

## Studies on the Impact of Altitudinal Gradient on Ammonium Assimilatory Metabolism in *Glycine max* L. (Fabaceae)

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### ABSTRACT

Seedlings of *Glycine max* L. were grown, transplanted and acclimatized for 60 days at different altitudes (250, 400 and 1600 m). Response to shift in altitude was observed in the plants. Shoot length decreased with the increase in the altitude, while root length followed a reverse trend. Biomass accumulation in shoot and roots of *G. max* was the maximum at high altitude. Total soluble protein content was significantly high at low altitude in the shoot and the roots. Free tissue ammonia level in this species showed positive correlation with increasing altitude. Ammonium assimilatory enzymes viz., glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) were studied. GS/ GOGAT specific and total activity were altitude sensitive, whereas GDH activity exhibited inverse trend. Results indicate that there is a positive shift in ammonium assimilatory

pathway in plants growing at high altitude.

**KEYWORDS:** Ammonium Assimilatory Pathway; Glutamine Synthetase (GS); Glutamate Synthase (GOGAT); Glutamate Dehydrogenase (GDH); *Glycine max*.

## INTRODUCTION

Nitrogen metabolism in plants is a complex process, and is regulated by the form of nitrogen that is available to the plant (Magalhaes and Huber, 1989). Major source of nitrogen to the plant is ammonium, and is largely assimilated by the roots (Yoneyama and Kumazawa, 1974). Generally, plants prefer ammonium ( $\text{NH}_4^+$ ) compared to nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) as ammonia is the starting point for nitrogen assimilation in higher plants. Internal source of ammonia is photorespiration and amino acid catabolism (Srivastava and Singh, 1987). However, high level of ammonia is toxic, therefore is converted to amino acids in plants (Mifflin and Lea, 1980). Enzymes namely Glutamine synthetase (GS) (EC 6.3.1.2)/ Glutamate synthase (GOGAT) (EC 1.4.1.14) and Glutamate dehydrogenase (GDH) (EC 1.4.1.2-4) play a vital role in ammonia assimilation and detoxification. Of the three enzymes, GDH occupies a key role in plant metabolism.

The ability of plant to acquaint to new environmental conditions depends upon its morphological adaptation and physiological response. In mountainous environment, variation in altitude offers wide variety of environmental conditions. In general, with increase in elevation, stressors such as temperature, pressure, light intensity, rainfall, partial pressure of metabolic gases are known to influence plant metabolism (Woodcock, 1976; Purohit, 1977). Like any other metabolic process, nitrogen metabolism is significantly influenced by variation in the altitude. Increased nitrogen content has been reported in plants with the increase in the altitude (Korner, 1989). This implies that that plants which are least influenced by altitudinal changes are much resistant to multiple stresses.

Several workers have compared morpho-physiological response of plants to the change in the altitude (Bhadula *et al.*, 1985; Rajasekaran *et al.*, 1998; Rajasekaran, 2000). Ammonium assimilation play an important role in growth and development and has been extensively studied in various species.

However, studies on ammonium assimilation in plants from the various climatic zones have been limited and no studies have been undertaken in Shervaroyan hills, part of Eastern Ghats, Tamil Nadu, India. The present work is an attempt to study growth behavior and analyze the activity of ammonium assimilation enzymes in *Glycine max* (L.) an annual leguminous food crop, grown at different altitudes in Shervaroyan hills.

## MATERIALS AND METHODS

Seeds of *Glycine max* L. (Fabaceae) were sown in farmyard manure and garden soil in a ratio of 1:2 at

