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Biochemical Evaluation of Cadmium on Calli of *Solanum melongena* L.

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A B S T R A C T

Accumulation of cadmium in vegetables poses serious threats to human health; therefore it is necessary to study the accumulation of cadmium in the edible parts. Eggplant has a higher tendency for cadmium accumulation, plants grown in soil system cannot project the true picture of cadmium tolerance, due to the interaction of edaphic factors. Therefore in vitro study on cadmium toxicity in cotyledon and hypocotyl derived differentiating and non-differentiating stages of calli of *Solanum melongena* L. was conducted. In this study metabolism of proteins, amino acids, and different enzymes like Malate dehydrogenase (MDH), Glutamate dehydrogenase (GDH), Nitrate reductase (NR), and Peroxidase activity was evaluated.

Introduction

Cadmium a major environmental pollutant (Li *et al.*, 2014) causes major health hazards. Studies by Shen *et al.*, (2017) have revealed that the major source of cadmium accumulation in the human body originates from the intake of vegetables. The major source of cadmium intake in a food chain is controlled directly or indirectly by plants. It accumulated in various plant parts which affects the growth directly or indirectly (Chugh *et al.*, 1992, Grant *et al.*, 2008). Generally, resistance to heavy metal is achieved either by avoidance or tolerance i.e. plants tolerate stress by adapting themselves to the toxic concentration.

To understand the mechanism of tolerance, plants grown in soil cannot project the true picture because of the interaction of the metal with the soil system. So a more realistic approach for heavy metal tolerance may be made through in vitro culture - which can be easily monitored and successfully employed to develop metal resistance lines. The following study is in context to develop the metal tolerant lines in eggplant (*Solanum melongena* L.) which is a vegetative cash crop grown in the

outskirts of cities and is irritated with sewage sludges. Eggplant is a vegetable of great economic and nutritional value (Saini and Kaushik, 2019). Investigations suggested that eggplants had high cadmium accumulation potential in the fruit which exceeds the national food safety standards (Li *et al.,* 2014; Yuan *et al.,* 2019). It is obvious that during adaptability to stress, there is a quantitative and qualitative change in metabolites to adapt tolerance to cadmium. In the present investigation, efforts have been made to find out the adaptation of various metabolites in cadmium-tolerant calli of egg plant at differentiated and non-differentiated stages of growth.

Materials and Methods

Seedlings of *Solanum melongena* (egg plant) were raised aseptically on MS medium (Murashige and Skoog, 1962) at 25±1^{0}C. Explants like cotyledon and hypocotyl were excised from 15 days old seedlings and inoculated on MS medium supplemented with 5.38µM NAA (Naphthyl acetic acid)+4.44 µM BAP 6-Benzyl amino purine). Thirty days old calli were divided into three lots for further studies.

In the first lot, calli were subcultured and maintained on the same medium which served as control. In the second lot, calli were subcultured on differentiating medium containing MS+2.86 µM IAA (Indole -3-acetic acid) + 13.65 µM Kinetin as reported by Jyoti *et al.,* (1994). In the third lot, calli were subcultured on a medium adjuncted with 125 µM Cadmium Chloride which was inhibitory for callus growth (Jyoti *et al.,* 1995).The stability in altered response in metabolites due to cadmium treatment was tested by subculturing calli on a medium devoid of cadmium and also subcultured for differentiation on differentiating medium.

This served as passage l. In passage ll these calli were further grown on MS medium supplemented with cadmium salt, these calli were again grown on medium without cadmium and subcultured on differentiating medium which is shown in the flow chart (Fig.1). All the calli were subcultured for 45 days and sampling of calli was done at differentiating and non-differentiating Stage.

Explants like cotyledon and hypocotyl were analyzed for various metabolites. One hundred and fifty milligram of callus was homogenized in 80% ethanol (v/v). The homogenate was refluxed for 15 minutes in water bath and centrifuged. The residue was further refluxed thrice with 80% ethanol. This supernatant was used for the estimation of amino acids by Yemm and Cocking (1955). Pellets were hydrolysed with 0.2 N $HClO_4$ and hydrolysates were used for the estimation of proteins by Lowry *et al.,* (1951).

For extraction of Enzymes - One gram of callus was taken from all stages. Then callus was homogenized in chilled glass mortar with pestle using acid washed sand as an abrasive. The extraction medium contained 0.1 M Tris HCl pH7.4; 0.25mM EDTA; 2.5 mM cysteine HCl and 2.5% polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 x g for 10 minutes at 4°C in a refrigerated centrifuge. The supernatant was used for estimation of activity of various enzymes like Peroxidase (1.11.1.7) by the method of Seevers *et al.,* (1971); Malate Dehydrogenase (MDH) (1.1.1.37) by the method of Davies (1969); Glutamate dehydrogenase (GDH) (1.4.1.4) by the method of Joy (1969). For the estimation of Nitrate reductase activity 250 mg of callus was cut into pieces & amp; were suspended in screw cap vials containing 5 ml. of the incubation medium.

The vials were sealed and kept at 30°C for 4 hours, then Nitrate reductase(NR) (1.6.6.1) activity was determined by the method of Jaworski (1971).

