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Research Article

BIOCHEMICAL STUDIES IN RELATION TO GLUTAMATE DEHYDROGENASE IN *Piper Nigrum* (L.)

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ABSTRACT

Black pepper, the king of spices, the most important and widely used spice in the world is the dried mature berries of *P. nigrum* L. Inorganic nitrogen is assimilated into amino acids glutamine, glutamate, asparagines and aspartate which serve as important nitrogen carriers in plants. The enzymes glutamine synthetase (GS) EC. 6.3.1.2, glutamate synthase (GOGAT) E.C. 1.4.1.14, glutamate dehydrogenase (GDH) E.C.1.4.1.4, aspartate aminotransferase and asparagine synthetase are responsible for biosynthesis of these amino acids. Although extensive studies have been carried out on the epidemiology of the diseases and agronomical aspects of *P. nigrum*, very few studies have been made on the enzymological aspects especially that associated with the nitrogen assimilation. A better understanding of the various properties of the enzyme will be valuable in the future studies concerned with Nitrogen metabolism in *P. nigrum*. Main objective of present study was to standardize the extraction and study the properties of GDH from *P. nigrum* L. Effect of additives and its optimum concentration were determined, standardized homogenizing medium was formulated, standardized assay system formulated, different properties like pH optimum, Substrate saturation and km value determined; it's all in agreement with the previously reported values, result indicate $NADP^+$/NADPH is the better substrate i.e. the enzyme activity is contributed by chloroplastic form of GDH than by the mitochondrial form.

Keywords: *Piper nigrum L., GDH* (Glutamate dehydrogenase), Km value.

INTRODUCTION

Nitrogen assimilation is a vital process controlling plant growth and development. GDH activity has been found in most species tested. The existence of two distinct GDH enzymes in higher plants is now well documented.

a) A mitochondrial enzyme which specifically requires co-enzyme NAD^+
b) A chloroplast enzymes which specifically requires co enzyme $NADP^+$

NADH-dependent form (NADH-GDH: E.C.1.4.1.2) found in the mitochondria and an NADPH-dependent form (NADPH-GDH: E.C.1.4.1.4) localized to the chloroplast. The GDH enzyme is abundant in several plant organs. In plants, the enzyme can work in either direction depending on environment and stress. Transgenic plants expressing microbial GDHs are improved in tolerance to herbicide, water deficit, and pathogen infections. They are more nutritionally valuable. The enzyme represents a key link between catabolic and metabolic pathways, and is, therefore, ubiquitous in eukaryotes.

GDH appears to catalyse the oxidation of glutamate in response to deficiency of carbon. The catabolic role for GDH implies an important regulatory function in carbon and nitrogen metabolism. Its capacity to fix ammonium in vitro utilising the organic acid 2-oxoglutarate to synthesise

glutamate led a number of authors to propose that GDH could operate in the direction of ammonium assimilation (Oaks, A, 1995). However, all the 15N labelling experiments performed in plants or using intact mitochondria have demonstrated that GDH operates in the direction of glutamate deamination (Robinson *et al*., 1992). It was therefore concluded that GDH is rather involved in the recycling of carbon molecules by supplying 2-oxoglutarate to tissues becoming carbon limited rather than in assimilation of ammonium when it is in excess (Stewart *et al*., 1995; Salsac *et al*., 1987).

Although a number of papers have been published to assess the role of GDH during plant C and N assimilation and remobilization, its physiological function still remains largely speculative. Another alternative is that the enzyme does not play any significant role in the C and N metabolic network but is rather involved in sensing the redox status of the plant and as such may represent a stress monitoring protein as already suggested by Loulakakis and Roubelakis-Angelakis (1992). Relevance of the study is when investigating a plant enzyme researchers often encounter a number of problems associated with the isolation and study of plant enzymes. Toughness of the tissue in many cases, the presence of chlorophyll and other pigments and acidity of the tissue are some of the factors to be taken into account. But a more serious problem encountered is with respect to the high phenolic content of many tissues. Keeping in mind the above, the author carried out several preliminary experiments. The rationable behind these is briefly discussed below.

The inhibition of enzyme activities by phenolics was observed by several earlier workers. (Anderson, 1968; Loomis and Battalle, 1966; Hulme and Jones, 1963). Tannin was shown to be inhibitory to banana aldolase (Young, 1965), and polyphenol oxidase (Badran and Jones, 1965). Khanna (1971) showed that the inhibition of glucan phosphorylase in the leaves of *Dendrophthoe falcata* could be considerably reduced by incorporating cysteine and EDTA in the homogenizing medium. A detailed review on enzyme extraction from plant tissues rich in phenols / phenolases was made by Krishnan (1969). While studying the activities of a number of enzymes from the leaf, fruit, pseudostem and root tissues of banana, Baijal *et al*., (1972) gave a detailed account of the effect of supplementing the homogenizing medium with PVPP and Triton. Haising and Schipper (1975) reported that PVPP and 2-mercaptoethanol alone could stabilize malate dehydrogenase from many plants. Guttenberger *et al*., (1994) could minimize the effect of interferences by the phenolic compounds on glucose-6-phospate dehydrogenase and other dehydrogenases in *Picea abies* roots by incorporating PVPP during extraction. There are many other reports, which stress the need for the proper formulation of the homogenizing medium by incorporating reducing agents and chelating agents such as EDTA.

