Isolation of a fruit ripening-related tonoplast aquaporin (GjTIP) gene from *Gardenia jasminoides*

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Published online: 26 July 2013

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Abstract Aquaporins are membrane water channels that play critical roles in controlling the water content of cells and tissues. In this work, a full-length cDNA encoding putative aquaporins was isolated from *Gardenia jasminoides* fruit cDNA library. The GjTIP cDNA is 1188 bp, contains a predicted 774 bp open reading frame that encodes 257 amino acids. A phylogenetic analysis conducted with previously characterized aquaporins from other plant species indicates that the cDNA encode putative tonoplast aquaporins (TIPs), and proposed that GjTIP has a tendency to be a mixed function aquaporin similar to the TIP1s from *Arabidopsis* and *Gossypium raimondii*. A typical “hourglasses” three-dimensional model of GjTIP was built. The expression of the GjTIP transcripts at fruits during maturation was conducted by RT-PCR analysis. The data revealed that the transcript levels of GjTIP have increased during fruit maturation.

Keywords *Gardenia jasminoides* · Tonoplast aquaporin · Expression · Fruits

Introduction

Aquaporins is a class of major intrinsic proteins (MIPs), which enables fast and controlled translocation of water and small neutral solutes across cellular membranes (Borgnia et al. 1999). Aquaporins have been found in bacteria, fungi, animals and plants. The relevance of water channels in cell physiology arise from the fact that aquaporins can give to the cell rapid and reversible changes in its hydraulic conductance by modulating membrane water permeability. Proteins of the MIP family are abundant in plant cells, the proteins vary in length

from 230 to 350 amino acids, they are classified into five groups (Danielson and Johanson 2008): the tonoplast intrinsic proteins (TIPs) localized to the vacuolar membranes, the plasma membrane intrinsic proteins (PIPs) localized to the plasma membranes, the NOD-26-like MIPs (NIPs) mainly localized in the nodules of nitrogen fixing legumes, the small basic intrinsic proteins (SIPs) and the X intrinsic proteins (XIP) appear to localize to the endoplasmic reticulum. Some of PIPs, TIPs and NIPs have been found to transport glycerol as well as water (Maurel et al. 2008). Substantial evidence is now available from a number of plant species that shows differential gene expression of aquaporins in different development stages and in response to salt, drought, cold and light, some aquaporins have also been characterized as tissue-specific in various plants (Bessorer et al. 2012; Liu et al. 2012; Péret et al. 2012).

The broad's genome sequence analyses have identified aquaporin genes and their structure in a number of plant species. There were 35 aquaporins genes in *Arabidopsis* genome (Johanson et al. 2001), 33 aquaporins genes in rice (Sakurai et al. 2005), 28 aquaporins genes in grape (Fouquet et al. 2008), 71 aquaporins genes in upland cotton, the cotton aquaporins consist of 28 PIP, 23 TIP, 12 NIP, 7 SIP members and one XIP member (Park et al. 2010). Analysis of the AQP family of maize based on complete sequences of the cDNAs obtained after sequencing a set of unique expressed sequence tags (ESTs) show that there are 31 aquaporins genes in maize (Chaumont et al. 2001). A majority of plant aquaporins have been found to share essential structural features with the human aquaporin and exhibit water-transporting ability in various functional assays. Human aquaporin family members include at least 12 aquaporins (Ishibashi 2009), and the structures of aquaporins have been studied (Fu et al. 2000; Sui et al. 2001), all members are tetramers of four functional monomeric channels. Each monomer contains six transmembrane α helices (H1-H6) connected by five loops, and two half-

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membrane-spanning α helices (HB and HE) around a central channel containing a conserved Asn-Pro-Ala signature sequences (NPA motifs), with the N and C termini that face the cytosol. A model representing the aquaporins as “hour-glasses” has been proposed. In the model, the channel pore is constituted by the junction of HB and HE that overlap midway between the leaflets of the membrane.