Estimation of Cadmium

For determination of cadmium concentration in different cadmium-treated calli, 100 mg of dried sample was digested with a mixture of perchlorate acid and sulphuric acid in the ratio of 1:4. A Known volume was made with distilled water and cadmium

concentration was determined by Atomic Absorption Spectrophotometer (Perkin-2308).

Results and Discussion

There was a decline in protein content in cadmium treated cotyledon (fig.2a) and hypocotyl (fig.2b) derived calli & this decline progressed with the cadmium passages, however the protein content was more in differentiating over non-differentiating stage. Free amino acids decreased significantly with cadmium treatment in both calli of cotyledon (fig.3a) and hypocotyl (fig.3b) and reduction was more pronounced in hypocotyl derived calli at passage ll. At differentiating stage free amino acids decreased in both cadmium treated and untreated Calli. At passage l, content increased as compared to untreated and exposure of calli to Cadmium at passage ll. As cadmium treatment lowered the protein content (Khadijah *et al.,* 2011) which might be due to the adverse effect of cadmium on Nitrate and ammonium assimilation thus hampering the synthesis and supply of free amino acids as reported by Griger and Lindberg (1986) in sugarbeet. In the present investigation increase in protein content and decline in free amino acids in the control calli at differentiation might be due to immediate utilization of amino acids was also reported in *Solanum surattense* (Swarankar *et al.,* 1986). Nitrate reductase activity (fig.4a & 4b) declined in the calli which in turn reduces the free amino acids and ultimately decreases the protein synthesis (Chiraz *et al.,* 2004). It is observed that cadmium treatment causes a markedly increase in the specific activity of GDH which suggests that cadmium selectively promotes the synthesis of GDH (fig.5a & 5b) as compared to other cellular proteins. In cadmium exposed tissue, GDH plays a significant role in the assimilation of ammonia. An increase in GDH has been reported in cadmium treated seedling by Chugh *et al.,* (1992) and supports the role in the assimilation of ammonia as heavy metal exerted a pronounced deleterious effect on GS and GOGAT. At differentiation increase in GDH activity may be related to the supply of NADH, which is required for organogenetic differentiation as reported by Kumar and Maherchandani (1988) in *Nicotiana tabacum.* Cadmium treatment decreased (fig.6a & 6b) MDH activity on per gram tissue basis but its specific activity was hardly affected. This decrease in MDH activity may be due to the general decrease in the rate of photosynthesis which require further investigation. Cadmium inhibits cytosolic or mitochondrial MDH. Chugh (1991) reported a decrease in MDH activity in pea seeds germinating in presence of Cadmium. Cadmium treatment resulted in the senescence of peripheral cells due to proteolysis. It is also found that cadmium accumulated in the cotyledon derived calli (Table 1) at passage l than at passage ll. This may be due to the inherent capacity of the explant as cotyledon being the storage tissue and hypocotyl is the site of metabolites activities (Jyoti *et al.,* 1994) in egg plant. Peroxidase activity increased markedly in untreated differentiated Cotyledon derived calli (Fig.7a), whereas at passage l there was a marginal increase. A decline in peroxidase activity at passage ll was observed. All the calli at differentiating stage showed maximum increase in peroxidase activity to its non-differentiating Stage. In the hypocotyl (Fig.7b) derived untreated calli there was a substantial increase in peroxidase activity.

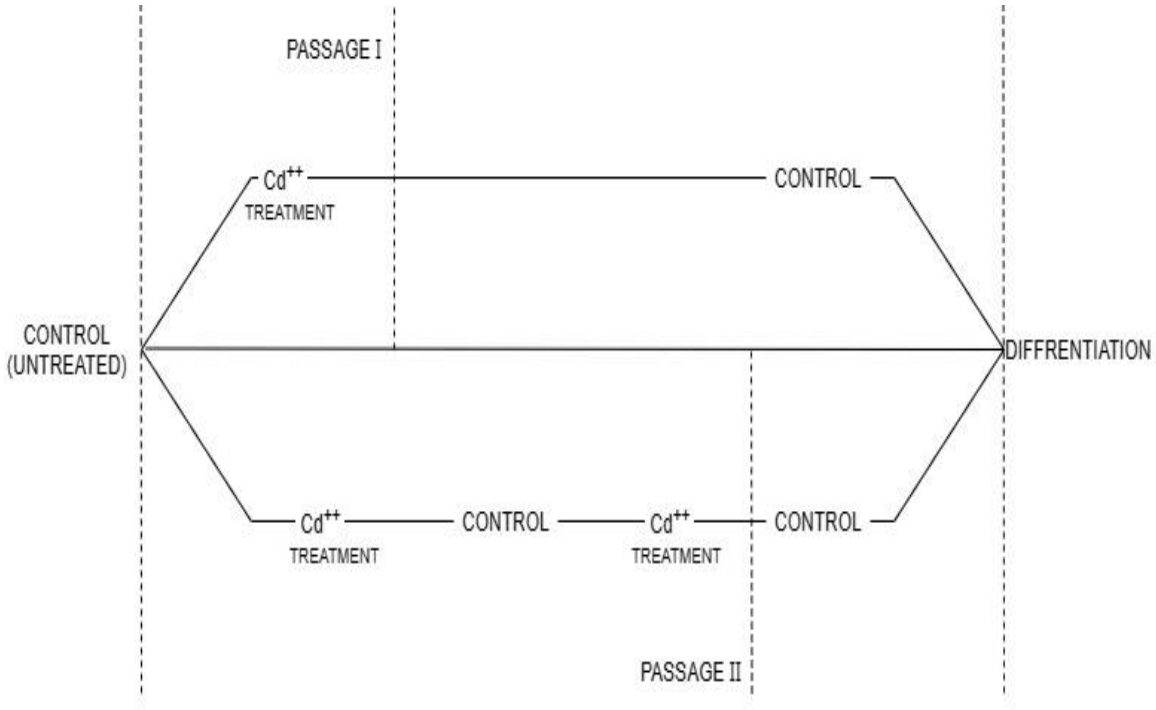
Although an apparent increase in cadmium treated calli at differentiating stage was observed which is much less than that of untreated calli. The cadmium treatment also resulted in slight browning of callus indicating the senescence of peripheral cells probably due to proteolysis in duckweed as observed by Srivastva and Jaiswal (1989). Swarankar *et al.,* (1986) also observed an increase in peroxidase activity at differentiation stage. This increase in peroxidase activity prior to initiation of organ is hypothesized to be indicative of lower requirement of endogenous auxin to bring about a suitable auxin-cytokinin ratio (Khadijah *et al.,* 2011) which might have resulted in high frequency of multiple shoot production. Our results will provide useful information for understanding the biochemical mechanism in eggplant under cadmium stress, which could be exploited in future for food safety purposes in human beings.

