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Relationship analysis of *FaPYR1* and *FaCHS* genes encoding fruit ripening of three species of strawberries (*Fragaria* spp.) fruit

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Abstract. *Aristya GR, Dyatama GR, Zuyyina C, Maulina NTA, Arif MF, Musthofa A, Kasiamdari RS. 2023. Relationship analysis of FaPYR1 and FaCHS genes encoding fruit ripening of three species of strawberries (*Fragaria *spp.) fruit. Biodiversitas 24: 87-97.* The difference in the phenotypes characters and gene expression of strawberries (*Fragaria* spp.) is influenced by changes in hormone signaling mechanisms to receptors due to the adaptation to various types of environments. Abscisic acid (ABA) is one of the phytohormones that plays a role in the growth and development of strawberries, response to environmental stress, and fruit ripening. One of the ABA receptors is the *PYR1* protein which is encoded by *FaPYR1* gene. ABA also plays a role in inducing the *FaCHS* gene associated with pigmentation in strawberry fruit. This study aimed to analyze the relationship between *FaPYR1* and *FaCHS* gene sequences in three strawberry species encoding fruit ripening. The *FaPYR1* (585-633 bp) and *FaCHS* (90-93 bp) genes from three strawberries species namely *Fragaria moschata*, *Fragaria vesca* L. 'Californica', and *Fragaria* x *ananassa* 'Festival' were successfully amplified. The results of the gene family relationship analysis of *FaPYR1* gene sequences showed a close relationship with the comparative three species to GenBank, with a genetic distance value of 0.01-0.02. Meanwhile, *FaCHS* gene sequences from three species of strawberries showed a close relationship with the comparable *FaCHS* gene sequence from GenBank, with a genetic distance of 0.1-0.2.

Keywords: ABA receptors, *FaPYR1*, *FaPYR1*, *Fragaria*

INTRODUCTION

Strawberry plants (*Fragaria* spp.) have been widely cultivated in Indonesia. In their distribution, there are many various characters of strawberry fruits that have high commercial value and are very popular fruit in societies (Kilic et al. 2021). However, the high demand for strawberry fruits was not followed by the fruit quality and production (Roussos et al. 2012). Moreover, the problems faced by the farmers were the fruits failed to form and have a small size, and the fruits rapidly decomposed after harvesting (Giordano et al. 2020). The decreasing in fruit production can be caused by changes in environmental conditions (Felicia et al. 2022). Unfavorable environmental conditions will be responded to abscisic acid (ABA) (Ali et al. 2020).

Abscisic acid (ABA) is a phytohormone that plays a role to respond environmental stress, growth, and development, such as seed maturation, which prevents premature germination and induces dormancy, and plays role in the process of fruit ripening (Nguyen et al. 2021). One of the ABA receptors is encoded by the *FaPYR1* gene which is involved in the early growth and maturation of strawberry fruit by initiating the expression of seed maturation genes and fruit ripening (Chai et al. 2011; Fidler et al. 2022). Abscisic acid is also one of the phytohormones that induces the *FaCHS* gene which is a reporter gene that first play role in the biosynthesis pathway of flavonoids (Gao et al. 2018). Flavonoids such as anthocyanins are secondary metabolites that have a function of color biosynthesis in strawberry fruit maturation (Härtl et al. 2017; Warner et al. 2021). Chai et al. (2011) reported that ABA plays a role in the regulation of the maturation of non-climacteric fruits, such as strawberries (Tong et al. 2021). Jiroutova et al. (2021) stated that abscisic acid (ABA) has a role in increasing the sugar uptake into the fruit in apples. Feng et al. (2021) also stated that ABA plays a role in increasing sugar levels in citrus plants during the fruit formation process. The process of fruit ripening is closely related to changes in sugar metabolism, softening, and discoloration (Carvalho et al. 2016; Balestrini et al. 2021).

The increasing production and quality of strawberries can be done through genetic improvement. Genetic improvement by analyzing gene relationships will provide information related to the evolutionary character of genes. In addition, gene relationship analysis can be used as an initial step in linking information on environmental

conditions with molecular changes in an organism. this study aimed to analyze the relationship of the *FaPYR1* and *FaCHS* genes from three species of strawberries, namely *Fragaria moschata* Duchesne ex Weston, *Fragaria vesca* L. 'Californica', and *Fragaria* x *ananassa* (Duchesne ex Weston) Duchesne ex Rozier 'Festival' which is a strawberry developed in Indonesia. This research is expected the development of the *FaPYR1* and *FaCHS* genes to be able to produce strawberry plants that form fast fruit, resistant to fruit rot, and withstand environmental stress. We also revealed the *FaPYR* and *FaCHS* gene sequences of three different species of strawberries clade with comparable *FaCHS* gene sequences from GenBank. Then we made a comparative study between the gene family analysis of both genes from three species of strawberries with several genes which is closely related to GenBank.

MATERIALS AND METHODS

Plant materials

Six Samples were strawberries of species *Fragaria moschata*, *Fragaria vesca* ('Californica', and *Fragaria* x *ananassa* ('Festival', 'Kristal', 'Rosa Linda', 'KP Brite') originating from the Agrowisata Banyuroto strawberry farm, Magelang, Central Java, Indonesia.

Procedures

Isolation of total RNA

RNA was extracted using CTAB3-LiCl method following the protocol described by Yu et al. (2012) with modifications. A total of 0.5 g of fresh strawberry fruits was mashed and 0.02 g of PVP was added. Subsequently, 700 µL of CTAB3-LiCl extraction buffer was added and centrifuged at 15,700 g for 5 minutes. The formed supernatant was added with chloroform at the same volume and centrifuged at 15,700 g for 10 minutes. The supernatant was added with 4 M of LiCl at the same volume and then subjected to overnight precipitation at 4°C. The mixture precipitated overnight was subsequently centrifuged at 15,700 g for 15 minutes at 4°C. The pellets formed were then washed with 100 µL of 75% ethanol and centrifuged at 15,700 g for 5 minutes at 4°C. The formed pellets were washed twice. It was centrifuged at 5,900 g for 5 minutes at 4°C and subsequently, 30 µL of nuclease-Rnase-free water was added to dissolve RNA. RNA isolates were then subjected to qualitative and quantitative RNA tests by electrophoresis and spectrophotometry.

