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Full Length Article

Nickel Metal Stress Induces Alterations in Expression of Amylase and Acid-phosphatase Isozymes in *Cenchrus ciliaris*

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Abstract

When plants of a specific area grow in conditions that are far from optimum state, the first effect of such conditions is limitation in plant growth due to cellular damage. These sub optimal or imbalanced environmental conditions constitute stress. Conversely nature always tries to manage the unbalanced state because protective cellular, metabolic and molecular adaptations occur in plants to deal with these stresses that alter physiological reactions of the plants. Whole mechanistic details of these processes are still unknown. The present work focuses on the defensive role of two key enzymes amylase and acid phosphatase in plants under abiotic stress. Our study covers stress related alterations in expression pattern of isozymes of acid phosphatase (Apase) and amylase in *Cenchrus ciliaris*, a desert plant after treatment of Ni metal stress. The plants were grown hydroponically in the presence of different concentrations of Nickle (Ni) metal (0.3, 3, 10, 20 mg/L). Results of Spectrophotometry and stain specific native PAGE showed that expression pattern of various isoforms of amylase and acid phosphatase varied with change in intensity of Ni metal stress in *C. ciliaris*. Thus Nickle (Ni) metal stress induces alterations in carbohydrate and phosphorus metabolism and *C. ciliaris* uses it as adaptive strategy to cope stress conditions.

Keywords: Acid phosphatase; Inorganic phosphate; Nickle; *Cenchrus ciliaris*; Amylase; Isoforms

Introduction

Plant homeostasis is very important for normal metabolic processes, including chloroplastic, mitochondrial, and plasma membrane-linked electron transport systems for transport but sometimes it gets disturbed due to different environmental disasters (Carvalho *et al.*, 2015). Various abiotic stresses including continuous light, dark, heat, cold, drought, salinity, heavy metal toxicity, wound stress and UV stresses affect plant growth and limit productivity (Nouri and Komatsu, 2013).

Heavy metal stress is one of the major stresses that adversely affect crop plants as well as grasses (Nasim *et al.*, 2015). In the present study on *C. ciliaris*, our focus was on Nickel (Ni) metal stress because Nickel (Ni) metal is an essential trace element and also required for certain enzymes such as urease (Polacco *et al.*, 2015). Many biochemical and physiological processes are maintained by Ni. It is also involved in various cellular oxidation and reduction reactions (Yusuf *et al.*, 2011) but its high concentration is toxic for plant growth and development. Biochemical changes associated with exposure to stress and stress tolerance are not clearly understood (Nouri and Komatsu, 2013; Ali *et al.*, 2015).

One of the important aspects of these biochemical changes is variation in the expression of hydrolyzing enzymes that play a vital role in mobilization of food from plant food reserves (carbohydrate, fat, protein) by hydrolyzing them (Kuriakose and Prasad, 2008; Pandey *et al.*, 2015).

Vegetation of Cholistan desert is facing severe problem of drought and salinity (Arshad *et al*., 2007). *Cenchrus ciliaris* is a perennial bunch grass belonging to the family Poaceae. Its common names are buffle grass, African foxtail grass and anjan grass. *Cenchrus* is a hardy, drought resistant, non-native grass, widely planted as pasture grass in many parts of the world. This widely distributed perennial grass is resistant to harsh environmental conditions. It can withstand strong winds, low annual rainfall, acute erosion and nutrition depletion (Syamaladevi *et al*., 2016). *C. ciliaris* is a naturally growing grass in Cholistan due to its tolerance to many drastic conditions and to some abiotic stress factors (Arshad *et al*., 2007).

Amylase causes starch degradation in order to balance the supply of energy. Climatic conditions and unsuitable environments affect amylase activity (Kondhare *et al.*,

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2015). In many higher plants, sucrose and starch are the primary photosynthetic end products. Sucrose is the main carbohydrate translocated from leaves to sink tissues while starch is temporary carbohydrate storage in the chloroplast. Both these components act as osmoprotectants during abiotic stress. Using the stored starch as substrate, amylases produce the precursors for energy supply and building components of cell like glucose and oligosaccharides respectively (Gupta and Kaur, 2005; Kashefi *et al.,* 2012). Carbohydrate metabolism and sugar signaling pathways have been suggested to interact with the stress pathways to modulate metabolism (Buchanan *et al.*, 2015).

