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Note

Transcriptional responses of laticifer-specific genes to phytohormones in a suspension-cultured cell line derived from petioles of *Hevea brasiliensis*

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Abstract *Hevea brasiliensis* is the key source of latex for commercial natural rubber production. Genetic improvement of *H. brasiliensis* is required to enhance natural rubber production, although the biosynthetic mechanism has not been fully elucidated. In this study, we established a cell suspension culture from petiole explants of *H. brasiliensis* clone RRIM 600 for basic research on the biosynthesis of natural rubber. Calli were induced from petiole explants on callus induction medium supplemented with 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.05 mg l^{-1} 6-benzylaminopurine (BA). Then, the calli were suspension cultured in MS basal medium supplemented with 2 mg l^{-1} 2,4-D and 2 mg l^{-1} BA. Although transcripts of some laticifer-specific genes were detected in the cultured cells, their levels were much lower than those in the other laticifer-containing tissues. Additionally, there was no detectable activity of rubber transferase in this cell line. The laticifer-specific genes in the cell line showed transcriptional responses to phytohormone treatments. Among them, up-regulations of *Rubber Elongation Factor* by the ethophon treatment were concordant with those in laticifers, suggesting that these cells retained at least some of the cytochemical properties of laticifers. The cell line established in this study could be useful for biochemical and molecular studies on natural rubber biosynthesis.

Key words: *Hevea brasiliensis*, latex, natural rubber, suspension-cultured cell.

The Para rubber tree (*Hevea brasiliensis*) is the key commercial source of natural rubber, one of the most important raw materials in the world. This species produces high yields of natural rubber with excellent physical properties (Cornish 2001). In *H. brasiliensis*, natural rubber is obtained from latex, the milky cytoplasm of specialized vascular cells known as laticifers (Hagel et al. 2008). To meet the increased demands for natural rubber, new technologies to improve natural rubber production are required. In addition, it is required to establish a natural rubber over-producing system that is not affected by environmental conditions, because *H. brasiliensis* can only be cultivated in tropical or subtropical regions.

Plant cell cultures are an attractive alternative to whole plants as sources of high-value secondary metabolites, used as organic materials, pharmaceuticals and food additives (Ramachandra Rao and Ravishankar 2002). The advantage of plant cell cultures is that the cells can be grown under strictly controlled growth conditions. Therefore, cell cultures are not affected by geographical and seasonal variations or by environmental factors. Furthermore, plant cell suspension cultures are also used as a convenient tool for research on a wide range of phenomena, including early responses to various environmental stimuli and regulation of secondary metabolism (Moscatiello et al. 2013). Accordingly, the establishment of *H. brasiliensis* cell cultures will be useful not only for industrial applications, but also for research on molecular mechanisms for natural rubber biosynthesis. To date, several studies have focused on establishing calli from *H. brasiliensis*. Arokiaraj et al. (1994; 1996) established anther-derived calli, and genetically transformed them via particle gun and *Agrobacterium tumefaciens* infection methods. *Agrobacterium*-mediated gene transfer has also been developed with friable calli from immature fruit, and several factors affecting the efficiency of transformation

Abbreviations: BA, 6-benzylaminopurine; CIM, callus induction medium; 2,4-D, 2,4-dichlorophenoxyacetic acid; HRT, Hevea rubber transferase; IPP, isopentenyl diphosphate; JA, jasmonic acid; MeJA, methyl jasmonate; REF, rubber elongation factor; SRPP, small rubber particle protein.

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of calli were identified (Blanc et al. 2006; Montoro et al. 2003; Montoro and Teinseree 2000; Rattana et al. 2001). Furthermore, efficient calli regeneration procedures have been established for producing transgenic *H. brasiliensis* plantlets (Blanc et al. 2006; Lardet et al. 2007). However, there are no reports on the generation of stable suspension-cultured cell lines from vascular tissues that contain many laticiferous cells. Inducing calli from vascular tissues, for example, stems or petioles, is a promising approach to establish cultures of cells with the properties of laticifer cells. In this study, we established a suspension cultured cell line from petioles of *H. brasiliensis*, and analyzed the transcript levels of some laticifer-specific genes to evaluate the properties of the cells.