Salem (250m) to raise seedlings. 40 day-old seedlings were transferred to experimental sites: Salem (250m), Kurumbapatti (400m) and Yercaud (1600m) in Shervaroyan hills of Salem, Tamil Nadu. Experimental plants acclimatized for 60 days at respective sites and growth performance was analyzed (shoot and root length and dry weight). For biochemical analysis, plant samples were frozen in liquid nitrogen after collection. Samples were homogenized in 0.1 M, Tris-HCl pH 7.5 containing 2 mM EDTA and 0.1% -mercaptoethanol, PMSF was added to prevent proteolysis. All the studies were conducted at 4°C. Total soluble protein was estimated using Bradford (1976) method. Free tissue ammonia level was estimated using method of Chaney and Marbach (1983). GS activity was determined by the method of Truax *et al.* (1994). One hundred µl of supernatant was incubated with 900 µl of assay buffer (0.1 M - imidazole-HCl, 65 mM - L- Glutamate, 4 mM - MnCl<sub>2</sub>, 0.75 ADP, 33 mM sodium arsenate and 17mM hydroxylamine, pH 6.8) at 30°C for one hour. The reaction was terminated by adding 1ml of stop solution (0.37 M FeCl<sub>3</sub>, 0.2 M TCA in 0.67 N HCl). After centrifugation, absorbance was determined at 540nm. Glutamylhydroxamate (Sigma Chemical Co, USA) was used to develop standard curve. A modified method of Dougall (1974) for the assay of NADH-GOGAT and NADH-GDH by following the rate of reduction of NADH at 340nm for 5 min. GOGAT assay mixture contain 20 mM Tricine, pH 7.5, 12.5 mM, α- ketoglutarate, 12.5 mM L- Glutamine and 0.15 mM NADH. Assay mixture for GDH contained 20 mM Tricine, pH 7.5, 1 mM α- ketoglutarate, 1 mM CaCl<sub>2</sub>, 100 mM NH<sub>4</sub>Cl and 0.15 mM NADH. All the estimations were carried out in triplicate (n = 3) following standard methods.

## RESULTS AND DISCUSSION

Plant growth, biochemical and enzymes of ammonium assimilation in *G. max* were estimated. In *G. max* shoot and root length from three different altitudes is summarized in Fig. 1. Shoot and root length decreased with increase in altitude. Similar observation has been reported for different plant species by Nautiyal and Purohit (1980), Rajasekaran *et al.*, (1998), Rajasekaran (2000). However, Bhatt and Purohit (1984) and Pankaj Prasad (1997) reported more root length than shoot at high altitudes due to non-availability of water in higher altitude and other factors such as soil, temperature. Sharma, (1980) reported dwarfism as an environment stress at high altitudes. Decrease in cumulative height and growth rate was steep in low land species than high land species (Todaria, 1980). Whereas shoot and root dry weight increased with the increase in the altitude (Fig 2). This could be due to higher concentration of carbon dioxide in the atmosphere (Purohit, 1998).

Total soluble protein (TSP) content decreased with increasing altitude (Fig 3). However, free tissue ammonia (FTA) in *G. max* increased with increasing altitude (Fig 4). In roots maximum FTA was

recorded at high altitude and minimum at low altitude. The present observations are in agreement with previous studies on *Selinum vaginatum* (Rajasekaran, 2000). Specific and total activities of GS are presented in Fig 5-6. GS specific and total activities of both the parts showed an inverse relation with the increasing altitude. Maximum activity was observed at the low and minimum at high altitudes. Variation in activity of GS under certain environmental conditions has been attributed to reassimilation of ammonia released during photorespiration (McNally *et al.*, 1983; Wallsgrave *et al.*, 1983). However, seasonal variation in GS of temperate deciduous tree leaves strongly indicated that decline in light intensity and temperature in late season accounted for drop in GS activity (Pearson and Ji, 1994), similar has already been reported in *G. max* and *S. vaginatum* (Rajasekaran *et al.*, 1998; Rajasekaran, 2000). Decrease in GS activity under water stress in *Albizia stipulata* and *Oeugenia dalbergioides* indicates that GS in both the plants is sensitive to water stress (Pankaj Prasad, 1997; Purohit, 1998), although in some plant species GS has been insensitive to water stress (Becana *et al.*, 1984). FTA accumulation reflected in decreased GS activity in shoot and root (Figs. 4-6). Kamachi *et al.*, (1992) reported that environmental conditions may induce FTA accumulation that may affect GS activity at high altitude. This is in accordance with previous reports (Cren and Hiral, 1999; Rajasekaran, 2000). This could be due to the fact that GS is less effective under high ammonia accumulation.

