Cloning and characterisation of JAZ gene family in Hevea brasiliensis

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Keywords
Expression profiles; Hevea brasiliensis; jasmonate ZIM-domain; yeast two-hybrid.

INTRODUCTION

Jasmonic acid (JA) and its derivatives are volatile compounds that are cyclic fatty acid-derived regulators. JA plays an important role in regulating plant responses to stress and development, including host responses to insects, pathogens and abiotic stresses such as UV radiation, ozone and salinity. JA is also involved in production of specific metabolites, control of vegetative growth rate, stamen development, senescence, trichome patterning and anthocyanin accumulation (Qi et al. 2011; Song et al. 2011; Wang et al. 2013). The broad activities of this hormone highlight the versatility of JA as a small molecule regulator for diverse biological systems. Elucidation of the plant JA signalling pathway predominantly depends on identification of mutants that are deficient in JA synthesis or perception (Berger et al. 1996; Xie et al. 1998; Staswick & Tiryaki 2004), of which, coronatine insensitive 1 (coi1) is the most important. The coi1 mutant is highly insensitive to JA and defective in most JA responses. COI1 encodes an F-box protein that associates with other proteins, SKP1 and CULLIN, to form the SCF<sup>COI1</sup> ubiquitin-ligase complex (Xie et al. 1998; Xu et al. 2002). This complex uses the F-box protein to bind target substrates, which are then polyubiquitinated and degraded by the 26S proteasome (Chini et al. 2007; Thines et al. 2007). The JAZ proteins directly interact with JA-responsive transcription factors (e.g. MYC2), and function as repressors of JA signalling (Chini et al. 2007; Melotto et al. 2008). After induction by wounding or developmental cues, JA is conjugated with isoleucine. This active form serves as ‘glue’ to mediate the interaction between COI1 and the JAZ family of repressor proteins, leading to the ubiquitination and an activating response. The COI1-JAZ-MYC2 is therefore suggested as the first core signalling module in the JA pathway (Chini et al. 2009). As the SCF<sup>COI1</sup> complex is very conservative in plants (Devoto et al. 2002; Xu et al. 2002; Chini et al. 2009), the tissue- and temporal-specific expression of JAZ gene family members, as well as their possible roles in repressing different transcription factors, may account for the specific responses of plants to the JA signal (Chini et al. 2009). In addition to the model plant Arabidopsis, specialised tissues that are JA responsive in other plant species should serve as excellent models for elucidating how specific JA responses are regulated (Chung et al. 2009).

Rubber (cis-1,4-polyisoprene) is synthesised by over 2,000 plant species distributed among 300 genera of seven families. Among these, the rubber tree (Hevea brasiliensis Mull. Arg.) is the sole commercial source of natural rubber because of its

ABSTRACT

Mechanical wounding or treatment with exogenous jasmonates (JA) induces differentiation of the laticifer in Hevea brasiliensis. JA is a key signal for latex biosynthesis and wounding response in the rubber tree. Identification of JAZ (jasmonate ZIM-domain) family of proteins that repress JA responses has facilitated rapid progress in understanding how this lipid-derived hormone controls gene expression and related physiological processes in plants. In this work, the full-length cDNAs of six JAZ genes were cloned from H. brasiliensis (termed HbJAZ). These HbJAZ have different lengths and sequence diversity, but all of them contain Jas and ZIM domains, and two of them contain an ERF-associated amphiphilic repression (EAR) motif in the N-terminal. Real-time RT-PCR analyses revealed that HbJAZ have different expression patterns and tissue specificity. Four HbJAZ were up-regulated, one was down-regulated, while two were less effected by rubber tapping treatment, suggesting that they might play distinct roles in the wounding response. A yeast two-hybrid assay revealed that HbJAZ proteins interact with each other to form homologous or heterogeneous dimer complexes, indicating that the HbJAZ proteins may expand their function through diverse JAZ–JAZ interactions. This work lays a foundation for identification of the JA signalling pathway and molecular mechanisms of latex biosynthesis in rubber trees.

