The *Hevea brasiliensis* AP2/ERF superfamily: from ethylene signalling to latex harvesting and physiological disease response

Superfamili AP2/ERF pada Hevea brasiliensis*: dari sinyalisasi etilen hingga penyadapan lateks dan respons terhadap penyakit fisiologis*

Riza Arief PUTRANTO[1)*] & Pascal MONTORO[2)]

[1)] Indonesian Research Institute for Biotechnology and Bioindustry, Jl. Taman Kencana No. 1, Bogor 16128, Indonesia
[2)] Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Avenue d'Agropolis, Montpellier 34398, France

Abstrak

   Etilen merupakan hormon yang dikenal karena perannya dalam proses penyadapan lateks di tanaman karet (Hevea brasiliensis). Hormon tersebut memfasilitasi aliran lateks melalui aktivasi metabolisme endogen dalam sel lateks beranastomosis yang disebut latisifer. Etilen memiliki peran ganda yaitu menguntungkan karena memacu produksi lateks dan tidak menguntungkan pada tingkat tertentu sehingga menyebabkan munculnya penyakit fisiologis yang disebut sebagai kering alur sadap (KAS). Beberapa penelitian mendalam telah dilakukan untuk mengungkap aktor molekuler dalam biosintesis dan sinyalisasi etilen pada Hevea brasiliensis. Salah satu superfamili yang penting dan terlibat sebagai faktor transkripsi terakhir yang diketahui pada sinyalisasi etilen adalah APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF). Saat ini, 114 sekuen unik yang menyandi superfamili AP2/ERF pada Hevea telah diidentifikasi dan dikarakterisasi. Karakterisasi spesifik pada saat kondisi stres penyadapan dan kejadian KAS telah berhasil mengidentifikasi 36 marka ekspresi gen (GEMs). Delapan belas GEMs diprediksi memiliki ortologi dengan 19 gen AP2/ERF pada Arabidopsis. Meskipun karakterisasi ini difokuskan pada regulasi tingkat transkripsi, regulasi pasca-transkripsi dan pasca-translasi potensial dari HbAP2/ERFs juga diprediksi. Dikarenakan akumulasi transkrip yang tinggi pada latisifer dan dalam respon terhadap multi-stres abiotik, tiga grup HbERF (HbERF-VII, HbERF-VIII dan HbERF-IX) diduga memiliki peran penting dalam toleransi Hevea selama produksi lateks. Untuk beberapa gen kunci, analisis fungsional lanjut perlu dilakukan untuk sepenuhnya memahami regulasi dari HbAP2/ERFs. Akhirnya, penanda molekuler dalam kaitannya untuk pemuliaan tanaman karet kemungkinan dapat dikembangkan dari superfamili ini.

[Kata kunci : etilen, pohon karet, faktor transkripsi, analisis ekspresi, kering alur sadap].

Abstract

   Ethylene is a hormone known for its involvement in the process of latex harvesting in *Hevea brasiliensis*. It facilitates latex flow by activation of endogenous metabolism in the anastomosed latex cells called laticifers. In regard to its ambivalent role, ethylene is both favourable to the latex production and unfavourable to a certain level, to the apparition of a physiological disease termed as tapping panel dryness (TPD). Comprehensive researches have been carried out to reveal the molecular actors in ethylene biosynthesis and signalling pathways in *Hevea brasiliensis*. One of the most important superfamily implicated as the last transcription factor known in plant ethylene signalling is the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF). Currently, 114 unique sequences related to the *Hevea AP2/ERF* gene superfamily have been identified and characterized. Specific characterizations under the condition of harvesting stress and the occurrence of TPD have identified 36 gene expression markers (GEMs). Eighteen of these GEMs were predicted as ortholog with 19 *Arabidopsis AP2/ERF* genes. The characterization was mainly focused on transcriptional regulation, whilst potential post-transcriptional and post-translational regulations of *HbAP2/ERF* genes were formerly predicted. Three HbERF groups (HbERF-VII, HbERF-VIII and HbERF-IX) were hypothesized to have an important role in *Hevea* tolerance during latex production as they highly accumulated in laticifers and in response to multiple abiotic stresses. Further functional analysis of several key genes is suggested in order to fully understand the regulation of *HbAP2/ERFs*. Finally, the molecular markers for future *Hevea* breeding could be possibly developed from this superfamily.

[Keywords: ethylene, rubber tree, transcription factor, expression analysis, tapping panel dryness].

*)Penulis korespondensi: rizaputranto@iribb.org

Introduction

Ethylene is a hormone known for its involvement in the process of latex harvesting in *Hevea brasiliensis*. It facilitates latex flow by activation of endogenous metabolism in the anastomosed latex cells called laticifers (Pujade-Renaud *et al.,* 1994). Latex regeneration disrupts the metabolism homeostasis in laticifers and triggers the production of ROS in particular the O_2 form by lutoidic NADPH oxidase. In extreme cases of stress, this leads to an oxidative burst provoking coagulation of the rubber particles (d'Auzac *et al.,* 1993). This condition will cause the onset of a physiological disease known as tapping panel dryness (TPD). Thus, the ethylene is subjected to an ambivalent role, both favourable for the latex production and unfavourable in certain level leading to the apparition of TPD. For several years, comprehensive researches have been carried out to reveal the molecular actors in ethylene biosynthesis and signalling pathways in *Hevea brasiliensis*.

One of the most important superfamily implicated as the last actor known in plant ethylene signalling is the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF). Members of the AP2/ERF superfamily encode transcription factors involved in the regulation of biological processes, including floral and ovum development, responses to phytohormones, and adaptation to biotic and abiotic stress (Mizoi *et al.,* 2012; Licausi *et al.,* 2013a, Li *et al.,* 2012; Xu *et al.,* 2011). These transcription factors were characterized by a conserved DNA-binding AP2 domain of about 60 amino acids (Ohme-Takagi & Shinshi, 1995). The AP2 domain was able to interact specifically with GCC-box (Ohme-Takagi & Shinshi, 1995) or dehydration-responsive element/C-repeat (DRE/CRT) *cis*-acting elements in the promoter region of target genes (Ito *et al.,* 2006; Sakuma *et al.,* 2006). The AP2/ERF superfamily was classified according to the number of AP2 domain. The AP2 family consists of proteins with a double domain of AP2 in repeated tandem (Okamuro *et al.,* 1997). The ERF family encodes transcription factors containing a single AP2 domain. Finally, the Related to AB1/VP1 (RAV) family encodes proteins with a single AP2 and an additional domain of B3-type (Kagaya *et al.,* 1999). There are different classifications of the superfamily. Sakuma *et al.* (2002) have described five subfamilies consisting of the APETALA2 (AP2), Related to AB1/VP1 (RAV), Dehydration Responsive Element Binding Proteins (DREB) and Ethylene-Responsive Element Binding Proteins (EREBP) also called the Ethylene Response Factor (ERF). By contrast, Nakano *et al.* (2006) have categorized these proteins in three families consisting of the AP2, ERF and RAV.

The current *state-of-the-art* of ethylene research in *Hevea brasiliensis* especially in relation to the characterization of AP2/ERF superfamily involved in diverse physiological mechanisms of *Hevea brasiliensis* is reviewed in this paper. The main ideas discussed are consisted of (1) characterization of the ethylene biosynthesis and signalling pathways in rubber tree, (2) identification of AP2/ERF gene expression markers related with potential function in rubber tree, (3) potential post-transcriptional and post-translational regulations of *Hevea AP2/ERF* genes, (4) potential important role of *Hevea AP2/ERF* genes in reversible tapping panel dryness generated by oxidative stress, and (5) future perspective for functional analysis of *AP2/ERF* candidate genes.

