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The *CYP707A* Gene Family in Strawberry (*Fragaria × ananassa*)

Hugo Carlos Bolzon Gonzalez[1]
https://orcid.org/0000-0002-9551-6012

Vanessa Galli[1]*
https://orcid.org/0000-0002-5735-9749

[1]Federal University of Pelotas, Technological Development Center, Pelotas, Rio Grande do Sul, Brazil.

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*Correspondence: vane.galli@yahoo.com.br; Tel.: +55-53-32757350 (V.G.)

HIGHLIGHTS

- Abscisic acid is catabolized by CYP707A enzymes
- *CYP707A* gene family was characterized in strawberry
- Phylogenetic, network interaction and gene expression analysis were performed
- *CYP707A* genes are involved in the salt stress response in strawberry.

Abstract: Abscisic acid (ABA) is a plant hormone that plays several roles in plant development. The de novo synthesis and the reversible inactivation of ABA have been largely described in the literature; however, the degradation of ABA, promoted by the enzymes Abscisic Acid 8'-Hydroxylase, encoded by the *CYP707A* gene family, is still poorly elucidated. Strawberry (*Fragaria x ananassa*) has been used as a model to study the ABA-dependent maturation process of non-climacteric fruits, and the ABA-dependent response to abiotic stress. However, the *CYP707A* genes from this species have not been fully described and characterized. In this perspective, *FaCYP707A* sequences were identified from strawberry fruit transcriptome and several structural and comparative genomic analyzes were performed. Moreover, the expression of the *FaCYP707A* sequences identified was investigated in fruits under salt stress and ABA application. Four putative *FaCYP707A* were identified and the structural analysis confirmed the identity of three of them. The phylogenetic analysis allowed to determine their homologous in other plant species and to predict their evolutionary history; and the expression profile of the *FaCYP707As* demonstrated that *FaCYP707A3* seems to be involved in the response against salt stress in an ABA-dependent manner. Moreover, the interaction network analysis pointed out proteins involved in the ABA metabolism, heavy metal homeostasis and detoxification, and cell wall dissemble. This study characterized for the first time the *CYP707A* gene family in *F. ananassa*; this information will guide future studies in order to develop biofortified fruits and stress tolerant plants.

Keywords: gene identification; phylogenetic analysis; stress response; non-climacteric fruits; abscisic acid.

INTRODUCTION

The strawberry (*Fragaria χ ananassa* Duch.) is a pseudo fruit of economic importance because its cultivation, processing and commercialization involves several workers. Its high demand is related to the sensorial characteristics, including taste, aroma and color, it is also rich in bioactive compounds, particularly ascorbic acid and phenolic compounds [1-3]. These compounds show health beneficial properties, as antioxidant, antioncogenic, neuroprotective, antimicrobial activity, among others [4].

Despite the high demand of this crop, strawberry is very sensitive to salt stress, with variation on the tolerance rate between different cultivars. Salinity has been pointed as cause for reduction on plant growth and fruit yield, as well as leaf edge burn, necrosis or nutritional imbalance. These effects are influenced by the increasing levels of salinity and by the cultivar tolerance. For example, a recent study [5] compared seven different cultivars of strawberry under salt stress and indicated that “Camino Real”, “Benicia”, “Chandler”, and “Radiance” were less tolerant, while “Albion”, “Camarosa”, and “San Andreas” were more tolerant.

Previous studies have reported the role of abscisic acid (ABA) in the response of strawberry against salt stress [6-8]. Understand how the content of ABA is regulated in plant cell represents an opportunity to develop more resistant plants. The ABA metabolic pathways can be divided in three: the de novo synthesis pathway is widely described, and is promoted by the NCED enzymes (9-cis epoxycarotenoid dioxigenase); the reversible inactivation by glycosylation pathway is promoted by the enzymes BGs / GTs (β-glucosidases / UDP-glucosyltransferases) and have also been described; and the irreversible inactivation pathway is promoted by the enzymes CYP707A (Abscisic Acid 8 '- Hydroxylase 4), and is still poorly characterized. In the irreversible inactivation pathway, ABA is converted to phaseic acid (PA) and later to dehydrophaseic acid (DPA) [9-12].

Regulation of *CYP707A* has been shown to affect the dynamic balance of ABA [13, 14]. The increase or loss of function of the *CYP707A2* gene in Arabidopsis affects the level of ABA in seedlings, affecting their sensitivity to exogenous glucose [14]. Moreover, the genes *PacCYP707A1* and *PacCYP707A3* in cherry are positively regulated under water stress and ABA treatment [15]. In Arabidopsis, as in other species, different *CYP707A* genes plays different roles in plant metabolism, and are regulated according to the tissue, developmental stage, and environmental stimuli [13-16].

For *Fragaria ananassa*, only one complete sequence of *CYP707A* gene had been described in the literature by Ji and coauthors [17]. Recently, our research group carried out the assembly and annotation of the transcriptome of strawberry fruits submitted to saline stress and water deficit [6]. Through a deep search in this transcriptome, in the present study we identified four *CYP707A* genes in *Fragaria x ananassa*. Our analyses provided detailed information on these *FaCYP707As*, including phylogenetic tree, genomic structures and network interaction. In addition, the expression profile of *FaCYP707A* genes was investigated in response to salt and ABA application, providing new information for further studies and biotechnology applications.