To induce somatic callus formation from petiole explants, petioles (5- to 10-cm long) of young leaves were collected from the *H. brasiliensis* clone RRIM 600. The petioles were sterilized in 70% (v/v) ethanol for 30 s, then in 1% (v/v) sodium hypochlorite solution for 5 min, and then rinsed three times with sterile distilled water. The explants were cut into 10- to 15-mm transverse sections and sliced longitudinally to expose the vascular cambium. The petiole explants were then placed with the inner side in contact with callus induction medium (CIM) containing various concentrations and combinations of phytohormones, to determine the optimal callus induction conditions. The CIM consisted of 0.5 g l^{-1} MES (pH 5.7), 3.3 g l^{-1} Gamborg's B5 medium salt mixture (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Gamborg's vitamin mixture (Sigma-Aldrich, St. Louis, MO, USA), 3% (w/v) sucrose, 2.5 g l^{-1} gellan gum, and various concentrations of the auxins 2,4-dichlorophenoxyacetic acid (2,4-D, Sigma-Aldrich) and 1-naphthalenacetic acid (Sigma-Aldrich) and the cytokinin 6-benzylaminopurine (BA, Sigma-Aldrich). After incubation for 2 weeks under continuous darkness or illumination of ≈60 μmol s^{-1} m^{-2} at 28°C, callus initiation was observed on the cut edges of explants. Eventually, the highest rates of calli induction and growth were recorded on CIM containing 2 mg l^{-1} 2,4-D and 0.05 mg l^{-1} BA under continuous darkness (Figure 1a). These calli were maintained under the same conditions as those used for callus induction, and were subcultured onto fresh CIM each month. To establish suspension-cultured cell lines, 6-week-old calli were divided into small pieces and transferred into 20 ml MS basal liquid medium [0.5 g l^{-1} MES (pH 5.7), 3.3 g l^{-1} Murashige and Skoog salt mixture (Wako Pure Chemical Industries, Ltd.), Gamborg's vitamin mixture, 3% (w/v) sucrose, and different concentrations of phytohormones, 2,4-D and BA]. The cultures were incubated at 28°C in the dark with shaking at 130 rpm. The optimal phytohormone conditions were determined to be 2 mg l^{-1} 2,4-D and 2 mg l^{-1} BA, based on observations that the cells