A major advance in our understanding of the molecular mechanism of JA action came from characterisation of the first SCF<sup>COI1</sup> targets, which were identified as the jasmonate ZIM-domain (JAZ) family of protein in two independent studies (Chini et al. 2007; Thines et al. 2007). The JAZ proteins directly interact with JA-responsive transcription factors (e.g. MYC2), and function as repressors of JA signalling (Chini et al. 2007; Melotto et al. 2008). After induction by wounding or developmental cues, JA is conjugated with isoleucine. This active form serves as ‘glue’ to mediate the interaction between COI1 and the JAZ family of repressor proteins, leading to the ubiquitination and an activating response. The COI1-JAZ-MYC2 is therefore suggested as the first core signalling module in the JA pathway (Chini et al. 2009). As the SCF<sup>COI1</sup> complex is very conservative in plants (Devoto et al. 2002; Xu et al. 2002; Chini et al. 2009), the tissue- and temporal-specific expression of JAZ gene family members, as well as their possible roles in repressing different transcription factors, may account for the specific responses of plants to the JA signal (Chini et al. 2009). In addition to the model plant Arabidopsis, specialised tissues that are JA responsive in other plant species should serve as excellent models for elucidating how specific JA responses are regulated (Chung et al. 2009).

Rubber (cis-1,4-polyisoprene) is synthesised by over 2,000 plant species distributed among 300 genera of seven families. Among these, the rubber tree (Hevea brasiliensis Mull. Arg.) is the sole commercial source of natural rubber because of its
high production and rubber quality (Priyadarshan & Goncalves 2003). In *H. brasiliensis*, laticifers produce and accumulate rubber particles. The laticifers consist of contiguous cells that are arranged in rings parallel to the vascular cambium. A laticifer network structure developed from increased anastomoses between adjoining laticifers allows drainage of latex from a large area of bark at a single tapping (Hao & Wu 2000; Sando et al. 2009). Farmers harvest latex by regularly tapping the bark at 2- to 3-day intervals; rubber trees therefore represent the most frequently wounded plants in the world. Mechanical wounding induces laticifer differentiation and latex production (Hao & Wu 1982). Latex drainage accelerates laticifer differentiation in *H. brasiliensis*. The exploited trees have two- to three-fold laticifer rings than the unexploited trees (Hao & Wu 1984). Additionally, treatment with exogenous JA or linolenic acid, a precursor of JA biosynthesis, induces secondary laticifer differentiation in stems of epicormic shoots (Hao & Wu 2000). All these data suggest that laticifer differentiation is mediated by JA signalling. Two genes, *HbCOI1* and *HbJAZ1*, were recently cloned and preliminarily characterised (Peng et al. 2009; Tian et al. 2010); however, other key genes of the JA signalling pathway in *H. brasiliensis* have not been reported, and many gaps in the *Hevea* JA signalling pathway remain. The *Hevea* JAZ gene family has many members, each of which might have a specific function. In this work, the JAZ gene family of *H. brasiliensis* was cloned using an *in silico* procedure and then characterised. This will provide a basis to elucidate the JA signalling pathway and the molecular mechanism of latex biosynthesis in rubber trees.

**MATERIAL AND METHODS**

Plant material and treatment

*Hevea brasiliensis* cultivar Reyan 7-33-97 was used in this study, and planted in Qionghai, Hainan Province, in 2003. To study tissue-specific expression of *HbJAZ*, bark, latex and leaves were collected from 10-year-old mature trees that had been tapped for the last 2 years. To analyse the effect of tapping and wounding on *HbJAZ* expression, 10-year-old mature virgin (untapped) plants were selected and tapped successively at 2-day intervals, and latex collected for RNA extraction during the first five tapings.

