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Molecular Characterisation of the Sucrose Transporter HbSUT1B *in Relation to Rubber Production in Latex Cells*

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The rubber synthesis in latex cells requires sucrose. These particular cells are heterotrophic ones and are apoplastically connected to other tissues. Such features imply that the rubber yield may depend on the latex cell efficiency to absorb and to use the imported sucrose. This study investigates the sucrose transporters as key genes for rubber biosynthesis involved in the ethylene-stimulated rubber yield. Seven putative sucrose transporters had been isolated from a latex-derived cDNA library of PB 217. Expression profiling of these sucrose transporters under ethylene stimulation in PB 217 underlined the role of one sucrose transporter: HbSUT1B (*Hevea brasiliensis* Sucrose Transporter 6) *(Dusotoit-Coucaud* et al*., 2007). This paper reports the next stage in* HbSUT1B, *molecular characterisation. Its expression in different parts of the rubber tree was studied. It was preferentially expressed in leaves and bark. This result was completed by* in-situ *hybridisation experiments. In addition, promoter cloning and analysis revealed some clues for the wounding regulation of* HbSUT1B. *The* HbSUT1B *expression was analysed in response to different treatments in latex or bark tissues.* HbSUT1B *was considerably up-regulated by hormones and wounding in bark but not in latex. This regulation appeared to be tissue-specific.*

Keywords: sucrose transporters; *HbSUT1B*; rubber production; latex cells; molecular regulation

Laticifers are highly specialised cells from the bark of *Hevea brasiliensis*. Their cytoplasm is called latex and contains rubber particles[1,2]. After tapping (to collect rubber), the latex flows out of the laticifers under pressure. Laticifers have to completely regenerate their cytoplasm (except nuclei and mitochondria) to synthesise rubber particles. Latex cells consist of a heterotrophic sink, they have to import sucrose to meet their high carbon and energy demand[3–6]. Ultrastructural studies have shown that laticifers organised in an isolated network, do not have plasmodesmata[7,8]. Electrophysiological studies have indicated

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that sucrose influx into the latex protoplasts leads to a plasma membrane depolarisation[9]. Based on these observations, it has been postulated that sucrose uptake may be mediated by plasma membrane sucrose transporters.

In herbaceous species, many sucrose transporters have been isolated and well-characterised over the last decade[10]. In woody species, however, few sucrose transporters have been cloned to date. These share significant homology with those previously isolated from herbaceous species. In general, the sucrose transporters, belonging to the MFS family (Major Facilitative Superfamily), are encoded by a multigenic family and exhibit 12 transmembrane helices. According to their specificity and loading capacity in heterologous systems, sucrose transporters are divided into three distinct groups. The SUT1 group is characterised by a low capacity and high affinity (Km < 3mM). The SUT4 group has a relatively low affinity (5mM < Km < 11.6mM) but a high capacity and the SUT2 group includes putative sucrose sensors (*AtSUT2*, *Arabidopsis thaliana* Sucrose Transporter 2, *LeSUT2*, *Lycopersicon esculentum* Sucrose Transporter 2[11]) and functional sucrose transporters (*PmSUC3*, *Plantago major* Sucrose Carrier 3[12]).

Sucrose transporters are known to be controlled at the transcriptional[13], post-transcriptional[14] and post-translational[15] levels. They are regulated by several exogenous and endogenous factors like hormones[16,17]. Sucrose transporters are expressed in source and sink organs (*Arabidospis thaliana* Sucrose Carrier 5[18], *Juglans regia* Sucrose Transporter 1[19]). For example, *AtSUC1* and *AtSUC2* are expressed in young and mature leaves in *Arabidopsis thaliana* but only *AtSUC1* is expressed in the roots[20]. In addition, *AtSUC2* transcripts are detected in the phloem, specifically in the companion cells, whereas *AtSUC1* is mainly expressed in non-phloem leaf cells[21].

