SHORT-TERM WATERLOGGING-INDUCED CHANGES IN PHOSPHATASE ACTIVITIES IN SHOOTS AND ROOTS OF SORGHUM SEEDLINGS: ROLE OF PHOSPHATASES DURING WATERLOGGING IN RELATION TO PHOSPHORUS

Arun Dev Sharma*, Neha Singh and Jagjeet Kaur Kang

Department of Biotechnology, Lyallpur Khalsa College, G T Road, Jallandhar-144001, Punjab, India

Received: February 07, 2005

Abstract. Short-term waterlogging stress-induced changes in phosphatase (P-ase) activities in relationship with phosphorus were studied in sorghum (*Sorghum bicolor*). Waterlogging was imposed by watering 3-day-old grown seedlings with water. The shoots and roots were harvested and P-ase activities were estimated. Significant waterlogging stress-induced increase in shoot and root P-ase activities coupled with low phosphorus level was observed at various time points. The shoot and root P-ase activities confer adaptation to plants under waterlogging stress.

Keywords: Phosphatase (acid and alkaline) activities, *Sorghum bicolor*, waterlogging

INTRODUCTION

Plants, like animals are obligate aerobes, but due to their inability to move, have evolved adaptation mechanisms that enable them to survive adverse conditions such as those occurring after heavy rain or waterlogging. The specific plant responses vary with many factors including plant species and genotype, age of plants, time, duration of waterlogging and tissue type (Kozlowski, 1997). Under waterlogging conditions, the activity of several metabolic pathways is reduced or altered (Kozlowski, 1997 and references therein). Shifts occur in carbohydrate, protein, organic acid and lipid metabolism. Exposures to waterlogging (min for 2 hrs) can have striking effects on

*Corresponding author; e-mail: goldi77700@yahoo.com

the composition and quantity of proteins and can increase or decrease the activities of the vital enzymes involved (Subbaiah and Sachs, 2003). The mechanisms by which flood-tolerant plants survive waterlogging are complex and involve interactions of morphological, anatomical and physiological adaptations (Hook, 1984). Metabolic adaptation to waterlogging is associated with several metabolic adjustments which lead to the modulation of different enzymes viz: glucose-phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, sucrose synthase etc. (for review, Sachs et al., 1996). Phosphatases (P-ases), classified either acid- or alkaline-, constitute an enzyme group, which is presumed to catalyze the hydrolysis of several organic phosphate-monoesters for the regulation and maintenance of soluble Pi, required for growth (Vance, 2003). The intracellular phosphatases, present in cytosol, plastids and vacuoles, are responsible for the Pi-hydrolysis from organic compounds during seed germination, favoring internal Pi mobilization and translocation from senescent tissues (Lee, 1988; Duff et al., 1994). There is prevailing hypothesis about the role of phosphatases in plants and its relation to plant nutritional status i.e. plants adapted to Pi stress would present high leaf or root P-ase 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 plants parts (Lee, 1988; Barret-lenard et al., 1993). Abiotic stresses like salt, osmotic and water stress, have been reported to increase acid or alkaline phosphatase activity by maintaining a certain level of inorganic phosphate in the plant cells (Olmos and Hellin, 1997). However, the variation that occurs in phosphatase activities during very early waterlogging conditions is poorly understood and information on physiological events involved in this process is scarce. Therefore, in the continuation of our recent study we aim to find out the role of P-ases against abiotic stresses (Sharma et al., 2004). In the present study, we report the role of phosphatases in sorghum plants under waterlogging stress.

MATERIALS AND METHODS

Seed germination and growth conditions

Seeds of *Sorghum bicolor* (L.) Moench were surface sterilized with 1% (w/v) mercuric chloride followed by 70 % (v/v) ethanol (Sharma et al., 2004). Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in petriplates containing sterile filter sheets, moistened with water. The plates were incubated at 37 ±1°C in a seed germinator in darkness and allowed to grow. Intact plants reaching the 3-day-old stage were used for the experiment. The petriplates with the seedlings were waterlogged in water according to Hsu et al. (2000). Each of the petriplates received 50 ml of water during the waterlogging period and the roots were completely submerged. At various waterlogging intervals (0,1,2,3,4 h), tissues (shoots and roots) from intact plants were harvested and pooled for further analysis.