**In silico cloning procedure**

All expressed sequence tags (EST) and nucleotide sequences, except genomic sequences of *H. brasiliensis* deposited in NCBI database (http://www.ncbi.nlm.nih.gov/) were downloaded in FASTA file format and de novo assembled as unigenes using illumina paired-end sequencing technology; 9860 (accession Nos. EC600050–EC609910; Chow et al. 2007), 37,432 (GSE26514) and 22,756 (JR344291–JR366936) Sanger-based EST sequences from latex. A local library for *Hevea* EST and nucleotide sequences was created using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Then each JAZ protein sequence of *Arabidopsis thaliana* was locally Blasted against the *Hevea* EST library using the tblastn program. This allowed overlapping partial cDNA sequences to be combined into full-length cDNA sequences. According to predicted sequences, a primer set was designed (Table S1). The full-length cDNA was RT-PCR amplified and sequenced. Furthermore, the protein sequences of *HbJAZ* genes were aligned with 12 *Arabidopsis* JAZ proteins using the multiple sequence alignment program ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Based on the phylogenetic tree and similarity to *Arabidopsis* JAZ genes, newly cloned *HbJAZ* genes were named and submitted to the NCBI GenBank (Accession Nos.: KJ001643–KJ001648).

**RNA extraction and quantitative real-time PCR**

*Hevea* RNA was extracted according to methods described in the literature (Xia et al. 2011). All RNA samples were treated with RQ1 RNase-free DNase I (Promega, Madison, WI, USA) to remove DNA contamination. The quality and concentration of the DNaseI-treated total RNA were checked using agarose gel electrophoresis and measured with spectrophotometry. A total of 2 μg DNase I-treated total RNA was used as template for the first-strand cDNA synthesis according to the manufacturer’s instructions (RevertAid First Stand cDNA Synthesis kit; Fermentas, Vilnius, Lithuania). Quantitative real-time (RT-)PCR assay was performed on an ABI-7500 Real-Time PCR apparatus with the dye SYBR Green I (Takara, Tokyo, Japan). The cDNA encoding 18S rRNA was chosen as reference using GeNorm software (Czechowski et al. 2005). The efficiency of each primer pair was evaluated before PCR, and primers are listed in Table S1. PCR reactions were performed as follows: 3 min at 95 °C, followed by 40 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 58 °C and extension for 20 s at 72 °C. The relative abundance of transcripts was automatically calculated as $2^{-ΔΔCt}$ using the ABI-7500 software and 18S rRNA gene as internal standard. All experiments were performed with three independent biological replicates and three technical repetitions. Calculation of the standard error and ANOVA were used for statistical significance analyses.

**Yeast two-hybrid assay**

The cloned full-length coding DNA sequences (CDS) of *HbJAZ* genes were first inserted into the pGBKKT7 vector to generate a bait vector. The bait vector was transformed into yeast Y2H gold strain to test toxicity and auto-transcription activity, according to the manufacturer’s instructions (Cat. No. 630489; Clontech, Mountain View, CA, USA). Subsequently, the CDS of *HbJAZ* genes were further fused into the pGADT7 vector to generate a prey plasmid. The bait vector and prey vector were transformed into Y187 and Y2H gold strains, respectively. The yeast two-hybrid was obtained by mating Y187 and Y2H gold strains. Protein interaction was assessed by expression of different reporters under control of GAL4-responsive promoters.

