Gene 563 (2015) 87–93

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Cloning and expression analysis of *CaPIP1-1* gene in pepper (*Capsicum annuum* L.)

Yan-Xu Yin [a,1], Shu-Bin Wang [b,1], Huai-Xia Zhang [a], Huai-Juan Xiao [a], Jing-Hao Jin [a], Jiao-Jiao Ji [a], Hua Jing [a], Ru-Gang Chen [a], Mohamed Hamed Arisha [a,c], Zhen-Hui Gong [a,*]

[a] College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China
[b] Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, P. R. China
[c] Horticulture Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 16 January 2015
Accepted 5 March 2015
Available online 11 March 2015

Keywords:
Capsicum annuum L.
CaPIP1-1
Gene expression
Abiotic stresses
Biotic stress

ABSTRACT

Plant aquaporins are responsible for water transmembrane transport, which play an important role on abiotic and biotic stresses. A novel plasma membrane intrinsic protein of *CaPIP1-1* was isolated from the pepper P70 according to transcriptome databases of *Phytophthora capsici* inoculation and chilling stress library. *CaPIP1-1*, which is 1155 bp in length with an open reading frame of 861 bp, encoded 286 amino acids. Three introns, exhibited CT/AC splice junctions, were observed in *CaPIP1-1*. The numbers and location of introns in *CaPIP1-1* were the same as observed in tomato and potato. *CaPIP1-1* was abundantly expressed in pepper fruit. Increased transcription levels of *CaPIP1-1* were found in the different stresses, including chilling stress, salt stress, mannitol stress, salicylic acid, ABA treatment and *Phytophthora capsici* infection. The expression of *CaPIP1-1* was downregulated by 50 μM $HgCl_2$ and 100 μM fluridone. The pepper plants silenced *CaPIP1-1* in cv. Qiemen showed growth inhibition and decreased tolerance to salt and mannitol stresses using detached leaf method.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Water is indispensable to life, and sufficient water supply is also essential for plants to survive. For horticultural crops, water supply has become an important restriction factor of high yield. About 70% of water was consumed on agriculture every year, according to statistics of UNFAO (Reuscher et al., 2013). In recent years drought has become more and more serious in Chinese agriculture, especially in the northwest area, in which drought even ruins the crop up.

Water supply has been studied extensively during the last few years. Water transport in different tissues mainly relies on plasma membrane intrinsic proteins (PIPs) (Zhou et al., 2007). Chaumont found that plant aquaporins (AQPs) play an important role in water transmembrane transport and improving efficiency of water use (Steudle, 2000; Chaumont et al., 2001). Further research revealed that the AQPs are not only responsible for water transport, but also transport some solutes across cell membranes (Soto et al., 2012). The AQP1 in animal cells can transport CO_2 in *Xenopus* oocyte, which may be controversial. Moreover, Uehlein et al. found that transmembrane transport CO_2 in tobacco is possible (Uehlein et al., 2003). Now more and more evidences show that AQPs also play a critical role in plant regeneration, cell elongation, opening stomata, fruit ripening and seed germination (Forrest and Bhave, 2007). Besides, AQPs have a positive effect on abiotic and biotic stresses of plants. Sreedharan et al. had reported that content of malondialdehyde (MDA) was lower compared with controls, but relative water content (RWC), proline content and photosynthetic efficiency were higher in transgenic *MusaPIP1;2* banana under abiotic stress. Furthermore, the transgenic lines also showed better recovery ability after different abiotic stresses (Sreedharan et al., 2013). Results of Siefritz et al. showed that inhibition of *NtAQP1* expression can decrease root hydraulic conductivity and result in lower capability of plant tolerance to water stress. All these results demonstrated the important role of AQPs in the water supply by the symplast pathway in plants (Siefritz et al., 2002). Yu et al. found that *OsPIP1-1* and *OsPIP2-1* might play a crucial role in recovery of water balance after chilling stress (Yu et al., 2006).

