Transcriptional and post-transcriptional regulation of the jasmonate signalling pathway in response to abiotic and harvesting stress in *Hevea brasiliensis*

Julien Pirrello¹, Julie Leclercq¹, Florence Dessailly¹, Maryannick Rio¹, Piyanuch Piyatrakul¹², Kuswanhadi Kuswanhadi³, Chaorong Tang⁴ and Pascal Montoro¹*

**Abstract**

**Background:** Latex harvesting in *Hevea brasiliensis* amounts to strong abiotic stress that can cause a halt in production in the most susceptible clones. Although the role of jasmonic acid has been suggested in laticifer differentiation, its role in latex production and in the response to harvesting stress has received very little attention. Only a few key genes acting in the COI-JAZ-MYC module have been isolated and studied at transcriptional level.

**Results:** Use of a reference transcriptome obtained on rubber clone PB 260 covering a large number of tissues under different environmental conditions enabled us to identify 24 contigs implicated in the jasmonate signalling pathway in the rubber tree. An analysis of their expression profile by qPCR, combined with hierarchical clustering, suggested that the jasmonate signalling pathway is highly activated in laticifer cells and, more particularly, in the response to harvesting stress. By comparison with their genomic sequences, the existence of regulation by alternative splicing was discovered for JAZ transcripts in response to harvesting stress. Lastly, positive transcriptional regulation of the *HbJAZ_1405* gene by MYC was demonstrated.

**Conclusion:** This study led to the identification of all actors of jasmonate signalling pathway and revealed a specific gene expression pattern in latex cells. In-depth analysis of this regulation showed alternative splicing that has been previously shown in *Arabidopsis*. Interestingly, genotypic variation was observed in *Hevea* clones with contrasting latex metabolism. This result suggests an involvement of jasmonate signalling pathway in latex production. The data suggest that specific variability of the JA pathway may have some major consequences for resistance to stress. The data support the hypothesis that a better understanding of transcriptional regulations of jasmonate pathway during harvesting stress, along with the use of genotypic diversity in response to such stress, can be used to improve resistance to stress and rubber production in *Hevea*.

**Keywords:** Latex, Tapping panel dryness, Jasmonic acid, Alternative splicing, Rubber, Transcriptional regulation

---

**Background**

Jasmonic acid (JA) is a key hormone in the development of plant responses to abiotic stress, such as wounding [1]. This plant hormone also plays a key role in the biosynthesis of secondary metabolites [2]. It is particularly involved in the response to oxidative stress since it activates the ascorbate-glutathione cycle for the reduction of these major antioxidants [3,4].

The jasmonate biosynthesis and signalling pathways have been very widely studied and described in *Arabidopsis* using the analysis of mutants [5]. The *jar1* mutant is deficient in certain responses to JA, including ascorbate production to prevent oxidative stress [6]. The JAR1 enzyme catalyses the conjugation of jasmonate to isoleucine (JA-Ile) [7]. JA-Ile is the bioactive form of JA [8]. It was demonstrated recently that COI1 is the JA receptor and can interact directly with JA-Ile [9]. For its part, the *coi1* mutant also shows insensitivity to JA, thereby causing male sterility, resistance to the inhibition of root development by JA, and a defect in the expression of genes regulated by JA.
Latex is harvested by tapping the tender bark (phloem tissues). After latex flow, rubber particles agglomerate and clog the tapping zone. Latex is regenerated within a few days. In order to stimulate latex production 2.5% ethephon (an ethylene releaser) is applied to the tapping panel. Tapping and stimulation frequency is usually adapted to the metabolic activity of *Hevea* clones. In extreme cases of environmental or man-made stress due to rubber tree tapping, an oxidative burst occurs in the laticifers. That oxidative stress leads to peroxidation of lipids in the membrane of lutoids (poly dispersed lysosomal vacuome), which contain agglutinins such as heveins. The release of these factors leads to the in situ coagulation of rubber particles: this is the physiological syndrome known as Tapping Panel Dryness (TPD) [29]. TPD causes substantial NR production losses (10-40%) and economic models predict that NR production will not be sufficient by 2020. The genetic variability existing within the different cultivated rubber clones shows that the intrinsically most productive and fast growing clones are also the most susceptible to abiotic stress and TPD [30]. Some rubber clones with a slow laticifer metabolism are more tolerant to TPD. To date, few selected clones have the latter characteristics [31]. For instance, clone PB260 is a clone with an active metabolism that is highly susceptible to TPD, while INC53 and RIM600 are clones with a slower metabolism and are more resistant to TPD. The physical wounding caused by tapping, the osmotic shock within the laticifers due to latex flow, and metabolic activation linked to latex regeneration between two tappings amount to harvesting stress which causes diverse responses, including the production of hormones such as JA. In fact, wounded tissues produce systemin, which induces JA production [32]. Interestingly, jasmonate and wounding are also involved in laticifer differentiation [33]. It was recently shown that Jasmonate ZIM Domain (JAZ) proteins are involved in the defense of plants against environmental stress [10]. COI1 is an F-box protein [11] forming, with some other partners, a complex of the E3 ubiquitin ligase type, SCFCOI1 [12,13]. This type of complex is involved in the ubiquitination of target proteins, leading to their subsequent degradation by the 26S proteasome [14]. JASMONATE ZIM DOMAIN (JAZ) proteins are recognized by the SCFCOI1 complex. JAZ degradation enables the release of transcription factors such as MYC2,3 and 4 [15]. The latter bind to cis-acting elements of the G-box type [16] present in the promoters of JA response genes and they initiate their transcription [17-19]. In addition to the MYC transcription factors, JAZ are able to interact with other transcription factors of the MYB, EIN3, EIL, ERF, GAI, RGA and RGL1 types, suggesting that they play a role in the interaction of the JA signalling pathway with those of other hormones [20,21]. In Arabidopsis, JAZ proteins comprise 12 members which are characterized by the presence in the C-terminal position of the highly conserved domains, Jas and ZIM (TIFY). The Jas domain enables interaction with COI1 and with transcription factors, while the TIFY domains involved in dimerization and in the interaction with NINJA [21-23]. At the moment, the most likely model concerning the repression of genes induced by JA is an interaction of JAZ with TOPLESS (TPL). This interaction may necessitate the presence of the NINJA protein as an adapter, unless the JAZ protein possesses an EAR domain (ERF-associated amphiphilic repressor) to which TPL binds [1]. The transcriptional regulation of JAZ can occur via MYC2 [16], but other components might be involved. Indeed, in the *myc2* mutant, most JAZ were expressed following infection with *Pseudomonas syringae* [24]. The sub-unit of the mediator complex, MEDIATOR25 (MED25), was recently identified as an integrative node of JA-dependent gene expression [25]. At post-transcriptional level, most JAZ genes can be regulated by alternative splicing. In Arabidopsis, it has been found that intron/exon organization in the region of the Jas domain is conserved in the majority of JAZ genes [26]. In Arabidopsis, the spliced form of JAZ10 has a Jas domain that is partially (JAZ10.3) or totally truncated (JAZ10.4) [23]. Ectopic expression of JAZ10.3 and JAZ10.4 affords dominant insensitivity to JA as a consequence of the stabilization of the JAZ protein [19,23]. The physiological role of isoforms with a truncated Jas domain in attenuating hormonal response has been suggested by various studies [19,27]. Many studies have shown the importance of alternative splicing in plant development, but also in the response to stress (for review [28]), though that link has never been demonstrated in the case of post-transcriptional regulation of JAZ genes.

