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Expressions of Three Wheat S-like RNase Genes Were Differentially Regulated by Phosphate Starvation

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Abstract: In plants, RNase can degrade RNA to release Pi in senescent organs, which can be reused by young organs. The expression of many S-like RNase genes were up-regulated by phosphate starvation. Using the ESTs, three S-like RNase gene cDNAs were isolated from common wheat by RT-PCR. The predicated amino acid sequences of the three sequences were found having high similarities with those S-RNases and S-like RNases in other plant species. The expression of *WRN1* was down-regulated by phosphate starvation and leaf senescence, while *WRN2* and *WRN3* were up-regulated by phosphate starvation.

Key words: Phosphate starvation; Common wheat (*Triticum aestivum* L.); S-like RNase

三个类核糖核酸酶基因在磷饥饿条件下的表达

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摘 要:核糖核酸酶(RNases)可以将衰老的植物组织中的核糖核酸降解释放出磷元素,使它能够运送到幼嫩部位被重新利用。许多核糖核酸酶基因的表达受磷饥饿的正调控。利用已有的 EST 序列,从普通小麦"小偃 54"中分离了 3 个核糖核酸酶基因的 cDNA 序列。这 3 个基因预测的氨基酸序列与 S-核糖核酸酶和 S-like 核糖核酸酶(类核糖核酸酶)的氨基酸序列有较高的同源性。*WRN1* 的表达受磷饥饿和衰老的负调控,而 *WRN2* 和 *WRN3* 受磷饥饿的正调控。

关键词:磷饥饿;普通小麦;类核糖核酸酶

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Phosphate (Pi) is one of the key substrate in energy metabolism and biosynthesis of nucleic acids and membranes. It also plays an important role in photosynthesis, respiration and regulation of a number of enzymes[1]. Ribonucleases (RNases) can degrade RNA and DNA in senescent plant organs releasing Pi to immature organs, and improve the Pi utilizing rate in plants[2]. The first identified plant RNase is S2-glycoprotein from tobacoo (*Nicotiana alata*)[3]. It has close relation to self-incompatibility[4]. RNase NE, also isolated from *Nicotiana alata*, expressed in styles, petals and immature anthers but not in the vegetative tissues under normal growth conditions[5]. Under Pi-limited conditions, RNase NE expression was induced in roots but not in leaves[5]. Besides tobacoo, RNase genes had also been isolated from *Pyrus pyrifolia*[6], *Malux* × *Domestica* Borkh[7] and *Pyrunnus dulcis*[8], respectively. Although the amino acid sequences of RNase genes isolated from self-compatibility plants (S-like RNase) were highly identical to those isolated from self-incompatibility plants (S-RNases), many differences existed in expression patterns and functions between the two types of RNases. S-like RNases did not take part in the control of self-imcompatibility. The expressions of S-like RNase genes were induced by specific stimuli[9~11].

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S-like RNases take part in the reactions of stresses, senescence and RNA metabolisms. The activities of RNases in tomato leaves increased with age[12]. The expressions of RNase LE and LX genes were induced in leaves during an advanced stage of senescence in tomato[13]. The S-like RNase LE gene was also induced by Pi starvation in cultured tomato cells[14]. In *Arabidopsis*, three S-like RNase genes, *RNS1*, *RNS2* and *RNS3*, were each induced to different extents during leaf senescence. *RNS1* was induced only slightly[15], whereas *RNS2* and *RNS3* were more strongly induced[15, 16]. However, their responses to Pi starvation differed, *RNS1* and *RNS2* were induced by Pi starvation, whereas *RNS3* was not[15,16]. Antisense inhibition of *RNS1* or *RNS2* elevated the accumulation of anthocyanins, the production of which is often associated with several forms of stress, including Pi starvation. This suggested that S-like RNases may participate in the related processes of nutrient recycling during senescence and scavenging Pi sequestered in RNA, in combination with the actions of phosphatases during starvation for Pi[17].

In a previous study, we have isolated a wheat S-like RNase (*WRN1*) gene, and found that its expression was down-regulated by senescence[18]. In this study, two new S-like RNase genes from wheat were isolated, and the expressions of them, together with formally isolated *WRN1*, under the conditions of Pi starvation were analyzed.