Synthesis of cDNA and amplification of FaPYR1 and FaCHS

RNA isolate was then converted into cDNA for it to be more stable and used for the target gene amplification stage. The cDNA was synthesized by using the reverse transcriptase-polymerase chain reaction (RT-PCR) using the iScript™ cDNA Synthesis RT-PCR kit. The cDNA synthesis reaction contained 2 µL of total RNA and 18 µL of iScript™ cDNA Synthesis kit. Furthermore, incubation was performed using the PCR according to the protocol kit, consisting of priming at 25°C for 5 minutes, reverse transcriptase at 45°C for 20 minutes, RT inactivation at 95°C for 1 minute, and optional step at 4°C for ∞.

Results of cDNA synthesis from strawberry RNA were used as a template for gene amplification using the polymerase chain reaction (PCR). The PCR reaction was performed in a volume of 25 µL containing 2 µL of DNA template, 12.5 µL of MyTax™ HS Red Mix BIOLINE, 1 µL of forward primer, 1 µL of reverse primer, and 8.5 µL of ddH_2O. Referring to Chai et al. (2011), the primers used were a forward primer, 5'-ATG GAG AAA CCA TCA TCG GC-3' and a reverse primer, 5'-TCA GAC CTG GGG AGT TAG CG-3') and Actin (forward, 5'-TGG GTT TGC TGG AGA TGA T-3'; dan reverse, 5'-CAG TAG GAG AAC TGG GTG C-3'). Referring to Muñoz et al. (2011), the primers *used* for *FaCHS* were a forward primer, 5'-GCC TTT GTT TGA GCT GGT CT-3' and a reverse primer, 5'-CCC AGG AAC ATC TTT GAG GA-3') and *26S-18S RNA Housekeeping* (forward, 5'-ACC GTT GAT TCG CAC ATT TGG TCA TCG-3' and reverse, 5'-TAC TGC GGG TCG GCA ATC GGA CG-3'). Gene amplification was performed using a Bio-Rad thermocycler with an initial denaturation at 95°C for 3 minutes, followed by 39 cycles consisting of denaturation at 95°C for 30 seconds, annealing (*FaPYR1* and *Actin*: 60°C; *FaCHS* and *26S-18S Housekeeping*: 54°C) for 30 seconds, and extension at 72°C for 40 seconds. Subsequently, it proceeded with the post-extension stage at 72°C for 10 minutes and was held at 12°C for ∞ (optional). The PCR results were then tested qualitatively using electrophoresis with 2% agarose and run at 50 volts for 40 minutes.

The successfully amplified target genes were subsequently subjected to the sequencing stage. Sequencing was performed by sending the amplified samples along with the primers used to the 1st BASE Malaysia, a sequencing service provider.

Data analysis

Sequencing results were then processed using the GeneStudio software to edit the contig. Furthermore, the BLAST (Basic Local Alignment Search Tool) search at the NCBI's (National Center for Biotechnology Information) GenBank, was performed to find gene sequences that matched *FaPYR1* and *FaCHS*. The alignment process was carried out using the Mesquite software (version 3.31). Results of alignment were then exported in the (.fas) format and a multiple sequence alignment was made using the Clustal Omega on EMBL-EBI (The European Bioinformatics Institute) to produce a multiple sequence alignment capable of showing different sequences in one gene family. Reconstruction of phylogenetic trees and analysis of genetic distances were performed using the MEGA X software. The phylogenetic trees were reconstructed using the Neighbor-Joining approach (Hong et al. 2021) and using the Kimura-2-Parameter analytical model with a bootstrap test 1,000 times (Mahadani et al. 2022).

RESULTS AND DISCUSSION

The quantitative and qualitative of RNA's strawberry

The qualitatively tested total RNA isolates were converted into cDNA and used to serve as a template for amplification using the PCR. Isolation of the total RNA of three species of strawberries produced concentrations ranging from 10.55 ng/µL to 358.43 ng/µL with a purity of 1.05 to 2.16. RNA purity was indicated by the Å260/Å280 ratio with a good ratio of 2.0±0.1 and the Å260/Å230 ratio of 2.0-2.4 (Table 1). Several contaminations indicated that phenolic compounds, thiocyanates, or other organic acids would lead the value of the Å260/Å280 ratio to be lower than 2.0. Meanwhile, polysaccharide or *β*-mercaptoethanol contamination would lead the Å260/Å230 ratio to be lower than 2.0.

Amplification using the primer *FaPYR1* showed a thin band, while amplification using the primer *FaCHS* showed a thicker band (Figure 1). Results of electrophoresis showed that *FaPYR* conformed with the target gene size, which was approximately 627 bp. Similarly, results of electrophoresis showed that *FaCHS* also conformed with the target gene size of 127 bp. *FaPYR1* is 627 bp as indicated by the electropherogram band that is slightly above the 600-bp marker. *Actin* is 261 bp with the electropherogram band almost close to the 300-bp marker. *FaCHS* is 127 bp with the electropherogram band slightly above the 100-bp marker. Meanwhile, *26S-28S RNA Housekeeping* is 146 bp with the electropherogram band in between the 100-bp and 200-bp markers (Figure 1). *FaPYR1* and *FaCHS* that were successfully amplified were subsequently subjected to the sequencing stage to determine the amplicon sequences of the three strawberry species. The housekeeping genes used to serve as control were *Actin* and *26S-18S RNA housekeeping*. Actin was used as the control for *FaPYR1*, while *16S-18S RNA Housekeeping* was used as the control for *FaCHS*. *Actin* and *26S-18S RNA Housekeeping* were used as the control or reference genes since these two genes are always expressed in any condition with a constant level of expression and are not affected by the treatment given. Furthermore, the sequence data were analyzed using the GeneStudio software to edit the contig. A homology search was conducted at the NCBI's (National Center for Biotechnology Information) GenBank to find out the similarity with several sequences of *FaPYR1* and *FaCHS* family members. Results of the homology search showed that *FaPYR1* and *FaCHS* had a high similarity.

Relationship between *FaPYR1* and *FaCHS* genes family members

A homology search was conducted at the NCBI's (National Center for Biotechnology Information) GenBank to find out the similarity with several sequences of *FaPYR1* and *FaCHS* family members. Results of the homology search showed that *FaPYR1* and *FaCHS* had a high similarity (Tables 2-7). Table 2 shows the comparison between seqence *FaPYR* gene in *F. moschata* with 3 samples from NCBI which have high similarity values, as well as for *F. vesca* 'Californica' (Table 3) and *F.* x *ananassa* 'Festival' (Table 4) in different NCBI samples.