Members of the aquaporin family are composed of functionally distinct subgroups that include transmembrane water-conducting channels (aquaporins, AQPs) and glycerol-conducting channels (aquaglyceroporins, AQGPs). The aquaglyceroporins conduct both water and amphipathic solutes such as glycerol, urea, ammonia or boric acid. The *Escherichia coli* genome encodes one member of each subfamily, aquaporin Z (AqpZ), and the glycerol facilitator (GlpF). The molecular mechanisms of substrate selectivity and permeation are intensively investigated. Two highly conserved structural features within the channel were proposed as filters that exclude the passage of solutes larger than water and of charged molecules. A central constriction point is formed by NPA motifs (Zardoya 2005). The second selective structure is ar/R selectivity filter (aromatic/arginine motifs), it is located below the channel mouth and is narrower than the central NPA motifs (de Groot and Grubmüller 2005). The conserved difference between the AQPs and AQGPs occurs at the ar/R selectivity filter. In water-selective AQPs, this region is more polar and contains a conserved histidine, usually formed by four amino acids Phe-His-Cys-Arg. In AQGPs, it is more hydrophobic, with two conserved aromatic residues, usually tryptophan and tyrosine (Savage et al. 2010), and the ar/R selectivity filter is wider due to replacement of histidine by a glycine (Fu et al. 2000). Besides, the Froger's position P1–P5, was identified as the five conserved amino acid positions that differ consistently between the AQPs and AQGPs (Froger et al. 1998). P1 located in the terminal part of the H3 segment; P2 and P3 are located in HE, behind the second NPA motif; P4 and P5 are located in the H6 segment. P1 is an aromatic residue in the GlpF cluster, it is not aromatic in the AQP cluster. P2 and P3 correspond respectively to an acidic then a basic residue in the GlpF cluster and to two small uncharged residues in the AQP cluster. P4 and P5 can be defined as two aromatic residues in the AQP cluster compared with a proline followed by a nonaromatic residue in the GlpF cluster.

Gardenia jasminoides originates in Asia and has been in cultivation for at least a thousand years. The fruit of *G. jasminoides* is used in Asian countries as a natural colorant, and as a traditional herbal medicine. Crocin, crocetin and geniposide are the main secondary metabolites in the fruit (Chen et al. 2010). The orange or red colors of the fruit are caused by apocarotenoids, mainly crocin and crocetin. Geniposide is a predominant iridoid compound present in gardenia fruits. Crocin, crocetin and geniposide exhibit a wide range of pharmacological activities (Liu et al. 2013).

Little is known about the aquaporin gene in *G. jasminoides*. In this paper, we identified and analyzed the expression pattern of GjTIP in fruit during maturation.

Materials and methods

Plant and growth conditions

G. jasminoides plants cultivated at Guangdong Pharmaceutical University were used as materials. Fruits were collected at 4 development stages from the 8th to the 36th week after flowering date, the sampling times were the 8th, 16th, 24th, and the 36th weeks. StageI, closed with green exocarp and colorless mesocarp; stageII, closed with yellowish green exocarp and orange mesocarp; stageIII, closed with orange exocarp and red mesocarp; stageIV, the fully ripening stage, closed with half-dried fruit (red exocarp and deep red mesocarp). The samples were stored at -80°C until required.

cDNA library construction and ESTs sequencing

Total RNA was extracted from gardenia fruit (stageII), using a modified CTAB (hexadecyl trimethyl ammonium bromide) based extraction protocol (Bekesiova et al. 1999). From total RNA, the cDNA library construction and amplification were performed following the users manual of the CreatorTM SMARTTM cDNA Library construction Kit (Clontech, USA). The SMART cDNAs were ligated into *Sfi*I-digested pDNR-LIB vector and transformed into *Escherichia coli* strain DH5 α . Colonies were randomly picked, inoculate each colony to a separated PCR reaction solutions. The colony was lysed by heating the mixed solutions at 95°C in a PTC-200 Thermocycler (MJ Research, USA) for 5 min. After then, went to PCR amplification procedure with M13 primers provided by the CreatorTM SMARTTM cDNA Library construction Kit. The amplified PCR products (ESTs, expressed sequence tags) were analyzed by 1.2 % agarose gel electrophoresis. When the amplified PCR products were longer than 1000 bp, incubated the isolated colonies and sequenced the ESTs. There are 40 ESTs were sequenced. After sequencing and analysis, the colony containing the predicted pDNR-LIB-GjTIP was isolated.

Cloning of GjTIP ORF

After sequencing and analysis, the colony containing pDNR-LIB-GjTIP was isolated. According to the predicted full-length cDNA sequence of GjTIP, specific primers were designed to amplify the deduced ORF (open reading frame) of GjTIP gene, the forward primer A: 5'-ATGCCTGAAGTGATCGTGGTA CCGT-3' and the reverse primer B: 5'-CTAGTAATCTGC AACGGGCACGGC-3'. After PCR reaction, the amplified ORF of GjTIP was isolated.