Characterization of the Ethylene Biosynthesis and Signalling Pathways in Rubber Tree

The ethylene biosynthesis may be induced by the presence of endogenous or exogenous ethylene in *Hevea brasiliensis*. The aminocyclopropane-1-carboxylic acid (ACC) synthase activity or ACS and the ACC oxydase or ACO are the last two enzymes of the ethylene biosynthetic pathway (Yang & Hoffman, 1984). The genes involved in the ethylene biosynthesis pathway have been identified and partially characterized (Kuswanhadi, 2006). Three *HbACO* genes (*HbACO1, HbACO2,* and *HbACO3*) and three *HbACS* genes (*HbACS1, HbACS2,* and *HbACS3*) have been identified (Kuswanhadi *et al.* 2007; Kuswanhadi *et al.,* 2010). The transcript of *HbACO1* was the most abundant among three *HbACOs* in bark tissue of 3-month-old budded plants. However, it was reduced upon stimulation by ethylene in leaves and bark (Kuswanhadi, 2006). The *HbACO2* and *HbACO3* genes were transiently induced in response to ethylene. The application of an ethylene inhibitor, i.e. 1-MCP, abolished the induction of ethylene in both of the mentioned genes demonstrating the positive feedback regulation. All *HbACOs* genes were expressed at all developmental stages. All these works have suggested that *HbACO1* gene was responsible for the ethylene biosynthesis regulating at the basal level while the *HbACO2* and *HbACO3* genes were regulated in response to external factors (Kuswanhadi *et al.,* 2010, Kuswanhadi *et al.,* 2007).

The molecular actors involved in ethylene perception and signalling were also studied in *Hevea brasiliensis* (Duan *et al.,* 2010). Two genes of ethylene perception, *Hevea brasiliensis* Ethylene Receptor 2 (*HbETR2*) and *Hevea brasiliensis* Ethylene-insensitive 2 (*HbEIN2*), and a gene of ethylene signalling (*HbEIN3*) were differentially regulated by ethylene treatment. The transcript of *HbETR2* gene was rapidly

accumulated upon stimulation of ethylene while the transcripts of *HbEIN2* and *HbEIN3* genes were significantly reduced. This early induction *HbETR2* was suppressed by an ethylene inhibitor, 1-MCP. In this work, Duan *et al.* (2010) have shown the effect of ethylene and wounding treatments on the downstream-response genes of ethylene signalling pathway. Thus, it opened the perspective of crosstalk signalling between phytohormones in *Hevea brasiliensis*. In search of transcription factors involved in this mechanism, the *Hevea* AP2/ERF superfamily has been the focus subject of study. Based on known previous knowledge in model plants such as *Arabidopsis thaliana* and *Oryza sativa*, these transcription factors were involved in the hormonal response of various biotic and abiotic aspects.

Duan *et al.* (2013) have classified 142 members of *Hevea* AP2/ERF superfamily based on the AP2 domain sequence from full length transcripts of RNA sequencing from rubber clone PB 260. The authors have classified HbERF family into 10 functional groups. The transcriptomic database was generated from somatic embryo tissue, leaves, bark, latex, and roots. The work of Duan and colleagues has further focused on the study of transcription factors involved in the ethylene and jasmonate signalling. Among those Ethylene Response Factors, two *Arabidopsis* genes (*ERF1* and *ORA59*) were found at the intersection of both hormonal signalling (Huang *et al.* 2015, Pre *et al.* 2008). In *Hevea*, the *HbERF-IXc4* and *HbERF-IXc5* genes were the orthologue to *ERF1* while the *HbERF-IXc6* gene was the orthologue of *ORA59* (Duan, 2011). A further study revealed that *HbERF-IXc4* and *HbERF-IXc5* genes behaved like the *Arabidopsis ERF1*. Both of genes were induced by ethylene and methyl jasmonate (MeJA). When all the treatments such as MeJA and ethylene were combined, the transcript abundance has multiplied. A preliminary functional study using trans-activity and subcellular localization experiments was performed for *HbERF IXc4* and *HbERF IXc5* genes. The HbERF-IXc4 and HbERF-IXc5 proteins were able to bind to the promoter of *AtPDF1.2* gene and were localized in the nucleus (Duan, 2011).

The *Hevea brasiliensis* AP2/ERF super-family was further characterized by Piyatrakul and colleagues in 2012. Firstly, the work has focused in the role of AP2/ERF transcription factors in the developmental aspect of rubber tree. The transcript accumulation of AP2/ERF genes was analyzed during the process of somatic embryogenesis from callus lines with different regeneration capacity in various vegetative and reproductive tissues (Piyatrakul *et al.,* 2012). Secondly, Piyatrakul *et al.* (2014) focused the experiment on the search for ERF family having

an important role in the laticifers related to the latex production. The transcriptomic database was improved by supplementing RNA samples from reproductive tissues (immature and mature male flowers, immature and mature female flowers, and zygotic embryos). The conducted work has confirmed 114 contigs encoding *Hevea* AP2/ERF genes. These transcription factors were further confirmed by *in silico* analysis of genomic sequences from rubber clone CATAS-7-33-97. The transcriptomic and genomic database showed a difference in the number of AP2/ERF genes. Potential isoforms of the genes were hypothesized since the clones used for sequence comparison were unlike. In addition, the absence of certain tissues in the transcriptomic database (such as cambium and xylem) could explain the absence of several *Hevea* AP2/ERF genes. However, the work has identified the *Hevea* ERF Group VII as gene expression markers in latex suggesting a potential role in the hypoxia regulation in laticifers. Functional analysis by trans-activity and subcellular localization has confirmed that members of *HbERF-VII* was an activator-type transcription factor (Piyatrakul *et al.,* 2014).

In parallel with the work of Piyatrakul, the characterization of *Hevea* AP2/ERF superfamily was similarly addressed by studying their stress response as well as during the occurrence of tapping panel dryness (Table 1) (Putranto *et al.,* 2015b, Putranto *et al.,* 2015a). A polyclonal test was initiated in 2003 at the Sembawa Research Station, Palembang, under collaboration of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and the Indonesian Rubber Research Institute (IRRI). The first tapping of mature trees was carried out in 2010. Since, latex diagnostic have been regularly measured in order to monitor the physiological state of rubber tree under different harvesting system. Latex and bark samples under the effect of harvesting system, including tapping and ethephon stimulation, and tapping panel dryness were then collected. The *HbERFs* as gene expression markers (GEMs) was identified using high-debit qPCR analysis from the juvenile plant samples which were generated from the somatic embryogenesis prepared in CIRAD (Putranto *et al.,* 2015a).

Identification of AP2/ERF Gene Expression Markers Related with Potential Function in Rubber Tree

The identification of gene expression markers was focused on the strongly expressed genes during latex harvesting and during the occurrence of tapping panel dryness. In regard to known function and well-characterized AP2/ERF superfamily, the *Arabidopsis* database was selected as comparison to *Hevea brasiliensis*.

Duan *et al.* (2013) was also used this database to construct the classification of *Hevea AP2/ERF* transcription factors. The bibliographic and phylogenetic analyses were able to reveal the potential functions of these GEMs (Putranto *et al.* 2015a, Piyatrakul *et al.* 2014). However, these GEMs may or may not have all important function in rubber tree. Putranto *et al.* (2015b) and Putranto *et al.* (2015a) have identified 36 *HbAP2/ERF* genes including 5 genes of AP2 family and 31 genes of ERF family as GEMs (Table 1). Eighteen GEMs were predicted as ortholog with 19 *Arabidopsis AP2/ERF* genes. Nevertheless, several GEMs without known-ortholog could have a specific and important function in *Hevea*. For now, the *HbERF-IXb2* gene is linked to the occurrence of tapping panel dryness. It was the sole gene induced by the disease in laticifers.

In general results, high relative abundance of transcripts were detected in several groups of *HbAP2/ERFs* (Putranto *et al.* 2014; 2015a; 2015b). Although some members of these groups have not proven to be GEMs, high level of expression leads us to suggest their involvement in some regulation at the molecular level. The transcripts of the HbERF-I, HbERF-VII, HbERF-VIII and HbERF-IX were highly accumulated in various responses such as development process, latex harvesting and tapping panel dryness. Among members of ERF group I, WOUND INDUCED DEDIFFERENTIATION 1 (WIND1) and RELATED TO AP2.4 (RAP2.4) proteins have known functions in *A. thaliana*. The *WIND1* gene was associated with the control of wounding-induced cellular dedifferentiation (Iwase *et al.,* 2011a; 2011b). This gene was rapidly induced in the wounded site promoting cell dedifferentiation leading to cell proliferation until a mass of pluripotent cells called callus were formed (Iwase *et al.,* 2013). The *RAP2.4* gene was highly expressed in stems and roots under abiotic stress such as cold, dehydration and osmotic stress (Lin *et al.,* 2008). It has been shown that the abundance of RAP2.4 protein was regulated by a BR-C, ttk and bab/Pox virus Zinc finger (BTP/POZ-MATH) or briefly known as BPM protein family (Weber & Hellmann, 2009). In addition, it is suggested that this gene plays a role in the regulation of water homeostasis (Rae *et al.,* 2011). The *HbERF-Ib7* gene is a GEM in response to latex harvesting stress but it has no known ortholog in *Arabidopsis*.