MATERIAL AND METHODS

Identifying possible *FaCYP707A* sequences in the strawberry transcriptome

Nineteen *CYP707A* sequences from three different species (*Pyrus pyrifolia*, *Prunus avium*, and *Malus x domestica)* were searched and selected in GenBank database (*https://www.ncbi.nlm.nih.gov/genbank/*) and are shown in Table 1. The sequences selected were blasted via Multiblast tool into *Fragaria ananassa* Camarosa transcriptome obtained previously, aiming to identify possible homologous sequences, using CLC Genomics Workbench 10 – Qiagen® (*https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-clc-genomics-workbench/*).

Table 1. Table showing the list of genes selected from GenBank database to be used in the search for *CYP707A* homologous in the strawberry transcriptome

Species	NCBI Identifier
Prunus avium	>GU559990.1
Prunus avium	>GU559989.1
Prunus avium	>GU559988.1
Prunus avium	>GU559987.1
Pyrus pyrifolia	>JF825450.1
Pyrus pyrifolia	>LC155802.1
Pyrus pyrifolia	>KP723487.1
Pyrus pyrifolia	>KP723486.1
Pyrus pyrifolia	>KP723485.1
Pyrus pyrifolia	>KP723484.1
Pyrus pyrifolia	>KP723483.1
Pyrus pyrifolia	>KP279631.1
Pyrus pyrifolia	>KP279630.1
Pyrus pyrifolia	>KP162149.1
Pyrus pyrifolia	>KP162148.1
Pyrus pyrifolia	>KP057206.1
Pyrus pyrifolia	>JN602256.1
Malusχ domestica	>AB593331.1
Malus χ domestica	>AB593330.1

Open reading frame (ORF) identification

The biggest contigs identified in the transcriptome as putative *FaCYP707A* homologous were submitted to the ORFfinder tool *(https://www.ncbi.nlm.nih.gov/orffinder/),* using as parameters: Minimal ORF length (nt): 150 nt; Genetic code: 1. Standard; ORF start codon to use: 'ATG' only; and Ignore nested ORFs: Not marked. This methodology was applied to obtain the possible open reading frames (ORFs) for each sequence.

Characterization of putative *FaCYP707A* sequences

The ORF sequences were used as query in the SmartBlast *(https://blast.ncbi.nlm.nih.gov/smartblast/smartBlast.cgi)* and BlastP *(https://blast.ncbi.nlm.nih.gov/Blast.cgi)* tools, aiming to analyze the existence of conserved protein domains, super domains and any other information that may help to identify the protein families, as compared to the sequences already indexed in the NCBI database.

The amino acid sequence for each *FaCYP707A* was predicted using MEGA X software *(https://www.megasoftware.net/)*. SignalPHMM 5.0 *(http://www.cbs.dtu.dk/services/SignalP/)*, HMMSmart *(http://smart.embl-heidelberg.de/)* and TargetP 2.0 *(http://www.cbs.dtu.dk/services/TargetP/)* were then used to search for the presence of any signal peptide and to predict the possible subcellular localization of the proteins.

Alignment of putative *FaCYP707A* sequences

The sequences obtained from the ORFfinder were aligned using ClustalW algorithm in the CLC Genomics Workbench 12.0.2.

CYP707A gene sequences search using the *Fragaria ananassa* Genome

The same ORF sequences were blasted against the *Fragaria ananassa* Camarosa Genome, which is assembled as scaffolds and is available at *Genome Database for Rosaceae*. The CLC Genomics Workbench 12.0.2 software was used to perform this analysis and the sequences found with the highest homology were aligned in the same software, using ClustalW algorithm.

Phylogenetic analysis

To evaluate the evolutionary history of the *CYP707A* sequences, the same 19 *CYP707A* sequences from *Pyrus pyrifolia*, *Prunus avium* and *Malus x domestica* described in Table 1, as well as *CYP707A*

sequences from *Arabidopsis thaliana* (AT4G19230, AT2G29090, AT5G45340 and AT3G19270), and the putative *CYP707A* from strawberry were aligned using the ClustalW algorithm in the MEGA X software. Then, a phylogenetic tree was built using the neighbor joining method, with bootstrap values of 1000.

Network Interaction

STRING 11 (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database that aim to provide analysis of known and predicted protein-protein interactions in an organism, also known as protein network, allowing the study of interactions including direct (physical) as well as indirect (functional) association, on a global scale.

Therefore, a specific interaction network was constructed using textmining, databases, co-expression, neighborhood, gene fusion, co-occurrence and experimental evidence in the online STRING 11 (http://string-db.org/), using *Fragaria×vesca* homologous from the putative *FaCYP707A* genes.

