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EFFECT OF ENVIRONMENTAL POLLUTANTS AND THERMAL STRESS ON NITROGEN METABOLISM OF *LABLAB PURPUREUS* SEEDLINGS

Jeetendra Kumar Chouhan
Stress Physiology Laboratory, Department of Botany, J.N.V University, Jodhpur – 342005
Email - jeetendrachouhan2017@gmail.com

ABSTRACT:
Heavy metals like Cadmium and Lead has no nutritional value for plants. The presence of Cd and Pd in plants results in many physiological alterations affecting nitrogen metabolism. The effect of $CdCl_2$ 10^{-6}, 10^{-4} mol/l and Pb $(NO_3)_2$ 10^{-7}, 10^{-4} mol/l and thermal stress were investigated in *L. purpureus* seedlings *invitro. Invitro* studies showed stimulatory effect of ALT (Alanine aminotransaminase) activity with respect to control in Heavy metals(($PbNO_3)_2$ and $CdCl_2$) under normal condition whereas thermal stress treated seedlings had an inhibitory effect on ALT activity. However, AST (Aspartate aminotransaminase) activity was increased when the seedlings were treated with $CdCl_2$ only under normal condition whereas Pb $(NO_3)_2$ decreased AST activity. Thermal stress-imposed *L. purpereus* showed decreased AST activity when compared to control. NR (Nitrate reductase) activity was found to be inhibited with Pb $(NO_3)_2$ but treatment of $CdCl_2$ stimulated NR activity under normal condition. Metabolites like Protein and Proline was found to be declined when treated with Pb $(NO_3)_2$ and $CdCl_2$ under normal conditions but thermal stress stimulated Proline contents in *LabLab* seedlings which may be attributed to stress tolerance mechanism.

Keywords- Heavy Metals, Thermal Stress, Stress Tolerance, Alanine aminotransaminase, Aspartate aminotransaminase, Nitrate reductase'

INTRODUCTION:

Plants suffer the ups and downs of temperature of their environment, while animals often regulate their temperature, either by movement or metabolism. Therefore, global warming may affect plants more than animal and there are indications that plants experience substantial damage from high temperature stress. Estimate range up to a 17% decrease in crop yield for each degree Celsius increase in average growing season temperature (Lobell and Asner,2003). Plants growing in heavy metal – contaminated sites generally accumulate higher amounts of heavy metals, and thus, contamination of food chain occurs. Contaminated food chain acts as a primary route for the entry of heavy metals into animal and human tissues, making them prone to several diseases. Due to increasing pollution cadmium and lead levels are rising in plants and people are exposed to cadmium toxicity mainly by eating grains and vegetables that are grown in contaminated soils. Thus, it is necessary to decrease cadmium levels in plants to eliminate this health risk.

Since pollution is widespread and hard to control, research efforts are currently trying to establish how cadmium reacts with soils and plant growth, in a bid to control toxicity, by altering agricultural practices.

Thus, transitory or constant high thermal stress and heavy metal pollutants like cadmium and lead causes an array of morphoanatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield. Therefore, plants have evolved various avoidance and tolerance mechanism for thriving under higher prevailing temperatures and metal toxicity.

For several years it has been known that plants respond to thermal stress by undergoing biochemical adaptive process such as ion transport and by accumulating different compounds, named compatible osmolytes. These osmolytes, which are known to increase under osmotic stress, include proline, they accumulate to high concentrations without interfering with cell metabolism (Bray,1993). As well as proline, other nitrogen compound could be accumulating in plants in response to thermal and drought stress (Good and Zaplachinski,1994; Chiang and Dandekar,1995). The amino acid metabolism may play an important role in plant stress tolerance, by osmotic adjustment through to accumulation of compatible osmolytes, by detoxification of active oxygen species, xenobiotics and heavy metals and by intracellular pH regulation (Rhodes et al.,1999; Alia et al.,2001)

During thermal stress, protein residues may be altered by chemical processes; some proteins are irreversibly damaged by the effect of thermal stress and are degraded by proteases. It has been suggested that proteases mobilize amino acids from proteins to the synthesis into compatible osmolytes (Campalans et al.,1999).