**RESULTS**

**Cloning JAZ gene family of *Hevea brasiliensis***

*Arabidopsis* has 12 and rice has 15 JAZ genes. So far, only one JAZ gene was reported in *H. brasiliensis* (Tian et al. 2010). In order to globally identify the JAZ gene family of *H. brasiliensis*,...
all Hevea EST and nucleotide sequences deposited in the NCBI database were downloaded and searched using A. thaliana JAZ protein sequences as query template. Through prediction and in silico assembly, at least 12 JAZ genes were predicted in *H. brasiliensis*. After PCR amplification and sequencing validation, only seven full-length cDNAs of the JAZ genes containing the ZIM domain and Jas domain (including the reported *HbJAZ1*) were identified. To further analyse the sequence similarity, JAZ protein sequences of *H. brasiliensis* (*HbJAZ*) and 12 *Arabidopsis* JAZ proteins were aligned and a phylogenetic tree created using the multiple sequence alignment program ClustalW2. Alignment showed that the TIFYXG motif and Jas domain are highly conserved in all sequences tested, although there were different gene lengths and great sequence diversity (Figure S1). The distribution of the ZIM domain, Jas domain and the LxLxL type EAR (ERF-associated amphiphilic repression) motif, are represented in colour (Fig. 1A). According to nomenclature for JAZ genes of *Arabidopsis*, the phylogentic tree, and properties of the ZIM domain, Jas domain and EAR domain, the *HbJAZ* were termed *HbJAZ1, HbJAZ2, HbJAZ7, HbJAZ8, HbJAZ9, HbJAZ10* and *HbJAZ11*. As shown in Fig. 1B, the α-helix regions of the Jas domain in all *HbJAZ* genes tested are relatively conserved, but the loop regions vary. *HbJAZ1, HbJAZ2, HbJAZ10* and *HbJAZ11* have a short conserved LPIAR motif, which was reported to seal JA-Ile into its binding pocket at the COI1-JAZ interface (Shyu et al. 2012). Like AtJAZ8, *HbJAZ7* and *HbJAZ8* lack this motif (Fig. 1B) and contain an EAR motif in the N-terminal (Fig. 1C). EAR binds the co-repressor TOPLoss and represses transcriptional activation. This type of JAZ protein is unable to associate strongly with COI1 in the presence of JA-Ile and is stabilised against JA-mediated degradation. The repression does not require the ZIM domain, which in other JAZ proteins recruits TOPLoss through the EAR motif-containing adaptor protein NINJA (Pauwels et al. 2010). *HbJAZ7* and *HbJAZ8* are considered independent of COI1 and constitutively repress JA responses in *H. brasiliensis*.

**Tissue expression patterns of the *HbJAZ* gene family**

In order to evaluate expression patterns of the *HbJAZ* gene family, RNA was isolated from latex, leaves and bark. Gene expression was analysed with real-time RT-PCR. The expression levels of *HbJAZ1, HbJAZ7* and *HbJAZ11* are relative higher than those of *HbJAZ2, HbJAZ8, HbJAZ9* and *HbJAZ10*. Generally, most *HbJAZ* genes show higher expression in leaves than in latex and bark (Fig. 2), suggesting that *HbJAZ* genes have different expression patterns and tissue specificity.

**Effect of tapping wounds on expression of the *HbJAZ* gene family**

To harvest latex, farmers regularly tap the trunk bark of rubber trees, normally at 2- to 3-day intervals. Tapping is mechanical wounding, and latex leakage is a wounding response of the rubber tree. We examined how mechanical tapping affects the expression of *HbJAZ* family genes. Real-time RT-PCR showed that expression patterns of *HbJAZ* genes varied after mechanical wounding. *HbJAZ1, HbJAZ2, HbJAZ7* and *HbJAZ11* were up-regulated after tapping treatment, *HbJAZ9* was down-regulated, and *HbJAZ8* and *HbJAZ11* were less affected. Additionally, the up-regulated peak of expression of *HbJAZ1, HbJAZ2* and *HbJAZ7* occurred at the third tapping, whereas expression of *HbJAZ11* increased continuously and peaked at the fifth tapping (Fig. 3). These results suggest that each of the *HbJAZ* genes had different regulation of gene expression and might play distinct roles in the wounding response.