Plant materials and stress treatments

The study was conducted on a greenhouse, using the cultivar Camarosa. The seedlings with similar crown size (approximately 9 mm diameter) were planted and grown in 9 L pots, containing soil (Ultisoil) and vermiculite (in a proportion of 3:1). The fertilization and irrigation were performed according to Galli and coauthors. [18]. The relative humidity in each pot was monitored using a hygrometer in order to maintain between 16% and 19%, without water leaching. The experiment was composed by four treatments with six replicates per treatment and ten plants per replicate. The plants were subjected to the following treatments: C - Control; SS – Salt Stress; ABA+SS – exogenous application of 200 µM ABA + Salt Stress (SS); ABA - exogenous application of 200 µM ABA. For SS, 50 mL of 400 mM NaCl was applied once a week. The stress level was based on preliminary experiments. ABA was obtained by Sigma Aldrich and was applied weekly by foliar spraying. The ABA and SS treatments were performed from the beginning of the flowering stage (45 days after transplanting) to the end of the experiment (95 DAT). An approximate pool of ten ripe fruits (fully red, according to Jia and coauthors. [19]) by replicate were harvested. All samples were immediately frozen in liquid nitrogen and stored at -80 °C until further analysis.

Gene expression analysis

To analyze the effects of salt stress and/or ABA exogenous application on *CYP707As* gene expression, the total RNA from strawberry mature fruits was isolated using CTAB (cetyltrimethylammonium bromide) according to Galli and coauthors. [18]. Meanwhile, aiming to evaluate the pattern of expression in the different development stages, samples were collected in six periods, representing: 7 (small green, SG), 14 (big green, BG), 18 (degreening, DG), 21 (white, W), 24 (partial red, PR), and 28 (full red, FR) days after anthesis (DAA), as previously reported [19]. Fifteen uniformly sized fruits at each stage were collected and separated into three pools (5 fruits for each repetition). Samples of Leaf (L) and Root (R) from five different plants were also collected, immediately frozen in liquid nitrogen and stored at -80 °C until analysis. The RNA concentration was quantified using NanoVue®. Total RNA was treated with DNAse and reverse transcribed using the M-MLV enzyme and oligo-dT primers, according to the manufacturer's instructions (Invitrogen). cDNA was obtained according to Galli and coauthors. [18]. The reference genes PIRUV_DESCARB (pyruvate decarboxylase), DBP (DNA binding protein), and HISTH4 (histone H4) were used to normalize transcription levels, as proposed by Galli and coauthors. [18]. The primers used are presented the same as Perin and coauthors. [7]. The relative expression data were calculated according to the $2^{-\Delta\Delta Cq}$ method. The analysis was performed for four biological replicates and three analytical replicates. The results were submitted to analysis of variance (ANOVA) and when significant ($p \leq 0.05$) were submitted to a mean comparison using Tukey test at 5% of probability. Statistical analyzes were performed using SAS software.

RESULTS

Identifying possible *FaCYP707A* sequences in the strawberry transcriptome

The search for *FaCYP707A* genes using homologous sequences from other species allowed the identification of four putative coding sequences for *FaCYP707A* in strawberry: *CL15525Contig1 (FaCYP707A1), CL17770Conting1 (FaCYP707A2), CL18454Contig1 (FaCYP707A3)* and *CL24483Contig1 (FaCYP707A4).* The *CL15525Contig1 (FaCYP707A1)* sequence was previously described by Jia and coauthors. [19].

ORF identification

In the ORFfinder tool, only the biggest ORF sequence for each contig found in the transcriptome were selected. The *FaCYP707A1* had an ORF sequence of 1446 nt, and was the longest sequence identified. The shortest sequence was from *FaCYP707A4* which had an ORF sequence of 291 nt. Each *FaCYP707A* and their respective longest ORF sequence were described in Table 2.

Table 2. The ORF information for each *FaCYP707A* sequence.

CONTIG	TOTAL NT[1]	FRAME	Initial	End	ORF NT[2]	ORF AA[3]
FaCYP707A1	1753	-3	1670	225	1446	481
FaCYP707A2	1603	-3	1520	225	1296	431
FaCYP707A3	895	-2	840	>1	840	279
FaCYP707A4	653	-2	484	194	291	96

1 – Total Nucleotides; 2 – ORF Nucleotides; 3 – ORF amino acids.

Characterization of putative *Fa**CYP707A* sequences

By using the SmartBlast, it was possible to identify the sequences with higher identity and coverage among the sequences available in the database. *FaCYP707A3* showed higher similarity with a sequence from *Arabidopsis thaliana,* while *FaCYP707A1* and *FaCYP707A2* showed higher similarity with a *Glicine max* sequence.

The length of *FaCYP707A1* and *FaCYP707A2* sequences, compared to similar sequences in the Genbank database, suggests that they represent complete sequences. Moreover, for all sequences the leading match was with Abscisic Acid 8' – Hydroxylase, assuring their identification as *CYP707A* sequences. The matching sequences shown by SmartBlast are described in Table 3.

Table 3. Data shown by the SmartBlast search for each *FaCYP707A* sequence.