The systematic assessment of the effect of additives such as 2-mercaptoethanol, EDTA and PVPP by the present author resulted in obtaining a suitable homogenization medium with minimum interference from the endogenous phenolics. The present author took into consideration the above points while standardizing the extraction conditions for measurements of enzyme activity and protein determination. The results obtained from this study might serve as a valuable indicator to link the activities of glutamate dehydrogenase in the presence and absence of high nitrogen content. A better understanding of the various properties of the enzyme will be valuable in the future studies concerned with nitrogen metabolism in *P. nigrum*.

Materials and methods

For all the experiments outlined below, *Piper nigrum* L. var. Panchami was used. Authentic plants were obtained from the Indian Institute of Spices Research (IISR), Kozhikode, Kerala, India. These plants were raised in the net house of the Botanic Gardens of the Department of Botany, St. Joseph's College, Devagiri and used in the present study.

Selection of tissue.

Enzymatic studies were carried out in the leaf tissue of *P. nigrum* L. The leaf samples were collected between 9 and 10 am every time. Care was taken to prevent the loss of moisture by keeping the samples immediately on collection in polyethylene bags and storing in thermocol box containing cracked ice. Enzyme extraction was conducted within 10 minutes after the collection of samples. The leaves were washed in tap water followed by distilled water and blotted with filter paper to remove the adhering water. The petiole was removed and the tissue was cut into small pieces and was randomized. The required quantity of tissue was

accurately weighed in a chemical balance.

Studies on Glutamate dehydrogenase in the leaves of *P. nigrum* L.

Standardization of the homogenizing medium

Tissue homogenisation

A 10 % (w/v) homogenate was usually prepared. *P. nigrum* is rich in phenolics. Crude tissue homogenates without the supplements such as 2-mercaptoethanol and ethylenediamine tetraacetate showed browning due to phenolic oxidation. Hence the following experiments were carried out.

Medium for homogenization

For the preliminary experiments, to standardize the homogenizing medium in order to minimize the effect of phenolics on enzyme activity measurements, an ice-cold basal medium consisting of 20mM Tris-HCl buffer pH 7.5, 20mM 2-mercaptoethanol, 50 mg polyvinyl polypyrrolidone (PVPP) / g tissue and 10mM ethylenediamine tetraacetate (EDTA) was used. The tissue was homogenized in a pre-chilled porcelain mortar using a pestle. Acid-washed sand was used as an abrasive to increase the efficiency of grinding. The mortar was kept in a plastic bowl surrounded by cracked ice to prevent inactivation of the enzyme due to increase in temperature. The finely ground tissue was then squeezed through two layers of muslin and the volume of the filtrate was made up to 20 ml. All steps during extraction were conducted at 4 to 8^0 C. The filtrate was centrifuged at 19000 X *g* for 15 minutes at 0^0 C in a Plastocraft Rota 4R-V/Fm table top refrigerated centrifuge and the supernatant was collected.

Effect of additives during homogenization

The standardized medium of homogenization for *Piper nigrum* leaf tissue was formulated after evaluating the effects of different additives such as 2-mercaptoethanol, ethylenediamine tetraacetate (EDTA) and polyvinyl polypyrrolidone (PVPP) on the activity of glutamate dehydrogenase. For this purpose, different concentrations of the above-mentioned additives were incorporated systematically in the homogenizing medium and the enzyme activities were monitored. The optimum concentrations of 2-mercaptoethanol (has been widely used as an antioxidant/reducing agent and to prevent the oxidation of phenolics) EDTA (for complexing with divalent cations and with higher valence metal ions) and PVPP (insoluble PVP, which effectively fixes the phenols), during homogenization were found out and subsequent experiments were performed using the optimized medium.

SephadexG-25 Gel filtration

This step became necessary following the observation that spectrophotometric measurements of enzyme activity using the 19000 X *g* supernatants of extracts of *P. nigrum* gave very high background absorption. This was due to the presence of substances endogenously present in the supernatant, which absorbed strongly in the UV region. By the removal of the endogenous, UV-absorbing molecules by gel filtration, the assays could be rendered sensitive and hence this step was resorted to. The following is the protocol of gel filtration.

