Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A tomato proline-, lysine-, and glutamic-rich type gene *SpPKE1* positively regulates drought stress tolerance

Jinhua Li, Yaling Wang, Juanjuan Wei, Yu Pan, Chenggang Su, Xingguo Zhang*

Key Laboratory of Horticulture Science for Southern Mountainous Regions, Ministry of Education, College of Horticulture and Landscape Architechture, Southwest University, No.2 Tiansheng Road, Beibei, Chongqing, 400715, China

ARTICLE INFO

Article history:
Received 28 March 2018
Accepted 30 March 2018
Available online xxx

Keywords:
Abiotic stress
Drought stress
Proline-
lysine
Glutamic-rich protein
Wild species
Tomato

ABSTRACT

Plant abiotic resistance in cultivated species features limited variability. Using genes of wild species serves as a valid approach for improving abiotic resistance of cultivated plants. In this study, we uncovered a previously uncharacterized proline-, lysine-, and glutamic-rich protein gene (*SpPKE1*), which was isolated from drought-resistant wild tomato species *Solanum pennellii* (LA0716). When M82, which is a drought-sensitive tomato cultivar, was engineered to overexpress *SpPKE1*, its tolerance under drought stress was significantly improved by the accumulation of more chlorophyll, proline, and limited malondialdehyde compared with that in RNA interference (RNAi)-suppression lines, which were more sensitive than the wild-type plants. Several ion transporter genes, abiotic-related transcriptional factors, and reactive oxygen species-scavenging genes were upregulated in *PKE1* overexpression (OE) lines but downregulated in RNAi plants. OE of *SpPKE1* enhanced drought tolerance in tobacco. Screening results of yeast two-hybrid protein−protein interaction revealed that SpPKE1 can bind to an F-box protein that plays an important role in plant drought resistance. We posited that *PKE1* enhanced drought tolerance by modulating the expressions of stress-responsive genes and interacting with the F-box protein.

1. Introduction

Abiotic stresses, such as drought, salinity, or cold, are defined as any change in the surrounding environmental factors that can adversely affect crop quality and/or yield. Therefore, improved stress tolerance is an important breeding trait [1]. Plant exposure to drought or salt stress triggers numerous common reactions [2]. Drought is one of the major abiotic stresses limiting plant production, and it also affects morphological, physiological, and biochemical progress in plant growth and development. Global climate change and water shortage also highlight the need to improve plant drought resistance [3]. Tomato (*Solanum lycopersicum* L.) is a tropical plant that is well adapted to almost all climatic regions worldwide; however, environmental stress factors, specifically drought, act as major constraints of the potential yield of this crop [4,5].

Cultivated tomato (*S. lycopersicum*) is sensitive to drought [6]. However, *Solanum pennellii* is a drought-resistant wild tomato adapted to the coastal cliffs of Peru and is indigenous to arid and semi-arid environments in South America [7]. Genetic engineering of the expression of stress-responsive genes is a key approach for improving abiotic stress resistance, and the best gene sources for cultivated tomato include other wild species of the genus [8]. Overexpression (OE) of *SpWRKY1*, which is isolated from the wild tomato species *S. pimpinellifolium*, promoted tolerance to salt and drought stress in transgenic tobacco [9]; an early response to the dehydration gene *SpERD15* was cloned from *S. pennellii*, in which heterologous OE in tobacco enhanced multiple abiotic resistance [10]. Considerable evidences suggest that transgenic plants with engineered endogenous genes produce abiotic stress-tolerant phenotypes. *S. lycopersicum* ethylene response factor B.3 (SlERF.B.3) antisense transgenic plants exhibited salt- and cold stress-dependent growth inhibition [11]; abscisic acid (ABA)-responsive element binding protein (AREB1), which is a tomato bZIP transcription factor, is involved in ABA signals that increase tolerance to drought stress in tomato [12]. Other functional proteins are also used to improve abiotic stresses tolerance. The tomato plasma membrane Na+/H+ antiporter SlSOS1 is essential for salt tolerance [13], whereas the tomato K^+/H^+ antiporter NHX2 confers salt tolerance and plays an important role in response to salt stress by improving K^+ accumulation [14,15].

* Corresponding author. Tel: +86 23 68250974; fax: +86 23 68251274
E-mail address: zhangdupian@swu.edu.cn (X. Zhang).

https://doi.org/10.1016/j.bbrc.2018.03.222
0006-291X/

In the present study, a proline-, lysine-, and glutamic-rich protein gene (*PKE1*) was isolated from *S. pennellii* LA0716. *PKE1* was differentially expressed after exposing *S. lycopersicum* cv. M82 and *S. pennellii* LA0716 to drought treatment [6]. To date, the function of *PKE1* in plant remains unclear. Therefore, functional analysis was performed on this gene in tomato and tobacco. *SpPKE1* OE can significantly enhance drought resistance in tomato and tobacco. The SpPKE1 protein physically interacted with an F-box protein, which is reportedly associated with drought tolerance. To the best of our knowledge, this study is the first to reveal the positive regulating effect of tomato *PKE1* on drought tolerance.

2. Materials and methods

2.1. Gene isolation, vector construction, and tomato transformation

The tomato *SpPKE1* coding sequence (CDS) was amplified with polymerase chain reaction (PCR) from *S. pennellii* LA 1706 cDNA by using gene-specific primers (GSPs: *PKE1*-OE), as shown in Supplementary Table S1, on the basis of unigene sequences (*SpPKE1*: Sopen05g032700, http://solgenomics.net/). The resulting product was inserted into the binary vector pMV (pBI121 reformed) to yield the overexpressing construct with *SpPKE1*, which was driven by a cauliflower mosaic virus 35S promoter.

