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Sucrose importation into laticifers of *Hevea brasiliensis*, in relation to ethylene stimulation of latex production

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• *Background and Aims* The major economic product of *Hevea brasiliensis* is a rubber-containing cytoplasm (latex), which flows out of laticifers (latex cells) when the bark is tapped. The latex yield is stimulated by ethylene. Sucrose, the unique precursor of rubber synthesis, must cross the plasma membrane through specific sucrose transporters before being metabolized in the laticifers. The relative importance of sucrose transporters in determining latex yield is unknown. Here, the effects of ethylene (by application of Ethrel®) on sucrose transporter gene expression in the inner bark tissues and latex cells of *H. brasiliensis* are described.
• *Methods* Experiments, including cloning sucrose transporters, real time RT-PCR and *in situ* hybridization, were carried out on virgin (untapped) trees, treated or untreated with the latex yield stimulant Ethrel.
• *Key Results* Seven putative full-length cDNAs of sucrose transporters were cloned from a latex-specific cDNA library. These transporters belong to all SUT (sucrose transporter) groups and differ by their basal gene expression in latex and inner soft bark, with a predominance of *HbSUT1A* and *HbSUT1B*. Of these sucrose transporters, only *HbSUT1A* and *HbSUT2A* were distinctly increased by ethylene. Moreover, this increase was shown to be specific to laticifers and to ethylene application.
• *Conclusion* The data and all previous information on sucrose transport show that *HbSUT1A* and *HbSUT2A* are related to the increase in sucrose import into laticifers, required for the stimulation of latex yield by ethylene in virgin trees.

Key words: *Hevea brasiliensis*, laticifers, latex production, ethylene, sucrose transporters.

INTRODUCTION

Natural rubber (*cis*-polyisoprene) is the main economic product of *Hevea brasiliensis* and is widely used industrially. *cis*-Polyisoprene synthesis takes place in the cytoplasm of highly specialized cells, known as laticifers (latex cells), and is their main metabolic function. Because sucrose is the unique precursor for rubber synthesis, its import into the laticifers may be an important limiting factor in latex production.

Laticifers are periodically differentiated from the cambium and arranged in an isolated network in the inner bark of *H. brasiliensis* (Hébant and de Faÿ, 1980; de Faÿ and Jacob, 1989). The cytoplasm of laticifers, known as latex, is expelled when the bark is wounded or deliberately regularly cut (tapped) to obtain the latex (Gomez, 1976; Gomez and Moir, 1979). Rubber particles represent 30–50 % of the latex in volume and 90 % in dry weight, and therefore laticifers have to regenerate their cytoplasm between two consecutive tappings. Much research has shown that rubber synthesis is brought about through the mevalonate-dependent metabolic pathway (Hepper and Audley, 1969), but more recently, transcriptome studies have suggested that synthesis could also follow a mevalonate-independent pathway (Ko *et al.*, 2003). Both pathways coexist in laticifers and need sucrose as a precursor (d'Auzac, 1964; Chow *et al.*, 2007). In addition, sucrose contributes, together with polyols and potassium, to the generation of the high turgor pressure (0·9–1·5 MPa) in the laticifers and their surrounding cells in the soft bark (liber), which is important for latex flow (Buttery and Boatman, 1966). Moreover, the cytosolic sucrose concentration is used to evaluate metabolic activity of laticifers (Jacob *et al.*, 1988, 1989).

Laticifers are regarded as a strong sink for sucrose and need to be adequately supplied exogenously with sucrose to meet their high carbon and energy demands (Tupy, 1973; Eschbach *et al.*, 1986; Silpi *et al.*, 2007). Based on a few circumstantial observations, active transport across the plasma membrane of laticifers had been hypothesized as the main route of sugar uptake. Ultrastructural studies on mature laticifers showed that they have no plasmodesmata and are therefore apoplastically isolated from adjacent cells (Hébant, 1981; de Fay and Jacob, 1989). Electrophysiological research carried out on isolated laticifers and protoplasts indicates that sucrose is able to depolarize the plasma membrane and this process is sensitive to H^+-ATPase inhibitors (Bouteau *et al.*, 1991, 1992, 1999), suggesting the existence of an energy-dependent, H^+/sucrose symporter in the plasma membrane. However, there are no reports, based on molecular evidence, of the presence of such sucrose transporters in the laticifers.

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Sucrose transporter (SUT) cDNAs have been isolated mainly from herbaceous plants, but also from some woody plants (Lemoine, 2000; Sauer, 2007). Generally, these sucrose transporters belong to the major facilitative superfamily (MFS; Marger and Saier, 1993) and are encoded by a multigenic family. Their activity was reported to be finely controlled (Sakr *et al.*, 1997; Chiou and Bush, 1998; Roblin *et al.*, 1998; Vaughn *et al.*, 2002; Schneidereit *et al.*, 2008) and modulated by exogenous (Juergensen *et al.*, 2003; Meyer *et al.*, 2004; Decourteix *et al.*, 2006) as well as endogenous signals such as sucrose (Chiou and Bush, 1998) and hormones (Chincinska *et al.*, 2008). Phylogenetic analyses have shown that sucrose transporter proteins fall into three independent phylogenetic clades, called SUT1, SUT4 and SUT2 (Barker *et al.*, 2000; Weise *et al.*, 2000; Barth *et al.*, 2003).

Sucrose transporters play a key role in many physiological processes. This is true both for source and for sink organs. For example, *AtSUC5*, specifically expressed in the endosperm, is required for the supply of sucrose to seeds during early stages of development (Baud *et al.*, 2005). Based on comparison of expression patterns of three sucrose transporters of the grape berry (*VvSUC11*, *VvSUC12* and *VvSUC27*), Davies *et al.* (1999) suggested that only two sucrose transporters (*VvSUC11*, *VvSUC12*) might have a significant role in ripening-related sucrose import. Inhibition of *LeSUT2* in tomato by antisense expression resulted in reduction of the number of fruit and the number of seed fertilized per fruit. This phenotype is due to the inability of these organs to download sucrose from phloem, and supports the central role of this sucrose transporter in fruit and seed development (Hackel *et al.*, 2006). *JrSUT1* (from *Juglans regia* sucrose transporter), isolated from xylem parenchyma cells, is involved in sucrose retrieval from the xylem vessel after freezing and thawing (Decourteix *et al.*, 2006) and in providing sucrose to bursting vegetative buds (Decourteix *et al.*, 2008). Although sucrose is the predominant soluble sugar in *H. brasiliensis* latex and is required for rubber synthesis and for latex cell cytoplasm regeneration between tappings, no study has yet focused on the involvement of sucrose transporters in these processes.

As ethylene was shown to be an efficient stimulant of latex yield (Abraham *et al.*, 1968; d'Auzac and Ribaillier 1969), Ethrel® and ethylene gas are commonly used to increase rubber yield in plantations worldwide. This stimulation may occur in several ways: through an increase in sugar metabolism (Tupy, 1973; Tupy and Primot, 1976; Silpi *et al.*, 2006) and the consecutive increase in energy (ATP) availability (Amalou *et al.*, 1992), by increased sugar import (Lacrotte *et al.*, 1985) and by a hyperpolarization of the laticifer's plasma membrane (Bouteau *et al.*, 1992), suggesting a possible stimulation of H^+-ATPase plasma membrane by ethylene application, activity of which is required for a plasma membrane H^+-symporting sucrose transporter.

Rubber biosynthesis is the major metabolic feature of laticifers, and requires exclusively sucrose as precursor. These cells are a metabolically active sink but are apoplastically isolated from neighbouring cells. Therefore, to meet adequately their substantial carbon and energy needs, they have to import sucrose through hypothetical plasma membrane sucrose/H^+ symporters. Our hypothesis is that these kinds of transporters (sucrose/H^+ symporters) may be localized on the plasma membrane of laticifers and are positively regulated by ethylene application to increase sucrose import, required for the stimulation of latex production by ethylene. The present study was carried out on unexploited (virgin) trees treated or untreated with the latex yield stimulant Ethrel. Mature virgin trees were used to analyse independently the direct effects of ethylene *per se* upon a first tapping, and then upon a second tapping to study the combined effects of ethylene and of the metabolic changes due to the previous tapping (latex regeneration demand) on sucrose transporter gene expression. In addition, study was made of the clone PB217, which is characterized by medium rubber production and metabolic activity without ethylene stimulation, but with high latex yield when treated with ethylene (Gohet, 1996; Gohet *et al.*, 1997, 2003).

