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YUCCA type auxin biosynthesis genes encoding flavin monooxygenases in melon: Genome-wide identification and developmental expression analysis

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ABSTRACT

Auxin, the first plant hormone identified more than 80 years ago, is critical for nearly all aspects of plant life. As its diverse action and signaling in plants, auxin biosynthesis also has manifold facets. Recently, a simple two-step pathway of auxin biosynthesis called the TAA/YUC pathway has been proposed to be the main source of IAA in plants. Melon, a global crop, holds a key position in the Cucurbitaceae family for its high economic value and as a model to study biologically relevant characters. Here, we identified the *YUCCA* (*YUC*) family in this model of genetic system for Cucurbitaceae species. The genome of melon contains at least 9 loci for YUC-like FMOs and 7 members of the family have been successfully isolated in this study. Quantitative RT-PCR analysis shows that *CmYUCs* are differentially expressed in nearly all melon young organs. All seven *CmYUCs* were found to be expressed in certain stage(s) of melon fruits. Of the family, *CmYUC6* and *CmYUC11* are quite noticeable due to their high expression in seeds and mesocarp of fruits, respectively. In sum, this work supports that the TAA/YUC pathway to IAA may have a role in melon fruit development.

1. Introduction

Auxin, the first hormone identified in plant (Went, 1935), is known to be involved in nearly all aspects of plant life. During the growth and development of plants, auxin functions in apical dominance, tropic responses, root and shoot architecture, vascular differentiation, embryo patterning, and so on (Teale et al., 2006). In particular, auxin plays a critical role in fruit development, beginning with flower formation and patterning of the gynoecium, through fruit set, fruit enlargement, fruit ripening and abscission (Sundberg and Østergaard, 2009; Pattison et al., 2014). It has been proposed that auxin might act as a kind of cellular currency, permitting many different "transactions" to occur (Stewart and Nemhauser, 2010). Auxin might actually *do nothing*, but it is impetus and rather motivates everything (Bennet and Leyser, 2014). The manifold facets of auxin are reflected not only in its action and signaling, but also in its biosynthesis (Sauer et al., 2013; Brumos et al., 2014; Tivendale et al., 2014; Zhao, 2014).

Despite indole-3-acetic acid (IAA), naturally occurring compounds with auxin activity described in plants consist also of phenylacetic acid (PAA) (Okamoto et al., 1967), 4-chloroindole-3-acetic acid (4-Cl-IAA) (Engvild, 1980), and indole-3-butyric acid (IBA) (Ludwig-Muller and Epstein, 1991). IAA, the main natural auxin in plants, can be produced from de novo biosynthesis, released from IAA conjugates, or converted from IBA (Brumos et al., 2014). Previous biochemical studies suggested the existence of two general routes for IAA biosynthesis: the tryptophan (Trp) dependent and Trp-independent routes (Normanly et al., 1993). Four interconnected Trp-dependent IAA biosynthetic pathways have been proposed, each named after the intermediate immediately downstream of Trp—the IAOx (indole-3-acetaldoxime), IAM (indole-3-acetamide), TAM (Tryptamine), and IPyA (indole-3-pyruvic acid) pathways (Tivendale et al., 2014). However, the relative biological significance of these pathways is still uncertain. It is believed that even if one pathway is found to operate in a particular organ of a particular species, it is often not possible to extrapolate that finding directly to other species, or even to other organs of the same species (Tivendale et al., 2014).