The ERF-VII was characterized by a protein motif MCGGAI(I/L) at the N-terminus (van Veen *et al.,* 2014; Piyatrakul *et al.* 2014; Nakano *et al.* 2006). This N-end rule regulation was associated with the targeted protein degradation by the proteasome (Licausi *et al.,* 2011). The elimination of the methionine at N-terminal by methionine aminopeptidase (MAP) left the cysteine residue to

be exposed and destabilized. Under the normal condition the oxygen rate called normoxia, this cysteine was oxidized by oxygen or nitric oxide (Gibbs *et al.,* 2014). This oxidized cysteine induced the addition of argynil residue at the N-terminal end which promotes polyubiquitination leading to the degradation of ERF-VII (Licausi *et al.,* 2013b). The ERF-VII in *Arabidopsis* has been reported as a group of hypoxia- or anoxia-responsive genes such as *HYPOXIA RESPONSIVE 1* and *2* (*HRE1* and *HRE2*), *RELATED TO AP2.2 (RAP2.2)* and *RELATED TO AP2.12 (RAP2.12)* (Seok *et al.,* 2014; Licausi *et al.,* 2010b). The ethylene-induced overexpression of *RAP2.2* gene improves the plant survival under hypoxia (Hinz *et al.,* 2010). By contrast, the overexpression of *HRE1* gene increased tolerance to anoxia (Licausi *et al.,* 2010b). Its role as repressor of ethylene during hypoxia has been demonstrated (Yang *et al.,* 2011). The constitutive regulation of *RAP2.12* and *RAP2.3* genes involving a membrane protein named Acyl-CoA-binding protein (ACBP) has been shown (Bailey-Serres *et al.,* 2012; Licausi *et al.,* 2011). In rubber tree, a hypoxic condition has been suggested in the laticifers since the fermentative metabolism of pyruvate is the main degradation pathway of sugar in the latex cytosol (Tupý, 1989). The intermediate product, pyruvate can be used by both aerobic and anaerobic pathways to generate acetyl-CoA (Jacob *et al.,* 1989a). The transcripts of *HbERF-VIIs* were accumulated in laticifers, suggesting a potential role of regulation to hypoxia in *Hevea brasiliensis.* Based on a phylogenetic analysis, the *HbERF-VIIa12* and *HbERF-VIIa20* genes were orthologous with *Arabidopsis* RAP2.12 and RAP2.3, respectively (Piyatrakul *et al.,* 2014).

The ERF-IX group probably contains the transcription factors whose involvement in the response to pathogens has been most extensively studied (Licausi *et al.,* 2013a; Zarei *et al.,* 2011; Moffat *et al.,* 2012). Members of this group were characterized by a protein motif of activator-type EDLL in the C-terminal (Tiwari *et al.,* 2012). The involvement of ERF-IX (*ERF1, ORA59, ERF5,* and *ERF6* genes) in the ethylene and jasmonate signalling was clearly demonstrated in *Arabidopsis* (Cheng *et al.,* 2013a; Moffat *et al.,* 2012; Zarei *et al.,* 2011). The *ERF1* and *ORA59* genes belong to the subgroup of ERF-IXc while *ERF5* and *ERF6* genes are within the subgroup of ERF-IXb. Recently, it was shown that ERF5 also called AAL MODULATOR OF CELL DEATH (MACD1) was upregulated in response to programmed cell death via the ethylene signalling during the attack of pathogens (Mase *et al.,* 2013). In *Hevea*, the *HbERF-IXa3, HbERF-IXb3, HbERF-IXc4, HbERF-IXc5* and *HbERF-IXc6* genes were orthologous to *AtERF1, ERF5/MACD1, ERF1* and *ORA59* genes in *Arabidopsis*, respectively (Piyatrakul *et al.,* 2014).

Table 1. The AP2/ERF gene expression markers (GEMs) during latex harvesting and under the occurrence of tapping panel dryness (TPD) in *Hevea brasiliensis*. The upregulated GEMs were shown in red meanwhile the downregulated GEMs were in blue colour. LH = latex harvesting; T = tapping; Et- = no ethephon treatment; Et+ = ethephon treatment. The GEMs were extracted from Putranto *et al.* (2015a, 2015b).

Tabel 1. Marka ekspresi gen (GEMs) dari AP2/ERF saat penyadapan lateks dan kejadian kering alur sadap (KAS) pada Hevea brasiliensis. *Regulasi positif dari GEMs ditunjukkan dengan warna merah, sedangkan regulasi negatif ditunjukkan dengan warna biru. LH = penyadapan lateks; T = penyadapan; Et- = tanpa perlakuan ethephon; Et+ = perlakuan ethephon. Marka-marka tersebut disarikan dari Putranto et al. (2015a, 2015b).*

Gene / *Gen*	GEMs				Ortholog / *Ortolog*	*Arabidopsis* accession / Aksesi	Known function / *Fungsi yang diketahui*	Reference / *Referensi*
	LH		TPD					
	T	Et+	Et-	Et+				
HbAP2-3	red							
HbAP2-6	red	blue		red	*WRI1*	At3g54320	Seed development	(Focks & Benning, 1998; Cernac *et al.* 2006)
HbAP2-7	red							
HbAP2-10	red	red		red				
HbAP2-13		red						
HbERF-Ib7	red			red				
HbERF-IIb2	red	red	red		*ORA47*	At1g74930	JA biosynthesis	(Pauwels *et al.,* 2008)
HbERF-IIIb1		red						
HbERF-IVa3	red			red				
HbERF-Va2				red	*ESE3*	At5g25190	Salinity stress response	(Zhang *et al.,* 2011)
HbERF-VI1	red	blue						
HbERF-VI3	red							
HbERF-VI5	red			red				
HbERF-VI-L3		red			*CRF10*	At1g68550.1 At1g68550.2	Leaf development	(Rashotte & Goertzen, 2010)
HbERF-VI-L4	red	blue		red				
HbERF-VIIa12	red	blue			*RAP2.12*	At1g53910	Hypoxia response	(Bailey-Serres *et al.,* 2012)
HbERF-VIIa20	red	blue			*EBP/RAP2.3*	At3g16770	Defence against pathogen	(Büttner & Singh, 1997)
HbERF-VIIIa4	red			red	*ERF3*	At1g50640	Programmed cell death response	(Ohta *et al.,* 2001; Ogata *et al.,* 2013)
HbERF-VIIIa8								
HbERF-VIIIa9	red	blue						

Table 1. The AP2/ERF gene expression markers (GEMs) during latex harvesting and under the occurrence of tapping panel dryness (TPD) in *Hevea brasiliensis*. The upregulated GEMs were shown in red meanwhile the downregulated GEMs were in blue colour. LH = latex harvesting; T = tapping; Et- = no ethephon treatment; Et+ = ethephon treatment. The GEMs were extracted from Putranto *et al.* (2015a, 2015b) (cont.).