**Homo- and heteromeric interaction of *HbJAZ* proteins**

Most *A. thaliana* JAZ exhibit homo- and heteromeric interactions mediated by the TIFY motif (TIFY/XYG) within the ZIM domain (Chung & Howe 2009). All *HbJAZ* proteins contain a conserved TIFY motif in the ZIM domain. We therefore used a yeast two-hybrid (Y2H) system to determine whether the *HbJAZ* proteins interact with each other to form homo- or heterodimers. First, each full-length *HbJAZ* was fused separately to the GAL4 BD domain to generate a bait plasmid, which was further transformed into Y2H Gold-competent cells and plated on SD-Trp/-His/-Ade agar. There was no autoactivation or toxicity of *HbJAZ* proteins (Figure S2). Then the full-length *HbJAZ* were separately fused to the GAL4 AD domain to generate prey vectors. Homo- or heterodimeric interactions were assessed as expression of reporter genes under control of the GAL4-responsive promoters. As shown in Fig. 4, the *HbJAZ* showed universal homo- or heterodimeric interactions, determined as strong X-a-gal reporter activity. *HbJAZ1, HbJAZ7, HbJAZ8, HbJAZ9* and *HbJAZ10* strongly interacted as homodimers. Strong heteromeric interactions were observed among *HbJAZ1, HbJAZ2, HbJAZ7, HbJAZ8, HbJAZ9, JAZ10* and *HbJAZ11*, whereas several combinations showed no interaction, indicating that the absence of an interaction cannot be attributed to lack of protein expression.

**DISCUSSION**

Jasmonic acid regulates a series of plant responses to biotic and abiotic stresses. JA is involved in biosynthesis of specific metabolites, such as anthocyanin and nicotine (Qi et al. 2011; Shoji and Hashimoto 2011; Zhang et al. 2012). Anthocyanins are responsible for plant colour, attracting insects to pollinate flowers, protecting plants from UV radiation and stress damage, and function as antimicrobial agents against insect attack and pathogen infection (Gould 2004). JAZ proteins interact with bHLH and R2R3 MYB transcription factors to repress JA-regulated anthocyanin accumulation (Qi et al. 2011). Tobacco (*N. tabacum* L.) produces an array of alkaloids that play an important role in plant defence against herbivore and insect attack. The main alkaloid in cultivated tobacco is nicotine (Steppuhn et al. 2004). Tobacco JAZ proteins interact with NtMYC2/a/b that bind a G-box-containing promoter and regulate expression of putrescine N-methyl transferase (PMT), a key enzyme in nicotine formation (Shoji & Hashimoto 2011; Zhang et al. 2012).

Rubber (cis-1,4-polyisoprene) has been an undeniably beneficial commodity for the past 100 years. Natural rubber attracts much attention as an extremely useful industrial polymer because no current synthetic substitute has comparable physical properties, but little attention has been paid to the biological nature of rubber. A growing body of evidence suggests that JA is also involved in latex biosynthesis, a specific metabolite protecting the rubber tree from insect attack and pathogen
infection. Since the 1980s, Hao and Wu have examined laticifer differentiation and found that JA plays a key role in this process. In 2004, they were awarded gold medals by the International Rubber Research and Development Board (IRRDB). Much evidence suggests that JA is an important regulator of latex biosynthesis. First, some key enzyme genes, e.g. for gernylgeranyl diphosphate synthase, small rubber particle protein and rubber prolong factor, farnesyl diphosphate synthase, are induced by mechanical wounding or exogenous JA treatment (Adiwilaga & Kush 1996; Oh et al. 1999; Sookmark et al. 2002). Second, isoprenoid biosynthesis and taxane accumulation are induced by methyl jasmonate in *Taxus baccata* cell cultures (Laskaris et al. 1999). Additionally, many genes involved in the isoprenoid pathway are induced through JA or mechanical wounding (Duan et al. 2010). As described, wounding or JA induce laticifer differentiation and latex biosynthesis, but over-exploitation (too much tapping and drainage stimulated by ethephon) cause Tapping Panel Dryness (TPD) syndrome (Faridah et al. 1996). JA signalling plays an important role in both latex biosynthesis and TPD formation in *H. brasiliensis*. JAZ proteins are key regulators of the JA signalling pathway. Cloning and characterisation of the JAZ gene family might help us to understand the molecular mechanism in *H. brasiliensis*.