Both, specific and total activity of NADH-GOGAT at three different altitudes is shown in Fig 7-8. NADH-GOGAT specific and total activity in shoot and root followed similar trend as in GS. NADH-GOGAT activity in shoots showed similar trend in low and middle altitudes. A decrease of 34% was observed at high altitude. Likewise, with the increase in the altitude decrease in shoot and root GOGAT specific and total activity was observed (Fig. 7-8). This indicates that FTA content in the tissues has complex regulatory effects on GOGAT activity as several stresses have been reported to be operative along different altitudes (Bhadula and Purohit, 1994; Pankaj Prasad, 1997; Purohit, 1998; Rajasekaran, 2000). Specific and total activities of GDH-amination in *G. max* (shoot and root) showed a positive correlation with increase in altitude (Fig. 9-10). Increased NADH-GDH activity has been reported along an altitudinal gradient (Pankaj Prasad, 1997; Rajasekaran *et al.*, 1998; Rajasekaran, 2000). However, increased GDH activity is an indicator of detoxification of ammonia released during the breakdown of proteins and amino acids (Becana *et al.*, 1984). In the present investigation, GDH-amination activity increased with the increase in the altitude (Fig. 9-10). Srivastava and Singh, (1987) reported that GDH pathway is active under certain nutritional and environmental conditions. Although, GS/GOGAT pathway is predominant in ammonia assimilation in higher plants, plants do switch over to GDH pathway under certain conditions of low energy and high ammonia (Yamaya, 1999; Rajasekaran *et al.*, 1998; Rajasekaran, 2000).

Specific activity of GS decreased at high altitude (Fig. 5), whereas at these altitudes GDH specific

activity increased (Fig. 9). GDH plays a complementary role to GS/GOGAT cycle in synthesis of glutamate (Srivastava and Singh, 1987; Rajasekaran *et al.*, 1998; Rajasekaran, 2000) or could catalyze the oxidation of glutamate to provide carbon-skeletons to the TCA cycle. At high altitude, FTA levels also were found to increase (Fig. 4). Increased levels of ammonia may have resulted in increase in *de novo* synthesis of GDH (Srivastava and Singh, 1987; Loulakakis and Angelakis 1990a; Watanebe *et al.*, 1992). This may be one of the reasons for enhancement of GDH activities at high altitudes. However, investigations on isoforms, isoform patterns of ammonium assimilatory enzymes, *in-vitro* studies using inhibitors of ammonium assimilatory enzymes along an altitudinal gradient is required to elucidate mechanism for this behavior.

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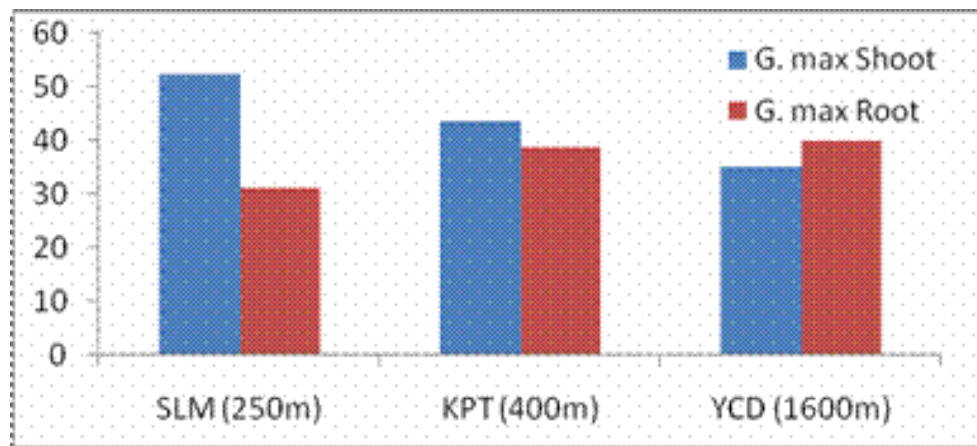
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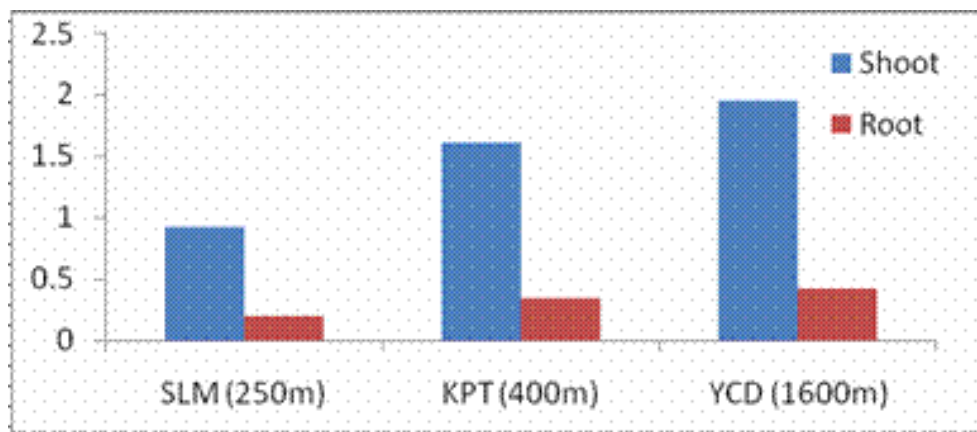
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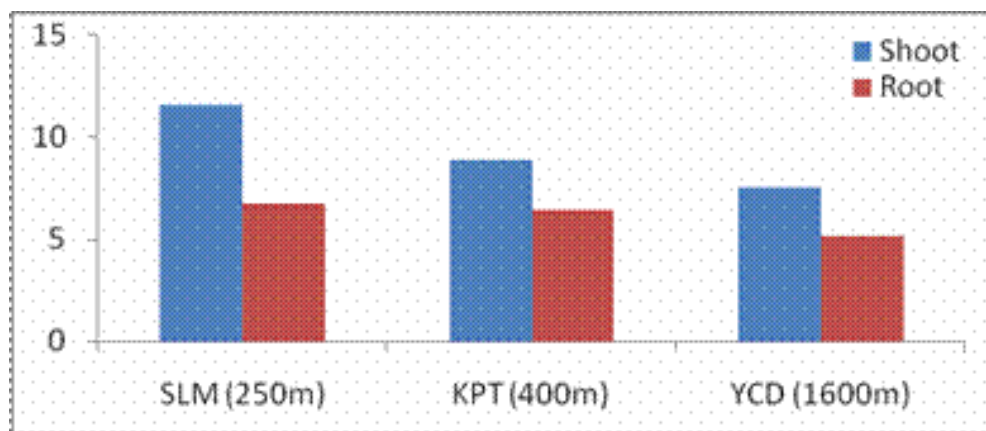
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**Fig. 1.** Changes in shoot and root growth patterns of *G. max* acclimatized at different altitudes.