SephadexG-25 (coarse) was allowed to swell overnight in excess double distilled water. The suspension was stirred and the fine particles were removed by successive washing and decantation. Water was drained away and the swollen gel was suspended in a medium consisting of 20mM Tris-HCl, pH 7.5 and 5mM 2-mercaptoethanol. After stirring and equilibration overnight in the cold, the gel was packed into a glass column, 20 cm in length and 1.8 cm in diameter. The gel was packed to a height of 10cm leaving about 10cm space above (total bed volume was about 25ml). The column was fixed vertically and the top layer of the gel was made perfectly horizontal. A filter paper disc was placed above the gel surface and the column was transferred to a refrigerator and allowed to cool. The equilibration medium above the filter paper disc was drained off by operating the stopcock. The void volume was determined using **Blue Dextran**. 2.0 ml of the 19000 X *g* supernatant of enzyme extract was carefully layered above the filter paper using a pipette and an equal volume of the equilibration medium was drained off. The top of the column was carefully rinsed with a small quantity of equilibration medium and the same was drained off as before. Proteins were eluted from the column using excess equilibration medium. A total effluent volume equal to the void volume was drained off at a flow rate of 0.5 ml / minute. Proteins were collected as a single fraction of 4.0 ml following the exclusion of the void volume, with one-fold dilution. The gel filtrate thus obtained which was almost colorless and served as the source of enzyme for the preliminary studies.

Assay system

Assay systems for both forward and reverse reactions were designed separately

An assay system patterned by Robinson *et al.*, (1991), with minor modifications was initially used for preliminary standardization experiments of glutamate dehydrogenase.

Standardized medium for homogenization

From the above experiments it was possible to formulate a medium for homogenization suitable for eliciting maximum activity of glutamate dehydrogenase. The optimum concentration of each additive like 2-mercaptoethanol, EDTA, and PVPP, which were determined separately were kept constant and incorporated into the basal Tris-HCl buffer medium.

Enzyme activity substrate concentration relationship

The activity of glutamate dehydrogenase at different concentrations of the substrates was tested.

$NADP^+$: Final $NADP^+$ concentrations in the assay system ranging from 3mM to 0.125 mM (3.0mM, 2.0, 1, 0.5, 0.25, and 0.125mM) at a fixed concentration of 2.0mM glutamate were employed for determining the relationship between the enzyme activity and substrate concentration. Activity measurements were carried out for six minutes for each assay and substrate saturation curve for $NADP^+$ was plotted and the concentration of $NADP^+$ at which maximum velocity was obtained was determined. This saturating concentration was employed in the subsequent experiments.

The same was done by using NAD^+, NADH, NADPH, Glutamate (at comcentrations,0.125,0.25,0.5,1.0,2.0,3.0mM at a fixed concentration of 1mM NAD^+), α-ketoglutarate (same concentrations at a fixed concentration of 1mM NADH).

Determination of optimum pH

The optimum pH for glutamate dehydrogenase was determined using the SephadexG-25 gel filtrate in the experiments. Tris-maleate buffer in the pH range 7.0 to 7.6 and Tris-HCl in the pH range 7.6 to 8.8 were used. The assay system consisted of 0.4 ml 200mM buffer of various pH values, 0.2 ml enzyme preparation, 0.1 ml 10mM $CaCl_2$, 0.1 ml 200mM glutamate, 0.1 ml 1.0mM $NADP^+$, and 0.1 ml water in a total volume of 1.0 ml. The reaction was initiated by $NADP^+$. The measurements of enzyme activity were done by direct spectrophotometry as explained earlier. The change in optical density in three minutes was measured. The optimum pH was determined by plotting a graph with activities on the Y-axis and pH on the X-axis.

In a similar manner the pH optimum of glutamate dehydrogenase was studied using NAD^+ as the substrate. The result of a typical experiment is recorded in the results.

Activity of glutamate dehydrogenase under conditions of high nitrogen availability

Levels of activity of glutamate dehydrogenase under conditions of high nitrogen availability was studied by supplying 1% urea solution. The control plants did not receive any urea supplement. The urea solution was poured into the soil and also sprayed on the leaves. This work has been carried out for the comparison of activity of the enzyme glutamate dehydrogenase in the presence of high nitrogen availability. The selection of tissue was done in the same way as explained above. The sample tissue was collected after 48 hours. Tissue homogenization was done by using the standardized homogenizing medium. The comparisons of activity of the enzyme glutamate dehydrogenase in the control and in the experimental (urea treated) were discussed in the results.

Standardized assay system

After the standardization of optimum conditions for glutamate dehydrogenase activity from the leaves of *P. nigrum*, an assay system fulfilling all these parameters was finalized and followed in the subsequent studies.