*Hevea brasiliensis* is the only commercial source of natural rubber (NR). NR is synthesized in the rubber particles of latex cells. Those cells differentiate from the cambium then anastomose to form an articulated network: laticifers.
In this study, the availability of *Hevea* transcriptomic and genomic resources made it possible to identify all the different genes acting in the jasmonate signalling pathway and characterize their implication during development, and in response to abiotic stress. Among the transcriptomes sequenced on different tissues (latex, bark, leaves and stem tips) [43-48], a reference transcriptome covering a large number of tissues and environmental conditions has been published for rubber clone PB 260 [35]. An analysis of the structure of JAZs genes based on the genomic sequences of rubber clone CATAS 7-33-97 (BIG-CATAS Project, unpublished data), and an analysis of the corresponding transcripts revealed the existence of a mechanism of alternative splicing of JAZ gene transcripts. Thus, this study suggested that the stress caused transcriptionally and post-transcriptionally by latex harvesting regulates the jasmonate signalling pathway in *Hevea*.

**Methods**

**Plant material**

For the transcript analyses, plant material of clone PB 260 grew in accordance with the conditions described in Duan and coll. [35]. Samples corresponding to reproductive tissues (immature and mature, male and female flowers, zygotic embryos) were added to this study. Flowers were collected from 15-year-old trees. Zygotic embryos were collected from 5-year-old trees. *In vitro* plantlets of clone PB 260 were obtained by somatic embryogenesis with line CI07060. The plantlets were acclimatized and grown for 3 months in a greenhouse at a temperature of 27°C with 45% humidity. Several treatments mimicking abiotic stress were carried out, such as wounding, methyl jasmonate (MeJA), ethylene (ET) and dehydration at 1, 4, 8 and 24 hours. Drought stress treatment was controlled by following *HbERF1a* (orthologue to DREB2A) transcript accumulation during this stress [49], whereas efficiency of wounding, ethylene and MeJA treatment were controlled using *HbERF-IXc4* and *HbERF-IXc5* (orthologue to ERF1) [37,50]. The bark was wounded every 0.5 cm by scarification with a razor blade. The ET and MeJA treatments were carried out by placing the plants in a 300-l open-door Plexiglas box overnight before treatment. Five parts per million of pure ET gas (1.5 ml/300 l) was injected into the sealed air-tight box. A concentration of 100 μl of liquid ≥95% MeJA solution (Sigma, St Louis, MO) was diluted in 500 μl of absolute ethanol and then placed on Whatmann paper inside the box for gas release. Control plants used for the ET and MeJA treatments were placed in the box and exposed to air only. The dehydration treatment was carried out by taking the plants out of their pots and leaving them to dry under laminar air flow. Each sample was collected 1 h, 4 h, 8 h and 24 h after treatment.

RNA samples were collected and prepared at the CRRC, RRIT, Sanam Chaikhet District, Chachoengsao 24160, Thailand (13.39N latitude and 101.26E longitude). These locations and our activities did not require any specific permission. The field studies did not involve endangered or protected species.

**Total RNA extraction**

Total RNAs were extracted from one gram of fresh matter using the caesium chloride (CsCl) cushion method adapted from Sambrook and coll. (Sambrook et al. [51]) by Duan and coll. (Duan et al. [37]). DNA contamination was checked by PCR amplification using primers of the actin gene.

**Primer design and transcript abundance analysis by qPCR**

Total RNA integrity was checked by electrophoresis. For each candidate gene a blast was performed against the transcriptome of PB 260, to define highly conserved regions. The qPCR primers were designed outside these regions using the Primer 3 module of Geneious Pro software version 5.3.6 (Biomatters Ltd., New Zealand). Each primer pair was blasted against the transcriptome library. qPCR and the fusion curve of the PCR amplicon were done using a mix of cDNAs. In addition, the specificity of each primer pair was checked by sequencing the PCR amplicon. The sequences of the primers used are listed in Additional file 1.

cDNAs were synthesized from 2 μg of total RNA to a final reaction volume of 20 μl using a Revert AidTM M-MuLV Reverse Transcriptase (RT) kit according to the manufacturer’s instructions (MBI, Fermentas, Canada). Full-length cDNA synthesis was checked for each sample by PCR amplification of the actin. Quantitative gene expression analysis was carried out by qPCR using a Light Cycler 480 (Roche, Switzerland) as described by Putranto and coll. [52]. Real-time PCR was carried out for eleven housekeeping genes in order to select the most stable gene as the internal control for all the compared tissues (HbelF1Aa, HbUBC4, HbUBC2b, HbYLS8, HbRH2b, HbRH8, HbUBC2a, HbalphaTub, Hb40S, HbUbi, HbActin). HbRH2b was selected as the best reference gene due to its stability in the different tested tissues [39] (Additional files 2 and 3). The transcript abundance of each gene was relatively quantified by normalization relative to the transcript abundance of the *HbRH2b* reference gene. All the normalized ratios corresponding to transcript accumulation were calculated automatically by Light Cycler software version 1.5.0 provided by the manufacturer. Heatmap representation was carried out on normalized and centered ΔCt values, using the heatmap2 function of the R software gplots package [53,54].