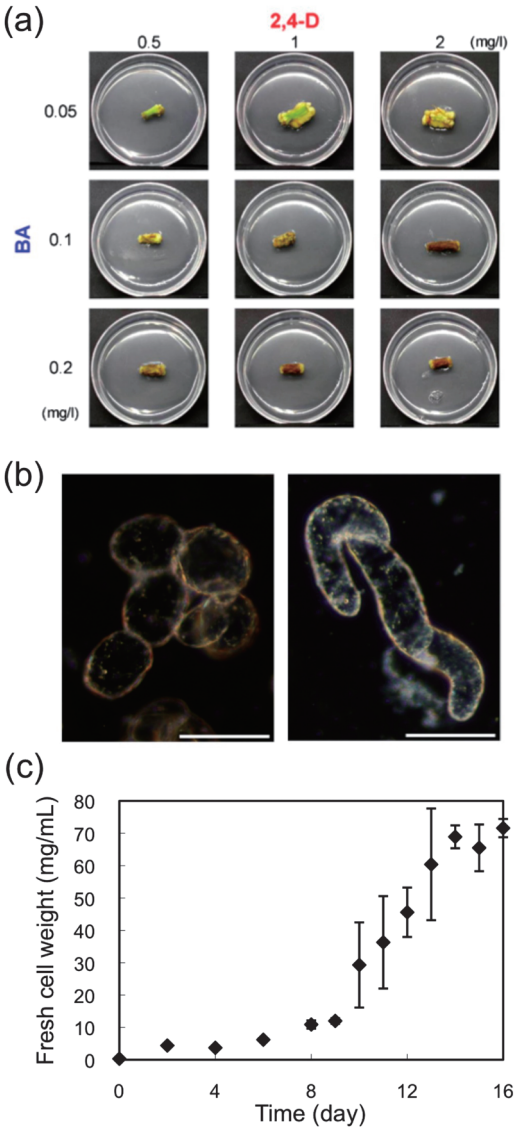

Figure 1. Establishment of the suspension-cultured cell line from petioles of *H. brasiliensis*. (a) A typical results of the somatic callus induction from petiole explants on CIM containing various concentration of 2,4-D and BA for 2 weeks under continuous darkness. (b) The cells in the suspension culture grown in the MS basal liquid medium containing 2 mg l^{-1} 2,4-D and 2 mg l^{-1} BA for 2 weeks after subculturing. Bars, 10 μm. (c) Growth of the suspension-cultured cell line determined by fresh weight of the cells after removing the liquid medium by centrifugation.

proliferated faster, and formed smaller clumps, under these conditions. The resulting suspension-cultured cells were subcultured at 2-week intervals by transferring 1 ml culture solution into 20 ml fresh media. After subculturing several times, a stably growing cultured cell line was established. This culture consisted of spherical cells (7–10 μm) that formed small clumps (Figure 1b, left panel) and smaller populations of elongated cells (10–20 μm) (Figure 1b, right panel). In the typical growth curve of suspension cells, the cell proliferation rate was initially slow during the first 9–10 days. Then, cell growth increased markedly over the following 10 days, as

indicated by the accumulation of fresh weight. Typically, the maximum fresh weight was recorded at day 20. At this point, the fresh weight was more than 30-times greater than the initial fresh weight (Figure 1c). The cells remained in the stationary phase for a further 20 days and then the fresh cell weight declined (data not shown).

To analyze rubber transferase activity in this cell line, crude proteins were prepared as follows: cells collected from the suspension culture were ground in liquid nitrogen with a mortar and pestle, and homogenized in protein extraction buffer [50 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 5% (w/v) glycerol, 3 mM dithiothreitol, a suitable amount of Complete Protease Inhibitor Cocktail without EDTA according to the provider's instruction (Roche, Basel, Switzerland), and 5% (w/v) Polyclar VT (Sigma-Aldrich)]. Cell lysates were centrifuged at 300×*g* for 5 min at 4°C to remove intact cells and insoluble Polyclar VT, and then the supernatants were further centrifuged at 20,000×*g* for 30 min at 4°C to separate the insoluble and soluble crude fractions. The rubber transferase assay was conducted using an aliquot of the crude soluble or insoluble fraction and the substrates, farnesyl diphosphate and [1-^{14}C]isopentenyl diphosphate (IPP) (58 Ci mol^{-1}, GE Healthcare, Little Chalfont, UK), as reported previously (Takahashi et al. 2012). The reaction mixture was incubated at 30°C for 16 h, and then the reaction products were extracted by stepwise extraction with diethylether, 1-butanol, and toluene/hexane (1 : 1) (T/H). The molecular size distribution of the radioactive products in the T/H extracts was analyzed by gel permeation chromatography as reported previously (Takahashi et al. 2012). The incorporation of [1-^{14}C]IPP into the T/H extracts, in which high-molecular weight polyisoprenoids were included, was quite low, and no radioactive products corresponding to natural rubber were detected in the assays with either insoluble or soluble crude fractions from the cells (data not shown).