Contig	Species match	Description	Cover	Identity	E-value
FaCYP707A1	Glycine max	abscisic acid 8'-hydroxylase 4-like	99%	71%	0.0
FaCYP707A2	Glycine max	abscisic acid 8'-hydroxylase 4	99%	64%	0.0
FaCYP707A3	Arabidopsis thaliana	cytochrome P450, family 707, subfamily A, polypeptide 4	100%	73%	1e-154
FaCYP707A4	Glycine max	abscisic acid 8'-hydroxylase 4	97%	80%	1e-50

The BlastP and HMMSmart search was able to identify the presence of a P450 super domain and a CypX domain in the sequences of *FaCYP707A1*, *FaCYP707A2* and *FaCYP707A3,* showing a gap in the middle of the P450 super domain from *FaCYP707A2* sequence.

The SignalPHMM 5.0 and TargetP 2.0 query showed the presence of a signal peptide in *FaCYP707A1* and *FaCYP707A2* among with a transmembrane region between the amino acid 2 and 21 of the sequences.

Alignment of putative *FaCYP707A* sequences

FaCYP707A2 has a gap of 150 nucleotides compared to *FaCYP707A1*. This gap is located between nucleotide 556 and 705 of the major sequence. On the other hand, *FaCYP707A3* and *FaCYP707A4* sequences show a region of homology with *FaCYP707A1* and *FaCYP707A2*, from nucleotide 743 to 1415; in the rest of these sequences, only a few dispersed homology nucleotides were observed throughout the sequences.

FaCYP707A gene sequence search using the *Fragaria ananassa* Genome

The search for *FaCYP707A* sequences in the *Fragaria ananassa* cultivar Camarosa Genome showed that *FaCYP707A1* and *FaCYP707A2* matched with almost the same sequences, while *FaCYP707A3*

matched with completely different sequences. *FaCYP707A4* matched with some of the same sequences as *FaCYP707A1* and *FaCYP707A2* and with other sequences not matched by the other *FaCYP707A*. The matches for each *CYP0707A* in the *Fragaria ananassa* Genome are shown in Table 4.

Table 4. Each *FaCYP707A* sequence and their respective matches in the *Fragaria ananassa* Genome.

CYP707A	IDENTIFIER
CYP707A1	>ORD_ID5978 >ORD_ID65946 >ORD_ID87415 >ORD_ID86795
CYP707A2	>ORD_ID5978 >ORD_ID65946 >ORD_ID87415 >ORD_ID86795
CYP707A3	>ORD_ID90279 >ORD_ID67618 >ORD_ID3487
CYP707A4	>ORD_ID5978 >ORD_ID87415 >ORD_ID86795 >ORD_ID65946 >ORD_ID5966

Phylogenetic analysis

As shown in Figure 1, *FaCYP707A1* and *FaCYP707A2* are in the same clade as *PaCYP707A4* (>GU559989.1) from *Prunus avium* and *PpCYP707A2* from *Pyrus pyrifolia* of 2 different cultivars (Mixue and Huanghua - >KP723484.1 and >KP162148.1 respectively). Otherwise, *FaCYP707A3* is in the same clade as *MdCYP707A1* (>AB593330.1) and *PpCYP707A1* from *Pyrus pyrifolia* of the 2 different cultivars (Mixue and Huanghua - >KP723483.1 and >KP057206.1, respectively). These results suggest that *FaCYP707A3* diverged in the evolution history before *FaCYP707A1* and *FaCYP707A2*. The placing of *FaCYP707A4* next to the *Arabidopsis thaliana* sequence (>NC_003071.7) seems to represent a mistake in the align algorithm because the *FaCYP707A4* is the smallest sequence used in the analysis, and the *A. thaliana* sequence is the biggest, suggesting that in the lack of a true match with other *CYP707A* sequence, the algorithm understood that those sequences are close related.