**Statistical analyses**

qPCR reactions were carried out with 3 biological replicates. The statistical analyses were ANOVAs carried out
on raw data after logarithmic transformation. An ad-hoc Tukey HSD (Honestly Significantly Different) test was carried out for the analysis of transcripts in the different organs and different clones. Values with the same letter were not significantly different. For the analysis of transcripts in response to abiotic stress and to hormone treatments, a comparison of means test (Student or Wilcoxon test, depending on the data) was carried out between the control and the treatment at each point of the kinetics. If, at one point, at least one significant difference was found an s was indicated, otherwise ns was indicated.

Phylogenetic analysis of JAZ genes
Multiple alignment was carried out on the total protein sequences of the JAZs of Arabidopsis and Hevea. Alignment with Gblocks software [55] led to the isolation of conserved blocks at least 10 amino acids long. The blocks were then used to construct the phylogenetic tree using PhyML software [56] which implements a maximum likelihood tree reconstruction method using the LG + gamma model, starting from a BioNJ tree [57]. A RAP-Green analysis was carried out using the original tree from PhyML to predict duplications [58]. The final tree was visualized with the Archaeopteryx program [59].

Test of transcriptional activity by transient expression in BY-2 cells of tobacco
Transactivation experiments were carried out following the procedure described by Chaabouni and coll. [60]. The pJAZ_1405(−267) and pJAZ_1405(−955) promoters were cloned to the pMDC107 vector [61], thereby controlling the GFP reporter gene. The MYC_771 and MYC_94937 genes were cloned under control of the 35S promoter to the pMDC32 vector. Transactivation assay was carried with several controls. First, a window excluding debris and define auto-fluorescence was defined from protoplast lysis was carried out using the original tree from PhyML to predict duplications [58]. The final tree was visualized with the Archaeopteryx program [59].

Accession numbers
Reads from this library are those published by Duan and coll. (Accession: PRJNA235297 ID: 235297) available on NCBI database.

Results
Identification of the different genes acting in the jasmonate signalling pathway
The different genes acting in the jasmonate signalling pathway were identified from the comprehensive transcriptome published by Duan and coll. [35] using TBLASTN with the corresponding Arabidopsis sequences as the query. For each gene, we selected the one that came out with the best score. For each gene identified in that way, we checked for the conservation of the domains characteristic of each family using INTERPROSCAN. We thus identified 6 contigs corresponding to JAR, 2 for COI, 10 for JAZ and 3 for MYC. The JARs belonged to the GH3 multigene family [62]. GH3s are generally characterized by the presence of 3 small conserved motifs [63]. Motif III was found to be highly conserved in the 6 Hevea sequences. Motifs I and II were found in JAR_5108, JAR_14894 and JAR_59958, while JAR_20347 and JAR_20244 did not display motif I. The absence of motif I in the N terminal part of the proteins deduced from the JAR_20347, JAR_21367 and JAR_20244 contigs was linked to the fact that the contig sequences were incomplete at 5' (Additional file 5).

An analysis of the protein sequences revealed that the two HbCOL, HbCOL_2304 and HbCOL_3058, were characterized by the presence of an F-box domain, along with 3 leucine-rich repeat (LRR) domains (Additional file 6). All of the JAZ proteins identified had the 2 characteristic domains, TIFIY and Jas (Additional file 7) [64]. HbMYC_424, HbMYC_771 and HbMYC_94937 all had a bHLH DNA binding domain, along with the 4 conserved regions (I to IV) (Additional file 8) [65]. An analysis of the transcriptome of rubber clone PB260 also enabled us to identify the partners HbNIN/JA_6328 (Additional file 9) and HbTPL_7591 (Additional file 10). HbNIN/JA displayed 46% of identical residues with At4g28910, while HbTPL displayed very strong homology with At1g15750 as 85% of the amino acids were identical. We were also able to identify an orthologue of AtMED25, HbMED25_16787, which displayed 50% identity (Additional file 11).

Expression profile of the different genes acting in different developing tissues
In order to characterize the JA signalling pathway in different Hevea tissues, the transcript abundance of all the genes identified was analysed by qPCR in zygotic embryos (embryo body and cotyledon), roots (TR: taproot and SLR:secondary lateral root), bark, leaves, latex, and male and female flowers at the immature and mature stages (Figure 1). Heatmap representation combined with
an analysis of variance (ANOVA) was used to class the genes according to their level of expression in each tissue (Additional file 12). In addition, hierarchical clustering was used to identify tissues that displayed the same transcriptional signature. The different parts of the embryo displayed the same transcriptional landscape. Overall the jasmonate-related genes implicated in flowers were those that were not expressed in the embryo. Male and female flowers, whether mature or immature, were grouped together, suggesting that the same jasmonate-related genes were involved, whatever the flower gender and development stage. The transcriptional signature in the taproot (TR) was grouped with the embryo, while most of the genes expressed in the secondary lateral roots (SLR) were also expressed in bark. Surprisingly, latex displayed an original profile, with a large number of over-expressed signalling genes (JAZ_14313, COL_3058, COL_2304, JAZ_1660, JAZ_1405, JAZ_17062, MYC_771, MYC_424, MYC_85795, MYC_94937). These results confirmed the importance of the jasmonate pathway in latex. In addition, it was interesting to see that TPL_7591 and NINJA_6328, which, a priori function together, were in the same cluster. MED25_16787 had an expression profile similar to that of JAZ_14313.