Although the precise biosynthetic pathway of natural rubber has not been fully elucidated, many genes have been suggested to participate in natural rubber biosynthesis. Most of these genes are expressed specifically in laticifers (Aoki et al. 2014). No detectable rubber transferase activity in the cultured cell line derived from laticifer-containing tissues suggested that there was very low transcription of natural rubber biosynthesis-related genes, and that the properties of laticiferous cells had been lost during dedifferentiation. To test this hypothesis, we compared the transcript levels of four natural rubber biosynthesis-related genes, expressed specifically in laticifers (Aoki et al. 2014), between the suspension-cultured cell line and various tissues of *H. brasiliensis*; leaves, stems, and latex, the cytoplasm of laticifers obtained by tapping of the bark of the tree trunks. The targets selected for these analyses were *Rubber Elongation Factor* (*REF*) (Dennis and Light 1989), *Small Rubber Particle Protein* (*SRPP*) (Oh et al. 1999), *Hevea Rubber Transferase 1* (*HRT1*) and *Hevea Rubber Transferase 2* (*HRT2*) (Asawatreratanakul et al. 2003). GenBank accession numbers for each target gene are as follows: *REF* (X56535), *SRPP* (AF051317), and *HRT2* (AB064661). REF and SRPP are well-studied latex-abundant proteins localized on the surface of rubber particles, and are thought to be related to natural rubber biosynthesis (Dennis and Light 1989; Shamsul Bahri and Hamzah 1996; Yeang et al. 1996). HRT1 and HRT2 are *cis*-prenyltransferases considered to function as key enzymes in natural rubber biosynthesis (Asawatreratanakul et al. 2003; Takahashi et al. 2012). Total RNA was extracted from cultured cells grown for 10 days after subculturing based on the phenol/SDS method (Palmiter 1974). Contaminating DNA was removed from the total RNA sample by treatment with DNase I (RNase-free; Takara Bio, Ohtsu, Japan) at 37°C for 30 min. Total RNAs from other tissues of *H. brasiliensis* clone RRIM600 were prepared as reported elsewhere (Aoki et al. 2014). Reverse-transcription quantitative PCR analyses were carried out using a One-step SYBR RT-PCR kit (Takara Bio), according to the manufacturer's instructions, with the following gene-specific primers: REF-S (5′-TTG TTT CAG CAA TTC GAG CT-3′) and REF-A (5′-TCA ACA CTC AGG ATG AGA AC-3′) for *REF*; SRPP-S (5′-CGG TAG CTC AAG ATG CTC CA-3′) and SRPP-A (5′-GGC ACA ACT ACA TTT GCC AC-3′) for *SRPP*; HRT1-S (5′-GTA TTT TGT CTT TTT TGA ATT TTG GC-3′) and HRT1-A (5′-CGG TAC TTG AGC TCA TCT ACA G-3′) for *HRT1*; HRT2-S (5′-CCT CTA AGG ATA AAT TGA AAT CCG-3′) and HRT2-A (5′-AGT TGC TCA GAC GGG TCT C-3′) for *HRT2*; and 18SrRNA-S (5′-CAA AGC AAG CCT ACG CTC TG-3′) and 18SrRNA-A (5′-TGC TTT CGC AGT TGT TCG TC-3′) for *18S rRNA*, which served as the control gene. Among the laticifer-specific genes, transcripts for *HRT1* could not be detected in the cultured cells (data not shown), consistent with the fact that *HRT1* transcripts are detectable only in latex (Aoki et al. 2014). Although transcripts of the other three genes were detected in the cultured cells, their levels were much lower in the suspension cells than in the other tested plant tissues (Figure 2). Because leaves and stems of *H. brasiliensis* contained latex, the transcripts detected in each tissue include those in the laticifers of each tissue. Relatively higher transcript levels of each laticifer-specific gene in the stems might reflect the higher accumulation levels of latex in the stems than in leaves. It was inferred from the low transcript levels of the laticifer-specific genes that the suspension cell population contained very few laticifer-derived cells, or that the cells had lost the properties of laticifers. Therefore, their ability to biosynthesize natural

rubber was very poor.