Extraction and assay of acid and alkaline phosphatases

Both acid- and alkaline-phosphatases were extracted from the tissues according to Sharma et al. (2004). Briefly, the tissue was ground with mortar and pestle at 0-4°C using 50 mM sodium acetate buffer (pH 5.0) for acid phosphatase and 50 mM glycine NaOH buffer (pH 10.5) for alkaline phosphatase. The homogenate was centrifuged at 8000 g for 15 min, and the supernatant was collected. Phosphatase activities were assayed by measuring the amount of p-nitrophenol produced. Phosphatase activities were measured spectrophotometrically at 410 nm in a final volume of 1 ml. The reaction mixture contained 300 µl of enzyme extract, 0.05 M buffer [Sodium acetate (pH5.0) for acid phosphatase and Glycine-NaOH (pH10.5) for alkaline phosphatase], 0.1 M NaCl and 0.2 mg/ml BSA, with 5 mM para-nitrophenylphosphate (pNPP) as a substrate. 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 (U) of phosphatase (acid and alkaline) is equivalent to the amount of enzyme liberating 1 µmol of product per min under assay conditions.

Extraction and assay of total phosphorous (P)

For total soluble Pi determination, only fresh tissue samples were used. They were homogenized with 5 ml of 10% (v/v) $HClO_4$ at 4 ^oC. After centrifugation at 5000 g at 4 ^oC, the supernatant was collected for Pi analysis. Pi content of the resultant soluble fraction was measured by the formation of a blue molybdenum complex according to Tsvetkova and Georgiev (2003). Briefly, appropriate aliquots were mixed with 5 ml 0.1 M acetate buffer pH 4.0, 0.5 ml 1% (w/v) ammonium molybdate in 0.05 N H_2SO_4, 0.5 ml 1% (w/v) Na-ascorbate. To avoid the delay in the conversion of the blue color of molybdate- phosphoric complex, 1 mM $CuSO_4.5H_2O$ was added into the ascorbate solution. The blue color of the complex was obtained after 10 min and the absorption was determined using spectrophotometer at 620 nm.

Statistical analysis

Data obtained were subjected to ANOVA and student's *t*-test.

RESULTS AND DISCUSSION

Physiological conditions under waterlogging stress

Under waterlogging stress, as compared to the control seedlings, no significant differences in shoot or root fresh weight as well as dry weight were observed (data not shown). When compared to the control, shoot as well as root lengths were hardly

affected under waterlogging treatment. Thereby, no significant change in root to shoot ratio was observed (data not shown).

Phosphatase activities (acid- and alkaline-) under waterlogging conditions

Phosphatases (P-ases) are reported to be induced under phosphorous (Pi) deficiency conditions during abiotic stresses (salt, osmotic and water) in order to maintain certain level of Pi inside the cells (Barrett-Lennard et al., 1982; Olmos and Hellin, 1997). However, the precise role of phosphatases during early waterlogging is still not known. Our studies revealed a significant enhancement in phosphatase activities (both acid- and alkaline-) in a spatial and temporal manner under waterlogging conditions. Under control conditions, shoot acid-phosphatase activity remained relatively unchanged during the 4 h of waterlogging treatment (Fig. 1A). However, imposition of waterlogging treatment resulted in a significant linear increase in acid phosphatase activity from 2 h to 4 h, after the start of waterlogging treatment. Generally, a significant increase in shoot acid phosphatase activity was observed under waterlogging treatment at each time interval, except at 1 h, where, no significant difference in the activity was observed. Similar to acid phosphatase, alkaline phosphatase activity under non-waterlogging control conditions revealed no significant differences at various time points (Fig. 1B). However, under waterlogging treatment, a marked increase in alkaline phosphatase activity was observed from 1 h to 2 h, thereafter, it attained constant levels. Like shoot P-ase activities, as compared to control, a significant increase in root acid phosphatase activity was observed from 1 h to 2 h of waterlogging treatment, thereafter revealing constant levels (Fig. 2A). On the contrary, alkaline phosphatase activity increased linearly after 1 h of waterlogging treatment (Fig. 2B). Between both tissues (shoot and root), roots depicted higher activities (both acid- and alkaline-) under waterlogging conditions, indicating tissue specific induction of P-ases. Similar observation on tissue specific variations in the different mechanisms of waterlogging stress tolerance in roots and shoots have been reported in Arabidopsis (Ellis *et al*., 1999). Overall, results obtained suggest that the increase in P-ase activities in both tissues may be due to the fact that under waterlogging conditions, the delivery of phosphate (Pi) is impaired, thus, resulting in activation of the cellular phosphatases. Phosphatases release soluble phosphate from its insoluble compounds inside or outside of the cells, thus modulating the osmotic adjustment by free phosphate uptake mechanism. Nevertheless, Olmos and Hellin (1997) observed that acid phosphatases are known to act under salt and water stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force. Moreover, our earlier study (Sharma et al., 2004) suggested that phosphatases are modulated by salt stress and to the application of phytohormones like abscisic acid (ABA) or gibberellin (GA_3). In the present study, we found that P-ases are also induced under waterlogging conditions, suggesting functional overlap

