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Genome-wide identification and analysis of FK506-binding protein family gene family in strawberry (*Fragaria* × *ananassa*)

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ABSTRACT

The FK506 binding proteins (FKBPs) are abundant and ubiquitous proteins belonging to the large peptidyl-prolylcis-trans isomerase superfamily. FKBPs are known to be involved in many biological processes including hormone signaling, plant growth, and stress responses through a chaperone or an isomerization of proline residues during protein folding. The availability of complete strawberry genome sequences allowed the identification of 23 *FKBP* genes by HMMER and blast analysis. Chromosome scaffold locations of these *FKBP* genes in the strawberry genome were determined and the protein domain and motif organization of FaFKBPs analyzed. The phylogenetic relationships between strawberry FKBPs were also assessed. The expression profiles of *FaFKBPs* genes results revealed that most *FaFKBPs* were expressed in all tissues, while a few *FaFKBPs* were specifically expressed in some of the tissues. These data not only contribute to some better understanding of the complex regulation of the strawberry *FKBP* gene family, but also provide valuable information for further research in strawberry functional genomics.

1. Introduction

The FK506 binding proteins (FKBPs) belong to the superfamily of peptidyl-prolylcis-trans isomerase (PPIase, EC5.2.1.8) which is present in all organisms and almost all subcellular compartments (Galat, 2004; He et al., 2004). The PPIase catalyzes the isomerization of the peptide bond between a proline and a generally bulky residue (Barik, 2006; Fanghänel and Fischer, 2004). This naturally slow reaction occurs after protein synthesis, which produces peptide bonds on the amino acid of the proline in the open trans-conformation (Kay, 1996). The cis-trans interconversion accelerated by PPIase is significant for the final protein structure because cisproline introduces bends within the protein (Harrar et al., 2001).

The FKBPs are a distinct set of cellular receptors which bind the immunosuppressive drugs FK506 and rapamycin. The complexes formed by the FKBPs and their ligands are the functional modules for immunosuppression and therefore the *FKBPs* are named also immunophilins (Harding et al., 1989; Siekierka et al., 1989). When FKBPs bind these drugs, their peptidyl-prolylcis-trans isomerase activity is inhibited (Magiri et al., 2006; Schreiber et al., 1991).

FKBPs are defined by the presence of at least one 'FK506-binding domain' (FKBd), a conserved sequence of approximately 110 amino acids (Galat, 2000; Somarelli and Herrera, 2007). Structural modeling of FKBPs from organisms separated by large evolutionarily distances, e.g., mammals and plants, has demonstrated that the FKBd forms a highly conserved tertiary structure (Somarelli et al., 2008) that provides the active site for substrate isomerization (PPIase activity) (Heitman et al., 1991; Siekierka et al., 1990). Significant size variation exists among members of the FKBP family, ranging from single domain (SD) isoforms comprising a single FKBd to large (>100 kDa) complex multi-domain (MD) proteins (Geisler and Bailly, 2007; Nigam et al., 2008). The most extensively characterized SD isoform is the mammalian FKBP12. In mammals, FKBP12 binds the macrolides FK506 and rapamycin forming a complex that inhibits the phosphatase activity of calcineurin and/or the kinase activity of TOR (target of rapamycin) leading to inhibition of downstream signal transduction (Breiman and Camus, 2002; Geisler and Bailly, 2007; He et al., 2004). MD FKBPs (high-molecular weight) contain up to three FKBds. The well-known MD FKBP in mammals is FKBP52 that possesses two successive FKBds, a tetratricopeptide repeat (TPR) domain and a C-terminal calmodulin-binding domains (CaM-BDs) (Callebaut et al., 1992; Radanyi et al., 1994) and is associated with the native glucocorticoid receptor complex. FKBP51, the homologous of FKBP52, is considered to compete with FKBP52 for binding to HSP90 (heat streak protein 90) via their TPR acceptor site to regulate hormone dependent recruitment of transcription factors to the nucleus (Davies et al., 2005). Most of the FKBP52 subcellular expression was reported in the nucleus with the rest being localized to microtubules in