Among seven sucrose transporter cDNAs identified, the relative transcript level of two isoforms (*HbSUT1A* and *HbSUT2A*) is found to be increased by ethylene application. Further characterization showed that they were induced specifically by ethylene. In addition, their localization in the inner bark tissues and especially in the latex cells was confirmed by *in situ* hybridization. The data show that these two isoforms could play an essential role in sucrose import into laticifers of virgin trees, necessary for ethylene-stimulated latex production.

MATERIALS AND METHODS

Plant material

Latex and bark samples were collected from the trunk of mature virgin rubber trees (about 8 years old) of the PB217 clone in the Bongo/SAPH plantation (Côte d'Ivoire). Latex samples were collected as described by Pujade-Renaud *et al.* (1994). Briefly, after discarding the first 20 drops, the latex samples were collected as a mixture of 2 mL from three trees per treatment in an equal volume of 2× fixation buffer. The samples were immediately deep-frozen in liquid nitrogen and stored at $-80\,^{\circ}C$.

Back-cutted trees (cut 6 months earlier) grown in a controlled environment chamber at CPN (Composants Naturels, Michelin Group, Clermont-Ferrand, France) were used to analyse the gene expression patterns of the sucrose transporter in different organs (mature leaves, bark and xylem) and for *in situ* hybridization (stems). These young trees were grown with 12 h daylight of 40 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetically active radiation, at 27–30 °C, with 70 ± 10 % relative humidity, and at 25 °C, with 90 % relative humidity (night). Each pot of 160 L, filled with potting mix, was connected to a watering system and sprayed to maintain high atmosphere and soil moisture.

Field experiments of ethylene treatments

Seven batches of three homogeneous (in growth) mature virgin trees were selected as described by Pujade-Renaud *et al.* (1994) and Sookmark *et al.* (2002): two as a control (treated with palm oil only) and five treated with 1 mL of 2·5 % Ethrel® emulsion (approx. 70 mM ethephon) in crude palm oil on a 1-cm-wide, slightly scraped bark band and just beneath the next tapping cut, for 4, 8, 16, 24 and 40 h,

respectively, before the first sampling. To avoid the influence of possible weather variations, in all experiments the trees were opened (tapped for the first time) on a half spiral with a tapping knife, and the latex and the bark samples were collected for analysis on the same day and at the same time. The homogenous depth (approx 1 mm from the cambium) of tapping was tested with a sharp calibrated punch.

After a 3-d rest (without further stimulation), latex and bark samples from the same trees were collected again and analysed as the second tapping (Tap2).

Hormones and other bark treatments

As the relative transcript level of sucrose transporters was reported to be regulated by hormonal treatments such as gibberellins (Chincinska *et al.*, 2008), ethylene (Chincinska *et al.*, 2008), auxin (Harms *et al.*, 1994) and cytokinin (Harms *et al.*, 1994; Ehness and Roitsch, 1997), we wanted to check whether various hormone or wounding treatments could induce any effect on laticifer SUT expression. To do this, a protocol similar to that for ethylene application was used. Briefly, two batches were used as controls (treated with 1 g palm oil containing 0·05 % Tween 20), and four others were treated with 1 g of 2 % jasmonic acid (JA), 1 % salicylic acid (SA), 1·5 % 4-chlorophenoxyacetic acid (CPA, auxin analog) and 2 % abscissic acid (ABA) palm oil emulsion with 0·05 % Tween 20, and a last batch was mechanically wounded by sealing 15 nails up to the cambium (Nails), on an approx. 1-cm-wide, slightly scraped bark band, just beneath the next tapping cut, for 4, 8 and 16 h, respectively, before the first tapping. The latex and bark samples were collected on the same day and analysed as the first tapping (Tap1) in the same way as for the Ethrel experiment. After a 3-d rest, samples from the batches of the 4-h-treated trees were collected again and analysed as the second tapping (Tap2).

*Molecular cloning of sucrose transporter (*HbSUTs*) full-length cDNA*

Four latex and bark sucrose transporter probes were obtained by polymerase chain reaction (PCR) directly on latex or bark cDNA libraries, kindly provided by the team of Dr P. Kongsawadworakful, Unchera Viboonjun and H. Chrestin (Mahidol University, Bangkok, Thailand). For this purpose, degenerate primers were designed from conserved regions of published plant sucrose transporter cDNAs (EMBL data library). One primer couple was used for each SUT group: SUT1 group [primer SUT1F (5′-TA/TC/TA/GA/GC/TACA/C/TGAC/TTGGA/TTG/TGG-3′) and primer SUT1R (5′-TA/TC/TYACA/C/TGAYTGGA/TTKGG-3′)], SUT2 group [primer SUT2F (5′-CATTTA/GCCA/C/TCCC/G/TGCA/TATGCA-3′) and primer SUT2R (5′-ACTCCG/TATA/C/TGCCAAA/G/TCCTTGC/TCC-3′)] and SUT4 group [primer SUT4F (5′-AA/GATC/TTATGGCGGTGAACC-3′) and primer SUT4R (ACA/GCCCAA/TA/TGAC/TAAGCCTTGA/TCC)]. Amplifications were carried out in a thermal cycler (PTC-200; MJ Research, Watertown, MA, USA) using standard protocols (Decourteix *et al.*, 2006). The different amplified fragments were cloned and sequenced, and then used as SUT homologous probes to screen the latex cDNA libraries under low-stringency conditions. Seventeen clones were obtained and fully sequenced. Their nucleotide sequence alignment showed that they corresponded to seven different isoforms, referred to as *HbSUT1A*, *HbSUT1B*, *HbSUT2A*, *HbSUT2B*, *HbSUT2C*, *HbSUT4* and *HbSUT5* (*H. brasiliensis* sucrose transporters). In addition, the sequences of five of them (*HbSUT1A*, *HbSUT2A*, *HbSUT2B*, *HbSUT4* and *HbSUT5*) were identical to *H. brasiliensis* SUT sequences, concomitantly registered in the EMBL/GenBank/DDBJ under accession numbers DQ985466, DQ985467, DQ985465, EF067335 and EF067333, respectively (Yang *et al.*, 2006, direct submission). The *HbSUT1B* and *HbSUT2C* sequences were registered in the EMBL/GenBank/DDBJ under accession numbers AM492537 and AM491808, respectively.

RNA extraction

Total RNAs were extracted from latex as described by Pujade-Renaud *et al.* (1994, 1997) and developed by Sookmark *et al.* (2002). Total inner bark RNA extraction from mature trees used the caesium chloride cushion method, adapted from Sambrook *et al.* (1989), using approx. 2 g of inner bark ground under liquid nitrogen.

Deep-frozen leaves, bark and xylem samples collected from back-cutted trees were ground in liquid nitrogen and total RNAs were extracted using CTAB extraction buffer (Chang *et al.*, 1993) and then treated with RNase-free RQ1 DNase (Promega, Madison, WI, USA). mRNAs were spectrophotometrically quantified and checked by agarose gel electrophoresis.

Specific primer for real-time RT-PCR

Primer couples specific to each isoform were designed for real-time RT-PCR: *HbSUT1A* [primer SUT1AF (5′-CAG CTTTTGTGGTGGGGGCGA-3′) and primer SUT1AR (5′-T GCACATCTATATGATCACATCCA-3′)], *HbSUT1B* [primer SUT1BF (5′-CAGCTTTTGTGGTGGGAGGGG-3′) and primer SUT1BR (5′-CCAATTTTTGGCCATTGATGCCC-3′)], *HbSUT2A* [primer SUT2AF (5′-GGTTTTCATTTTGGCTAACGA CTG-3′) and primer SUT2AR (5′-TGATAAAGCACTCATCT TTTTACA-3′)], *HbSUT2B* [primer SUT2BF (5′-GGCTTTCC TCTTGCTATTACG-3′) and primer SUT2BR (5′-GTAAACTC AATTGAAGTGTTTCAGTC-3′)], *HbSUT2C* [primer SUT2CF (5′-TCCTTTGAAAGCATGCGCTAAT-3′) and primer SUT 2CR (5′-GTAAACTCAATTGAAGTGTTTCAGTC-3′)], *HbSUT4* [primer SUT4F (5′-GCAGTTCTTGGTGTTCCGT-3′) and primer SUT4R (5′-TCAATGGACTGTTATCTGCAAA-3′)], *HbSUT5* [primer SUT5F (5′-GCAGTTCTTGGTGTTCCAC-3′) and primer SUT5R (5′-ATGCTGGCATCCAATCGGATG-3′)]. The specificity of each primer pair was tested by semi-quantitative and real-time RT-PCR on each full-length cDNA cloned in pGEM-T easy vector (Promega). PCR reactions were performed as described below for real-time RT-PCR.