The standard assay system consisted of 0.4 ml 200mM Tris-HCl buffer pH 8.2, 0.2 ml enzyme, 0.1 ml 10mM $CaCl_2$, 0.1 ml 200mM glutamate, 0.1 ml 1.0mM $NADP^+$, and 0.1 ml water in a total volume of 1.0 ml in the case of reverse reaction. The reaction was started by the addition of $NADP^+$/NAD and the activity for 3 minutes was measured by direct spectrophotometry at room temperature. In the case of forward reaction the standard assay system consisted of 0.4 ml 200Mm Tris-HCl buffer pH 8.2, 0.2 ml enzyme, 0.1 ml 10mM CaCl2, 0.1 ml 200mM α- ketoglutarate, 0.1 ml 1.0Mm NADH/NADPH and the activity for 3 minutes was measured by direct spectrophotometry at room temperature.

Unless and otherwise specified, the above assay system was employed for the various experiments.

Verification of endogenous activity:

Since glutamate dehydrogenase activity was measured as

change in optical density at 340 nm it was felt necessary to verify whether the Δ O. D. observed was solely due to the enzyme reaction or whether non-enzymatic and / or other endogenous reactions also contributed to the overall increase in the optical density. This was verified by withholding one or the other substrate in the assay system. The change in the optical density was measured for 3.0 min. In one experiment, only glutamate was added to the assay system withholding $NADP^+$ and the change in O.D. was monitored. In a similar experiment, only $NADP^+$ was supplied withholding glutamate. No change in O.D was noted when one or the other substrate was withheld; the reaction commenced only in the presence of both the substrates.

Substrate specificity: The substrate of choice i.e. NAD^+/NADH or $NADP^+$ / NADPH for the enzyme glutamate dehydrogenase were also verified. The assay system was the same as explained earlier. The change in optical density was insignificantly low and confirmed that $NADP^+$ /NADPH is the substrate of choice.

Determination of Michaelis-Menten constant (Km value)

The activities of sephadexG-25 gel filtrated glutamate dehydrogenase at different concentrations of $NADP^+$, NAD^+, NADPH, NADH in the assay system ranging from 0.125 to 0.3mM (0.125, 0.25, 0.5, 1.0, 2.0, 3.0mM) with fixed concentration of glutamate, Glutamate (were fixed concentration of NAD^+), α- ketoglutarate (were fixed concentration of NADH) were measured. The standardized assay system was used. The Km value for all these are determined using the double-recipfrocal method of Lineweaver-Burk (Dixon and Webb, 1979).

RESULTS

Standardization of the homogenizing conditions

The effects of additives like 2- mercaptoethanol, EDTA and PVPP in the homogenization medium were tested to optimise the homogenizing medium for the activity measurements of glutamate dehydrogenase. The results of the above are presented below.

Determination of optimum concentration of 2-mercaptoethanol in the homogenization medium.

Tissue homogenates, which were prepared without 2-mercaptoethanol, showed browning due to phenolic oxidation and consequent protein precipitation as a result of phenolic-protein interaction. The protective effect of 2-mercaptoethanol at different concentrations in the homogenization medium was tested and the results of a typical experiment are recorded in table 1.

There was no demonstrable activity of glutamate dehydrogenase in the absence of 2-mercaptoethanol. Incorporation of 2-mercaptoethanol in the homogenization medium resulted in an increase in enzyme activity. Compared to the activity at 10mM concentration of 2-mercaptoethanol, there was a marked increase at 20mM (45 %) and there is only marginal increase of at 50mM. Based on the results, the optimum concentration of 2-mercaptoethanol was fixed at 20mM for a 10 % (w/v) fresh tissue homogenate.

Determination of optimum concentration of ethylene diamine tetra acetate in the homogenization medium

Incorporation of neutralized EDTA in the homogenization medium as a chelating agent for divalent metal ions was tested at final concentrations of 5.0, 10 and 20mM. Increase in activity of about 47 % at 5.0mM and 66 % at 10mM were noted when compared to the control. The increase was only 64 % at 20mM. The optimum concentration of EDTA was fixed at 10mM and was used in all subsequent experiments. The result of a typical experiment is represented in table 2.

Determination of optimum concentration of polyvinyl polypyrrolidone during homogenization

Different concentrations (50, 100, and 150 mg / g fresh tissue) of polyvinyl polypyrrolidone were incorporated during homogenization of tissue and the enzyme activities were compared with the control (without PVPP). Supplementation of PVPP during homogenization resulted in a higher enzyme activity when compared to the control experiments. At 50 mg / g and 100 mg / g fresh tissue there was 38 % and 66 % increase in activity respectively. At 150 mg / g tissue, the maximum concentration tested, there was no further increase in activity. In all future experiments, the concentration of PVPP was maintained at 100 mg / g fresh tissue. It may be mentioned, inter alia, that the incorporation of PVPP during homogenization has also resulted in a decrease in the background absorption of assay system at 340 nm. The results from a typical experiment are recorded in Table 3.