Table 1. quantitative and qualitative tests of RNA strawberry (Aristya 2020)

Samples	Concentration (ng/µL)	Å260/Å280 ratio	Å260/Å230 ratio
F. moschata	358.43	2.16	2.09
F. vesca 'Californica'	11.09	1.48	0.27
F. x *ananassa* 'Festival'	15.99	2.11	0.06
F. x *ananassa* 'Kristal'	10.55	1.33	0.31
F. x *ananassa* 'Rosa Linda'	15.47	1.19	0.32
F. x *ananassa* 'KP Brite'	61.52	1.05	0.63

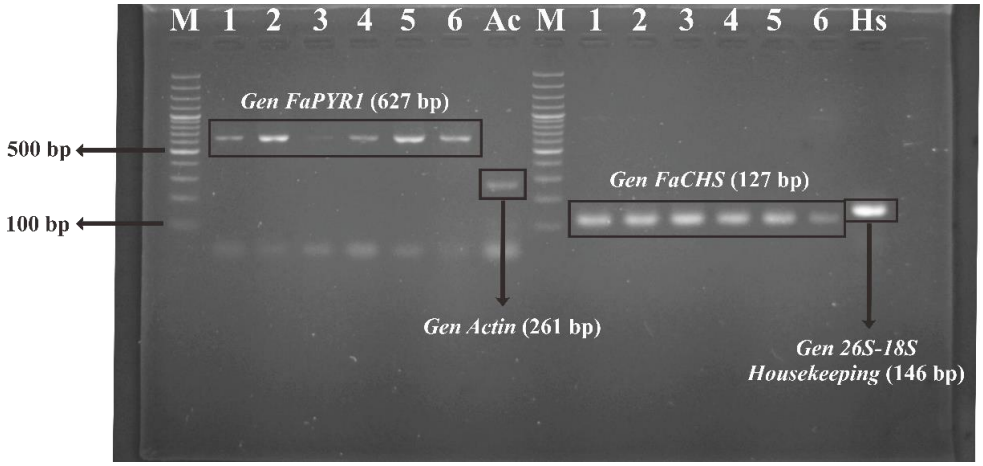

Figure 1. Electropherogram of the PCR products of *FaPYR1*, *Actin*, *FaCHS*, and *26S-18S RNA Housekeeping* in three species of strawberries. M: Marker 10kb, (1) *F. moschata*; (2) *F. vesca* 'Californica'; (3) *F.* x *ananassa* 'Festival'; (4) *F.* x *ananassa* 'Kristal'; (5) *F.* x *ananassa* 'Rosa Linda' and (6) *F.* x *ananassa* 'KP Brite' (Aristya et al. 2020)

Table 2. Similarity of sequence BLAST *FaPYR1* gene from *F. moschata* (Dyatama 2019)

Sample	NCBI sample	Max score	Query cover (%)	Accession number	Similarity values (%)
F. moschata	*F. vesca* subsp. vesca *PYR1-like*	1044	98	XM011466857	99.31
	F. vesca subsp. vesca *PYR1-like*	1051	100	XM004300193	99.15
	F. x *ananassa PYR1*	1040	100	JF268669	98.80

Table 3. Similarity of sequence BLAST *FaPYR1* gene from *F. vesca* 'Californica' (Dyatama 2019)

Sample	NCBI sample	Max score	Query cover (%)	Accession number	Similarity values (%)
F. vesca 'Californica'	*F. vesca* subsp. vesca *PYR1-like*	1118	99	XM004300193	99.35
	F. vesca subsp. vesca *PYR1-like*	1110	98	XM011466857	99.35
	F. x *ananassa PYR1*	1107	99	JF268669	99.03

Table 4. Similarity of sequence BLAST *FaPYR1* gene from *Fragaria* x *ananassa* 'Festival' (Dyatama 2019)

Sample	NCBI sample	Max score	Query cover (%)	Accession number	Similarity values (%)
F. x *ananassa* 'Festival'	*F. vesca* subsp. vesca *PYR1-like*	1114	98	XM011466857	98.88
	F. vesca subsp. vesca *PYR1-like*	1116	99	XM004300193	98.73
	F. x *ananassa PYR1*	1105	99	JF268669	98.41

The similarity values between *F. moschata* with *F. vesca* subsp. vesca PYR1-like, *F. vesca* subsp. vesca PYR1-like and *F.* x *ananassa* PYR1 were 99.31%, 99.15% and 98.80%, respectively (Table 2). The similarity values between *F. vesca* 'Californica' with *F. vesca* subsp. vesca PYR1-like, *F. vesca* subsp. vesca PYR1-like and *F.* x *ananassa* PYR1 were 99.35%, 99.35%, and 98.03, respectively (Table 3). The similarity values between *F.* x *ananassa* 'Festival' with *F. vesca* subsp. vesca PYR1-like, *F. vesca* subsp. vesca PYR1-like and *F.* x *ananassa* PYR1 were 98.88%, 98.73% and 98.41, respectively (Table 4). This value indicates high similarity which indicates the sequence of genes being compared has high similarity. Moreover, the high similarity value of three strawberries formed in this study (*F. moschata*, *F. vesca* 'Californica' and *F.* x *ananassa* 'Festival') to the linked PYR1- like gene of *F. vesca* and *F. x ananassa* reveal homogeneousness genetics in the consistency of ABA receptor vector. Furthermore, the comparison between sequence *FaCHS* gene in *F. moschata* with 8 samples from NCBI (Table 5) which is having lower similarity values rather than in *FaPYR* gene sequence, as well as for *F. vesca* 'Californica' (Table 6), and *F.* x *ananassa* 'Festival' (Table 7) in different NCBI samples. They also have high similarity values between Indonesia strawberries samples with eight strawberries from the GenBank for *FaCHS* gene family. It reveals that homogeneousness genetics in both genes have high potency and chance to combine for the evolutionary process.

The alignment configuration between *FaPYR1* and *FaCHS* genes family members

The sequence data of *FaPYR* and *FaCHS* families used to serve as the reference were those with high similarity. A DNA sequence can be considered identical if they have a similarity of 91 to 100%. DNA sequences' similarity of 60 to 100% is considered similar, 20 to 60% is considered closely related, and less than 20% is considered different (Kolondam et al. 2012). Upon subjection of *FaPYR1* and *FaCHS* sequences to the NCBI's BLAST, alignment was carried out with family genes obtained from the GenBank (Figures 2 and 3). Upon subjection of *FaPYR1* and *FaCHS* sequences to the NCBI's BLAST, alignment was carried out with family genes obtained from the GenBank. Nucleotide alignment was performed using the Mesquite version 3.31 software. Results of alignment were then exported in the (.fas) format and a multiple sequence alignment was made using the Clustal Omega on EMBL-EBI (The European Bioinformatics Institute) to produce a multiple sequence alignment capable of showing different sequences in one gene family (Figures 2 and 3).