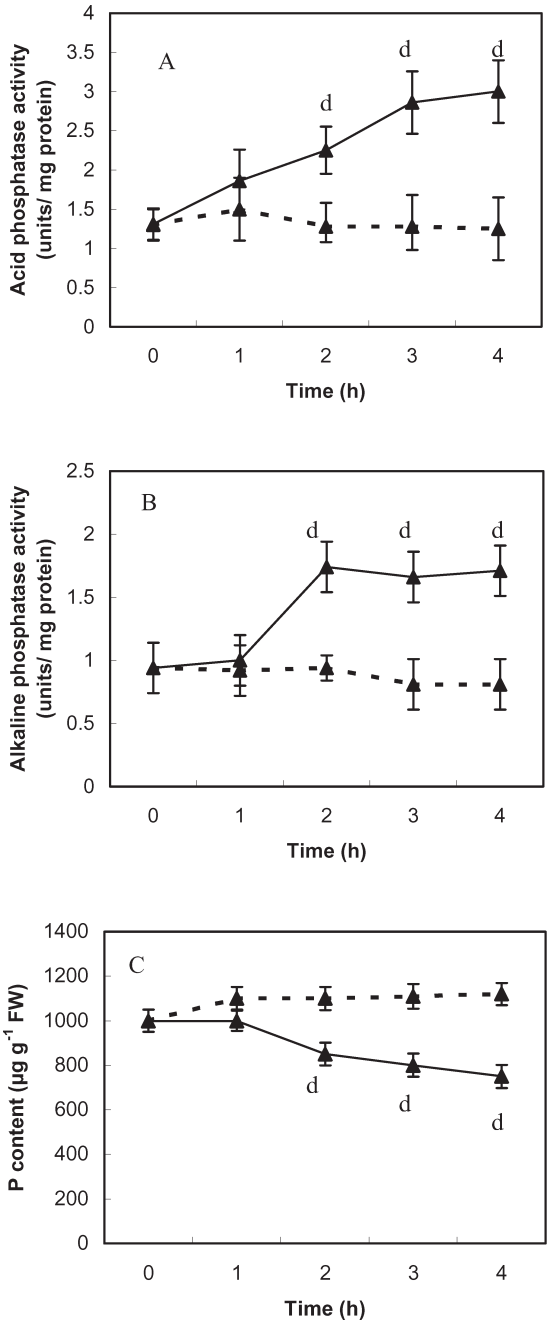

Fig. 1. Waterlogging-induced changes in shoot acid phosphatase activity (A), alkaline phosphatase activity (B) and phosphorus content (C) in sorghum seedlings during a 4-h period. Data shown are average ± SD of three replicates. Values in common letter are significantly different (P≤0.05 d) with respect to the non-flooded control.

