Mutations in *PmUFGT3* contribute to color variation of fruit skin in Japanese apricot (*Prunus mume* Sieb. et Zucc.)

Xiaopeng Ni, Zhaojun Ni, Kenneth Omondi Ouma and Zhihong Gao*

**Abstract**

**Background:** Japanese apricot (*Prunus mume* Sieb. et Zucc.) is popular for both ornamental and processing value, fruit color affects the processing quality, and red pigmentation is the most obvious phenotype associated with fruit color variation in Japanese apricot, mutations in structural genes in the anthocyanin pathway can disrupt the red pigmentation, while the formation mechanism of the red color trait in Japanese apricot is still unclear.

**Results:** One SNP marker (PmuSNP_27) located within *PmUFGT3* gene coding region was found highly polymorphic among 44 different fruit skin color cultivars and relative to anthocyanin biosynthesis in Japanese apricot. Meanwhile, critical mutations were identified in two alleles of *PmUFGT3* in the green-skinned type is inactivated by seven nonsense mutations in the coding region, which leads to seven amino acid substitution, resulting in an inactive UFGT enzyme. Overexpression of the *PmUFGT3* allele from red-skinned Japanese apricot in green-skinned fruit lines resulted in greater anthocyanin accumulation in fruit skin. Expression of same allele in an Arabidopsis T-DNA mutant deficient in anthocyanidin activity the accumulation of anthocyanins. In addition, using site-directed mutagenesis, we created a single-base substitution mutation (G to T) of *PmUFGT3* isolated from green-skinned cultivar, which caused an E to D amino acid substitution and restored the function of the inactive allele of *PmUFGT3* from a green-skinned individual.

**Conclusion:** This study confirms the function of *PmUFGT3*, and provides insight into the mechanism underlying fruit color determination in Japanese apricot, and possible approaches towards genetic engineering of fruit color.

**Keywords:** *Prunus mume*, Anthocyanin, UFGT, SNP, Mutation

**Background**

Japanese apricot (*Prunus mume* Sieb. et Zucc.), an ornamental plant and fruit tree originating from Southwest China and is widely cultivated in all of East Asia and Japan. According to its various applications, Japanese apricot is divided into two groups, i.e. fruiting-mei and flowering-mei. Fruiting-mei has been cultivated and consumed in China for more than 7000 years. China, being the origin of Japanese apricot, is rich in good quality germplasm, and approximately 190 fruiting cultivars have been recorded [1]. The fruit is usually processed into many value-added products, including salted-mei, mei wine, and juice, and is considered to have high nutritional and medicinal value [2]. Fruit colour works as a significant factor to evaluate fruit value and quality, and attracts customer’s attention to increase sales [3].

Plentiful fruit colors result from the accumulation of various anthocyanins [4], which are the main water-soluble pigments belong to parent class of flavonoids [5], synthesized through the phenylpropanoid pathway [6], accumulated and localized in vacuoles in different plant tissues such as fruit, flowers, leaves, roots, stems [7–9]. The accumulation of anthocyanins leads to multiple
characteristics: red, purple or blue hue which depends on pH of the vacuole, not only improve ornamental value of merchandise, but also has physiological healthy benefits on several disease, Alzheimer [10], Parkinson [11], diabetes [12], and has anticancer importance [13].

The anthocyanins biosynthetic pathway has been widely reported in various fruits, including grape [14], apple [15], peach [16], pear [17] and plum [18]. The phenylpropanoid and flavonoid pathway involve in the biosynthesis of anthocyanins, several essential structural genes encode enzymes such as cinnamic acid 4-hydroxylase (C4H), phenylalanine ammonia-lyase (PAL) and 4-coumarate-CoA ligase (4CL), chalcone isomerase (CHI), chalcone synthase (CHS), dihydroflavonol 4-reductase (DFR), flavanone 3-hydroxylase (F3H), flavanone 3'-hydroxylase (F3' H), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose flavonoid-3-O-glycosyltransferase (UFGT) participated in regulation process [19]. UFGT works as the final structural gene to catalyze the 3-O-glucosylation of anthocyanidins in the biosynthesis process [20, 21]. UFGT was only been detected and expressed in red grapes, and it is not found in white grapes which appear to lack anthocyanins, even same as other different tissues [14]. UFGT expression was consistent with accumulation of anthocyanids and flavonols in the pericarp of litchi, and plays as a key factor to influence the anthocyanins biosynthesis and fruit color in litchi [22], AcUFGT3 is found as the key gene to regulate the biosynthesis and accumulation of anthocyanins in red-fleshed ‘Hongyang’ kiwifruit [23].

Mutations in structural genes in the anthocyanin pathway can disrupt the red floral pigmentation [24]. Both no-sense and mis-sense mutations in the chalcone flavonone isomerase (CfI) resulting in the absence of anthocyanin in barley and rice [25]. Mutations in the sequence of dihydroflavonol 4-reductase resulted in the absence of anthocyanins and proanthocyanins of barley [26]. One single-base deletion in the flavanone 3'-hydroxylase which represents the T gene of soybean controls gray pubescence color [27]. Frameshift mutation was characterized in the UDP-glucose flavonoid-3-O-glycosyltransferase in Japanese (Ipomoea nil) and the common (J. purpurea) morning glory which caused about 80% reduction of anthocyanin accumulation, such defects cause pale flower pigmentation [28]. Although these studies have provided valuable insights into the mechanism of coloration, the regulatory mechanism of anthocyanin biosynthesis and the cause for fruit color difference between two lines in Japanese apricot remain unclear.