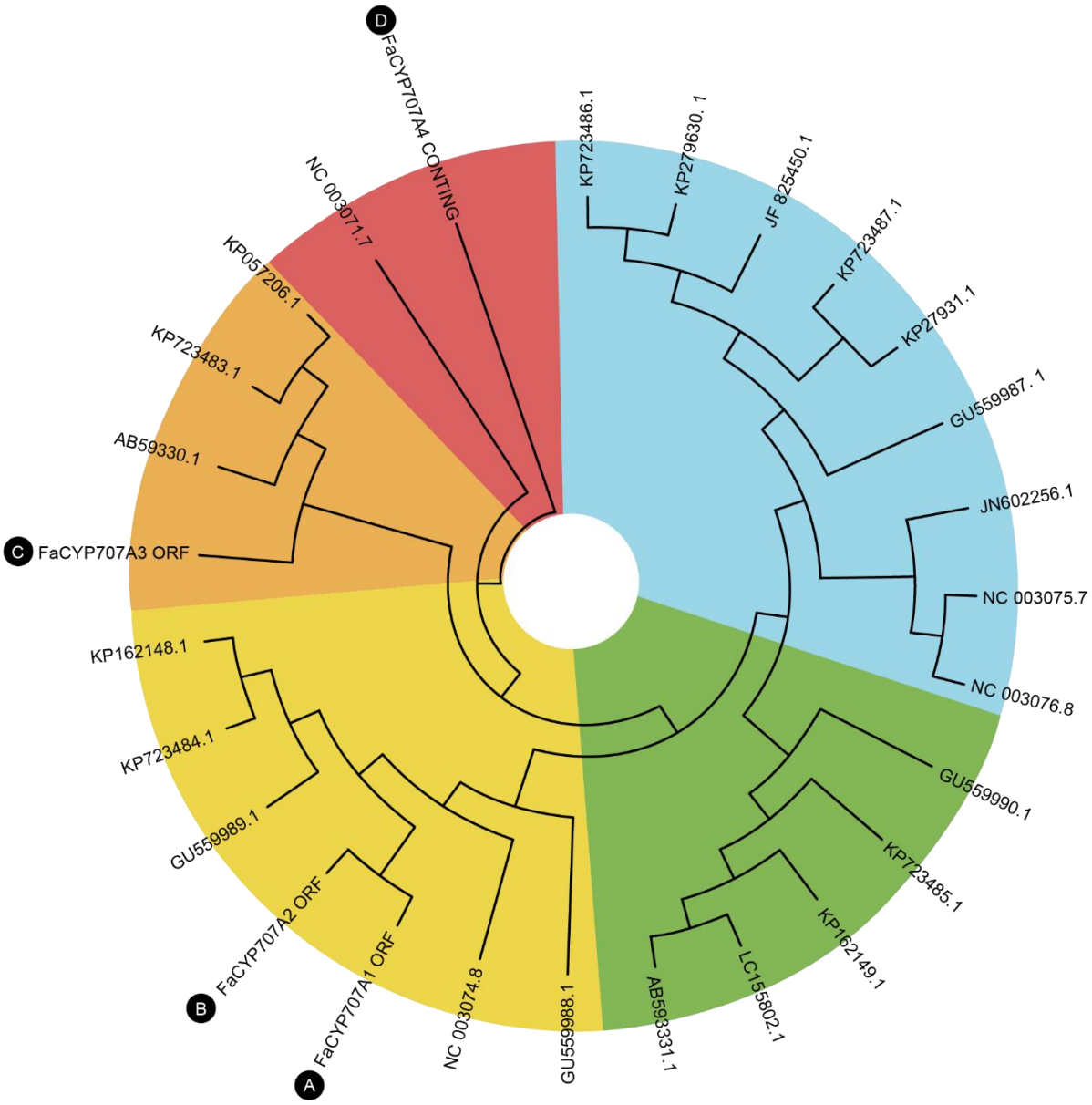

Figure 1. Phylogenetic tree of *FaCYP707A* genes built with ClustalW algorithm and Neighbor Joining method.

Network Interaction

When the amino acid sequences of *Fa*CYP707A1, *Fa*CYP707A2, *Fa*CYP707A3 and *Fa*CYP707A4 from *Fragaria ananassa* were submitted to STRING 11.0 Database, the *Fragaria vesca* sequences with greater homology for each sequence were presented. *Fa*CYP707A1 and *Fa*CYP707A2 showed greater identity with the sequence Abscisic Acid 8'-Hydroxylase 3 (XP_004291107.1), being 97% for *Fa*CYP707A1 and 87% for *Fa*CYP707A2. *Fa*CYP707A3 showed greater homology (99%) with the Abscisic Acid 8'-Hydroxylase 4 sequence (XP_004294805.1). *Fa*CYP707A4 showed no homology with any sequence present in the database. In addition, *Fa*CYP707A1, *Fa*CYP707A2 and *Fa*CYP707AA3 showed partial homology with two other sequences: Abscsic Acid 8'-Hydroxylase 1 (XP_004300683.1) and Abscisic Acid 8'-Hydroxylase 2 (XP_004295971.1). The interactome obtained in this study is presented in Figure 2.