Inorganic Phosphate (Pi) is a critical macronutrient and acts as one of the major growth-limiting factors for plants in many natural ecosystems (Rouached *et al*., 2010; Onaga *et al.,* 2013). It is a structural element of many biological molecules, such as DNA, RNA and cell membrane phospholipid bilayer (Buchanan *et al.,* 2015). Plants absorb Pi from the soil but its availability depends upon the rate of conversion of organic phosphate to Pi in the soil. A significant amount (20%–80%) of the nutrients may be found in the organic forms. In response to persistent Pi deficiency, plants have developed many adaptive mechanisms to enhance the availability and the uptake of Pi. One such adaptive mechanism is the production and secretion of phosphatases to release Pi from organic forms (Baldwin *et al.,* 2001). Acid phosphatase (Apase) is known to hydrolyze the phosphomonoesters and is a major marker enzyme which is involved in production, transport and Pi recycling, crucial for cellular metabolism and bioenergetics (Antonyuk *et al.,* 2014). Many physiological processes including the activity of many hydrolytic, lysosomal enzymes are inhibited by heavy metals even though these metals may also activate certain enzymes. Heavy metals accumulate in a relatively high concentration in lysosomes, destabilizing its single membrane. Phosphatases are often classified as acid or alkaline phosphatases, depending on whether their optimal pH for catalysis is below or above 7.0 (Chatterjee *et al.,* 2006; Jayakumar *et al*., 2008). Plant Apase is induced under various environmental and developmental conditions, including salt, metal stress, flowering, fruit ripening, and seed germination (Kuang *et al.*, 2009; Pervez *et al.*, 2009).

Main objective of the research being presented was to investigate the activity and expression of isozyme profile of some hydrolyzing enzymes i.e., Amylase and acid phosphatase (Apase) in *C. ciliaris* under Ni stress that induce synthesis, accumulation and clearance of some defense-related secondary metabolites.

Materials and Methods

C. ciliaris was collected from Cholistan desert. Identity of the plant was established through physical examination. The chemicals and solvents used were of analytical grade and were purchased from Merck, Sigma, Fermentas etc.

Cutting and Sterilization of Stubs

C. ciliaris stubs obtained by cutting shoots and the specific parts of the plant having uncut eyes were used to regenerate the plant. Approximately equal length (about 5 cm) stubs taken from fresh plants were used. The stubs were surface sterilized for 20 min in 1% (v/v) sodium hypochlorite, followed by 70% ethanol washing several times with distilled water.

Growth Conditions and Regeneration of Plant

Surface sterilized stubs were hydroponically regenerated in plastic pots supplemented with Hoagland solution (Hoagland and Arnon, 1950). Plant propagation was initiated in November and climatic conditions were recorded periodically. Minimum and maximum temperatures during the course of every set of experiment were noted (Table 1).

Experimental Design and Ni Treatment

The experimental set comprised two groups where every group contained 12 plants. Group I designated as Control (0 mg/L) was given no treatment. Group II was designated as the test group which received Ni metal treatment (0.3, 3, 10 and 20 mg/L) in the form of $NiCl_2$ solution. Dates and temperatures of the germination period were recorded (Table 1).

For imposition of treatments 1000 mg/L Stock solution of Ni was prepared by using the following formula:

Gram wt of Ni in 1L distilled water = 1000 mg/L= molecular wt of $NiCl_2$/molecular wt of Ni.

Test concentrations 0.3, 3, 10, 20 and 30 mg/L were used to carry out the metal treatment. However 30 mg/L treatment was found to be lethal for the plant growth and was therefore diminished from all the subsequent analysis. Hoagland Solution (6 L) was kept constant in each experimental vessel. Nutrient solution without Ni was used in the control (0 mg/L) experiment.

Harvesting

Plants were harvested on maturity (45th day) and washed thoroughly with distilled water to remove any particulate matter. Leaves, stem and roots were separated and stored at -80ºC for future analysis.

Analytical Procedures

Moisture content: The plant moisture contents were determined using the AOAC oven dry method.

$$\text{Percent moisture content} = \frac{\text{Wt. of sample lost during drying}}{\text{Fresh wt. of sample before drying}} \times 100$$

Table 1: Chronology of Plants grown hydroponically under Nickel metal stress

Temperature (°C)	Start of the experiment March, 14	Sprouting 1st root March 17	Sprouting 1st shoot March 18	Sprouting 1st ear April 3	Maturity April 12	Metal Treatment April 15	Harvesting May 1
Early morning	27	28	28	19	17	16	19
Day time	36	37	36	35	34	34	35
Night time	31	31	31	22	20	19	21