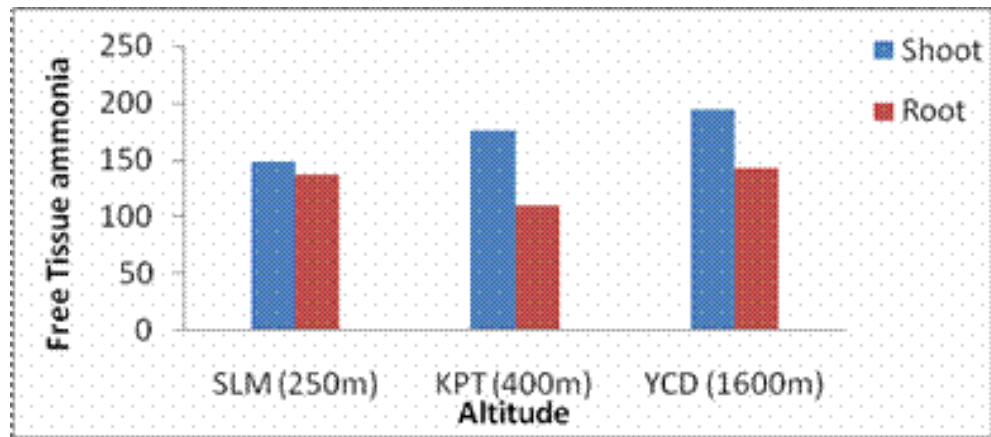


**Fig. 2.** Changes in shoot and root dry weights of *G. max* acclimatized at different altitudes

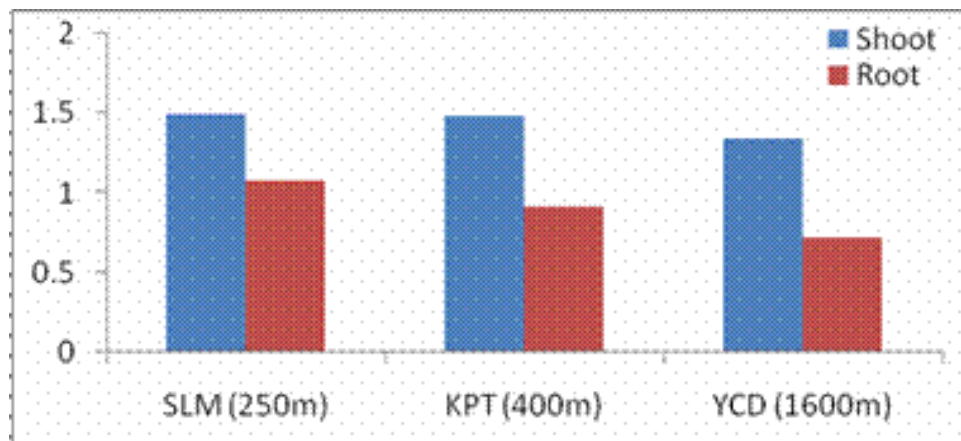


**Fig. 3.** Changes in total soluble protein of shoot and root of *G. max* acclimatized at different altitudes.

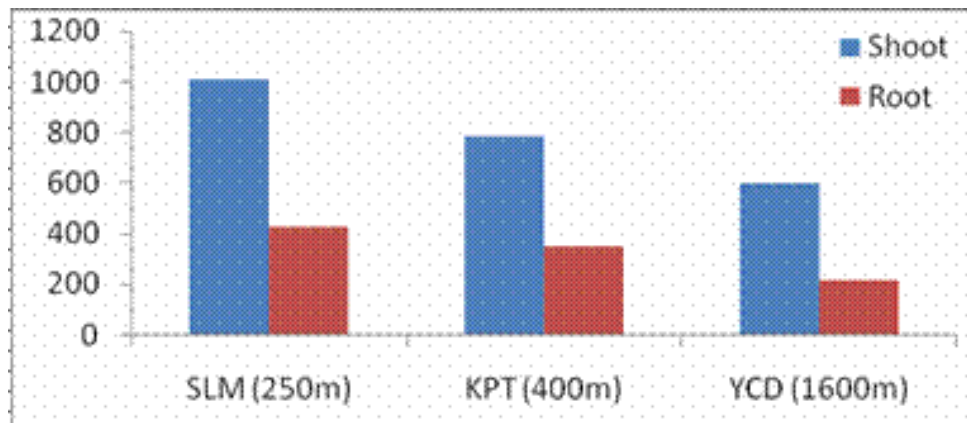