Transcriptional analysis of GjTIP

Total RNA was extracted from fruits at development stage I to III (at stage IV, fruits are half-dried, could not be analyzed). cDNA was synthesized from total RNA using the Primescript one step RT-PCR kit for RT-PCR (TaKaRa, Japan). Primers C 5'-CTTGCGGTAACATTACCCTATT-3' and D 5'-CGGTGGCATAGACTGTGTAAACT-3' were designed for the reverse transcription and amplification of GjTIP cDNA. The levels of constitutively expressed RPS25-1 coding genes (Genbank accession no. GU797554) was assayed as controls. It was amplified in the same conditions, using primers E: 5'-CAGAAGAAGAAGAAGTGGAGCAA-3' and F: 5'-GTTCTTGAACCACTTGATGGTCT-3'. The amplification procedure by PCR consisted of an initial denaturing step at 95 °C for 2 min, followed by 32 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C. The amplified PCR products were analyzed by 1.2 % agarose gel electrophoresis. These experiments were repeated twice. To correct the initial mRNA levels, each cDNA band intensity was normalized to the intensity for the RPS25-1 gene amplification.

Results and discussion

Structure analysis of GjTIP

To identify gene encoding aquaporins in *G. jasminoides*, a cDNA library of *G. jasminoides* fruit was constructed and screened with primers M13. The inserts of about 40 clones were sequenced and analyzed. After search over the NCBI database, identified the full-length GjTIP cDNA (Genbank accession No. HQ599861). The cDNA is 1188 bp, contains a predicted 774 bp ORF that encodes 257 amino acids, a 88 bp non-coding region at 5' end and a 326 bp of non-coding region flank at 3' end. The predicted amino acid sequence of GjTIP specifies a polypeptide with a molecular mass of 26.7 kDa. Sequence comparison indicates that the deduced GjTIP protein is quite similar to other known aquaporins (Fig. 1), where GjTIP protein shared 67 % to 69 % amino acid identity with tonoplast aquaporins from 8 monocotyledonous and dicotyledonous plants (*Olea europaea*, *Arabidopsis thaliana*, *Brassica oleracea*, *Daucus carota*, *Gossypium hirsutum*, *Nicotiana tabacum*, *Vitis vinifera* and *Zea mays*), and 31 % amino acid identity with rabbit Aqp4m23 (PDB code 2d57_A, from 1 to 254 residue range). The highest degree of conservation is in the HB and HE that form the aqueous pore and contain the two NPA motifs. A phylogenetic tree was constructed by including GjTIP and 21 TIPs from *Arabidopsis thaliana* and *Gossypium raimondii*, indicated that the GjTIP belongs to TIP1 subfamily (Fig. 2a). TIP1 corresponds to the highly expressed and active γ -TIPs found in many plants (Chaumont et al. 1998).

The 3D (three dimension) model structure of GjTIP was predicted using SWISS-PDB software (Fig. 2b, c), the electron crystallography at resolutions down to 3.2 Å in three dimensions of rabbit Aqp4m23 (PDB code 2d57_A) was used as template. The amino acid sequence of GjTIP (from 23 to 246 residue range) has 34 % amino acid identity with Aqp4m23 (from 31 to 254 residue range). The quaternary structure was successfully built as tetramer. The analysis of the GjTIP amino acid sequence shows that the protein shares the following features with other reported aquaporins: have six α -helical transmembrane domains; have two highly conserved NPA (Asn-Pro-Ala); have the ar/R selectivity filter Tyr-Ile-Ala-Val, all TIPs in the phylogenetic tree have His-Ile-Ala-Val, the four amino residues was indicated in Fig. 2b, c. 21 TIPs from *Arabidopsis* and *Gossypium raimondii* (Genbank accession no. are shown in Fig. 2), Aqp4m23 from rabbit (AQPs), AqpZ (AQPs, Genbank accession no. U38664), GlpF (AQGP, Genbank accession no. P11244) and GjTIP were analyzed to check the ar/R selectivity filter and Froger's position, as shown in Table 1. From the comparison, confirms that many residues present a perfect correspondence with the Froger's position rule. The exception concerns the H2 position in ar/R selectivity filter, it's Y instead of H, F or W, whereas the H5, HB and HE positions are very well conserved in all TIP1s. Conservation in the ar/R selectivity filter and five Froger's positions provides that the GjTIP likely perform similar biological functions as other TIP1 aquaporins.