Sucrose transporters are implicated in numerous different physiological processes. For example, *AtSUC5* is specifically expressed in the endosperm and is involved in the filial tissue nutrition during early seed development in *Arabidopsis*[18]. Several sucrose transporters are known to be involved in phloem loading: *LeSUT1* (*Lycopersicon esculentum* Sucrose Transporter 1[22]); *DcSUT1* (*Daucus carota* Sucrose Transporter 1[23]) and *OsSUT1* (*Oryza sativa* Sucrose Transporter 1[24]).

Although sucrose acts as a precursor in rubber synthesis in laticifers, the role of its corresponding transporter in the latex yield has only been investigated very recently. In fact, seven isoforms of sucrose transporters have been cloned by screening a latex cDNA library from PB 217 trees by Dusotoit-Coucaud *et al*.[25] and direct submission by Yang *et al*[38]. These genes have been found to be homologous to the sucrose transporters previously cloned in herbaceous and woody species. Two isoforms have been identified as belonging to SUT1 group (*HbSUT1, HbSUT1B*); two others to SUT4 group (*HbSUT4, HbSUT5*) and three to SUT2 group (*HbSUT2A, HbSUT2B, HbSUT2C*). This work also indicated that the major sucrose transporter in the laticifers, *HbSUT1B*, may be involved in latex production. *HbSUT1B* is the most highly expressed isoform in the latex of mature unstimulated trees and show very high and prolonged upregulation upon ethylene treatment.

The present work completes our first study on latex sucrose transporters and their expected implication in the latex yield. Our attention focuses here on the regulation of *HbSUT1B*, which has already been reported as playing a potential role in latex production. This study was monitored with young or mature virgin trees of PB 217 and PB 260. A series of experiments were carried out to bring into light elements in *HbSUT1B* expression pattern in leaves, stem and bark.

EXPERIMENTAL

Material

Plant Material. The trees used for RNA extraction, *in situ* hybridisation and promoter cloning came from a controlled environment room (Michelin, Ladoux, France). The trees were cut back six months earlier. Trees of PB 217 were used for the expression analysis in different cells and for *in situ* hybridisation. For the promoter cloning, trees of PB 217 and PB 260 were used.

The latex and bark samples for RNA extraction were collected after hormonal treatments from rubber trees of PB 217 which had been ½ spiral-tapped for two years and selected for their medium, homogenous growth and yield from the Bongo/SAPH plantation (Ivory Coast).

Controlled environment chamber at Composants Naturels (CPN) Ladoux, France. The culture conditions of young trees were determined as follows: 12h daylight, at 27–30ºC, with 70 ± 10% relative humidity, light at 40 µE m^{-2} s^{-1} (day) and at 25ºC, with 90% relative humidity (night). Each pot was connected to the watering system and sprayed twice a day for 3 mins. 15 gL^{-1} fertilizer was added to the water.

Field experiments (Bongo/SAPH). Five batches of three virgin mature trees were set-up. One batch as a control (untreated) and four others were treated on a 1 cm wide slightly scraped bark band, just beneath S/2 tapping cut: 2.5% jasmonic acid (JA), 625 µM salicylic acid (SA), 1% 4-chlorophenoxyacetic acid (CPA, auxin analog), 2.5% abscissic acid (ABA) (1.5 mL in ethanol 0.25% final, 0.05% Tween 20 in water) or nails (N) for 4, 8 and 16 hours, respectively, before the first tapping and in accordance with Kongsawadworakul *et al*.[26]. The latex and total inner soft bark samples were collected on the same day and analysed as the 1st tapping (R1). After 3 days without treatment, samples from these same trees were collected again and analysed as the second tapping (R2).

Methods

Latex collection and RNA extraction. Latex and bark collection and total RNA extraction were performed according to the method adapted from Pujade-Renaud *et al*.[27] and developed by Kongsawadworakul *et al.*[26].