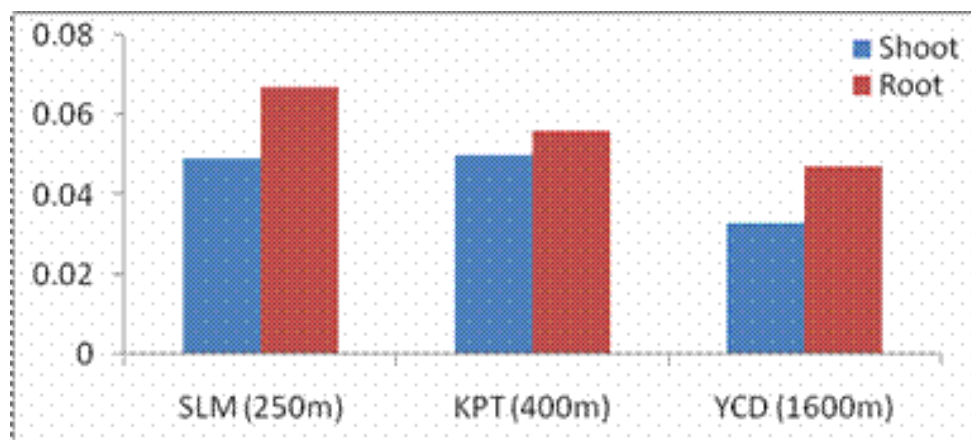
**Fig. 4.** Changes in shoot and root free tissue ammonia levels of *G. max* acclimatized at different altitudes



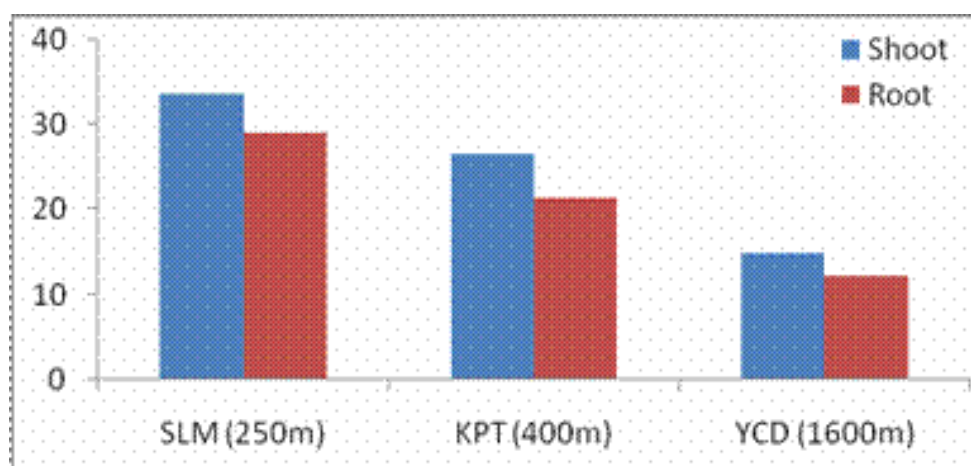
**Fig. 5.** Changes in shoot and root GS (Specific) activity of *G. max* acclimatized at different altitudes.