In recent years, molecular markers developed based on resequencing data have received extensive attention and research, especially the third generation of DNA molecular markers represented by single nucleotide polymorphisms (SNPs), which are abundant in plant genomes and widely recognized as a large number, genetically stable and easily detectable high-efficiency molecular marker, with an estimated more than one SNP per thousand bases [29], and is the most common type of genomic variation among different individuals within a species [30]. SNP molecular markers have been applied in a variety of research areas in Japanese apricot, such as germplasm resource identification [31, 32], genetic evolutionary analysis [33, 34], genetic map construction [35, 36] and flower color gene localization [37, 38] et al. Fang et al. identified 95 SNPs among 67 reliable sequences in 13 homologous sequence groups by amplifying fragment length polymorphism fragments (AFLP) of several varieties, and the high quality and low error rate of the repeat sequencing results demonstrated that the development of SNP molecular markers based on AFLP of Japanese apricot could be used to identify Chinese and Japanese germplasm resources [31]. Li et al. investigated a total of 68 Japanese apricot cultivars genetic relationships between two categories of flowering and fruiting mei cultivars. 92 SNPs were detected from the DNA of all cultivars by nine pairs of PCR primers with a distribution frequency of one SNP per 32 bp. Using these SNPs, mei cultivars were clustered and analyzed into 13 groups, different from the original classification of flowering and fruiting group, proving that flowering and fruiting mei cultivars are genetically close to each other, indicating that SNP molecular markers are an effective tool to reveal the genetic evolutionary analysis of Japanese apricot [33]. Kitamura et al. developed the first high-density SNP genetic map of Japanese apricot based on genotyping-by-sequencing (GBS) technology, and used parents with different cooling requirements and their hybrid progeny to analyze the phenotype of dormancy-related traits. PmDAM6 was identified by quantitative trait locus (QTL) analysis as a dose-dependent inhibitor of dormancy breaking to control bud dormancy of Japanese apricot [36]. Zhang et al. identified 10 floral trait QTLs and genomic regions including petal color, stigma color, calyx color, and bud color by genome-wide association study (GWAS), and found that MYB108 may played a key role in regulating the genetic control and evolution of petal color in Prunus [38], but molecular markers related to fruit skin color in Japanese apricot have been reported.

In this study, we investigated the mechanism underlying fruit skin color variation in Japanese apricot. Recently, a cDNA coding the UDP-glucose: flavonoid- 3-O-glycosyltransferase gene (PmUFGT3) was isolated from fruit skin from a red-skinned variety and shown to function in anthocyanin biosynthesis in vitro, and by complementation of a gene mutation in Arabidopsis. Meantime, according to resequencing data of previous study [39],
one key nonsense point mutation on chromosome 4 linked to *PmUFGT3* was found and worked as highly polymorphic PmuSNP_27 SNP molecular maker, transversion between T and G at bp1332 in the CDS resulting in one amino acid substitution D to E in green-skinned fruits of Japanese apricot compared with red-skinned cultivars, and propose a working model for *PmUFGT3* to modify cyanidin 3-O-glucoside in Japanese apricot.

### Results

**Fruit skin color variation in Japanese apricot**

The red-skinned varieties were significantly distinguished from the green-skinned varieties due to the red pigmentation of the epidermis by observation of fruit skin phenotype (Fig. 1A). 'XZM' was the cultivar with the largest coloring area and the highest anthocyanin content, which was significantly higher than 'RHM' and 'ZHM' these two red-skinned cultivars, while there were no anthocyanins detected in all green-skinned cultivars. Based on phenotypic indicators of flank diameter, vertical diameter and width, green-skinned cultivar ‘QJM’ and all red-skinned cultivars showed no significant difference in fruit size, but the other two green-skinned cultivars, ‘SKM’ and ‘YLM’ were significantly lower than these four cultivars (Fig. 1B).

**Anthocyanin content between red- and green-skinned fruits**

In terms of anthocyanin content, red-skinned fruits contained high levels while no anthocyanin was detected in green-skinned fruits (Fig. 1B). Fruit from the ‘XZM’ cultivar exhibited the highest anthocyanins content, more than two folds higher than the other two red-skinned cultivars ‘RHM’ and ‘ZHM’.

**Genetic diversity**

The number of effective alleles is the reciprocal of gene homozygosity, which is an indicator of population genetic variation, which indicates the degree of distribution in the allele population. For *PmUFGT3* gene, there was one non-synonymous SNP (PmuSNP_27) in the CDS at bp1332 (T/G), two different fruit skin groups (green and red-skinned) and total 44 accessions of Japanese apricot cultivars were used for this SNP validation, there were total 88 alleles among 44 varieties, average two alleles per SNP site of each cultivar. The number of observed alleles and effective alleles of PmuSNP_27 were 2 and 1.97, respectively (Table 1).

Shannon's information index (*I*) of PmuSNP_27 was 0.69, Nei’s gene diversity (*He*) was 0.49, gene flow (*Nm*) is 0.16. Fixation index (*Fst*) of PmuSNP_27 was 0.6, Table 1 Parameter values of PmuSNP_27 SNP polymorphism in 44 Japanese apricot genotypes

| Markers   | Na | Ne   | I    | He   | Fst | Nm  | PIC  |
|-----------|----|------|------|------|-----|-----|------|
| PmuSNP_27 | 2  | 1.97 | 0.69 | 0.49 | 0.6 | 0.16| 0.581|

*Na* Observed number of alleles, *Ne* Effective number of alleles, *I* Shannon’s information index, *He* Nei’s gene diversity, *Fst* Fixation index, *Nm* Gene flow, *PIC* Polymorphism information content
which indicated that there is significant genetic difference between green and red-skinned cultivars group. More importantly, polymorphic information content (PIC) is an indicator of the diversity of species. PIC > 0.5 in the population indicates that the site is highly polymorphic. In this study, PmuSNP_27 was highly polymorphic (0.581). PmuSNP_27 was highly congruent relative to identifying genotypes and for estimating population genetic differences, meanwhile marker PmuSNP_27 (PmUFGT3) was also the candidate gene involved in the biosynthesis of anthocyanin, suggesting that it is the potential vital regulatory gene for the biosynthesis of anthocyanin in Japanese apricot.