Besides sucrose transporters, the expression pattern of the glutamine synthetase gene (*HbGS*) was monitored by using the specific primer pair HbGS_F (5′-GCTGGCATCAACATT AGTGG-3′) and HbGS_R (5′-CAACGCCCCATAAGAAAG TG-3′)]. *HbGS* has been reported to be a good marker of the ethylene effect on gene expression in latex (Pujade-Renaud *et al.*, 1994, 1997). For bark tissue, the expression pattern of the ethylene-induced gene ACC oxidase (1-aminocyclopropane-

1-carboxylate oxidase) was determined as a positive control of the response to ethylene (Kongsawadworakul *et al.*, 2004). The specific primer pair used was: HbAccOx_F (5′-ATGGACACA GTTGAGAGGATGAC-3′) and HbAccOx_R (5′-AGGTGG CGGAGGAAGAAGG-3′)].

Quantification of sucrose transporter transcripts by real-time RT-PCR

After treatment with DNase I, 2 μg of total RNA was used as the template in the first-strand cDNA synthesis reaction, according to the manufacturer's instructions (SuperScript-III, Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed using the generated cDNA as target template, a fluorescent dye SYBR-Green and an iCycler system (Bio-Rad, Hercules, CA, USA). cDNA encoding actin was used as the internal control gene. PCR reactions were performed as follows: 5 min at 95 °C for denaturation, 20 s at 95 °C, 20 s at 58 °C and 20 s at 72 °C for amplification, and 10 min at 72 °C for final extension. ΔCt was calculated from the formula $\Delta Ct = Ct_{(\text{treated sample})} - Ct_{(\text{control sample})}$. The normalized expression ratio (Q_r) was calculated using the comparative Ct method, with the formula: $Q_r = E^{-\Delta\Delta Ct}$ (E = efficiency of the primer couple, $\Delta\Delta Ct = \Delta Ct_{\text{sucrose transporter}} - \Delta Ct_{\text{actin}}$). This method makes it possible to visualize the increase ($Q_r > 1$) and decrease ($Q_r < 1$) of genes (up- and downregulation, respectively). The efficiency of each primer pair was previously evaluated and found to be between 1·75 and 1·85. To compare the relative abundance of transcripts in different organs, expression was calculated from: Expression $= E^{\text{Ct(actin)}-\text{Ct(sugar transporter)}}$ as given in the Bio-Rad real-time PCR Application Guide.

Statistical analysis

As empirical errors in Q_r increased with Q_r values, consistent with the above exponential formulation, statistical procedures were performed on log-transformed data. Two samplings, each consisting of two independent plants, were used to estimate an unbiased biological error (with two degrees of freedom) for comparison of means using Student's *t*-test. Results were considered to be statistically different at $P < 0{\cdot}01$.

In situ *hybridization*

Tissue preparation was done according to Brunel *et al.* (2002). Transverse sections, 12 μm thick, were cut with a rotary microtome, mounted on SuperFrost Plus slides (Fisher Scientific, Elancourt, France) and dried at 42 °C for 2 d. Paraffin was removed via two baths (10 min each) in Histoclear II. Slides were progressively rehydrated in an ethanol series and then washed for 5 min in DEPC water and twice in 1× phosphate-buffered saline (PBS). Slides were treated with proteinase K at 10 μg ml^{-1} in 1× PBS at 37 °C for 15 min and then incubated in glycine at 0·2 % in 1× PBS at room temperature for 2 min. After two washes in 1× PBS, slides were fixed in 4 % paraformaldehyde (in 1× PBS) for 10 min, rinsed in 1× PBS and then acetylated using 0·5 % acetic anhydride in 0·1 M triethanolamine for 10 min and rinsed in 1× PBS. Before hybridization, sections were dehydrated in an ethanol series. Sections were hybridized with equal concentrations (1·5 ng μL^{-1}) of either sense or antisense probes in 1× Denhart's, 10 % dextran sulfate, 1 mg ml^{-1} tRNA, 50 % formamide, 1× salts (300 mM NaCl, 10 mM Tris–HCl, pH 6·8, 10 mM NaH_2PO_4, 10 mM Na_2HPO_4 and 5 mM EDTA), and incubated at 50 °C overnight in a humid chamber. Sections were washed twice in 2× saline sodium citrate (SSC), six times in 0·1× SSC for 15 min at 42 °C each and twice in 1× PBS for 10 min at room temperature. Detection of digoxigenin (DIG)-labelled probes used an anti-digoxigenin alkaline phosphatase conjugate (Poupard *et al.*, 2001). After suitable colour development, the reaction was stopped by rinsing in water and the sections were dried and mounted in Eukitt. Sections were observed under a Zeiss Axioplan 2 microscope and with an AxioCam HR camera (Zeiss) with AxioVision digital imaging software.

RESULTS

Latex production

Application of Ethrel® under the tapping-cut significantly stimulated latex yield (Fig. 1), especially for the first tapping (Tap1). In comparison with the control of Tap1, latex production increased strongly by 16 h and reached its maximum value 40 h after treatment (27-fold more than in the control). The standard deviation for the marked increase in yield was very large, suggesting substantial heterogeneity in the response between trees. Nevertheless, the treatment differed significantly from the control. For the second tapping (Tap2), latex production increased only weakly at 4 h (three-fold) and remained constant until 40 h after treatment.

Cloning cDNA of putative sucrose transporters from latex

Degenerated oligonucleotide primers from conserved regions of each group of sucrose transporters (SUT1, SUT2 and SUT4) were used and allowed cloning of four different amplification products from latex and bark libraries. These four probes were used for screening a latex cDNA library, which led to the identification of seven full-length cDNA-encoding putative sucrose transporters. Based on their sequence homologies, two isoforms (*HbSUT1A* and

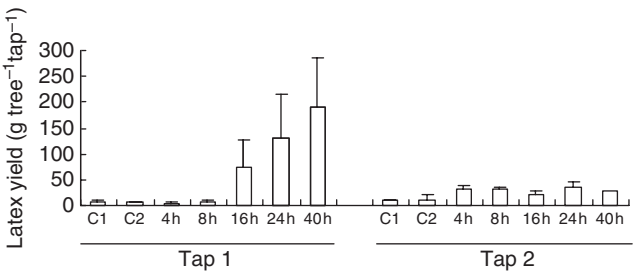

FIG. 1. Latex yield of virgin PB217 trees after ethylene stimulation. Batches of three homogenous mature virgin trees were treated with 5 % Ethrel® (ethylene) 4, 8, 16, 24 or 40 h before the first simultaneous tapping (Tap1). After 3 d without any further treatment, the same trees were tapped again (Tap2). C1 and C2 represent two batches of three trees, which were un-stimulated as controls. Latex yield is expressed in grams of fresh latex per tree per tapping. Bars represent the biological standard deviation.

HbSUT1B) were found to belong to the SUT1 group, three (*HbSUT2A*, *HbSUT2B* and *HbSUT2C*) to the SUT2 group and two others (*HbSUT4* and *HBSUT5*) to the SUT4 group.

Further investigations showed that *HbSUT1A*, *HbSUT2A*, *HbSUT2B*, *HbSUT4* and *HBSUT5* were identical to those concomitantly registered by Yang *et al.* (direct submission, 2006) in the EMBL/GenBAnk/DDBJ under accession numbers DQ985466, DQ985467, DQ985465, EF067335 and EF067333, respectively. The new *HbSUT1B* and *HbSUT2C* full-length nucleotide sequences were registered in the EMBL/GenBank/DDBJ under accession numbers AM492537 and AM491808, respectively.

All these putative transporters belonged to the MFS and shared main characteristics with the previously cloned sucrose transporters. Their nucleotide sequence contained an open reading frame ranging from 1·5 to 1·9 kb that encoded 500 to 633 amino-acid polypeptides. In addition, their predicted amino-acid sequences exhibited highest identity to those of *Ricinus communis* (about 83 %) and *Manihot esculenta* (about 90 %), both of which belong, like *H. brasiliensis*, to the Euphorbiaceae family. According to the proposed classification of sucrose transporters (Sauer, 2007), these were divided into three groups (Fig. 2; accession numbers of the sequences presented are given in the Appendix). *HbSUT1A* and *HbSUT1B* belong to Group II, which contains sucrose transporters involved in phloem loading and sucrose import into different sink organs. *HbSUT2A*, *HbSUT2B* and *HbSUT2C* fall into Group III, which contains more amino acids than all the other sucrose transporters, due to the presence of an extended N-terminal domain and a central cytoplasmic loop. *HbSUT4* and *HbSUT5* belong to Group IV, with sucrose transporters characterized by low affinity/high capacity and specific localization in either the plasma membrane (Weise *et al.*, 2000) or the tonoplast (Endler *et al.*, 2006). For the following, we refer to the classification comprising the SUT1, SUT2 and SUT4 groups.