Aminotransferases (ALT and AST) are group of enzymes that involves the transfer of amino group from one amino acid to the keto group of keto acid. Glutamic acid is the main amino acid from which other amino acid are formed through transamination. Alterations in the activities of ALT and AST was reported in many plants under thermal stress.

Nitrate reductase is a substrate inducible enzyme of high molecular weight that has been identified as a rate-limiting step in nitrate assimilation. The activity of this enzyme is considerably affected by salt, moisture and heat stress (Sinha and Nicholas,1981). Its activity also coordinated with the role of photosynthesis and the availability of C-skeleton by both transcriptional and post translational control.

The present study was conducted to evaluate the impact of $CdCl_2$, $Pb(NO_3)_2$ and thermal stress on metabolites and enzymes of nitrogen metabolism in seedlings of *Lablab purpureus* plant. *Dolichos lablab*, a member of Fabaceae, of a high protein content, displays wide range of pharmacological values. Despite its label as “underutilized”, substantial cultivation of lablab bean is seen in certain tropical regions, either as sole crop or in mixed production system. The data obtained in the present study will provide insights that might help in enhancing stress tolerance. Therefore, some suggestions were made for further research in this field at the molecular level and particularly at the transcription level of the Nitrogen transporters.

MATERIAL AND METHODS:

Viable seeds of *L.pupureus* (L.)var L.P.-1461 were selected for this investigation. The seeds were sterilized by 0.1% mercuric chloride ($HgCl_2$) solution for five minutes and then thoroughly washed with double distilled water 4-5 times. The seeds were then germinated in petri dishes lined with Whattman filter paper and were kept at 28° ± 2℃ in BOD incubator. A cool fluorescent light of 34.1 µ mol/m^2/s PAR was given to the seed. There were three replications for the stressed and unstressed

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seedlings taken for analyzing different parameters. Moisture stress was induced by placing the dried filter paper instead of wet one in the petri dishes for two hours before analysis.

Test solution of $CdCl_2$ 10^{-6},10^{-4} mol/l and $Pb(NO_3)_2$ of 10^{-7},10^{-4}mol/l were added to each petri dish. After 5th day, seedlings were excised for studying its impact on metabolites and enzymes of nitrogen metabolism.

Alanine (ALT, EC.2.61.2) and Aspartate (AST, EC.2.61.1) aminotransferases activities were assayed using Bergmeyer's (1974) method. Nitrate reductase (NR, EC.1.6.6.2) was assayed following Wray and Filner's (1970) method. Soluble protein were estimated calorimetrically by using the commassie brilliant blue dye binding method (Bradford,1976) and free proline was assayed by Bates et al. (1973) method.

RESULTS AND DISCUSSION:

Aminotransferase play an important role in distribution of the assimilated nitrogen into protein amino acids. In higher plants the transamination reaction are responsible for the synthesis of all amino acids. Studies carried out in *L. purpureus* seedlings *invitro* showed stimulatory effect of ALT with respect to control in heavy metals ($CdCl_2$and $Pb(NO_3)_2$) under normal condition suggesting higher conversion of alanine to pyruvate. The result are in accordance with (Elshintinawy and Elansary 2000) who reported increased ALT activity in Cd treated roots in Soyabean seedlings. However in present investigation thermal stress treated seedlings had an inhibitory effect effect on ALT activity in *L.purpureus* seedlings (Table-1). However, AST activity was increased when the seedlings were treated with $CdCl_2$ only under normal condition whereas $Pb(NO_3)_2$ decreased AST activity. Thermal stress imposed seedlings also showed decreased AST activity when compared to control.

The reduction of nitrate to nitrite catalysed by nitrate reductase (NR) is considered to be the rate limiting step of n-assimilation. The activity of this enzyme is considerably affected by salt, moisture and heat stress. (Sinha and Nicholas, 1981).

In present investigation NR activity was enhanced in *L.purpureus* grown in *invitro* with $CdCl_2$ under normal conditions but decreased under thermal stress condition. Lead nitrate displayed decreased NR activity under normal and thermal stress with respect to control in *L. purpureus* seedlings. Similarly NR activity was inhibited in cadmium treated plants of *V.radiata* (Keshan and Mukhherji 1994), in *P. vulgaris* with lead acetate treatment (Jain and Gadre, 1997) and a gradual decline in NR activity was obtained with lead nitrate treatment in *V. radiata* (Tomar et al,2000),NR activity also declined in *S. squarrosum* with Pb treatment (Saxena and Saxena,2002).