Tabel 1. Marka ekspresi gen (GEMs) dari AP2/ERF saat penyadapan lateks dan kejadian kering alur sadap (KAS) pada Hevea brasiliensis. *Regulasi positif dari GEMs ditunjukkan dengan warna merah, sedangkan regulasi negatif ditunjukkan dengan warna biru. LH = penyadapan lateks; T = penyadapan; Et- = tanpa perlakuan ethephon; Et+ = perlakuan ethephon. Marka-marka tersebut disarikan dari Putranto et al. (2015a, 2015b) (lanjutan).*

Gene / *Gen*	GEMs				Bibliographical and phylogenetic analyses / *Analisis bibliografi dan filogenetik*			
	LH		TPD		Ortholog / *Ortolog*	*Arabidopsis* accession / Aksesi	Known function / *Fungsi yang diketahui*	Reference / *Referensi*
	T	Et+	Et-	Et+				
HbERF-VIIIa10	red	red		red	*ERF11*	At1g28370	Repressor of ethylene biosynthesis	(Li *et al.,* 2011)
HbERF-VIIIa12	red							
HbERF-VIIIa13	red	red			*ERF12*	At1g28360	Repressor of ABA and ET	(Yang *et al.,* 2005)
HbERF-VIIIa14	red		blue					
HbERF-VIIIb1	red	blue			*DRN-LIKE*	At1g24590	Regeneration of apical meristem	(Kirch 2003; Chandler *et al.,* 2007)
HbERF-IXa3	red	red			*AtERF1*	At4g17500	Division of vascular cells	(Etchells *et al.,* 2012)
HbERF-IXb1	red							
HbERF-IXb2			red	red		At5g07580		(Nakano *et al.,* 2006)
HbERF-IXb3	red				*ERF5/MACD1*	At5g47230	Programmed cell death response	(Moffat *et al.,* 2012; Mase *et al.,* 2013)
HbERF-IXc1	red							
HbERF-IXc4	red	red			*ERF1*	At3g23240	Defense against pathogen	(Lorenzo *et al.,* 2003; Cheng *et al.,* 2013a)
HbERF-IXc5	red							
HbERF-IXc6	red				*ORA59*	At1g06160	Defense against pathogen	(Pre *et al.,* 2008; Zarei *et al.,* 2011)
HbERF-Xa2	red				*RAP2.6-LIKE*	At5g13330	Multiple abiotic stress response	(Krishnaswamy *et al.,* 2011)
HbERF-Xa8	red	red		red	*ABR1*	At5g64750	Repressor of ABA response	(Pandey *et al.,* 2005)
HbERF-Xb1	red	red		red	*RRTF1*	At4g34410	Redox homeostasis	(Khandelwal *et al.,* 2008)

Taken together, the result of differential gene expression analysis by Duan *et al.* (2013) and Putranto *et al.* (2015a) has brought into conclusion that *HbERF-IXc4* and *HbERF-IXc5* could have a major role in the regulation of latex production as they were highly expressed in bark during latex harvesting.

The ERF group VIII contains an ERF-associated amphiphilic repression (EAR)-motif known as the transcriptional repressors (Ohta *et al.,* 2001). These ERFs of repressor-type were demonstrated to be able to induce a programmed cell death (Ogata *et al.,* 2011; 2013). The multifunctional co-repressors, TOPLESS (TPL) and TPL-related (TPR) interact directly with the EAR motif of ERF group VIII. The interaction of TPL/TPR and other transcription factors was co-opted several times to modulate the expression of genes in various biological processes, including hormonal signalling and stress responses (Causier *et al.,* 2012). The *Arabidopsis ERF3, ERF4, ERF7, ERF10, ERF11, ERF12* and *ESR1/DRN* genes belong to the ERF-VIII. However, these genes showed differential expression and distinct roles in plant growth and development as well as in response to environmental stress. For instance, the *ERF4* and *ERF7* genes play an important role in the response to ABA (Yang *et al.,* 2005; Song *et al.,* 2005). The overexpression of ERF7 increased the sensitivity to ABA in guard cells (Song *et al.,* 2005). The suppression of ERF-VIII also requires interactions with other transcription factors. The interaction of *ERF11* gene with the bZIP-type transcription factor, HY5 has modulated the ethylene biosynthesis *via* ABA signalling (Li *et al.,* 2011). The *ESR1/DRN* genes were involved in the development of petiole, the structuration of embryo and the development of cotyledons (Chandler & Werr, 2011; Cole *et al.* 2013). The high abundance of HbERF-VIII transcripts in bark and latex under harvesting stress suggest a post-transcriptional regulation of other *HbERFs* by their role as repressor (Putranto *et al.,* 2015a).

Two GEMs such as *HbERF-IIIB1* and *HbERF-IVa3* did not have orthologs in *Arabidopsis*. However, these genes could have an important role in osmotic stress response especially linked to the production of latex. In the classification of Sakuma (2002), the *HbERF-III* genes were included in the CBF/DREB1 subfamily. The *CBF/DREB1* genes have been extensively characterized in plants linked to the cold stress (Kurepin *et al.,* 2013; Rehman & Mahmood, 2015). In *Hevea*, a small number of Cold Binding Factor/Dehydration Responsive Element Binding 1 (CBF/DREB1) genes were identified (Duan *et al.,* 2013). This could be explained by a minor adaptation of *Hevea* tree to the cold zone. Additionally, *HbERF-IV* genes correspond to the DREB2 subfamily involved in the response to dehydration. The expression of *HbERF-IVa3* gene was induced by tapping, ethephon stimulation and tapping panel dryness (Putranto *et al.* 2015b; Putranto *et al.,* 2015a). The expression of *HbERF-IVa3* gene was consistent with the hypothesis since laticifers are subject to a recurrent osmotic stress during the exploitation of rubber tree. In the future, these ERF groups, which have a strong expression but no known functions, deserve a deep characterization to determine their involvement in cell processes, including latex production.

Potential Post-transcriptional and Post-translational Regulations of *Hevea* AP2/ERF Genes

The characterization of AP2/ERF superfamily in *Hevea brasiliensis* was focused on transcriptional regulation. Yet, previous research works in different plant species has demonstrated that the AP2/ERF genes can be regulated at the post-transcriptional level, including the regulation by alternative splicing, control of protein stability, acetylation and nitrosylation (Licausi *et al.,* 2013a). A strong accumulation of transcripts was observed for *HbERF-I, HbERF-VII, HbERF-VIII* and *HbERF-IX* members under harvesting stress and tapping panel dryness in bark and latex tissues (Putranto *et al.,* 2015a; 2015b). The constitutive expression of several *HbERFs* led us to consider a post-transcriptional regulation controlling the activity of these proteins. Yet, it has been shown in grapevine, tomato and *Arabidopsis* as ERF groups (ERF-II, ERF-III, ERF-VII and ERF-IX) were strongly expressed in different tissues (Licausi *et al.,* 2010a; Pirrello *et al.,* 2012; Bailey-Serres *et al.,* 2012). A high abundance transcript in non-stressed condition was potentially a default defence system being present before the stress condition (Cheng *et al.,* 2013b).

Post-transcriptional regulation of Dehydration Responsive Element Binding Proteins 2 (DREB2) genes by alternative splicing has been reported in *Arabidopsis*, tomato and wheat (Rehman & Mahmood, 2015; Pirrello *et al.,* 2012; Lata & Prasad, 2011; Lucas *et al.,* 2011; Mizoi *et al.* 2012). In the case of alternative splicing, two types of transcripts may exist. The first is an inactive portion having a transcribed intron sequence which generates a stop codon before the DNA binding domain. The second is an active transcript encoding the full-length protein. This type of regulation was also observed in the ERF-VII in tomato and *Arabidopsis* (Pirrello *et al.,* 2006 , Licausi *et al.,* 2010b). Furthermore, post-transcriptional regulation of *AP2/ERF* genes by microRNAs was also demonstrated. The *JcDREB* gene in *Jatropha*

curcas has been targeted by miR156 (Galli *et al.,* 2014) while a repression of expression of *OitaAP2* gene in *Orchis italica* was controlled by miR172 (Salemme *et al.,* 2013). In some cases, the action of miRs can be specific. In *Arabidopsis*, the miR319b targeted transcript isoform of RAP2.12 containing an intron in order to inactivate the gene (Sobkowiak *et al.,* 2012). In *Hevea*, several microRNAs including the well-known miR172 were predicted to target AP2/ERF transcripts (Duan *et al.,* 2013). Although the inhibition mode was being achieved mainly by cleavage of target RNA messenger, translation inhibitions has been predicted for two genes (*HbERF-VI5* and *HbERF-IXc5*).

The AP2/ERF genes are likewise regulated at the post-translational level. For now, the DREB2 belonging to ERF-IVa contains a rich region of Ser/Thr is a potential site of phosphorylation. A deletion of a Ser/Thr motif led to a constitutively active form of DREB2A in *Arabidopsis* (Sakuma *et al.,* 2006). This finding was validated by overexpression of DREB2A in *Arabidopsis* and *Oryza* that did not result to an induction of downstream genes in normal conditions. Post-translational modification was necessary to activate this phosphorylation site (Dubouzet *et al.,* 2003; Liu *et al.,* 1998). Phosphorylation regulation *via* histidine kinases has also been suggested for the ERF-VI (Raines *et al.,* 2016). Another ERF belonging to the subgroup of ERF-IXb, *AtERF104* required an interaction with the mitogen-activated protein kinase 6 (MPK6) for its stability (Bethke *et al.,* 2009). The stability of ERF proteins occasionally involves the 26S proteasome. Once more, the ERF-VII gives an example of ubiquitin ligase involvement such as PROTEOLYSIS6 (PRT6) in the degradation of ERF-VII proteins *via* the N-end rule pathway (Gibbs *et al.,* 2011; Licausi *et al.,* 2011). There are not yet any reports about the post-translational regulation of *AP2/ERF* genes in *Hevea brasiliensis*. However, the low expression of *HbERFs* during the onset of TPD has brought a hypothesis in which a post-translational regulation was taken place (Putranto *et al.,* 2015b).