Proteins Extraction and Estimation

Plant sample cut in small pieces (5 g) was taken in pre-chilled 50 mL tubes kept on ice and homogenized in 6 mL of 0.1 M phosphate buffer (pH 7) containing phenyl methyl sulfonyl fluoride (PMSF, a proteinase inhibitor), Octylphenol Ethoxylate (Triton X-100, a detergent) and Ethylene diamine tetra acetic acid (EDTA) for protein extraction. Each sample was homogenized for at least 20 min to extract maximum proteins. The sample tubes were kept on ice during homogenization and the homogenized materials were filtered through cheese cloth to remove debris. The filtered extracts were centrifuged at 16000 rpm (Revolutions per minute) at 4°C for 20 min. The supernatants were collected and preserved at -70°C for further analysis. Total protein was estimated with Bradford reagent (Fermantas) using 0.1 M phosphate buffer (pH 7). Bovine serum albumin (BSA) was used to make the standard curve (Bradford, 1976).

Acid Phosphatase Activity

The assay mixture contained sodium acetate buffer (50 mM, pH 5.5), 3 mM pNPP (para-nitrophenol phosphate) and 10 µL of enzyme extract, in a final volume of 325 µL. After 10 min at 37°C, the reaction was stopped by adding 1 mL of 0.2 M NaOH. Absorbance was measured at 410 nm. One unit of acid phosphatase will hydrolyze 1 µmole of 4-nitrophenol phosphate per minute at pH 4.8 at 37°C. The activity was expressed in milliomoles of *p*-nitrophenol (pNP) formed/mg of protein/min, based on an extinction coefficient of 18,400 M^{-1} cm^{-1} at 410 nm (Schadeck *et al.*, 2000).

Amylase Activity

For amylase assay 0–100 µg of 0.1% gelatinized solution of soluble starch was prepared and placed in a 96 well plate wells. The volume was made up to 100 µL by adding distilled H_2O. The detecting reagent (Add 145 g iodine in 200 mL of 0.2M HCl solution containing 43.3 mM KI. Heat this solution on heat bath to dissolve iodine) 150 µL was added to each well and the mixture was incubated at 37°C for 30 min. Absorbance was measured at 490 nm using a plate reader to prepare the standard curve. Amylase activity in the sample solution was determined by mixing 70 µg of the starch as substrate, 10 µL enzyme extract and distilled H_2O to make up the volume to 100 µL. 150 µL detecting reagent was added in each well. The reaction mixture was incubated at 37°C for 30 min. Absorbance was taken at 490 nm and the value obtained was used to calculate the enzyme activity that was expressed as mg of starch hydrolysed/min/g fresh wt. The specific activity was expressed as mg of starch hydrolysed/min/mg protein. One amylase unit was defined as the amount of enzyme that hydrolyzes 1 mg of starch (0.1% w/v) in 10 min at 37°C and at pH 5.9 (Sunitha *et al.*,2012).

Acid Phosphatase Native PAGE

Separation of acid phosphatase isozymes was carried out by native PAGE on 10% separating and 4% stacking gel. Samples (Leaf, Stem or Root extract) containing 70 µg of protein was loaded per well and the gel was run at 150 V for 5 h (Perera *et al.*, 2008). The gel was washed three times in 100 mM sodium acetate buffer (pH 5). Enzymatic activity bands were visualized by diazo dye method using 1-naphthyl phosphate (0.1% w/v as a substrate in sodium acetate buffer 100 mM pH 5 and diazo blue (O- dianisidine tetra oxide 0.1% w/v) as the stain. A dark purple colour indicates phosphatase activity (Besford, 1979; Perera *et al.*, 2008).

Amylase Native PAGE

Separation of amylase isozymes was carried out by native PAGE on 7% resolving and 5% stacking gel system. Samples (leaf, stem or root extacts) containg 70 µg protein was loaded per well. The gel run at 180 V for 4 hrs (Hussain *et al.*, 2003; Dojnov *et al.*, 2010) was washed with distilled water to remove buffer and soaked in 200 mL of 2% gelatinized solution of soluble starch prepared in 0.1 M solution of sodium acetate buffer (pH 5) at 37°C for 2 h. After incubation, the gel was washed with distilled water to remove excess starch solution and incubated again in a capped container in moistened environment for 2 h at 37°C. The gel was stained in a solution containing 5.7 mM iodine (I_2) and 43.3 mM potassium iodide (KI) in 0.2 M HCl. Colourless activity bands of amylase appeared on light blue background within 5 min (Lin *et al.*, 1988).

Statistical Analysis

Biochemical parameters were statistically analyzed by applying completely randomized analysis of variance (ANOVA) with three replications of reading using Statistix 8.1 software.

