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REVIEW-PHYSIOLOGICAL AND BIOCHEMICAL ROLE OF GLUTAMATE DEHYDROGENASE IN PLANTS GROWTH AND REGULATION

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ABSTRACT:

In the present review importance of Glutamate dehydrogenase in the physiological and biochemical processing of plant has been reviewed. It shows that glutamate dehydrogenase play very important role in the ammonium assimilation as well as in the mobilization of nitrogen and carbon from storage materials to aspergiene in germinating seeds. It also help in regulation of some vital physiological cycle in plant.

KEY WORD: *Glutamate dehydrogenase, ammonium assimilation, germinating seeds, regulation and physiological cycles.*

INTRODUCTION:

Plants utilize nitrate, ammonium and dinitrogen (N_2) molecules as external nitrogen sources. Ammonium is the final form of inorganic nitrogen prior to the synthesis of organic nitrogen compounds. Ammonium is also produced via internal metabolic reactions including photo-respiration, hydrolysis of nitrogen carrying and storage molecules and amino acids conversion (Ireland and Lea, 1999). Although several biochemical reactions involving ammonium as a reactant are known (Miflin and Lea, 1980), the reductive amination of 2-oxoglutarate to glutamic acid has long been considered as a major route of ammonia assimilation. This reaction is catalyzed by the enzyme L-glutamate dehydrogenase {L-glutamate NAD^+/ $NAD(P)^+$ or $NADP^+$ oxidoreductase (deaminating) GDH, EC 1.4.1.2-4}. Since the discovery of glutamate synthase, GDH has been a subject of controversy (Miflin and Lea, 1976; Stewart *et. al.,* 1980; Srivastava and Singh, 1987). Now, it is accepted that ammonium is first assimilated into the Glu amide group which is then transferred to the position of 2-oxoglutarate, yielding two molecules of Glu by the concerted reaction of Gln synthetase (GS; EC 6.1.1.3) and Glu synthase (ferredoxin [Fd]- GOGAT; EC 1.4.7.1;

NADH-GOGAT; EC 1.4.1.14) (Miflin and Lea, 1982). L-glutamate dehydrogenase is thought not to function in the normal metabolic assimilation of ammonia but rather in glutamate catabolism, providing carbon skeletons for the tricarboxylic acid cycle and thus, acting as a link between carbon and nitrogen metabolism (Miflin and Lea, 1976; Goodwin and Mercer, 1983; Robinson *et. al.,* 1991). Numerous studies have been carried out to define the roles of enzymes in nitrogen assimilation and remobilization, tightly interrelated processes during plant growth and development (Miflin and Habash, 2002). However, physiological role of large amount of GDH present in the tissues of higher plants is still obscure. Our aim in present article is to review the characteristics, properties and behaviour of GDH and possible physiological role of this enzyme in the nitrogen metabolism.

OCCURRENCE AND DISTRIBUTION:

Glutamate dehydrogenase has been found to be of universal occurrence in almost all living organisms (Stewart *et. al.,* 1980; Goodwin and Mercer, 1983; Srivastava and Singh, 1987; Kwinta and Bielawski, 1998; Muro-Pastor and Florencio, 2003; Morel *et. al.,* 2006). In higher plants it exists into two distinct forms (i) a mitochondrial enzyme which is generally referred to as NAD linked and (ii) a chloroplast enzyme which is generally referred to as NADP linked (Stewart *et. al.,* 1980). Enzymes have been isolated and characterized from a number of plant tissues (Pahlich and Joy, 1971; King and Wu, 1971; Mazurova *et. al.,* 1980; Nagel and Hartmann, 1980; Scheild *et. al.,* 1980; Yamaya *et. al.,* 1984; Itagaki *et. al.,* 1986; Shargool and Jain, 1989; Loulakakis and Roubelakis-Angelakis, 1990). In higher plants NADH-GDH is a hexameric enzyme composed of two different subunits GDH1 and GDH 2 encoded by Gdh 1 and Gdh 2, respectively. These are located at two independent loci (Pryor, 1979; Goodman *et. al.,* 1980). NADH-GDH is found to be localized in bundle sheath cells (Mellor and Treguna, 1971). In C_4 plants there were equal distribution of 69-87% of NADH-GDH between mesophyll and bundle sheath cells (Rathnam and Edwards, 1976; Harel *et. al.,* 1977; Moore and Black, 1979; Yamaya and Oaks, 1988; Becker *et. al.,* 2000).