Potential Important Role of *Hevea* AP2/ERF in Reversible Tapping Panel Dryness Generated by Oxidative Stress

The occurrence of tapping panel dryness depends on various factors, such as plant material (genotype sensitivity and rootstock-scion interaction), environment aspects (harvesting system, biotic and abiotic stresses, and soil compaction) (Putranto *et al.,* 2015b). These factors result in a complex signalling involving molecular responses at transcriptional and post-transcriptional levels. Previous studies in *Hevea* have identified gene expression markers for TPD such as *HbMyb1*,

HbTOM20 and *HbTCTP* genes (Venkatachalam *et al.,* 2009; Peng *et al.,* 2011). These genes were negative regulators of genes that positively regulate the programmed cell death. The inhibition of these genes in TPD-affected trees thus causes the activation of programmed cell death. *HbTOM20* gene encodes a translocate protein of the outer mitochondrial membrane regulating the transport of nuclear proteins within mitochondria (Venkatachalam *et al.,* 2009). It is likely that inhibition of this protein results to the mitochondrial dysfunction leading to impaired respiration and a low level of ATP. *HbTCTP* gene encodes cytosolic calcium binding protein that exact function remains to be elucidated in rubber tree. The transcript of this gene was also 60% lower during the occurrence of TPD in comparison to normal condition (Venkatachalam *et al.,* 2007). In their study, Gébelin *et al.* (2013) predicted that the microRNA, *HbmiR159b* could target the Myb-like transcription factor (*HbMyb1*) and glutathione peroxidase (*HbGPX*) in *Hevea brasiliensis*. These post-transcriptional regulations may result in a change in the activity of target genes. Glutathione peroxidase is one of the key enzymes in the regulation of thiols in plants which function is to reduce the hydro-lipid peroxides in a form of alcohol as well as to hydrogen peroxide into H_2O (Noctor *et al.,* 2012).

The characterization of *Hevea AP2/ERFs* has led to the possible involvement of these transcription factors during the occurrence of TPD. In transcriptional level, Putranto *et al.* (2015b) has observed the induction of two *HbERF* genes (*HbERF-IIb2* and *HbERF-IXb2*) and the inhibition of *HbERF-VIIIa14* gene. The participation of jasmonate biosynthetic pathway could be suggested since the *HbERF-IIb2* gene (ortholog of *Arabidopsis ORA47*) was induced. The *ORA47* gene was demonstrated to induce the expression of *LOX3,* one of key genes to jasmonate (JA) biosynthesis (Khurshid 2012). Recently, Chen *et al.* (2016) has demonstrated the dual function of ORA47 protein in both JA and ABA biosynthesis leading to a clear involvement of ORA47 under osmotic stress. By contrast, the inhibition of *HbERF-VIIIa14,* a member of ERF-VIII during the occurrence of TPD suggests a control of the cell death regulation.

These responses at the molecular level generate a biochemical change in response to TPD. It is known that the thiol concentration and the enzymatic activities of catalase (CAS) and superoxide dismutase (SOD) were reduced during the occurrence of TPD (d'Auzac *et al.,* 1993). Some of these results have been confirmed in *Hevea* (Putranto *et al.,* 2015b). In addition, the reduction of the CAS and SOD activities was followed by

the increase of NAD(P)H oxidase activity (Chrestin, 1989). These parameters highlighted the appearance of oxidative burst that could also become a response signal of TPD. From this oxidative stress, Putranto *et al.* (2015a) hypothesized two events by known mechanisms which can be drawn. First, the peroxidation of lutoidic membranes releases coagulation factors leading to reactive oxygen species-type of TPD (ROS-TPD) (Jacob *et al.,* 1989b). Secondly, oxidative stress may lead to lipid peroxidation in the plasma membrane, release of linamarase and linamarin resulting to cyanide production (Kadow *et al.,* 2012). In a worse condition, a bark necrosis can occur resulting finally brown bast-type of TPD (BB-TPD).

Future Perspective for Functional Analysis of AP2/ERF Candidate Genes

HbAP2/ERF genes, which were responsive to multiple harvesting stress and TPD, have been identified and characterized in transcriptional level. Further characterization of several *HbAP2/ERF* candidate genes using transgenesis was suggested in order to fully understand their functions, especially in latex production of *Hevea brasiliensis*. Targeted candidate genes should be selected from HbERF-VII, HbERF-VIII, and HbERF-IX previously described as the most abundant and potentially important groups involved in the tolerance to harvesting stress and tapping panel dryness.

The metabolism activities of laticifers consume oxygen and generate reactive oxygen species. Interestingly, the GEMs of HbERF-VII are potential activators of downstream genes in response to hypoxia such as *HbADH* and *HbSUS1* (Piyatrakul *et al.,* 2014). Among the members of this group, *HbERF-VIIa12* gene, a potential ortholog of *RAP2.12*, could have an important role in the regulation of homeostasis of oxygen in the laticifers. In *Arabidopsis*, the RAP2.12 protein was known to be able to interact with alcohol dehydrogenase (ADH) and regulate their activity throughout the fermentation route. Further characterization of the gene, including a study of co-expression with response to hypoxia genes such as *HbADH*, a study of regulation by microRNAs and an analysis of transgenic line overexpressing or dowregulating *HbERF-VII* should clarify its involvement during a hypoxic condition in laticifers.

The rubber biosynthetic pathway belongs to the secondary metabolism (d'Auzac *et al.,* 1993). For this reason, the HbERF-IX group could play an important role controlling latex metabolism, e.g. during sucrose loading into laticifers. Among members of this group, two genes (*HbERF-IXc4* and *HbERF-IXc5*) were dramatically induced by the combination of ethylene and methyl jasmonate treatments in *Hevea* (Putranto *et al.,* 2015a). These genes are potential ortholog to *ERF1* famously known to be highly regulated at transcriptional level during multiple abiotic stresses. A further characterization of these genes requires the identification of their regulons (downstream target genes) by Chromatin Immunoprecipitation Sequencing (ChIP-seq) method and the functional analysis in transgenic lines. Transgenic lines overexpressing *HbERF-IXc4* and *HbERF-IXc5* genes have been regenerated (Lestari *et al.,* 2014). These lines showed a promising phenotype of highly tolerant to abiotic stress which will confirm the major role of both genes in latex production.

Another full characterization should be carried out on the ERF-VIII having an important role in the induction of programmed cell death biotic and abiotic stresses. In relation to TPD, members of this group should be considered as important candidate genes. An ortholog of *Arabidopsis ERF12*, *HbERF-VIIIa13* gene has been demonstrated to be repressor-type transcription factor (Putranto *et al.,* 2015a). In addition, *HbERF-VIIIa14* gene was a gene expression marker specifically induced under the occurrence of TPD. The protein-protein interaction analysis with known repressor network such as TOPLESS protein could be an important aspect to be elucidated.

Conclusion and Remarks

The ethylene biosynthesis and signalling pathway is one of the most studied aspects in *Hevea brasiliensis*. These studies have been driven by the practice of latex harvesting involving an ethylene releaser. Understanding the hormonal regulation of latex production in cellular and molecular levels is an important aspect in *Hevea brasiliensis*. However, the crosstalk of other phytohormones such as jasmonate and ABA with ethylene cannot be taken aside. Clear evidence has shown that their regulation could be also key factors in latex production. As a transcription factor, the *Hevea brasiliensis* AP2/ERF superfamily has shown its potency to be the master regulator of phytohormones related directly to latex production, regulator of tolerance to abiotic and biotic stress, and regulator of tolerance to physiological disease. In the near future, molecular markers could be developed from HbAP2/ERFs in order to select *Hevea* clones tolerant to abiotic and biotic factors.