1 Materials and Methods

1.1 Plant Materials and Growth Conditions

Common wheat (cv. Xiaoyan 54) seeds were surface-sterilized and germinated on filter paper. When the hypocotyls had emerged, the seeds were transferred to a thin layer of quartz saturated with 0.2 mmol/L calcium sulphate solution, and incubated at 25℃ for 5 days. Then the seedlings with the residual endosperms removed were transferred to complete nutrition solution in a growth cabinet kept at a constant (20 ± 2)℃, with a relative humidity of 75%, and illumination at a photon fluence rate of 280 – 300 μmol photons·m^{-2}·s^{-1} for 16 h a day. After one week, the seedlings were transferred to the nutrient solution deprived of Pi for 0, 3 and 7 days, then roots and leaves were collected for gene expression analysis. The reagents of the nutrient solution and the phosphate treatments were done according to the previous article[19]. During the hydroponic culture, solution were continuously aerated and changed every other day.

1.2 RNA Extraction and Isolation of Full-length cDNAs of Three WRNs

Total RNA samples from roots and leaves were isolated using a Trizol reagent (Gibco BRL). RNA samples were treated with DNase Ⅰ. Total RNA was used as template for synthesis of the first-strand cDNA. PCR reaction was carried out in 25 μL reaction volume, involving 1 μL first-strand cDNA as template, with total 35 cycles of denaturation for 30 s at 94℃, annealing for 30 s at 56℃, and elongation for 1.5 min at 72℃. According to the rice S-like RNase gene sequence (accession numbers AF495872), six wheat EST sequences (the accession number was BE591570, BM136217, BJ260079, BJ265782, BJ234043 and BJ239911, respectively) were found in GenBank. The first pair of primers (P1: 5′-CATTTCTGCTTGCAATACTTAGTAGA-3′; P2: 5′-CATGTATACACT TTTATTTATTCAACCATC-3′) was designed according to BE591570 (5′) and BM136217 (3′). PCR reaction was performed with total 35 cycles of denatuation for 30 s at 94℃, annealing for 1 min at 53℃, and elongation for 1 min at 72℃. The second pair of primers (P3: 5′-GCGAGATAGATAGCTCTGCAGCGAG-3′; P4: 5′-ATGAAATAGCAACCGGCTCCCTCC-3′) was designed according to BJ260079 (5′) and BJ265782 (3′). PCR reaction was carried out with total 35 cycles (94℃ 30 s, 65℃ 1 min, 72℃ 1 min). The third pair of primers (P5: 5′-TGAGGTTGCAGTGCTCCCTGGTCCT-3′; P6: 5′-CCTAAGTTTATTCTGCCGCGGCAAG-3′) was designed according to BJ234043 (5′) and BJ239911 (3′). PCR reaction was carried out with total 35 cycles (94℃ 30 s, 65℃ 1 min, 72℃ 1 min). The PCR products were recovered and cloned into pGEM T-easy vector (Promega) and sequenced, respectively.

1.3 Relative-quantitative Polymerase Chain Reaction (RQRT-PCR)

Samples of 200 ng of total RNA were used as templates for synthesis of the first-strand cDNA. RQRT-PCR was done for *WRN1* using the specific primers P1 and P2. P3 and P4 were used to identify the expression of *WRN2*. P5 and P6 were used for *WRN3*. PCR reaction was carried out as described above. As a control, amplifi-

cation by RT-PCR was performed using two primers (P7: 5′-AGAACACTGTTGTAAGGCTCAAC-3′ and P8: 5′-GAGCTTTACTGCCTCGAACATGG-3′) specific for the wheat *tublin* gene. PCR was performed for 30 cycles (94℃ 30 s, 55℃ 60 s, 72℃ 60 s).

2 Results

Using the known rice S-like RNase gene (accession number AF495872), six wheat ESTs were found. Three clones were got by RT-PCR. They were named as *WRN1* (accession number AF495872), *WRN2* (accession number AY517470) and *WRN3* (accession number AY528721), respectively. *WRN1* and *WRN2* were both full-length cDNAs, which had start and stop codons. They had an open reading frame containing 735 bp and 684 bp, respectively. *WRN3* was a partial cDNA. The predicated amino acid sequences of the three genes were highly similar with those encoded by S-like RNase genes (Fig.1).