Table.1 Accumulation of cadmium in the calli of Cotyledon and Hypocotyl at non-differentiating and differentiating stage of cadmium treated calli.

Accumulation of Cadmium (n moles)		
Stages ↓	**Cotyledon**	**Hypocotyl**
C. Control		
a) Non-differentiating Stage	-	-
b) **Differentiating Stage**	-	-
I.**Passage I**		
a) Non-differentiating Stage	68	95
b) Differentiating Stage	54	88
II. Passage II		
a) Non-differentiating Stage	80	76
b) **Differentiating Stage**	54	66

Accumulation of cadmium in the calli of Cotyledon and hypocotyl at non-Differentiating Stage and Differentiating Stage of cadmium treated calli

Fig.1 Flowchart of different passage treatments of Cadmium



DIAGRAMMATIC REPRESENTATION OF DIFFERENT PASSAGE TREATMENTS

Fig.2A Proteins

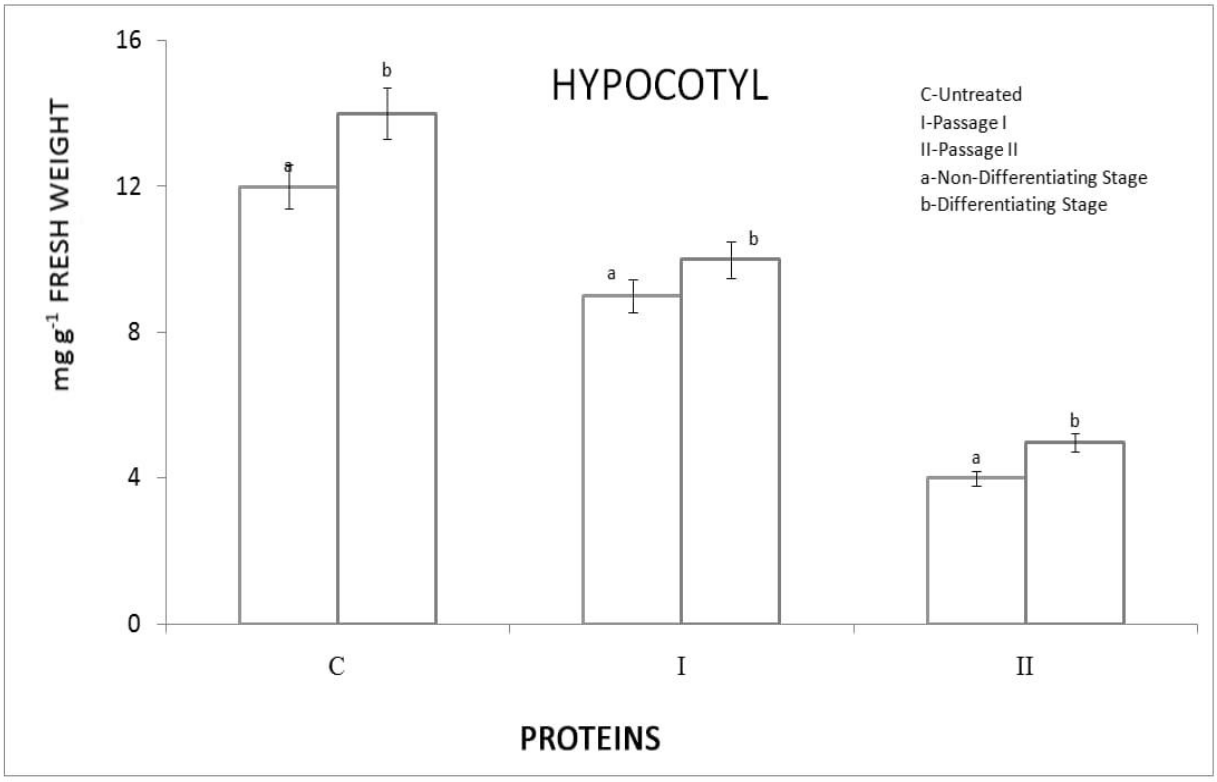


Fig.2B Proteins

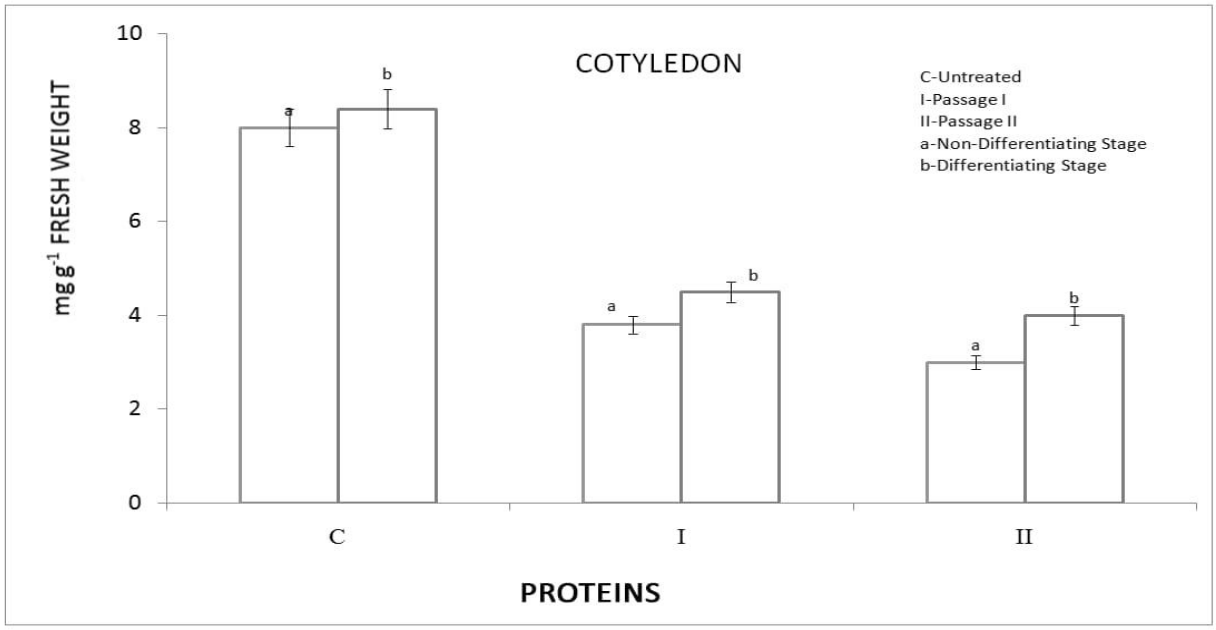


Fig.3A Free amino acids

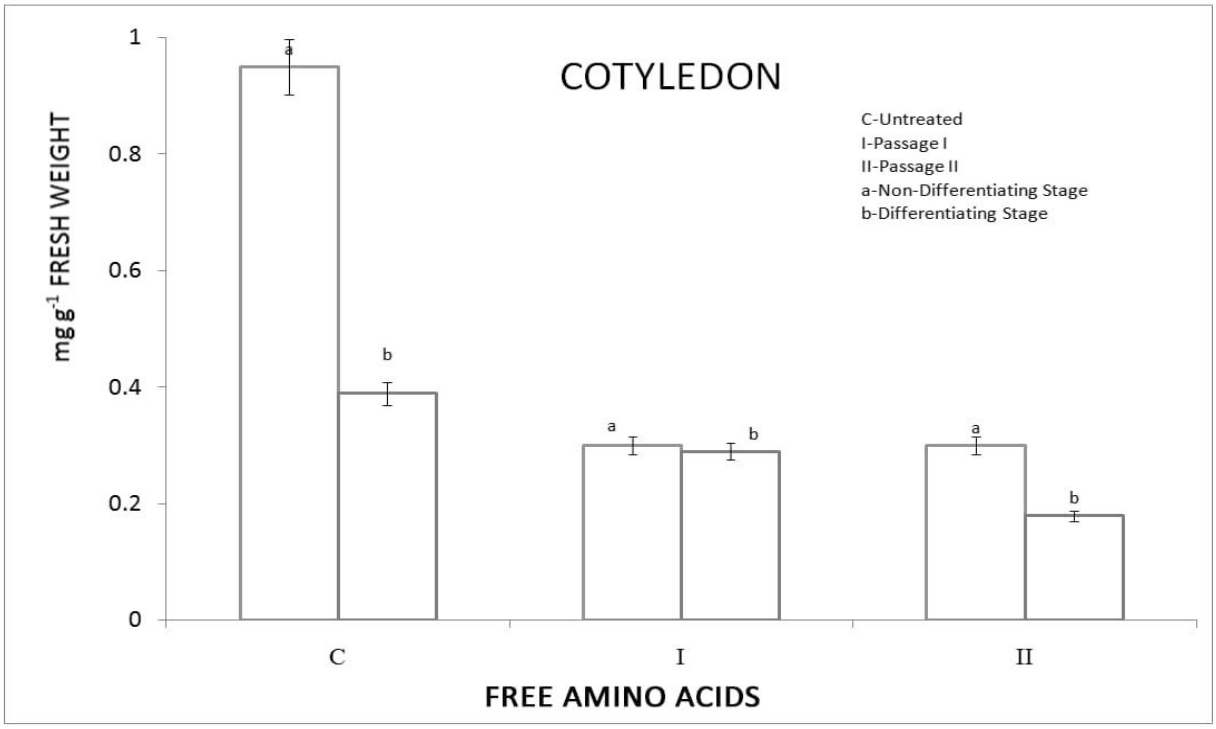


Fig.3B Free amino acids

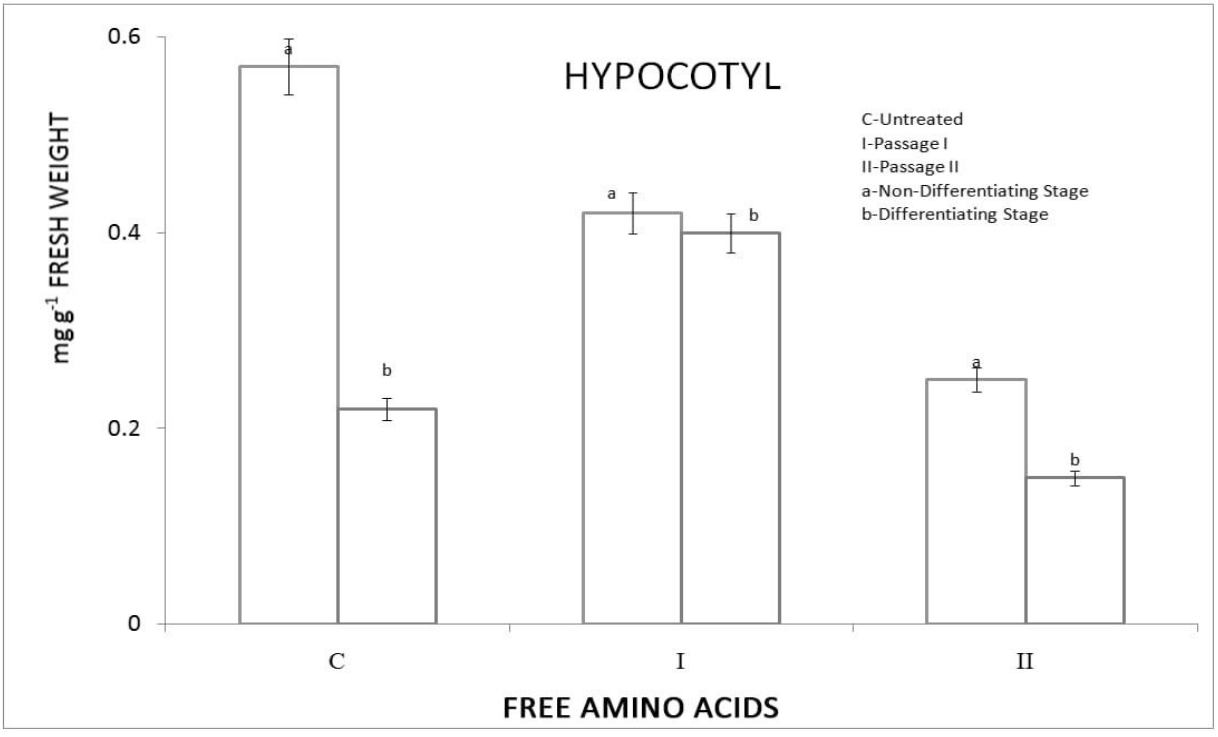


