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EFFECT OF PHOSPHORUS DEFICIENT CONDITIONS ON EXPRESSION OF ACID PHOSPHATASE (APASE) IN WHEAT TISSUES.

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ABSTRACT

The effect of phosphorus stress on changes in acid phosphatase (APase) activities was studied in wheat seeds and shoots. Six-day old wheat seedlings were exposed to low phosphorus (LP) and high phosphorus (HP) stress by providing two different concentrations of 10µM and 1000µM KH_2PO_4 .The tissues (seeds and shoots) were separated . Treated and untreated extracts of seeds and shoots were compared. The Acid phosphatase activity was measured by using p-nitrophenyl phosphate (pNPP) as substrate, results revealed that APase activity was strongly induced under phosphorus stress. NATIVE PAGE APase activity also revealed a similar type of induction under phosphorus stress. SDS PAGE Analysis revealed that many polypeptides in treated extracts disappeared as compared to untreated extracts Based on these results; role of acid phosphatase (APase) was studied.

Keywords: Acid Phosphatase Activity, phosphorus, wheat.

INTRODUCTION

Phosphorus (P) plays key roles in many processes, including energy generation, synthesis of nucleic acids and membranes, photosynthesis, glycolysis, respiration, enzyme activation/inactivation, redox reaction, signaling, carbohydrate metabolism and nitrogen fixation(Vance et al, 2003). In most agricultural soils, organic P comprises 30-80% of the total P (Dalal, 1978).The largest fraction of organic P, approx 50%, is in the form of phytin and its derivatives (Tarafdar and Claassen,1988). For organic P sources in the soils to be used, they must be first hydrolyzed by acid phosphatases. Free soluble phosphate reserves play a vital role in energy transfer, metabolic regulation, and prot ein and nucleotide phosphorylation or important structural constituent of biomolecules like phytin bodies in ungerminated seeds, (Fincher, 1989; Ehsanpour and Amini, 2003). Acid phosphatases (APases) form a group of enzymes catalyzing hydrolysis of a variety of phosphate esters in acidic environments. APases are believed to increase orthophosphate (Pi) availability under phosphorous deficient conditions (Vance et al., 2003). Although, there are many controversial issues with acid P-ase accumulation and stress resistance, but, it is believed that high levels of acid P–ases can be beneficial to stressed plants (Ehsanpour and Amini, 2003). Enhanced excretion of acid P-ases under phosphorus stress has been documented in a number of plants(Tarafdar and Claassen, 1988; Vance, 2003). A positive relation was reported between root APases and phosphorus uptake in beans (Helal, 1990) and barley (Asmar *et al*., 1995). However, a negative relationship was also observed between APases and phosphorus uptake under low phosphorus stress in wheat (Barrett- Lennard *et al*., 1982). There is a prevailing hypothesis about the role of phosphatases in plants and its relationship with plant nutritional status i.e. plants adapted to P stress would present a high leaf or root APase activity as a sign of hydrolyzing and remobilizing Pi, by root secretion and/or leaf synthesis, making Pi more available to plant, from soil or other plant parts (Lee, 1988; Barrett – Lennard *et a*l, 1993). Some stress-induced proteins are produced in response to various environmental stresses such as salt and cold and are highly hydrophilic and remain soluble even after boiling (Close *et al*. 1989), a characteristic that has been termed "boiling stability" (BSPs) (Close et al., 1989;Jacobsen and Shaw 1989). Some proteins also show heat stability but exits in cells under non stress conditions. In order to find role of BSPs, this study was designed to examine the role of boiling stable APases under phosphorus deficient conditions.

MATERIALS AND METHODS

Seed germination and Growth Conditions

The seeds of HD 2894 (cultivar of *Triticum aestivum),* were procured from PAU Ludhiana, Punjab, India. Seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70 % (v/v) ethanol (Sharma et al., 2007). Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in Petri plates containing sterile filter sheets, moistened with water. The plates having seeds were incubated at 25 ±1°C in a seed germinator in darkness and allowed to grow for 4 days. Germinated seeds were used for the experiment. All the experiments were performed on 3M Whatman filter paper. Stress was applied by giving treatment of KH_2PO_4 (10µM and 1000µM) in two sets of petri plates. Extraction of seeds and shoots was done at different time intervals (0, 2, 8, 24, 48 hrs). Tissue Water content (TWC) was measured after imposing stress treatments. Fresh weights were determined within 2 h after collection. Dry weights were obtained after oven drying the samples for 72 h at 70^0 C. TWC was calculated from given equation:

TWC (%) = fresh weight-dry weight/fresh weight x 100.