Basal expression of putative sucrose transporters in latex and bark of untreated virgin trees

Because 3′ untranslated region (UTR) sequences are the most divergent regions within isoforms (Lerchl *et al.*, 1995; Duval

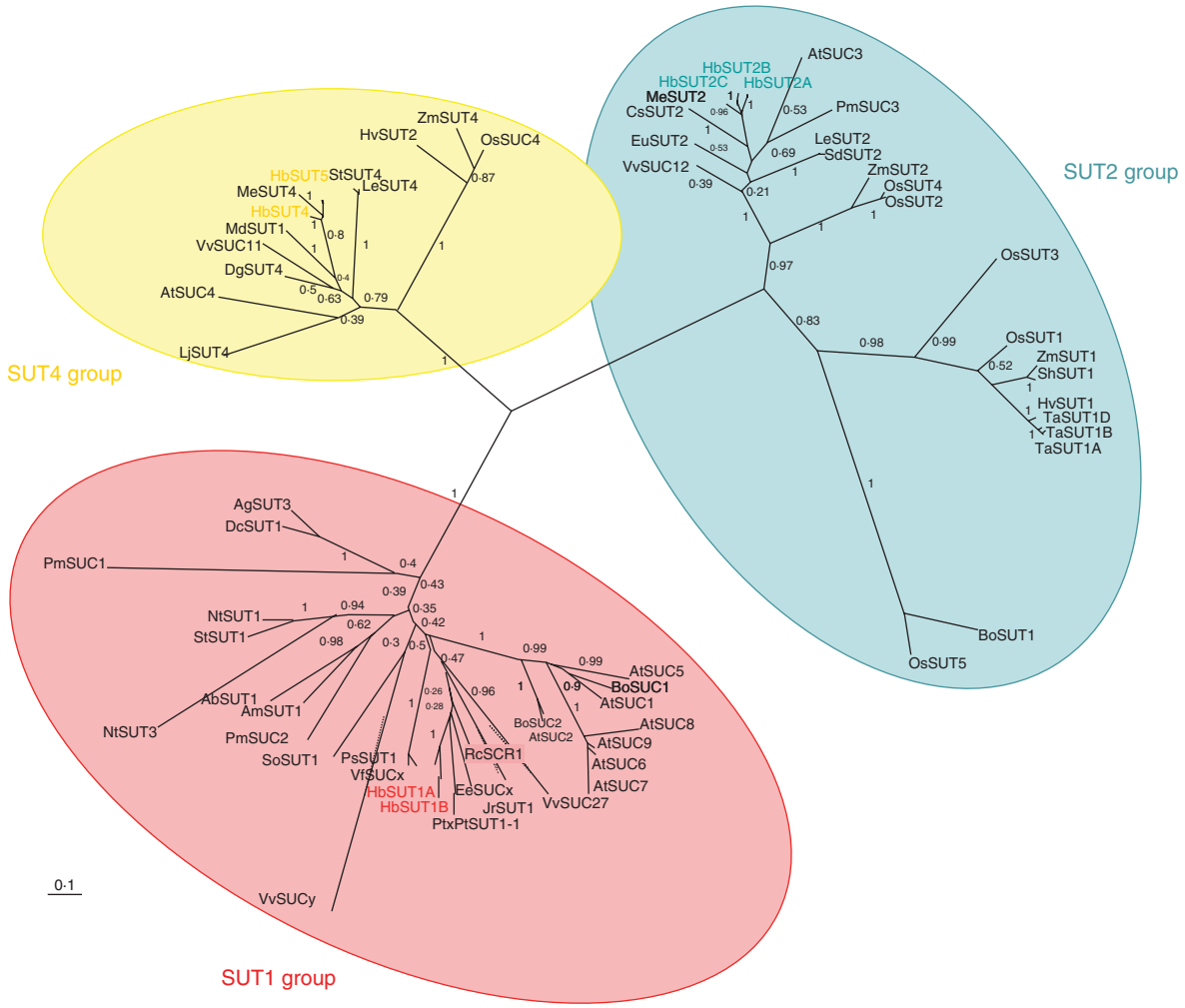

FIG. 2. Phylogenetic tree of plant sucrose transporters. Confirmed or predicted plant sucrose transporter sequences from public databases (EMBL/GenBank/DDBJ) were used to construct a phylogenetic tree. Deduced protein sequences were aligned using *MU*ltiple *S*equence *C*omparison by *L*og-*E*xpectation (http://www.ebi.ac.uk/Tools/muscle/). The tree was constructed using the program PhyML (http://www.phylogeny.fr/version2_cgi/one_task.cgi?task_type=phyml) and Treeview. Internal labels indicate bootstrap values (100 bootstraps).

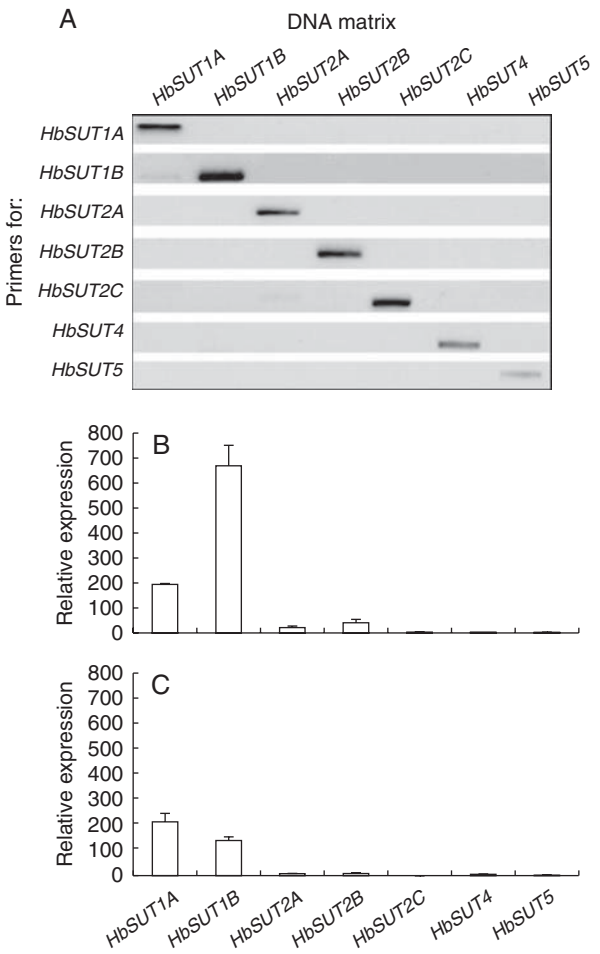

FIG. 3. Specificity of the different primers and basal expression of different sucrose transporters in latex and bark of virgin trees. (A) Specificity, and (B) basal expression of different *HbSUT* isoforms in latex and (C) in bark. The relative expression of *HbSUT*s was determined by real-time RT-PCR in bark and latex, using actin expression as control.

et al., 2002; Morey *et al.*, 2002; Sakr *et al.*, 2003; Decourteix *et al.*, 2006, 2008) most of the eight primer pairs were designed in this region to specifically amplify each of the eight isoforms. The specificity of each primer pair was verified by PCR amplification using the purified cDNA clone of each different isolated sucrose transporter from latex, such as templates. Figure 3A shows that each primer pair only recognized and amplified the cDNA of the sucrose transporter isoform that they were designed from. Such specificity allowed us to investigate the expression of each sucrose transporter in the latex and bark, in response to tapping and ethylene treatment.

First, the basal expression of different sucrose transporters from untreated virgin trees was investigated in latex. The sucrose transporter isoforms of the SUT1 group (*HbSUT1A* and *HbSUT1B*) were predominantly expressed in the resting latex cells (untapped, unstimulated), with *HbSUT1B* as the most abundant sucrose transporter isoform in latex (Fig. 3B). Compared with the SUT1 group, members of the SUT2 group, mainly *HbSUT2A* and *HbSUT2B*, showed very low basal expression. However, lowest expression was for the two isoforms (*HbSUT4* and *HbSUT5*) of the SUT4 group and one isoform (*HbSUT2C*) of the SUT2 group.