**Regulation by abiotic stress of genes acting in the jasmonate pathway**

The relative transcript abundance was monitored over time for the 24 contigs corresponding to the genes of the jasmonate signalling pathway in response to wounding, dehydration, ethylene and methyl jasmonate (MeJA) (Figure 2). In the aim to validate efficiency of treatments, Hevea orthologous of Arabidopsis genes, known to be regulated by wounding, dehydration, ethylene and MeJA were used as positive control. Orthologous to DREB2 and ERF1
Figure 2 (See legend on next page.)
were identified in *Hevea* by Piyatrakul and coll. [37]. Expression of the DREB2 genes is induced by drought stress and involved in drought-responsive gene expression [49]. HbERF-IXc3 is an orthologous of DREB2A and DREB2B [37]. ERF1 is induced by various stresses including wounding and hormonal treatments, acts at the crosstalk of jasmonate and ethylene signalling pathways. Its two putative orthologous genes in *Hevea*, HbERF-IXc4 and HbERF-IXc5 [37], were used to validate wounding, ethylene and jasmonate treatments in this study. The transcript abundance of all the genes of the jasmonate signalling pathway was modified between 1 h and 24 h, whatever the treatment. The transcripts of JAR_14894, JAR_20347, JAR_5108 accumulated significantly in response to wounding, while the transcript abundance of JAR_20244 was significantly lower than in the control (Figure 2A,B). Only the transcript of JAR_5108 accumulated significantly in response to dehydration. For each type of stress, at least one transcript of each partner of the COI-JAZ-MYC module was highly activated (Figure 2C). Interestingly, several genes acting in the signalling pathway were also regulated by ethylene (Figure 2D). Statistical analyses showed that JAR_5108, JAR_863, JAR_1405, JAR_2001, JAR_14313, JAR_26925 and MYC_94937 were regulated by ethylene. It should be noted that, among the JAR and COI, only the transcripts of JAR_5108 and COL_3058 were significantly regulated by JA (Figure 2E). Of the 10 JAZ genes studied, the transcripts of eight of them were significantly accumulated in response to JA (JAZ_863, JAZ_1229, JAZ_1405, JAZ_1660, JAZ_14313, JAZ_19967, JAZ_26925, JAZ_29511). The transcripts of MYC_771 and MYC_94937 were significantly accumulated in response to JA. TPL_7591, NINJA 6328 and MED25_16787 were regulated overall in the same manner. In response to wounding, their transcript abundance increased rapidly in 1 h, while in response to ET and MeJA, they only accumulated after 24 h. Taken together, these results suggest that the genes involved in JA signalling are expressed in response to abiotic stress, but it was not always the same JARs, COIs, JAZs and MYCs that were involved depending on the stress in that signalling.

**Genotypic regulation of genes involved in the JA pathway in response to harvesting stress in mature trees**

The transcript abundance of genes belonging to the COI-JAZ-MYC module were studied in the latex and bark of tapped rubber trees (Figure 3, Additional file 13). During harvesting, the transcripts of the COI-JAZ-MYC module were regulated in both latex and bark. Our results showed that the expression level for COI_2304 was significantly higher in latex than in bark (p < 0.01). In latex, the transcripts of COI_2304 were accumulated in response to tapping, while in bark the transcript abundance decreased in response to that stress (p < 0.01). Treatment with ethephon tended to reduce the transcript abundance of COL_3058 (p < 0.01) in latex and in bark. The results suggested that the transcript regulation of JAZ genes was specific to each gene and that no general tendency could be brought out for the family as a whole. For example, JAZ_1229, JAZ_2001 and JAZ_19967 were significantly more expressed in bark than in latex, while the reverse was true for JAZ_1405, JAZ_1660, JAZ_17062 and JAZ_29511. In latex, JAZ_1405 was the only JAZ to be significantly repressed by tapping (p < 0.01) (Additional file 13), while JAZ_863 (p < 0.01), JAZ_1660 (p < 0.01), JAZ_17062 (p < 0.01), JAZ_29511 (p < 0.01) and JAZ_26925 (p < 0.01) were induced by tapping when there was no ethephon treatment. Interestingly, the transcripts of JAZ_17062 and JAZ_26925 were accumulated in an opposite manner in latex and bark in response to tapping. In bark, JAZ_863 (p < 0.01), JAZ_1229 (p < 0.01), JAZ_19967 (p < 0.01), JAZ_1660 (p < 0.01), and JAZ_29511 (p < 0.01) were induced by tapping, while the expression of JAZ_2001 was repressed by tapping (p < 0.01). In the majority of cases, ethephon reduced JAZ transcript abundance. However, in latex, the transcripts of JAZ_29511 and JAZ_863 were accumulated in the presence of ethephon (p < 0.01 and p < 0.01, respectively). In bark, when the tree was...
tapped, the transcripts of *JAZ_26925* were accumulated in the presence of ethephon (p < 0.01). The 3 MYC factors tested displayed the same expression profile. In fact, they were expressed more in latex than in bark and were strongly induced by tapping. Together, these results suggested that JA played a role during latex harvesting.

### Clonal regulation of genes acting in the jasmonate signalling pathway

In order to find out whether the jasmonate signalling pathway might be involved in the differences in laticifer metabolism observed at clonal level, we studied the transcript abundance of each of the genes acting in the pathway in clones PB260, INC53 and RRIM600. Of the 24 genes studied, only 8 displayed a transcript abundance that was significantly different between clones (Figure 4, Additional file 14). *JAR_59958* and *MYC_771*, positive regulators of the JA signalling pathway, were highly expressed in INC53 and RRIM600. On the other hand, the transcripts of the *JAZ* negative regulators were more expressed in clone PB260. These results showed that the positive regulators of the JA pathway are preferentially accumulated in

---

Figure 3 Regulation of COI-JAZ-MYC module members in *Hevea* in response to harvesting stress in latex and in bark. The accumulation of each COI-JAZ-MYC member was studied in the latex (dark grey bar) and bark (light grey bar) of adult trees, during latex production. During latex production the trees were tapped (3,4) every 2 days, or not (1,2), and stimulated by 2.5% ethephon, once per month (2,4), or not (1,3). The data presented correspond to averages of 3 independent biological replicates.
clones with a slow laticifer metabolism, while the negative regulators are accumulated more in clones with a rapid laticifer metabolism.

