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RESEARCH ARTICLE

Characterization and expression analysis of a novel RING-HC gene, *ZmRHCP1*, involved in brace root development and abiotic stress responses in maize

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Abstract

RING is a really interesting new gene which plays important regulatory roles in many developmental processes as well as in plant-environment interactions. In the present report, the *ZmRHCP1* gene encoding a putative RING-HC protein was isolated from maize and characterized. The ZmRHCP1 protein contained 310 amino acid residues with a conserved RING-HC zinc-finger motif and two transmembrane (TM) domains. *ZmRHCP1* was expressed ubiquitously in various organs (root, stem, leaf, seedling, immature ear, and tassel), but its transcript levels were higher in vegetative organs than in reproductive organs. Moreover, the expression pattern of *ZmRHCP1* in brace roots indicated that *ZmRHCP1* functions in brace root initiation. In addition, *ZmRHCP1* expression was regulated by abiotic stresses. The expression results suggested that *ZmRHCP1* plays important roles in brace root development and abiotic stress responses. The findings of the present study provide important information to help us understand the function of *ZmRHCP1* in maize.

Keywords: RING-HC zinc-finger, brace root, expression, abiotic stresses, maize

1. Introduction

Zinc-finger proteins, which have a finger-like structure held together by one or more zinc ion(s), are abundant in eukaryotic genomes (Kam *et al*. 2007). Their zinc-finger motifs vary widely in structure and function, and are involved in DNA/RNA binding, protein-protein interactions and membrane association (Laity *et al*. 2001). *RING* is a really interesting new gene, its RING zinc-finger motif, which has a conserved pattern of cysteine and histidine residues, was defined as a novel zinc-finger domain (Freemont *et al*. 1991). RING zinc-finger proteins mediate protein-protein interactions and are necessary for E3 ligase activity in protein ubiquitination (Freemont 1993; Park *et al*. 2010). RING motifs are divided into three types, namely C3HC4 (RING-HC), C3H2C3 (RING-H2), and other minor modified variants, depending on the presence of a cysteine or histidine in the fifth position (Saurin *et al*. 1996; Jensen *et al*. 1998; Stone *et al*. 2005). Numerous RING-H2 proteins associated with plant development and defense have been identified (Schwechheimer *et al*. 2009). In *Arabidopsis*, STRF1 is a membrane trafficking-related ubiquitin ligase, involved in the response to

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salt stress through the monitoring of intracellular membrane trafficking and reactive oxygen species production (Tian *et al*. 2015), and *ALT2* is an auxin-inducible gene (Serrano *et al*. 2004). The protein BIG BROTHER (BB) controls organ size by limiting the duration of cell proliferative growth (Disch *et al*. 2006). The *OsRFPH2-10* gene, which was identified from rice, is associated with antiviral defense in the early stages of rice dwarf virus infection (Liu *et al*. 2014). In addition, *PtaRHE1* regulates the development of the secondary phloem fibers in *Populus* (Baldacci-Cresp *et al*. 2015), and over-expression of *XERICO* improved drought tolerance in maize (Ko *et al*. 2006). However, there have been few reports about RING-HC proteins. OsRHC1, a RING-HC protein in rice, plays an important role in defense responses to a broad-spectrum disease resistance (Cheung *et al*. 2007). Other RING-HC proteins from rice and apple have been analyzed at a genome-wide level, but there is little detailed functional information about each RING-HC protein (Lim *et al*. 2010; Li Y Z *et al*. 2011).

The maize root system includes embryonic primary roots, lateral roots, seminal roots and shoot borne roots that are formed during postembryonic development (Hochholdinger *et al*. 2004a). Shoot borne roots include crown roots formed at underground shoot nodes and brace roots born on consecutive above-ground nodes (Hochholdinger *et al*. 2004b). Brace roots contribute enormously to providing anchorage, water and nutrient uptake in the late growth and development stages of maize plants (Varney 1993; Wang *et al*. 1994). In a previous study, many transcription factors active in the early development of maize brace roots were found by deep sequencing, including RING zinc-finger proteins (Li Y J *et al*. 2011).

In this study, one mRNA preferentially expressed during the early development of brace roots encoding a RING-HC zinc-finger protein (designated ZmRHCP1) was characterized in detail. We first isolated and characterized the full-length cDNA of *ZmRHCP1*. Then, we examined the expression pattern of *ZmRHCP1* in different organs and in response to various abiotic stresses.

2. Materials and methods

2.1. Plant materials and growth conditions

Maize plants were grown in a growth chamber at (25±1)°C under long-day conditions (16 h light/8 h dark). For salt and drought treatments, the two-week-old seedlings were exposed to 250 mmol L^{-1} NaCl and 15% PEG6000, respectively. For the low and high temperature treatments, the seedlings were placed at (4±1)°C and (40±1)°C in a growth chamber, respectively. After 0, 1, 3, 6, 12, 24 and 48 h, the young leaves of the treated seedlings were collected for qRT-PCR analyses. The stem was harvested at the 25th day after seeding (V5 stage), and the primary roots, the 2nd leaves and the entire seedlings were as root, leaf and seedling sample, respectively, which were collected at the 14th day after seeding (V2 stage). The immature ears and tassels were collected at the 34th day after seeding (V7 stage). The samples to examining gene expression of brace root were obtained from separate successive phytomers of the V5 stage. All samples used in the expression analysis were from the maize (*Zea mays* L.) inbred line B73.

2.2. Phylogenetic analyses and *cis*-acting elements analyses

To determine the evolutionary relationships of the ZmRHCP1 and its homologous sequences in other plant species, the full-length protein sequences were first aligned with DNAMAN 7.0, and then a phylogenetic tree was constructed using the maximum likelihood (ML) method with 1 000 bootstrap replicates.

To analyze the promoter regions of *ZmRHCP1*, the 1.5-kb of genomic sequences upstream of start codon was screened against the PlantCare database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

2.3. RNA isolation and qRT-PCR expression analysis

Total RNA from each sample was extracted according to the instructions of Trizol Reagent (Invitrogen, Carlsbad, USA), and cDNA was synthesized and purified as described by Li *et al*. (2015). qRT-PCR analysis was conducted on a Bio-Rad CFX96 Real-Time Detection System (Bio-Rad, Hercules, CA, USA), with gene-specific primers, RING-F: 5′-AAGAGAGGCTTTGTGGTCTACC-3′ and RING-A: 5′-GGAATGTTGCGAGCACTTGG-3′, and the *18S rRNA* gene in maize was used as internal control. The gene expression at 0 h under each treatment was set to "1" and their relative expression levels at other time points were calculated based on these controls.

2.4. Histological analysis and mRNA *in situ* hybridization

Tissue samples were fixed in formalin/acetic acid/alcohol (FAA) overnight at 4°C. After fixation, the specimens were dehydrated through a graded ethanol series, and embedded in paraplast (Sigma, USA). Fixed tissues sections (8 μm) were stained with 1% (w/v) safranine/0.5% (w/v) fast green and then observed under an Olympus BH-2 microscope (Olympus, Japan).

For mRNA *in situ* hybridization, tissue samples were fixed in paraformaldehyde (PFA) in 100 μmol L^{-1} sodium