ENZYME EXTRACTION

The enzyme was extracted from the tissue described by Sharma *et al* (2007). Briefly, the tissue was ground with mortar and pestle at 0-4^0C using 50 mM sodium acetate buffer (pH-5.0). The homogenate was centrifuged at 10000 rpm for 10 minutes and the supernatant collected and boiled for 10 minutes in boiling water bath, then immediately kept in ice for 5 minutes and again centrifuged at 10000 rpm for 15 minutes, supernatant collected and stored at -20^0C and the extracted enzyme was used for further analysis. Total protein content of boiling stable fractions was determined by the Lowry method using BSA as a standard.

Enzyme Activity

Acid phosphatase (A-Pase) activities were measured spectrophotometrically at 410nm in a final volume of 1 ml. In this experiment, p-nitrophenyl phosphate was used as the substrate, since the product after ester hydrolysis; p-nitrophenol can be easily measured. The degree of hydrolysis of the substrate was determined by spectrophotometric measurement of the p-nitrophenol liberated in the reaction. In alkaline solution, the p-nitrophenolate ion is bright yellow. The reaction mixture contained 200µg of protein. The reaction mixture contains enzyme extract (for 60µg), 100 µl buffer, 140 µl substrate and added distilled water and made volume up to 1 ml. The time of reaction was 10 min. The reaction was stopped by adding 1.5 ml of 0.25 M NaOH. The liberated p-nitrophenol (pNP) was determined at 410 nm and calibration curve of pNP prepared in the same conditions. One unit of phosphatase is equivalent to the amount of enzyme liberating 1µM of product per minute under assay conditions.

Electrophoresis and Zymogram Analysis

The supernatant was resolved on SDS-PAGE on 12% (w/v) polyacrylamide gel and visualized by Coomassie brilliant blue. The proteins were separated by a non-denaturing 12% polyacrylamide gel electrophoresis.

When the electrophoresis was complete, the gel was washed three times in 100 mM

Sodium acetate buffer (pH 5.0). Activity was visualized by incubating the gel in sodium acetate buffer having sodium -1-napthyl phosphate (0.1% w/v), diazo blue B (1%) and 10 mM $MgCl_2$. Reaction was terminated in acetic acid: methanol: water mixture (1:5:5 v/v).

RESULTS AND DISCUSSION

Acid P-ases in plants are classes of enzymes that display considerable heterogeneity with regard to their kinetics and functions (Duff *et al*. 1994). This complexity may contribute to conflicting reports regarding role of acid P-ases in phosphorus nutrition. Acid P-ases are reported to be induced under phosphorus (Pi) deficiency, in order to maintain certain level of Pi inside the cells (Barrett-Lennard *et al*., 1982; Olmos and Hellin, 1997). Plants respond to Pi deficiency depending on persistence of stresses with coordinated adaptations on multiple levels comprising well documented morphological, physiological and biochemical changes. An integral part of the plant response to Pi deficiency is the induction of both extracellular and intercellular APases (Sharma *et al*, 2007). Alternation in protein expression is an important part of the ability of the plant to respond to the environmental stresses. Phosphorus crisis is the second major problem for agriculturists, which necessitate studying details at gene, and protein level which are responsible for regulating and maintaining P level under the water prone condition. Thus, understanding the responses of plants to phosphorus stress is of importance not only for basic research but also an attractive target for improving such stress tolerance. In the present study, the effect of phosphorus stress was studied on boiling stable acid phosphatase (A-Pase) in *Triticum aestivum*.

Six-day old *Triticum aestivum* seedlings were exposed to low phosphorus (LP) and high phosphorus (HP) stress by providing two different concentrations of 10µM and 1000µM KH_2PO_4 for different time intervals viz. 0h,2h,8h,24h and 48h. Extracts were boiled to obtain boiling stable proteins. We used chemical assay, SDS and native gel electrophoresis to determine the role of APases in the phosphorus stress.