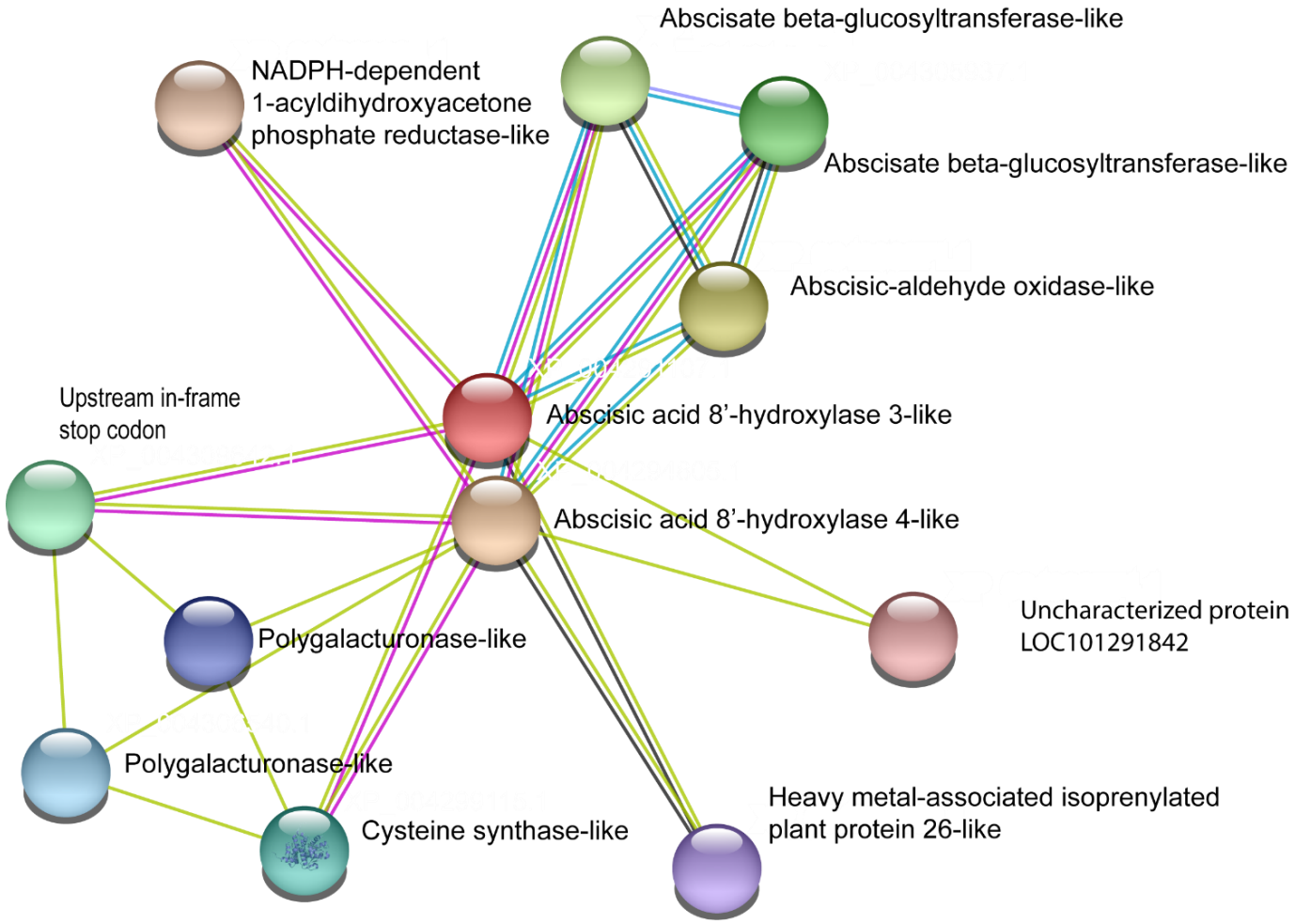

Figure 2. Network interactions of *Fragaria vesca* sequences homologous to *Fa*CYP707A1-A2-A3, obtained by STRING 11.0 DB tool.

Observing the constructed interactome, the homologous sequence for *Fa*CYP707A1, *Fa*CYP707A2 and *Fa*CYP707A3 in the *Fragaria vesca* database showed the same correlations with the other proteins present in the construct. Among those, it is interesting to note the textmining and co-expression relation with an HIPP - Heavy metal-associated isoprenylated plant protein 26-like; the textmining correlations with Abscisate beta-glucosyltransferase-like proteins, and Abscisic-aldehyde oxidase-like protein, whose putative homologs are mentioned for other species and the interaction is known in curated databases, assuring the association of the CYP707A sequences with the ABA metabolic pathway.

The interactome also shows textmining associations with Cysteine synthase-like protein, Polygalacturonase-like protein and NADPH-dependent 1-acyldihydroxyacetone phosphate reductase-like protein.

Gene expression analysis

It was possible to observe that the application of salt stress resulted in increased expression of the gene *FaCYP707A3* but had the opposite effect on *FaCYP707A1* and *FaCYP707A2*. When ABA was applied to plants subjected to stress, there was a reduction in the expression of *FaCYP707A1* and *FaCYP707A2,* while *FaCYP707A3* showed a similar expression profile as the stressed plants not subjected to the application of this phytohormone. Plants supplemented with ABA without stress application showed an increase in *FaCYP707A3* expression (Figure 3). The *FaCYP707A4* gene showed expression under the limits of RT-PCR detection in strawberry fruits, suggesting that its role may be related to other tissues and physiological processes, and is not represented in the graphs.