The results of *FaPYR1* and *FaCHS* alignment are presented in Figures 2 and 3, respectively. The asterisk shows similarities in nucleotides. These results show that *FaPYR1* and *FaCHS* have undergone mutations. Those mutations are generally in the form of substitution. Substitution is the replacement of one type of nucleotide with another. If the substitute is a nucleotide deriving from one group (e.g., purine), it is called a transition. Meanwhile, if the purine base is replaced by the pyrimidine base or vice versa it is referred to as transversion.

Phylogenetic analysis based on *FaPYR1* and *FaCHS* genes family members

Results of phylogenetic tree reconstruction using the Neighbor-Joining method show that *FaPYR1* sequences of three strawberry species were closely related to the *FaPYR*1 gene family sequences from GenBank (Figure 4).

The phylogenetic tree shows a division into two clades with two outgroups. After 1,000 times of bootstrap tests, *FaPYR1* sequences in three strawberry species have a close relationship with the three sequences of the *FaPYR1* family of the genus *Fragaria* (*F*. x *ananassa* JF268669; *F. vesca* XM004300193; and *F. vesca* XM011466857). These results were also corroborated by the similarity index (SI) value of BLAST results at NCBI, that *FaPYR1* sequences derived from three strawberry species had a high value (98 to 99%) with the sequences of *FaPYR1* family members of the genus *Fragaria* at the GenBank. A phylogenetic tree describes the history or evolutionary relationships between genes. The length of branches of the phylogenetic tree represents the many evolutionary changes (Kapliet et al. 2020).

PYR1 receptors rely heavily on the abscisic acid (ABA) signaling mechanism. Abscisic acid has a major role in overcoming the stress of biotic and abiotic environments. In addition, ABA is also required for plant growth and development in stressful conditions. Chai et al. (2011) showed that ABA plays a role in the regulation of maturation of non-climacteric fruits, such as strawberries. The current mechanism of ABA signaling involving its receptors constitutes the evolutionary results. This evolutionary process occurs as part of dynamic adaptation due to different environmental conditions, leading to the development of specific mechanisms of ABA signaling as a form of adaptation specific to each species (Hauser et al. 2011). This explains the distant relationship of *FaPYR1* sequence in strawberry species with those sequences of *FaPYR1* family members in other species (Figure 4).

Table 5. Similarity of sequence BLAST *FaCHS* gene from *F. moschata* (Dyatama 2019)

Sample	Sample NCBI	Max score	Query cover (%)	Accession number	Similarity value (%)
F. moschata	*F. vesca VrCHS*	108	76	AB250913	94.37
	F. x *ananassa CHS*	108	76	AY997297	94.37
	F. vesca subsp. vesca *PKS1*	108	76	XM004306494	94.37
	F. vesca subsp. vesca *PKS1*	102	76	XM004306495	92.96
	F. vesca VwCHS	102	76	AB250914	92.96
	F. x *ananassa FrCHS5*	97.1	76	AB201758	91.55
	F. x *ananassa FrCHS3*	97.1	85	AB201757	88.89
	F. x *ananassa FrCHS2*	97.1	85	AB201756	88.89

Table 6. Similarity of sequence BLAST *FaCHS* gene from *F. vesca* 'Californica' (Dyatama 2019)

Sample	Sample NCBI	Max score	Query cover (%)	Accession number	Similarity value (%)
F. vesca L. 'Californica'	*F. vesca* subsp. vesca *PKS1*	122	77	XM004306494	97.26
	F. vesca VrCHS	122	77	AB250913	97.26
	F. x *ananassa CHS*	117	77	AY997297	95.89
	F. vesca subsp. vesca *PKS1*	115	76	XM004306495	95.83
	F. vesca VwCHS	115	76	AB250914	95.83
	F. x *ananassa FrCHS5*	115	76	AB201758	95.83
	F. x *ananassa FrCHS3*	113	75	AB201757	95.77
	F. x *ananassa FrCHS2*	108	75	AB201756	94.37

Table 7. Similarity of sequence BLAST *FaCHS* gene from *Fragaria* x *ananassa* 'Festival' (Dyatama 2019)

Sample	Sample NCBI	Max score	Query cover (%)	Accession number	Similarity value (%)
F. x *ananassa* 'Festival'	*F. vesca* subsp. vesca *PKS1*	130	87	XM004306494	96.25
	F. vesca VrCHS	130	87	AB250913	96.25
	F. x *ananassa CHS*	124	87	AY997297	95.00
	F. x *ananassa FrCHS3*	117	83	AB201757	94.74
	F. vesca subsp. vesca *PKS1*	119	87	XM004306495	93.75
	F. vesca VwCHS	119	87	AB250914	93.75
	F. x *ananassa FrCHS5*	119	87	AB201758	93.75
	F. x *ananassa FrCHS2*	111	83	AB201756	93.42

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XM017362194   CTCTCTCTCTCTCACTCA-TGGAAAAGAGTCATGGCCAAGAGACCACCACAAGCCACCAC  59
XM024316987   ----CAGCAGCGGAGGGATCCATGGCGGCCGAGCGCGACCCGGAGAGGTCGCAGCACCAC  56
SEQFaPYR1-A   ------------------------------------------------TCTCAGCATCAC  12
SEQFaPYR1-B   ---------------ACATCATCGGCGGCGGAGCAAGACCCGGAGAGCTCGCAGCATCAC  45
SEQFaPYR1-C   ---TTATGGAGAAAAACATCATCGGCGGCGGAGCAAGACCCGGAGAGCTCGCAGCATCAC  57
XM011466857   ----CAATGGAGAAACCATCATCGGCGGCGGAGCAAGACCCGGAGAGCTCGCAGCATCAC  56
XM004300193   ----CAATGGAGAAACCATCATCGGCGGCGGAGCAAGACCCGGAGAGCTCGCAGCATCAC  56
JF268669      ------ATGGAGAAACCATCATCGGCGGCGGAGCAAGACCCGGAGAGCTCGCAGCATCAC  54
                                                                 *    ** ***