Results

When *C. ciliaris* plants were grown hydroponically and affected by the presence of Ni metal in the growth medium (Hoagland medium) it was noted that this desert plant is sensitive to the heavy metal stress. While the 30 mg/L Nickle concentration was found to be the most toxic and lethal resulting in the death of the growing plant, varying effects were noticed in the morphology of the growing plants at lower concentrations of the Ni metal. Survival of the plants under these conditions suggested that the plant was equipped with processes to tolerate the oxidative stress generated by this heavy metal and can therefore be used as model system to investigate and delineate the biochemical nature of the the tolerance mechanism involved. All experiments were done in triplicate along with proper controls. Plants were grown at 26 to 37ºC (Table 1). Moisture contents of the tissues analysed varied with changing Ni concentration. Roots are the first to come in contact with the Ni metal. They accumulate the highest moisture contents at the very first exposure to Ni metal at 0.3 mg/L but at 20 mg/L show minimum moisture contents. Leaves accumulate the higher amount of moisture only when the plant is exposed to the highest level of Ni stress (20 mg/L). However at 0, 0.3 and 3 mg/L, the leaves showed almost the same moisture contents. In stem 3 mg/L Ni treatment showed the maximum moisture contents. These results indicate that stress generated by Ni metal appears to follow a trickledown effect and there might be involvement of some dilution mechanism that helps plants to survive with increasing concentration of Ni (Fig. 1).

Maximum protein contents were observed at 0 and 10 mg/L Ni treatment, whereas minimum amount of protein was noticed at 0.3 and 3 mg/L, Ni treatment. A gradual increase in stem protein content was noticed from 0.3 to 10 mg/L while 20 mg/L showed a sharp decline in total protein content in stem. Roots of the treated plants showed a gradual decrease in total protein with increasing stress. Ni level of 0 and 0.3 mg/L showed maximum protein content in roots that declined in subsequent treatments (Fig. 2).

Two isoforms of amylase were observed in the *C. ciliaris* leaves, stem and root. However the 1st isoform of amylase showed comparatively more expression against Ni metal stress as compared to the 2nd isoform in all tissues (leaves, stem and root) at all treatments. Leaves of control plants (0 mg/L) showed maximum expression of these isoforms of amylase. It was only the 10 mg/L Ni metal treatment where leaves showed some prominent expression as compared to 0 mg/L (control), while at all other treatments very light bands appeared showing minimum expression of both isoforms (Fig. 3). In case of stem and roots, remarkably similar pattern of expression of both isoforms was observed against all stress treatments. The control (0 mg/L) plants and the plants at 0.3 mg/L Ni metal treatment showed almost similar expression, while at 3 mg/L, the expression significantly decreased. At 10 and 20

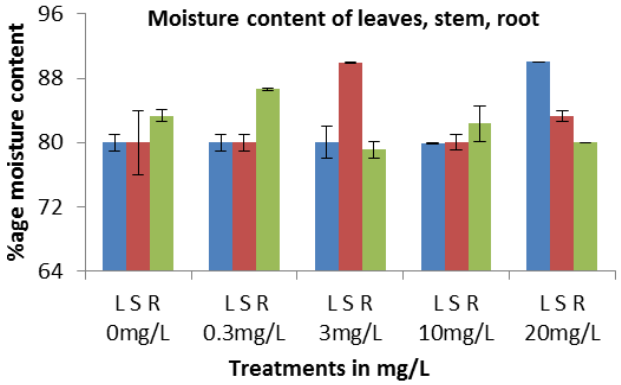

Fig. 1: Moisture content in leaves, stem and roots of *C. ciliaris* grown under Ni stress

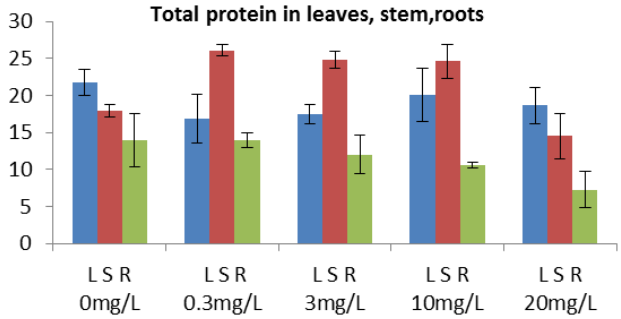

Fig. 2: Total protein in leaves, stem and roots of *C. ciliaris* grown under Ni stress

mg/L Ni metal concentrations, very sharp bands exhibiting maximum expression of both isoforms appeared in both tissues (stem and roots) (Figs. 4–5). Spectrophotometric estimations also showed variations in the quantity of enzyme activity. In control, leaves showed maximum relative activity and specific activity that decreased to the minimum at and 3 mg/L. In stem and roots maximum relative activity of amylase was seen in plants exposed to 0.3 mg/L and 10 mg/L Ni although maximum specific activity was seen in plants exposed to 10 and 20 mg/L Ni. On the other hand 3 mg/L Ni treatment showed minimum relative activity of amylase. Quantitative estimations also showed the same variations in activity as did native PAGE (Figs. 6–7).