Real time PCR. The real time PCR technique was used to study the transcript accumulation of *HbSUT1B*, using specific primer couples designed in the 3'-untranslated region[25]. After RNA samples were treated with DNase I, 2 µg total RNA were used as templates in the first strand cDNA synthesis reaction (SuperScript-III, Invitrogen). Real time PCR was carried out using the generated cDNA as template, a fluorescent SYBR-Green dye and an iCycler system (Bio-Rad). Rubber tree cDNA encoding actin RNA was used as internal control gene. The real time PCR were run at least as four individual experiments (two experiments on two different Reverse Transcription) and performed in triplicates. The raw results were given by the real time PCR machine as Ct value (threshold cycle) corresponding to the cycle number at which the amplified product accumulates to yield a detectable fluorescent signal at the beginning of the exponential stage of PCR amplification. ΔCt was calculated from the formula $\Delta Ct = Ct_{(treated\ sample)} - Ct_{(control\ sample)}$. The normalised expression ratio (Qr) could be calculated using the comparative Ct method, with the formula: $Qr = E^{-\Delta\Delta Ct}$ (E= efficiency of the primer couple, ΔΔCt = Sucrose transporter-Actin). The efficiency of the primer couple had been previously evaluated. To compare the abundance of *HbSUT1B* transcripts in different organs, the expression levels were

calculated from the formula:
Expression = $E^{(Ct(actin)-Ct(\textit{HbSUT1B}))}$

In situ hybridisation. Sense and antisense probes were synthesised as described by Brunel *et al.*[28].

Tissue preparation was carried out according to the method of Brunel *et al.*[28]. Transversal sections 12 µm thick were cut with a rotary microtome, mounted onto SuperFrost Plus slides (Fisher Scientific, Elancourt, France) and dried at 42ºC for two days. Paraffin was removed through two baths (10 mins each) of Histoclear II. Slides were progressively rehydrated in an ethanol series and then washed for 5 mins in DEPC-water and then twice in PBS 1X. Slides were treated with proteinase K at 10 µg mL^{-1} in PBS 1X at 37ºC for 15 mins then incubated in glycine at 0.2% in PBS 1X at room temperature for 2 mins. After two washes in PBS 1X, slides were fixed in 4% paraformaldehyde (in PBS 1X) for 10 mins, rinsed in PBS 1X and then acetylated using 0.5% acetic anhydride in 0.1 M triethanolamine for 10 mins and rinsed in PBS 1X. Before hybridisation, sections were dehydrated in an ethanol series. Sections were hybridised with equal concentrations (1.5 ng μL^{-1}) of either sense or antisense probes in 1X Denhart's, 10% dextran sulfate, 1 mg mL^{-1} tRNA, 50% formamide, 1X salts (300 mM NaCl, 10 mM Tris-HCl pH 6.8, 10 mM NaH_2PO_4, 10 mM Na_2HPO_4, 5 mM EDTA) and incubated at 50ºC overnight in a humid chamber. Sections were washed twice in 2X SSC, six times in 0.1X SSC for 15 mins at 42ºC each and twice each in 1X PBS for 10 mins at room temperature. The detection of DIG-labeled probes was performed as reported by Poupard *et al.*[29] using an anti-digoxigenin alkaline phosphatase conjugate. After suitable colour development the reaction was halted by rinsing in water and the sections were fixed onto slides, dried and mounted in Eukitt. Observations were performed under a Zeiss Axioplan 2 microscope and using an AxioCam HR camera (Zeiss) with the AxioVision digital imaging software.