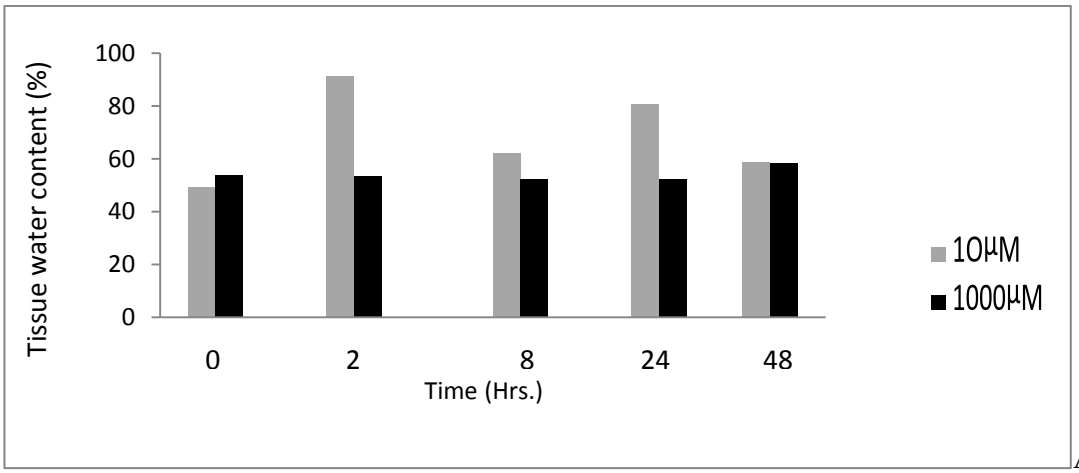
BIOCHEMICAL CHANGES IN SEEDS AT DIFFERENT TIME INTERVALS

Tissue water content (TWC) and protein content were measured in the seed extracts under LP and HP conditions. Under LP conditions, after 2h, a significant increase in TWC was observed (Fig 1-A). Under HP conditions, no significant difference was observed at different time intervals. Protein content (Fig.2-A) was significantly higher under HP conditions at 2h, 8h, 24h. However at 48h, protein content was higher under LP conditions. As compared to HP conditions, APase activity accompanied by specific activity was higher at 0h under LP conditions (Fig.2-B and 2-C). No significant difference was observed between two treatments at all stages. SDS PAGE was done for both treated and untreated enzyme extracts. SDS PAGE analysis revealed the induction of several polypeptides of different molecular weight in the seed extract taken at 0h, 2h, 8h, 24h and 48h of phosphorous stress imposed on seedlings. Many polypeptides in treated extracts disappeared as compared to untreated extracts. In treated seeds (Fig.4-B), protein bands of 16.04KDa and 25.9KDa were observed at all time intervals, however, bands of MW 32.5KDa and 45.8KDa were obtained at 0h(LP) . Zymogram Analysis and NATIVE PAGE analysis (5-A and 5-B) were carried out for seed extracts at different time intervals. When proteins containing APases from phosphorus stressed seeds were separated by Native PAGE Analysis, it revealed the induction of different boiling stable polypeptides of different molecular weight 14.42, 26.7, 58.07 and 77.6 KDa in enzyme extracts of seeds. Zymogram analyses were conducted for seed extracts taken at different time intervals and a high MW band was observed at 0h under LP conditions.

