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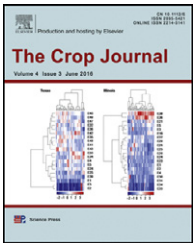

Molecular characterization and expression analysis of three homoeologous *Ta14S* genes encoding 14-3-3 proteins in wheat (*Triticum aestivum* L.)

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ABSTRACT

The purpose of this study was to characterize *Ta14S* homoeologs and assess their functions in wheat seed development. The genomic and cDNA sequences of three *Ta14S* homoeologous genes encoding 14-3-3 proteins were isolated. Sequence analysis revealed that the three homoeologs consisted of five exons and four introns and were very highly conserved in the coding regions and in exon/intron structure, whereas the cDNA sequences were variable in the 5′ and 3′-UTR. The three genes, designated as *Ta14S-2A*, *Ta14S-2B* and *Ta14S-2D*, were located in homoeologous group 2 chromosomes. The polypeptide chains of the three *Ta14S* genes were highly similar. These genes were most homologous to Hv14A from barley. Real-time quantitative PCR indicated that the three *Ta14S* genes were differentially expressed in different organs at different developmental stages and all exhibited greater expression in primary roots of 1-day-old germlings than in other tissues. Comparison of the expression patterns of the three homoeologous genes at different times after pollination also revealed that their expression was developmentally regulated. The transcription of *Ta14S-2B* was clearly higher during seed germination, whereas expressions of *Ta14S-2A* and *Ta14S-2D* were up-regulated at the beginning of seed imbibition (0–12 h), but declined thereafter. The results suggested that the three *Ta14S* homoeologous genes have regulatory roles in seed development and germination.

1. Introduction

The 14-3-3 proteins are a family of highly conserved regulatory proteins found in virtually all eukaryotes. The N- and C-ends of these proteins are highly variable whereas the core structures are highly conserved [1]. These molecules are small acidic soluble proteins with a molecular mass of approximately 30 kDa. They usually form homo- and hetero-dimers to interact with diverse target proteins by specific phosphoserine/phosphothreonine-binding activity [2,3]. To date, there are over three hundred proteins identified as their interacting proteins [4,5], and the outcomes of binding are diverse, including alteration in conformation, subcellular localization and stabilization of the interacting proteins. They also mediate formation

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of protein complexes [6–9]. The physiological function of these proteins in plants has been the focus of considerable research. Various 14-3-3 isoforms have been isolated from diverse species including fifteen known protein isoforms in *Arabidopsis* [10], eight in rice [11], five in barley [12] and 7 potential isoforms in wheat [13–17]. Plant 14-3-3 proteins can be divided into two classes, the epsilon and the non-epsilon groups, based on sequence similarity and phylogenetic analyses of sequences obtained from *Arabidopsis* [3]. Functional analysis revealed that increased or decreased expression of 14-3-3 protein genes caused a number of phenotypic, developmental and stress tolerance changes. For example, in rice plants transformed with an over-expression construct of the maize 14-3-3 protein gene, *GF14–6*, tolerance to drought stress and response to pathogen infection were changed [18]. When overexpressed in *Arabidopsis* plants wheat 14-3-3 protein caused shorter primary roots, delayed flowering and retarded growth rates [16]. The overexpression of cotton 14-3-3 protein *Gh*14-3-3 *L* promoted fiber elongation, leading to an increase in mature fiber length. By contrast, the suppression of the expression of *Gh*14-3-3 *L*, *Gh*14-3-3 *e* and *Gh*14-3-3 *h* in cotton slowed down fiber initiation and elongation [19]. These results indicated that some 14-3-3 protein-coding genes have roles in plant development and stress response.

Wheat is a globally important crop, accounting for 20% of the calories consumed by humans [20]. Research that focuses on mechanisms of developmental regulation at the molecular level has potential to accelerate progress of wheat improvement. Although 14-3-3 proteins are increasingly implicated as key factors in developmental regulation [21–24], little is known about such genes in wheat seed development. Because hexaploid wheat contains three sets of chromosomes, *Ta14-3-3* genes are likely to occur as homoeologous triplicates. Previously, we isolated a wheat cDNA designated as *Ta14S* with an open reading frame encoding a putative 14-3-3 protein [15]. To further clarify a regulatory function in wheat seed development, we isolated the genomic and cDNA sequences of *Ta14S* homoeologs in hexaploid wheat and located the genes to chromosomes using Chinese Spring nullisomic–tetrasomic lines. In addition, we analyzed their expression patterns in different tissues and at different seed developmental and germination stages.