All of the amino acid sequences of active RNases reported had two conserved histidine sites[20]. They may play important roles in keeping RNase activity. WRN 2 and WRN3 both had the two conserved histidine sites, but the two sites in WRN1 were replaced (indicated by arrows in Fig.2).

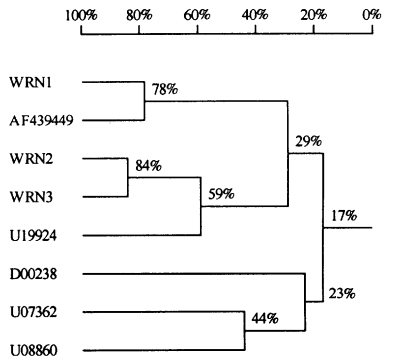

Fig.1 Phylogenetic tree of three wheat S-like RNase amino acid sequences

The amino acid sequences of the three S-like RNases were aligned with that of *Oryza sativa* S-like RNase (AF439449), Zinnia RNase Ⅱ (U19924), *Nicotiana alata* RNase Rh (D00238) and S2-RNase (U08860), *Petunia hybrida* S1 (U07362). The phylogenetic tree was created using the software DNAMAN.

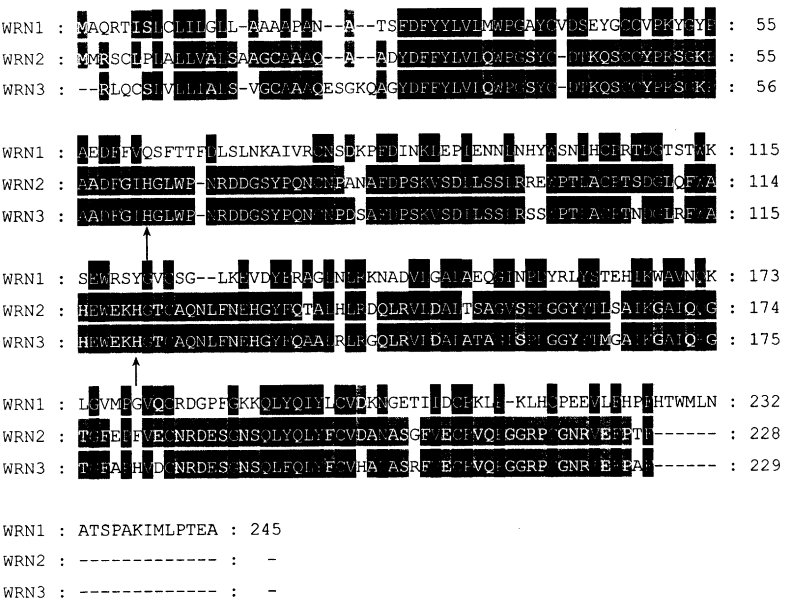

Fig.2 Aligment of three wheat S-like RNase amino acid sequences

The two arrowheads showed the conserved histidine residues in all of the active S-RNases and S-like RNases isolated now. The alignment was done using the software ClustalW at http://www.ch.embnet.org/software/ClustalW.html.

Using RQRT-PCR, we found that all the three genes mainly expressed in leaves. Their expressions in roots were much weaker than those in leaves (Fig.3). The responses of these three genes to Pi starvation were quite different. The mRNA levels of *WRN2* and *WRN3* were greatly increased in leaves and roots, while that of *WRN1* were decreased in leaves by Pi starvation (Fig.3). No obvious change was observed for the expression of *WRN1* in roots under Pi starvation.

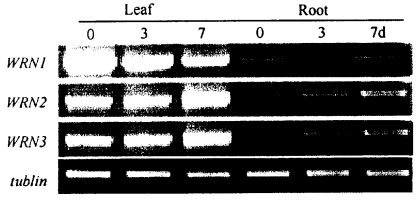

Fig.3 Expression of three wheat S-like RNase genes in response to Pi starvation

RQRT-PCR was carried out with the leaves and roots cut from seedlings treated with Pi starvation for 0, 3 and 7 days, respectively. The bottom line showed the corresponded expression of *tublin*. Reaction products were analyzed by agarose gel electrophoresis.