**Isolation of full-length PmUFGT3 cDNAs**

The full length of the PmUFGT3 gene sequence of both red- and green-skinned ‘RHM’ and ‘QJM’ fruits was 1509 bp. This sequence was submitted to GenBank under

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**Fig. 2** Multiple sequence alignments of nucleotide sequences of PmUFGT3 from red and green-skinned fruits of Japanese apricot.
the accession number xxx (uploading). A comparison of the full-length genomic sequence of \textit{PmUFGT3} with that of the cDNA isolated from the corresponding apricot fruits revealed one exons of 1509 bp, without introns. It shared 99.8% homology with the \textit{PmUFGT3} gene of \textit{Prunus mume} (GenBank accession number 103328988).

A total of 23 SNPs were identified by comparing \textit{PmUFGT3} cDNA sequences between red and green-skinned fruits of Japanese apricots (Fig. 2). Among them, transition substitutions (69.6%) were more frequent than transversions (30.4%). Among the base transitions, 9 transitions between G and A were higher than 7 transitions between C and T. For the transversions, the substitutions were as follows (in decreasing order of frequency): 3 times for T and G, including the PmuSNP_27 site located at bp1332 (T/G), 2 times for A and G, and 1 time for both G and C with T and A.

Characterization of \textit{pmUFGT3} cDNA and the encoded protein

The SNPs at positions 664, 669–670, 764, 1014, 1318–1319, 1332, 1441–1442 bp were classified as seven nonsense mutations, resulting in seven amino acid substitution in green-skinned fruits of Japanese apricot, while SNPs at the 13 other positions were classified as synonymous mutations and did not affect the protein sequence.

The full-length cDNA of \textit{pmUFGT3} from red fruits of Japanese apricot had a length of 1509 bp, encoding a polypeptide of approximately 502 amino acid residues, which corresponds to a molecular mass of 55.82 kDa, with a theoretical pl of 5.1 (Fig. 3). Analysis of the secondary structure of the predicted \textit{PmUFGT3} protein revealed that all examined \textit{PmUFGT3} contained a Glycosyltransferase_GTB-type super family binding domain (accession: c10013) (Additional file 1). Furthermore, secondary structure analysis of the predicted \textit{PmUFGT3} revealed that this predominantly consisted of 216 alpha-helices (43.03%), followed by 190 random coils (37.85%), 72 extended strand (14.34%) and 24 beta turn (4.78%) (Additional file 2).

The phylogenetic tree based on the amino acid sequences of \textit{PmUFGT3} in red fruits of Japanese apricot and other nucleic acid sequences of UGTs of various plant species are shown in Fig. 4. Although the in vitro substrate specificities and in vivo functions of flavonoid UGTs cannot be accurately predicted using amino acid sequences alone (Fig. 4). Amino acid sequences of \textit{PmUFGT3} (GenBank accessions: XP008229637.1) has close relationship with \textit{Prunus persica} and \textit{Malus domestica}.

Construction of the plant expression vector and transient transformation of Japanese apricot skins

To confirm that \textit{PmUFGT3} regulates anthocyanin biosynthesis in Japanese apricot, a transient assay of anthocyanin production was primarily developed in immature Japanese apricot. The full-length \textit{PmUFGT3} cDNA was expressed under the control of the constitutive cauliflower mosaic virus 35S promoter in pCAMBIA1301, injection of \textit{A. tumefaciens} trains (GV3101) in Japanese apricot fruit.

As can be seen from Fig. 5, transient expression of \textit{PmUFGT3} from red-skinned cultivars ‘RHM’ showed red coloration around the injection site of immature ‘QIM’ fruit skin by transient expression in the shape of a

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**Fig. 3** Multiple sequence alignments of amino acid sequences of \textit{PmUFGT3} between red and green-skinned fruits of Japanese apricot using the DNAMAN program. The M in the leader sequences are underlined while stop codons are marked with an F.
circle, however, green-skinned cultivars ‘QJM’ PmUFGT3 did not show red coloration around the injection site of immature ‘QJM’ fruit skin. The results showed that the PmUFGT3 gene of red-skinned PmUFGT3-R could promote the synthesis and accumulation of anthocyanin in the peel of ‘QJM’ fruit skin, while the green-skinned PmUFGT3-G gene could not promote the synthesis and accumulation of anthocyanin in the fruit skin of ‘QJM’.

The fine structure of transient expression of green and red-skinned PmUFGT3 in immature green-skinned ‘QJM’ pericarp was observed by somatic microscopy (Fig. 6). There was no significant difference in the brown wounds caused by PmUFGT3 at the injection site, while red-skinned PmUFGT3-R transient expression around the injection hole had a ring of red coloration, which is dark red in color and also accumulates red within the

Fig. 4 Unrooted phylogenetic tree of PmUFGT3 (GenBank accessions: XP008229637.1) and flavonoid glycosyltransferase amino acid sequences. The number adjacent branches indicate maximum parsimony bootstrap values for the corresponding node. The scale bar indicates the number of differences per 100 residues derived from the ClustalW alignment. The phylogenetic tree was generated using TreeView software.
white villi. In contrast, the epidermal microstructure of red-skinned PmUFGT3-G did not have any changes.