In present investigation, heavy metals ($Pb(NO_3)_2$ and $CdCl_2$) in *L. purpureus* seedlings grown in *invitro* condition had inhibitory effect on the accumulation of protein content. Similar results were obtained in *Vigna* seedlings when treated with $PbCl_2$ and $CdCl_2$ (Bhattacharya and Choudhary,1995). $Pb(NO_3)_2$ treatment also resulted in decreased protein content in leaves stem and root of *V.radiata* (Tomar et al.,2000).

Proline is an important metabolic constituent and can easily be converted to key amino acids, glutamate. It has been suggested the proline protects plant tissue against osmotic stress because it is an osmosolute, a source of nitrogen compounds, a protectant for enzymes and cellular structures (Stewart and Lee, 1974).

It was reported that constitute production of proline could confer osmotolerance in transgenic tobacco plants (Kishore et al,1995).

In present study, proline was found to be declined when treated with $Pb(NO_3)_2$ and $CdCl_2$ under normal condition but thermal stress stimulated proline content in *Lablab* seedlings which may be attributed to stress tolerance mechanism. It has been speculated that proline accumulation could represent a compensatory mechanism for better plant survival during a period of thermal stress, a protector of enzyme denaturation, a reservoir of nitrogen and carbon sources or even as a stabilizer of the machinery for protein synthesis. Similar reported in bean seeds where proline was found to be increased under temperature stress. (Nelson et al., 2004).

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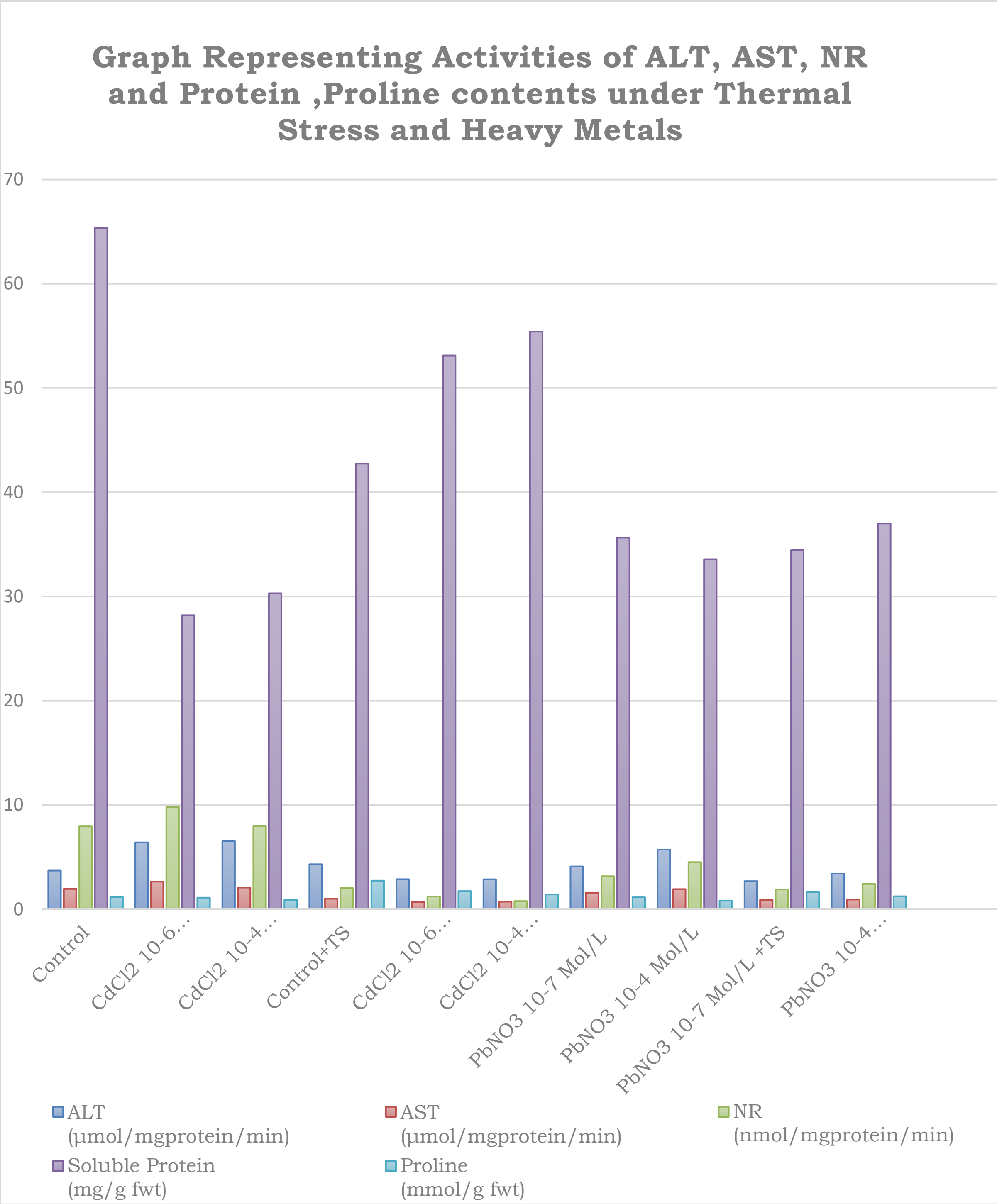
Graph Representing Activities of ALT, AST, NR and Protein ,Proline contents under Thermal Stress and Heavy Metals
70
60
50
40
30
20
10
0
Control
CdCl2 10-6…
CdCl2 10-4…
Control+TS
CdCl2 10-6…
CdCl2 10-4…
PbNO3 10-7 Mol/L
PbNO3 10-4 Mol/L
PbNO3 10-7 Mol/L +TS
PbNO3 10-4…
ALT (µmol/mgprotein/min)
AST (µmol/mgprotein/min)
NR (nmol/mgprotein/min)
Soluble Protein (mg/g fwt)
Proline (mmol/g fwt)