Conservation of the structure HbJAZ genes and that of their Arabidopsis orthologue
A study of the phylogeny and structure of the JAZs of Hevea was carried out on 7 JAZs (JAZ_1660, JAZ_19967, JAZ_1405, JAZ_29511, JAZ_26925, JAZ_863, JAZ_1229) which were of interest because they are regulated both by JA and by tapping. A phylogenetic analysis based on the protein sequences of the HbJAZ sand AtJAZs revealed an organization primarily in three sub-classes B, C and D previously defined by Chung [26], with sub-class A not being represented by any JAZ member of Hevea (Figure 5A). AtJAZ10, previously assigned to sub-class C, revealed stronger homology with HbJAZ_29511 to form a new sub-class that we called sub-class E. HbJAZ_26925 was not grouped in the previously described sub-classes and formed a new sub-class. The gene structure of the 7 JAZs of interest was analysed using the scaffold sequences corresponding to the genome of rubber clone CATAS 7-33-97 (Figure 5B). Most rubber tree JAZs possess an intron in the Jas domain, which is similar to what has been described in Arabidopsis. The sequence analysis predicted that retention of the 5′ region of the Jas intron splicing site during pre-mRNA processing of JAZ_1660, JAZ_29511 and JAZ_26925 generated a premature termination codon immediately or very rapidly (Figure 5C). By using both primers overlapping the splicing zone and primers inside the intron, it was possible to confirm alternative splicing by sequencing of the qPCR amplicons for JAZ_1229, JAZ_863, JAZ_1660, JAZ_29511 and JAZ_26925.

Alternative splicing of HbJAZ is regulated by tapping stress
In order to gain a clearer understanding of JAZ post-transcriptional regulation in response to tapping stress in rubber trees, we used qPCR to measure the spliced

Figure 4 Expression of genes acting in the jasmonate pathway in 3 Hevea clones. The transcript abundance of the 24 identified genes was studied by qPCR on latex from PB 260, INC 53 and RRIM 600. According to an analysis of variance 8 transcripts were significantly over-represented in one or two clones (Additional file 14). The data presented correspond to averages of 3 independent biological replicates. Different letters correspond to significantly different values after the Tukey HSD test (p < 0.05).
form/non-spliced form ratio of transcripts in the latex and bark of tapped or untapped rubber trees, treated or not with ethephon (Figure 6, Additional file 15). Despite the existence of non-spliced forms for the transcripts of JAZ_29511, JAZ_863 and JAZ_26925, this was not detected by qPCR in the samples tested. The alternative splicing of JAZ_1229 and JAZ_1660 was significantly regulated by tapping and by ethephon treatment. Our results suggested opposite regulation between the splicing of JAZ_1229 and JAZ_1660. In fact, tapping induced the non-spliced form of JAZ_1229, suggesting an increase in the repressive form of that JAZ, while the same stress induced the spliced form of JAZ_1660.

**MYC_771 and MYC_94937 regulate the transcription of JAZ_1405**

The transcripts of certain JAZs were not subjected to alternative splicing like JAZ_1405. Hierarchical clustering...
grouped MYC_771, MYC_424, JAZ_1405 and JAZ_17062 in the same cluster. Given that the transcripts of MYC_424 and JAZ_17062 were not significantly regulated by jasmonate, we focused on the transcriptional regulation of JAZ_1405 by MYC_771 and MYC_94937 which, in response to JA, displayed the same expression profile. To that end, we cloned 2 versions of the JAZ_1405 promoter (Figure 7A): a short sequence (pJAZ_1405(−267)) which contained 267 bp upstream of the ATG and which contained a G-box, known to be regulated by MYC factors [16] and a longer sequence (pJAZ_1405(−955)) with a size of 955 bp upstream of the ATG and which contained 2 G-boxes. The GFP reporter gene under the control of promoters pJAZ_1405(−267) or pJAZ_1405(−955) was co-transformed with 35S::HbMYC_771 or 35S::HbMYC_94937 (Figure 7A,B). The results showed that the 2 MYCs had a transcriptional activity that was twice as strong with the pJAZ_1405(−955) promoter than with the pJAZ_1405(−267) promoter (Figure 7C).

Discussion

Laticifer cells display a transcript pattern for genes acting in the jasmonate pathway that differs from the other tissues

To date, data regarding the jasmonate signalling pathway have been limited in latex plants to Hevea. Although a few genes acting in the jasmonate signalling pathway (COI1, JAZ, MYC) have been identified in Hevea [40-42], our study led to the identification of all the factors (6 JAR, 2 COI, 10 JAZ, 3 MYC, 1 NINJA, 1 TPL, 1 MED25). Recently, a sequence of HbCOI1 was isolated from clone RRIM600 (EU136026) [40]. Our analyses enabled us to identify an allele of this gene in clone PB260, HbCOI_2304. HbCOI_2304 displayed 95% identity with the sequence of RRIM600. So far, in Hevea brasiliensis, only one JAZ sequence has been identified (HbJAZ1: GQ369508) in clone RY-7-33-97 [41]. An analysis of the transcriptome of clone PB260 resulted in identification of the HbJAZ_1660 sequence, which displayed 100% homology with HbJAZ1. In addition, the same laboratory isolated 2 transcription
factors, HbMYC1 and HbMYC2 (HbMYC1: GU434304 and HbMYC2: HM061097) in clone RY-7-33-97, which have been described as orthologous to AtMYC2 [42]. An analysis of the transcriptome led to the identification of these 2 genes. MYC_424 displayed 99% identity with HbMYC1, like MYC_771 orthologous to HbMYC2. The number of COI and JAZ gene models is similar in Hevea, rice, Arabidopsis, poplar, grapevine and Brachypodium,
but the number of JARs is larger in Hevea (6) than the one or two genes found in the other species. However, given the diversity of GH3s, it is highly likely that not all of them are involved in JA conjugation with Ile (Table 1).

Laticifers cells displayed an original signature of transcripts for the JA signalling pathway since they stood out from all the other tissues tested (Figure 1). Transcript analyses, obtained on PB260, confirmed the results published by Peng and coll. on RRIM 600 [40] showing that HbCOI_2304 is preferentially expressed in latex. In addition, HbCOI_3058 displayed a similar profile (Figure 1). In clone RX-7-33-97, Zhao and coll. showed that the transcripts of HbMYC1 and HbMYC2 were preferentially accumulated in latex [42]. Our results, obtained on clone PB 260 confirmed that tendency (Figures 1 and 2). However, our results did not confirm an abundance of MYC_424 transcripts in response to JA observed in clone RX-7-33-97 [42] (Figure 2E). To date, no study has shown the potential implication of JAZs in the laticifer metabolism. An analysis of the HbJAZ expression profile showed that the transcripts of 5 HbJAZs were more highly accumulated in latex than in the other tissues. For instance, in addition to the transcripts of COI, JAZ and MYC, the transcripts of the JAR_59958 and JAR_20347 genes were highly accumulated in this tissue. All these results suggested that the jasmonate signalling pathway in laticifer cells involved specific actors. This confirmed the importance of studying this pathway in latex production but also in response to harvesting stress.