All tissues (Leaves, Stem, Root) of test plants were found to express four isoforms of Apase but their expression pattern varied at different stress treatments. The control (0 mg/L), 0.3 and 10 mg/L treatments showed almost the same expression in leaves except two new isoform that appeared at 0.3 mg/L treatments. At 3 and 20 mg/L concentration the expression of Apase was comparatively low (Fig. 8). In stem the expression of Apase was variable regarding number of isoforms and intensity of bands because expression increased in low stress treatments i.e., 0.3 and 3 mg/L but decreased at 10 mg/L and again at 20 mg/L the expression increased. In stem at 0.3 mg/L Ni treatment two

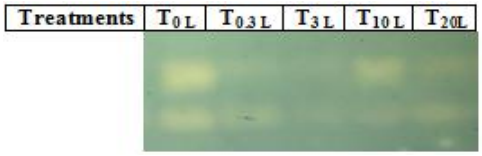

Fig. 3: Expression of amylase in leaves of *C. ciliaris* under Ni metal stress

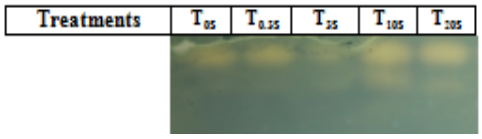

Fig. 4: Expression of amylase in stem of *C. ciliaris* under Ni metal stress

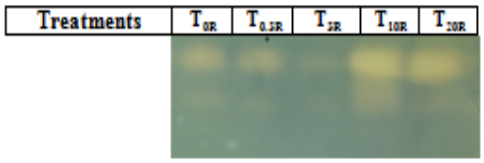

Fig. 5: Expression of amylase in roots of *C. ciliaris* under Ni metal stress

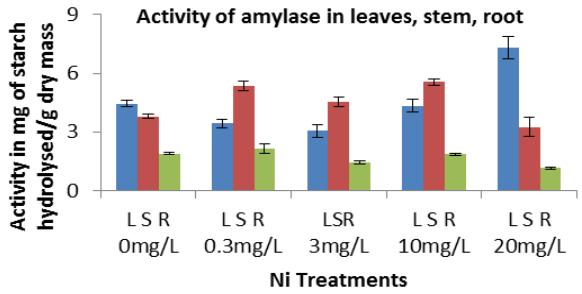

Fig. 6: Activity of amylase in leaves, stem and roots of *C. ciliaris* grown under Ni stress

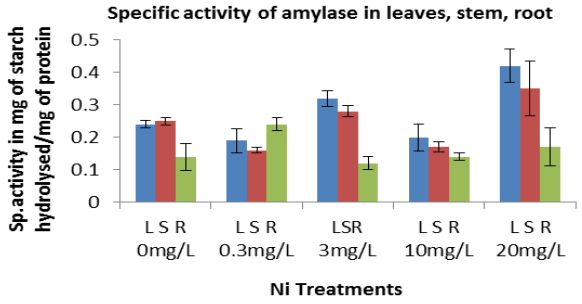

Fig. 7: Specific activity of amylase in leaves, stem and roots of *C. ciliaris* grown under Ni stress

additional bands of low and high intensity were appeared resulted in increased expression of Apase (Fig. 9). In roots expression of Apase isoforms gradually increased with increase in stress treatments (0.3, 10 and 20 mg/L) except 3

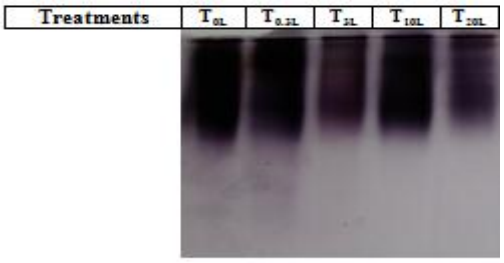

Fig. 8: Expression of acid phosphatase in leaves of *C. ciliaris* under Ni metal stress