In recent years, many researchers have revealed the positive effect of PIPs, Aharon et al. found that PIP1b could enhance the vitality of transgenic tobacco under favorable growth condition, while it had some negative influence on plants under salt and drought stresses (Aharon et al., 2003). The activity of PIP1s subfamily was less than that of PIP2s in *Xenopus* oocyte (Jang et al., 2004), which might result from different gene structures, or the regulation of phosphorylation. In our previous study, we obtained some stress-related expressed

Abbreviations: AQPs, aquaporins; ESTs, expressed sequence tags; hpi, hour post inoculation; MDA, malondialdehyde; PIPs, plasma membrane intrinsic proteins; qRT-PCR, quantitative real-time PCR; RACE-PCR, rapid amplification of cDNA ends PCR; RWC, relative water content; SA, salicylic acid; SE, standard error; SSH, suppression subtractive hybridization; VIGS, virus induced gene silencing.

* Corresponding author.
E-mail address: zhgong@nwsuaf.edu.cn (Z.-H. Gong).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.gene.2015.03.012
0378-1119/© 2015 Elsevier B.V. All rights reserved.

sequence tags (ESTs) from the suppression subtractive hybridization (SSH) cDNA library (Guo et al., 2013) and the transcriptome after *Phytophthora capsici* infection, combined with the database of gene expression under chilling stress (Hwang et al., 2005). Then we selected stress-related EST and cloned the full-length of *CaPIP1-1* (GenBank accession no. JX402929) by rapid amplification of cDNA ends PCR (RACE-PCR). So far there were few reports on the expression characterization under stress and function of PIPs in pepper. In this study, we analyzed expression patterns of *CaPIP1-1* gene under different stresses by quantitative real-time PCR (qRT-PCR). Additionally, we also reported the function of *CaPIP1-1* under osmotic stresses with virus induced gene silencing (VIGS) method. This study will certainly contribute to explore the roles of PIPs genes in abiotic and biotic stresses.

2. Materials and methods

2.1. Plant materials and seedling treatment

Seeds of *Capsicum annuum* L. cv. P70 and Qiemen were used in this study. The seedlings were grown in a growth chamber at 25 °C with a 16 h light/8 h dark photoperiod cycle. Different stress [chilling stress, salt stress (0.15 M NaCl), mannitol stress (0.3 M), ABA treatment (100 μM), 5 mM salicylic acid (SA), 50 μM $HgCl_2$ and 100 μM fluridone] treatments were performed as previously described (Yin et al., 2014a). Leaves were harvested at 0, 1, 3, 6, 12 and 24 h, quickly frozen with liquid nitrogen and stored at −80 °C. Pepper plants were used for *P. capsici* infection (HX-9 strain) at the six-true-leaf stage with root-drenching method, and as described by Wang et al., the control plants were inoculated treated with sterile distilled water. All the infection plants were cultured according to the protocols (Wang et al., 2013b). The *P. capsici*-infected pepper leaves and roots were collected at 0, 6, 12, 24, 48 and 72 h after inoculation and stored at −80 °C for RNA isolation.

2.2. Cloning the CaPIP1-1 gene in pepper

The EST of transcriptome databases of *P. capsici* inoculation was used as the query probe to search against the pepper EST database at GenBank. According to the nucleotide sequences (GenBank accession no. GO345801, GD065213, BM064594 and CK901684), primers of *CaPIP1-1*F and *CaPIP1-1*R (Supplemental Table 1) were designed to identify if they come from the same gene. After that, the special gene primers were designed to clone the 5′ and 3′ DNA sequence using Smart RACE cDNA amplification kit. The primers of *CaPIP*F3 and *CaPIP*R3 were used to clone the full cDNA of the pepper *CaPIP1-1* gene with *pfu* polymerase.

2.3. RNA isolation and quantitative real-time PCR analysis

Reverse transcription was performed using the Primescript™ first strand cDNA Synthesis Kit (TaKaRa, Dalian, China). qRT-PCR was performed as described by Guo et al. (2012). qRT-PCR cycling conditions were as follows: 95 °C for 1 min and 45 cycles of 95 °C for 15 s, 52 °C for 20 s and 72 °C for 30 s. Fluorescence data were collected during the 52 °C step. The ubiquitin-conjugating protein (*CaUbi3*, accession no. AY486137.1), actin mRNA (*CaActin2*, accession no. AY572427) and translation elongation factor 1α (*CaTEF1A*, accession no. AF242732) were used as pepper reference genes (Wan et al., 2011; Wang et al., 2012).