Fig.4A Nitrate reductase

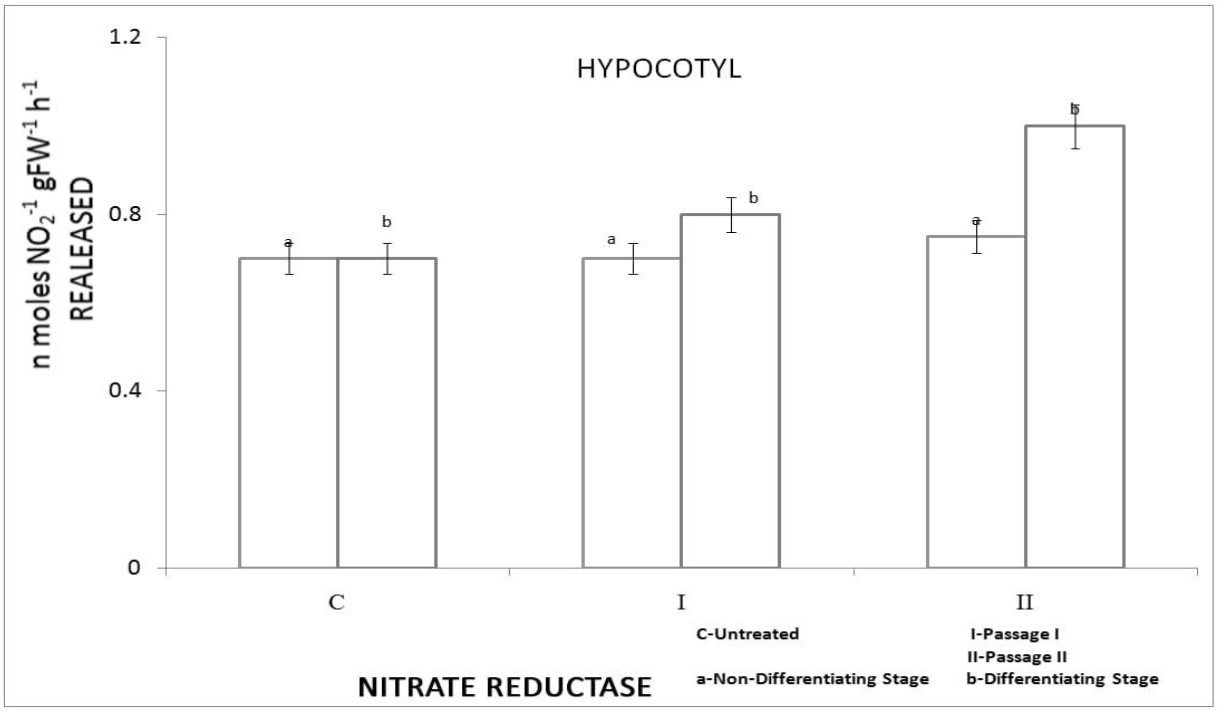


Fig.4B Nitrate Reductase

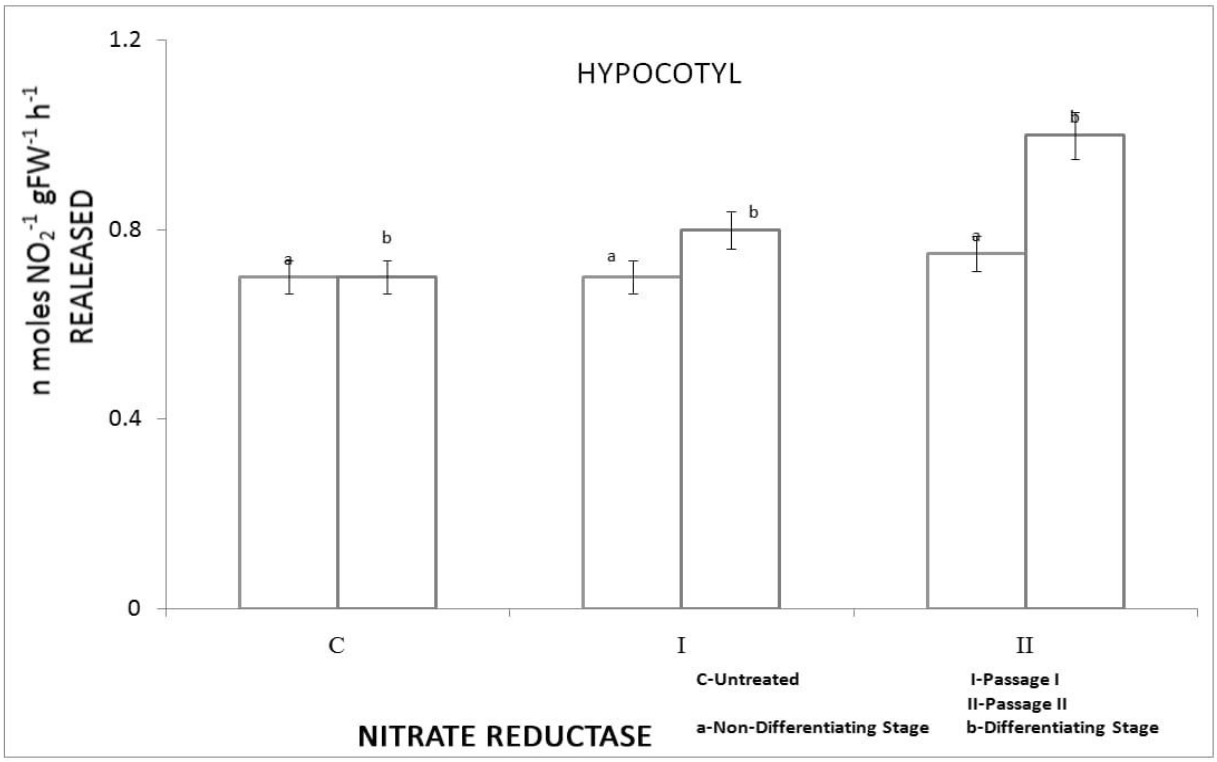


Fig.5A Glutamate Dehydrogenase

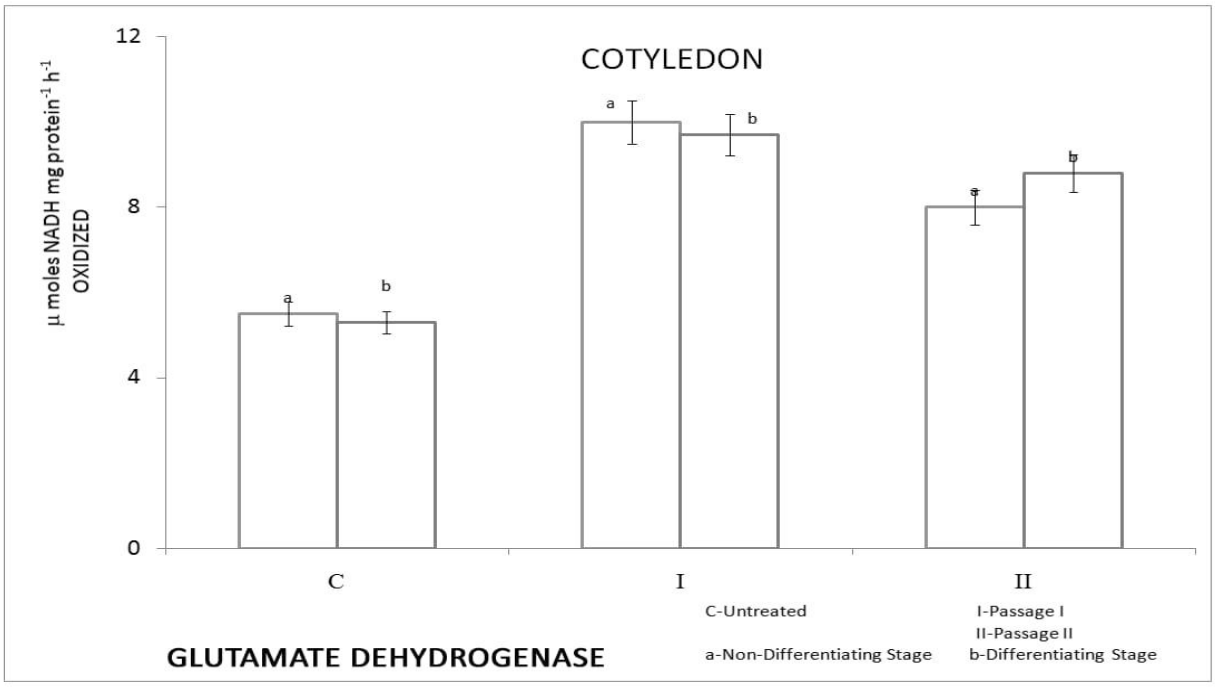


Fig.5B Glutamate Dehydrogenase