Promoter cloning and sequence analysis. To clone the promoter of the *HbSUT1B* gene, the GenomeWalker Universal kit (Clontech, Mountain View, USA) was used according to

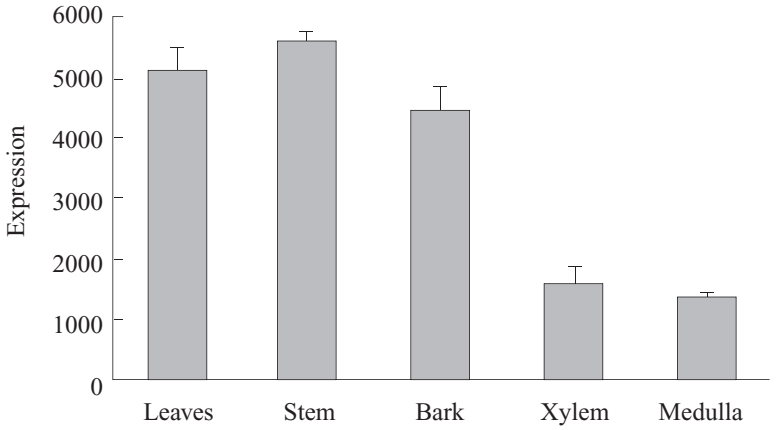

Figure 1. Expression pattern of HbSUT1B *sucrose transporter in several organs of young* Hevea *trees of PB 217. All trees were cut back 6 months before and kept in a controlled room.*

the manufacturer's instructions. The sequences obtained were *in silico* analysed with the website PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html).

RESULTS

HbSUT1B Transcript Accumulation in Different Organs of PB 217

The level of *HbSUT1B* transcripts was monitored in different organs of young rubber trees (cut back 6 months earlier) from PB 217 grown in a controlled environment room. The leaves, stem, bark, xylem and medulla were harvested in order to provide a picture of the *HbSUT1B* expression pattern in the tree. It is known that sucrose transporters belonging to the SUT1 group (like *HbSUT1B*) are not organ-specific but most of them are expressed in sink tissues[18,19,30].

As shown in *Figure 1*, *HbSUT1B* was expressed in both sources namely photosynthetic leaves and sink (stem) organs. In the stem tissue, *HbSUT1B* transcripts were feebly accumulated in xylem and medulla, but highly accumulated in bark tissue where latex cells are located and rubber biosynthesis occurs.

RNAs Localisation by *in-situ* Hybridisation

In order to provide insight into the spatial accumulation of *HbSUT1B* transcripts, *in-situ* hybridisation experiments were carried out on the same sample used for RT-PCR.

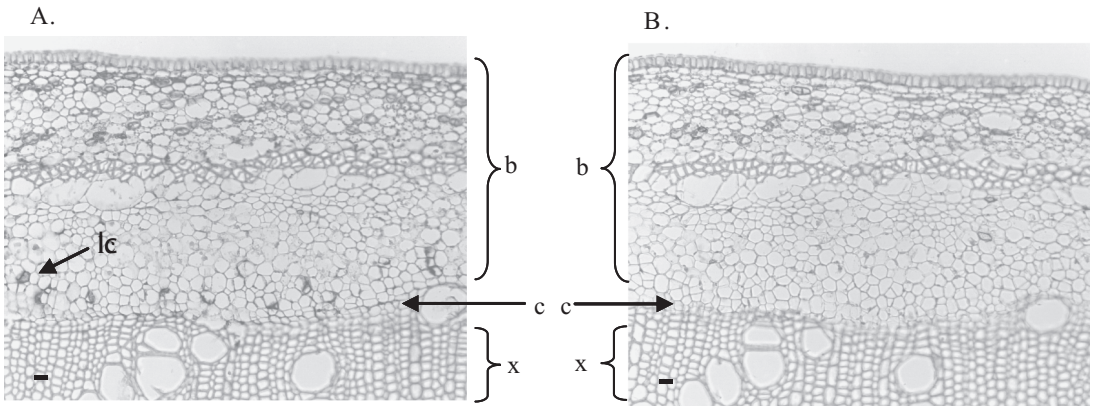

Figure 2. In-situ *localisation of* HbSUT1B *transcripts in stem of young* Hevea *(cut back 6 months before) grown in a controlled room.*
Transversal sections were obtained and hybridised with (A) HbSUT1B *antisense mRNA probe, (B)* HbSUT1B *sense mRNA probe as a negative control.*
Positive hybridisation signals are visualised by violet staining using a digoxigenin-labelled RNA immunodetection system as described in materials and methods.
Bark (b), xylem (x), latex cells (lc), cambium (c).
Bars in figures A and B = 50 µm.