The first problem was how to define JAZ proteins. JAZ are members of the TIFY family, but not all TIFY proteins are JAZ. The ZIM domain has a highly conserved TIFYXG motif. Apart from a 28 amino acid ZIM domain near the N-terminal, JAZ proteins contain a Jas domain at the C-terminal. In *A. thaliana*, TIFY proteins are encoded by 18 genes, 14 of which containing the Jas domain: 12 JAZ and two PEAPOD (PPD; Chung et al. 2009). An obvious difference between JAZ and PPD is that JAZ have a conserved PY terminal instead of the

Fig. 1. Comparison of JAZ genes of *H. brasiliensis* and *A. thaliana*. A: Multiple sequence alignment and phylogenetic tree created using ClustalW. The relative positions of ZIM domain, Jas domain and EAR domain are shown in colour. B: Comparison of Jas domains. C: Comparison of LxLxL type EAR motifs of AtJAZ8, HbJAZ7 and HbJAZ8.
KK terminal of PPD in the Jas motif (Chung et al. 2009). JAZ exert their effects on gene expression through physical interaction with transcription factors, but PPD can bind to the promoter region of a gene (Lacatus & Sunter 2009). Through in silico gene cloning, more than 12 JAZ genes were predicted in *H. brasiliensis*, but only seven JAZ genes were successfully amplified with PCR in this work. One potential reason for this might be allelic differences among cultivars. The cDNA sequences used for in silico prediction come from many cultivars, but the cDNA template used for PCR is from the cultivar

**Fig. 2.** Tissue-specific expression profiles of *HbJAZ*. RNA was isolated from the pooled samples of latex, leaves and bark, and expression levels were measured with real-time RT-PCR and normalised to the constitutive actin control. Results are given as the mean of three independent experiments. y-axis is relative expression level. Asterisks denote significant difference (*P* < 0.05).

**Fig. 3.** Effect of tapping wounds on expression of *HbJAZ*. Latex samples from successively tapped trees were collected 5 min after the first (1), second (2), third (3), fourth (4) and fifth (5) tapping at 2-day intervals. RNA was isolated from the pooled latex of five trees per tapping, expression levels were measured with real-time RT-PCR and normalised to the constitutive actin control. Results are given as the mean of three independent experiments. y-axis is relative expression level and x-axis is successive tapping number. Asterisks represent significant difference (*P* < 0.05).
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Chong & Howe 2009; Chung JAZ interactions have expanded the repertoire (Yan [pGBKT7-Lam] and Y187 [pGADT7-T].

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ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of China (Grant Nos. 31370608, 31260170, 31060107), Hainan Major Research Project for Science and Technology (ZDZX2013023) and the Research Fund for the Doctoral Program of Higher Education (Nos. 20104601110003 and 20114601110003).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. CLUSTAL 2.1 multiple sequence alignment of JAZ gene family of A. thaliana and H. brasiliensis.

Figure S2. Autoactivation and toxicity test of HbJAZ proteins in bait vector.