It was supposed that the mechanisms of substrate selectivity and permeation are mainly involved in NPA motif and ar/R selectivity filter. Froger's position is another proposal that concern about the channel selectivity. The two highly conserved Asn-Pro-Ala motifs participate in one of the main pore constrictions. There are five well-oriented waters in the AqpZ channel, forming a chain of water nearly the length of the channel, the waters form hydrogen-bond to side chains of several amino acids including amino acids in NPA motif and ar/R selectivity filter. Asn63 and Asn186 of the NPA motif form hydrogen bonds to the central water by their NH₂ moieties (Savage et al. 2003). Another constriction is ar/R selectivity filter, it is formed on the extracytoplasmic side of the membrane by a spatial arrangement of aromatic and other amino acids (usually have an arginine residue). The pore structures at ar/R constrictions have been investigated. It was found that smaller, hydrophilic channels conduct water and larger, hydrophobic channels conduct solutes such as glycerol, urea, nitrate and CO₂ (Gaspar et al. 2003; Loqué et al. 2005; Hanba et al. 2004; Kojima et al. 2006). In water-selective AQPs, this region usually formed by four amino acids Phe-His-Cys-Arg, some AQPs have Phe-His-Ala(Thr)-Arg (Plasencia et al. 2011). The conservation of arginine, histidine and phenylalanine side chains at their respective locations within the constriction region in the known water channels is a strong indicator of channel water specificity. The selectivity filter of AqpZ is the narrowest point in it's water channel, it is formed by the side

Fig. 1 Alignment of GjTIP with other aquaporins (ClustalW2). The abbreviation and Genbank accession number are: Gja (*Gardenia jasminoides*, AEF59492); Oeu (*Olea europaea*, ABB76813); Ath (*Arabidopsis thaliana*, P25818); Bol (*Brassica oleracea*, ABD64981); Dca (*Daucus carota*, ACV52008); Ghi (*Gossypium hirsutum*, ACP28878); Nta (*Nicotiana tabacum*, BAF95576); Vvi (*Vitis vinifera*, XP_002267788); Zma (*Zea mays*,

NP_001104896); Rno (*Rattus norvegicus*, 1–254 range, 2d57_A). The predicted conserved sites: NPA sequences are in **bold**; star indicates selectivity filter; # indicate Froger's position, residues on a black background are consensus. Helix: α -helix secondary structure of GjTIP predicted by Swiss-Model from the 23–246 residue range; α -helix are shown with "h" below the alignment

chains of Phe43, His174, Arg189 and the carbonyl of Thr183, the water in channel forms hydrogen-bond to the carbonyls of T183 (Savage et al. 2003). AQGs usually have Trp-Gla-Phe-Arg, some AQGs have His (Phe)-Ile(His)-Ala(Thr)-Val (Ma et al. 2004), their selectivity filters usually are more hydrophobic than in AQPs. GlpF has a much lower conductivity for water than to water channels, the selectivity filter is formed by Trp48, Glu191, Phe200 and Arg206, the phenyl ring of Phe200 and the indole of Trp48 form the hydrophobic corner, they have Van der Waals interaction with the alkyl chain of glycerol, and successive hydroxyl groups form hydrogen bonds with Arg206 and Phe200, and groups of several other amino acids (Fu et al. 2000). There are researches concerns about the relationship of amino acids in selectivity filter with the channel specificity. The functions of mutants with changed amino acids were investigated. The function of the amino acids in the ar/R selectivity filter (Phe56, His180, Cys189 and Arg195) in rat water-selective

AQP1 was analyzed by Beitz (Beitz et al. 2006), individual or joint replacement of His180 and Arg195 by alanine and valine residues respectively, the mutants enlarged the maximal diameter of the ar/R selectivity filter and enabled glycerol to pass, without affecting water permeability, the result is in accordance with the rule. While, Fu (Fu et al. 2000) found that the conservation of amino acids in ar/R selectivity filter sometimes can not be used as an indicator of channel specificity. When replaced three ar/R selectivity filter residues in AqpZ by their counterparts in GlpF, the mutant F43W/H174G/T183F was obtained, the mutant had a water permeability that was much less than GlpF but with a lack of increase in glycerol permeability. Using X-ray crystallography, the channels of AqpZ and mutant channels were determined, and revealed similar channel sizes, suggest this result could be partly attributed to differences in helix-helix packing (Savage et al. 2010).