As latex comes from the laticifers that are embedded in the inner soft bark tissues, basal expression of these transporters in bark was also investigated (Fig. 3C). All the transporters were detected, to varying degrees, in the inner bark. Again, *HbSUT1A* and *HbSUT1B* were the most highly expressed isoforms. *HbSUT1B* was weakly expressed in the inner bark compared with latex, suggesting a preferential expression of these two transporters in the laticifers.

Ethylene effect on sucrose transporter expression profiles in latex

To confirm that ethylene treatment of bark stimulated gene expression in the laticifers, the relative transcript level of *HbGS* was investigated as a positive ethylene-responsive gene in these cells (Pujade-Renaud *et al.*, 1994, 1997). *HbGS* increased in the latex cells, starting within 16 h of ethylene treatment, with a maximum (18-fold) after 24 h (Tap1, Fig. 4A). This response was prolonged for the second tapping, regardless of when the ethylene treatment was applied, with a maximum (ten-fold expression compared with the control) at 8 and 16 h (Tap2, Fig. 4A). As shown in Fig. 4, the effect of ethylene treatment not only differed between the sucrose transporter isoforms, but also between the first (Tap1) and the second (Tap2) tapping. The transcripts of *HbSUT1A* (Fig. 4B) and, to a lesser extent, of *HbSUT2A* (Fig. 4C) were found to be the most responsive to ethylene treatment.

No significant effect was observed at the Tap1 within the first 8 h following ethylene treatment. At 16 h, the transcripts of *HbSUT1A* and *HbSUT2A* increased 16- and six-fold, respectively (Fig. 4B, C). This accumulation was abrupt and transient for *HbSUT1A*, whereas it was progressive and maximum after 40 h for *HbSUT2A*. Transcripts of *HbSUT2B* and *HbSUT2C* were very slightly and transiently increased by ethylene. No significant ethylene effect was detected for *HbSUT4* and *HbSUT5* (Fig. 4D). Of all these sucrose transporters, *HbSUT1B* was the only isoform for which expression was decreased by ethylene stimulation after 24 h treatment (Fig. 4B).

In contrast to Tap1, there was hardly any regulation of the sucrose transporter transcripts during Tap2. However, a slight and transient induction was observed for *HbSUT1A* between 8 and 16 h after ethylene treatment (Fig. 4B–D).

Ethylene effect on the expression profiles of the putative sucrose transporters in the inner bark

First, ethylene treatment was confirmed to induce an early (4 h) and strong (up to 200-fold) accumulation of ACC oxidase transcripts (Kongsawadworakul *et al.*, 2004) during the first and second tappings (Fig. 5), which validated the ability of the ethylene bark treatment to regulate gene expression in the soft inner bark.

At Tap1, most of the sucrose transporter transcripts either remained constant or decreased (Fig. 5B–D). Only the *HbSUT2B* isoform was weakly and transiently increased at 40 h (Fig. 5C). During Tap2, *HbSUT1A*, *HbSUT2B* and *HbSUT4* were transiently increased with a maximum around 16 h (Fig. 5C, D). The increase in transcripts was strongest

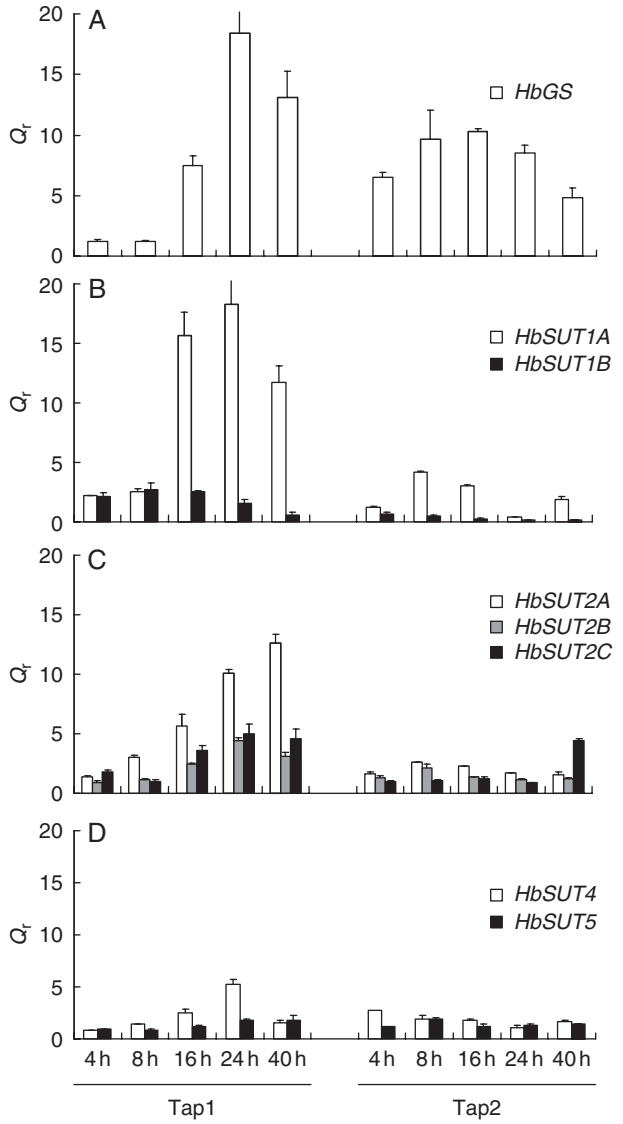

FIG. 4. Transcript accumulation of glutamine synthetase and different sucrose transporters after ethylene stimulation in virgin tree latex. Relative transcript levels of glutamine synthetase (A), and sucrose transporters of SUT1 group (B), SUT2 group (C) and SUT4 group (D) were monitored in latex of ethylene-treated virgin trees. Latex mRNA from mature virgin trees (PB217) was used for real-time RT-PCR. Q_r was obtained by the $E^{-\Delta\Delta Ct}$ methods. Bars represent the technical standard deviation.

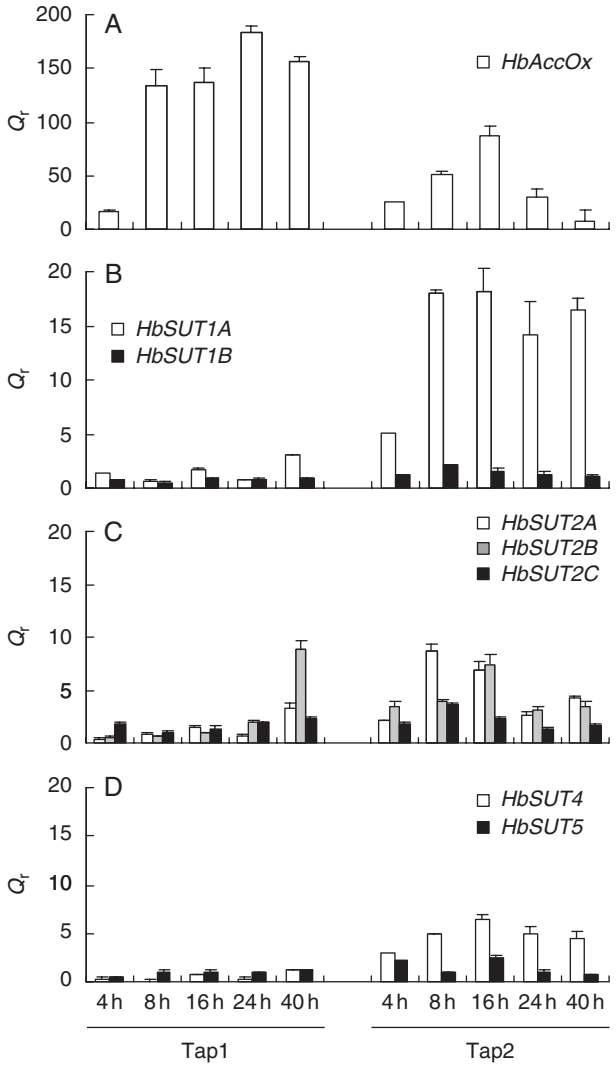

FIG. 5. Transcript accumulation of ACC oxidase and different sucrose transporters after ethylene stimulation in bark of mature virgin trees. Relative transcript levels of ACC oxidase (A) and sucrose transporters of SUT1 group (B), SUT2 group (C) and SUT4 group (D) were monitored in bark of ethylene-treated virgin trees. Bark mRNA samples from virgin mature trees (PB217) were used for real-time RT-PCR. Q_r was obtained by the $E^{-\Delta\Delta Ct}$ method. Bars represent the technical standard deviation.

for the *HbSUT1A* isoform, which was markedly increased at 4 h, and reached a plateau between 8 and 40 h (Fig. 5B).