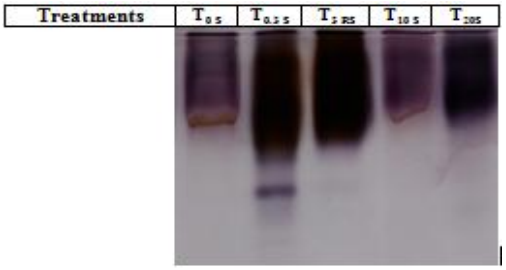

Fig. 9: Expression of acid phosphatase in stem of *C. ciliaris* under Ni metal stress

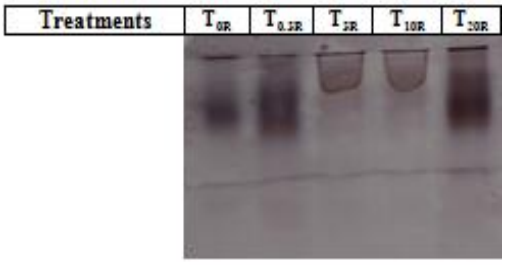

Fig. 10: Expression of acid phosphatase in root of *C. ciliaris* under Ni metal stress

mg/L treatment, where decreased expression of Apase was observed (Fig. 10). Quantitative estimations also showed the same variation in activity as native PAGE results. When quantitative estimations of Apase were done to measure relative activity and specific activities, 0 mg/L showed maximum relative activity while it was less in 3 mg/L and 20 mg/L but 0.3 and 10 mg/L show almost same and average relative activity. On the other hand, 0, 0.3 and 10 mg/L showed maximum specific activity but 3 mg/L showed medium specific activity and 20 mg/L showed minimum specific activity. In stem the relative activity and specific activity results were nicely matched. Stem at 0.3 mg/L showed maximum and 3 mg/L showed medium relative and specific activity but minimum relative and specific activity was seen at 0, 10 and 20 mg/L. Roots showed variations in Apase measurement i.e. 0.3 mg/L showed maximum relative activity but 0 and 20 mg/L Ni treatments showed medium relative activity. Maximum

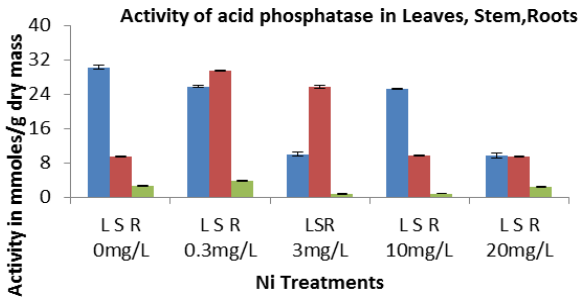

Fig. 11: Activity of acid phosphatase in leaves, stem and roots of *C. ciliaris* grown under Ni stress

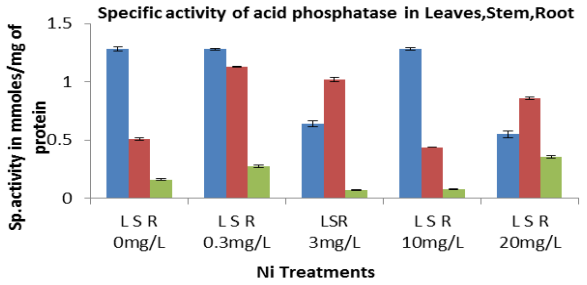

Fig. 12: Specific activity of acid phosphatase in leaves, stem and roots of *C. ciliaris* grown under Ni stress

specific activity was seen at 20 mg/L and medium specific activity was seen at 0.3 mg/L and the minimum one at 3 mg/L. Quantitative estimations also demonstrated the same variation in activity as seen in native PAGE (Figs. 11–12).