Many studies have shown that certain phytohormones are involved in laticifer development or affect latex yields in *H. brasiliensis*, making them important factors in natural rubber production. Previous studies showed that ethylene stimulates latex production (Coupé and Chrestin 1989) and jasmonic acid (JA) induces differentiation of lactiferous cells (Hao and Wu 2000). A recent study showed that treatment of bark with ethephon, an ethylene releaser, induced the expression of *REF*, and the transcript level of *REF* was correlated with latex yield (Priya et al. 2007). The ethylene and JA signaling pathways have been intensively studied to explore the molecular mechanisms regulating laticifer differentiation and natural rubber metabolism. Therefore, the suspension-cultured cell line established in this study can serve as a model for such studies, if the cells retain their phytohormone signaling pathways. To investigate the responsiveness of the laticifer-specific genes to phytohormones in the cultured cell line, we analyzed the transcript profiles of *REF* and *HRT2* after treatment with ethylene, JA, or methyl jasmonate (MeJA). For these analyses, suspension cells were subcultured into maintenance media supplemented with 100 μM ethephon or 50 μM JA/MeJA, and then collected at each time point. The ethylene and MeJA treatments stimulated transcription of *REF*, and MeJA had a stronger promoting effect than did ethylene (Figure 3a). The *REF* transcript levels were immediately up-regulated as early as 2 h, followed by a decline to the control level within 5 h after the MeJA treatment, while they slowly increased at least until 10 h and then declined after the ethephon treatment. The ethylene-induced transcription of *REF* is concordant with the results of a previous report, in which the *REF* transcript levels in latex are shown to be increased until 48 after the ethephon treatment on barks by spreading 2.5 or 5% (v/v) ethephon in palm oil over the tapping cut after scraping (Priya et al. 2007). More persistent increase of the *REF* transcripts in latex than those in the cultured cells may be due to sustained releases of ethephon treated on barks. As far as we know, this is the first report showing that MeJA

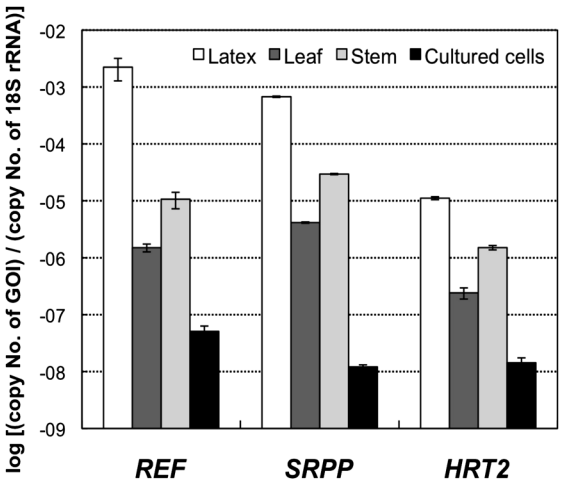

Figure 2. Transcript levels of the laticifer-specific genes in various tissues and the suspension cultured cells. The transcript levels of genes of interest were determined as the copy number in an amount of total RNA extracted from latex, leaves, stems, and cultured cells and then normalized as relative copy numbers by the level of the 18S rRNA in corresponding samples. The results are presented as the means of three-independent determinations ±SDs.