REGULATION:

GDH activity is regulated by a variety of exogenous (Lu-BinBin, 2005) and endogenous factors including age, nutritional and environmental conditions etc. Plants GDH level is affected by age. Several workers have reported increased enzymatic activity in roots, hypocotyls, cotyledons, shoots and leaves (Masurowa *et. al.,* 1980; Fawola, 1977; Kennedy, 1980; Groat and Vance, 1981& 1982; Storey and Beevers, 1978; Smirnoff and Stewart, 1978; Loyola-Vargas and Jimenez, 1984; Puranik, 1985; Sairam *et. al.,* 1975; Quetz *et. al.,* 1982; Nicklisch *et. al.,* 1976; Street *et. al.,* 1979; Cammaert and Jacobs, 1985). The enzyme is least studied in mature plants; in citrus trees, GDH levels increase at the time of flowering and fruiting when the plants are supplied with nitrate as an N-source (Ramamurthy and Ludders, 1982). An increase

in GDH activities has also been reported during senescence of plant tissues (Lauriere *et. al.,* 1981; Cammaerts and Jacobs, 1985; Thomas, 1978; Kang and Titus, 1980; Simpson and Dalling, 1981; Guello and Sabater, 1982; Kar and Feierebend, 1984; Garg and Srivastava, 1992). It has been suggested that ammonia produced by proteolysis during senescence could be responsible for de novo synthesis of the enzyme (Lauriere and Daussant, 1983; Postius and Jacobs, 1976; Thomas, 1978; Givan, 1979).

Several workers have shown an induction of GDH activity by high levels of ammonia (Kanamori *et. al.,* 1972; Shepherd and Thurman, 1973; Singh and Srivastava, 1982; Lauriere and Daussant, 1983; Prunkard *et. al.,*1986; Srivastava and Singh, 1987; Jain and Shargool, 1987; Shargool and Jain, 1989; Bahadula and Shargool, 1991; Senger and Srivastava, 1995; Magalhaes *et. al.,* 1995; Chopra *et. al.,* 2003). GDH also seems to play an important role during plant development when nitrogen compounds are being mobilized (Srivastava and Singh, 1987). The effect of ammonium varies in magnitude with different concentrations of ammonium in most cases (Kanamori *et. al.,* 1972; Kindt *et. al.,* 1980; Caldas and Caldas, 1976). It is generally believed that GDH is the primary aminating enzyme when the source of inorganic nitrogen is the ammonium (Singh and Srivastava, 1982; Cammaerts and Jacobs, 1985) while glutamine synthetase and glutamate synthase pathway operates primarily when nitrogen supply is in the form of nitrate (Rhodes *et. al.,* 1975; Loyola-Vargas and sanchez de Jimenez, 1986; Magalhaes and Huber, 1989). Ammonium may increase enzyme activity by increasing the amount of enzymic protein or / and by modulating the activity of existing enzyme molecules. *De novo* synthesis of GDH in response to ammonium has been demonstrated in various plants (Kanamori *et. al.,* 1972; Barash *et. al.,* 1975; Lauriere and Daussant, 1983; Cammaerts and Jacobs, 1985; Shepherd and Thurman, 1973). The effect of nitrate on GDH activity varies with species, tissues, time of incubation etc. (Oaks *et. al.,* 1980; Singh and Srivastava, 1982; Ramamurthy and Ludders, 1982; Loyola Vergas and de Jimemez, 1984). During short term supply of nitrate to excised tissues, it either inhibit or has no effect on enzyme activity (Singh and Srivastava, 1982; Levis *et. al.,* 1982; Oaks *et. al.,* 1980; Ehmkey and Hartmann, 1976; Kanamori *et. al.,* 1972; Ingle *et. al.,* 1966). However, long term supply to intact seedlings increases enzyme activity in various plants (Singh and Srivastava, 1982; Quetz *et. al.,* 1982; Stulen *et. al.,* 1981). The effect of nitrate is not as pronounced as that of ammonium (Singh and Srivastava, 1982; Quitz *et. al.,* 1982).

Aminating and deaminating activities of GDH is also affected by amino acids (Nauen and Hartmann, 1980; Singh and Srivastava, 1983; Rhodes *et. al.,* 1976; Chopra *et. al.,* 2003). The response of amino acids in different tissues possibly due to differences in the regulatory nature of enzyme. It is also dependent on the concentration, period and procedure of application (Loyola-Vergas and Jimenez, 1984; Singh and Srivastava, 1983; Ratazczak *et. al.,* 1981; Rhodes *et. al.,* 1976). Some amino acids analogs have been reported to be important in determining the path of ammonium assimilation in higher plants. Exogenously supplied sugars inhibit the activity of GDH in most of the cases (Sahulka, 1975; Nauen and

Hartmann, 1980; Cammaerts and Jacobs, 1985; Sahulka *et. al.,* 1975; Tassi *et. al.,* 1984). However, the effect of glucose is variable, it increases enzyme activity of *Lemna* and lowers it in *Pisum* root and shoot (Sahulka and Lisa, 1980; Nauen and Hartmann, 1980). Besides glucose, fructose and sucrose are found to inhibit GDH activity in *Pisum* shoot and *Lupinus* embryonic axis, respectively. Low concentration of IAA increased GDH activity in excised maize leaves (Awasthi and Garg, 2006).

