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## Short Communication

# Drought-induced changes in acid phosphatase activities in wheat in relationship with phosphorus

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**Abstract:** The effect of drought stress on changes in acid phosphatase (APase) activities in relationship with phosphorus was studied in wheat embryos and endosperm. Drought stress was imposed by withholding water irrigation to the 12 h germinated seeds. The tissues (embryos and endosperm) were separated and APase activity was estimated. After drought stress, a significant increase in embryo and endosperm APase activity was observed. Native-PAGE APase activity also revealed a similar type of induction under stress conditions. Imposition of drought stress caused no significant change in phosphorous level in endosperm; however, a significant decrease in the embryos phosphorus level was observed after 6 h of stress, indicating tissue specific key role of APase activity under drought stress in maintaining Pi level.

**Key words**: Acid phosphatase activiy, drought, wheat.

# تغيرات مستحثة بواسطة الجفاف في أنشطة الفوسفاتيز في القمح وعلاقتها بالفسفور

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**الملخص:** تم دراسة تأثير الجفاف على التغيرات في أنشطة حمض الفوسفاتيز(APase) وعلاقتها بالفوسفات في أجنة و إندوسبرم القمح. تم إحداث الجفاف بمنع الري بالماء عن البذور النابتة لمدة 12 ساعة. تم فصل الأنسجة (الأجنة والإندوسبرم) ثم تقدير حمض الفوسفاتيز. بعد إحداث الجفاف لوحظت زيادة معنوية في أنشطة حمض الفوسفاتيز في الجنين و الإندوسبرم، كما كشفت أنشطة الفوسفاتيز بواسطة تقنية Native- PAGE عن مستحثات مشابهة تحت ظروف ضغط الجفاف. الجفاف سبب تغير معنوي في مستوى الفوسفور في الإندوسبرم، غير أن حدوث نقص معنوي في مستوى الفوسفور حدث بعد 6 ساعات من ضغط الجفاف، مما يدل على وجود دور أساسي لأنشطة حمض الفوسفاتيز تحت ضغط الجفاف في حفظ مستوى الفوسفور.

**الكلمات المفتاحية:** نشاط حمض الفوسفاتيز،الجفاف، القمح.

## Introduction

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). 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 phosphatases. Free soluble phosphate reserves play a vital role in energy transfer, metabolic

regulation, and protein and nucleotide phosphorylation or important structural constituent of biomolecules like phytin bodies in ungerminated seeds, (Finche, 1989; Ehsanpour and Amini, 2003). Enhanced excretion of APases under P-deficient conditions has been documented in a number of plants (Tarafdar and Claassen, 1988; Vance et al. 2003). A positive relation was reported between root APases and phosphorous uptake in beans (Helal, 1990) and barley (Asmar et al., 1995). However, a negative relationship was also observed between APases and phosphorous uptake under low phosphorous stress in wheat (Barret-Lennard et al., 1982). Salt and osmotic stresses have also been reported to increase APase activity (Barret-Lennard et al., 1982; Szabo-Nagy et al., 1992). However, it has also been demonstrated that induction of APase under osmotic and salt stress was not accompanied by a decrease in Pi level. Hence, the role of APases against abiotic stresses is still a matter of conjuncture. Wheat is one of the most important crops in arid and semi arid areas worldwide and is sensitive to drought and temperature stress. Therefore, this study was designed to examine the role of APase under drought stress in relationship with Pi in wheat.

## Materials and Methods

Seed germination and growth conditions

The wheat seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70 % (v/v) ethanol (Sharma et al., 2004). The seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in Petri dishes 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 for 12 h. Intact 12 h germinated seeds were used for the experiment. Drought experiments were performed on 3 M Whatman filter paper. The seeds were shifted to new Petri dishes having filter paper without any water irrigation. At various time intervals (0, 2, 6 h), tissues (embryos and endosperm) were harvested and pooled for further analysis.

Enzyme extraction

The enzyme was extracted from the tissues as described by Sharma et al. (2004). Briefly, the tissue was ground with a pestle and mortar at 0-4°C using 50 mM sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 8000 g for 15 min, and the supernatant collected. Phosphatase activities were assayed by measuring the amount of p-nitrophenol produced. Phosphatase activities were measured spectrophoto-metrically 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)], 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 is equivalent to the amount of enzyme liberating 1 µmole of product per min under assay conditions.

Extraction and assay of phosphorous (Pi)

For determination of total soluble Pi, only fresh tissue samples were used, and were homogenized with 5 ml of 10% (v/v) $HClO_4$ at 4 ºC. After centrifugation at 5000 g at 4ºC, the supernatant was collected for analysis of Pi. The Pi content of the resultant soluble fraction was measured by the formation of a blue molybdenum complex as described by 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.

