Journal of Integrative Agriculture 2016, 15(6): 1239–1255

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RESEARCH ARTICLE

Phenylalanine ammonia-lyase gene families in cucurbit species: Structure, evolution, and expression

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Abstract

Phenylalanine ammonia-lyase (PAL), the first enzyme of phenylpropanoid pathway, is always encoded by multigene families in plants. In this study, using genome-wide searches, 13 *PAL* genes in cucumber (*CsPAL1–13*) and 13 *PAL*s in melon (*CmPAL1–13*) were identified. In the corresponding genomes, ten of these *PAL* genes were located in tandem in two clusters, while the others were widely dispersed in different chromosomes as a single copy. The protein sequences of CsPALs and CmPALs shared an overall high identity to each other. In our previous report, 12 *PAL* genes were identified in watermelon (*ClPAL1–12*). Thereby, a total of 38 cucurbit *PAL* members were included. Here, a comprehensive comparison of *PAL* gene families was performed among three cucurbit plants. The phylogenetic and syntenic analyses placed the cucurbit PALs as 11 CsPAL-CmPAL-ClPAL triples, of which ten triples were clustered into the dicot group, and the remaining one, CsPAL1-CmPAL8-ClPAL2, was grouped with gymnosperm PALs and might serve as an ancestor of cucurbit PALs. By comparing the syntenic relationships and gene structure of these *PAL* genes, the expansion of cucurbit *PAL* families might arise from a series of segmental and tandem duplications and intron insertion events. Furthermore, the expression profiling in different tissues suggested that different cucurbit *PAL*s displayed divergent but overlapping expression profiles, and the *CsPAL-CmPAL-ClPAL* orthologs showed correlative expression patterns among three cucurbit plants. Taken together, this study provided an extensive description on the evolution and expression of cucurbit *PAL* gene families and might facilitate the further studies for elucidating the functions of PALs in cucurbit plants.

Keywords: phenylalanine ammonia-lyase (PAL), gene family, cucurbit, evolution, expression

1. Introduction

The phenylpropanoid pathway is one of the most important secondary metabolism pathways in higher plants. This pathway engenders a vast of aromatic metabolites, such as flavonoids, isoflavonoids, anthocyanins, plant hormones, phytoalexins, and lignins (Vogt 2010). These metabolites are critically important for the growth, development, and environmental adaption of plants. Some of aromatic compounds have high economic value (Zhang and Liu 2015).

Received 27 May, 2015 Accepted 4 February, 2016
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doi: 10.1016/S2095-3119(16)61329-1

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.4) is the first committed enzyme in the phenylpropanoid pathway, catalyzing the non-oxidative deamination of phenylalanine to *trans*-cinnamate, a common substrate of various phenylpropanoid compounds (Ferrer *et al.* 2008). Increased PAL activity is always correlated with the increased production of phenylpropanoid products (Vogt 2010).

Over the decades, many studies have shown that PAL is encoded by a multiple gene family in plants. Several copies of *PAL* genes have been discovered, including four in *Arabidopsis* and tobacco (Fukasawa-Akada *et al.* 1996; Raes *et al.* 2003; Reichert *et al.* 2009), five in poplar (Hamberger *et al.* 2007), 12 in watermelon and rice (Dong and Shang 2013; Rawal *et al.* 2013), 16 in grapevine (Rawal *et al.* 2013), and more than 20 in tomato and potato (Joos and Hahlbrock 1992; Chang *et al.* 2008). The expansion of *PAL* gene families in plants is likely due to gene duplication, including tandem duplication, segmental duplication, and whole-genome duplication (Shang *et al.* 2012; Dong and Shang 2013).