XM017362194   CTAACACTCCCACCCGGCCTCACCCAGGAAGATTTCCTCGAGCTAAACCCCCTGATAACC  119
XM024316987   CTCACAGTCCCCTCCGGGTTGACTCAGTCCGAGTTCGAGGAGTTGAAGCCGAGCGTGGCC  116
SEQFaPYR1-A   CTCACAGTCCCCTCCGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  72
SEQFaPYR1-B   CTCACAGTCCCCTCCGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  105
SEQFaPYR1-C   CTCACAGTCCCCTCCGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  117
XM011466857   CTCACAGTCCCCTCTGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  116
XM004300193   CTCACAGTCCCCTCTGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  116
JF268669      CTCACAGTCCCCTCTGGACTGACCCAGTCCGAGTTCGACGACCTCAAGCCCGTCGTGGCC  114
              ** *** ****  * **  * ** ***   ** ***   **  * ** **     *  **

XM017362194   GAGTTCCACACCTACACCCTCACACCCTCCCAATGCTCCTCCCTCCTCTCCCAACGCATC  179
XM024316987   GAGTTCCACACGTACCGGGTAGGCCCGGGGTATTGTTCCTCGCTACTGGCGCAGCGAGTC  176
SEQFaPYR1-A   GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  132
SEQFaPYR1-B   GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  165
SEQFaPYR1-C   GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  177
XM011466857   GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  176
XM004300193   GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  176
JF268669      GAGTTCCACACCTACCGTGTGGGCCCTGGCCATTGCTCCTCCCTACTCGCGCAGCGAATC  174
              *********** ***    *    **     * ** ***** ** **  * ** **  **

XM017362194   CACGCGCCCCCACACGCCGTCTGGTCCATCGTCCGCAGCTTCCACAAGCCCCAACTGTAC  239
XM024316987   CACGCGCCGCTGGAGACGGTGTGGAGGGTGGTGCGGCGGTTCGACCGGCCGCAGACTTAC  236
SEQFaPYR1-A   CACGCGCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  192
SEQFaPYR1-B   CACGCGCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  225
SEQFaPYR1-C   CACGCTCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  237
XM011466857   CACGCGCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  236
XM004300193   CACGCGCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  236
JF268669      CACGCGCCGCTGGAGACGGTGTGGAAGGTGGTGAGGCGGTTCGATAAGCCGCAGACGTAC  234
              ***** ** *   *  * ** ***    * **  *  * *** *   *** **    ***

XM017362194   AAACACTTCATCAAAAACTGCACCGTCAAGCCCACCTTCACAATGACCGTCGGCGACACA  299
XM024316987   AAGCACTTCATCCGGAGCTGCACGGTCAGAGAAGGGTTTGTAATGAGCGTGGGATGCACG  296
SEQFaPYR1-A   AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  252
SEQFaPYR1-B   AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  285
SEQFaPYR1-C   AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  297
XM011466857   AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  296
XM004300193   AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  296
JF268669      AAGCACTTCATCCGGAGCTGCACAGTGAAAGAAGGCTTCGTAATGAGCGTGGGATGCACG  294
              ** *********   * ****** ** *        **   ***** *** **   ***

XM017362194   CGCGACGTCAACGTCATCTCCGGATTACCGGCTGCCACCAGTACCGAGAGGCTCGACATA  359
XM024316987   AGGGAGGTGAATGTAATTTCGGGGCTACCAGCGGCGACGAGCACGGAGAGGCTGGACTTG  356
SEQFaPYR1-A   AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  312
SEQFaPYR1-B   AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  345
SEQFaPYR1-C   AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  357
XM011466857   AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  356
XM004300193   AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  356
JF268669      AGGGACGTGAATGTAATTTCCGGGCTGCCGGCGGCGACGAGCACGGAGAGGCTGGACTTG  354
               * ** ** ** ** ** ** **  * ** ** ** ** ** ** ******** *** *

XM017362194   CTCGACGATGACCGACACGTCACGGGGTTCAGTATCATAGGAGGCGAACATAGGTTAAGG  419
XM024316987   CTGGACGATGACCGACACGTCACCGGGTTCAGTATTACAGGGGGAGAGCACCGGCTGAGG  416
SEQFaPYR1-A   CTGGACGAGGAGAGGCACGTGACTGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  372
SEQFaPYR1-B   CTGGACGAGGAGAGGCACGTGACTGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  405
SEQFaPYR1-C   CTGGACGAGGAGAGGCACGTGACTGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  417
XM011466857   CTGGACGAGGAGAGGCACGTGACTGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  416
XM004300193   CTGGACGAGGAGAGGCACGTGACTGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  416
JF268669      CTGGACGAGGAGGGGCACGTGACCGGTTTCAGTATTATAGGAGGGGAGCACAGGTTGAGG  414
              ** ***** **  * ***** ** ** ******** * *** ** ** **  ** * ***

XM017362194   AATTACAGGTCCGTGACGACAGTGCATGGAGTGGAGCGTGAGGGGAAGATGTTGAGCGTT  479
XM024316987   AATTACCGGTCGGTGACCACCGTGCATGGGTTCGAGCGCGACGGGAGGATCTGCACGGTC  476
SEQFaPYR1-A   AATTACCGGTCGGTGACCACCGTGCATGGGTTAGAGCGCGACGGGAGGATCTGGACCGTT  432
SEQFaPYR1-B   AATTACCGGTCGGTGACCACCGTGCATGGGTTCGAGCGCGACGGGAGGATCTGGACCGTT  465
SEQFaPYR1-C   AATTACCGGTCGGTGACCACCGTGCATGGGTTCGAGCGCGACGGGAGGATCTGGACCGTT  477
XM011466857   AATTACCGGTCGGTGACCACCGTGCATGGGTTTGAGCGCGACGGGAGGATCTGGACAGTT  476
XM004300193   AATTACCGGTCGGTGACCACCGTGCATGGGTTTGAGCGCGACGGGAGGATCTGGACAGTT  476
JF268669      AATTACCGGTCGGTGACCACCGTGCATGGGTTTGAGCGCGACGGGAGGATCTGGACAGTT  474
              ****** **** ***** ** ********  * ***** ** **** *** *  *  **
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XM017362194   GTGTTGGAGTCGTACGTGGTGGATGTGCCGGAGGGGAACACGGAGGAGGATACGAGGTTG  539
XM024316987   GTTTTGGAATCGTATGCGGTGGATGTGCCGGAGGGGAATAGCGAAGAGGAAACGCGACTG  536
SEQFaPYR1-A   GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  492
SEQFaPYR1-B   GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  525
SEQFaPYR1-C   GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  537
XM011466857   GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  536
XM004300193   GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  536
JF268669      GTTCTGGAATCGTACGTGGTGGATGTGCCGGAGGGGAACAGCGAGGAGGACACGCGGCTG  534
              **  **** ***** * ********************* *  ** ***** *** *  **