**Harvesting stress can take both common and distinct wounding and dehydration pathways**

Latex harvesting amounts to strong abiotic stress that can lead to a physiological syndrome such as TPD. A study of transcript abundance in response to wounding, dehydration, and to hormonal treatments, ethylene and jasmonate, helped to determine what types of abiotic stress and signalling pathways occurred during tapping. The jasmonate signalling pathway was regulated by all the types of stress tested, suggesting the involvement of that pathway in the response to each of the types of stress. Although the comparison was difficult, as the tissues and times tested were not exactly the same, our results confirmed that the transcript abundance of HbMYCI and HbMYC2, corresponding to MYC_424 and MYC_771, decreased after 6 h of MeJA treatment, then rose from 24 h onwards [42] (Figure 2E). The results suggested that the signalling pathways of abiotic and harvesting stress may activate the same genes. Indeed, JAZ_863, repressed by ethylene in the stem and by ethephon in bark, was at the same time induced by dehydration and tapping (Figures 2D and 3). In addition, JAZ_29511 and JAZ_14313 were induced by both wounding and tapping, while JAZ_1229 was induced by dehydration and tapping (Figures 2 and 3). Despite that, the results in this study suggested that some independent wounding, dehydration and ethylene pathways were activated during latex harvesting. Indeed, JAZ_2001, JAZ_14313 and JAZ_26925 were induced by ethylene at 24 h, 8 h and 1 h respectively, but were repressed by ethephon in bark (Figures 2 and 3). In latex, the transcripts of COI_2304, MYC_424 and MYC_771 were accumulated in response to tapping (Figure 3), which confirmed the results in the literature [40,42]. Nevertheless, JAZ members are functionally redundant to some extent [17-19] and the diversity in their structures suggests specific roles for some of them. This hypothesis is corroborated by the diverse expression patterns displayed by HbJAZ in response to various types of abiotic stress.

**HbMYC_771 and MYC_94937 can regulate the HbJAZ genes containing a G-box type cis-element**

In addition to negative regulation of JAZs on MYCs, it has been shown that there exists a positive regulation of JAZ transcription by MYCs via the G-box cis-element [16]. Hierarchical clustering grouped MYC_771, MYC_424, JAZ_1405 and JAZ_17062 in the same cluster (Figure 1). Given that the transcripts of MYC_424 and JAZ_17062 were not significantly regulated by jasmonate, we focused on the regulation of JAZ_1405 by MYC_771 and MYC_94937 which, in response to JA, displayed the same expression profile (Figure 2). Transactivation experiments showed that the 2 MYC factors tested were able to regulate the transcription of JAZ_1405 (Figure 7). It is highly likely that this activation of JAZ_1405 by MYC factors occurs via the G-box present in the promoter of that gene. Among the promoters isolated from scaffold

| Genes | Contigs | Number of genes (data from http://rice.plantbiology.msu.edu) |
|-------|--------|-------------------------------------------------------------|
|       |        | Rice          | Arabidopsis     | Poplar     | Grapevine      | Brachypodium |
| JAR   | 6      | 1             | 1               | 2          | 1              | 1            |
| COI   | 2      | 2             | 1               | 2          | 1              | 2            |
| JAZ   | 10     | 15            | 12              | 14         | 9              | 11           |

**Table 1 Number of identified members of JAR, COI and JAZ genes in the rubber tree, rice, Arabidopsis, poplar, grapevine and Brachypodium**
sequences, we were able to show that the promoters of HbJAZ_1229 and HbJAZ_19967 also possessed a G-box, suggesting transcriptional regulation of these 2 JAZs by MYCs. This last result may explain the grouping of MYC_771 and JAZ_1405 in the same cluster, but also a similar expression profile for MYC_94937 and JAZ_1405 in response to JA.

**JAZ_1229 and JAZ_1660 are regulated by alternative splicing in the laticifer cells of tapped trees**

The regulation of alternative splicing provides flexibility at transcriptome and proteome level, which helps plants adapt to their environment [66]. However, while the misregulation of alternative splicing has been associated with many human diseases, its biological relevance in plant systems is just beginning to be decipher [67]. Many studies have shown the importance of AS and splicing factors (SF) in the response to abiotic stress (for review, [28]). With the alternative splicing of JAZ proteins, recent studies suggest that it makes it possible to establish a negative feedback loop to attenuate the response to JA in the event of over-stimulation of the signalling pathway [26,27]. To date, this regulation of JAZs has never been linked to the response to abiotic stress. An analysis of the structure of HbJAZs, along with the sequencing results, confirmed the existence of alternative splicing of the HbJAZ_1229 and HbJAZ_1660 genes (Figure 5). An analysis of the exon/intron junction of these 2 HbJAZs showed that this splicing led to the introduction of a premature termination codon (PTC). In Arabidopsis, the introduction of that PTC leads to the production of a JAZ protein, whose jas domain is absent [26]. In general, the introduction of a PTC engages the transcript in the nonsense-mediated decay (NMD) pathway to degrade it [68,69]. According to the literature, these results suggest that truncated forms of HbJAZ_1229 and HbJAZ_1660 are dominant negative regulators [26,27,70].

For the first time, our results brought out a link between the regulation of JAZ alternative splicing and abiotic stress. Indeed, our results showed that alternative splicing of JAZ_1229 and JAZ_1660 could be regulated by tapping in latex. Curiously, that regulation was opposite for the transcripts of JAZ_1229 and JAZ_1660 (Figure 6). An analysis of the abundance of the 2 forms of transcripts of these 2 HbJAZs showed that the transcript abundance of the spliced form remained at least 500 times greater than the non-spliced form (Figure 6). This final observation tallied with what is generally observed in Arabidopsis since, although intron retention is the most common form of AS (~40%), many of these transcripts are not very abundant at all [71]. Taken together these results suggested a compensatory phenomenon and functional redundancy of HbJAZ_1229 and HbJAZ_1660, which is all the more likely in that they were classed in the same cluster (Figure 1).