Abbreviations: FKBPs, FK506-binding proteins; FKBd, FK506-binding domain; PPIase, peptidyl-prolylcis-trans isomerase; TPR, tetratricopeptide repeat; CaM-BDs, calmodulin-binding domains; SD, single-domain; MD, multidomain; TOR, target of rapamycin; HSP90, heat streak protein 90; CDS, coding sequence; RT-PCR, semi-quantitative reverse transcription PCR; ER, endoplasmic reticulum.

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the cytoplasm. Similarly, plant MD FKBPs also contain tetratricopeptide repeat (TPR) domains and C-terminal calmodulin-binding domains (CaM-BDs), both are known to mediate protein–protein interactions (Breiman and Camus, 2002; He et al., 2004; Romano et al., 2005; Shangguan et al., 2013).

With the development of bioinformatics and release of plant genome sequences, *FKBP* family genes have been discovered in different species such as in *Arabidopsis thaliana*, *Zea mays* L, *Oryza sativa* and *Vitis vinifera* (Ahn et al., 2010; Gollan and Bhave, 2010; He et al., 2004; Nigam et al., 2008; Shangguan et al., 2013; Yu et al., 2012). Four *FKBP* genes are expressed in *Saccharomyces cerevisiae* (Dolinski et al., 1997) and 15 in human (Rulten et al., 2006). The plant genome encodes the largest *FKBP* gene family described so far, with 23 identified in *A. thaliana* (He et al., 2004) and 29 in the monocot rice (Gollan and Bhave, 2010). *Arabidopsis* 23 *FKBP* genes include 16 single-domain *FKBPs* and seven multidomain *FKBPs*. FKBPs have been isolated from the cytosol (Xu et al., 1998), nucleus (Carol et al., 2001) and ER (Luan et al., 1996) of plants, and may also occur in the mitochondrion (Breiman et al., 1992), however, the majority of isoforms in plants is targeted to the chloroplast thylakoid (Friso et al., 2004; Peltier et al., 2002; Schubert et al., 2002). Although historically linked to immunosuppression and proline bond rotation, the physiological importance of FKBPs extends beyond FK506-binding and general protein folding, to signal transduction, chloroplast function, DNA transcription, protein trafficking, apoptosis, and fertility (Gopalan et al., 2004; Heitman et al., 1992; Kang et al., 2008; Luan et al., 1994; Meiri et al., 2010; Yu et al., 2012). Through various interacting protein partners, *FKBP12* has been shown to regulate the cell cycle (Aghdasi et al., 2001; Vespa et al., 2004) and participate in control of pollen tube growth direction (Yu et al., 2011). The wheat FKBP73 and FKBP77 proteins and the closely related *Arabidopsis* FKBPs, FKBP62 (ROF1), and FKBP65 (ROF2), were induced by wounding, NaCl stress (Vucich and Gasser, 1996) and malondialdehyde treatment, which induces the expression of genes involved in abiotic stress responses (Weber et al., 2004). The AtFKBP42 was shown to regulate efflux of auxin (Bailly et al., 2008). In maize and *Chlamydomonas reinhardtii* but *Arabidopsis* (Menand et al., 2002), FKBP12 binds rapamycin (Agredano-Moreno et al., 2007; Crespo et al., 2005), forming a complex that inhibits the TOR kinase, a powerful regulator of plant germination and development. Although the FKBP family is large, in higher eukaryotes the specific biological role of each member is unique and one member cannot completely complement the absence of another one (Breiman et al., 2002). Therefore, it will be necessary to characterize independently each member of FKBP family for revealing their unique functions.