Effect of hormones and wounding on expression of HbSUT1A, HbSUT2A *and* HbSUT2B *in the latex*

As *HbSUT1A*, *HbSUT1B* and *HBSUT2A* were found to be significantly regulated by ethylene, the effects of various hormonal and wounding treatments applied to bark on their respective expression in latex were also investigated. For *HbSUT2A*, no significant increase was observed, regardless of the treatment, whereas *HbSUT1A* exhibited an early, transient and low stimulation in the presence of SA and CPA (Fig. 6A, C). CPA and, to a lesser extent, the other treatments (especially JA) were found to induce a significant accumulation of *HbSUT1B* at Tap1 (from 8 h after treatment) and Tap2 (Fig. 6B).

HbSUT1A, HbSUT1B *and* HbSUT2A *expression in different organs*

HbSUT1A, *HbSUT1B* and *HbSUT2A* gene expression analysis was carried out by real-time PCR, using RNAs from leaves, bark and xylem of young back-cutted trees. These three transporters were detectable to varying degrees in the source and sink organs (Fig. 7). *HbSUT1A* was more highly expressed in sink organs (xylem and bark) than in leaves. *HbSUT1B* was predominantly expressed in leaves and bark, but relatively weakly in xylem. A similar pattern but of a reduced magnitude was found for *HbSUT2A*.

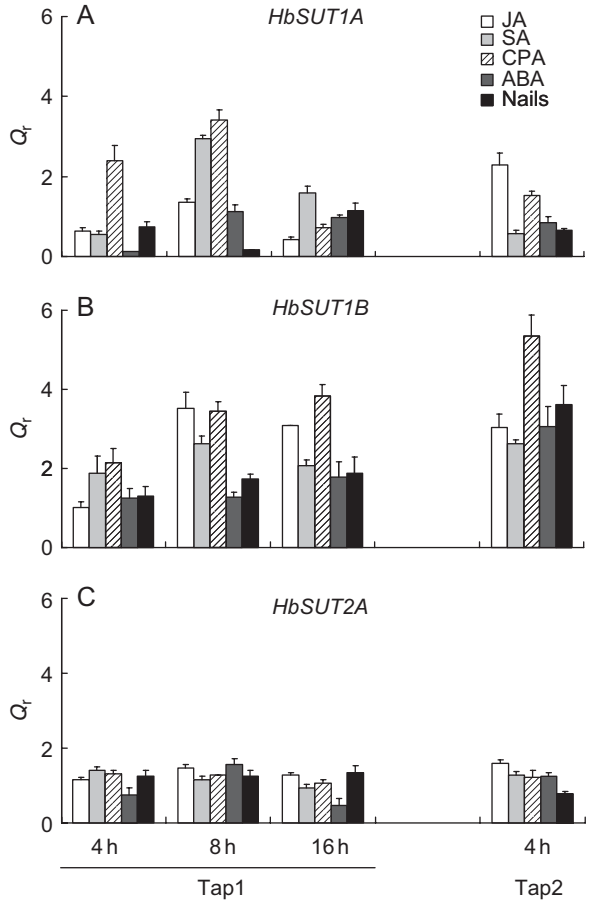

FIG. 6. Transcript accumulation of ethylene-modulated *HbSUT1A*, *HbSUT1B* and *HbSUT2A* after hormonal treatment or wounding in latex of mature virgin trees. Relative transcript levels of *HbSUT1A* (A), *HbSUT1B* (B) and *HbSUT2A* (C) were monitored in latex of virgin trees treated with jasmonic acid (JA), salicylic acid (SA), 4-chlorophenoxyacetic acid (CPA, an auxin analogue), abscissic acid (ABA) or nails planting. Latex mRNA samples from these trees were used for real-time RT-PCR analysis of *HbSUT1A*, *HbSUT1B* and *HbSUT2A* transcripts. Q_r was obtained by the $E^{-\Delta\Delta Ct}$ method. Bars represent the technical standard deviation.

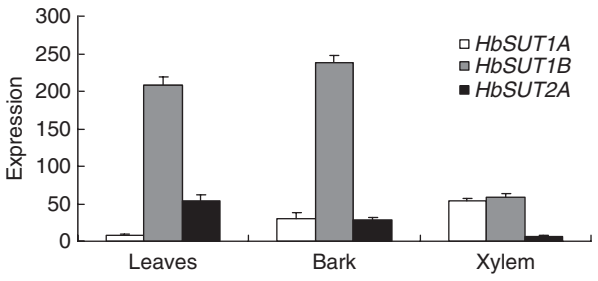

FIG. 7. Transcript accumulation of *HbSUT1A*, *HbSUT1B* and *HbSUT2A* in different organs of young virgin trees. cDNA from leaves, bark and xylem of young back-cutted trees (cut-back 6 months earlier) were used as matrix for real-time RT-PCR analysis of *HbSUT1A*, *HbSUT1B* and *HbSUT2A* transcripts. Expression = $E^{Ct(\text{actin})-Ct(\text{sugar transporter})}$. Bars represent the technical standard deviation.

In situ *hybridization*

Experiments were carried out with two types of probes: the first was specific to *HbSUT1A* and *HbSUT1B* (SUT1 group), which exhibit strong sequence identity, and the second was quite *HbSUT2A*-specific. As shown in Fig. 8A and B, young stems of *H. brasiliensis* contain some isolated laticifers exclusively localized in the phloem region, near cambial cells.

A typical colouration (violet staining) was only detected with the antisense probe (Fig. 8E, F, I, J), whereas no signal was observed with the sense probe (Fig. 8C, D, G, H), suggesting that the colouration indicates the presence of transcripts of *HbSUT1s* or *HbSUT2A*.

With regard to *HbSUT1* mRNAs, colouration was found in inner bark tissue, more precisely in young phloem, laticifers and the cambial zone. No colouration was found in xylem tissue, implying limited or no expression of these transporters. Similar data were found for *HbSUT2A*, for which a positive hybridization signal was also mainly confined to the cambial zone and the phloem tissue. However, a weaker colouration than for the *HbSUT1* probe was observed in the fusing young latex cells.

DISCUSSION

Due to their active metabolism in the synthesis of rubber, laticifers are a strong sink and require substantial and rapid sucrose import to meet their carbon and energy demands. Ethrel® is often used as latex yield stimulant (Tupy, 1973). A series of field and molecular experiments were conducted, for the first time, to assess if sucrose transporters are involved in the ethylene-induced increase in latex yield. These experiments were performed with mature virgin trees, at the very first tapping, to study the direct effect of ethylene, and then upon a second tapping. The PB217 rubber clone was used because it responds well to Ethrel yield stimulation.

Laticifers contain transcripts of many putative sucrose transporters

It is normally very difficult to access the pure cytoplasm of one single cell type. This is not the case for laticifers, from which the out-flowing cytoplasm (latex) can be collected by simple bark tapping. Moreover, the presence of polysomes and mRNA in the expelled latex (Coupé *et al.*, 1977; Kush *et al.*, 1990; Pujade-Renaud *et al.*, 1994; Ko *et al.*, 2003; Chow *et al.*, 2007) makes it possible to study the expression of any gene expressed in this kind of cell. Therefore, to identify the sucrose transporters expressed in laticifers, a latex-specific cDNA library was screened using four PCR-amplified homologous probes. Seven putative sucrose transporter isoforms were cloned from these cells, indicating that sucrose transporters are also encoded by a multigenic family in *H. brasiliensis*, as is the case for many other species. For example, nine sucrose transporter isoforms were identified from *Arabidopsis thaliana* (Sauer and Stolz, 1994; Barker *et al.*, 2000; Gottwald *et al.*, 2000; Ludwig *et al.*, 2000; Meyer *et al.*, 2000, 2004; Sauer *et al.*, 2004), three from tobacco (Burkle *et al.*, 1998; Ward *et al.*, 1998; Lemoine *et al.*, 1999), three from tomato (Barker *et al.*, 2000; Weise *et al.*, 2000), four from *Vitis vinifera* (Davies *et al.*, 1999; Ageorges *et al.*, 2000), two from citrus (Li *et al.*, 2003) and three from *Oryza sativa* (Scofield *et al.*, 2007; Sun *et al.*, 2008).