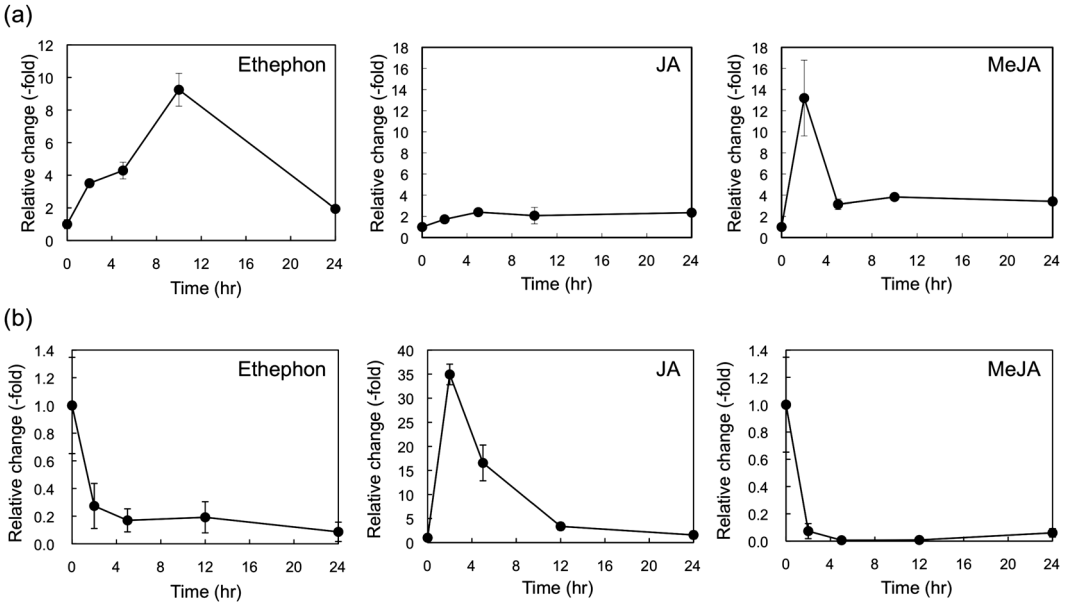

Figure 3. Changes in transcription levels of *REF* (a) and *HRT2* (b) in the suspension-cultured cells grown for 2 weeks after subculturing by the treatments with phytohormones, ethephon, JA and MeJA. The results are expressed as relative changes, with relative copy numbers (GOI/18S rRNA) at zero time of treatments taken to be 1.0. The results are presented as the average of three independent determinations with ±SDs.

also transcriptionally activates *REF*. In a search for possible *cis*-acting elements in a 5′-upstream sequence of *REF* (accession number: AB861873) against a database of Plant Cis-acting Regulatory DNA Elements (PLACE) (Higo et al. 1999), we could find out three motifs corresponding to "AWTTCAAA" (PLACE ID: S000037), an ethylene responsive element identified in 5′ flanking sequences of *E4* in tomato (Montgomery et al. 1993) and *GST1* encoding glutathione-S-transferase in carnation (Itzhaki et al. 1994). We also found a motif corresponding to "AACGTG" ((PLACE ID: S000458), which was identified as a T/G-box motif recognized by the MeJA-inducible bHLH-Leu zipper proteins, JAMYC2 and JAMYC10 (Boter et al. 2004). These motifs in the 5′-upstream sequence of *REF* may be attributed to the up-regulation of *REF* transcription in response to the phytohormone treatments.

Interestingly, the transcript levels of *HRT2* were inversely related to those of *REF*; that is, there was a fast and significant down-regulation of *HRT2* in response to ethephon and MeJA treatments and up-regulation in response to JA treatment (Figure 3b). Opposite effects of JA and MeJA on the transcript levels of *HRT2* were inconsistent with the well-studied positive correlation between the effects of JA and MeJA on gene expression (Sasaki-Sekimoto et al. 2005; Taki et al. 2005). In JA signaling, a JA-Ile conjugate ((+)-7-*iso*-jasmonoyl-L-isoleucine) plays a pivotal role as the most bioactive ligand of JA receptor (Fonseca et al. 2009). Based on the fact that JAR1, an ATP-dependent adenylate-forming enzyme that catalyzes conjugation of JA to Ile, accepts JA but not MeJA as a substrate (Staswick et al. 2002), MeJA-induced responses appear to involve initial hydrolysis of MeJA to JA, followed by formation of JA-Ile by JAR1 (Wasternack and Hause 2013). The difference between the effects of JA and MeJA on the *HRT2* transcripts suggested relatively slow conversions between MeJA and JA and involvement of a JA-Ile-independent MeJA signaling mechanism in the cultured cells.

In higher plants, such as *Arabidopsis thaliana* (Kera et al. 2012; Surmacz and Swiezewska 2011) and *Solanum lycopercicum* (Akhtar et al. 2013), *cis*-prenyltransferases are encoded by a multigene family, and their transcripts are induced in response to various abiotic stimuli. These reports suggest that some *cis*-prenyltransferases and their products functions in different tissues and/or participates in different cellular processes in response to various environmental stimuli. However, transcriptional regulation of *cis*-prenyltransferase via phytohormone signaling is not yet understood well. The significant responses of *HRT2* transcript levels to phytohormones in the cultured cells may reflect, at least in part, strict phytohormonal regulation of natural rubber biosynthesis in laticiferous cells, correlated with a probable role of natural rubber in latex as plant defensive agent. The suspension cell line obtained in this study retained the ability to respond to phytohormonal stimuli, and has the potential to be a model system for studying complex signaling pathways related to natural rubber biosynthesis.