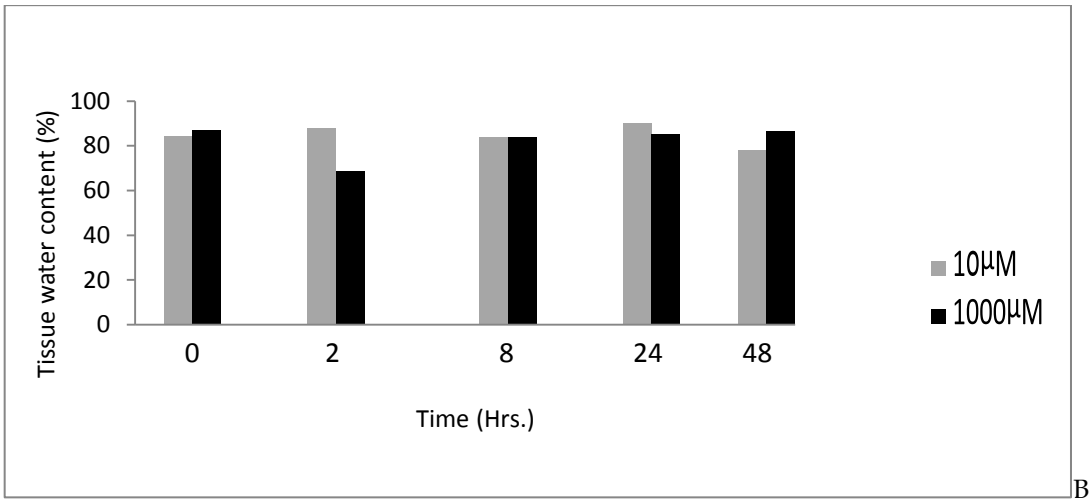
BIOCHEMICAL CHANGES IN SHOOTS AT DIFFERENT TIME INTERVALS

In shoots, no significant difference of Tissue water content (TWC) under LP and HP conditions for different time intervals was observed (Fig.1-B). Protein content (Fig.3-A) of extracts under HP conditions was higher at different time intervals from 0h to 24h than LP conditions. However, protein content in case of LP conditions was higher than HP conditions at 48h. As compared to HP conditions, APase activity accompanied by specific activity (Fig.3-B and 3-C) was significantly higher under LP conditions at 0h, 2 h and 24h. However, APase activity under HP conditions was higher than LP conditions at 48h. In SDS-PAGE analysis of treated shoots (Fig. 6), scarcely traceable bands were observed at all the time intervals. However, at 2h under HP conditions,

a band of MW 27.7 KDa was observed. No significant expression of APase in treated shoots was observed under NATIVE and Zymogram analysis.



A

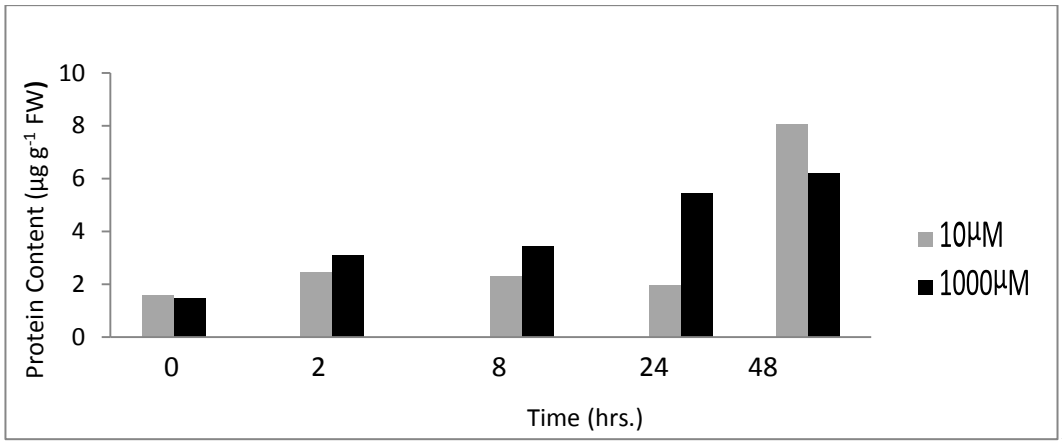


B

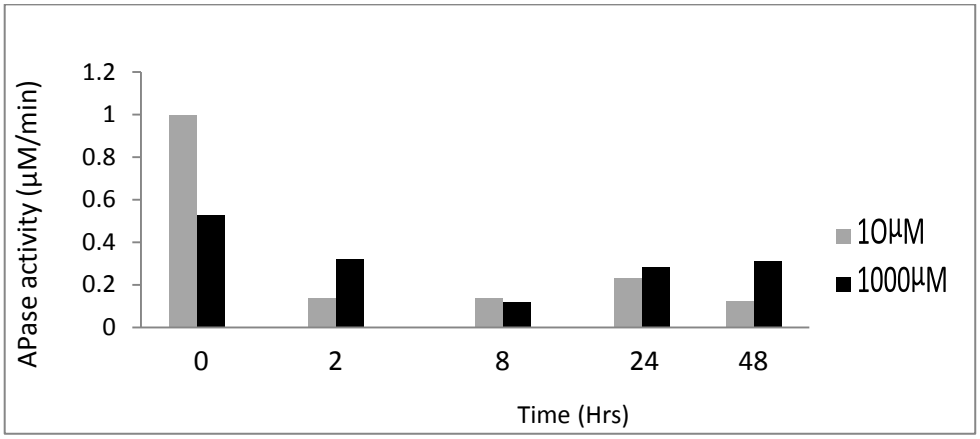
Figure 1. Tissue Water Content (TWC) of seeds (A) and shoots (B) under LP and HP conditions at different time intervals viz. 0h, 2h, 8h, 24h, 48h.

