Journal of Integrative Agriculture 2018, 17(2): 348–358

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Identification and expression of the *CEP* gene family in apple (*Malus×domestica*)

LI Rui[1], AN Jian-ping[1], YOU Chun-xiang[1], SHU Jing[2], WANG Xiao-fei[1], HAO Yu-jin[1]

[1] National Key Laboratory of Crop Biology, National Research Center for Apple Engineering and Technology/College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an 271018, P.R.China
[2] Shandong Agriculture and Engineering University, Jinan 251100, P.R.China

Abstract

Plant peptide hormones play important roles in plant growth and development. Among these hormones, the C-TERMINALLYENCODED PEPTIDE (CEP) belongs to a newly found peptide family that regulates root development in *Arabidopsis* as well as in other species. However, nothing is known about the *CEP* genes in apple (*Malus×domestica*, *MdCEP*). In this study, a total of 27 apple *CEP* genes were identified through a genome-wide analysis and were phylogenetically divided into three classes (I, II and III). The predicted *MdCEP* genes were distributed across 10 of 17 chromosomes with different densities. Next, the gene structures and motif compositions of the *MdCEP* genes were analyzed. Subsequently, the expression analysis suggested that the *MdCEP* genes were highly activated in roots and that *MdCEP23* may play an important role in regulating the growth and development of roots. Moreover, all of the *MdCEP* genes were responsive to multiple abiotic stresses, indicating that *MdCEP* genes may be involved with various aspects of physiological processes in apple. Nearly one-third of *MdCEP* genes had a significant response to low nitrogen treatment. Most of the *MdCEP* genes were up-regulated under stress, including mannitol, polyethylene glycol (PEG) and abscisic acid (ABA), suggesting that *MdCEP* genes may be involved in the drought stress response. This study provides insight into the putative functions of the *MdCEP* genes using a genome-wide analysis of the *CEP* gene family.

Keywords: peptide signals, *CEP* gene family, expression analysis, apple

1. Introduction

The majority of signaling peptides are small cleavage products of precursor peptides. Several of these precursors must be post-translationally modified to form mature peptides, which usually contain 20 amino acids (Butenko *et al*. 2009; Murphy *et al*. 2012). In the *Arabidopsis* genome, over 1 000 putative small signaling peptides have been predicted, and some of them have been functionally identified, including C-TERMINALLY ENCODED PEPTIDE1 (CEP1), CLAVATA3 (CLV3), CLV3/EMBRYO SURROUNDING REGIONRELATED (CLE), and RAPID ALKALINIZATION FACTOR (RALF) (Czyzewicz *et al*. 2013). Small and secreted regulatory peptides are a growing class of signaling molecules that are involved in regulating plant developmental programs and adapting to extreme environment *via* cell-to-cell communica-

---

Received 2 February, 2017 Accepted 7 May, 2017

LI Rui, E-mail: liruisdau@163.com; Correspondence HAO Yu-jin, E-mail: haoyujin@sdau.edu.cn; WANG Xiao-fei, E-mail: xfwang2004@163.com

doi: 10.1016/S2095-3119(17)61653-8

tion (Katsir *et al*. 2011; Murphy *et al*. 2012). Several families of regulatory peptides have been functionally identified and have been found to participate in this process (Butenko *et al*. 2009; Czyzewicz *et al*. 2013; Lee *et al*. 2015; Song *et al*. 2016).

Among the peptide families, the C-TERMINALLYENCODED PEPTIDE (*CEP*) genes contain a conserved 15-amino acid peptide domain at or near the C-terminus and are characterized in *Arabidopsis* (Ohyama *et al*. 2008). The post-translationally modified CEP family members contain an N-terminal secretion signal (NSS) and one or more conserved CEP domains (Mohd-Radzman *et al*. 2015). *CEP* genes are widely present among gymnosperm and angiosperm plants, but absent in land plants that lack true branching roots or root vasculature, indicating that their emergence coincides with the evolution of seed plants (Delay *et al*. 2013; Ogilvie *et al*. 2014). Members of the *CEP* family have already shown to regulate plant lateral root and root nodule development as well as root/shoot growth (Delay *et al*. 2013; Roberts *et al*. 2013; Mohd-Radzman and Laffont 2016). For example, overexpression of *AtCEP1* or treatment with chemically synthesized CEP1 peptide in *Arabidopsis* results in a reduction in the number of emerged lateral roots and the inhibition of primary root growth (Ohyama *et al*. 2008). In *Medicago*, overexpression of *MtCEP1* increases nodulation by promoting rhizobial infections and exhibits repression in lateral root development (Imin *et al*. 2013; Mohd-Radzman and Laffont 2016). In addition, *CEP* genes are reported to be negative regulators that mediate environmental influences on plant development (Delay *et al*. 2013). The *AtCEP3* loss-of-function mutant enhances root development under adverse environmental conditions (Delay *et al*. 2013).