Table S1. PCR primer sets of HbJAZ.

degradation signal (degron) includes a short conserved LPIAR motif that seals JA-Ile into its binding pocket at the COI1-JAZ interface. JAZ8 lacks this motif and thus is unable to associate strongly with COI1 in the presence of JA-Ile. As a consequence, JAZ8 is stabilised against jasmonate-mediated degradation. Additionally, JAZ8 has an LxLxL-type EAR motif at the N-terminus that interacts directly with the TPL and represses transcriptional activation. JAZ8-mediated repression does not require the ZIM domain, which, in other JAZ proteins, recruits TOPLESS through the EAR motif-containing adaptor protein NINJA (Shyu et al. 2012). HbJAZ7 and HbJAZ8 lack the LPIAR motif (Fig. 1B), and contain an EAR motif in N-terminal (Fig. 1C). Like JAZ8 in Arabidopsis, HbJAZ7 and HbJAZ8 might be independent of COI1 and constitutively repress JA responses, and might have distinct roles in H. brasiliensis.

Although HbJAZ genes may have functional redundancy, each of HbJAZ proteins has individual specificity, as indicated by the specific tissue expression patterns (Fig. 2), different response to wounding treatment (Fig. 3) and combinatorial diversity of JAZ–JAZ interactions (Fig. 4). Expression of HbJAZ1, HbJAZ2, HbJAZ7 and HbJAZ10 is induced through tapping, suggesting that HbJAZ proteins are directly correlated with the wounding response. The Jas motif of JAZ proteins interacts with a broad array of transcription factors that promote expression of JA response genes and control specific downstream processes (Chini et al. 2007; Melotto et al. 2008; Fernandez-Calvo et al. 2011; Qi et al. 2011; Song et al. 2011). It is essential to understand which transcription factors interact with these HbJAZ proteins, and whether the HbJAZ-interacting transcription factors bind to the promoter or to latex biosynthesis-related genes to control latex biosynthesis in the rubber tree. Such a screening experiment should be undertaken in the future.

Fig. 4. Homo- and heteromeric interactions of HbJAZ proteins. The full-length HbJAZ were fused to pGADT7 and pGBKT7 to generate prey and bait vectors separately, which were further transformed in Y187 and Y2H gold yeast strains, respectively. Then the Y187 and Y2H gold yeast strains were combined and cultivated. The mating cell was screened on synthetic drop-out DDO plates (SD-Trp/-Leu) and Y2H interactions were assessed on QDO/ X/A plates (SD-Trp/-Leu/Ade/-His/X/A). Positive control mating (+): Y2HGold [pGBKT7-53] and Y187 [pGADT7-T], Negative control mating (−): Y2HGold [pGBKT7-Lam] and Y187 [pGADT7-T].

Reyan 7-33-97. Another possible reason for no PCR product is the low expression level of some HbJAZ in the tissues tested. If more samples from different tissues and from different developmental stages were used for RT-PCR, more JAZ might be identified in the future.

Arabidopsis contains 12 JAZ genes, but alternative splicing of the JAZ genes and potential combinatorial diversity of JAZ–JAZ interactions have expanded the repertoire (Yan et al. 2007; Chung & Howe 2009; Chung et al. 2010). Each JAZ might have an individual or redundant function. For example, three bHLH transcription factors, EGL3, GL3 and TT8, interact with eight JAZ proteins (JAZ1, JAZ2, JAZ5, JAZ6, JAZ8, JAZ9, JAZ10 and JAZ11) but not with the remaining four JAZ proteins (JAZ3, JAZ4, JAZ7 and JAZ12). Two MYB factors, MYB75 and GL1, interact with JAZ1, JAZ8 and JAZ11 (Qi et al. 2011). A major challenge is to determine the contribution of individual JAZ isoforms, such as their targets, specificity, function and regulation, in JA signalling. However, the domain/motif existing in JAZ proteins can exactly reflect the role of a specific function. The ZIM domain and its associated TIFY motif mediate JAZ interaction with an adaptor protein called NOVEL INTERAC-
TOR OF JAZ (NINJA), which functions to recruit the transcription co-repressor TOPLESS (TPL) and TPL-related proteins (TPR) (Pauwels et al. 2010; Pauwels & Goossens 2011). NINJA contains an EAR motif that is necessary and sufficient for TPL interaction (Pauwels et al. 2010). The JAZ1
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