In summary, we successfully established a cell suspension culture from petioles of *H. brasiliesis*. We evaluated the cytochemical characteristics of the cells, including the transcript profiles of laticifer-specific genes. Currently, we are developing an optimized *Agrobacterium*-mediated transformation technique for the suspension-cultured cell line. This will provide not only the means to genetically modify *H. brasiliensis* to enhance natural rubber production, but also a useful molecular biological tool for in-depth studies on natural rubber biosynthesis.

References

Akhtar TA, Matsuba Y, Schauvinhold I, Yu G, Lees HA, Klein SE, Pichersky E (2013) The tomato *cis*-prenyltransferase gene family. *Plant J* 73: 640–652

Aoki Y, Takahashi S, Takayama D, Ogata Y, Sakurai N, Suzuki H, Asawatreratanakul K, Wititsuwannakul D, Wititsuwannakul R, Shibata D, et al. (2014) Identification of laticifer-specific genes and their promoter regions from a natural rubber producing plant *Hevea brasiliensis*. *Plant Sci* 225: 1–8

Arokiaraj P, Jones H, Cheong KF, Coomber S, Charlwood BV (1994) Gene insertion into *Hevea brasiliensis*. *Plant Cell Rep* 13: 425–431

Arokiaraj P, Jones H, Jaafar H, Coomber S, Charlwood BV (1996) *Agrobacterium*-mediated transformation of Hevea anther calli and their regeneration into plantlets. *Journal of Natural Rubber Research* 11: 77–87

Asawatreratanakul K, Zhang YW, Wititsuwannakul D, Wititsuwannakul R, Takahashi S, Rattanapittayaporn A, Koyama T (2003) Molecular cloning, expression and characterization of cDNA encoding *cis*-prenyltransferases from *Hevea brasiliensis*. A key factor participating in natural rubber biosynthesis. *Eur J Biochem* 270: 4671–4680

Blanc G, Baptiste C, Oliver G, Martin F, Montoro P (2006) Efficient *Agrobacterium tumefaciens*-mediated transformation of embryogenic calli and regeneration of *Hevea brasiliensis* Müll Arg. plants. *Plant Cell Rep* 24: 724–733

Boter M, Ruíz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. *Genes Dev* 18: 1577–1591

Cornish K (2001) Similarities and differences in rubber biochemistry among plant species. *Phytochemistry* 57: 1123–1134

Coupé M, Chrestin H (1989) Physico-chemical and biochemical mechanisms of hormonal (ethylene) stimulation. In: Auzac Jd, Jacob JL, Chrestin H (eds) *Physiology of Rubber Tree Latex: The Laticiferous Cell and Latex: A Model of Cytoplasm*. Boca Raton, CRC Press, pp 295–319

Dennis MS, Light DR (1989) Rubber elongation factor from *Hevea brasiliensis*. Identification, characterization, and role in rubber biosynthesis. *J Biol Chem* 264: 18608–18617

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

Hagel J, Yeung E, Facchini P (2008) Got milk? The secret life of

laticifers. *Trends Plant Sci* 13: 16–22

Hao B, Wu J (2000) Laticifer differentiation in *Hevea brasiliensis*: induction by exogenous jasmonic acid and linolenic acid. *Ann Bot (Lond)* 85: 37–43

Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res* 27: 297–300

Itzhaki H, Maxson JM, Woodson WR (1994) An ethylene-responsive enhancer element is involved in the senescence-related expression of the carnation glutathione-*S*-transferase (*GST1*) gene. *Proc Natl Acad Sci USA* 91: 8925–8929

Kera K, Takahashi S, Sutoh T, Koyama T, Nakayama T (2012) Identification and characterization of a cis,*trans*-mixed heptaprenyl diphosphate synthase from *Arabidopsis thaliana*. *FEBS J* 279: 3813–3827