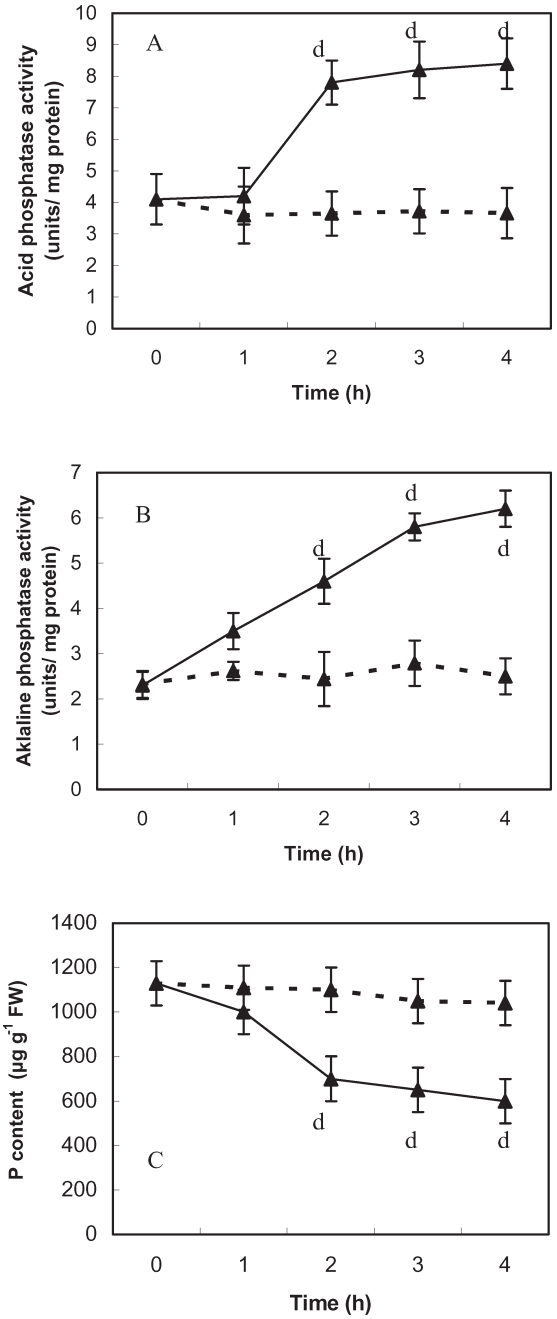

Fig. 2. Waterlogging-induced changes in root acid phosphatase activity (A), alkaline phosphatase activity (B) and phosphorus content (C) in sorghum seedlings during a 4-h period. Data shown are average ± SD of three replicates. Values in common letter are significantly different (P≤0.05 d) with respect to the non-flooded control.

of phosphatases between different stresses (like cold, salinity and waterlogging). It is likely that waterlogging stress response may consist of different set of genes, with different regulatory and functional properties in the response, and some of these genes could be shared by other stress responses.In Arabidopsis, AtMYB2 a transcription factor of the Myb family, has previously been shown to be induced by drought, salt stress and exogenous ABA (Urao et al., 1993). Later studies show that AtMYB2 is also induced by waterlogging, with mRNA levels, peaking after 2-4 h of waterlogging (Hoeren et al., 1998).

Phosphatases in relationship with phosphorous (Pi) under short-term waterlogging stress

Depending on the persistence of stresses, plants respond to Pi deficiency 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 P-ases. To understand whether waterlogging-induced increases in phosphatase activities in shoots and roots were by a low level of Pi or Pi deficiency, the effect of waterlogging treatment on the level of Pi was determined at different time intervals. As indicated in Fig. 1C and 2C, by comparison to control, Pi level of shoots and roots was strongly reduced after 1 h of waterlogging, coupled with a significant increase in P-ase activities. In contrast to earlier reported results, indicating that phosphatase activities are independent of phosphate levels (Szabo-Negy et al., 1992; Fernandez and Ascencio, 1994), induction of P-ases activities in the present study is likely to be caused by the low level of Pi under waterlogging treatment. These results suggest a dependence of the enzyme level on Pi availability as a signal for induction of P-ase activities in sorghum. Similar reports on the increase in P-ase activities in inverse reproportion to the low level of Pi has been demonstrated in numerous species and plant parts viz: wheat leaves and roots (Barret-Lennard et al., 1982; Mclachlan and Demarco, 1982), maize leaves (Elliot and Lauchli, 1986); sorghum roots (Furlani et al., 1984) and common beans roots (Helal, 1990). The expression of higher P-ase activities in both tissues (shoots and roots) is suggestion of its global role in enhancing Pi availability and possibility recycling of organic Pi compounds.

In conclusion, our present study results suggest that under short-term waterlogging stress P-ases play very important role to sustain the adverse environmental conditions in correlation to low phosphorus levels. In addition, results provide valuable information necessary to develop screening marker tools for selecting lines with tolerance to flooding stress and phosphorus status, thus improving filed emergence and survival percentage of plants.

Acknowledgement: We would like to thank management committee, Lyallpur Khalsa College, Jallandhar, Punjab, India, for providing financial assistance.