3 Discussion

RNA is the most abundant form of organic phosphorus in plant cells, but has a comparatively low rate of turnover[21]. RNases could degrade RNA molecules in the extracellular environment with the released nucleotides then acting as a source of Pi or reallocate Pi resources in internal pools[5]. All of the amino acid sequences of active RNases reported had two conserved histidine sites[20], indicating the importance of these two histidine amino acids in keeping RNase activity. In present study, we found that the expressions of *WRN2* and *WRN3* with the two conserved histidine residues was up-regulated in leaves and roots of wheat, while that of *WRN1* without the conserved histidine residues was down-regulated in leaves by Pi starvation. This suggests *WRN2* and *WRN3* may participate the related processes of nutrient recycling during scavenging Pi sequestered in RNA when wheat plant is suffered from Pi starvation. In *Arabidopsis*, antisense inhibition of *RNS1* or *RNS2* elevated anthocyanin accumulation, the production of which is often associated with Pi starvation, demonstrating that diminishing the amounts of either *RNS1* or *RNS2* leads to the effects that cannot be compensated for by the actions of other RNases, even though *Arabidopsis* contains a large number of different RNase activities[17]. Whether *WRN2* and *WRN3* had the similar roles of *RNS1* and *RNS2* needs further studies.

It is not clear how many types of RNases are in plants now. There were at least 20 types identified from *E. coli*[22]. Yen and Baenziger (1993) isolated and identified 15 types of wheat RNases whose molecular weights were from 16.3 kD to 40.1 kD[23]. Three types of RNases whose molecular weights were 20 kD, 26 kD, and 26 kD respectively were also identified from wheat[24]. The predicted proteins of WRN2 and WRN3 were both 25 kD or so. The RNase types they encoded were yet unclear now.

One difference of *WRN1* from *WRN2* and *WRN3* is that *WRN1* did not have the two conserved histidine residues essential for keeping the RNase activities. Replacement of Either the two histidine residues of RNase Rh by other amino acids would make the RNase activity completely or nearly completely lose[25]. This suggests that *WRN1* is possibly not active as an RNase. The expression patterns of *WRN1* were also different from those of *WRN2* and *WRN3*. Although *WRN1* was mainly expressed in leaves, it was down-regulated by Pi starvation. In a previous study, the expression of *WRN1* was found to decrease during the natural senescence in field and induced senescence in laboratory[18]. The expression patterns of *WRN1* were quite similar to that of the barley S-like RNase gene *RSH1*, whose the two conserved histidine residues were replaced by serine residues. *RSH1* mainly expressed in young, developing leaf cells and the expression declines well before senescence[20]. Our results, together with the results of *RSH1* from barley, suggest RNase without the two conserved histidine residues may not involve in reallocation of Pi sequestered in RNA during senescence.

References

[1] Raghothama K G. Phosphate acquisition. *Annu Rev Plant Physiol Plant Mol Biol*, 1999, 50: 665-693

[2] Shimizu T, Inoue T, Shiraishi H. A senescence-associated S-like RNase

in the multicellular green alga *Volvox carteri*. *Gene*, 2001, **274**: 227 – 235

[3] Anderson M A, McFadden G I, Bernatzky R, Atkinson A, Orpin T, Deman H, Tregear G, Fernley R, Clarke A E. Sequence variability of three alleles of the self-incompatibility gene of *Nicotiana alata*. *Plant Cell*, 1989, **1**: 483 – 491

[4] Clarke A E, Newbigin E. Molecular aspects of self-incompatibility in flowering plants. *Annu Rev Genet*, 1993, **27**: 257 – 279

[5] Dodds P N, Clarke A E, Newbigin E. Molecular characterization of an S-like RNase of *Nicotiana alata* that is induced by phosphate starvation. *Plant Mol Biol*, 1996, **31**: 227 – 238

[6] Norioka N, Norioka S, Ohnishi Y, Ishimizu T, Oneyama C, Nakanishi T, Sakiyama F. Molecular cloning and nucleotide sequences of cDNAs encoding S-allele specific stylar RNases in a self-incompatible cultivar and its self-compatible mutant of Japanese pear, *Pyrus pyrifolia* Nakai. *J Biochem* (Tokyo), 1996, **120**: 335 – 345

[7] Broothaerts W, Janssens G A, Proost P, Broekaert W F. cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. *Plant Mol Biol*, 1995, **27**: 499 – 511