GUS expression activity was detected in both green and red-skinned PmUFGT3 transient expression in fruit skin, and most of the green-skinned PmUFGT3 transient GUS expression was on the cell membrane with low activity. The red-skinned PmUFGT3 transient GUS expression was found in both cell membrane and cytoplasm and was more active. The results showed that red-skinned PmUFGT3 transient expression
functioned normally as UFGT, while green-skinned PmUFGT3 transient expression was slightly weaker (Fig. 6).

**Transient transformation analysis in Arabidopsis mutant line Atufgt**

To confirm that PmUFGT3 regulates anthocyanin biosynthesis in Japanese apricot, the full-length PmUFGT3 cDNA was expressed under the control of the constitutive cauliflower mosaic virus 35S promoter in the UFGT mutant, Atufgt. A total of ten independent transgenic plants were obtained and verified for transgene integration by PCR using primers designed to detect the binary vector pCAMBIA1301. The T2 transgenic Arabidopsis plant injected by PmUFGT3 from red-skinned cultivars showed complementation of the red-skinned phenotype, determined by visual examination of the hypocotyl color (Fig. 7). While the transgenic plant injected by PmUFGT3 from green-skinned cultivars showed complementation of the green-skinned phenotype, determined by visual examination of the hypocotyl color (Fig. 7), no color pigmentation compare with red-skinned PmUFGT3.

Anthocyanins were detected in leaves and hypocotyl edonary axis from red-skinned, which without SNP mutation induce anthocyanin accumulation in Arabidopsis thaliana leave. PmUFGT3 is a galactosyltransferase, catalyzing the glycosylation of cyanidin and used the UDP-galactose more efficiently than UDP-glucose.

**Point mutation at the conserved UFGT domain-encoding region abolishes the function of PmUFGT3 at an early stage of anthocyanin accumulation**

The PmUFGT3-GM1, PmUFGT3-GM2, PmUFGT3-GM3, PmUFGT3-GM4, PmUFGT3-GM5, PmUFGT3-GM6, and PmUFGT3-GM7 mutations were generated by target primers, which were designed according to the instructions in the site-directed mutagenesis kit. In additional file 3, codon D222 (AAG) was replaced by CAG (E) for PmUFGT3-GM1, codon D224 (ATC) was changed to CTC (L) for PmUFGT3-GM2, codon D338 (TCG) was changed to TGG (Trp) for PmUFGT3-GM4, codon D225 (GAT) was replaced by AAT (N) for PmUFGT3-GM3, codon D225 (GAT) was replaced by AAT (N) for PmUFGT3-GM3, codon D338 (TCG) was changed to CCG (P) for PmUFGT3-GM4,
codon D440 (GAC) was replaced by AGC (S) for \textit{PmUFGT3-GM5}, codon D444 (GAG) was changed to GAT (D) for \textit{PmUFGT3-GM6}, and the codon D481 (AAG) was substituted with GCG (A) for \textit{PmUFGT3-GM7}.

The transient expression of seven site-directed mutagenesis \textit{PmUFGT3} from green-skinned cultivars ‘QJM’, which only one \textit{PmUFGT3-GM6} mutagenesis induced the formation of red cells in the skin, the transgenic plant showed complementation of the red-skinned phenotype as \textit{PmUFGT3-R} from red-skinned cultivars ‘RHM’, determined by visual examination of the skin color (Fig. 8) while other six site-directed mutagenesis \textit{PmUFGT3} still have the same phenotype as green-skinned cultivars which no red-pigmentation on the fruit skin.

The different position of \textit{PmUFGT3} of Green-skinned fruit expression in Japanese apricot and Arabidopsis thaliana \textit{Atufgt}

To further confirm that \textit{PmUFGT3} regulates anthocyanin biosynthesis in Japanese apricot, the full-length seven site-directed mutagenesis \textit{PmUFGT3} from green-skinned cultivars ‘QJM’ was expressed under the control of the constitutive cauliflower mosaic virus 35S promoter in the \textit{UFGT} mutant, \textit{Atufgt}. A total of ten independent transgenic plants were obtained and verified for transgene integration by PCR using primers designed to detect the binary vector pCAMBIA1301. The transgenic plant injected by \textit{PmUFGT3-GM6} mutagenesis showed complementation of the red-skinned phenotype as \textit{PmUFGT3-R} from red-skinned

![Fig. 9 Overexpression of PmUFGT3 site-directed mutagenesis in Arabidopsis mutant line Atufgt](image)

![Fig. 10 Overexpression of PmUFGT3 site-directed mutagenesis in Arabidopsis mutant line Atufgt (hypocotyl)](image)
accumulation in Japanese apricot. While the transgenic plant injected by other six PmUFGT3-GM1 to GM5 and GM7 from green-skinned cultivars showed complementation of the green-skinned phenotype determined by visual examination of the green hypocotyl color (Fig. 10), no color pigmentation compare with red-skinned PmUFGT3-R. Anthocyanins were detected in leaves and hypocotyl edonary axis from red-skinned, which site-directed mutagenesis mutation of codon D444 (GAG) was changed to GAT (D) for PmUFGT3-GM6 induce anthocyanin accumulation in Arabidopsis thaliana hypocotyl.

Discussion
Non-synonymous mutations occurring in the coding region of UFGT, a structural gene that catalyzes the final step of anthocyanin biosynthesis, have been shown to affect anthocyanin synthesis in a variety of plants [40, 41], yet the function of UFGT has still not been reported in Japanese apricot. The objectives of this study were twofold: firstly, to confirm that UFGT is a key gene for anthocyanin synthesis in Japanese apricot fruit skin through transient expression and Arabidopsis mutant complementation experiments; Secondly, to explore the key mutational SNPs that lead to UFGT dysfunction in anthocyanin biosynthesis by targeted mutagenesis, which will provide a basis for future analysis of anthocyanin accumulation in Japanese apricot.