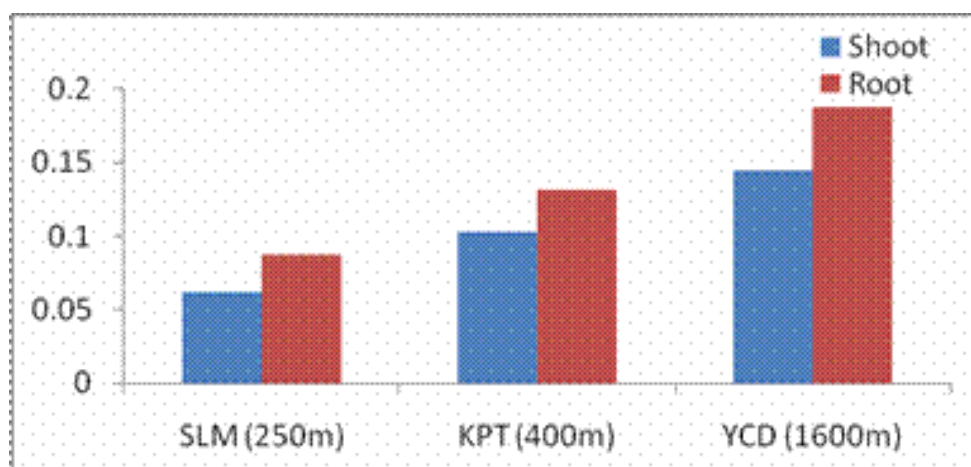
**Fig. 6.** Changes in shoot and root GS (total) activity of *G. max* acclimatized at different altitudes.



**Fig. 7.** Changes in shoot and root GOGAT (Specific) activity of *G. max* acclimatized at different altitudes.

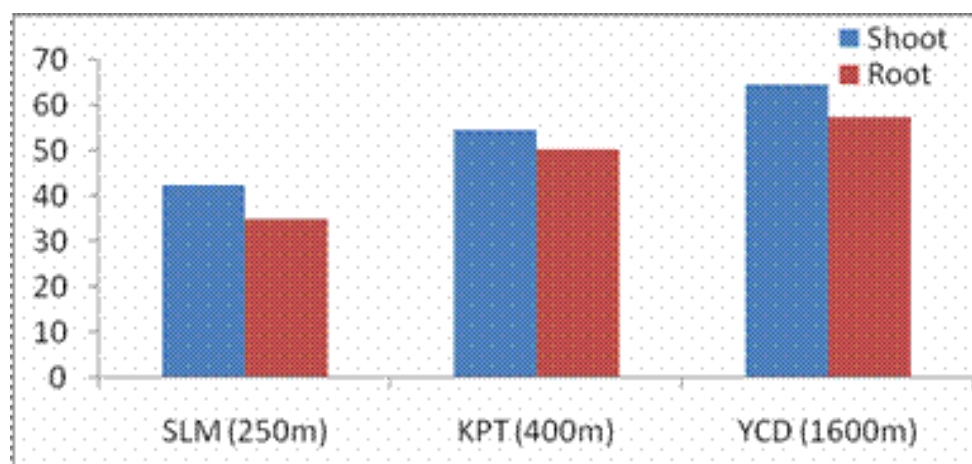


**Fig. 8.** Changes in shoot and root GOGAT (total) activity of *G. max* acclimatized at different altitudes.



**Fig. 9.** Changes in shoot and root GDH-amination (specific) activity of *G. max*

acclimatized at different altitudes.



**Fig. 10. Changes in shoot and root GDH-amination activity of *G. max* acclimatized at different altitudes.**