References

Bailey-Serres J, T Fukao, DJ Gibbs, MJ Holdsworth, SC Lee, F Licausi, P Perata, LACJ Voesenek & JT van Dongen (2012). Making sense of low oxygen sensing. *Trends in plant science,* 17**,** 129-138.

Bethke G, T Unthan, JF Uhrig, Y Poschl, AA Gust, D Scheel & J Lee (2009). Flg22 regulates the release of an ethylene response factor substrate from MAP kinase 6 in *Arabidopsis thaliana* via ethylene signaling. *Proceedings of the National Academy of Sciences,* 106**,** 8067-8072.

Büttner M & KB Singh (1997). *Arabidopsis thaliana* ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an ocs element binding protein. *Proceedings of the National Academy of Sciences,* 94**,** 5961-5966.

Causier B, M Ashworth, W Guo & B Davies (2012). The TOPLESS interactome: a framework for gene repression in *Arabidopsis. Plant Physiol,* 158**,** 423-38.

Cernac A, C Andre, S Hoffmann-Benning & C Benning (2006). WRI1 is required for seed germination and seedling establishment. *Plant Physiol,* 141**,** 745-57.

Chandler J & W Werr (2011). The role of Dornroschen-like in early floral organogenesis. *Plant Signaling & Behavior,* 6**,** 1244-1246.

Chandler JW, M Cole, A Flier, B Grewe & W Werr (2007). The AP2 transcription factors DORNROSCHEN and DORNROSCHEN-LIKE redundantly control *Arabidopsis* embryo patterning via interaction with PHAVOLUTA. *Development,* 134**,** 1653-62.

Chen HY, EJ Hsieh, MC Cheng, C-Y Chen, S-Y Hwang & T-P Lin (2016). ORA47 (octadecanoid-responsive AP2/ERF-domain transcription factor 47) regulates jasmonic acid and abscisic acid biosynthesis and signaling through binding to a novel cis-element. *New Phytologist***,** n/a-n/a.

Cheng MC, PM Liao, W-W Kuo & T-P Lin (2013a). The Arabidopsis ETHYLENE RESPONSE FACTOR1 Regulates Abiotic Stress-Responsive Gene Expression by Binding to Different cis-Acting Elements in Response to Different Stress Signals. *Plant Physiology,* 162**,** 1566-1582.

Cheng WN, JX Lei, WL Rooney, TX Liu & K Zhu-Salzman (2013b). High basal defense gene expression determines sorghum resistance to the whorl-feeding insect southwestern corn borer. *Insect Science,* 20**,** 307-317.

Chrestin H (1989). Biochemical aspects of bark dryness induced by overstimulation of rubber trees with ethrel. In *Physiology of Rubber Tree Latex,* eds. J. d'Auzac, J. L. Jacob & H. Chrestin, 431–439. Boca Raton, Florida: CRC Press Inc.

Cole M, B Jacobs, L Soubigou-Taconnat, S Balzergue, JP Renou, JW Chandler & W Werr (2013). Live imaging of DORNROSCHEN and DORNROSCHEN-LIKE promoter activity reveals dynamic changes in cell identity at the microcallus surface of *Arabidopsis* embryonic suspensions. *Plant Cell Rep,* 32**,** 45-59.

d'Auzac J, F Bouteau, H Chrestin, A Clément, JL Jacob, R Lacrotte, JC Prévot, V Pujade-Renaud & JP Rona. (1993). Stress Ethylene in *Hevea brasiliensis*: Physiological, Cellular and Molecular Aspects. In *Cellular and Molecular Aspects of the Plant Hormone Ethylene,* eds. J. Pech, A. Latché & C. Balagué, 205-210. Springer Netherlands.

Duan C (2011). Etude de l'interaction entre l'éthylène et le jasmonate, hormones impliquées dans la production de caoutchouc naturel chez *Hevea brasiliensis*. In *Sciences et Techniques du Languedoc*, 181. Montpellier: Montpellier Sup-Agro.

Duan C, X Argout, V Gébelin, M Summo, J-F Dufayard, J Leclercq, K Hadi, P Piyatrakul, J Pirrello, M Rio, A Champion & P Montoro (2013). Identification of the *Hevea brasiliensis* AP2/ERF superfamily by RNA sequencing. *BMC Genomics,* 14**,** 30.

Duan C, M Rio, J Leclercq, F Bonnot, G Oliver & P Montoro (2010). Gene expression pattern in response to wounding, methyl jasmonate and ethylene in the bark of *Hevea brasiliensis. Tree Physiol,* 30**,** 1349-59.

Dubouzet JG, Y Sakuma, Y Ito, M Kasuga, EG Dubouzet, S Miura, M Seki, K Shinozaki & K Yamaguchi-Shinozaki (2003). OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *The Plant Journal,* 33**,** 751-763.

Etchells JP, CM Provost & SR Turner (2012). Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. *PLoS Genet,* 8**,** e1002997.

Focks N & C Benning (1998). wrinkled1: A novel, low-seed-oil mutant of *Arabidopsis* with a deficiency in the seed-specific regulation of carbohydrate metabolism. *Plant Physiology,* 118**,** 91-101.

Galli V, F Guzman, LFV de Oliveira, G. Loss-Morais, AP Körbes, SDA Silva, MMAN Margis-Pinheiro & R Margis (2014). Identifying MicroRNAs and Transcript Targets in *Jatropha* Seeds. *PLoS ONE,* 9**,** e83727.

Gébelin V, J Leclercq, Kuswanhadi, X Argout, T Chaidamsari, S Hu, C Tang, G Sarah, M Yang & P Montoro (2013). The small RNA profile in latex from *Hevea brasiliensis* trees is affected by tapping panel dryness. *Tree Physiology,* 33**,** 1084-1098.

Gibbs DJ, SC Lee, NM Isa, S Gramuglia, T Fukao, GW Bassel, CS Correia, F Corbineau, FL Theodoulou, J Bailey-Serres & MJ Holdsworth (2011). Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature,* 479**,** 415-8.

Gibbs Daniel J, NM Isa, M Movahedi, J Lozano-Juste, Guillermina M. Mendiondo, S Berckhan, N Marín-de la Rosa, J Vicente Conde, C Sousa Correia, SP Pearce, GW Bassel, B Hamali, P Talloji, DFA Tomé, A Coego, J Beynon, D Alabadí, A Bachmair, J León, JE Gray, FL Theodoulou & MJ Holdsworth (2014). Nitric Oxide Sensing in Plants Is Mediated by Proteolytic Control of Group VII ERF Transcription Factors. *Molecular Cell,* 53**,** 369-379.

Hinz M, IW Wilson, J Yang, K Buerstenbinder, D Llewellyn, ES Dennis, M Sauter & R Dolferus (2010). *Arabidopsis* RAP2.2: an ethylene response transcription factor that is important for hypoxia survival. *Plant Physiol,* 153**,** 757-72.

Huang PY, J Catinot & L Zimmerli (2015). Ethylene response factors in *Arabidopsis* immunity. *Journal of Experimental Botany.*

Ito Y, K Katsura, K Maruyama, T Taji, M Kobayashi, M Seki, K Shinozaki & K Yamaguchi-Shinozaki (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol,* 47**,** 141-53.

Iwase A, N Mitsuda, M Ikeuchi, M Ohnuma, C Koizuka, K Kawamoto, J Imamura, H Ezura & K Sugimoto (2013). *Arabidopsis* WIND1 induces callus formation in rapeseed, tomato, and tobacco. *Plant Signaling & Behavior,* 8**,** e27432.

Iwase A, N Mitsuda, T Koyama, K Hiratsu, M Kojima, T Arai, Y Inoue, M Seki, H Sakakibara, K Sugimoto & M Ohme-Takagi (2011a). The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis. Curr Biol,* 21**,** 508-14.

Iwase A, M Ohme-Takagi & K Sugimoto (2011b). WIND1: A key molecular switch for plant cell dedifferentiation. *Plant Signaling & Behavior,* 6**,** 1943-1945.

Jacob JL, JC Prévôt & RGO Kekwick (1989a). General metabolism of *Hevea brasiliensis* latex (with the exception of isoprenic anabolism). In *Physiology of Rubber Tree Latex,* ed. J.-L. J. J d'Auzac, H Chrestin, 101-144. Boca Raton, Florida: CRC press, Inc.