The simple two-step IPyA pathway is also called the TAA/YUC pathway, where IPyA produced from Trp by TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1(TAA1) and TAA1 related (TAR) is converted to IAA by the YUCCA (YUC) family of flavin monooxygenases (Mashiguchi et al., 2011; Stepanova et al., 2011; Abu-Zaitoon et al., 2012). The TAA/YUC pathway is highly conserved in the plant kingdom and is probably the main auxin biosynthesis pathway, because disruption of this pathway affects many developmental and responsive events

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in several species (Gao and Zhao, 2014). Unlike overexpression of *YUC*s, overexpression of *TAA*s does not lead to any obvious developmental phenotypes, suggesting that TAAs-catalyzed reaction might not be a rate-limiting step in auxin biosynthesis (Stepanova et al., 2008; Tao et al., 2008). YUC was first identified as a key enzyme catalyzing a rate-limiting step in tryptophan-dependent auxin biosynthesis from *Arabidopsis* (Zhao et al., 2001). Biochemical studies on YUC-mediated auxin biosynthesis revealed that YUC enzymes use NADPH and molecular oxygen to catalyze the oxidative decarboxylation of IPA to generate IAA (Mashiguchi et al., 2011; Dai et al., 2013). Auxin from the TAA/YUC pathway is important for plant growth and development in many species, namely *Arabidopsis* (Zhao et al., 2001; Cheng et al., 2006, 2007; Zhao, 2010; Wang et al., 2011; Bai et al., 2013), rice (Woo et al., 2007; Yamamoto et al., 2007; Fujino et al., 2008), maize (Gallavotti et al., 2008; Bernardi et al., 2012), petunia (Tobeña-Santamaria et al., 2002), tomato (Expósito-Rodríguez et al., 2007, 2011), and strawberry (Liu et al., 2012, 2014). Recently, a wide range of progresses have been achieved in metabolic and transcriptional regulation of YUCs mediated IAA biosynthesis (Zhao, 2014).

Melon (*Cucumis melo* L.) belongs to the *Cucumis* genus, *Cucurbitaceae* family, which includes several other vegetables of economic importance such as cucumber, watermelon (Pitrat, 2008). It is a global crop and has one of the highest polymorphic fruit types and botanical varieties (Nuñez-Palenius et al., 2008). Melon has a key position in the Cucurbitaceae family for its high economic value and as a model to study the biologically relevant characters, such as fruit ripening and sex determination (Ezura and Owino, 2008; Pech et al., 2008; Martin et al., 2009). International Cucurbit Genomics Initiative (ICuGI) was launched in 2005, in which melon became a model species for Cucurbit genomics research (http://www.icugi.org). Most cultivated melons are diploid. Diploid melon has 24 chromosomes (haploid stage n = x = 12), and 83.3% of its genome (estimated, 454 Mbp/1C) has been sequenced and released (Arumuganathan and Earle, 1991; Garcia-Mas et al., 2012).

Fruits are the most commonly eaten part of the melon plant, being appreciated for their unique flavor (in particular the sweetness) and nutritious qualities. Among the different parts of a melon plant, fruits have the highest diversity in size, form, external ornamentation, and internal and external color (Kirkbride, 1993).The fruit is generally classified as an indehiscent "pepo", which is a modified berry, with three ovary sections or locules; the fleshy fruit often has a leathery, non-septate rind derived from an inferior ovary (Lin et al., 1991). The edible flesh is derived from the placenta or mesocarp tissue. Melon fruit development is a complex, integrated biological process. However, two general phases have been recognized: early development characterized by tight spatial and temporal regulation of cell division and cell expansion and subsequently, the fruit ripening phase characterized by important nutritional changes, including taste, flavor and texture (Gillaspy et al., 1993).

Auxin-derivative plant hormones, such as *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and naphthaleneacetic acid, as well as chemicals with cytokinin activity such as 6-benzylaminopurine and CPPU promote melon fruit set (Hayata et al., 2000, 2002). Changes in the endogenous IAA activity during melon fruit development were inconsistently reported: Lingle and Dunlap (1991) indicated that IAA content reached the highest levels before anthesis, and then decreased until melon fruit harvest; Lee et al. (1997) suggested that IAA levels remained constant throughout melon fruit development. Examination of the endogenous IAA content in different parts of melon fruit (cv. Crest Earl's) via GC-MS-SIM obtained probably more precise results: endogenous IAA levels in the seeds of pollinated fruit were the highest at 10 DAA, then decreased, and increased again after 30–45 DAA; the changing patterns in the placenta and mesocarp were very similar to those in the seeds, although IAA levels in the later two parts of fruit were lower than those in the seeds (Li et al., 2002).