An increase in growth temperature, salinity, water stress, pollutant leads to an increased level of GDH. Under stress conditions GDH is more stable enzyme than Glutamine synthetase and play a major role in determining the plant response to potentially toxic levels of ammonia (Srivastava and Singh, 1987; Senger and Srivastava, 1995; Gadi and Bohra, 2001). Pollutants may increase enzyme activity through altered membrane permeability.

GDH exists as a number of isoenzymes which vary in number according to environmental conditions (Sood *et. al.,* 1999) and developmental stages. GDH iso-enzymes have been found localized in the mitochondrial, chloroplastic, plastidial and cytoplasmic fractions (Srewart *et. al.,* 1980; Srivastava and Singh, 1987). Since GDH in different sub-cellular compartments is subjected to a particular micro-environment, it is difficult to explain how the activity of different iso-enzymes is co-ordinated within the plant cell. The changes in GDH isoenzymes pattern under environmental and developmental conditions and the compartmentalization of GDH suggests that there are distinct GDH genes present in plant tissues. Changes in GDH iso-enzymes pattern under different growth conditions have also been reported for other iso-enzymic systems associated with nitrogen metabolism such as glutamine synthetase (Gebharlt *et. al.,* 1986). In higher plants NADH-GDH is a hexameric enzyme composed of two different subunits, GDH1 and GDH2 (in maize), that are encoded by Gdh 1 and Gdh 2, respectively which are located at two independent loci (Pryor, 1979; Goodman *et. al.,* 1980). The mobility of GDH1 is lower than that of GDH2 during PAGE on non-denaturing gels. The seven iso-enzymes of NADH-GDH can change during development, ripening (Loulakakis *et. al.,* 1994), dark stress (Cammaerts and Jacobs, 1985) and upon feeding of a source of nitrogen (Loulakakis and Roubelakis Angelakis, 1991). The levels of the more anodal iso-enzymes are increased by de-novo synthesis of the α-subunit (Loulakakis and Roubelakis-Angelakis, 1992) which corresponds to the GDH 2 subunit in maize.Although good progress has been made to dissect and better understand both the main steps and the regulation of inorganic nitrogen assimilation in higher plants, the role of alternative metabolic pathways which are potentially able to incorporate ammonium into organic molecules is still not fully understood. One of them is the reaction catalysed by the mitochondrial enzyme glutamate dehydrogenase (NAD(H)-GDH, EC 1.4.1.2) which is either able to incorporate ammonium into 2-oxoglutarate to form glutamate or to function in the opposite direction to oxidise glutamate. Although it has been clearly demonstrated by the means of ^{15}N- or ^{13}C-labelling experiments that the later reaction occurs in the cell, it has been argued that under certain

physiological conditions, when the ammonium concentration reaches a certain threshold, the enzyme is able to function in the aminating direction. More recently, it has been found that in grapes, a high proportion of the protein is located in the mitochondria of the phloem companion cells and that a significant amount of enzyme is present in the cytosolic fraction of senescing flowers. Using cytoimmunochemistry, we confirmed in the present study that, in other higher plant species, GDH protein is localised in the mitochondria of the phloem companion cells and in the cytosol of senescing organs or tissues. These findings open, therefore, new perspectives toward a better understanding of the function of GDH, particularly in relation to stress and plant development. Both transgenic studies performed in the past and the quantitative genetic approach presented in this paper strongly suggest that the reaction catalysed by NAD(H)-GDH is of major importance in the control of plant growth and productivity.

CONCLUSION:

This review shows that glutamate dehydrogense role in nitrogen assimilation and remobilization, tightly interrelated processes during plant growth and development .However, physiological role of large amount of GDH present in the tissues of higher plants is still obscure. The changes in GDH isoenzymes pattern under environmental and developmental conditions and the compartmentalization of GDH suggests that there are distinct GDH genes present in plant tissues. L-Glutamate dehydrogenase catalyses the reversible conversion of 2-oxoglutarate and L-glutamate for the entry of ammonium into the organic cycle and for its release as well. Various isozymes of GDH are present ubiquitously in higher plant tissues. The enzyme, with a molecular weight of 208 000 to 270 000, is composed of four to six subunits, contains a free -SH group at the active centre, and is associated with metal ions. Some isozymes of GDH are inducible and vary according to the nutritional and environmental status of the tissues. The level and activity of enzyme is either direction is regulated by age, light/dark regime, inorganic and organic nitrogen, carbon and energy status, growth regulators and some other factors. The enzyme seems to be important in assimilation of ammonia under stress conditions such as dark starvation, high temperature, salinity, water stress, environmental pollution, senescence and other abnormalities.

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