Electrophoresis

Soluble proteins were extracted as described above. The proteins were separated by a non-denaturing 12% polyacrylamide gel electrophoresis. When the electrophoresis was complete, the gel was washed three times in 50 mM sodium acetate buffer (pH 5.0). Activity was visualized by the diazo-dye method using 1-napthyl phosphate (0.1% w/v) as the substrate in the same buffer and diazo green as the stain. Proteins were detected by staining with Coomassie Blue R-250.

**Statistical analysis**

The data obtained was submitted to analysis of variance using Duncan's test at $P \leq 0.05$.

**Results and Discussion**

Phosphatase activity under drought conditions

APases in plants are a 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 the role of APA in phosphorous nutrition. APases are reported to be induced under phosphorous (Pi) deficiency, in order to maintain a certain level of Pi inside the cells (Barrett-Lennard et al., 1982; Olmos and Hellin, 1997). However, the precise role of phosphatases during early drought is still not known. Our studies revealed a substantial enhancement in acid phosphatase activities in a temporal manner under drought stress. We used both chemical assay and native gel electrophoresis to determine the role of APases in the water stress. As compared to 0h, a significant increase in APase activity was observed up to 6 h of stress in embryos (Figure 1B). Like embryo APase activity, in endosperm a significant increase in activity was observed from 0 h to 6 h of stress treatment, as compared with 0 h. Thereafter constant levels (Figure 2B) was revealed. Overall, results obtained suggest that the increase in APase activities in both the tissues may be due to fact that under conditions of drought, delivery of phosphate (Pi) is impaired, thus resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds inside or outside of the cells, thereby modulating osmotic adjustment by a free phosphate uptake mechanism. Olmos and Hellin (1997) also 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. When proteins containing APases from water stressed embryos were separated by Native PAGE, a strong APase staining band was markedly expressed at 2 and 6 h of drought stress (Figure 1A), which could not be seen in untreated protein preparations. Similarly, in endosperm (Figure 2A), a strong band was observed at 2h of drought treatment, which disappeared thereafter.

