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Short Communication

Expression analysis and Cloning of TaPase phosphatase gene in wheat (*Triticum aestivum*)

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Abstract: The present study was designed to clone gene encoding phosphatase and its regulation by abiotic stress treatments. The *genomic* DNA was isolated, *TaPase* DNA was amplified and cloned from wheat (*Triticum aestivum*). Expression studies by northern blot analysis was carried out by isolating RNA from tissues under different abiotic stress treatments. Clustal-W analysis of *TaPase* (assession no EU. 723832) with all the reported phosphatase genes revealed that codon encoding tryptophan (TGG) residue was conserved. Northern blot analysis revealed that under abiotic stress treatments like osmotic, salt and heat, the expression of *TaPase* was induced, indicating modulation of *TaPase* under stress treatments. Based upon these results, a possible physiological role of *TaPase* in wheat is discussed.

Keywords: Abiotic stresses, phosphatases, *TaPase*, wheat

التحليل للجينات والاستنساخ من الفوسفاتيز فى نبات القمح

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الملخص:صممت هذه التجربة لاستنساخ الجين المعلم بالفوسفاتيز والمنظم من قبل معالجات الإجهاد اللا إحيائية . تم عزل الحمض النووي الجيني (*TaPase*) وقد تم تضخيم الحمض النووي (*TaPase*) واستنساخه من نبات القمح . وقد أجريت دراسات التعبير عن طريق (northern blot) من خلال عزل الحمض النووي أو حمض (RNA) من أنسجة تحت عدة معالجات إجهاد لا إحيائية . كشف تحليل (Clustal-W) و assession no EU. 723832 عن كل الجينات الفوسفاتيز وان ترميز المعلم (tryptophan TGG) المتبقي كان محفوظا. وكشف تحليل (northern blot) تحت المعالجة بالإجهاد اللا إحيائي مثل الملح والحرارة والتناضح بان التعبير عن (***Triticum aestivum***) كان مستحفزا مشيرا على أن التعديل على (***Triticum aestivum***) تحت المعالج تحت المعالجات الاجهادية . وبناء على هذه النتائج فان احتمال الدور الوظائفى الفسيولوجي لنبات القمح (***Triticum aestivum***) تم تناوله.

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Introduction

In most agricultural soils, organic phosphorus (P) comprises 30-80% of the total P and the largest fraction of organic P, approx 50%, is in the form of phytin and its derivatives (Lin et al., 2009; Burke, 2003; Cashikar and Rao, 1996). For organic P sources in the soils to be used, they must be first hydrolyzed by acid phosphatases. Acid phosphatases (acid P-ases) form a group of enzymes catalyzing hydrolysis of a variety of phosphate esters in the acidic environments. These are believed to increase orthophosphate (Pi) availability under phosphorous deficient conditions (Vance et al., 2003). Pi play a vital role in many biological processes including photosynthesis, respiration, enzyme regulation, energy transfer, metabolic regulation, important structural constituent of biomolecules like phytin bodies in the ungerminated seeds, protein and nucleotide phosphorylation. 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 phosphorous stress has been documented in a number of plants (Vance et al., 2003). A positive relation was reported between root acid P-ases and phosphorous uptake in bean and barley (Asmar et al., 1995). However, a negative relationship was also observed between acid P-ases and phosphorous uptake under low phosphorous stress in wheat (Barret-Lennard et al., 1982). Hence, role of P-ases against phosphorous stress is still a matter of conjuncture. In addition to act as a Pi scavenger, several possible physiological roles also have been attributed to supraoptimal level of acid P-ases such as: seed dormancy, embryo germination, and cell wall regeneration (Sharma et al., 2004; Olczak and Watorek, 2003). We have previously studied the effect of drought and other abiotic stresses in drought tolerant and susceptible cultivars of wheat (Sharma and Kaur, 2007, 2008) and observed that drought induced enhancement was cultivar dependent. In this report we describe the cloning of acid phosphatase DNA (*TaPase*) and its reaction to different abiotic stress treatments. Enhanced expression of *TaPase* was observed under various abiotic stresses, suggesting that *TaPase* may be playing some adaptive role under stress condition.

Material and methods

Plant material and DNA isolation

The wheat seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70 % (v/v) ethanol (Sharma et al., 2008). 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 were incubated at 37 ±1°C in a seed germinator in darkness and allowed to grow for 5 days. The shoots were harvested and used for DNA isolation. DNA was isolated from the pooled shoots as per Sharma et al. (2002).