In *Figure 2*, there is clearly a difference between the two pictures (A and B) respectively depicting the hybridisation with anti sense and sense probes. A typical colouration (violet staining) was detected in *Figure 2A*, whereas no signal could be observed in *Figure 2B*, demonstrating that this coloration was specific to *HbSUT1B* mRNA.

Figure 2A shows the specific accumulation of *HbSUT1B* mRNA in young phloem and cambial zone. Furthermore, some cells were completely violet. These cells may be latex ones because they were localised in the phloem, regularly differentiated and their full colouration indicated the absence of large vacuoles as in the case of laticifers.

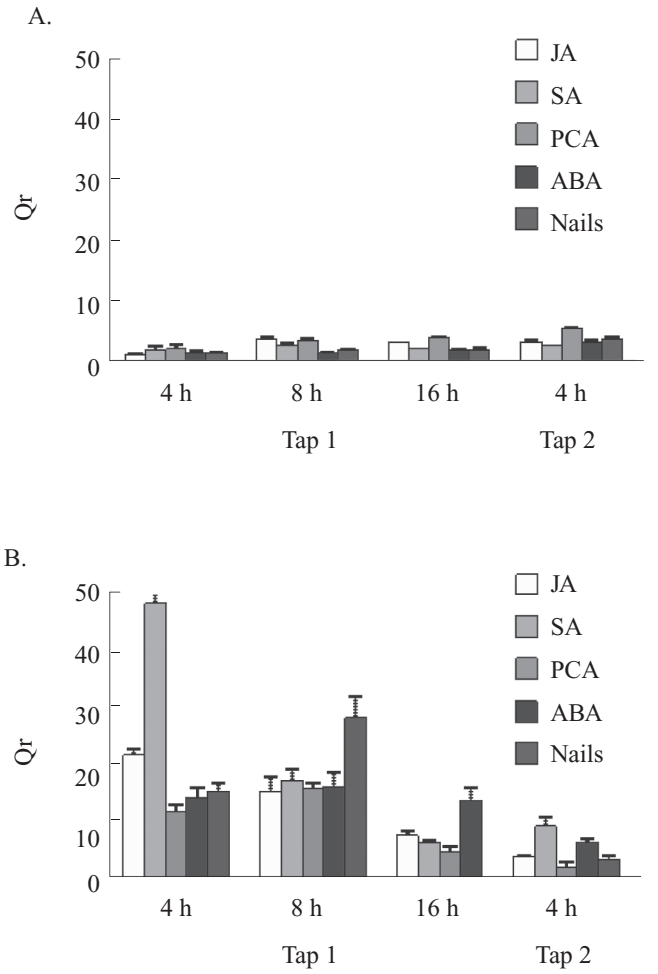

Figure 3. Chronology of the relative expression of the sucrose transporter 6 gene (HbSUT1B) *(A) in the latex and (B) in the bark of PB 217 mature virgin trees, in response to different hormonal treatments, compared to untreated trees. Actin cDNA was used as internal control.*

HbSUT1B Transcript Accumulation under Hormonal Treatment

In order to enhance the characterisation of *HbSUT1B* regulation, several field hormonal treatments were performed on mature trees. Our previous data showed that *HbSUT1B* is regulated by wounding and ethylene[25]. The present study further analyses whether *HbSUT1B* transcript accumulation is responsive to certain other hormones.