PmUFGT3 is the key gene and encoded enzyme related to anthocyanin accumulation
The recessive mutations of UDP-glucose: flavonol glucosyltransferase at the bronze locus were firstly reported and cloned using the transposable controlling element Activator (Ac) from maize in 1984 [42], which conducted the study of UFGT. Since then, UFGT enzymes were confirmed using uridine diphosphate-D-glucose (UDPG) as glucosyl-donor to catalyse the glucosylation of cyanidin and isolated and purified from red cabbage (Brassica oleracea) [43] and Haplopappus gracilis (Nutt.) [44], followed by soybean (Glycine max L.) [45] and red campion (Silene dioica) [46], those studies can validate the red color pigmentation depends on the function of UFGT. At the molecular level, the full length cDNA of UFGT was cloned to understand the molecular biology and biochemistry of anthocyanins biosynthesis from Gentiana triflora [47]. In particular, its important role in fruits has been valued, UFGT was reported as an enzyme responsible for a late step of anthocyanin biosynthesis in grapevive (Vitis vinifera), to glucosylate anthocyanidins in red fruit during ripening [48]. The biosynthesis of anthocyanins was induced by the introduction of UFGT regulated by MybA genes, resulted in reddish-purple spots in embryos. The activities and properties of UFGT enzymes were demonstrated related to the high amounts of anthocyanins accumulation in cultivated strawberry (Fragaria x ananassa) [49]. A Citrus paradisi UFGT gene was cloned and introduced in recombinant expression system to investigate the function of anthocyanin glucosylation [50], Liu et al. reported the functional characterization of two second UFGT genes, AcUFGT6b and AcUFGT7c from ‘Hongyang’ kiwi- fruit, which cluster with other plant GGTs. 3-O-glycosylated anthocyanins were recognized by the recombinant protein of AcUFGT6b, and resulted in the new anthocyanins accumulation under the co-expression of other two genes, AcMYBF110 and AcUFGT3a, also the increased content of cyanidin 3-O-xyl-galactoside was observed after the overexpression of AcUFGT6b together with AcMYBF110 and AcUFGT6b7c-RNAi, which showed AcUFGT6b was responsible for the end-product of the anthocyanin biosynthesis pathway in red-fleshed kiwifruit [23].

In this study, a striking difference in PmUFGT3 gene was detected in two Japanese apricot lines. In all populations of Japanese apricot species with green-skinned fruit, PmUFGT3 is a nonfunctional gene; however, in all populations of Japanese apricot species with red-skinned fruit, PmUFGT3 is a functional gene. It indicated a relationship between fruit skin color difference and loss of functional PmUFGT3 gene.

The key SNP mutation of PmUFGT3 associated with red pigmentation.
UFGT, as the structural genes in the anthocyanin biosynthesis pathway are highly conserved among flowering and fruit species [51, 52]. SNPs have been found as the most common genetic mutations in the genome of eukaryotic species, mutations in structural genes can affect the biosynthesis pathway of anthocyanin, then resulted in diverse phenotypes [24, 53].

One SNP s30 on UFGT was associated with the skin and pulp color phenotypes based on RP-HPLC analysis and visual characterization in Vitis vinifera [54]. One indel marker of UFGT was used to constructed carrot linkage maps controlling the anthocyanin pigmentation [55]. The SNPs of coding region sequence of UFGT prompted the development of molecular markers which enabled the discovery of discriminant SNPs (1/34 bp) and the reconstruction of 130 V. vinifera distinct genotypes, and infer SNP-based genotypes of grapevine for assessing the genetic identity including different color pigmentation cultivars [56]. A UFGT high resolution melting (HRM) assay presents 58 SNPs within 22 grapevine varieties and produced differentiated melting curves
for 18 haplotypes which is efficient in grapevine varietal discrimination [57]. Wu et al. found the UFGT gene had a significant higher expression in red flower than white flower in Japanese apricot, in addition, there were SNPs resulted nonsynonymous mutations which may affect the enzyme activity [37].

In this study, we found that CDS sequences of PmUFGT3 showed high similarity between two different-colored skin fruit, but there were 23 SNPs were detected in a genomic cDNA PmUFGT3 gene comparison between red and green-skinned fruits of Japanese apricot, 16 Transition substitutions were more common than 7 transversions (with a ratio of transitions to transversions close to 2.3:1) among all SNPs, SNPs at positions 201, 660, 789, 894, 1009, 1017, 1050, 1066, 1149, 1336, 1477 were classified as synonymous mutations. For a long time, people have believed that synonymous SNPs are irrelevant, because the main sequence of the protein is preserved [58, 59]. Although synonymous mutations may change the structure, function, and expression levels of proteins strongly associated with CpG islands, several mechanisms are now being elucidated. Studies have shown that synonymous polymorphisms can affect messenger RNA splicing, stability and structure, and protein folding [60, 61]. These changes can have a significant impact on the function of the protein, but we did not detect such a CpG island, and the higher frequency of A-G SNPs transitions occurred, which showed these synonymous mutations show no function associated with anthocyanin pigmentation.