XM017362194   TTTGCCGATACGGTGGTGAAGTTGAATCTGCAGAAGCTTGCGGTTGTTAGTGAAGGAAAG  599
XM024316987   TTCGCCGATACGGTGGTCAGGTTGAATCTGCAGAAGCTGGCGTCGGTCACGGAAGCCATG  596
SEQFaPYR1-A   TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  552
SEQFaPYR1-B   TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  585
SEQFaPYR1-C   TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  597
XM011466857   TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  596
XM004300193   TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  596
JF268669      TTCGCGGATACGGTGGTGAGGTTGAATCTGCAGAAGCTGGCGTCGGTGACGGAAGCCATG  594
              ** ** *********** * ****************** ***   ** *  ****  * *

XM017362194   TCTGAATAATCTCAATAACATTTCTCGCAACTAGAGAAAA   639
XM024316987   GCCCGTGACGGTGACGCTAATTTCCAGGTCTGATCT----   632
SEQFaPYR1-A   GCCCGTGACGGTGACGCTAACTCCCCAGGTCTG-------   585
SEQFaPYR1-B   GCCCGTGACGGTGACGCTAACTCCCAAGGTCTGAAA----   621
SEQFaPYR1-C   GCCCGTGACGGTGACGCTAACTCCCAAGGGTCTGAA----   633
XM011466857   GCCCGTGACGGTGACGCTAACTCCCAGAGGAAGAAA----   632
XM004300193   GCCCGTGACGGTGACGCTAACTCCCAGGTCTGAGTT----   632
JF268669      GCCCGTGACGGTGACGCTAACTCCCAGGTCTGA-------   627
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Figure 2. Multiple sequence alignment of *FaPYR1* gene in three strawberry species with 8 family members of *FaPYR1* from NCBI. Information for each accession number refers to an organism that has abscisic acid receptor of *PYR1* for XM017362194 (*Daucus carota* subsp. *sativus*), XM024316987 (*Rosa chinensis*), three samples from this study (SEQFaPYR1-A, SEQFaPYR1-B, SEQFaPYR1-C), two samples of *Fragaria vesca* subsp. *vesca* (XM011466857 and XM004300193), and JF268669 (*Fragaria* x *ananassa*)

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SEQFaCHS-A    -------------GTACTTTTACGCTCTTCTGACGTGAGGTGCATCGACGGTCATCTTCG  47
AY048707      -------GCGGCCCAAACTATTCTTCCTGACAGTGACGGGGCCATCGACGGGCATCTTCG  53
AB201757      GCAGCCCAAACTATCCTTCCTGATAGTGACGGAGCCAT-------CGACGGGCATCTTCG  53
XM024313136   -------GCAGCCCAAACTATCCTTCCTGACAGTGACGGAGCCATCGACGGGCATCTTCG  53
AB201758      -------GCAGCCCAAACTATCCTTCCCGATAGTGACGGAGCCATCGACGGGCACCTTCG  53
AB201756      -------GCGGCCCAAACTATCCTTCCTGATAGTGACGGGGCCATCGACGGGCATCTTCG  53
XM004306494   -------GCGGCCCAAACTATCCTTCCCGATAGTGACGGAGCCATCGACGGGCATCTTCG  53
AB250913      -------GCGGCCCAAACTATCCTTCCCGATAGTGACGGAGCCATCGACGGGCATCTTCG  53
AY997297      -------ACGGCCCAAACTATCCTTCCCGATAGTGACGGGGCCATCGACGGGCATCTTCG  53
XM004306495   -------GCGGCCCAAACTATCCTTCCCGATAGTGACGGGGCCATCGACGGGCATCTTCG  53
AB250914      -------GCGGCCCAAACTATCCTTCCCGATAGTGACGGGGCCATCGACGGGCATCTTCG  53
SEQFaCHS-B    -------CCCGCTCCGACTGGCAATCATCGATGTGACGGAGCCATCGACGGGCATCTTCG  53
SEQFaCHS-C    -------------------------TCTCGATATGACGGAGCCATCGACGGGCATCTTCG  35
                                                ****** ** *****

SEQFaCHS-A    TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCTGGG        90
AY048707      TGAAGTTGGG-CTCACATTTCACCTCCTGAAGGATGTTCCCGGG        96
AB201757      TGAAGTCGGG-CTCACATTTCACCTCCTCAAAGATGTTCCCGGG        96
XM024313136   TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCCGGG        96
AB201758      TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCCGGG        96
AB201756      TGAAGTTGGG-CTCACATTTCACCTCCTTAAAGATGTTCCCGGG        96
XM004306494   TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCTGGG        96
AB250913      TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCTGGG        96
AY997297      TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCTGGG        96
XM004306495   TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCCGGG        96
AB250914      TGAAGTTGGG-CTCACATTTCACCTCCTCAAAGATGTTCCCGGG        96
SEQFaCHS-B    TGAAGTTGGA-CTCACATTTCACCTCCTCAAAGATGTTCCT---        93
SEQFaCHS-C    TGAAGTTGGGACTCACATTTCACCTCCTCAAAGATGTTCCTGGG        79
              ****** **  ***************** ** ********
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Figure 3. Multiple sequence alignment of *FaCHS* in three strawberry species with 14 family members of *FaCHS* at NCBI. Information for each accession number refers to an organism that has abscisic acid receptor of PYR1 for three samples from this study (SEQFaCHS-A, SEQFaCHS-B, and SEQFaCHS-C), AY048707 (*Rubus idaeus*), AB201757 (*Fragaria* x *ananassa*), XM024313136 (*Rosa chinensis*), AB201758 (*Fragaria* x *ananassa*), AB201756 (*Fragaria* x *ananassa* complete cds), XM004306494 (*F. vesca* subsp. *vesca*), AB250913 (*F. vesca* cultivar Alexandria), AY997297 (*Fragaria* x *ananassa* complete gene), XM004306495 (*F. vesca* subsp. *vesca*, AB250914 (*F. vesca*)

Results of the phylogenetic tree reconstruction for *FaCHS* sequences of three species of strawberries with the GenBank's *FaCHS* family is shown in Figure 5. The phylogenetic tree reconstruction produced three clades. *FaCHS* sequences in three species of strawberries showed a distant relationship with the family members of *FaCHS* in both different strawberry species and species. *FaCHS* sequences in *Fragaria* spp. from the GenBank are more closely related to *Rosa* sp. and *Rubus idaeus*. The Sequences of *FaCHS* originating from *F. vesca* (SEQ*FaCHS*-B) has a close relationship with *FaCHS* sequence originating from *F*. x *ananassa* (SEQ*FaCHS*-C) with a bootstrap value of 85%. Meanwhile, *FaCHS* sequences originating from *F. moschata* show a distant relationship with all members of *FaCHS* family. This is in contrast to the results of NCBI's homology search showing that *FaCHS* sequences deriving from three strawberry species had a high similarity index (SI) value of 88 to 94%. This result can occur because SI compares the similarity of nucleotides. Meanwhile, a phylogenetic tree compares the level of relationship through the evolutionary history and the level of mutation.