Mature virgin trees (about 7 years old, neither stimulated nor tapped) from PB 217 were used. Several phytohormones were directly applied on bark: (i) jasmonic acid (JA) which is a signal molecule involved in abiotic and biotic stress responses, and specially in wounding response[31] (ii) Salicylic acid (SA) which is known to play a key role in pathogen responses[32] (iii) Auxin (CPA) which is involved in most physiological processes such as wounding[33], and (iv) abscissic acid (ABA) because of its utility in both plant water status and abiotic stress responses[34]. Finally, to analyse the direct effects of wounding, another treatment was performed that consisted in nail sealing in the bark (in the tapped area) as far as the cambium. After all these different treatments, bark and latex samples were collected.

In latex samples (*Figure 3A*), the four tested hormones and the wounding treatment did not appear to induce any significant transcript accumulation of *HbSUT1B*, contrary to ethylene[25]. In bark samples, there was a clear and early induction by the five treatments (*Figure 3B*). JA, CPA, ABA, nails and especially SA, considerably increased the quantity of *HbSUT1B* transcripts accumulated in bark 4h after the treatment. This induction seemed to decrease with time. The effect of ABA was the only one that decreases more slowly.

Promoter Cloning

The purpose of this procedure was to obtain preliminary ideas about the molecular mechanism regulation of *HbSUT1B* transcription in response to different treatments (hormones and wounding) and to check its differential regulation observed between PB 217 and PB 260 that could reflect a difference in the promoter sequence.

The "pattern matching" approach was used to identify putative *cis*-acting regulatory elements from the promoter sequences of *HbSUT1B*. This consists in searching for each query sequence for motifs identical with or similar to previously reported *cis*-elements registered in the PLACE database.

HbSUT1B transcripts accumulation is regulated by hormones or mechanical wounding in bark tissue. An *in silico* analysis of *HbSUT1B* promoter (PHbSUT1B) indicated the presence of *cis*-acting elements putatively recognized by ABA, SA, auxin and wounding. These elements are described in detail in *Table 1*.

It has been previously shown that *HbSUT1B* transcription was responsive to ethylene treatment (Ethrel®). *HbSUT1B* exhibited a huge and early transient up-regulation in latex of both PB 217 and PB 260 after ethylene treatment[25]. PB 217 is characterised by a low-medium metabolism and a high sugar loading, whereas PB 260 presents a high metabolism and a medium sugar loading[25,36–38]. The comparison of *HbSUT1B* promoter sequences from PB 217 and PB 260 over a sequence fragment comparable in length reveals that both promoter sequences are strictly identical (data not shown). The difference of *HbSUT1B* transcript accumulation between PB 217 and PB 260 may not be explained by a transcriptional regulation[25].

TABLE 1. POTENTIAL *CIS*-ACTING REGULATORY ELEMENTS IDENTIFIED IN PHbSUT1B FROM PB 217 AND PB 260 BY *IN SILICO* "PATTERN MATCHING" SEARCH AGAINST THE PLACE DATABASE

Cis-element	Associated transcription factor	Number of copies	Expected function	Reference
MYC	ATMYC2/ ICE1	10	ABA-regulated expression	40
MYB		12	ABA-regulated expression	40
E-box		10	Light/ABA-regulated expression	41
DOF	Dof protein (1 Zn finger)	8	Auxin-regulated expression	42
GT-1	GT-1 like factor	7	Light/Salicylic acid-regulated expression	43
WRKY/W-box	WRKY	20	Wounding/Salicylic acid/ sugar-regulated expression	44
AGAA-motif		5	Expression in pollen	45
CTCTT-motif		8	Expression in root nodules	46

Several unexpected *cis*-acting elements were found following the *in silico* analysis: AGAAA-motifs (specific expression in pollen) and CTCTT-motifs (specific expression in root nodules). These *cis*-acting elements have already been identified in the promoter of hevein gene[39].