The SNP at 664, 669–670, 764, 1014, 1318–1319, 1332, 1441–1442 bp was classified as nonsense mutations, resulting in 7 amino acid substitutions in red-skinned fruits of Japanese apricot compared with green-skinned. Among codon D222 (AAG) was replaced by CAG (E) for PmUFGT3-GM1, codon D224 (ATC) was changed to CTC (L) for PmUFGT3-GM2, codon D255 (GAT) was replaced by AAT (N) for PmUFGT3-GM3, codon D338 (TCG) was changed to CCG (P) for PmUFGT3-GM4, codon D440 (GAC) was replaced by AGC (S) for PmUFGT3-GM5, codon D444 (GAG) was changed to GAT (D) for PmUFGT3-GM6, and the codon 481 (AAG) was substituted with GCG (A) for PmUFGT3-GM7. Which only one PmUFGT3-GM6 mutagenesis induced the formation of red cells in the skin, the transgenic plant showed complementation of the red-skinned phenotype as PmUFGT3-R from red-skinned cultivars “RHM”, determined by visual examination of the skin color (Figs. 8 and 9) while other six site-directed mutagenesis PmUFGT3 still have the same phenotype as green-skinned cultivars which no red-pigmentation on the fruit skin. In conclusion, the PmUFGT3-GM6 SNP is a candidate genetic marker for red-skinned color. Obviously, it would be classified as a non-conservative amino acid substitution and might have a major impact on protein function.

Here, we propose a working model for PmUFGT3 to modify cyanidin 3-O-glucoside in Japanese apricot (Fig. 11). The enzyme PmUFGT3-R then attracts the UDP-xylose to the cyanidin 3-O-glucoside generating cyanidin 3-O-xyl-glucoside. While PmUFGT3-G, does not have this ability due to its structural variation.

Conclusions
In this study, gene catalyzing the final step, namely UDP-glucose: flavonoid 3-O-glycosyltransferase (PmUFGT3) involved in anthocyanin synthesis were identified from red-skinned of Japanese apricot. Our investigations revealed that PmUFGT3 is a functional glucosyl-transferase structure–function of anthocyanin synthesis. The fruit color difference between red and green-skinned may be related to the variation of PmUFGT3 gene. Key point
mutation may be one nonsense mutation, Transition between T and G resulting in one amino acid substitution D to E in green-skinned fruits of Japanese apricot compared with red-skinned cultivars, and propose a working model for PmUFGT3 to modify cyanidin 3-O-glucoside in Japanese apricot.

**Methods**

**Plant materials**

Six cultivars of Japanese apricot were used in this study and collected from the National Field Gene Bank for *Prunus mume* at the agricultural research station, Nanjing Agricultural University, Nanjing, P.R. China. Based on fruit skin pigmentation, the cultivars were classified into two groups: green-skinned and red-skinned. Green-skinned cultivars included ‘Qingjia No.2’ (QJM), ‘Yanglao’ (YLM) and ‘Shinano Koume’ (SKM). The red-skinned cultivars included ‘Ruantiao Hongmei’ (RHM), ‘Xiaoye Zhugan’ (XZM) and ‘Zhonghong’ (ZHM). Each three individual plants with immature green-skinned and red-skinned fruits were collected in May 2017.

Total 44 accessions of Japanese apricot cultivars were used for SNPs validation listed in Table 2 by Sanger sequencing, including a set of 15 diverse genotypes (set A) of different origins, which were identified as red-skinned cultivars group based on morphology, a second set of 29 genotypes (set B) were identified as green-skinned cultivars group.

**Experimental validation of SNPs**

A subset of 30 randomly selected SNPs from genome re-sequencing identified used for validation by sanger sequencing. Primer No.27 were designed to obtain amplicons of 300–1,000 bases containing at least one putative SNP (Additional file 4). Two µl of DNA was amplified with 1 µM of each primer, 0.2 mM dNTP (TaKaRa), 3 mM MgCl2, and 2 U Taq DNA polymerase (TaKaRa), using the following cycling program: 5 min at 94 °C, then 40 cycles of 30 s at 94 °C, 90 s at 60 °C, 90 s at 72 °C and final extension 10 min at 72 °C. PCR products were detected by 2% agarose gel electrophoresis and ethidium bromide staining. Purified PCR products were subjected to Sanger sequencing performed by TSINGKE Biological Technology (Nanjing, China). Genotype calling and subsequent SNP calling were examined chromatogram trace files and detect variants by extracting or comparing signals in the peaks of traces, using the NovoSNP software [62].

**Investigation of physiological indicators and agricultural traits**

Fruit physiological traits were measured at the same ripening time point (Fig. 1). Vertical height, flank diameter

| Table 2 | Phenotypic and cultivar data for 44 Japanese apricot cultivars used in this study |
|---------|----------------------------------------------------------------------------------|
| Phenotype | No | Cultivars | Phenotype | No | Cultivars |
| Red-skinned | 1 | Jiuzhongmei | Green-skinned | 23 | Huaxiangshi |
| Red-skinned | 2 | Wanhong | Green-skinned | 24 | Henghe |
| Red-skinned | 3 | Ruantiao Hongmei | Green-skinned | 25 | Xiyeqing |
| Red-skinned | 4 | Xiaoye Zhugan | Green-skinned | 26 | Yuying |
| Red-skinned | 5 | Hongnong | Green-skinned | 27 | Gucheng |
| Red-skinned | 6 | Daqiandi | Green-skinned | 28 | Pinzimei |
| Red-skinned | 7 | Zhonghong | Green-skinned | 29 | Longyan |
| Red-skinned | 8 | Zaozhong | Green-skinned | 30 | Guangdong Huangpi |
| Red-skinned | 9 | Dalizhong | Green-skinned | 31 | Siyuemei |
| Red-skinned | 10 | Weishanzhong | Green-skinned | 32 | Yunnan Hongmei |
| Red-skinned | 11 | Hongding | Green-skinned | 33 | Taoxingmei |
| Red-skinned | 12 | Nanhong | Green-skinned | 34 | Yanglao |
| Red-skinned | 13 | Kaidi | Green-skinned | 35 | Changnong 17 |
| Red-skinned | 14 | Daye Zhugan | Green-skinned | 36 | Shuangtaomei |
| Red-skinned | 15 | Huangxiaoda | Green-skinned | 37 | Yanhua |
| Green-skinned | 16 | Yeliqing | Green-skinned | 38 | Xuemei |
| Green-skinned | 17 | Sichuan Baimei | Green-skinned | 39 | Fenban Guomei |
| Green-skinned | 18 | Xinnong Xiaomei | Green-skinned | 40 | Daroumei |
| Green-skinned | 19 | Tonglv | Green-skinned | 41 | Danfenghou |
| Green-skinned | 20 | Lve | Green-skinned | 42 | No.67 |
| Green-skinned | 21 | Sichuan Qingmei | Green-skinned | 43 | Qingjia No.2 |
| Green-skinned | 22 | Yueshijie | Green-skinned | 44 | Dongqing |
and width were measured with a Vernier calliper (Guanglu, China). Single fruit weight was measured with an electronic balance (METTLER TOLEDO, Switzerland). Pulp from peeled Japanese apricot was squeezed in a cheesecloth, and the filtered, homogenized juice was used for determination of soluble solids using a digital refractometer (ATAGO, Japan).