Discussion

Plant growth depends upon balanced nutrient uptake, environmental conditions and internal mechanism working jointly to maintain homeostasis. Disturbance in both above conditions triggers endogenous mechanisms (metabolic, defensive) that help plant to grow better against rising abiotic stress (Cabello *et al.*, 2014). It is very interesting to note that not only the defensive mechanism but the whole metabolism becomes active for plant's existence. Understanding these mechanisms is therefore of considerable interest (Barkla *et al.*, 2013). One important group of enzymes that play vital role in food mobilization by hydrolyzing biomolecules (carbohydrate, protein, fat) includes hydrolytic enzymes like proteases, acid phosphatases, amylases, and lipases (Kuriakose and Prasad, 2008). It has also been reported that *C. ciliaris* is equipped with some machinery that assists in the metal-specific stress tolerance to some extent (Nasim *et al.*, 2015). In the present research we have used a proteomics approach to study the role of two hydrolyzing enzymes i.e., amylase and acid phosphatase. We utilized a proteomics technology (Native PAGE) to ascertain the role of theses enzymes in *C. ciliaris* tolerance against Ni metal stress. Our approach has generated data about stress-related responses in different tissues to check plant tolerance mechanism and expression of different proteins in the form of enzymes. Our results using *C. ciliaris* as a model plant suggest that the plant roots when exposed to Ni absorb the metal however the roots would also absorb more moisture to cope with the metal generated stress through some kind of dilution mechanism. With the increasing amount of Ni around the root Ni uptake also increases, but at some stage the roots start translocating the metal to the stem. As a result the stem accumulates more moisture to cope with the metal generated stress. Still at a higher metal concentration, the stem translocates accumulated Ni to the leaf tissues which also tries to cope with the metal generated stress through dilution by accumulating more moisture (Fig. 1). A second probable reason may be the growth phenomenon. Since the model plant was grown hydroponically, the Ni metal stress may cause water stress and stomatal closure (Singh and Pandey, 2011; Ghasemi *et al.*, 2013), while the roots are continuously absorbing water. One more possible reason is that *C. ciliaris* is a desert grass and its natural anatomy allows it to accumulate maximum water in its tissues that helps to do osmotic adjustments in stressed conditions (Nawazish *et al.*, 2006; Akram *et al.*, 2007). Few reports are available about Ni stress tolerance mechanism in *C. ciliaris*. We noted that *C. ciliaris* is competent to mobilizing and up taking Nickel metal from soil, its translocation from roots to shoots (Nasim *et al.*, 2015). The primary toxic impact of heavy metals is the reduced transpiration rate and shutting of stomatal apertures has earlier been reported (Nawazish *et al.*, 2006).

Total protein estimation in different tissues of *C. ciliaris* (Fig. 2) revealed that by increasing stress, protein expression decreased indicating that stress halts the cellular life of a plant (Sheng *et al.*, 2007). When plants are grown under stress conditions, their adaptation needs a change in metabolism to help to survive. One of the important adaptations is sugar metabolism (Thakur and Sharma, 2005). Starch metabolism is very sensitive to changes in the environment because during harsh conditions the main sugar stores i.e., glucose polymers hydrolyze to provide soluble sugars to the stressed plants. The plants then rapidly mobilize these sugars and secure protection against stress (Saeed and Duke, 1990). *C. ciliaris* under Ni metal stress showed different patterns of expression of amylase (Figs. 3–5). Two isoforms of amylase were expressed in leaves and the expression is more at 0 and 3 mg/L. It was seen that expression of amylase in leaves was less as compared to 0 mg/L (control) (Fig. 3). Amylase expression in leaves was less than in other tissues. Since leaf is the main site of photosynthesis to ensure availability of starch to meet plant need, amylase expression is decreased especially under the condition of stress. This possibility is supported by literature, where it has been reported that aerial or up ground tissues with low chloroplast concentrations such as stems often have higher amylase activity than leaves. Such results

are also reported earlier e.g., in pea petals and stems for example the amylase exhibit elevated levels of activity over leaf tissues (Saeed and Duke, 1990). Third main reason may be that amylase expression is tissue specific and sugar metabolism shows signaling of amylase expression (Gana *et al.,* 1998). In stem two isoforms appeared but at 10 and 20 mg/L Ni both isoforms were expressed more and at 3 mg/L the expression was very less (Fig. 4). Two isoforms of amylase appeared in roots and 1st isofrom showed more response against this 10 and 20 mg/L but 2nd isoform expressed more at 10 mg/L but lesser at 3 mg/L Ni (Fig. 5). Variation in amylase expression was also observed in different plant species under different metal stresses e.g. In *Sorghum bicolor* (L.) under Ni metal stress the activity of amylase is halted. Amylase activity increase at low concentration of Hg and Zn *Macrotyloma uniflorum* grown under Hg stress but its expression declined under Cd stress (Kuriakose and Prasad, 2008: Naji and Varadahally, 2016) similar increase in amylase activity was also seen in soyabean under Pb stress but on the other hand it was observed that Ni, Cu, Cd, Pb toxicity decrease amylase activity in germinating seeds (Sharma and Rama, 2005; Sethy and Ghosh, 2013). Amylase hydrolyses starch and release glucose and other sugars. In stress condition increased activity of amylase helps the plant to alleviate stress by enhancing food mobilization. And halted activity showed that the plant internal mechanisms are halted due to increased toxicity of metals. Sugars are not only used as food, there are also many other functions like many sugars are known to have antioxidant activity. Interactions between various pathways have also been suggested in stress relief. For example O-methyl inositol (OMI) during stress responses interact with sugars to alleviate stress (Elsayed *et al.,* 2013). Soluble sugars which are mobilized and accumulate in response to stress act as osmolytes to keep cell turgor normal and have the ability to protect cellular membranes and proteins from stress damage. These soluble sugars are called as osmoprotectants due to their indirect role in osmotic adjustment (Saeed and Duke, 1990).