2. Materials and methods

2.1. Plant sample preparation

Common wheat (*Triticum aestivum* L.) cultivar (cv.) Luohan 2 was used for gene cloning and expression analysis. After being sterilized, seeds were germinated on moist filter paper in growth chambers at 25 °C under 12-h light/12-h dark conditions and transplanted into pots in a naturally lit glasshouse with normal irrigation and fertilization until mature. Dry seed embryos, primary roots and shoots at 1 day post-germination, roots and fully expanded leaves at 10 days post-germination, leaves at the tillering stage, stems at jointing, flag leaves, young panicles at heading, and developing seeds at 5, 10, 15, 20, 25, 30 and 35 DAP (days after pollination) were sampled. For germination treatment, mature seeds were surface-sterilized and then imbibed water from moist filter paper in Petri dishes in a temperature-controlled cultivation chamber (16 h photoperiod at 25 °C). Seeds were collected at 0, 6, 12, 24, 36 and 48 h after initiation of imbibition.

All collected plant materials were frozen in liquid nitrogen immediately after collection and stored at −80 °C until used. Three biologically independent replicates were assayed to ascertain reproducibility. A set of Chinese Spring (CS) nullisomic–tetrasomic lines, kindly provided by Dr. Xianchun Xia, Chinese Academy of Agricultural Sciences, was used to determine the chromosomal locations of *Ta14S*.

2.2. DNA extraction, primer design, PCR and sequencing

Genomic DNA was isolated from wheat seeds using a CTAB method [25]. Gene-specific primers were designed based on the sequence of *Ta14S* using Primer Premier 5.0 software (http://www.premierbiosoft.com/) and synthesized by Beijing Liuhe Huada Gene Technology Co., Ltd. (http://www.bgitechsolutions.cn/). PCR were performed in a Biametra-T3000 thermal cycler in total volumes of 20 μL, including 2 μL 10 × PCR buffer, 100 mmol L^{-1} of each of dNTP, 5 pmol of each primer, 1 unit of *Taq* DNA polymerase (TIANGEN Biotech Co., Ltd., Beijing, http://www.tiangen.com/) and 100 ng of template DNA. Reaction conditions were 95 °C for 3 min followed by 35 cycles of 94 °C for 30 s, annealing at 55 °C for 1 min and 72 °C for 1–2 min, with a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis in 1.0% agarose gels. Targeted fragments of expected size were recovered and cloned into the pMD18-T vector and sequenced by Beijing Liuhe Huada Gene Technology Co., Ltd. (http://www.bgitechsolutions.cn/). To ensure sequencing accuracy PCR and DNA sequencing were repeated at least three times.

2.3. RNA extraction and first-strand cDNA synthesis

Total RNA extractions from embryos and seeds were carried out using a hot-phenol method [26] and from leaves and roots using TRIzol Reagent (Invitrogen, Shanghai) according to the manufacturer's instructions. Quality and concentrations of total RNA were measured by spectrophotometer (NanoDrop ND-1000, Wilmington, USA) and agarose gel electrophoresis. Equal amounts (2 μg) of total RNA were transcribed into cDNA in a 20 μL reaction system containing 50 mmol L^{-1} Tris–HCl (pH 8.3), 75 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} DTT, 50 mmol L^{-1} dNTPs, 200 U M-MLV reverse transcriptase (Promega, Madison, WI) and 50 pmol Oligo-dT_{15} anchor primer. Reverse transcription was performed for 60 min at 42 °C with a final denaturation step at 95 °C for 5 min.

2.4. Rapid amplification of cDNA ends (RACE)

mRNA was purified through oligotex chromatography (Clontech, Beijing) from total RNA and 3′-RACE and 5′-RACE were performed using a SMART-RACE cDNA amplification kit (Clontech, Beijing) according to the manufacturer's protocol. Gene-specific primers used for PCR were 3′-GSP (3′-RACE) and 5′-GSP (5′-RACE), respectively (Table 1). PCR was performed according to the manufacturer's protocol (Clontech). PCR conditions were 94 °C for 4 min, followed by 6 cycles of 94 °C