Lardet L, Martin F, Dessailly F, Carron M-P, Montoro P (2007) Effect of exogenous calcium on post-thaw growth recovery and subsequent plant regeneration of cryopreserved embryogenic calli of *Hevea brasiliensis* (Müll. Arg.). *Plant Cell Rep* 26: 559–569

Montgomery J, Goldman S, Deikman J, Margossian L, Fischer RL (1993) Identification of an ethylene-responsive region in the promoter of a fruit ripening gene. *Proc Natl Acad Sci USA* 90: 5939–5943

Montoro P, Rattana W, Pujade-Renaud V, Michaux-Ferrière N, Monkolsook Y, Kanthapura R, Adunsadthapong S (2003) Production of *Hevea brasiliensis* transgenic embryogenic callus lines by *Agrobacterium tumefaciens*: roles of calcium. *Plant Cell Rep* 21: 1095–1102

Montoro P, Teinseree N, Rattana W, Kongsawadworakul P, Michaux-Ferriere N (2000) Effect of exogenous calcium on *Agrobacterium tumefaciens*-mediated gene transfer in *Hevea brasiliensis* (rubber tree) friable calli. *Plant Cell Rep* 19: 851–855

Moscatiello R, Baldan B, Navazio L (2013) Plant Cell Suspension Cultures. In: Maathuis FJM (ed) *Plant Mineral Nutrients*. Humana Press, pp 77–93

Oh SK, Kang H, Shin DH, Yang J, Chow K-S, Yeang HY, Wagner B, Breiteneder H, Han K-H (1999) Isolation, characterization, and functional analysis of a novel cDNA clone encoding a small rubber particle protein from *Hevea brasiliensis*. *J Biol Chem* 274: 17132–17138

Palmiter RD (1974) Magnesium precipitation of ribonucleoprotein complexes. Expedient techniques for the isolation of undergraded polysomes and messenger ribonucleic acid. *Biochemistry* 13: 3606–3615

Priya P, Venkatachalam P, Thulaseedharan A (2007) Differential expression pattern of rubber elongation factor (REF) mRNA transcripts from high and low yielding clones of rubber tree (*Hevea brasiliensis* Muell. Arg.). *Plant Cell Rep* 26: 1833–1838

Ramachandra Rao S, Ravishankar GA (2002) Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol Adv* 20: 101–153

Rattana W, Teinseree N, Tadakittisarn S, Pujade-Renaud V, Mongkolsook Y, Montoro P (2001) Characterization of factors involved in the tissue growth recovery and the sustainability of the GUS activity in *Hevea brasiliensis* friable calli transformed by *Agrobacterium tumefaciens*. *Thai Journal of Agricultural Science* 34: 195–204

Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Suzuki H, Hirai MY, Noji M, Saito K, et al. (2005) Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. *Plant J* 44: 653–668

Shamsul Bahri A, Hamzah S (1996) Immunocytochemical localisation of rubber membrane protein in *Hevea* latex. *J Nat Rubb Res* 11: 88–95

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

Surmacz L, Swiezewska E (2011) Polyisoprenoids—Secondary metabolites or physiologically important superlipids? *Biochem Biophys Res Commun* 407: 627–632

Takahashi S, Lee H-J, Yamashita S, Koyama T (2012) Characterization of *cis*-prenyltransferases from the rubber producing plant *Hevea brasiliensis* heterologously expressed in yeast and plant cells. *Plant Biotechnol* 29: 411–417

Taki N, Sasaki-Sekimoto Y, Obayashi T, Kikuta A, Kobayashi K, Ainai T, Yagi K, Sakurai N, Suzuki H, Masuda T, et al. (2005) 12-Oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis. *Plant Physiol* 139: 1268–1283

Wasternack C, Hause B (2013) 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 (Lond)* 111: 1021–1058

Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chewa NP, Hamidb S, Hamilton RG, Cardosa MJ (1996) The 14.6 kd rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 98: 628–639