Gen. Physiol*. 45: 553-565.

Vezina LP, Hope HJ and Joy KW (1987) Isoenzyme of glutamine synthetase in roots of pea and alfalfa. *Plant Physiol*. 83: 58-62.

Zweig G and Whitaker JR (1967) Paper chromatography and electrophoresis vol. I. Electrophoresis in stabilizing media. Academic Press, New York, London.