PCR and Cloning

For cloning *TaPase* DNA, we used total DNA as indicated above. PCR reactions were carried out by using 50 ng of DNA, according to the manufacturer instructions of magic amplification kit (Banglore Genei, India). The *TaPase* was amplified using forward 5'-CAAGGATGCGGGTTGTGTTGC-3' and reverse 5'- CATGCTCACAGC TTCATCAACAAG-3' primers. The reaction was carried out as per following conditions: initial denaturation 5 min, followed by 35 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec), and extension (72°C, 3 min) and final incubation (72°C, 10 min). The PCR product was run on 2% agarose gel and the desired band of about 500 bp was excised, eluted and purified as per manufacturer's protocol of spin gel extortion kit (Banglore

Genei, India). The PCR product was cloned into TA vector and sequenced (pGEMT®-Easy, Promega) (Figure 1). The sequence data has been deposited at Genbank under accession no EU 723832. The homology analysis by using different reported phosphatases genes were performed by using Clustal-W analysis (www.ebi.ac.uk/index.html).

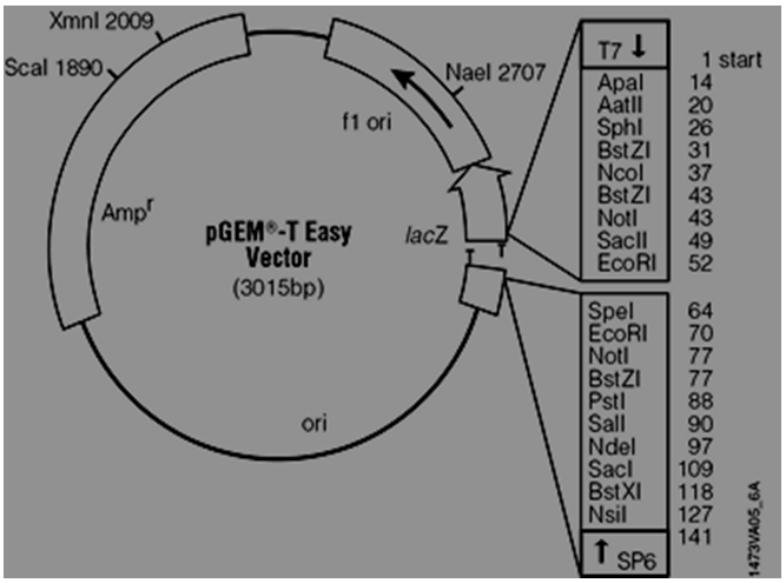

Figure 1. TA cloning vector (pGEMT®-Easy) used to clone *TaPase*.

Stress treatments and Northern blot analysis

The wheat seeds were surface sterilized and imbibed for 6 h. After imbibition, seeds were placed in Petri plates 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 5-day-old stage were used for the experiment. Stress treatments were performed on 3 M Whatman filter paper. Different stress treatments viz: Heat stress (42°C), mannitol (0.75 M) and NaCl (0.42M), were performed as described in Sharma et al. (2001). The tissues (shoots) from all treatments and control (seedlings irrigated with distilled water and kept at 37 ±1°C) were harvested and pooled for further analysis. Relative water content (RWC) was measured after imposing stress conditions. Immediately tissues were sealed in a plastic bag and quickly transferred to the laboratory. Fresh weights were determined within 2 h after collection. Turgid weights were obtained after soaking leaves in distilled water in test tubes for 16 to 18 h at room temperature under low light conditions. After soaking, leaves were quickly and carefully blotted dry with tissue paper in determination of turgid weight. Dry weights were obtained after oven drying the samples for 72 h at 70^{o} C. RWC was calculated from given equation: RWC(%) = fresh weight-dry weight/turgid weight-dry weight x 100. RNA was isolated from control and stress treated tissues as described in Sharma et al (2003). Total RNA (20ug) was separated on 1.5% formaldehyde agarose gel and blotted on to Hybond N+ nylon membrane (Amersham, NJ). Cloned *TaPase* DNA (approx 500 bp) was labelled with Gene Images Alk Phos Direct labeling and detection system (Amersham, USA). The Hybridization and detection was performed as per protocol of

northern blotting kit (Banglore Genei, India).

Results and discussion

A 516 bp DNA was amplified and cloned into TA vector (pGEMT®-Easy, Promega) (Figure 1) and submitted to GeneBnak database (accession no Eu732823). Interestingly, Clustal-W analysis of *TaPase* with various reported P-ase gene sequences revealed that TGG codon (underlined) encoding tryptophan residue is conserved in *TaPase* along with all the reported gene sequences (Figure 2), indicating that tryptophan residue may be involved in catalytic activity of *TaPase* encoding proteins. Earlier reports also indicated that tryptophan's are found as part of the phosphate binding sites in a number of phosphatase proteins in plants and animals (Cashikar and Rao, 1996; Zhang et al., 1997).