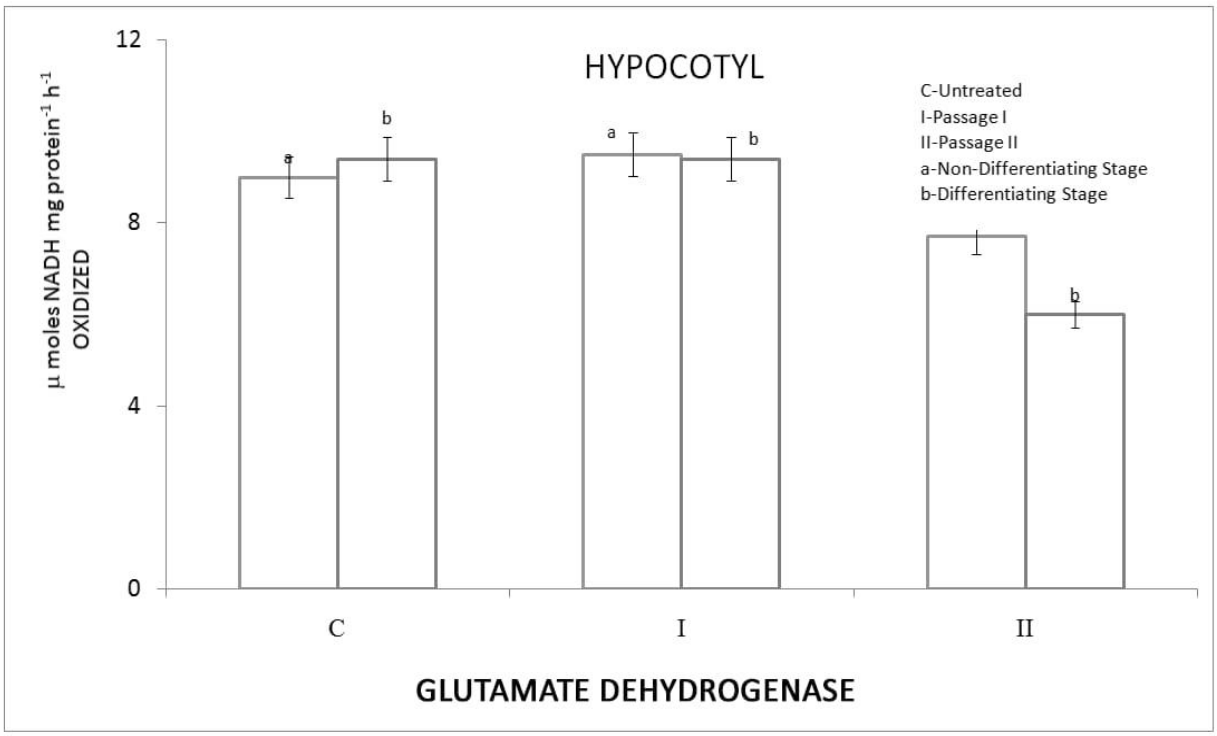


Fig.6A Malate Dehydrogenase

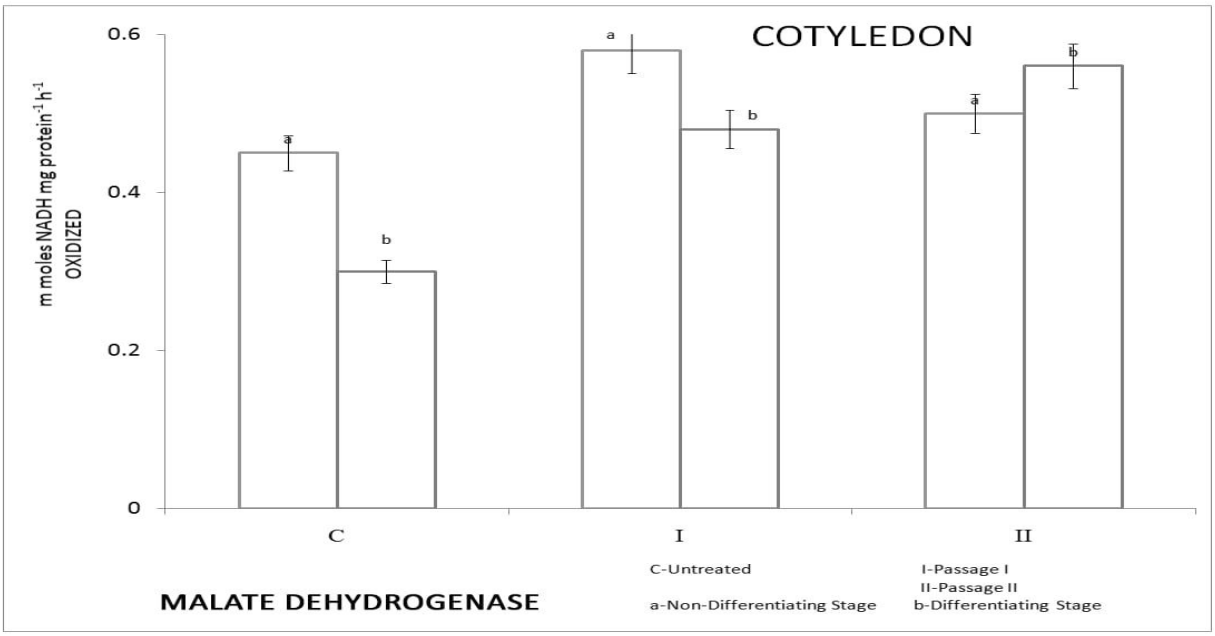
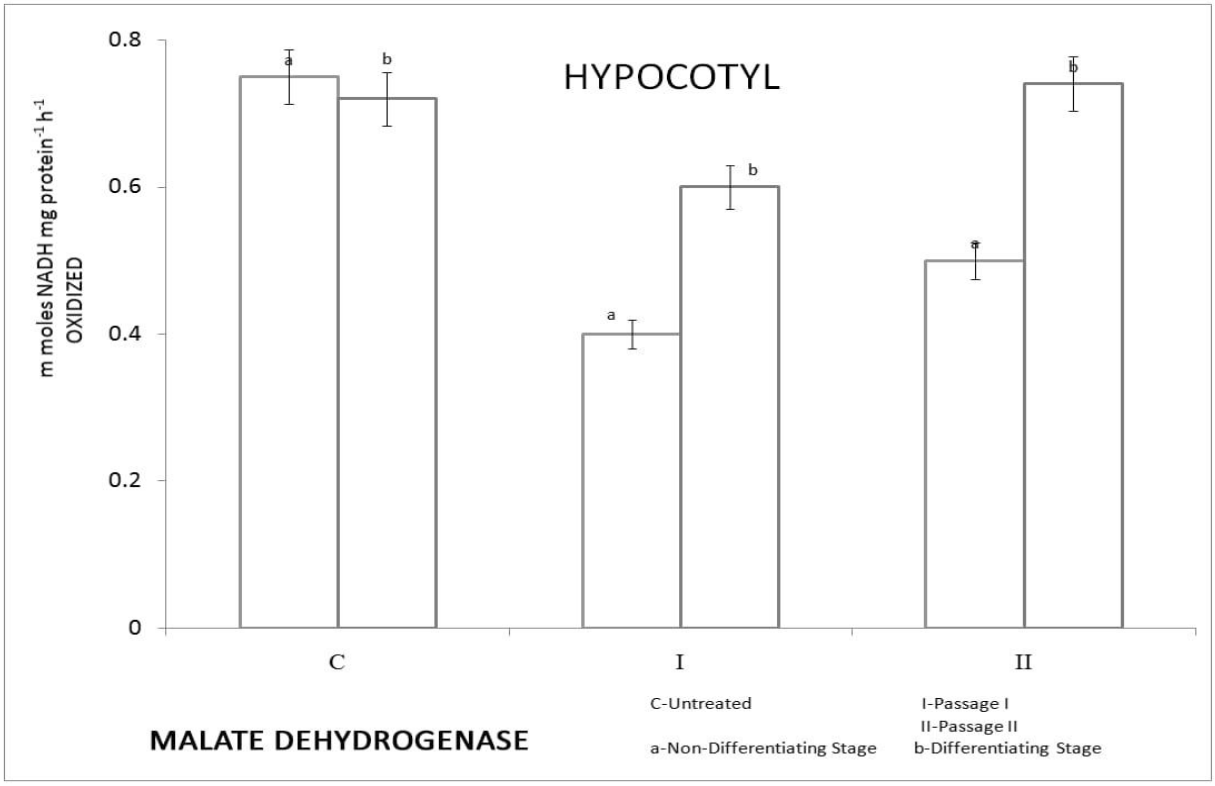


Fig.6B Malate Dehydrogenase



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