DISCUSSION

Previous studies reported that sucrose transporters are involved in a variety of physiological processes in herbaceous species[22,18–47] as well as in woody species[19]. Moreover, other studies have shown that the presence of sucrose transporters is essential for laticifers functioning[35,48–50]. As an initial step towards exploring the involvement of sucrose transporters in latex yield, the ethylene effects on the expression of seven sucrose transporters isoforms have been analysed[25]. We hypothesised that *HbSUT1B* could be linked to latex yield. As a result, we provided initial insight into the molecular study of the *HbSUT1B* gene regulation. Our present goal was to identify factors regulating *HbSUT1B*, which may have an impact on latex production.

Firstly, the profiling accumulation of *HbSUT1B* transcripts in virgin *Hevea* trees was investigated, using RT-PCR on cDNA from several organs. Our data showed that *HbSUT1B* transcripts were abundantly expressed in leaves and stems (*Figure 1*). Several other plant sucrose transporters have been described to be expressed in both source and sink organs. *AgSUT1* transcripts (*Apium graveolens* Sucrose Transporter 1) are detected in leaves and roots[51] or *StSUT1* transcripts (*Solanum tuberosum* Sucrose transporter 1) are present in leaves, roots and flowers[52]. In addition, with regards to stem tissue, the *HbSUT1B* transcripts were preferentially localised in bark (*Figure 1*). *In-situ* hybridisation analysis indicated that these *HBSUT1B* transcripts were localised in young phloem, cambial zone and presumably in latex cells of young stems (*Figure 2*). According to these results,

HbSUT1B transcripts seem to be preferentially localised in latex synthesis sites (latex cells), and might be involved in the production of rubber. Similarly, *HEV2.1* (Hevein 2.1 gene of *Hevea brasiliensis*) known to be implied in rubber biosynthesis, has been reported as being specifically expressed in latex cells according to *in situ* hybridisation data[53].

We have previously shown that *HbSUT1B* transcript level in latex is sensitive to ethylene treatment[25]. To check whether this response is ethylene-specific, the impact of various hormones on transcript level of *HbSUT1B* was analysed by real time RT-PCR, on virgin mature PB 217 trees. *HbSUT1B* expression was not regulated by the hormones tested in latex cells, but significantly increased by JA, PCA, ABA, Nails and especially SA in bark. Although latex cells are localised within the bark, our data indicates that the regulation mechanisms of *HbSUT1B* differ between these two compartments. In fact, *HbSUT1B* was found to be up-regulated only by ethylene[25] in latex cells, whereas it was sensitive to all hormonal and wound treatments in bark. There are limited data about differential regulation of genes between latex and bark tissues. However, there is the noticeable case of glutamine synthetase, in that this gene is up-regulated by ethylene only in latex[54]. Another example is the rubber elongation factor regulation, *i.e*., this gene is regulated by the development state of the tree, but differentially in latex and in bark[55].

The first purpose of the promoter cloning was to obtain preliminary ideas about molecular mechanism regulation of *HbSUT1B* in response to hormonal or wounding treatments (*Figure 3*). *HbSUT1B* transcripts are accumulated in bark tissue after hormonal treatments. After *in silico* analysis of the PHbSUT1B sequence (Promoter of *HbSUT1B*), no *cis*-acting elements directly implied in response to hormones (like ABRE or JASE) was identified. However, some other *cis*-acting elements might be involved in the *HbSUT1B* response to hormones. Firstly, MYB and MYC have already been depicted as ABA-response elements, for example, the *AtMYC2* and *AtMYB2* function as transcriptional activators in ABA signalling[40]. Secondly, DOF boxes are found in PHbSUT1B and they could intervene in auxin response, like in *Nicotiana tabacum*[56]. In addition, some GT-1 (GT-1 consensus) and WRKY (WBOXATNPR1) elements have been presented as SA-regulated elements[57–58]. Finally, a large number of wounding boxes (W-box and WRKY) were identified in PHbSUT1B. In conclusion, the promoter analysis appeared to indicate that *HbSUT1B* transcripts are directly regulated by wounding whereas their regulation by hormones might be indirect and complex. In short, these probably involve a great deal of hormonal cross-talk pathways.