LP-Low phosphorus (10µM)

HP-High phosphorus (1000µM)



A



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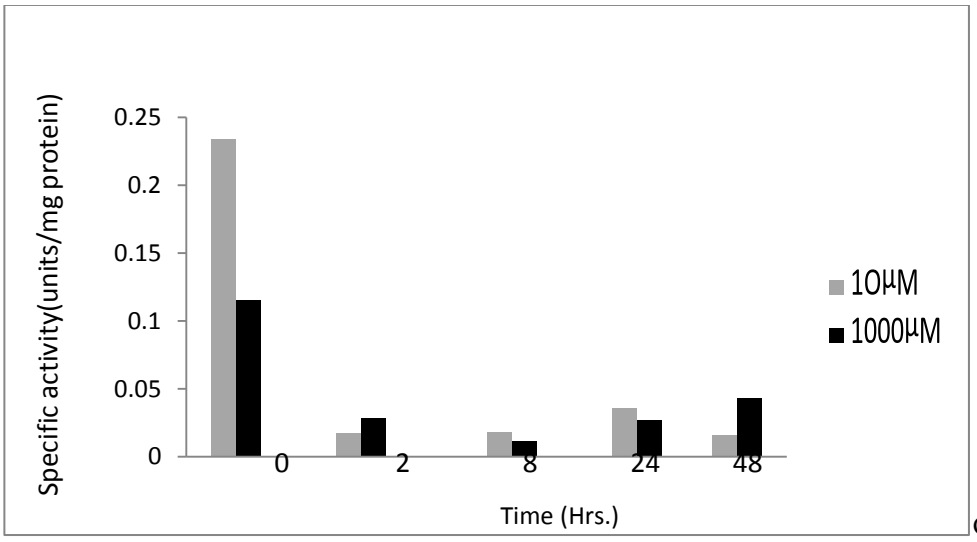
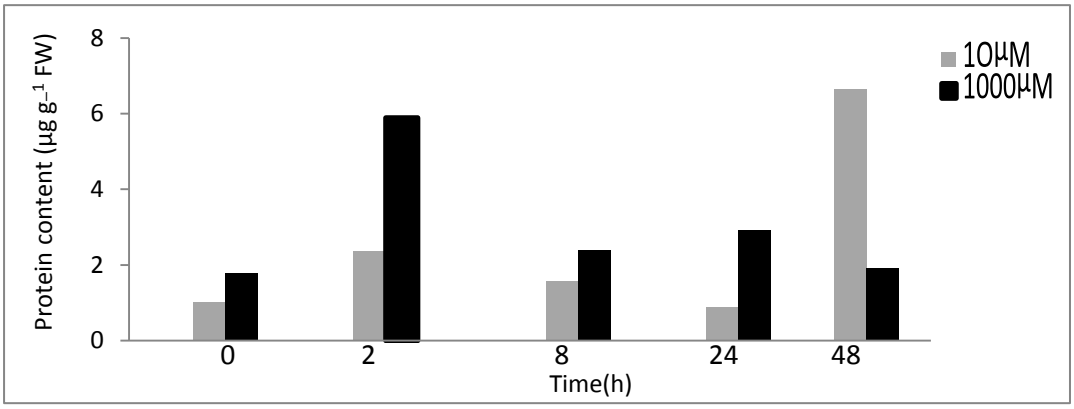
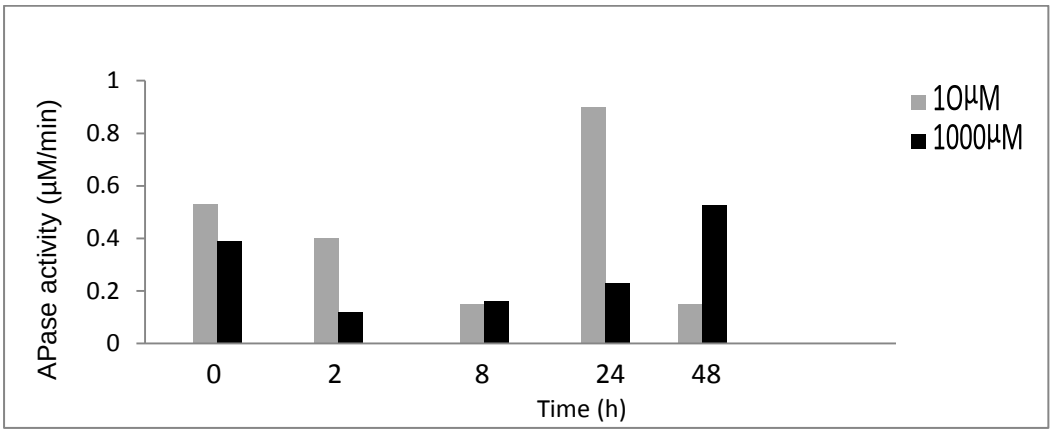