**Genes acting in the JA pathway are expressed differentially in the latex of different Hevea clones**

The results presented here show that the JA signalling pathway was regulated differently depending on the clones tested. Indeed, the negative JAZ regulators were overexpressed in PB260, while the transcripts of MYC factors and the JAR enzyme, which are positive regulators of the JA pathway, were accumulated preferentially in clones INC 53 and RRIM600 (Figure 4). These results suggested activation of the JA target genes in clones with a slow laticifer metabolism, more resistant to TPD.

The identification of signalling pathways involved in TPD resistance should make it possible to isolate the master regulators controlling resistance to this syndrome. The identification of JA transduction pathway members and knowledge of their expression pattern open up new ways of improving the TPD-tolerance of Hevea clones. The JA signalling pathway is very widely distributed and very highly conserved during evolution. However, a study on extra floral nectar (EFN) excretions by central American acacias revealed that resistance to biotic stress via the jasmonate pathway might be induced or constitutive in some phylogenetically very close species [72]. Thus, that study suggests that specific variability of the JA pathway may have some major consequences for resistance to stress.

**Conclusion**

The present study provides some molecular clues on how the Jasmonate pathway can be involved in harvesting stress in Hevea brasiliensis through (i) the specific expression pattern of jasmonate pathway actors in latex, (ii) their transcriptional regulation in response to harvesting stress, (iii) differential expression depending on the Hevea clone, (iv) their putative alternative splicing regulation in response to harvesting. A better understanding of transcriptional regulations during harvesting stress, along with the use of clonal diversity in response to such stress, are therefore a major challenge for improving resistance to stress and rubber production in Hevea.

**Availability of supporting data**

The authors confirm that all data underlying the findings are fully available without restriction. Character Matrix and phylogenetic tree have been deposited in treebase (ID: 16569) and data are available at the following URL: http://purl.org/phylo/treebase/phylo/study/TB2:S16569.

**Additional files**

Additional file 1: Sequences of the primers used for qPCR analysis.

Additional file 2: Comparison of Cp values, standard deviation and coefficient of variance for gene expression analysis by real-time RT-PCR of 11 housekeeping genes in in abiotic stress conditions.
Additional file 3: Comparison of Cp values, standard deviation and coefficient of variance for gene expression analysis by real-time RT-PCR of 11 housekeeping genes in harvesting stress conditions.

Additional file 4: Sequences of the primers used for cloning of MYC_771, MYC_94937 and pJAZ_1405.

Additional file 5: Amino acids sequence alignment of HbJAR1 with AtJAR1.

Additional file 6: Amino acids sequence alignment of HbCOI with AtCOI.

Additional file 7: Amino acids sequence alignment of HbJAZ with AtJAZ.

Additional file 8: Amino acids sequence alignment of HbMYC with AtMYC2.

Additional file 9: Amino acids sequence alignment of HbNINJA with AtNINJA.

Additional file 10: Amino acids sequence alignment of HbTPL with AtTPL.

Additional file 11: Amino acids sequence alignment of HbMED25 with AtMED25.

Additional file 12: Analysis of variance of gene expression according the tested tissues.

Additional file 13: Analysis of variance (ANOVA) to test effect of tapping, ethephon treatment, tissue and cross effects on the expression of the different gene of the JA signaling pathway.

Additional file 14: Analysis of variance of gene expression according the tested clone.

Additional file 15: Analysis of variance (ANOVA) to test effect of tapping, ethephon treatment, tree age and cross effect on the ratio spliced/unspliced of JAZ_1229 and JAZ-1660.

Competing interests
The authors declare that they have no competing interests.

Authors contributions
JP participated in the design of the study, performed the experiments. PP and MR carried out the total DNA isolation and cDNA synthesis from samples. FD carried out the transcriptional activity experiments in the single cell system. K supervised the plant culture experiments. CT supervised the plant culture experiments. JP, JL and PM drafted the manuscript. PM coordinated the plant system. K supervised the plant culture experiments. CT supervised the plant cell system.

Received: 15 April 2014 Accepted: 19 November 2014
Published online: 02 December 2014

References
1. Wasternack C, Hause B: Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development: an update to the 2007 review in Annals of Botany. Ann Bot 2013, 111:1021-1058.

2. De Geyter N, Gholami A, Goormachtig S, Goossens A: Transcriptional machineries in jasmonate-elicited plant secondary metabolism. Trends Plant Sci 2012, 17:349-359.

3. Wolucka B, Goossens A, Inz D: Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. J Exp Bot 2005, 56:2527-2538.

4. Sasakii-Seikiimoto Y, Tani K, Obayashi T, Aino M, Matsumoto F, Sakurai N, Suzuki H, Hira M, Noji M, Saito K, Masuda T, Takakiya K, Shibata D, Ohta H: Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. Plant J 2005, 44:553-668.

5. Browse J: The power of mutants for investigating jasmonate biosynthesis and signaling. Phytochemistry 2009, 70:1539-1546.

6. Staswick P, Tiryaki I, Rowe M: Jasmonate response locus JAR1 and several related Arabidopsis genes encode enzymes of the first firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell 2002, 14:1405-1415.

7. Suza W, Staswick P: The role of JAR1 in Jasmonoyl-λ -isoleucine production during Arabidopsis wound response. Plants 2008, 227:1211-1232.

8. Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramer R, Miersch O, Wasternack C, Solano R: (+)-7-iso-Jasmonoyl-λ -isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol 2009, 5:344-350.

9. Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, Wang Z, Xie D: The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell 2009, 21:2220-2236.

10. Feys BJF, Benedetti C, Penfold C, Turner J: Arabidopsis mutants selected for resistance to the phytoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell 1994, 6:751-759.

11. Xie D, Feys B, James S, Nieto-Rostro M, Turner J: COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science 1998, 280:1091-1094.

12. Xu L, Liu F, Lechner E, Genschik P, Crosby W, Ma H, Peng W, Huang D, Xie D: The SCFCOI1 ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell 2002, 14:1919-1935.

13. Ren C, Pan J, Peng W, Genschik P, Hobbie L, Hellmann H, Estelle M, Gao B, Peng J, Sun C, Xie D: Point mutations in Arabidopsis COP1 reveal its essential role in jasmonate response. Plant J 2005, 42:154-152.