Currently, an enormous number of possible peptide ligand-receptors (kinases) has been identified and several receptor-like proteins such as CLV1/2, FER, and CEPR1/2 have been found to be involved with root development through interaction with peptides (Kondo *et al*. 2011; Du *et al*. 2016; Roberts *et al*. 2016). The leucine-rich repeat (LRR) receptor kinases CEP RECEPTOR 1 (CEPR1; At5g49660) and CEP RECEPTOR 2 (CEPR2; At1g72180) have been shown to be the receptor for CEP1 and other CEPs (Bryan *et al*. 2012; Tabata *et al*. 2014; Roberts *et al*. 2016). Further studies may reveal that CEPs and CEPR1 participate in the N-dependent responses in long-distance systemic signaling pathways (Okamoto *et al*. 2016).

Apple (*Malus*×*domestica*) is one of the most widely cultivated fruit crops worldwide and is the most economically important woody plant in temperate regions (Dimick and Hoskin 1983; Lee *et al*. 2007). The draft genome sequence of apple has been completed, which allows genome-wide analyses of specific gene families (Velasco *et al*. 2010). Genome-wide analyses of the *CEP* genes have been reported in *Arabidopsis thaliana* (Delay *et al*. 2013) and *Oryza sativa* (Sui *et al*. 2016), and several gene families have been identified in apple. However, there is no genome wide data regarding the apple *CEP* genes. In brief, small signaling peptides play an essential role in all stages of plant growth and development. The characterization of apple CEP peptides provides insight into the molecular mechanism of apple root growth and the responses to different environmental factors.

In this study, a genome-wide analysis of the *CEP* gene family was conducted using the apple genome database, and the chromosome locations and gene structures of the putative *CEP* genes were analyzed. Next, the motif compositions of the *MdCEPs* were obtained using the MEME Program. Subsequently, the expression patterns of *MdCEP* genes in different tissues and in response to abiotic stresses were analyzed. This study provides a foundation for future research into the functional roles of *MdCEP* genes.

2. Materials and methods

2.1. Identification and annotation *MdCEP* genes in apple

To identify members of the *CEP* gene family, all known *Arabidopsis* CEP protein sequences were retrieved from the database of Institute for Genomic Research (TIGR) and used as queries in BLASTP searches against the Genome Database for Rosaceae (GDR) (http://www.rosaceae.org/). Stand-alone versions of BLASP (http://blast.ncbi.nlm.nih.gov), which are available from NCBI, were used with the e-value cutoff of 1e-003. Then, the predicted *CEP* gene family sequences were downloaded from the GDR database. All of the protein sequences that were derived from the selected *MdCEP* candidate genes were examined with the domain analysis programs Pfam (http://pfam.sanger.ac.uk/) and Simple Modular Architecture Research Tool (SMART; http://smart.embl-heidelberg.de/), with the default cutoff parameters.

2.2. Chromosomal locations and gene structures of *MdCEP* genes

The chromosomal locations and gene structures were retrieved from the apple genome data that were downloaded from the GDR database. The chromosomal map showing the physical location of all of the *MdCEP* genes was generated with the MapDraw Software and the gene structures of the *MdCEP* genes were generated with GSDS (http://gsds.cbi.pku.edu.cn/). The isoelectric point (pI) and molecular weight of MdCEPs were obtained with the assistance of proteomics and sequence analysis tools on the ExPASy Proteomics Server (http://expasy.org/). All putative MdCEPs