Table: 1. Effect of 2-mercaptoethanol during homogenization on Glutamate dehydrogenase activity from the leaves of *P. nigrum*.

2-Mercaptoethanol, mM	Glutamate dehydrogenase activity, Δ OD / h	Percentage activity.
10	0.937	(100)
20	1.357	145
50	1.537	164

The data are from a typical experiment. 10.0 % (w/v) homogenate of *P. nigrum* was prepared. The basal medium which consisted of 20mM Tris-HCl buffer pH 7.5. The activity at 20 mM 2-mercaptoethanol is taken as reference and is represented in parenthesis. Details of assay are as reported in Materials and methods.

Table 2: Effect of EDTA during homogenization on Glutamate dehydrogenase activity from the leaves of *P. nigrum*.

EDTA, mM	Glutamate dehydrogenase activity Δ OD / h	Percentage activity.
0	1.090	(100)
5	1.602	147
10	1.812	166
20	1.792	164

The data are from a typical experiment. 10 % (w/v) homogenate of *P. nigrum* tissue was prepared in the basal medium, which consisted of 20mM Tris-HCl buffer pH 7.5, and 20mM 2-mercaptoethanol. Neutralized EDTA was incorporated at final concentrations as indicated. The value for control is represented in parenthesis. Details of assay are as reported in Materials and methods.

Table3: Effect of polyvinyl polypyrrolidone during homogenization on glutamate dehydrogenase activity from the leaves of *P. nigrum*.

PVPP, mg / g tissue	Glutamate dehydrogenase activity, Δ OD / h	Percentage activity.
0	1.426	(100)
50	1.975	138
100	2.369	166
150	2.341	164

The data are from a typical experiment. 10.0% (w/v) homogenate of *P. nigrum* leaf tissue was prepared in the basal medium which consisted of 20mM Tris-HCl buffer pH 7.5, 20mM 2-mercaptoethanol and 10mM EDTA. PVPP was incorporated at final concentrations as indicated. The value for control is represented in parenthesis. Details of assay are as reported in Materials and methods.

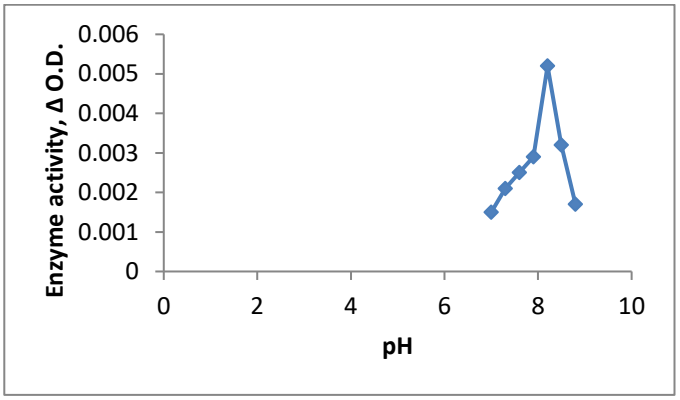

Fig.1 pH-activity relationship of Glutamate dehydrogenase with NADP$^+$ as one of the substrates from the leaves of *P. nigrum* (Details as described in Materials and methods).

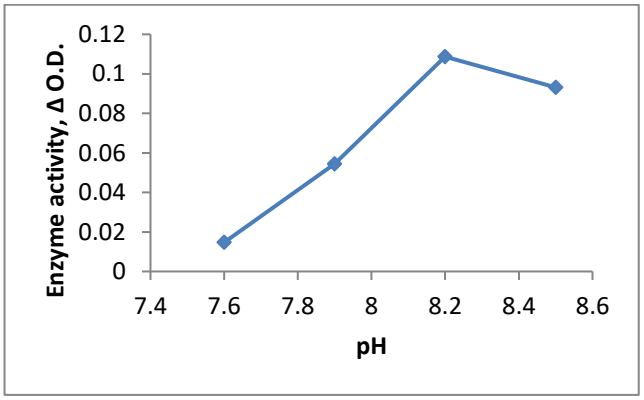

Fig.4 pH-activity relationship of Glutamate dehydrogenase with NADH as one of the substrates from the leaves of *P. nigrum* (Details as described in Materials and methods).