According to Yamaki and Asakura (1991), abscisic acid (ABA) has a role in increasing the sugar uptake into the fruit in apples. Kojima et al. (1995) also stated that ABA plays a role in increasing sugar levels in *Citrus* plants during the fruit formation process. It also plays a role in the regulation of anthocyanin biosynthesis pathways in fruits by inducing anthocyanin accumulation to bring out fruit colors. In addition, ABA also participates in plant tissue activation for defense through phenol biosynthesis, and acts as an antioxidant (Lacampagne et al. 2009). The process of fruit ripening is closely related to changes in sugar metabolism, softening, and discoloration. Genes or receptors related to sugars and pigments such as SigE, AMY, and CHS are regulated through ABA signal cascade reactions involved in ABA receptors (Rook et al. 2006).

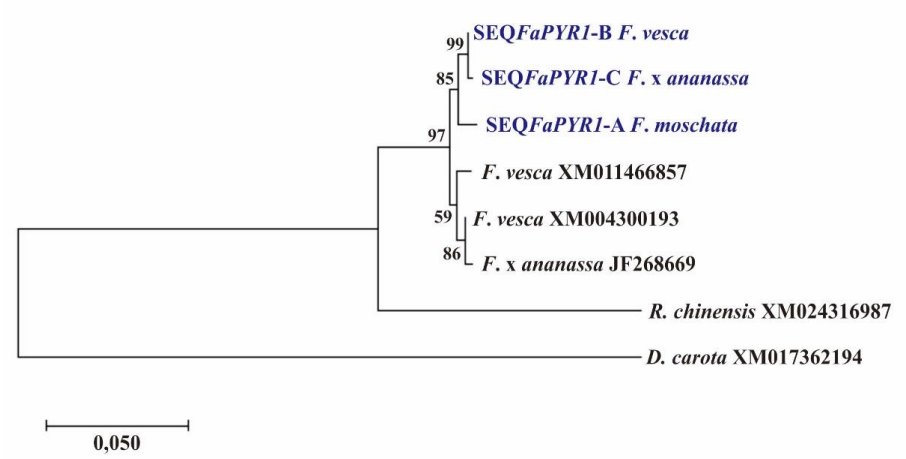

Figure 4. Relationship analysis of *FaPYR1* sequence in three strawberry species with the sequence of *FaPYR1* gene from NCBI (Dyatama 2019)

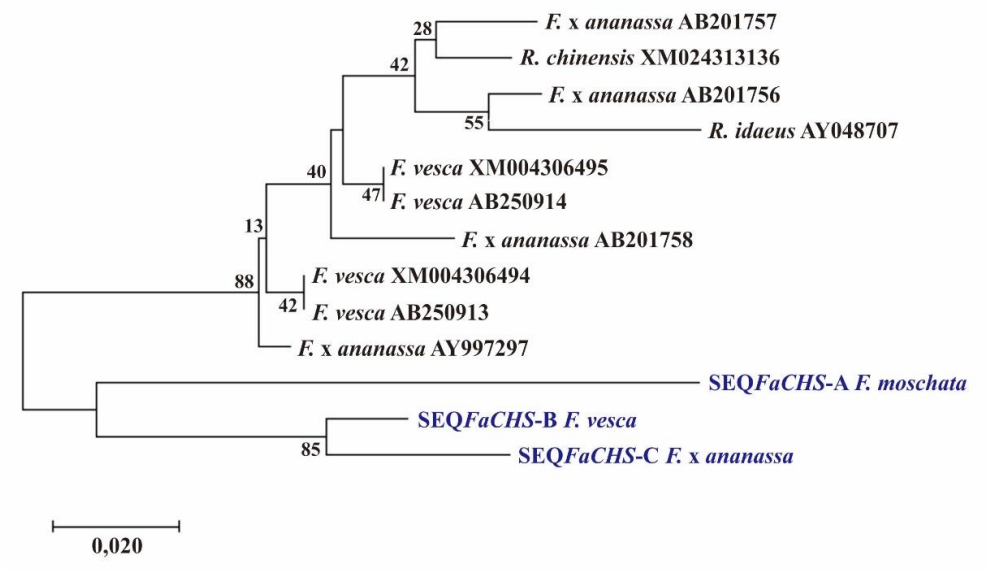

Figure 5. Relationship analysis of *FaCHS* sequences of three strawberry species with 14 sequences of *FaCHS* gene from NCBI (Dyatama 2019)

The pigmentation mechanism in strawberry fruits occurs when ABA hormone with metabolite compounds, hormones, enzymes and transcription factors work simultaneously to induce *FaCHS*, which in turn *FaCHS* catalyzes the formation of naringenine compounds and their derivatives. Naringenin compounds will activate another gene in the fruit, the chalcone isomerase (CHI), which will subsequently stimulate anthocyanin compounds to provide strawberry fruits with a color phenotype (pigment) (Carvalho et al. 2016). The mechanism of ABA signaling to induce *CHS* is through a complex regulatory process. Durbin et al. (1995) stated that *CHS* has a fast-evolving capability. The small changes in amino acids will change *CHS* function and evolution in *CHS* family members (*FaCHS* in *Fragaria*) producing copies of genes that vary greatly among plant lineages in response to functional differences and adaptations to different environments. This explains the distant relationship as indicated by the results of the relationship analysis of *FaCHS* sequences in three strawberry species with those sequences of *FaCHS* family members through phylogenetic tree reconstruction (Figure 5), despite the high similarity index (Table 3) as shown by the BLAST's homology search results at NCBI. A slight change in the sequence of *CHS* can change the amino acid. Small changes in amino acids can alter *CHS* function (Durbin et al. 1995).