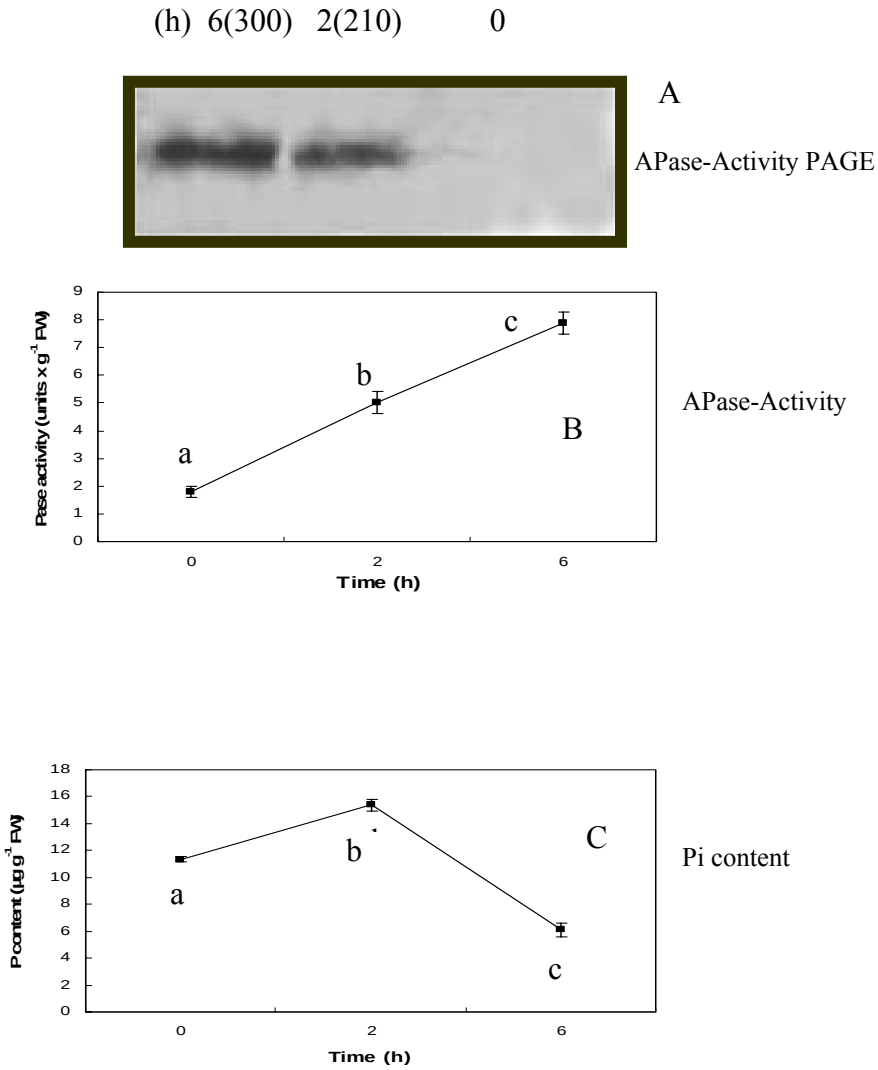


**Figure 1. Drought-induced changes in embryo APase activity stain of native PAGE (A), APase enzyme activity (B) and phosphorus content (C) in wheat seedlings during a 6-h period. Data shown are average ± SD of three replicates. Values with different letters (a, b, c) indicate significant difference at P≤ 0.05. Numerical values as shown in Panel A of this figure and subsequent, indicates relative band intensities, which were determined using Gel Visualization, Documentation and Analysis system (Bio-Rad, USA). Numerical comparisons are only valid within panels and cannot be made between panels. Each lane loaded with 60µg of total soluble proteins was resolved on 12% native PAGE.**

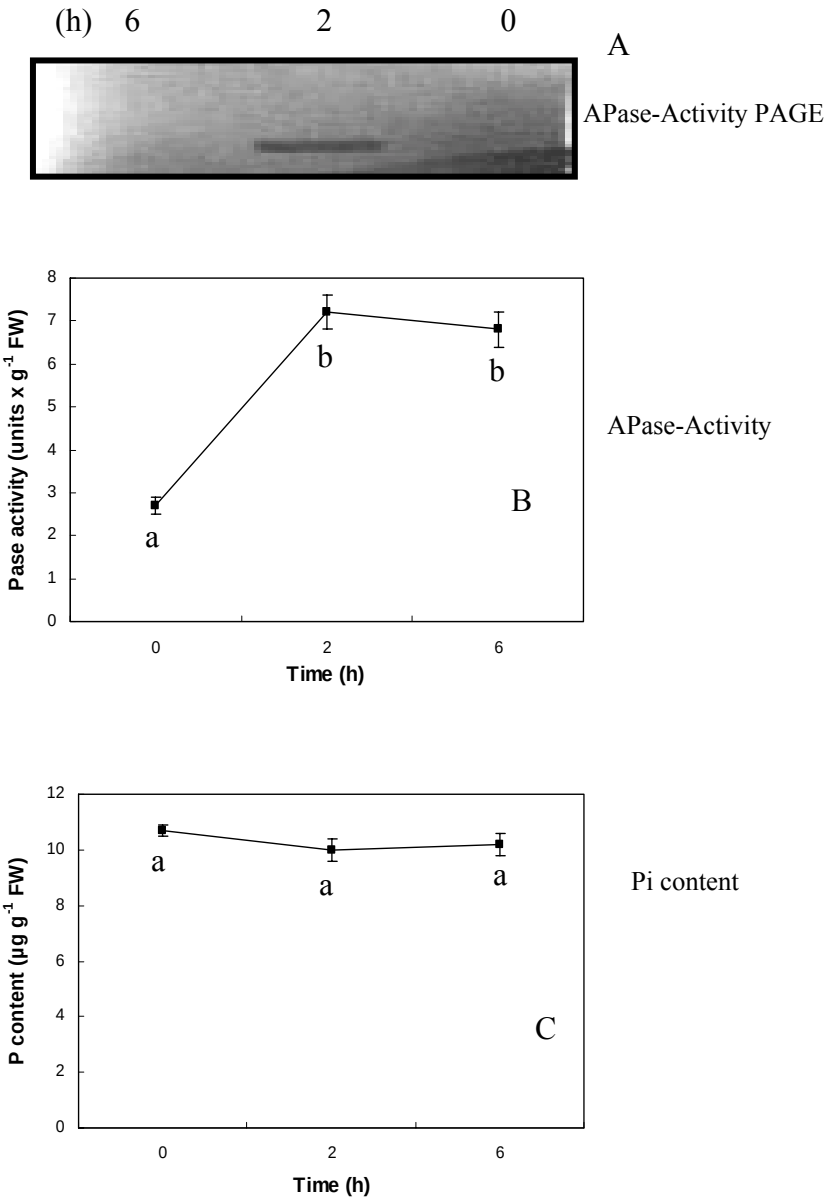


**Figure 2. Drought-induced changes in endosperm APase activity stain of native PAGE (A), APase enzyme activity (B) and phosphorus content (C) in wheat seedlings during a 6-h period. Data shown are average ± SD of three replicates. Values with different letters (a,b,c) indicate significant difference at P≤ 0.05.**