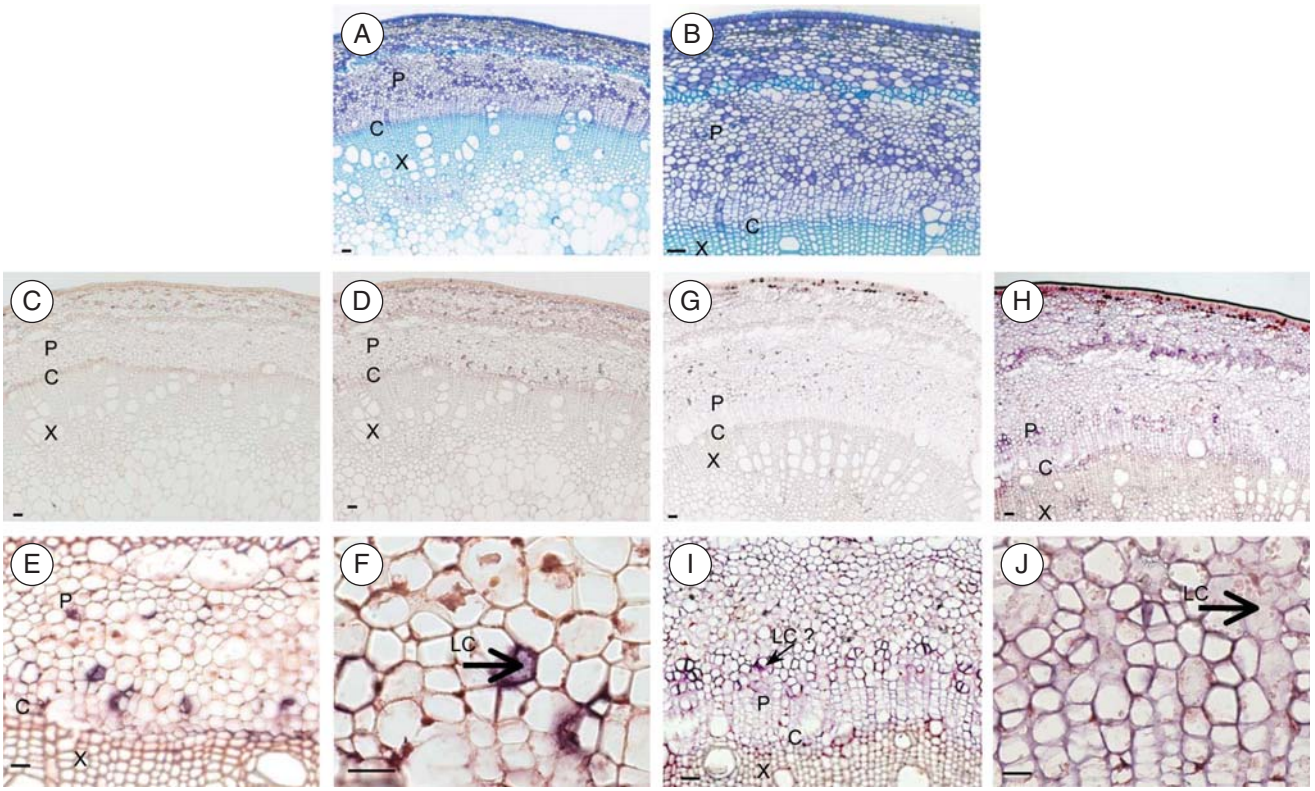

FIG. 8. *In situ* localization of *HbSUT1* and *HbSUT2A* transcripts in stems of young virgin trees. Transverse sections (12 μm thick) were obtained and hybridized with antisense probes specific for *HbSUT1* mRNA (E, F) and *HbSUT2A* mRNA (I, J); or sense probes as a negative control (*HbSUT1*: C, D; *HbSUT2A*: G, H). Positive hybridization signals are visualized by violet staining using a digoxigenin-labelled RNA immunodetection system. Other sections were stained with toluidine blue, to identify the cellular structures (A, B). Abbreviations: P, phloem; C, cambium; X, xylem; LC, laticiferous cells. Scale bar = 50 μm.

Based on their sequence homology, these sucrose transporters were found to be closely related to previously identified sucrose transporters from herbaceous as well as woody species (Lemoine, 2000; Sauer, 2007). The predicted amino-acid sequences of *Hevea* SUTs exhibited greatest identity (more than 83 %) with those of *Ricinus communis* and *Manihot esculenta*, which also belong to the Euphorbiaceae family. In addition, these sucrose transporters belong to all previously characterized groups of sucrose transporters, with two isoforms (*HbSUT1A* and *HbSUT1B*) in the SUT1 group, three (*HbSUT2A*, *HbSUT2B* and *HbSUT2C*) in the SUT2 group and two (*HbSUT4* and *HBSUT5*) in the SUT4 group.

Analysis of their basal transcript expression showed that these sucrose transporters were expressed to varying degrees in the latex, with greater expression of the SUT1 group (*HbSUT1A* and *HbSUT1B*) isoforms (Fig. 3B, C). Two isoforms of the SUT2 group (*HbSUT2A* and *HbSUT2A*) exhibited very low expression, while expression of the other SUT2 (*HbSUT2C*) and of the two SUT4 isoforms could barely be detected. On the basis of this transcript accumulation pattern, *HbSUT1A* and *HbSUT1B* may be considered as the main sucrose transporters involved in sucrose importation into the laticifers. Such a physiological role was supported by the *in situ* hybridization data, showing that these two transporters were expressed in the inner soft bark (liber) of young stems, including the young laticifers (Fig. 8).

To our knowledge, the presence of many isoforms of sucrose transporters in one single type of cell has only been reported once: this was for sieve elements with three isoforms (*LeSUT1*, *LeSUT2* and *LeSUT4*) proposed to be involved in sucrose retrieval from the apoplastic compartment (Barker *et al.*, 2000; Weise *et al.*, 2000; Kühn, 2003). Laticifers would therefore be expected to function as highly active sites of sucrose absorption.

Ethylene-induced increase in HbSUT1A *and* HbSUT2A *transcripts parallels the ethylene-induced stimulation in latex yield*

Much evidence in the literature indicates that an increase in sucrose transporter transcripts is often associated with a higher activity of the corresponding transporters, suggesting a major transcriptional regulation of these proteins (Sakr *et al.*, 1993, 1997; Chiou and Bush, 1998; Lemoine *et al.*, 1999; Decourteix *et al.*, 2006, 2008). Hence, as ethylene-stimulated latex production has been reported to occur through an increase in sugar metabolism (Tupy, 1973; Tupy and Primot, 1976; Silpi *et al.*, 2006), sugar influx (Lacrotte *et al.*, 1985) and hyperpolarization of the plasma membrane of laticifers (Bouteau *et al.*, 1999), an increased sucrose influx should be partly linked to an ethylene-induced stimulation of, at least, some of the putative cloned sucrose transporters. This should be particularly true for laticifers, as they are a very active sink cell type completely devoid of any starch reserve (Tupy, 1989).

In accordance with previously published data (Pujade-Renaud *et al.*, 1994), ethylene treatment below the tapping cut led to an

increase in *HbGS* expression in laticifers (Fig. 4A). This result confirmed that the ethylene signal was perceived by these cells. The expression pattern of the seven identified sucrose transporters was thus monitored on the same latex samples, using highly specific primer couples, designed from the UTR of their cDNAs (Fig. 3A). Only two transporters (*HbSUT1A*, *HbSUT2A*) were found to be, to varying degrees, significantly stimulated by ethylene. This stimulation was time-dependent as it peaked at 24 and 48 h after treatment for *HbSUT1A* and *HbSUT2A*, respectively. Note that *HbSUT1B*, which has the highest expression of any sucrose transporter isoform in the latex of untreated virgin trees, was significantly decreased when treated with ethylene. Under the same experimental conditions, the other isoforms of sucrose transporters were almost insensitive to ethylene. Taken together, these data suggest that the ethylene effect cannot be extended to all sucrose transporters initially cloned from the latex, and that the ethylene-induced sucrose influx into the laticifers (Lacrotte *et al.*, 1985) may be due, at least in part, to the ethylene stimulation of *HbSUT1A* and *HbSUT2A*. Moreover, this ethylene-induced transcript accumulation of *HbSUT1A* and *HbSUT2A* was correlated with the ethylene-stimulated latex production (Fig. 1). This potential role of *HbSUT1A* and *HbSUT2A* in ethylene-induced stimulation of latex production in virgin trees could be related to the fact that none of the sucrose transporters was found to be greatly increased during Tap2 (Fig. 4), which caused much less latex production (Fig. 1).

