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Short Communication

Glutamate Dehydrogenase Activity in Chickpea (*Cicer arietinum* L.) Seedlings

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Chickpea is a self-pollinated crop, needs intensive studies to exploit the existing variability. Chickpea improvement depends upon the magnitude of genetic variability present in base population. Bains and Mercer [1] observed the interdependence between the embryonic axis and cotyledons with regard to the development of several enzymatic activities. Glutamate dehydrogenase [GDH] is one of the important enzyme in the assimilation of ammonia in Kreb's cycle. Glutamate occurs at a metabolic crossroads and its metabolism plays a pivotal role in the integration of carbon and nitrogen metabolism in higher plants. GDH provides an assimilatory pathway for ammonium under some circumstances, for example, various stress conditions [2] and the contrary view that GDH has a catabolic role, catalyzing the oxidative determination of glutamate to facilitate the recycling of carbon and nitrogen [3& 4]. An assimilatory role for GDH have rare evidence for a quantitatively important contribution to ammonium assimilation. In contrast, metabolic studies have provided strong evidence for a catabolic role in germinating seedlings [5] and carbon-starved cells [3&4]. However, the catabolic role of GDH has generated controversy in the literature [6, 7, 8 & 9] and the function of GDH in higher plants still merit investigation. The glutamate dehydrogenase utilizes both nicotinamide nucleotide cofactors; NAD^+ in the direction of nitrogen liberation of $NADP^+$ for nitrogen incorporation. Glutamate dehydrogenase is important in converting free ammonia and a-ketoglutarate to glutamate, forming one of the 20 aminoacids required for protein synthesis. The growth of embryo in germinating seed is dependent on the supply of aminoacids for its protein synthesis [10]. The present work was conducted to study the glutamate dehydrogenase activity in five different chickpea cultivars.

Five cultivars ICCV 88503, ICCV 88504, ICCV 88511, PG-9425-5 and PG 96006 of chickpea were chosen. ICCV 88503, ICCV 88504 and ICCV 88511 germplasm were collected from ICRISAT, PG-9425-5 and PG96006 germplasm were collected from Pulses Improvement Research Center, Hyderabad. The specific activity of glutamate dehydrogenase was estimated in the seedling organs (leaf tissue). The chickpea cultivars were put for germination in the field. After 14^{lh} day, the seedling was excised and leaf tissues were used for further extraction. From these seedlings, 500mg of leaf tissue was taken for investigation. The leaf tissue was homogenized with 5ml of 100mM phosphate buffer pH 7.5 containing 1 per cent PVP and centrifuged at 10,000g for 30 minutes at 4°C. The supernatant was collected (separately from the residue) and used. The glutamate dehydrogenase activity was determined following the method of Doberty [11], The enzyme activity was analyzed at every 7^{th} day (interval period) in leaf tissue from all the cultivars.

The amount of NADH and NADPH oxidized is calculated from the following formula:

Molar extinction coefficient Nanomole of NAD (P) H oxidized/min/mg protein =

$$\frac{\text{A340 x vol. of assay solution x 1000}}{\text{6.22 x Time of incubation (min) x mg protein in enzyme extract used}}$$

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Table 1. Specific activity of GDH-NADH (n moles of NADH oxidized/min/mg protein) in leaf tissues of different chickpea cultivars

Variety	14th day	21st day	28th day	35th day	42nd day	49th day	Mean
ICCV 88503	9.643	6.538	2.515	2.516	1.863	0.3975	3.912
ICCV 88504	10.261	6.630	2.724	2.684	0.934	0.3767	3.935
ICCV 88511	14.256	6.662	3.563	2.894	1.237	0.3689	4.830
PG-9425-5	8.977	7.315	4.025	3.984	1.234	0.4430	4.329
PG-96006	12.541	7.137	2.083	3.056	1.008	0.4599	4.381

*Mean of triplicate determination

Table 2. Specific activity of GDH-NADPH (n moles of NADPH oxidized/min/mg protein) in leaf tissues of different chickpea cultivars

Variety	14th day	21st day	28th day	35th day	42nd day	49th day	Mean
ICCV 88503	2.1902	5.466	2.774	4.021	0.987	0.4910	2.655
ICCV 88504	2.2220	7.288	4.011	3.986	1.324	0.5066	3.223
ICCV 88511	2.6152	17.247	3.446	1.961	1.204	0.6652	4.517
PG-9425-5	4.0274	5.466	2.225	3.163	1.020	0.6313	2.755
PG-96006	2.0007	7.591	3.173	2.376	1.314	0.5586	2.835

*Mean of triplicate determination

In the present study, the analysis of glutamate dehydrogenase activity showed that there is a difference in both NADH and NADPH activity between young and aged seedlings in five cultivars of chickpea (Table 1 and 2). Due to aging (matured) the decreased activity of NADH (14.26 to 0.37 nmole/mg) and NADPH, (7.29 to 0.49 nmole/mg) was observed from 14th to 49th day. The activity was higher (17.25 nmole/mg) during 21st day and it gradually decreases (0.49 nmole/mg) due to aging. This gradual decrease in enzyme activity to feeding of more amino acids in the Kreb's cycle increase the respiratory activity of the seedlings. The growth of seedlings is dependent on the supply of aminoacids to its protein synthesis [10]. The supply of important aminoacids such as glutamate and glutamine from root to the leaves depends on the activity of GDH.

The activity of glutamate dehydrogenase was also higher (17.25 to 4.03 nmole/mg) in young seedlings (non-aged) compared to the matured (aged) seedlings (4.02 to 0.37 nmole/mg) in all the chickpea cultivars studied. This is in agreement with previous report in the seedling of *Brassica juncea* and *Brassica campestris* [12] and in roots and nodules of *Cajanus cajan* under stress conditions [13].

Among the five different chickpea varieties, the variety ICCV 88511 reported the maximum enzyme activity for the entire period of growth of the seedlings in both NADH and NADPH activity.

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