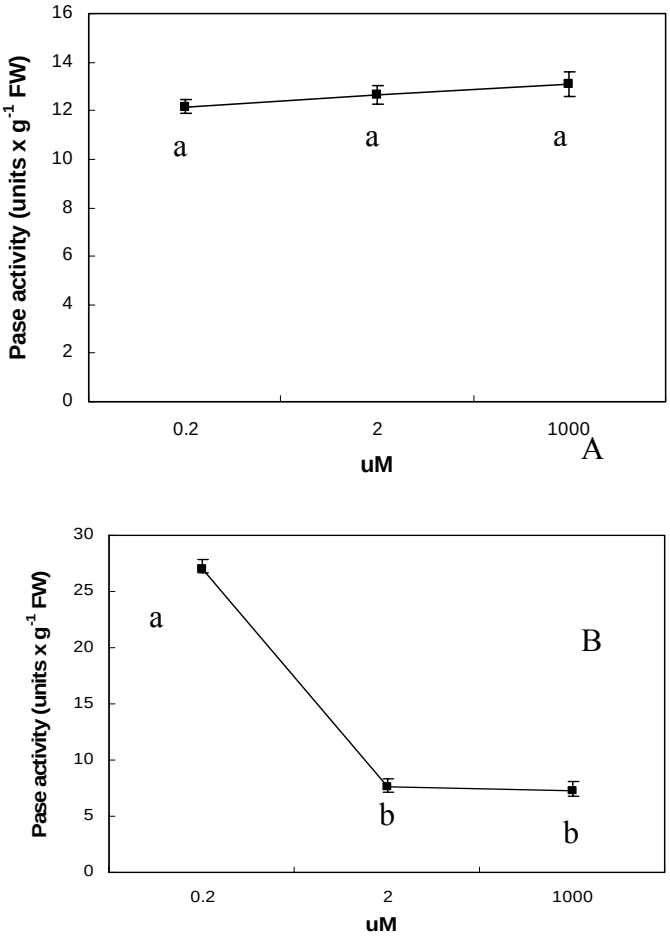


**Figure 3. The effect of phosphorous stress ($KH_2PO_4$, (0.2 µM, 2.0 µM and 1000 µM)) on endosperm APase activity (A) and embryo APase activity (B). APase activity was assayed after 6 h of Phosphorous stress. Data shown are average ± SD of three replicates. Values with different letters (a,b,c) indicate significant difference at P≤ 0.05.**

Phosphatases in relationship with phosphorous (Pi) under drought stress

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. To understand whether drought-induced increase in phosphatase activities in embryos and endosperm were caused by a low level of Pi or Pi deficiency, the effect of drought treatment on the level of Pi was determined at different time intervals. As indicated in (Figure 1C), with comparison to 0 h, the Pi level was dramatically increased at 2 h of drought, but it decreased substantially at 6 h of drought treatment. However, the APase activity (Figure 1A) increased linearly from 0h to 6h. So we have observed both positive and negative relations between enzyme activity and Pi concentration at 2h and 6h. Similar positive and negative correlations were also reported in scots pine trees (Kolari and Sarjala, 1995). However, in endosperm, Apase activity was appeared to be independent of Pi level (Figure 2C). These results are in general agreement with those reported by Szabo-Negy et al. (1992) and Fernandez and Ascencio (1994). To further determine the role of APase activity,

phosphorous stress was imposed by supplying different concentrations of $KH_2PO_4$ (Figure 3A and B). In endosperm, no significant effect on APase activity was observed; however, in embryos low phosphorous availability increased the APase activity dramatically. These results suggest a dependence of the enzyme level on Pi availability as a signal for induction of APase activities in wheat. Similar reports on the increase in APase activities in inverse relationship with 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 bean roots (Helal, 1990).

To conclude, the present study suggests that APases may be playing very important roles under drought stress in order to sustain adverse environmental conditions in correlation with low phosphorus levels. The expression of higher APase activities in both tissues is a suggestion of its global role in enhancing Pi availability and possibly in recycling of organic Pi compounds. In addition, the results provide valuable information to develop screening marker tools for selecting lines with tolerance to drought stress and phosphorus status, thus improving the filed emergence and survival percentage of plants.

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