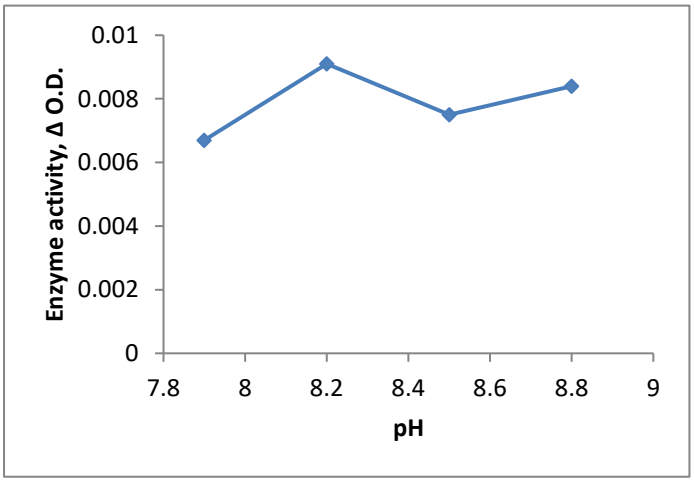

Fig.2 pH-activity relationship of Glutamate dehydrogenase with NAD$^+$ as one of the substrates from the leaves of *P. nigrum* (Details as described in Materials and methods).

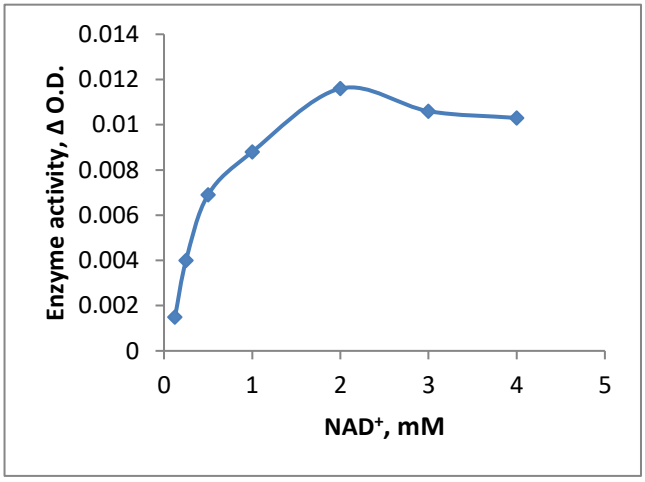

Fig.5 Enzyme activity NAD$^+$ concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

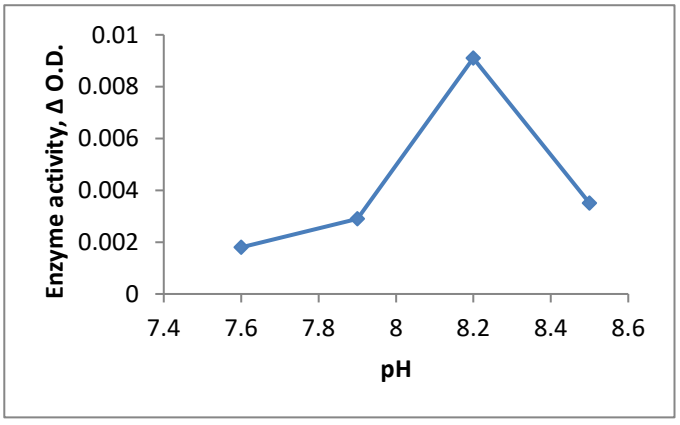

Fig.3 pH-activity relationship of Glutamate dehydrogenase with NADPH as one of the substrates from the leaves of *P. nigrum* (Details as described in Materials and Methods).

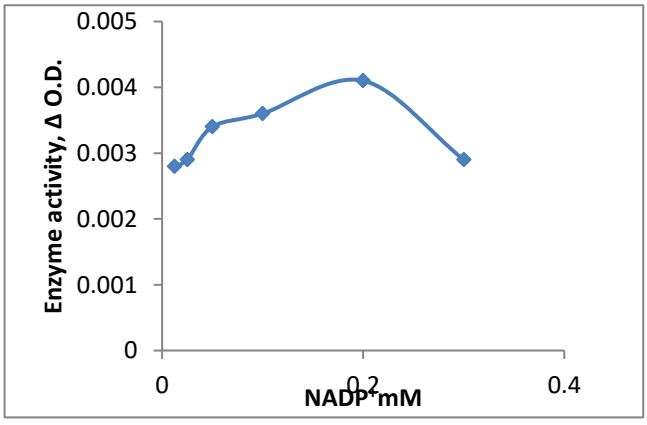

Fig.6. Enzyme activity NADP$^+$ concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