Acid phosphatases (E.C. 3.1.3.2) are a group of enzymes that catalyze hydrolysis of a variety of mono-ester soil organic phosphates (phosphate esters) in acidic (pH 4–7) environment (Gilbert *et al.,* 1999) under stress conditions. These are widely distributed in plants and are involved in phosphate supply and metabolism (Bieleski, 1973; Ethan *et al.,* 2012; Onaga *et al.,* 2013). Four isoforms of Apase expressed in all tissues (leaves, stem, root) of *C. ciliaris* under Ni metal stress but their expression varied i.e., in leaves 0.3 and 10 mg/L $NiCl_2$ treatments showed almost similar expressions like 0 mg/L (control). However two new isoform was seen at 0.3 mg/L. 3 mg/L and 20 mg/L show same expression of Apase but it was less than control and other treatments (Fig. 8). Stem showed gradual increase in expression from 0 to 3 mg/L concentration and at 10 mg/L treatment resulted in decrease in expression then increase in expression was seen at 20 mg/L treatment (Fig. 9). In roots a gradual decrease from 0 to 3 mg/L treatments increase was seen from 3 to 20 mg/L Ni treatment (Fig. 10). Quantitative estimations also showed the same variation in activity A number of metal ions are known to serve either as supressors or activators and behaved differently in different plants e.g. In case of *Macrotyloma uniflorum* Apase activity and expression was decreased at increasing concentration of Hg, Cd but increased under Zn stress. It is also reported that in cucumber Zinc (Zn) strongly inhibit the activity of acid phosphatase than Mercury (Hg) similarly As (III) acts as inhibitor of Apase activity in some plants (Kapoor *et al.,* 2015; Naji and Varadahally, 2016). Acid phosphatase activity is also reported to increase in plants under Cd stress (Kapoor *et al.,* 2015). In *Sorghum bicolor* (L.) under Ni metal stress some isoform of Apase had lost and some new isoform produce that showed that Apase is trying to tolerate Ni stress in sorghum. Overall a decrease in activity was seen (Kuriakose and Prasad, 2008). In Soybean plants grown in culture media containing 20–100 mg/L Pb showed increased activity of acid phosphatase, α-amylase (Sharma and Rama, 2005) Cu and Pb toxicity decrease the activity of acid phosphatase, α-amylase in plants and specifically in germinating seeds (Kuriakose and Prasad, 2008). In our study Nickel (Ni) acts as activator of Apase. Studies described that normally plants use phosphate (P) for many metabolic processes but when plant is under stress, the phosphate (P) pool in the cell is disturbed and as a result phosphate (P) level turns down that adversely affects phosphorus supply in plants. In such conditions acid phosphatases become active to fulfill "P" deficiency (Amal *et al.,* 2009) Under heavy metal stress the expression of phosphatase increases that showed that the Pi thus produced controls the cellular and biochemical activities by binding with heavy metals and by increasing the biosynthesis of amino acids and proteins (Hasan, 2007). Phosphatases are involved in Pi delivery not only to NMPs and NDPs but also to NAD^+ sugars and proteins (Nikel *et al.,* 2013).

Conclusion

We found that nickel stress affected plant metabolism. Sugars and Pi act as signaling molecules and help the plants in stress alleviation. Carbohydrates play their role as osmoprotectant whereas Pi plays its role in mobilization and synthesis of amino acids. They also constitute Nucleic acids to prevent DNA damage and recover the normal cellular activities. These hydrolyzing enzymes (Apase, amylase) act as stabilizing enzymes in case of Ni stress in *C. ciliaris* and exist in different parts of the cell and generate different threshold tolerance against heavy metals. Qualitative and quantitative analyses of activity of Apase and amylase enzymes in *C. ciliaris* under Ni metal stress were in nice agreement. In future, we can develop strategies to induce expression of these enzymes and defense-related secondary metabolites in plants facing abiotic stresses enabling them to tolerate stress.

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