Although it is evident that auxin biosynthesis is directly linked to melon fruit growth and development, the details of this connection at the molecular level remain completely obscure. Here, to understand how melon synthesizes auxin, we examined the existence of *YUC*-type auxin biosynthesis gene family in melon genome and characterized these genes at molecular and transcriptional levels. To the best of our knowledge, this is the first report on genes encoding key enzymes for auxin biosynthesis in melon, which will provide a good foundation for further investigation on auxin-regulated melon development at genetic and biotechnological levels.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of melon (commercial name Jingyu No. 352) used in this study were from local agricultural materials store. Based on the classification suggested by Pitrat et al. (2000) and Lin (2012), this cultivar belongs to *C. melo* var. *chinensis* Pangalo. It has been bred by National Engineering Research Center for Vegetables (Beijing, China). 'Jingyu' melon was grown in a plastic-mulched cultivation system at the trial station of Shanghai Academy of Agricultural Sciences (SAAS) in Zhuanghang Town, Fengxian District, Shanghai. In this system, 'Jingyu' melon produces fruits from May to July. Full maturity required 28 ± 2 days after anthesis (DAA). The ripe fruits are white, round, with strong fragrance, of 500 gram in average weight, and 9–10 cm in horizontal diameter (HD).

For expression analysis of *CmYUC*s in different organs, seven types of organs including roots (about 1 cm root apical tips), vines (about 2 cm vine apical tips), leaves (4–8 cm^2 in size), shoot apical meristems (SAM, about 0.5 cm in length), tendrils (about 4 cm apical part), Bs-Flowers (bisexual flowers at anthesis), and male flowers at anthesis were sampled from fruiting plants. In particular, fruits were selected and dissected at 4 consecutive developmental stages: Fruit-I (whole fruits at 3 DAA, about 1.5 cm in HD), Fruits-II (7 DAA, the beginning of fast enlargement stage, about 3.5 cm in HD), Fruit-III (14 DAA, about 5.5 cm in HD), and Fruit-IV (21 DAA, still in fast enlargement stage, about 7 cm in HD). For Fruit-I and -II, whole fruits were used for RNA analysis. For Fruit-III and IV, flesh (inner mesocarp tissue comprising the edible fruit flesh, and the mesocarp near the seed cavity and the rind-outer mesocarp tissue were removed) and seeds were separated and used for RNA isolation, respectively. All samples were immediately frozen in liquid nitrogen and stored at −74 °C before RNA isolation. Plant sampling was independently repeated twice.

2.2. Gene identification and phylogenetic analysis

To identify melon homologs of *YUC* genes, a BLAST search (default values) was performed against the *C. melo* WGS (whole-genome shotgun reads) database in NCBI using all 11 *Arabidopsis YUC* genes (Cheng et al., 2006) as query sequences. Thereafter, HMM-based gene structure prediction was carried out using the FGENESH program (default values) at http://linux1.softberry.com/berry.phtml. To confirm these candidates, the conserved FAD and NADPH binding motifs were identified at http://pfam.sanger.uk. These melon YUC loci were named following the guidance of phylogenetic analysis with their *Arabidopsis* homologs.

Multiple sequence alignment was first performed using the MUSCLE program at http://www. ebi.ac.uk/Tools/msa/muscle/ (Chenna et al., 2003). Based on the alignment result, phylogenetic analysis was conducted using MEGA 5.02 software (Tamura et al., 2011). The phylogenetic tree was constructed by the neighbor-joining method and bootstrap method was used for phylogeny test. The bootstrap values (%) of 1000 replicates were shown at the branching points. In addition to CmYUCs, other YUCs analyzed include YUC1–11 (AT4G32540, AT4G13260, AT1G04610, AT5G11320, AT5G43890,