The colour of all fresh-cut fruits was directly measured with a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan) using the Illuminant D75 and an observation angle of 10°, which had been calibrated with a standard white plate (Y = 94.00, x = 0.3158, y = 0.3322). Three readings of L* (lightness), a* (red-green chromaticity) and b* (yellow-blue chromaticity) coordinates were recorded for each Japanese apricot sunny surface. Each sample chose from 10 replicates by changing the position of the sunny surface at the maturity stage. Chroma and hue angle were calculated as Chroma = (a*2 + b*2)1/2 and Hue = tan−1(b*/a*) [63].

**Determination of anthocyanin accumulation**

Anthocyanin extraction and quantification was performed as previously described [64]. Briefly, 1 g fresh weight (FW) hypocotyl or fruit skin material was transferred into a tube containing 4.3 mL of extraction solution (1-propanol/HCl/distilled water, 18/1/81, v/v/v). The tubes were then placed in boiling water for 6 min and incubated in the dark overnight at room temperature. An additional 3.7 mL of extraction solution was then added to the mixture, the sample was mixed and centrifuged at 1,000 g for 5 min. The supernatant was filtered through a 0.45 μm filter (Millipore), and the amount of anthocyanin in the extracts was quantified using a spectrophotometer by reading at A535 and A650 and expressed as (A535-A650) in the extracts was quantified using a spectrophotometer.

**Isolation and sequencing of the PmUFGT3 gene**

Plant materials were harvested, frozen in liquid nitrogen, and then ground under RNase-free conditions. The RNA was extracted using the Trizol reagent (Invitrogen), following the manufacturer’s instructions, and then treated with DNase I at 37 °C for 30 min. The RNA was then reverse transcribed using the PrimeScript first-strand cDNA synthesis kit (TaKaRa), following the manufacturer’s instructions. A 10-mL aliquot of cDNA was diluted to 100 mL with water, and 2 mL (50 ng) of the diluted cDNA was used for PCR. The PmUFGT3 PCR product was cloned into pClone007 Blunt Vector (TSINGKE) and confirmed by sequencing the full open reading frame.

Full-length cDNAs of PmUFGT3 were obtained by RT-PCR using the primer pairs PmUFGT3-F/R (Additional file 5). The full-length cDNA sequence was used to search homologous sequences via the National Center for Biotechnology Information BLASTX as previously described [65]. Both the theoretical isoelectric point and molecular weight were calculated online (http://www.expasy.ch/tools/pi_tool.html). The ORF of the full-length PmUFGT3 was identified via Genscan (http://genes.mit.edu/GENSCAN.html) and motifs were found with Plantcare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). The secondary structure of the predicted PmUFGT3 protein was constructed with the Predict Protein tool (http://www.predictprotein.org/) and the three-dimensional structure of the predicted PmUFGT3 was modeled via SWISS-MODEL with Automated model (http://swissmodel.expasy.org/). A phylogenetic analysis of the PmUFGT3 protein was performed based on the deduced protein sequence using the NJ method, which was implemented in MEGA 5.0.

**Sequence alignment and phylogenetic analysis**

The amino acid sequences of PmUFGT3, obtained from full-length cDNAs, were aligned using the MEGA v 5.05 and ClustalW software as previously described [66]. Alignment parameters (gap opening penalty and gap extension penalty) used were 10.00 and 0.1 for pair-wise alignments, and 15.00 and 0.30 for multiple alignments. A phylogenetic tree was constructed and visualized using the neighbor-joining (NJ) method in MEGA v 5.0. The statistical significance of individual nodes was assessed by bootstrap analyses with 1,000 replicates.

**Construction of the plant expression vector and transient transformation of Japanese apricot fruit skins**

To generate the 35S:UFGT3 construct, the coding regions of PmUFGT3 were cloned into the pCAMBIA1301 binary vector driven by the CaMV 35S promoter. The binary Ti vector pCambia1301-PmUFGT3-35SN was used for transformation. It was constructed as follow. The cauliflower mosaic-virus (CaMV) 35S promoter and nopaline synthase terminator region were added into the pCambia1301 vector, the binary Ti vector pCAMBIA1301-35SN contains Kanamycin resistance for screening transgenic lines. After being digested with XbaI and SacI restriction enzymes, the cDNA of PmUFGT3 was inserted between the CaMV35S promoter and nopaline synthase terminator region of the Ti vector pCAMBIA1301-35SN, which has been digested with the same enzymes. The new construct, named pCambia1301-PmUFGT3-35SN, was electroporated into agrobacterium tumefaciens strain GV3101. The positive clones were selected on LB plates containing 50 mgL−1 kanamycin and identified by PCR amplification using PmUFGT3 specific primers. Separate strains containing PmUFGT3 and GUS fused to the 35S promoter in the
pCAMBIA1301 vector were infiltrated or co-infiltrated into the abaxial fruit surface. Each infiltration was performed using three fruits from the same plants. Photographs were taken 48 h after infiltration.