RNA interference (RNAi) vector was constructed by amplifying a 374 bp fragment from *SlPKE1* (Solyc05g054210) CDS by using GSPs with 5′-attB1 and 5′-attB2 extensions on forward and reverse primers, respectively (Supplementary Table S1). A recombination reaction was performed between PCR products and pHellsgate 2 vector (Invitrogen, USA) by using BP clonase (Invitrogen) in accordance with the manufacturer's instructions.

The plasmids were transformed into tomato cultivar M82 and tobacco (*Nicotiana nudicaulis*) by *Agrobacterium tumefaciens* (strain C58)-mediated transformation.

2.2. RNA isolation and quantitative reverse transcription–PCR (qRT-PCR)

RNA isolation and qRT-PCR were performed as previously described [16]. Each sample included three replicates, and each assayed sample represented three independently collected samples. Expressions of tomato abiotic-related genes were analyzed by real-time PCR in wild-type (WT) and transgenic plants. Supplementary Table S1 lists the qRT-PCR primer sequences.

2.3. Drought tolerance testing of transgenic plants

To evaluate the drought tolerance of transgenic lines, uniform-sized T_3 homozygous three-leaf stage tomato seedlings were transplanted individually into cylindrical pots (diameter: 8 cm, height: 15 cm) and nourished to grow up to the six-leaf stage for drought treatment. The seedlings in the pots were placed in plastic square plates (length × width × height: 60 cm × 40 cm × 10 cm). Drought stress was initiated by discharging water in the plastic square plates. After water deprivation for 14 days, which is a period that can typically cause serious drought stress (i.e., some leaves died, and most leaves completely rolled) in this environment, the plants were re-irrigated for recovery. Then, survival rate was investigated, and chlorophyll, malondialdehyde (MDA), and proline contents were measured. Chlorophyll content was measured by Lichtenthaler's method [17]. MDA was assayed for indirect evaluation of lipid peroxidation using thiobarbituric acid as previously described [18]. Proline was extracted using a previously described procedure [19]. The drought tolerance test for T_2 tobacco was similar to that for tomato, and the simulating drought treatment of seedlings was performed as previously described [16].

2.4. Yeast two-hybrid (Y2H) screening and assay

Full CDS of *SpPKE1* was obtained by PCR following amplification of the cDNA sequence with the use of primer SpPKE1BD (Supplementary Table S1). A PCR fragment was fused to the frame with GAL4 DNA-binding domain pGBKT7 vectors. The bait construct pGBKT7-SpPKE1 was transformed into the yeast strain Y187 through lithium acetate method. Interacting clones were screened through mating in accordance with manufacturer's instructions (Clontech, USA). Full-length CDS of the interacting gene was cloned into the pGADT7 vector to confirm the results of Y2H screening (Primer as shown in Supplementary Table S1). The resulting plasmid and pGBKT7-SpPKE1 plasmid were co-transformed into the yeast strain AH109 through lithium acetate method.

2.5. Statistical analysis

Drought treatment of transgenic lines and WT was performed twice with three replicates for each line. All studied plants displayed nearly identical results. The results of representative experiments were expressed as mean ± standard error (SE). Data were analyzed by analysis of variance using SAS software (version 8.0, SAS Institute, NC, USA), and statistical differences were compared using Fisher's least significant difference test.

3. Results

3.1. Isolation and characterization of PKE1 in tomato

On the basis of published comprehensive transcriptomes of two drought tolerant *S. pennellii* introgression lines, differential expression profile of the *PKE1* gene was observed between drought-tolerant introgression lines and M82 [6]. Full-length *PKE1* cDNAs were isolated from *S. pennellii* LA0716 by using RT-PCR and were labeled *SpPKE1*. Among the 319 amino acids of SpPKE1, proline accounted for 18.5% of the total amino acid residues, followed by lysine (16.3%) and glutamate (11.3%). These amino acid residues accounted for 46.1% of the total residues (Supplementary Table S2). A similar search of the GenBank database revealed that PKE1 in tomato shared a conserved N-terminus proline-, lysine-, and glutamic-rich repetitive abundance region. A significant degree of sequence identity was observed in seven cysteine motifs (7CM) following three repetitive “KEPEK” residues with other PKE1s from various species (Fig. 1).

3.2. OE of PKE1 in transgenic plants enhances tolerance to drought stress

For further functional analysis of *PKE1*, 22 *SpPKE1* OE and 16 RNAi-knockdown (Ri) transgenic plants were generated, and *PKE1* expression levels of these transgenic plants were examined. Then, the significant differentially expressed *PKE1* relative to WT M82, OE (OE2 and OE5), and knockdown *PKE1*-RNAi (Ri1 and Ri5) plants was selected for further analysis (Supplementary Fig. S1). Drought stress assays were conducted on these plants. At the beginning of drought stress (0 day), the transgenic plants showed no abnormal morphological phenotype compared with WT plants. Stress-induced symptoms with wilting of lower leaves were visible at 4 days after drought treatment. At day 7, the WT and RNAi plants exhibited wilting of the upper leaves, whereas the upper leaves of the OE plants grew well. After 14 days, all plants showed reduced growth and were completely chlorotic, implying the collapse of

Please cite this article in press as: J. Li, et al., A tomato proline-, lysine-, and glutamic-rich type gene *SpPKE1* positively regulates drought stress tolerance, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.222