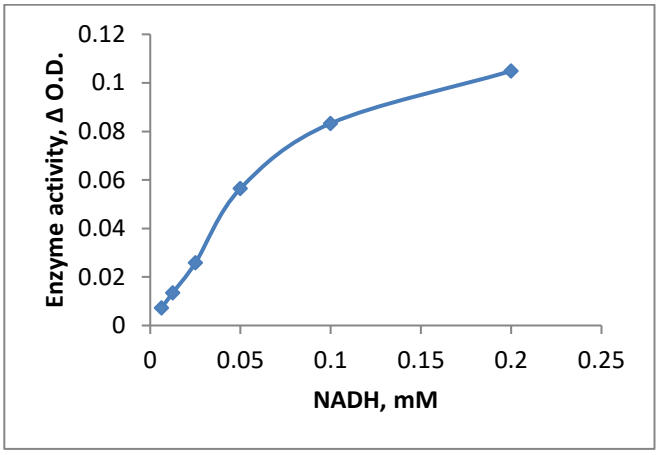

Fig.7. Enzyme activity NADH concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

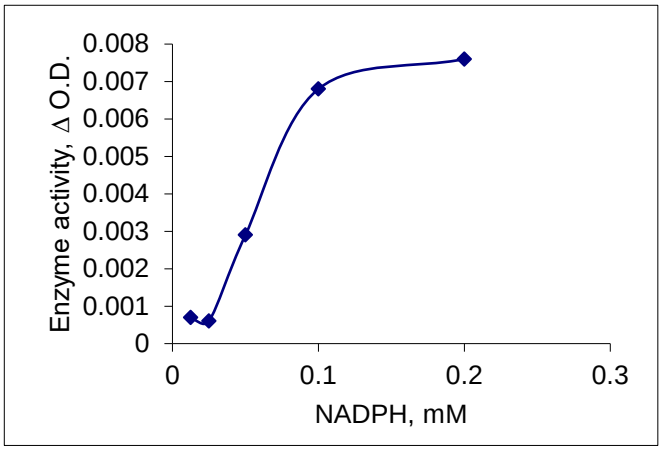

Fig.8. Enzyme activity NADPH concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

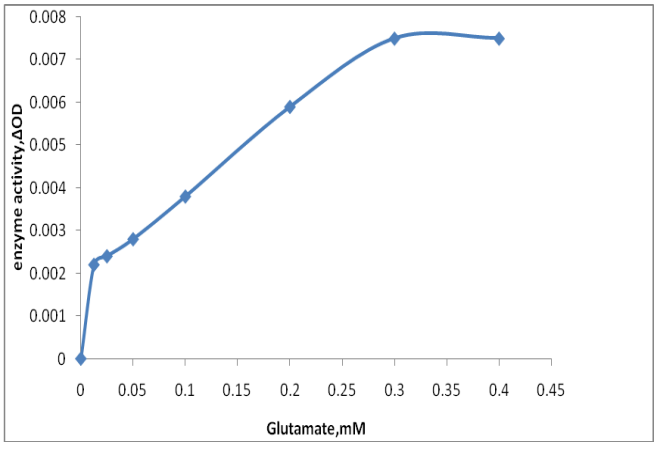

Fig.9 Enzyme activity Glutamate concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

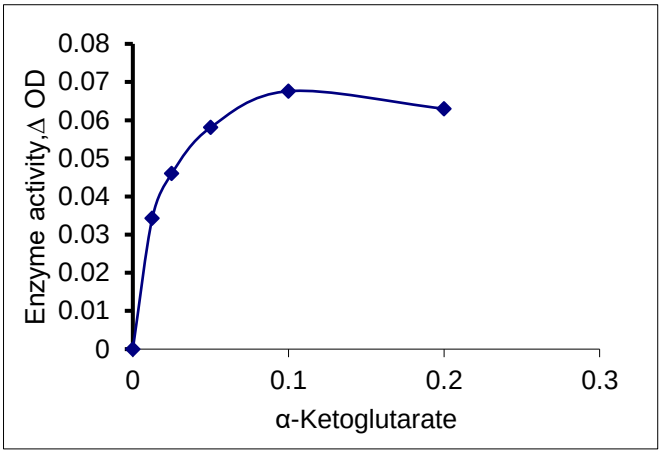

Fig.10 Enzyme activity α-ketoglutarate concentration relationship of glutamate dehydrogenase from the leaves of *P. nigrum* (Details as described in Materials and methods)

Standardized homogenizing medium for the assay of glutamate dehydrogenase :

From the results of the foregoing experiments, a standardized medium for homogenization was formulated for the detailed study of glutamate dehydrogenase from leaf tissue of *P. nigrum*. The standardized medium consisted of 20mM Tris HCl pH 7.5, 10mM EDTA, 20mM 2-mercaptoethanol and PVPP 50 mg/g fresh tissue. Using these conditions for a10% (w/v) homogenate of fresh tissue of *P. nigrum* leaves, optimum activity of glutamate dehydrogenase was elicited.

Standardization of assay system.