**Transient transformation analysis in Arabidopsis mutant line Atufgt**

Seeds of wild-type (Col-0) *A. thaliana* and overexpression lines (pCAMBIA1301-PmUFGT3-35SN) in the Col-0 background were grown on 1/2 MS medium as previously described [67]. The mutant line Atufgt (ugt78d2) were obtained from the European Arabidopsis Stock Centre (uNASC). Seeds were subjected to a chilling treatment at 4 °C for 72 h and then transferred to white light at 24 °C under long-day conditions (16-h light/8-h dark). Five-day-old Arabidopsis seedlings were used for hypocotyl measurements. At least 10 seedlings were imaged, and hypocotyl color were measured using ImageJ software (https://imagej.nih.gov/ij/).

**Site-directed mutagenesis of green-skinned PmUFGT3-3 mutant genes**

Site-directed mutagenesis was performed with the Easy Mutagenesis System (TransGen Biotech Ltd. Co., Beijing, China) and TransStart FastPfu DNA polymerase as previously described [68]. The mutated plasmid pCAMBIA1301-PmUFGT3-G template (methylated plasmid template) from green-skinned cultivars ‘QJM’ can be degraded by DMT digestive enzymes in vitro and DMT-competent cell in vivo, thereby screen performed effectively.

PCR was performed using plasmid Blunt-pCAMBIA1301-PmUFGT3-G as the template with two mutant primers for each reaction. Each mutated gene was linked to the surface display plasmid pKFS to form a fused gene with FS, and derived plasmids were named pKFSRm. *E. coli DH5α* strain was used as the host for propagation of plasmids containing mutated RML genes (pKFSRm). All plasmids were verified by Sanger sequencing by Sango (Shanghai, China).

**GUS assays**

To detect GUS activity in the calluses, histochemical staining was performed as described previously [69]. The callus tissue was immersed in GUS staining buffer (1 mM 5-bromo-4-chloro-3-indolyl-b-glucuronic acid solution in 100 mM sodium phosphate pH 7.0, 0.1 mm EDTA, 0.5 mm ferrocyanide and 0.1% Triton X-100), at 37°C. After staining, the calluses were photographed.

**Statistical analysis**

Genetic diversity of the whole collection was analyzed by calculating the observed number of alleles (Na), the effective number of alleles (Ne), Shannon's information index (I) [70] for each type of markers using POPGEN v.1.32 [71]. Nei's genetic diversity (He), gene flow (Nm), fixation index (Fst) and polymorphism information content (PIC) were calculated using PowerMarker 3.25 [72].

Analysis of variance (ANOVA) was performed to compare cultivar mean values using IBM SPSS Statistics 18 (SPSS Inc., Chicago, IL, USA). The least significant difference test was employed to determine differences between means at a 5% significance level. GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA) was used for graph plotting.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03693-8.

**Abbreviations**

C4H: Cinnamic acid 4-hydroxylase; PAL: Phenylalanine ammonia-lyase; 4CL: 4-Coumarate-CoA ligase; CHI: Chalcone isomerase; CHS: Chalcone synthase; DFR: Dihydroflavonol 4-reductase; F3'H: Flavanone 3-hydroxylase; F3'H; Flavonane 3'-hydroxylase; DDOX: Leucoanthocyanidin dioxygenase; UFGT: UDP-glucose flavonoid 3-O-glucosyltransferase; CR: Chalcone flavonone isomerase; SNPs: Single nucleotide polymorphisms; AFLP: Amplified fragment length polymorphism; GBS: Genotyping-by-sequencing; GWAS: Genome-wide association study; QTL: Quantitative trait locus; I: Shannon's information index; He: Nei's gene diversity; Nm: Gene flow; Fst: Fixation index; PIC: Polymorphic information content; QJM: Jingjia No.2; YLM: Yanglao; SKR: Shiniangoume; RHM: Ruantaohongmei; XKM: Xiaoye Zhuguan; ZHM: Zhonghong; qFT-PCR: Reverse transcription-quantitative polymerase chain reaction.

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**Authors’ contributions**

ZG conceived and designed the study, XN performed the experiments, analysed the data, and wrote the manuscript. ZN and KO participated in data analysis and material collection. All authors read and approved the final format of the manuscript.

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**Availability of data and materials**

Accession codes: The full length of the *PmUFGT3* gene sequence in this paper had uploaded to the GenBank (Submission ID is 2596019) and the accession numbers will be supplied once they are available. The BioProject is PRJNA371370. The sequence data for the ‘SKM’ cultivar have been deposited at BioSample under accession SAMN06298250. The sequence data for the ‘ZHM’ cultivar have been deposited at BioSample under accession SAMN06298251. The sequence data for the ‘SKM’ cultivar have been deposited at BioSample under accession SAMN06298252. The sequence data for the ‘YLM’ cultivar have
been deposited at BioSample under accession SAMN06298253. The sequence data for the ‘RMH’ cultivar have been deposited at BioSample under accession SAMN06298254. The sequence data for the ‘QJM’ cultivar have been deposited at BioSample under accession SAMN06298255.

**Declarations**

**Ethics approval and consent to participate**
The experiments did not involve endangered or protected species. The collection of plant material was carried out with permission of the National Field Gene Bank for Prunus mume, and complied with national or international guidelines and legislation.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no conflict of interest.

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