The ethylene effect is tissue-specific

As ethylene applied to the bark tapping area induced a pronounced increase in some SUTs in the laticifers, we verified whether a similar effect could be found in the surrounding inner liber tissues. In contrast to laticifers, no sucrose transporter was found to be significantly regulated by ethylene treatment on the first tapping (Fig. 5B–E). This lack of an ethylene effect could not be due to a failure of bark cells to perceive the ethylene signal (Fig. 5A), as ethylene did induce a marked stimulation of an ACC oxidase gene expression, which has been reported to behave as a positive marker of the response of bark tissues to ethylene treatment (Kongsawadworakul *et al.*, 2004). These data suggested that the expression of *HbSUT1A* and *HbSUT2A* induced by ethylene was limited to the laticifers, which are devoted to rubber synthesis and, thereby, latex production. A tissue-specific regulation was recently reported for *JrSUT1*, a putative plasma membrane-localized sucrose transporter, isolated from walnut tree xylem (Decourteix *et al.*, 2006). Analysis of its expression showed that *JrSUT1* was induced by freezing and thawing in xylem tissue but not in bark, indicating the occurrence of differential regulation mechanisms between these tissues.

Laticifers are considered to be apoplastic sinks which import sucrose from the phloem. The efficiency of phloem unloading is strongly related to the sink strength, which is defined as the capacity of cells to attract photoassimilates (Ho, 1988). This sink strength closely depends on the abundance and activity of plasma membrane transporters. Moreover, a close relationship between the transcript changes and the activity of sucrose transporters has been reported in some species (Chiou and Bush, 1998; Decourteix *et al.*, 2006, 2008). As ethylene induces a stimulation of two sucrose transporters in latex without having any effect on those of bark, this situation might reflect a diversion of carbon assimilates (sucrose) in favour of laticifers, where latex production is strongly stimulated by ethylene. This scenario emphasizes coordinated regulation between different sink organs of the stem.

Stimulation of HbSUT1A *and* HbSUT2A *in latex is ethylene-specific*

The transcript abundance of sucrose transporters in plants has been reported to be regulated by exogenous (Sakr *et al.*, 1993; Matsukura *et al.*, 2000; Meyer *et al.*, 2004; Decourteix *et al.*, 2006) and endogenous (Ehness and Roitsch, 1997; Chiou and Bush, 1998; Ward *et al.*, 1998; Matsukura *et al.*, 2000; Li *et al.*, 2003) stimuli. We have shown here that the ethylene effect was restricted to two sucrose transporters (*HbSUT1A* and *HbSUT2A*) and correlated with ethylene-induced stimulation production. It is noteworthy that ethylene decreased *HbSUT1B* expression (Fig. 4A) but it was significantly increased by most treatments, especially JA and CPA (an auxin-like chemical; Fig. 6B).

With regard to *HbSUT1A* and *HbSUT2A*, none of the hormonal treatments induced a stimulation of their respective transcript during Tap1 (Fig. 6B, C). In addition, as wounding (nail sealing, without latex exudation) did not lead to upregulation of either *HbSUT1A* or *HbSUT2A*, it can be hypothesized that either their response to ethylene is not due to a cross-talk between the ethylene and wounding pathway, or that the concentration of endogenous ethylene, probably induced by wounding in the rubber tree bark, might be too low to induce pronounced regulation of these two genes, as compared with the application of exogenous ethylene. This differential response of sucrose transporters means that laticifers are able to adapt the transcript amount of their sugar transporters to different environmental or developmental conditions.

The present study provides insight into the physiological role that sucrose transporters play in sucrose import to laticifers, in relation to ethylene-induced stimulation of latex production. We have described the potential involvement of two sucrose transporters, *HbSUT1A* and *HbSUT2A*, in this process. Indeed, *HbSUT1A* and *HbSUT2B* were increased by ethylene and this effect was positively correlated with ethylene stimulation of latex production. Moreover, this ethylene effect is specific to laticifers, in which *cis*-polyisoprene synthesis takes place. To elucidate how the expression of these transporters is stimulated by ethylene, their respective promoters will be isolated in future experiments to determine whether they harbour an ethylene-specific *cis*-element. Because latex yield is the most important trait in breeding programmes for *H. brasiliensis*, and rubber synthesis depends on sucrose importation in laticifers, the above two sucrose transporters may constitute a useful molecular tool to underpin latex yield in this plant. Thus, single nucleotide polymorphisms (SNPs) and microsatellites will be looked for in these SUT cDNA clones to map them in the *Hevea* gene maps (Low *et al.*, 1996; Guen *et al.*, 2004) and verify if they may co-localize with latex yield and/or growth quantitative trait

loci. This would be of major help in rubber breeding programmes.

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APPENDIX

Accession numbers of sucrose transporter sequences presented in Fig. 2: AbSUT1 (*Asarina barclaiana*; AAF04294), AgSUT3 (*Apium graveolens*; ABB89051), AmSUT1 (*Alonsoa meridionalis*; AAF04295), AtSUC1 (*Arabidopsis thaliana*; At1g71880), AtSUC2 (*Arabidopsis thaliana*; At1g22710), AtSUC3 (*Arabidopsis thaliana*; At2g02860), AtSUC4 (*Arabidopsis thaliana*; At1g09960), AtSUC5 (*Arabidopsis thaliana*; At1g71890), AtSUC6 (*Arabidopsis thaliana*; At5g43610), AtSUC7 (*Arabidopsis thaliana*; At1g66570), AtSUC8 (*Arabidopsis thaliana*; At2g14670), AtSUC9 (*Arabidopsis thaliana*; At5g06170), BoSUC1 (*Brassica oleracea*; AAL58071), BoSUC2 (*Brassica oleracea*; AAL58072), BoSUT1 (*Bambusa oldhamii*; AAY43226), CsSUT2 (*Citrus sinensis*; AAM29153), DgSUT4 (*Datisca glomerata*; CAG70682), DcSUT1 (*Daucus carota*; BAA89458), EeSUCx (*Euphorbia esula*; AAF65765), EuSUT2 (*Eucommia ulmoides*; AAX49396), HbSUT1A (*Hevea brasiliensis*; DQ985466), HbSUT1B (*Hevea brasiliensis*; AM492537), HbSUT2A (*Hevea brasiliensis*; ABJ51934), HbSUT2B (*Hevea brasiliensis*; ABJ51932), HbSUT2C (*Hevea brasiliensis*; AM491808), HbSUT4 (*Hevea brasiliensis*; EF067335), HbSUT5 (*Hevea brasiliensis*; ABK60189), HvSUT1 (*Hordeum vulgare*; CAB75882), HvSUT2 (*Hordeum vulgare*; CAB75881), JrSUT1 (*Juglans regia*; AAU11810), LeSUT2 (*Lycopersicum esculentum*; AAG12987), LeSUT4 (*Lycopersicum esculentum*; AAG09270), LjSUT4 (*Lotus japonicus*; CAD61275), MdSUT1 (*Malus domestica*; AAR17700), MeSUT2 (*Manihot esculenta*; ABA08445), MeSUT4 (*Manihot esculenta*; ABA08443), NtSUT1 (*Nicotiana tabacum*; X82276), NtSUT3 (*Nicotiana tabacum*; AAD34610), OsSUT1 (*Oryza sativa*; AAF90181), OsSUT2 (*Oryza sativa*; AAN15219), OsSUT3 (*Oryza sativa*; BAB68368), OsSUT4 (*Oryza sativa*; BAC67164), OsSUC4 (*Oryza sativa*; Q2QLI1), OsSUT5 (*Oryza sativa*; BAC67165), PmSUC1 (*Plantago major*; CAI59556), PmSUC2 (*Plantago major*; X75764), PmSUC3 (*Plantago major*; CAD58887), PtSUT1-1 (*Populus tremula* × *Populus tremuloides*; CAJ33718), PsSUT1 (*Pisum sativum*; AAD41024), RcSCR1 (*Ricinus communis*; CAA83436), SdSUT2 (*Solanum demissum*; AAT40489), ShSUT1 (*Saccharum hybridum*; AAV41028), SoSUT1 (*Spinacea oleracea*; Q03411), StSUT1 (*Solanum tuberosum*; CAA48915), StSUT4 (*Solanum tuberosum*; AAG25923), TaSUT1A (*Triticum aestivum*; AAM13408), TaSUT1B (*Triticum aestivum*; AAM13409), TaSUT1D (*Triticum aestivum*; AAM13410), VfSUCx (*Vicia faba*; CAB07811), VvSUCy (*Vitis vinifera*; AAL32020), VvSUC11 (*Vitis vinifera*; AAF08329), VvSUC12 (*Vitis vinifera*; AAF08330), VvSUC27 (*Vitis vinifera*; AAF08331), ZmSUT1 (*Zea mays*; BAA83501), ZmSUT2 (*Zea mays*; AAS91375), ZmSUT4 (*Zea mays*; AAT51689).