Determination of optimum pH

The optimum pH for the assay was found to be 8.2 and in the subsequent experiments the assays were conducted at the optimum pH of 8.2. The results from a typical experiment are recorded in figures 1, 2, 3 and 4.

Verification of endogenous activity: As it was explained earlier it was necessary to verify the contribution of endogenous reaction, which may affect the measurement of the activity of glutamate dehydrogenase. No change in absorption was observed when the substrate was withheld and the reaction commenced only in the presence of the substrate (Data not represented). This experiment ensured that the enzyme activities reported in the present work are truly due to the activity of glutamate dehydrogenase.

Enzyme activity-substrate concentration relationship: The reaction velocity with respect to the concentration of

substrate in the system was determined for glutamate, α-ketoglutarate, NAD^+, $NADP^+$, NADH, NADPH..

Using the different substrates at varying final concentrations in the range 0.125mM to 4.0mM in the assay system, the initial velocity showed an increase with an increase in the substrate concentration. And the velocity increased at higher substrate concentrations. For all future experiments the assays were carried out using the finalized concentration of substrate .The results from a typical experiments are recorded in figure 5, 6, 7, 8, 9, 10.

DISCUSSION

The main objective of the present investigation by the author was to standardize the extraction and properties of glutamate dehydrogenase from *P. nigrum* L. and to study the various properties of the enzyme-glutamate dehydrogenase. The studies of various properties of the enzyme are relevant. The effect of various additives like 2-mercaptoethanol, EDTA and PVPP were studied and the optimum concentrations of these compounds were determined and a standardized homogenizing medium was formulated which was used in all the subsequent experiments. A standardized assay system was formulated taking into considerations of the optimum pH, substrate saturation, enzyme concentration and incubation period.

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REFERENCES

1. Anderson JW, (1968) Extraction of enzymes and sub cellular organelles from plant tissues. Phytochemistry. 7: 1973-1988.
2. Badran AM, Jones DE. (1965) Polyethylene glycol-tannin interaction in extracting enzymes. Nature. 206: 633-624.
3. Baijal M, Singh S, Shukla RN, Sanwal GG. (1972) Enzymes of the banana plant: optimum conditions for extraction. Phytochemistry. 11: 929-936.
4. Dixon M, Webb EC: In the enzymes. Longsman group limited, London, 1979.
5. Guttenberger M, Schaeffer C, Hampp R. (1994) Kinetic and electrophoretic characterization of NADP-dependent dehydrogenases from root tissues of Norway spruce (Picea abies L.) employing a rapid one-step extraction procedure. Trees-struct. funct. 8: 191-197.
6. Haising BE, Schipper AL. (1975) Effect of nucleotide coenzymes and mercaptoethanol on stabilization of plant dehydrogenase activity in crude extracts. Physiol. Plant. 35: 249-255.
7. Hulme AC, Jones JD. (1963) Tannin inhibition of plant mitochondria. In enzyme chemistry of phenolic compounds. (J. B. Pridham. ed). Pergamon Press. 97-120.
8. Khanna SK, (1971) Glucan phosphorylase in the leaves of Dendrophthoe falcate: Purification and characterization of enzyme. Phytochemistry. 10: 555-559.
9. Krishnan PS. (1969) Enzyme extraction from plant tissues rich in phenols / phenolases. J. Scient. Indust. Res. 28: 93-98.
10. Loomis WD, Battalle J. (1966) Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry. 5: 423-438.
11. Loulakakis KA, Roubelakis-Angelakis KA. (1992) Ammonium-induced increase in NADH-glutamate dehydrogenase activity is caused by de-novo synthesis of the a-subunit. Planta. 187: 322-327.
12. Oaks A, (1995) Evidence for deamination by glutamate dehydrogenase in higher plants. Bot. 73: 1116-1117.
13. Robinson SA, Slade A, Fox GG, Phillips R, Ratcliffe RG, Stewart GR. (1991) The role of glutamate dehydrogenase in plant nitrogen metabolism. Plant Physiol. 95: 509-516.
14. Robinson SA, Stewart GR Phillips R. (1992) Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension cultures. Plant Physiol. 98: 1190-1195.
15. Salsac L, Chaillou S, Gaudry JFM, Lesaint C, Jolivet E. (1987) Nitrate and ammonium nutrition in plants. Plant Physiol. Biochem. 25: 805-812.
16. Stewart GR, Shatilov VR, Turnbull MH, Robinson SA Goodall R. (1995) Evidence that glutamate dehydrogenase plays a role in the oxidative deamination of glutamate in seedlings of Zea mays. Australian J. Plant Physiol. 22: 805–809.
17. Young RE, (1965) Extraction of enzymes from tannin bearing tissue. Arch. Biochem. Biophys. 111: 174-180.