Jacob JL, JC Prévôt, D Roussel, R Lacrotte, E Serres, J d'Auzac, JM Eschbach & H Omont (1989b). Yield limiting factors, latex physiological parameters, latex diagnosis, and clonal typology. In *Physiology of Rubber Tree Latex,* ed. J.-L. J. J d'Auzac, H Chrestin, 345-382 Boca Raton, Florida: CRC press, Inc.

Kadow D, K Voß, D Selmar & R Lieberei (2012). The cyanogenic syndrome in rubber tree *Hevea brasiliensis*: tissue-damage-dependent activation of linamarase and hydroxynitrile lyase accelerates hydrogen cyanide release. *Annals of Botany,* 109**,** 1253-1262.

Kagaya Y, K Ohmiya & T Hattori (1999). RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Research,* 27**,** 470-478.

Khandelwal A, T Elvitigala, B Ghosh & RS Quatrano (2008). *Arabidopsis* transcriptome reveals control circuits regulating redox homeostasis and the role of an AP2 transcription factor. *Plant Physiol,* 148**,** 2050-8.

Khurshid M (2012). Functional analysis of ORA47, a key regulator of jasmonate biosynthesis in *Arabidopsis*. Department of Plant Cell Physiology, Institute of Biology Leiden, Faculty of Science, Leiden University.

Kirch T (2003). The DORNROSCHEN/ ENHANCER OF SHOOT REGENERATION1 Gene of *Arabidopsis* Acts in the Control of Meristem Cell Fate and Lateral Organ Development. *The Plant Cell Online,* 15**,** 694-705.

Krishnaswamy, S, S Verma, MH Rahman & NN Kav (2011). Functional characterization of four APETALA2-family genes (RAP2.6, RAP2.6L, DREB19 and DREB26) in *Arabidopsis. Plant Mol Biol,* 75**,** 107-27.

Kurepin LV, KP Dahal, LV Savitch, J Singh, R Bode, AG Ivanov, V Hurry & NP Huner (2013). Role of CBFs as Integrators of Chloroplast Redox, Phytochrome and Plant Hormone Signaling during Cold Acclimation. *Int J Mol Sci,* 14**,** 12729-63.

Kuswanhadi (2006). Isolement et caractérisation des gènes *ACS* et *ACO* impliqués dans la biosynthèse de l'éthylène chez *Hevea brasiliensis*. In *Sciences et Techniques du Languedoc,* 146. Montpellier: Université Montpellier II.

Kuswanhadi, J Leclercq, M Rio, J Tregear, MN Ducamp-Collin & P Montoro (2010). Isolation of Three Members of The Multigene Family

Encoding ACC Oxidases in *Hevea brasiliensis* and Inverstigation of Their Responses to Ethylene Stimulation and Wounding. *Journal of Rubber Research,* 13**,** 185-205.

Kuswanhadi, J Leclercq, MA Rio, Sumarmadji & P Montoro (2007). Isolation and expression of ACC oxidase in *Hevea brasiliensis*. In *Advances in Plant Ethylene Research,* eds. A. Ramina, C. Chang, J. Giovannoni, H. Klee, P. Perata & E. Woltering, 31-34. Springer Netherlands.

Lata C & M Prasad (2011). Role of DREBs in regulation of abiotic stress responses in plants. *J Exp Bot,* 62**,** 4731-48.

Lestari R, RA Putranto, P Piyatrakul, C Duan, M Rio, F Martin, J Pirrello, F Dessailly, J Leclercq, Kuswanhadi & P Montoro (2014). Identification of signalling factors involved in the regulation of laticifer metabolism by tapping and Ethephon stimulation in *Hevea brasiliensis*. In *IRRDB International Rubber Conference,* ed. Irrdb, 1 p. PHL: s.n.

Li J, Y Zhang, J Gu, C Guo, S Wen, G Liu & K Xiao (2012). Molecular characterization and roles of AP2 transcription factors on drought tolerance in plants. *Frontiers of Agriculture in China,* 5**,** 463-472.

Li Z, L Zhang, Y Yu, R Quan, Z Zhang, H Zhang & R Huang (2011). The ethylene response factor AtERF11 that is transcriptionally modulated by the bZIP transcription factor HY5 is a crucial repressor for ethylene biosynthesis in *Arabidopsis*. *Plant J,* 68**,** 88-99.

Licausi F, FM Giorgi, S Zenoni, F Osti, M Pezzotti & P Perata (2010a). Genomic and transcriptomic analysis of the AP2/ERF superfamily in *Vitis vinifera*. *BMC Genomics,* 11**,** 719.

Licausi F., M. Kosmacz, D. A. Weits, B. Giuntoli, F. M. Giorgi, L. A. Voesenek, P. Perata & J. T. van Dongen (2011). Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature,* 479**,** 419-22.

Licausi F, M Ohme-Takagi & P Perata (2013a). APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. *New Phytologist,* 199**,** 639-649.

Licausi F, C Pucciariello & P Perata (2013b). New role for an old rule: N-end rule-mediated degradation of ethylene responsive factor proteins governs low oxygen response in plants. *J Integr Plant Biol,* 55**,** 31-9.

Licausi F, JT van Dongen, B Giuntoli, G Novi, A Santaniello, P Geigenberger & P Perata (2010b). HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. *Plant J,* 62**,** 302-15.

Lin RC, HJ Park & HY Wang (2008). Role of *Arabidopsis* RAP2.4 in regulating light- and ethylene-mediated developmental processes and drought stress tolerance. *Mol Plant,* 1**,** 42-57.

Liu Q, M Kasuga, Y Sakuma, H Abe, S Miura, K Yamaguchi-Shinozaki & K Shinozaki (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell,* 10**,** 1391-406.

Lorenzo O, R Piqueras, JJ Sánchez-Serrano & R Solano (2003). ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense. *The Plant Cell Online,* 15**,** 165-178.

Lucas S, E Durmaz, BA Akpinar & H Budak (2011). The drought response displayed by a DRE-binding protein from *Triticum dicoccoides*. *Plant Physiol Biochem,* 49**,** 346-51.

Mase K, N Ishihama, H Mori, H Takahashi, H Kaminaka, M Kodama & H Yoshioka (2013). Ethylene-responsive AP2/ERF transcription factor MACD1 participates in phytotoxin-triggered programmed cell death. *Mol Plant Microbe Interact,* 26**,** 868-79.

Mizoi J, K Shinozaki & K Yamaguchi-Shinozaki (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochim Biophys Acta,* 1819**,** 86-96.

Moffat CS, RA Ingle, DL Wathugala, NJ Saunders, H Knight & MR Knight (2012). ERF5 and ERF6 Play Redundant Roles as Positive Regulators of JA/Et-Mediated Defense against *Botrytis cinerea* in *Arabidopsis*. *PLoS ONE,* 7**,** e35995.

Nakano T, K Suzuki, T Fujimura & H Shinshi (2006). Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol,* 140**,** 411-32.

Noctor G, A Mhamdi, S Chaouch, YI Han, J Neukermans, B Marquez-Garcia, G Queval & CH Foyer (2012). Glutathione in plants: an integrated overview. *Plant, Cell & Environment,* 35**,** 454-484.

Ogata T, Y Kida, T Arai, Y Kishi, Y Manago, M Murai & Y Matsushita (2011). Overexpression of tobacco ethylene response factor NtERF3 gene and its homologues from tobacco and rice induces hypersensitive response-like cell death in tobacco. *Journal of General Plant Pathology,* 78**,** 8-17.

Ogata T, Y Kida, M Tochigi & Y Matsushita (2013). Analysis of the cell death-inducing ability of the ethylene response factors in group VIII of the AP2/ERF family. *Plant Sci,* 209**,** 12-23.

Ohme-Takagi M & H Shinshi (1995). Ethylene-Inducible DNA Binding Proteins That Interact with an Ethylene-Responsive Element. *The Plant Cell Online,* 7**,** 173-182.

Ohta M, K Matsui, K Hiratsu, H Shinshi & M Ohme-Takagi (2001). Repression Domains of Class II ERF Transcriptional Repressors Share an Essential Motif for Active Repression. *The Plant Cell Online,* 13**,** 1959-1968.