[8] Ushijima K, Sassa H, Tao R, Yamane H, Dandekar A M, Gradziel T M, Hirano H. Cloning and characterization of cDNAs encoding S-RNases from almond (*Prunus dulcis*): primary structural features and sequence diversity of the S-RNases in Rosaceae. *Mol Gen Genet*, 1998, **260**: 261 – 268

[9] Taylor C B, Green P J. Genes with homology to fungal and S-gene RNases are expressed in *Arabidopsis thaliana*. *Plant Physiol*, 1991, **96**: 980 – 984

[10] Jost W, Bak H, Glund K, Terpstra P, Beintema J J. Amino acid sequence of an extracellular, phosphate-starvation-induced ribonuclease from cultured tomato (*Lycopersicon esculentum*) cells. *Eur J Biochem*, 1991, **198**: 1 – 6

[11] Ide H, Kimura M, Arai M, Funatsu G. The complete amino acid sequence of ribonuclease from the seeds of bitter gourd (*Momordica charantia*). *FEBS Lett*, 1991, **284**: 161 – 164

[12] McHale J S, Dove L D. Ribonuclease activity in tomato leaves as related to development and senescence. *New Phytol*, 1968, **67**: 505 – 515

[13] Lers A, Khalchitski A, Lomaniec E, Burd S, Green P J. Senescence-induced RNases in tomato. *Plant Mol Biol*, 1998, **36**: 439 – 449

[14] Nürnberger T, Abel S, Jost W, Glund K. Induction of an extracellular ribonuclease in cultured tomato cells upon phosphate starvation. *Plant Physiol*, 1990, **92**: 970 – 976

[15] Bariola P A, Howard C J, Taylor C B, Verburg M T, Jaglan V D, Green P J. The *Arabidopsis* ribonuclease gene *RNS1* is tightly controlled in response to phosphate limitation. *Plant J*, 1994, **6**: 673 – 685

[16] Taylor C B, Bariola P A, Del Cardayre S B, Raines R T, Green P J. *RNS2*: A senescence-associated RNase of *Arabidopsis* that diverged from the S-RNases before speciation. *Proc Natl Acad Sci USA*, 1993, **90**: 5 118 – 5 122

[17] Bariola P A, MacIntosh G C, Green P J. Regulation of S-like ribonuclease levels in *Arabidopsis*: Antisense inhibition of *RNS1* or *RNS2* elevates anthocyanin accumulation. *Plant Physiol*, 1999, **119**: 331 – 342

[18] Chang S H, Ying J, Zhang J J, Su J Y, Zeng Y J, Tong Y P, Li B, Li Z S. Expression of a wheat S-like RNase (*WRN1*) cDNA during natural and dark-induced senescence. *Acta Botanica Sinica*, 2003, **45**: 1 071 – 1 075

[19] Davis T G E, Ying J, Xu Q, Li Z S, Li J, Gordon-Weeks R. Expression analysis of putative high-affinity phosphate transporters in Chinese winter wheats. *Plant, Cell and Environment*, 2002, **25**: 1 325 – 1 339

[20] Gausing K. A barley gene (*rsh1*) encoding a ribonuclease S-like homologue specifically expressed in young light-grown leaves. *Planta*, 2000, **210**: 574 – 579

[21] Bieleski R L, Ferguson I B. Physiology and metabolism of phosphate and its compounds. In: Luchli A, Bieleski R L eds. Encyclopedia of Plant Physiology. Springer-Verlag, Berlin, 1983, **15A**: 422 – 449

[22] Deutscher M P. Ribonuclease multiplicity, diversity, and complexity. *J Biol Chem*, 1993, **268**: 13 011 – 13 014

[23] Yen Y, Baenziger P S. Identification, characterization, and comparison of RNA-degrading enzymes of wheat and barley. *Biochem Genet*, 1993, **31**: 133 – 145

[24] Blank A, McKeon T A. Three RNases in senescent and nonsenescent wheat leaves. *Plant Physiol*, 1991, **97**: 1 402 – 1 408

[25] Huang S, Lee H S, Karunanandaa B, Kao T H. Ribonuclease activity of *Petunia inflata* S proteins is essential for rejection of self-pollen. *Plant Cell*, 1994, **6**: 1 021 – 1 028