The ar/R selectivity filter of GjTIP is Tyr-Ile-Ala-Val, it is hydrophobic than classic Phe-His-Cys-Arg (AQPs) and Trp-

Fig. 2 Phylogenetic analysis of GjTIP and predicted three-dimensional structure of GjTIP. **(a)** Phylogenetic analysis of GjTIP with 21 members of the cotton and *Arabidopsis* TIP family. Deduced amino acid sequences were aligned using ClustalW2 and the phylogenetic tree was generated using Bootstrap N-J tree (1,000 resamplings) method and Mega4 program. Gr(*Gossypium raimondii*); At(*Arabidopsis thaliana*); Gj(*Gardenia jasminoides*). The TIPs members and the Genbank accession number are

indicated. **(b)** And **(c)** three-dimensional model structure of GjTIP. **(b)** Ribbon representation of the monomeric GjTIP viewed from the periplasmic side. Helices are labeled H1 to H6, ar/R selectivity filter amino acids represented by spacefill. **(c)** Trace representation of the monomeric GjTIP with a stereo view (crossed eyes). The N and C termini that face the cytosol, HB, HE and ar/R selectivity filter amino acids are represented by ribbon

Gla-Phe-Arg (AQGP), and it is the same with TIP1s from *Arabidopsis* and *Gossypium raimondii* with the exception of Tyr (the TIP1s have His at the location), the Tyr at this location is rare in AQPs and AQGPs. AtTIP1;1 and several

TIPs from plants have mixed function, they can transport water and glycerol or urea. According to the report of Liu (Liu et al. 2003), AtTIP1;1 and AtTIP1;2 of *Arabidopsis* mediate the transport of water and conferred growth of a urea uptake-defective yeast mutant on 2 mM urea, the expression of AtTIP1;2 were up-regulated during early germination and under N deficiency in roots. Compare to AQPs, Tyr is larger and hydrophilic than Phe, Ile and Val is smaller and hydrophobic than His and Arg, respectively. Compare to AQGPs, Tyr is smaller and hydrophilic than Trp, and valine is hydrophobic than arginine. Compare to TIP1s of *Arabidopsis* and *Gossypium raimondii*, Tyr is larger and hydrophobic than His. Based on discussions above, there is not enough data to draw an unequivocal conclusion about the selectivity of GjTIP. We predicted that the GjTIP has a tendency to be a mixed function aquaporin similar to TIP1s from *Arabidopsis* and *Gossypium raimondii*. The predicted function of GjTIP needs to be verified through physiological assays.

Table 1 Comparing of ar/R selectivity filter and Froger's position of several aquaporins

Name	ar/R selectivity filter				Froger's position (P1-P5)				
	H2	H5	HB	HE	P1	P2	P3	P4	P5
GjTIP	Y	I	A	V	T	A	A	Y	W
TIP1	H	I	A	V	T	A,S	A	Y	W
Aqp4m23	F	H	A	R	T	S	A	Y	W
AqpZ	F	H	T	R	A	S	A	F	W
GlpF	W	G	F	R	Y	D	K	P	L

Froger's position is five conserved amino acids that concern about the channel selectivity. Froger's position of GjTIP is Thr-Ala-Ala-Tyr-Trp, it is the same with TIP1s from *Arabidopsis* and *Gossypium raimondii* and the amino acids present a perfect correspondence with the Froger's position rule for water-selective AQPs. As an experiment to verify the Froger's position rule, Lagrée (Lagrée et al. 1999) conducted function specialization on AQPcic (*Cicadella viridis*), Froger's position of AQPcic are Thr-Ser-Ala-Tyr-Trp, when replaced of the tyrosine and tryptophan by a proline and a leucine (conserved in AQGs) respectively, the water permeability was abolished and obtained a glycerol permeability. Since AtTIP1;1 and several TIPs from plants have mixed function, so, it seems the Froger's position rule has flaw, meanwhile the Froger's position of GjTIP suggested that the water transfer ability would be the main function of GjTIP. To our knowledge, GjTIP is the first TIP aquaporin described in *G. Jasminoides*, we suggested that the GjTIP would be a mixed function aquaporin similar to the TIP1s from *Arabidopsis* and *Gossypium raimondii*.