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Aj505579        ATCCGAATCATGA---TAATGTTAGGTGGGATACTTGGGGAAGGTTTATAGAAAGAAGTG 696
AF126255        ATCCATATCATGA---TAATGTTAGGTGGGACACCTGGGGAAGGTTTACAGAAAGAAGTG 2188
Af200824        ACCCAAATCATGA---TAACATTAGGTGGGATTCTTGGGGAAGGTTTACAGAAAGGAGTG 649
AJ001270        ACCCGAATCATGA---TAATGTTAGGTGGGATACTTGGGGAAGGTTTACAGAAAGGAGTG 773
Af200825        ATCCTAACCATGA---TAATGTAAGATGGGATACATGGGGTAGATTTGTTGAGAGAAGTA 655
Af200826        ATCCTAACCATGA---TAATGTAAGATGGGATACATGGGGTAGATTTGTTGAGAGAAGTA 655
Aj006224        ATCCTAACCATGA---TAATGTAAGATGGGATACATGGGGTAGATTTGTTGAGAGAAGTA 655
Ab039746        ACCCGGATCATGA---TAATGTAAGATGGGACACGTGGGGTAGGTTTGTTGAGAGAAGCA 670
Aj458943        ACAAGTACAATGATGTTGGTTTGCGATGGGACACATGGGGCCGGTTTGCCGAAAGGAGTA 752
Ay050812        ATCAGTATAATGACGTTGGTGTGAGATGGGATAGCTGGGGTCGTTTTGTGGAGCGTAGTA 719
Af356352        ATCAACATAATGA---TGGTGTTCGTTGGGATTCTTGGGGGCGGCTTGTGGAACGTAGTA 643
U48448          ACCCTAACCACGA---CAACAATAGATGGGATACTTGGGGAAGGTTCGTTGAGCGAAGTG 1430
AF492664        ACCCTAACCACGA---CAACAATAGATGGGATACTTGGGGAAGGTTCGTTGAGCGAAGTG 667
TaPase          AAGTGATTAAGGGATGCGGGTTGTGTTGGGGCAGAAGAACCAAGTCT---GATTCCAGTC 91
                *        * *            * ****      *            **    **

Aj505579        CTGCTTATCAACCTTGGATATGGACCGCAGGAAATCACGAAATTGATTTTGATCCACAAA 756
AF126255        CTGCTTATCAACCTTGGATATGGACCGCAGGAAACCATGAAATTGATTTTGATCTACAAA 2248
Af200824        TTGCTTATCAACCATGGATTTGGACTGCAGGCAACCATGAAAATCACTTTGCTCCAGAAA 709
AJ001270        TTGCATATCAGCCATGGATATGGACTGCAGGGAACCATGAAATTGAGTTTGCTCCAGAAA 833
Af200825        CTGCATATCAACCTTGGATTTGGACTGCAGGAAATCACGAGATAGATTTTGCCCCTGAAA 715
Af200826        CTGCATATCAACCTTGGATTTGGACTGCAGGAAATCACGAGATAGATTTTGCCCCTGAAA 715
Aj006224        CTGCATATCAACCTTGGATTTGGACTGCAGGAAATCACGAGATAGATTTTGCCCCTGAAA 715
Ab039746        CTGCATATCAACCGTGGATATGGACTACAGGAAACCACGAAATTGATTATGCTCCAGAGA 730
Aj458943        CAGCATATCAACCATGGATTTGGTCCGTTGGAAATCACGAAGTAGATTACATGCCTTACA 812
Ay050812        CCGCTTATCAACCGTGGCTTTGGTCTGCAGGAAATCATGAAGTAGATTACATGCCATACA 779
Af356352        CCGCTTATCAACCATGGATTTGGAGCGCTGGTAACCATGAAATTGAATACAGGCCTGATC 703
U48448          TTGCTTATCAACCTTGGATATGGACTGCTGGCAACCACGAAATCGACTTCGTTCCTGACA 1490
AF492664        TTGCTTATCAACCTTGGATATGGACTGCTGGCAACCACGAAATCGACTTCGTTCCTGACA 727
TaPase          CTTAGGAT-GACTAGGAAGCTGGGCTTGAGGGAA--ATCAACATCTCTCCTCTTCTGAAT 148
                      **   *   *    ***      ** **  *  *       *         *
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Figure 2. Clustal-W analysis of *TaPase* with different reported phosphatases genes. Aj505579: *Lupinus luteus*, AF126255:*Anchusa officilanus*; Af200824: *Glycine max*; AJ001270:*Phaesolus vulgaris*; Af200825: *Ipomoea batatas*; Af200826; *Ipomoea batatas*; Aj006224: *Ipomoea batatas*; Ab039746:*Spirodela oligrrhiza*; Aj458943: *Lupinus luteus*; Ay050812: *Arabidopsis thaliana*; Af356352; *Oriza sativa*; U48448: *Arabidopsis thaliana*; AF492664: *Arabidopsis thaliana*; TaPase: present study EU723832. Conserved nucleotide encoding tryptophan is underlined.* indicate identical residues.