Analysis of genetic distance between *FaPYR1* and *FaCHS* families genes

Gene relationship relations can be analyzed based on the genetic distance among *FaPYR1* and *FaCHS* family members. The present study analyzed the genetic distances of *FaPYR1* and *FaCHS* sequences to determine the similarity of the target gene sequences with those gene families originating from the GenBank (Tables 4 and 5). The lower the genetic distance means the higher the similarity of the gene sequences compared. The similarity of sequences shows closeness in terms of the relationship. An analysis of genetic distances of the sequences of *FaPYR1* and *FaCHS* families can show the relationship between the genes. According to Nei (1973), genetic distance ranging from 0.0 to 1.0, and distance D, ranging from 0.0 to infinity, were computed for each population pair.

Genetic distance of FaPYR1 gene sequences in three species of strawberries, namely SEQ*FaPYR1*-A, SEQ*FaPYR1*-B, and SEQ*FaPYR1*-C showed a genetic distance value of 0.01. Meanwhile, the genetic distance of *FaPYR1* gene sequences from three species of strawberries with the comparable *FaPYR1* gene sequence in strawberry species (*Fragaria* spp.) from GenBank also shows a genetic distance value of 0.01. This result shows that the gene sequences compared to have a close relationship because of their genetic distance value. *FaPYR1* gene sequences from three species of strawberries when compared with comparative *FaPYR1* gene sequences in other species show genetic distance values of 0.1 in *Rosa chinensis* (XM024316987) and 0.3 in *Daucus carota* (XM017362194. This result shows that *FaPYR*1 gene sequences from three species of strawberries have a relationship that is not close to the *PYR1* gene sequence in other species.

The mechanism of ABA signaling to induce *CHS* is through a complex regulatory process (Li et al. 2022). The small changes in amino acids will change *CHS* function and evolution in *CHS* family members (*FaCHS* in *Fragaria*) producing copies of genes that vary greatly among plant lineages in response to functional differences and adaptations to different environments. This result explains the distant relationship as indicated by the results of the relationship analysis of *FaCHS* sequences in three strawberry species with those sequences of *FaCHS* family members through phylogenetic tree reconstruction (Figure 5), despite the high similarity index (Table 3) as shown by the BLAST's homology search results at NCBI.

Table 4. Genetic distances between *FaPYR1* sequences and *FaPYR1* family from NCBI

	SEQ*FaPYR1*-A	SEQ*FaPYR1*-B	SEQ*FaPYR1*-C	XM004300193	XM011466857	JF268669	XM024316987	XM017362194
SEQ*FaPYR1*-A	0.000							
SEQ*FaPYR1*-B	0.010	0.000						
SEQ*FaPYR1*-C	0.012	0.002	0.000					
XM004300193	0.017	0.012	0.014	0.000				
XM011466857	0.016	0.014	0.016	0.009	0.000			
JF268669	0.021	0.016	0.017	0.003	0.012	0.000		
XM024316987	0.133	0.125	0.127	0.121	0.131	0.119	0.000	
XM017362194	0.377	0.377	0.380	0.383	0.377	0.383	0.440	0.000

Table 5. Genetic distances between *FaCHS* sequences and *CHS* family from NCBI

	SEQ*FaCHS*-A	SEQ*FaCHS*-B	SEQ*FaCHS*-C	XM004306494	AB250913	AY997297	XM004306495	AB250914	AB201758	AB201757	AB201756	XM024313136	AY048707
SEQ*FaCHS*-A	0.000												
SEQ*FaCHS*-B	0.153	0.000											
SEQ*FaCHS*-C	0.154	0.042	0.000										
XM004306494	0.151	0.103	0.119	0.000									
AB250913	0.151	0.103	0.119	0.000	0.000								
AY997297	0.135	0.119	0.136	0.014	0.014	0.000							
XM004306495	0.151	0.135	0.154	0.028	0.028	0.014	0.000						
AB250914	0.151	0.135	0.154	0.028	0.028	0.014	0.000	0.000					
AB201758	0.186	0.135	0.154	0.028	0.028	0.043	0.028	0.028	0.000				
AB201757	0.205	0.119	0.136	0.043	0.043	0.058	0.043	0.043	0.043	0.000			
AB201756	0.186	0.135	0.154	0.058	0.058	0.043	0.028	0.028	0.058	0.043	0.000		
XM024313136	0.186	0.103	0.119	0.043	0.043	0.058	0.043	0.043	0.043	0.028	0.043	0.000	
AY048707	0.204	0.152	0.171	0.089	0.089	0.073	0.057	0.057	0.089	0.073	0.042	0.042	0.000

FaCHS gene sequences in three species of strawberries show different values. *FaCHS* gene sequences from *F. vesca* L. (SEQ*FaCHS*-B) and *F.* x *ananassa* Duch. (SEQ*FaCHS*-C), showed a genetic distance value of 0.04. This result shows that the two samples have a close relationship. Meanwhile, *FaCHS* gene sequences from *F. moschata* (SEQ*FaCHS*-A) showed a genetic distance value of 0.1 with SEQ*FaCHS*-B and SEQ*FaCHS*-C. This shows that the *FaCHS* gene sequence from *F. moschata* has a relationship that is not close to the other two samples. *FaCHS* gene sequences from three species of strawberries showed a genetic distance value of 0.1 to 0.2 against all comparative samples from NCBI. This shows that *FaCHS* gene sequences from three species of strawberries are not closely related to all comparable *FaCHS* gene sequence samples from GenBank.

In conclusion, the sequenced *FaPYR1* gene has a length of 585-633 bp and the *FaCHS* gene has a length of 90-93 bp. The results of the identification of *FaPYR1* and *FaCHS* gene sequences in three species of samples strawberries and GenBank through the BLAST program in NCBI showed the results with similarity values of 98.41-99.35% and 88.89-97.26%, respectively. The results of phylogenetic tree reconstruction showed that *FaPYR1* gene sequences from three species of strawberries grouped in one clade with comparative *FaPYR1* gene sequences in *Fragaria* spp. from GenBank. The results of the family relationship analysis of *FaPYR1* gene sequences from three species of strawberries showed a close relationship with the comparative *FaPYR1* gene sequence in *Fragaria* spp. from GenBank, with a genetic distance value (0.01-0.02). Meanwhile, *FaCHS* gene sequences from three species of strawberries showed a close relationship with the comparable *FaCHS* gene sequence in *Fragaria* spp. from GenBank, with a genetic distance of 0.1-0.2.

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