C

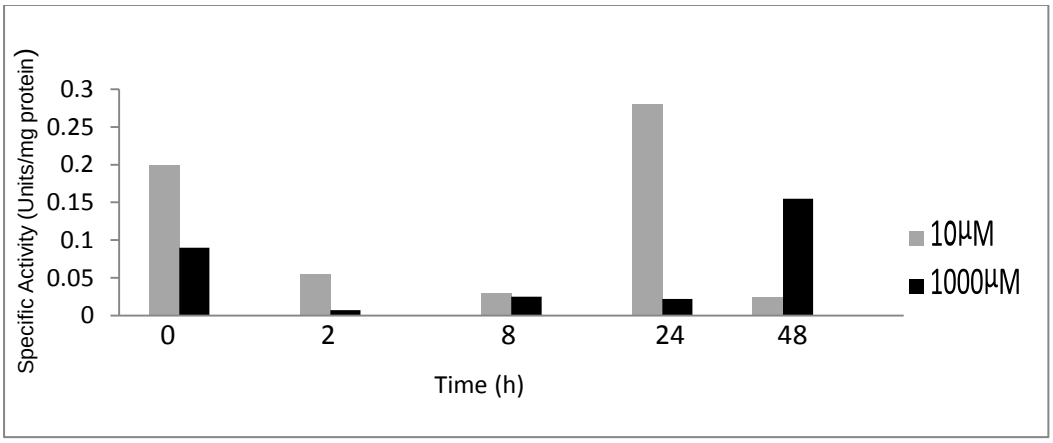
Figure 2. Protein Content (A), Acid phosphatase (APase) activity using para- nitrophenylphosphate (pNPP) as a substrate (B) and Specific Activity (C) under LP and HP conditions in Seeds of wheat. LP-Low phosphorus (10µM); HP-High phosphorus (1000µM).



A



B



C

Figure 3. Protein Content (A), Acid phosphatase (APase) activity using para-nitrophenylphosphate(pNPP) as a substrate (B) and Specific Activity (C) under LP and HP conditions in Shoots of wheat. LP-Low phosphorus (10µM); HP-High phosphorus (1000µM).