References

Barrett-Lennard, E.D., A.D. Robson, H. Greenway, 1982. Effect of phosphorus deficiency and water deficit on phosphatase activities from wheat leaves. J Expt. Bot., 33, 682-693.

de Bruxelles, G.L., W.J. Peacock, E.S. Dennis, R. Dolferus, 1996. Abscisic acid induces the alcohol dehydrogenase gene in Arabidopsis. Plant Physiol., 111, 381-391.

Duff, S.M.G., G. Sarath, W.C. Plaxton, 1994. The role of acid phosphatase in plant phosphorus metabolism. Physiol. Plantarum, 90, 791-800.

Elliot, G.C., A. Lauchli, 1986. Evaluation of an acid phosphatase assay for detection of phosphorus deficiency in leaves of maize (*Zea mays* L.). J. Plant Nutr., 9, 1469-1477.

Ellis, M.H., E.S. Dennis, W.J. Peacock, 1999. Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. Plant Physiol., 199, 57-64.

Fernandez, D.S., J. Ascencio, 1994. Acid phosphatase activity in bean and cowpea plants grown under phosphorus stress. J. Plant Nutr., 17, 229-241.

Furlani, A.M.C., R.B. Clark, J.W. Maranville, W.M. Ross, 1984. Root phosphatase activity of sorghum genotypes grown with organic and inorganic sources of phosphorus. J. Plant Nutr., 7, 1583-1595.

Helal, H.M., 1990. Varietal differences in root phosphatase activity as related to the utilization of organic phosphates. Plant and Soil, 123, 161-163.

Hoeren, F., R. Dolferus, Y. Wu, W.J. Peacock, E.S. Dennis, 1998. Evidence for a role for AtMYB2 in the induction of the arabidopsis alcohol dehydrogenase (*ADH1*) gene by low oxygen. Genetics, 149, 479-490.

Hook, D.D., 1984. Adaptations to flooding with fresh water. In: Kozlowski TT, ed. *Flooding and plant Growth*. Academic Press, Orlando, FL, 265-294.

Hsu, F.H., J.B. Lin, S.R. Chang, 2000. Effects of waterlogging on seed germination, electric conductivity of seed leakage and developments of hypocotyls and radicle in sudangrass. Bot. Bull. Acad. Sin., 41, 267-273.

Kozlowski, T.T., 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monograph, 1, 1-23.

Lee, R.B., 1988. Phosphate influx and extracellular phosphatase activity in barley roots and rose cells. New Phytol., 109, 141-148.

Mclachlan, K.D., D.G. Demarco, 1982. Acid phosphatase activity of intact roots and phosphorus nutrition in plants: III. Its relation to phosphorus garnering by wheat and a comparison with leaf activity as a measure of phosphorus status. Aust. J. Agric. Res., 33, 1-11.

Olmos, E., E. Hellin, 1997. Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium based in a salt-adapted cell line of *Pisum sativum*. J. Expt. Bot., 48, 1529-1535.

Orchard, P.W., R.S. Jessop, 1984. The response of sorghum and sunflower to short term waterlogging. I. Effects of stage of development and duration of waterlogging on growth and yield. Plant Soil**,** 81, 119-132.

Tsvetkova, G.E., G.I. Georgiev, 2003. Effects of phosphorous nutrition on the nodulation, nitrogen fixation and nutrient use efficiency of *Bradyrhizobium japonicum*-soybean (*Glycine max* L. Merr.) symbiosis. Bulg. J. Plant Physiol., special issue, 331-335.

Sachs, M.M., C.C. Subbaiah, I.N. Saab, 1996. Anaerobic gene expression and flooding tolerance in maize. J Expt. Bot., 47,1-15.

Sharma, A.D., M. Thakur, M. Rana, K. Singh, 2004. Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in *Sorghum bicolor* (L.) Moench seeds. African J. Biotechnol., 3, 308-312.

Subbaiah, C.C., M.M. Sachs, 2003. Molecular and cellular adaptations of maize to flooding stress. Annals of Bot. 90,119-127.

Szabo-Nagy, A.G., G. Galiba, E. Erdei, 1992. Induction of soluble phosphatases under ionic and non-ionic osmotic stress in wheat. J. Plant Physiol., 140, 329-633.

Urao, T., K. Yamaguchi-Shinozaki, S. Urao, K. Shinozaki, 1993. An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell, 5,1529-1539.

Vance, C.P., C. Uhde-Stone, D.L. Allan, 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol., 157, 423-447.