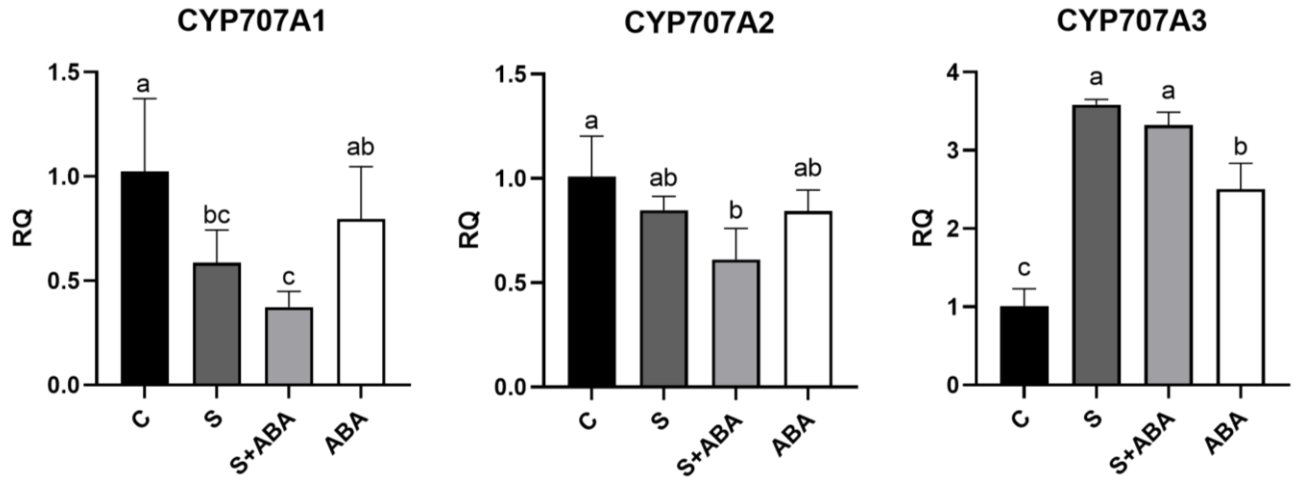

Figure 3. Relative expression of *FaCYP707A1, FaCYP707A2* and *FaCYP707A3* genes under salt stress and/or the application of ABA.

The expression of *FaCYP707A1* during strawberry fruit development was previously described [17]. In the present study we evaluated the expression of the other three *FaCYP707A* genes to compare with those reported for *FaCYP707A1*. *FaCYP707A2* and *FaCYP707A3* showed a significant expression level on day 7, which decreased along fruit development and ripening. The highest expression levels of *FaCYP707A2* was observed in fruits at day 7, while the expression of *FaCYP707A3* was higher in root samples, and *FaCYP707A4* showed the highest expression values in leaves, followed by roots. These results indicate a possible tissue-specific role of different *FaCYP707A* and during the strawberry fruit development.

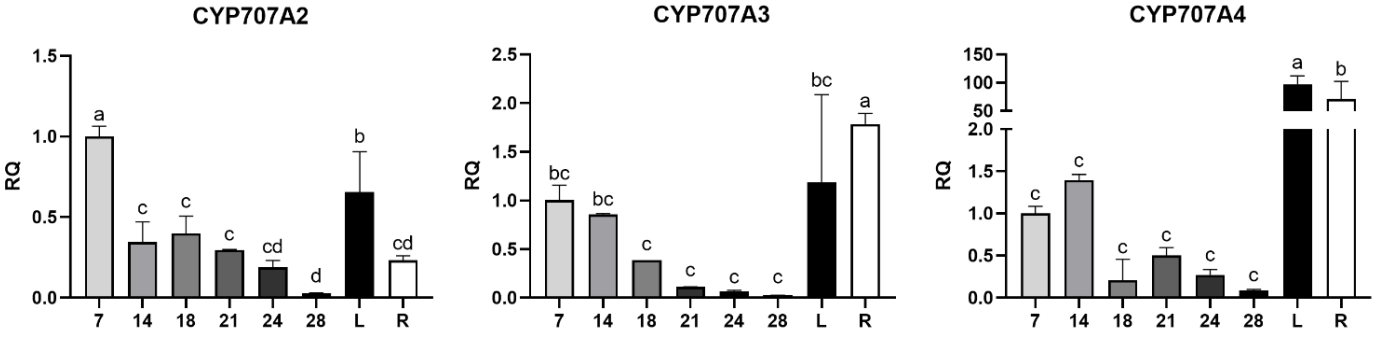

Figure 4. Relative expression of *FaCYP707A2, FaCYP707A3* and *FaCYP707A4* genes during different stages of fruit development and ripening (7, 14, 18, 21, 24 and 28 days after anthesis), and in two different tissues (L – Leaf and R – Root).

DISCUSSION

It is well-known that abscisic acid (ABA) plays important role in regulating the maturation of non-climacteric fruits and in the response against stress in several plant species [10, 19-20]. The enzymes and the role of the de novo synthesis of ABA in different physiological processes have been largely described in the literature; however, the catabolic pathway, where ABA is converted to PA and DPA by CYP707As enzymes is poorly elucidated. Through several analyzes of structural and comparative genomics, the present study describes the *FaCYP707As* from strawberry, determines their evolutionary relationships and predicts their functions to guide future studies of functional genomics.

According to the sequence alignment, it is possible to observe that *FaCYP707A2* is homologous to *FaCYP707A1*, but with a deletion of 150 nucleotides close to middle of the sequence. This result could represents an error during sequencing or assembly; however, it is also possible that these sequences are a result of alternative splicing that emerged during the process of genes differentiation, since both showed 100% homology to only one genomic sequence (>ORD_ID5978). More precise studies on these sequences are needed to validate these hypothesis.