Strawberry (*Fragaria* × *ananassa*) is one of the most economically important fruit crops worldwide and has a long history of cultivation as well as high value economically and nutritionally (Dong et al., 2012). With the release of strawberry genome sequence and development of bioinformatics tools and resources (http://bioinformatics.ca/links_directory/), mining of strawberry family genes is becoming possible, which is important to research on strawberry functional genomics. In this study, two sequence search tools and strawberry genome dataset were used for strawberry *FKBP* family gene identification, accompanied with sequence alignment and protein structural analysis. In addition, the reliability of predicted *FKBP* members was also analyzed. The results of this study can be useful in further functional analysis of the *FKBP* family genes in strawberry.

2. Materials and methods

2.1. Retrieval of FKBP gene sequences

Strawberry genome sequence has been completed, and filtered protein and CDS sequences have also become available. Several approaches were used to identify the *FKBP* genes from strawberry. Putative *FKBPs* of strawberry were identified via BLAST searching of the strawberry sequence database (http://www.rosaceae.org/) and the amino acid sequence translated from coding sequence of the archetypal FKBP12 in *A. thaliana* as query. HMMER 3.0 software was obtained from the HMMER website (http://hmmer.janelia.org/), and BLAST (Standalone) software was retrieved from NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/).

HMMER and BLAST were used to screen the putative *FKBP* genes from strawberry genome sequences using default search parameters. *FKBP* family gene domain model file (FKBP_C, pfam00254) was downloaded from the Sanger database (http://pfam.sanger.ac.uk/family/ PF00254#curationBlock), and mining all strawberry peptide sequences by HMMER3 software. All the putative strawberry FKBP peptide sequences were submitted to NCBI Conserved Domains Search website tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) to check whether they contained the FK506-binding domain. Finally, BLAST was used to remove the repetitive sequences of strawberry *FKBP* family genes.

2.2. Multiple sequence alignment and phylogenetic tree construction

All putative strawberry *FKBP* peptide sequence alignment was carried out in MacVector v11.04 (available at http://www.macvector.com/downloads.html) using the global alignment program ClustalW (Thompson et al., 1994) with the default parameters. The consequential alignment was used to create a phylogenetic tree in MEGA version 4.0 (available at http://www.megasoftware.net) using the Neighbor-joining method (Saitou and Nei, 1987), and bootstrapping set at 1000 replications.

2.3. Chromosome location and conserved domain search

Genomic locations of putative strawberry *FKBPs* were identified through the NCBI–BLAST function in the Genome Database for Rosaceae (GDR) website (http://www.rosaceae.org/bio/content?title=&url=http://app.bioinfo.wsu.edu/blast/blast_noheader.html&style=width:950px;height:950px;). Conserved FKBP, TPR and CaM-binding domains were identified in putative MD FKBPs using the Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and verified by manual alignment with those in corresponding *Arabidopsis* FKBPs (He et al., 2004).

2.4. Protein structure analysis

The number of amino acids, molecular weight, and theoretical pI of putative strawberry FKBPs were obtained from the Prot-Param analyses (http://cn.expasy.org/tools/protparam.html) (Guruprasad et al., 1990; Ikai, 1980; Kyte and Doolittle, 1982) on the basis of their sequence. Protein secondary structure element prediction was conducted using the SOPMA server (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) (Geourjon and Deléage, 1995). Putative amino acid sequences of various FKBPs were submitted to the SWISS-MODEL Workspace server (http://swissmodel.expasy.org) (Arnold et al., 2006). The proteins models were viewed and edited using DeepView/Swiss PdbViewer v4.0 software (http://spdbv.vital-it.ch).

2.5. Subcellular localization prediction

Computer programs were used to predict the subcellular locations with WoLF PSORT (http://wolfpsort.org) and TargetP 1.1 server (http://www.cbs.dtu.dk/services/TargetP).

2.6. Plant material

Strawberry cultivar was 'Ningyu', which was a new, early-maturing strawberry cultivar derived from 'Sachinoka' × 'Akihime', with disease resistance. Young leaves (10 days), mature leaves (50 days), flowers