Since plant acid P-ases display considerable heterogeneity with regard to their kinetics and functions (Zhang et al., 1997), hence this complexity may contribute to conflicting reports regarding role of acid P-ases gens under stress conditions. Acid P-ases are reported to be induced under phosphorous (Pi) deficiency, in order to maintain certain level of Pi inside the cells under stress conditions (Pant et al., 2008; Brini et al., 2007, Olmos and Hellin, 1997). However, the precise role of P-ases during drought stress is still unknown. So in order to study the integral role of *TaPase*, northern blot analysis studies were carried out under various abiotic stress treatments.

Imposition of osmotic stress (OS), salt stress (SS) and heat stress (HS) resulted in significant decrease in relative water content (RWC), indicating that seedlings were under stress (Figure 3A). Northern blot analysis revealed the modulation of *TaPase* transcript under various abiotic stress treatments (Figure 3B).

Osmotic, salt and heat stress treatments are depicted in Figure 3B. Compared to control, *TaPase* transcript level dramatically increased under all treatments, suggesting stress inducible nature of the gene. Overall, results obtained suggest that the increase of *TaPase* may be due to the fact that under stress conditions, phosphate (Pi) delivery is impaired, thus, resulting in the activation of the cellular phosphatase genes releasing soluble acid P-ases inside or outside of the cells thereby modulating osmotic adjustment by free phosphate uptake mechanism.

Olmos and Hellin (1997) also observed that acid phosphatases are known to act under salt stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force.

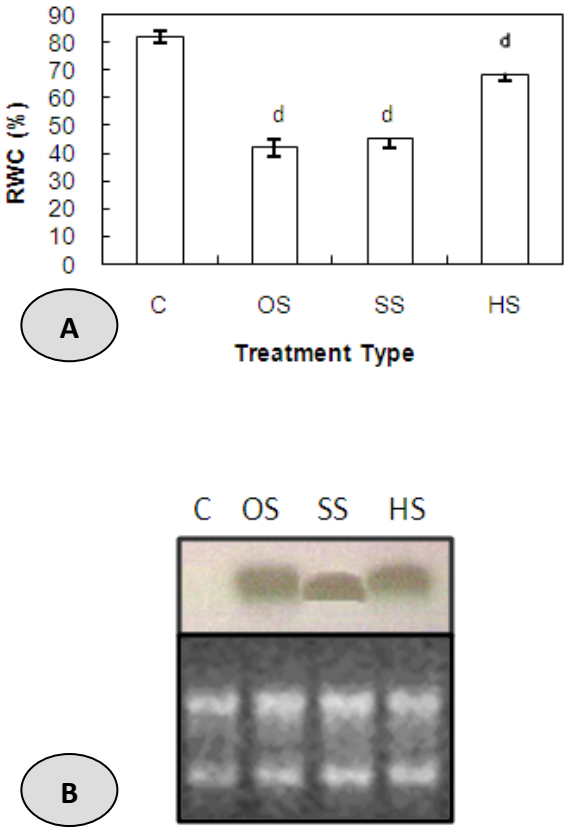

Figure 3. (A) Relative Water Content (RWC, %) of shoots under different stress treatments. Data shown are average ± SE of three replicates. [d]indicates significant difference vs. control at P≤ 0.05. (B) Changes in *TaPase* transcript (northern blot analysis) in response to osmotic stress (OS), salt stress (SS) and heat treatment (HT). Each lane contains 20ug of total RNA. Lower panel depicts ethidium bromide staining of RNA gel.

To conclude, it became apparent that in arid- and semi-arid areas of the world, the acid P-ases may be playing very important role under abiotic stresses in order to contrast adverse environmental conditions. The expression of higher *TaPase* suggests its global role in enhancing Pi availability. In addition, results provide valuable information to develop screening marker tools for selecting lines with tolerance to drought stress and phosphorus status, thus improving field emergence and survival percentage of plants.

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