Another point which could be discussed is the *HbSUT1B* regulation by ethylene. *HbSUT1B* transcripts have been shown to be up-regulated by ethylene in mature exploited PB 217 and PB 260 trees[25], whereas PHbSUT1B did not display any putative *cis*-acting regulatory elements from the ethylene response. Three explanations could be advanced. The cloned promoter sequence may be too small (490 bp) and there may be ethylene regulation elements upstream but it is often reported that the most significant *cis*-acting elements are in the first 500 bp. Another explanation could be that PHbSUT1B may contain unknown ethylene *cis* regulatory elements. The same case has already been observed: the *E4* gene from *Lycopersicon esculentum* is known to be regulated by ethylene, and the region of its promoter implied in this regulation has been identified. However, this promoter region did not contain any ethylene *cis*-element previously described[59]. Finally, as suggested by our *in silico* analysis,

it is possible that the PHbSUT1B does not contain any *cis*-acting element from ethylene regulation because the *HbSUT1B* transcript accumulation could be indirectly regulated by ethylene. Several wounding and stress boxes were detected in PHbSUT1B. This may mean that *HbSUT1B* is regulated by wounding and its overexpression observed after ethylene treatment may be due to the wounding and the stress caused by tapping. It could be an indirect activation by ethylene, as ethylene and wounding pathways are connected[60]. An argument in the favour of the last hypothesis is the presence of WBOXNTERF3 motifs in the PHbSUT1B. This *cis*-acting element has been described as a W box (activated by wounding) involved in ERF gene activation[61]. ERF are transcription factors involved in the ethylene pathway.

The promoter of *HbSUT1B* was cloned in two contrasted clones, *i.e*., PB 217 is characterised by a low-medium metabolism and a high sugar loading, whereas PB 260 presents a high metabolism and a medium sugar loading[35–38]. It has been shown that the expression of *HbSUT1B* was differentially regulated by ethylene in these two clones[25]. These new results demonstrated that there is no difference in terms of promoting sequences between PHbSUT1B from PB 217 and PB 260. Thus, the differences observed in transcript quantities after ethylene treatments between both clones could be explained by a post-transcriptional regulation (at the mRNA level) or by a differential regulation upstream in the signaling pathway, for example at the transcription factor level. In the literature, it is known that sucrose transporter could be regulated at post-transcriptional level but there exists little data concerning the mechanisms of this kind of regulation. It has been shown that sucrose transporter mRNA stability is often diurnally regulated, as is the case for *StSUT1* mRNAs[62].

In conclusion, this report extends our previous work concerning the sucrose transporters from *Hevea brasiliensis* in relation to latex yield. This is the first insight into molecular regulation mechanisms of *HbSUT1B*, a sucrose transporter potentially important for latex production. *HbSUT1B* transcripts were localised in phloem cells and more precisely in latex cells while strengthening the hypothesis of their involvement in latex production. In addition, *HbSUT1B* expression was increased by JA, SA, PCA, ABA, ethylene and nails in bark but not in latex (only by ethylene). *HbSUT1B* hormonal regulation was tissue-specific. This last feature could be explained by the presence of indirect hormonal *cis*-acting elements in *HbSUT1B* promoter. We also put forward a hypothesis to explain the ethylene regulation in the absence of ethylene *cis*-acting elements in PHbSUT1B. Lastly, it is suggested that ethylene could regulate *HbSUT1B* at the post-transcriptional level (in bark tissue and in latex).

To deepen the understanding of *HbSUT1B* regulation, it would be interesting to perform *Hevea* transformations with PHbSUT1B: GFP constructs (whole or deleted promoter). Moreover, a study of the expression and regulation of *HbSUT1B* protein, by western-blot analysis and immunolocalisation seems essential to further understand *HbSUT1B* functions in the rubber tree.

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