The transcriptional expression of GjTIP

Transcriptional expression of GjTIP in gardenia fruits during development stages was analyzed by reverse transcript (RT)-PCR. Fruits in stagel to III were analyzed (Fig. 3). Specific oligonucleotides for GjTIP mRNA were designed and used in the expression analysis. As shown in Fig. 3, a continuous increase in transcript levels of GjTIP during the fruit development stagel to III was found.

The fact that plants have large sized aquaporins gene family giving rise to close isoforms in a single species could be a way to control specific expression according to developmental and environmental conditions. For example, Sakurai (Sakurai et al. 2005) studied the expression levels of tonoplast aquaporins in rice organs, development stages and chilling treatment, to elucidate the physiological relevance of aquaporins in rice. The expression profile of OsTIP1;1 was differed with the development stages, it was highly expressed in roots at the early tillering stage and decreased at the panicle formation stage. Chilling treatment caused a decrease in the transcript level of OsTIP1;1 in roots, the decreased mRNA levels of OsTIP1;1 were recovered 9 h after warming. Using RNA interference(RNAi), Ma (Ma et al. 2004) reduced AtTIP1;1 to different extent in various

Arabidopsis lines, when most severely affected, miniature plants died, less severely affected lines produced small plants, early senescence, and showed lesion formation. Analysis of global expression profiling and metabolite profiles suggest a function for TIP1;1 in vesicle-based metabolite routing through or between pre-vacuolar compartments and the central vacuole.

The TIP1 subfamily corresponds to the highly expressed and active γ -TIPs found in many plants (Maurel et al. 1993; Chaumont et al. 1998). TIP1 subfamily is preferentially associated with the large lytic vacuoles (Jauh et al. 1998). *Arabidopsis thaliana* AtTIP1;1 was shown to accumulate in intravacuolar invaginations made of a double tonoplast membrane (Saito et al. 2002), and expression of the AtTIP1;1 is associated with cell enlargement in *Arabidopsis* roots, hypocotyls, leaves, and flower stems (Ludevid et al. 1992), the growth-promoting hormone gibberellic acid (GA3) would up-regulated the transcriptional level of AtTIP1;1(Phillips and Huttly 1994).

Aquaporin gene expression during fruit ripening might be associated with the main mechanisms involved in cell division and cell expansion, and fruit softening is also an important feature (Fouquet et al. 2008; Mut et al. 2008). The water channel expression pattern during ripening has been studied in grape berry, 3 putative tonoplast aquaporins and 6 plasma membrane aquaporins were found to be preferentially expressed in dividing and elongating cells and in cells involved in water and solutes transport, and have a global decrease gene expression during berry ripening. And in young berries, in dividing and elongating cells and in cells involved in water and solutes transport, aquaporin genes were highly expressed (Fouquet et al. 2008). Gene expressions of two strawberry aquaporins FaPIP1 and FaPIP2 in fruit increase in W (white) or 25 % R (25 % red) developmental stages in two cultivars, coincidently with the main firmness decrease that occurs at the early ripening stages, the aquaporins had a contribution to the loss of cell turgor (Villarreal et al. 2008). In this work, the expression of an isolated cDNA encoding supposed tonoplast aquaporins (GjTIP) was increased during the development. We suggested that the increased expression of GjTIP during fruit maturation could be due to contribute in dividing and elongating cells by increasing water and other small solutes flux into the cells and storage into and remobilization from the vacuole.

Water movements are crucial during plant development, and the separation of aquaporins in different subtypes and groups may reflect a specialization. The complete genome sequence of *G. jasminoides* is not available yet, so the full number and types of aquaporins present in *G. jasminoides* are still unknown. In conclusion, our results show the GjTIP is expressed in *G. jasminoides* fruit, and the increased expression during fruit maturation and the isolated GjTIP should be considered as aquaporin with mixed function similar to the TIP1s from *Arabidopsis* and *Gossypium raimondii*.

Fig. 3 Transcriptional expression of GjTIP gene in different fruit developmental stages (Ito III). The mRNA levels were determined by RT-PCR amplification of GjTIP. The levels of 40S ribosomal protein 25–1 coding gene were assayed as controls

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