14. Moon J, Pany G, Estelle M: The ubiquitin-proteasome pathway and plant development. Plant Cell 2004, 16:3181-3195.

15. Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-的结果显示，Coordinated activation of metabolic pathways for antioxidants and def...

16. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He S, Howe G, Browse J: The Arabidopsis JAZ family: regulators of the JASMONATE response. Science 2007, 319:5.83-6.83.
integrative hypothesis for the regulation of jasmonate-responsive gene expression in Arabidopsis. Plant Physiol 2012, 160:541 555.

26. Chung H, Cooke T, Depew C, Patel L, Ogawa N, Kobayashi Y, Howe G: Alternative splicing expands the repertoire of dominant JAZ repressors of jasmonate signaling. Plant J 2010, 63:613 622.

27. Moreno J, Shyu C, Campos P, Patel L, Chung H, Yao J, He S, Howe G: Negative feedback control of jasmonate signaling by an alternative splice variant of JAZ10. Plant Physiol 2013, 162:1006 1017.

28. Stagier D, Brown JWS: Alternative splicing at the intersection of biological timing, development, and stress responses. Plant Cell 2013, 25:3640 3656.

29. Chen Y, Banprat J, d'Auzac J, Jacob J, L RULET of the luteoinic tonoplast in the senescence and degradation of the laticifers of Hevea brasiliensis. Plant Physiol 1984, 84:114 1261.

30. Gohel E: La production de latex par Hevea brasiliensis. Relation avec la croissance: influence de différents facteurs: origine clonale, stimulation hormonale, réserves hydrocarbonées. In Physiologie végétale et développement. Montpellier: Universit Montpellier II; 1996.

31. Jacob JL, Pnt JC, Rousset D, Lacroute R, Serres E, d'Auzac J, Eschbach J-M, Omont H: Yield limiting factors, latex physiological parameters, latex diagnosis, and climatic typology. In Physiology of Rubber Tree Latex. Edited by d'Auzac J, Jacob JL, Chrestin H, Boca Raton, Florida: CRC press, Inc; 1989:345 382.

32. Sun J-Q, Jiang H-L, Li C-Y: Gene expression responses to tapping, mechanical wounding, ethrel and methyl jasmonate in bark of Hevea brasiliensis. Trees 2006, 20:779.

33. Piyatrakul P, Putranto R-A, Martin F, Rio M, Dessailly F, Leclercq J, Dufayard J-F, Lardet L, Montoro P: Laticifer differentiation in Hevea brasiliensis: induction of jasmonate-responsive gene expression in Arabidopsis. Plant Physiol 2014, 163:910 924.

34. Duan C, Argout X, Gobelin V, Summo M, Dufayard JF, Leclercq J, Omont H: Role of the lutoidic tonoplast in the senescence and degradation of the laticifers of Hevea brasiliensis. Physiologie végétale et développement. Montpellier: Universit Montpellier II; 1996.

35. Duan C, Argout X, Gobelin V, Summo M, Dufayard JF, Leclercq J, Omont H: Yield limiting factors, latex physiological parameters, latex diagnosis, and climatic typology. In Physiology of Rubber Tree Latex. Edited by d'Auzac J, Jacob JL, Chrestin H, Boca Raton, Florida: CRC press, Inc; 1989:345 382.

36. Sun J-Q, Jiang H-L, Li C-Y: Gene expression responses to tapping, mechanical wounding, ethrel and methyl jasmonate in bark of Hevea brasiliensis. Trees 2006, 20:779.

37. Piyatrakul P, Putranto R-A, Martin F, Rio M, Dessailly F, Leclercq J, Dufayard J-F, Lardet L, Montoro P: Some ethylene biosynthesis and AP2/ERF genes reveal a specific pattern of expression during somatic embryogenesis in Hevea brasiliensis. BMC Plant Biol 2012, 12:44.

38. Kuswanhadi, Leclercq J, Rio M, Jourdan C, Thaler P, Sabau X, Argout X, Montoro P: Differential gene expression in different types of Hevea brasiliensis roots. Plant Sci 2012, 183:149 158.

39. Team RC: Python programming tools for plotting data. 2110th edition; 2012.

40. Talalava G, Garestena I: Improvement of phylogeny after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 2007, 56:564 577.

41. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O: New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 2010, 59:307 321.

42. Gascuel O: BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 1997, 14:595.

43. Dufayard J-F, Duret L, Penel S, Gouy M, Rechenmann F, Penret G: Tree pattern matching in phylogenetic trees: automatic search for orthologs or paralogs in homologous gene sequence databases. Bioinformatics 2005, 21:596 603.

44. Han M, Zmasek C: phyloXML: XML for evolutionary biology and comparative genomics. BMC Bioinformatics 2009, 10:356.

45. Chaabouni S, Jones B, Delalande C, Wang H, Li Z, Mila I, Frasse P, Latche A, Takeda S: Bionej: an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 1997, 14:595.

46. Dufayard J-F, Duret L, Penel S, Gouy M, Rechenmann F, Penret G: Tree pattern matching in phylogenetic trees: automatic search for orthologs or paralogs in homologous gene sequence databases. Bioinformatics 2005, 21:596 603.

47. Han M, Zmasek C: phyloXML: XML for evolutionary biology and comparative genomics. BMC Bioinformatics 2009, 10:356.

48. Chaabouni S, Jones B, Delalande C, Wang H, Li Z, Mila I, Frasse P, Latche A, Takeda S: Bionej: an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 1997, 14:595.
70. Seo P, Hong S-Y, Kim S-G, Park C-M: Competitive inhibition of transcription factors by small interfering peptides. *Trends Plant Sci* 2011, 16:541–549.

71. Marquez Y, Brown J, Simpson C, Barta A, Kalyna M: Transcriptome survey reveals increased complexity of the alternative splicing landscape in *Arabidopsis*. *Genome Res* 2012, 22:1184–1195.

72. Hell M, Greiner S, Meimberg H, Kirger R, Noyer J-L, Heubl G, Linsenmair K, Roland W: Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature* 2004, 430:205–208.

doi:10.1186/s12870-014-0341-0

Cite this article as: Pirrello et al.: Transcriptional and post-transcriptional regulation of the jasmonate signalling pathway in response to abiotic and harvesting stress in *Hevea brasiliensis*. *BMC Plant Biology* 2014 14:341.