Table 1 - Effect of $CdCl_2$, Pb $(NO_3)_2$ and Thermal stress on the activities of ALT, AST, NR and on Protein and Proline contents in *L. purpureus* seedlings grown *invitro*

Treatments	ALT (µmol/mg protein /min)	AST (µmol/mg protein /min)	NR (nmol/mg protein/min)	Soluble Protein (mg/g fwt)	Proline (mmol/g fwt)
Control	3.706±0.000	1.944±0.034	7.944±0.082	65.353±0.656	1.173±0.013
$CdCl_2$ 10^{-6} Mol/L	6.405±0.378	2.646±0.213	9.816±0.598	28.202±0.127	1.107±0.002
$CdCl_2$ 10^{-4} Mol/L	6.535±2.309	2.076±0.625	7.957±0.351	30.307±0.143	0.899±0.001
Control+ TS	4.313±0.077	1.004±0.016	2.021±0.142	42.742±0.438	2.740±0.021
$CdCl_2$ 10^{-6} Mol/L +TS	2.875±0.019	0.689±0.007	1.219±0.012	53.119±0.151	1.732±0.007
$CdCl_2$ 10^{-4} Mol/L +TS	2.866±0.038	0.723±0.003	0.775±0.037	55.404±0.226	1.413±0.001
Pb $(NO_3)_2$ 10^{-7} Mol/L	4.101±0.106	1.583±0.032	3.161±0.077	35.648±0.231	1.138±0.004
Pb $(NO_3)_2$ 10^{-4} Mol/L	5.720±0.483	1.920±0.168	4.512±0.255	33.568±0.428	0.815±0.007
Pb $(NO_3)_2$ 10^{-7} Mol/L +TS	2.687±0.071	0.897±0.021	1.894±0.024	34.429±0.434	1.627±0.000
Pb $(NO_3)_2$ 10^{-4} Mol/L + TS	3.410±0.209	0.928±0.057	2.424±0.092	37.009±0.124	1.235±0.000

± = Values represent SD (Standard Deviation); TS = Thermal Stress