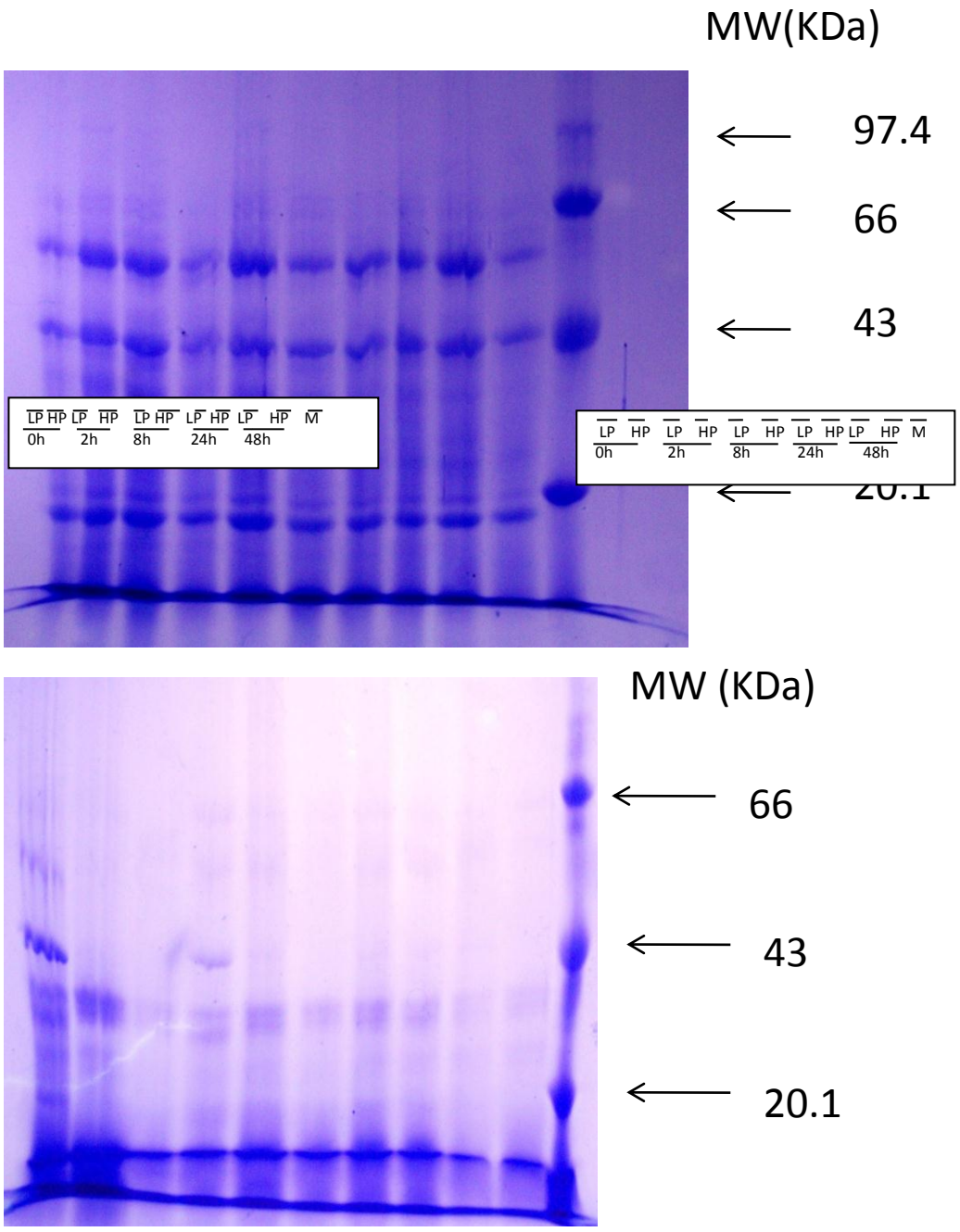


Figure 4. SDS-PAGE analysis of untreated seeds (A) and Treated seeds (B) under LP and HP conditions. Extracts obtained at different time intervals viz.0h, 2h, 8h, 24h, and 48h. Each lane loaded with 60µg of total soluble protein was resolved on 12% SDS – PAGE.

LP-Low Phosphorus (10µM KH_2PO_4)

HP-High Phosphorus (1000µM KH_2PO_4)