2.4. VIGS assay of CaPIP1-1 in pepper plants

The TRV-based VIGS system was used for gene silencing as described previously (Wang et al., 2013a). To generate the *CaPIP1-1*/TRV2 construct, a 309 bp fragment near the 3′-end of the *CaPIP1-1* gene was PCR amplified from pepper. The resulting product was cloned into TRV2 vector using the double digested method with enzymes *of Xba* I and *Kpn* I. *Agrobacterium tumefaciens* strain GV3101 harboring pTRV1 was respectively mixed with pTRV2 (as the negative control), TRV2-*CaPDS* (as the positive control) or TRV2-*CaPIP1-1* at 1:1 ratio, then, the mixtures were inoculated into the fully expanded cotyledons of the cv. Qiemen. After injection, all the seedlings were kept at 18 °C and 60% relative humidity for 2 days, and then cultured in a growth chamber according to the protocol. The gene-silenced leaf discs were used for 0.1 M salt or 0.3 M mannitol stress. The special primers (*CaPIP1-1*F4 and *CaPIP1-1*R4, Supplemental Table 1) near the 5′-end were used to determine *CaPIP1-1* expression levels in silenced pepper. The PCR product was sequenced in Sangon Biotech Company (Shanghai, China).

2.5. Statistical analysis

Statistical analysis was performed using Statistical Analysis System software (SAS 8.2, North Carolina State University, USA), and the mean separation was analyzed using the Duncan's multiple range test, taking $p < 0.05$ as a significant difference. Values were expressed as the mean ± standard error (SE). All experiments were performed and analyzed separately with three biological replicates.

3. Results

3.1. Cloning, sequence character and structural analysis of the CaPIP1-1 gene in pepper

3.1.1. Cloning of CaPIP1-1 gene

The cDNA of cultivar "P70" was used as template in the cloning of *CaPIP1-1* gene. Firstly we predicted a 1100 bp fragment from the comparative analysis of transcriptome databases of *P. capsici* inoculation and chilling stress cDNA library. Then a 611 bp 5′-end fragment was cloned by 5′ RACE and a 590 bp 3′-end cDNA sequence was amplified by 3′ RACE. Finally, a full-length cDNA, designated *CaPIP1-1* and consisted of 1155 bp, was assembled using ContigExpress software and verified by sequencing the fragment with primers designed according to the assembled sequence (Supplemental Table 1). It is predicted that *CaPIP1-1* encoded 286 amino acids (Supplemental Fig. 1). Because of the high homology to *Arabidopsis* PIP1 family, we named this gene *CaPIP1-1* (Fig. 1).

3.1.2. Analysis of phylogenetic and transmembrane structure of CaPIP1-1

The amino acid sequence alignment of PIPs in different plants, including tomato, potato, tobacco, *Arabidopsis* and petunia, is shown in

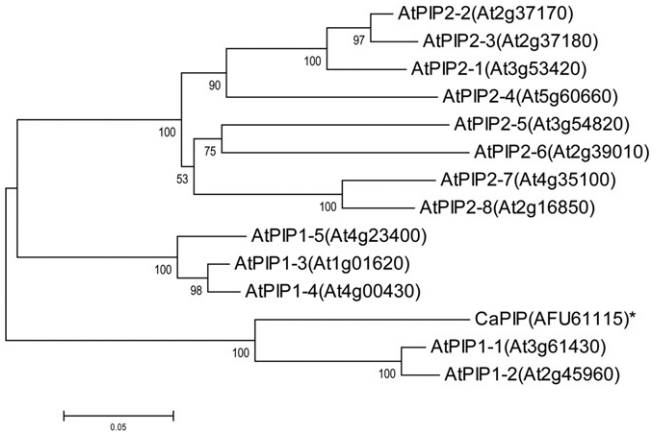

Fig. 1. Phylogenetic comparison of CaPIP1-1 (CaPIP) with PIP proteins from *Arabidopsis*. Phylogenetic tree generated using MEGA5.0 program. The rooted gene tree (majority-rule consensus from 1000 bootstrap replicates) resulted from heuristic searching in MEGA5.0. The amino acid sequences of 13 PIPs are from the *Arabidopsis* genome database. GenBank accession no. is in parentheses after each gene name. The asterisk indicates the CaPIP1-1 (CaPIP).

Download English Version:

https://daneshyari.com/en/article/5905446

Download Persian Version:

https://daneshyari.com/article/5905446

Daneshyari.com