The *FaCYP707A3* has correspondence with three sequences in the database that did not matched with *FaCYP707A1* / *A2* / *A4*, indicating that probably it is a true *FaCYP707A* sequence, with different evolutionary history and possibly performs specialized functions in this plant. When the amino acid sequence was

compared with known sequences from *Fragaria vesca*, the *FaCYP707A3* sequence shows the highest homology with *FvCYP707A4* (XP_004294805.1) with an identity of 99%, while *FaCYP707A1-A2* show higher homology with *FvCYP707A3* (XP_004291107.1) with an identity of 97% and 87%, respectively.

FaCYP707A4 is the shortest sequence of the four *CYP707As* identified, and do not presents a P450 domain. Additional studies are necessary using other transcriptomes and databases to evaluate whether this is an additional gene copy that was subjected to gene fragments deletions and possibly changed function during the course of the evolutionary process or it represents the result of alternative splicing, such as *FaCYP707A1-A2* sequences.

The sequence alignment with *CYP707As* from *Fragaria ves*ca has shown the existence of five other sequences of partial homology to the sequences identified in *Fragaria ananassa*, suggesting the existence of other *CYP707A* copies in *Fragaria ananassa* that were not identified via mRNA-Seq database. Therefore, further studies with other mRNA-Seq databases, obtained from different plant tissues and under different experimental conditions are needed to elucidate the existence of other *CYP707A* sequences in cultivated strawberry and the role of these sequences in different tissues and physiological processes.

The assembly of phylogenetic trees is a valuable tool to understand the origin and evolutionary drift of genetic sequences, providing information regarding the function of each sequence by comparing to sequences from other species whose function are better characterized. Ren and coauthors. [15] demonstrated that during the development of the *Prunus avium* fruit, the different *CYP707A* sequences show variation in the rate of gene expression, fulfilling overlapping roles over time. The exogenous application of ABA increased the expression of *PaCYP707A1* and *PaCYP707A3*, while the expression of *PaCYP707A4* (sequence > GU559989.1-with greater similarity with *FaCYP707A1* and *FaCYP707A2*) decreased. Kondo and coauthors. [21] demonstrated that *MdCYP707A1* (> AB593330.1-with greater similarity to *FaCYP707A3*) shows upregulation when subjected to water stress and dehydration in apple fruit. Moreover, when exposed to water stress, the *PaCYP707A1* and *PaCYP707A3* copies showed a significant increase in their expression rates, while no differences were observed in *PaCYP707A2* and *PaCYP707A4* in dehydrated and control fruits. The sequences also showed variations in gene expression rates associated with the analyzed tissue and the plant's development phase, as well as presenting a different response to water stress and exogenous ABA application. Similar behavior was observed in the present study, where the expression rate in response to water stress and exogenous ABA application ranged between gene copies. *FaCYP707A1* and *FaCYP707A2* are slightly downregulated when applying stress or exogenous ABA application, and are even more downregulated when subjected to stress and exogenous ABA application simultaneously. Meanwhile, *FaCYP707A3* shows upregulation under salt stress and/or exogenous ABA application indicating that the understanding of homologous enzymes in other species may provide an indicative of the role of the sequences being studied. Moreover, while previous study reported that *FaCYP707A1* showed increased level from 18 to 21 DAA, decreasing as it ripened [17], the present study showed that the expression of *FaCYP707A2, FaCYP707A3 a*nd *FaCYP707A4* are reduced along fruit development, suggesting a role during this process; and that *FaCYP707A3* are more expressed in root and *FaCYP707A*4 in roots and leaves, compared to fruits, corroborating the idea of particular/specialized responses and tissue-specific role of the sequences.

Interactome analyzes show textmining interactions between CYP707As and HIPP proteins, which are known for their involvement in homeostasis and detoxification of heavy metals in plants, especially cadmium, in addition to acting in response to thermal and water stress, suggesting that their relationship with CYP707As is related to the response to environmental changes. Also, interaction with Polygalacturonase is present, an enzyme that acts in the maturation process by degrading pectin, promoting structural changes and softening of the fruits. The relationship between polygalacturonases and the ABA pathway has already been demonstrated, where the exogenous application of ABA or Abz (abscinazole - *CYP707A* inhibitor) results in greater activity of polygalacturonases and reduced firmness of the treated fruits [22].

CONCLUSION

This study allowed the identification for the first time of *FaCYP707A* in *F. ananassa*. Four sequences were identified in RNAseq databanks and the genomic structure was characterized, confirming the identity of three of them. A phylogenetic analysis allowed to determine their homologous with other plant species and predicts their evolutionary history. The expression profile of the *FaCYP707As* demonstrated their specific role according to the physiological condition: *FaCYP707A3* seems to be involved in the response to salt stress in an ABA-dependent manner; and *FaCYP707A2, FaCYP707A3 and FaCYP707A4* play a role during fruit development, as their expression are reduced along fruit development. Moreover, the interaction network

analysis pointed out proteins involved in the ABA metabolism, heavy metal homeostasis and detoxification, and cell wall dissemble. Future functional genomic studies will allow elucidating the role of *FaCYP707Aa* in *F. ananassa* in order to develop biofortified fruits and stress tolerant plants.

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