M- Marker

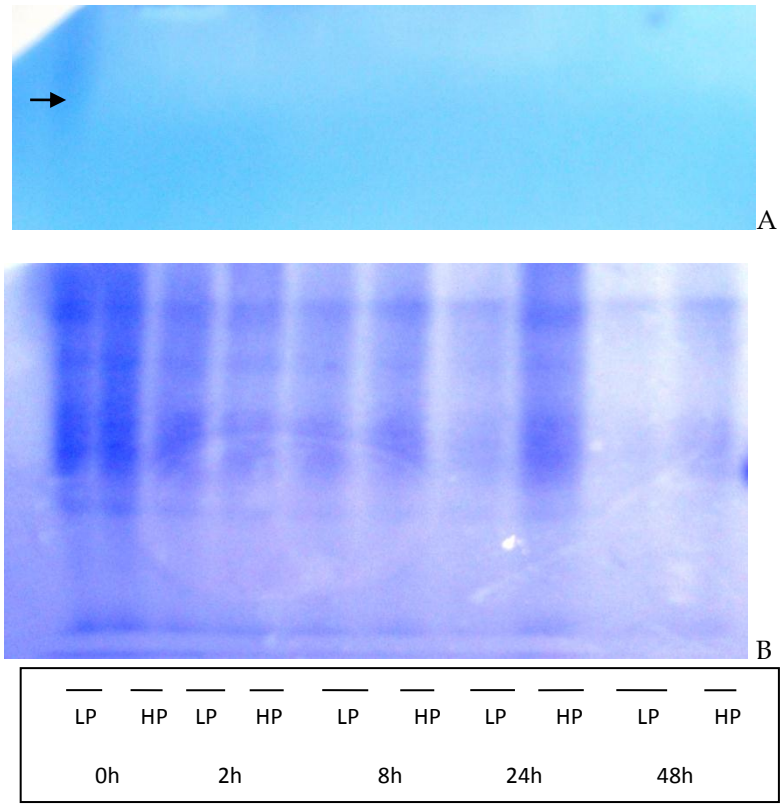


Figure 5. Zymogram analysis (A) and NATIVE PAGE analysis (B) of wheat seeds under LP and HP conditions. Extracts obtained at different time intervals viz. 0h, 2h, 8h, 24h and 48h. Each lane loaded with 60µg of total soluble protein was resolved on 12% gel.

→ Indicates high Molecular Weight (MW) band in Fig. A

LP-Low Phosphorus (10µM KH_2PO_4)

HP-High Phosphorus (1000µM KH_2PO_4)

REFERENCES

1. Asmar, F., T. Gahoonia and N. Nielsen. , 1995. Barley genotypes differ in activity of soluble extra cellular phosphates and depletion of organic phosphorous in the rhizosphere soil. Plant Soil, 172:117-122.
2. Barrett –Lennard, E. G., Robson, A.D. and Greenway, H., 1982. Effect of phosphorus deficiency and water deficit on phosphatase activities from wheat leaves. Journal of Experimental Botany, 33: 682-693.
3. Close, T. J., Fenton, K. A. A. and Chandler, P. M., 1989. A cDNA based comparisons of dehydration-induced proteins (dehydrins) in barley and corn. Plant Molecular Biol., 13: 95-108.
4. Dalal, R.C., 1978. Organic phosphorous. Advances in agronomy, 29: 83-117.
5. Duff, S. M.G., Sarnath, G. and Plaxton, W. C., 1994. The role of acid phosphatase in plant phosphorus metabolism. Physiologia Planatarum, 90:791-800.
6. Ehsanpour, A.A. and F. Amini, 2003. Effect of salt and drought stresses on acid phosphatase activities in alfalfa (Medicago *sativa* L.) explants under *in vitro* culture. Afr. J. Biotechnol., 2: 133-135.
7. Fincher, G. B., 1989. Molecular and cellular biology association with endosperm mobilization in germinating cereal grains. Annual Rev. Plant Physiol. Plant Mol. Biol, 40:305-346.

8. Helal, H. M., 1990. Varietal differences in root phosphatase activity as related to the utilization of organic phosphates. Plant and Soil, 123:161-163.
9. Jacobsen, J. V. and Shaw, D. C., 1989. Heat-stable proteins and Abscisic acid action in barley aleurone cells. Plant Physiol., 91*:* 1520-1526.
10. Lee, R. B., 1988.Phosphate influx and extracellular phosphatase activity in Barley roots and Rose cells. New Phytol., 109: 141-148.
11. Olmos, E. and Hellin, E. 1997. Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium- based in a salt adapted cell line of *Pisum sativum*. Journal of Experimental Botany, 48: 1529-1535.
12. Sharma, A.D.and Kaur, R., 2007. Drought- induced changes in acid phosphatase activities in wheat in relationship with phosphorus. Emir. J. Food Agric., 19(1): 31-38.
13. Tarafdar J. C.and Claassen, N., 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and micro organisms. Boil Fertise Soils, 5: 308-312.
14. Vance, C. P., Uhde- Stone,C. and Allan, D. L.,2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157: 423-447.