Functional differentiation follows the duplication events of plant *PAL* genes. The different isoforms of PAL contribute to the differential roles in plant development. For example, in *Arabidopsis*, *PAL1*, *PAL2*, and *PAL4* are strongly expressed in inflorescent stems, a tissue that is rich in lignifying cells, whereas the *PAL3* transcript is expressed at a very low level, suggesting the potential roles of *PAL1*, *PAL2* and *PAL4* in tissue-specific lignin synthesis (Raes *et al.* 2003). Both *PAL2* and *PAL4* are expressed in seeds, whereas only *PAL1* expression is localized to vascular tissues (Raes *et al.* 2003; Rohde *et al.* 2004). A phenotypic analysis with the single and multiple mutants of *PAL* genes also revealed similar results (Olsen *et al.* 2008; Huang *et al.* 2010). In raspberry (*Rubus idaeus*), two *RiPAL* genes have been identified, of which *RiPAL1* is associated with early fruit-ripening events, whereas *RiPAL2* is correlated more with later stages of flower and fruit development (Kumar and Ellis 2001). The expression of *PAL* can also be induced by a variety of environmental stresses, including pathogen infection (Liang *et al.* 1989; Huang *et al.* 2010; Kim and Huang 2014), wounding (Liang *et al.* 1989), nutrient depletion (Huang *et al.* 2010), UV irradiation (Huang *et al.* 2010), and extreme temperatures (Huang *et al.* 2010; Dong *et al.* 2014). The individual *PAL* gene may respond differentially to distinct environmental stimuli. Taken the four *Arabidopsis PAL* genes for example, *PAL1* and *PAL2* are co-induced by phytopathogenic pseudomonas, and in response to nitrogen depletion and low temperature, only *PAL1* and *PAL2* transcript levels strongly increase (Olsen *et al.* 2008). In bean, three *PAL* genes (*PAL1-3*) are activated by wounding, whereas only *PAL1* and *PAL3* can be induced by fungal infection (Liang *et al.* 1989).

The botanical family cucurbit includes many economically important cultivated plants, such as cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.)), and pumpkin (*Cucurbita spp*). Agricultural production of cucurbits utilizes nine million ha of land and yields 184 million t of vegetables, fruits, and seeds annually (http://faostat.fao.org). The cucurbit plants also serve as the ideal model systems for fruit ripening, vascular physiology, and comparative genomic studies (Pech *et al.* 2008; Zhang *et al.* 2010). In this family, cucumber and melon belong to the same genus, with their ancestors diverging 10.1 million years ago, while watermelon is a distant relative, with a speciation event occurring approximately 15–23 million years ago (Garcia-Mas *et al.* 2012; Guo *et al.* 2012). Noticeably, the draft genome sequences of these three cucurbit plants have been released (Huang *et al.* 2009; Garcia-Mas *et al.* 2012; Guo *et al.* 2012). In our previous reports, some primary analyses have been performed on cucumber and watermelon *PAL* gene families, and seven and 12 *PAL* genes were identified, respectively (Shang *et al.* 2012; Dong and Shang 2013). Recently, with the release of a new annotation version (2.0) of the cucumber genome, the cucumber *PAL* family might be updated. Also, to reveal the comprehensive evolutionary relationships of cucurbit *PAL* gene families, more information should be required, especially in melon whose genome sequences would facilitate our analyses.

In this study, *in silico* searches were conducted to the latest version of genomes of cucumber (ver. 2.0) and melon, and totally 13 *CsPAL* and 13 *CmPAL* genes are identified. These genes, together with the *ClPAL* genes from watermelon (Dong and Shang 2013), were used to explore their phylogenetic relationships and expansion histories of cucurbit *PAL* gene families. Then, the expression profiles of these *PAL* members in different tissues were compared. Our findings would contribute to understanding the evolution of *PAL* genes in cucurbit plants and provide a basis for further functional studies of cucurbit *PAL* genes.

2. Results

2.1. Identification of the *PAL* genes from cucumber and melon

To update the entire cucumber *PAL* (*CsPAL*) gene family, the cucumber genome database (ver. 2.0) was searched using the known *CsPAL* genes (Shang *et al.* 2012) as the queries. As a result, six more *CsPAL* genes were retrieved. Besides the previously identified *CsPAL* genes, a total of 13 potential *CsPAL* members were identified. According to their order on chromosomes 1, 4 and 6, we renamed these genes as *CsPAL1*–*CsPAL13* (Table 1). Of the 13 *CsPAL* genes, three (*CsPAL1*, *CsPAL5* and *CsPAL6*) were dispersed on