Okamuro JK, B Caster, R Villarroel, M Van Montagu & KD Jofuku (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis*. *Proc Natl Acad Sci U S A,* 94**,** 7076-81.

Pandey GK, JJ Grant, YH Cheong, BG Kim, L Li & S Luan (2005). ABR1, an APETALA2-domain transcription factor that functions as a repressor of ABA response in *Arabidopsis*. *Plant Physiol,* 139**,** 1185-93.

Pauwels L, K Morreel, E De Witte, F Lammertyn, M Van Montagu, W Boerjan, D Inze & A Goossens (2008). Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured *Arabidopsis* cells. *Proc Natl Acad Sci U S A,* 105**,** 1380-5.

Peng SQ, KX Wu, GX Huang & SC Chen (2011). HbMyb1, a Myb transcription factor from *Hevea brasiliensis*, suppresses stress induced cell death in transgenic tobacco. *Plant Physiol Biochem,* 49**,** 1429-35.

Pirrello J, F Jaimes-Miranda, MT Sanchez-Ballesta, B Tournier, Q Khalil-Ahmad, F Regad, A Latché, JC Pech & M Bouzayen (2006). Sl-ERF2, a Tomato Ethylene Response Factor Involved in Ethylene Response and Seed Germination. *Plant and Cell Physiology,* 47**,** 1195-1205.

Pirrello J, N Prasad, W Zhang, K Chen, I Mila, M Zouine, A Latche, JC Pech, M Ohme-Takagi, F Regad & M. Bouzayen (2012). Functional analysis and binding affinity of tomato ethylene response factors provide insight on the molecular bases of plant differential responses to ethylene. *BMC Plant Biology,* 12**,** 190.

Piyatrakul P, RA Putranto, F Martin, M Rio, F Dessailly, J Leclercq, JF Dufayard, L Lardet & P Montoro (2012). Some ethylene biosynthesis and AP2/ERF genes reveal a specific pattern of expression during somatic embryogenesis in *Hevea brasiliensis*. *BMC Plant Biology,* 12**,** 244.

Piyatrakul P, M Yang, RA Putranto, J Pirrello, F Dessailly, S Hu, M Summo, K Theeravatanasuk, J Leclercq, Kuswanhadi & P Montoro (2014). Sequence and Expression Analyses of Ethylene Response Factors Highly Expressed in Latex Cells from *Hevea brasiliensis*. *PLoS ONE,* 9**,** e99367.

Pre M, M Atallah, A Champion, M De Vos, CM Pieterse & J Memelink (2008). The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiol,* 147**,** 1347-57.

Pujade-Renaud V, A Clement, C Perrot-Rechenmann, JC Prevot, H Chrestin, JL Jacob & J Guern (1994). Ethylene-Induced Increase in Glutamine Synthetase Activity and mRNA Levels in *Hevea brasiliensis* Latex Cells. *Plant Physiology,* 105**,** 127-132.

Putranto RA., C. Duan, Kuswanhadi, T. Chaidamsari, M. Rio, P. Piyatrakul, E. Herlinawati, J. Pirrello, F. Dessailly, J. Leclercq, F. Bonnot, C. Tang, S. Hu & P. Montoro (2015a). Ethylene Response Factors Are Controlled by Multiple Harvesting Stresses in *Hevea brasiliensis*. *PLoS ONE,* 10**,** e0123618.

Putranto RA., E. Herlinawati, M. Rio, J. Leclercq, P. Piyatrakul, E. Gohet, C. Sanier, F. Oktavia, J. Pirrello, Kuswanhadi & P. Montoro (2015b). Involvement of Ethylene in the Latex Metabolism and Tapping Panel Dryness of *Hevea brasiliensis*. *International Journal of Molecular Sciences,* 16**,** 17885.

Putranto RA, Kushwanhadi & P Montoro (2014). Molecular response of *Hevea brasiliensis Ethylene Response Factors* (HbERFs) as expression marker genes in response to ethephon stimulation in rubber tree clones. *Menara Perkebunan,* 82**,** 70-80.

Rae L, NT Lao & TA Kavanagh (2011). Regulation of multiple aquaporin genes in *Arabidopsis* by a pair of recently duplicated DREB transcription factors. *Planta,* 234**,** 429-44.

Raines T, C Shanks, CY Cheng, D McPherson, CT Argueso, HJ Kim, JM Franco-Zorrilla, I López-Vidriero, R Solano, R Vaňková, GE Schaller & JJ Kieber (2016). The cytokinin response factors modulate root and shoot growth and promote leaf senescence in *Arabidopsis*. *The Plant Journal,* 85**,** 134-147.

Rashotte AM & LR Goertzen (2010). The CRF domain defines cytokinin response factor proteins in plants. *BMC Plant Biol,* 10**,** 74.

Rehman S & T Mahmood (2015) Functional role of DREB and ERF transcription factors: regulating stress-responsive network in plants. *Acta Physiologiae Plantarum,* 37**,** 1-14.

Sakuma Y, Q Liu, JG Dubouzet, H Abe, K Shinozaki & K Yamaguchi-Shinozaki (2002). DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochem Biophys Res Commun,* 290**,** 998-1009.

Sakuma Y, K Maruyama, Y Osakabe, F Qin, M Seki, K Shinozaki & K Yamaguchi-Shinozaki (2006). Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell,* 18**,** 1292-309.

Salemme M, M Sica, G Iazzetti, L Gaudio & S Aceto (2013). The *AP2*-Like Gene *OitaAP2* Is

Alternatively Spliced and Differentially Expressed in Inflorescence and Vegetative Tissues of the Orchid *Orchis italica*. *PLoS ONE,* 8**,** e77454.

Seok HY, V Tarte, SY Lee, HY Park & YH Moon (2014). *Arabidopsis* HRE1α, a splicing variant of AtERF73/HRE1, functions as a nuclear transcription activator in hypoxia response and root development. *Plant Cell Reports***,** 1-8.

Sobkowiak L, W Karlowski, A Jarmolowski & Z Szweykowska-Kulinska (2012). Non-canonical processing of *Arabidopsis* pri-miR319a/b/c generates additional microRNAs to target one RAP2.12 mRNA isoform. *Frontiers in Plant Science,* 3.

Song CP, M Agarwal, M Ohta, Y Guo, U Halfter, P Wang & JK Zhu (2005). Role of an *Arabidopsis* AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. *Plant Cell,* 17**,** 2384-96.

Tiwari SB, A Belachew, SF Ma, M Young, J Ade, Y Shen, CM Marion, HE Holtan, A Bailey, JK Stone, L Edwards, A. D. Wallace, R. D. Canales, L. Adam, O. J. Ratcliffe & P. P. Repetti (2012). The EDLL motif: a potent plant transcriptional activation domain from AP2/ERF transcription factors. *Plant J,* 70**,** 855-65.

Tupý J (1989). Sucrose supply and utilization for latex production. In *Physiology of Rubber Tree Latex,* eds. J. d'Auzac, JL Jacob & H Chrestin, 179-218. Boca Raton, Florida: CRC Press Inc.

van Veen H, M Akman, DCL Jamar, D Vreugdenhil, M Kooiker, P van Tienderen, LACJ Voesenek, ME Schranz & R Sasidharan (2014). Group VII Ethylene Response Factor diversification and regulation in four species from flood-prone environments. *Plant, Cell & Environment***,** n/a-n/a.

Venkatachalam P, A Thulaseedharan & K Raghothama (2007). Identification of expression profiles of tapping panel dryness (TPD) associated genes from the latex of rubber tree (*Hevea brasiliensis* Muell. Arg.). *Planta,* 226**,** 499-515.

Venkatachalam P, A Thulaseedharan & K Raghothama (2009). Molecular identification and characterization of a gene associated with the onset of tapping panel dryness (TPD) syndrome in rubber tree (*Hevea brasiliensis* Muell.) by mRNA differential display. *Mol Biotechnol,* 41**,** 42-52.

Weber H & H Hellmann (2009). *Arabidopsis thaliana* BTB/ POZ-MATH proteins interact with members of the ERF/AP2 transcription factor family. *FEBS J,* 276**,** 6624-35.

Xu ZS, M Chen, LC Li & YZ Ma (2011). Functions and application of the AP2/ERF transcription factor family in crop improvement. *J Integr Plant Biol,* 53**,** 570-85.

Yang CY, FC Hsu, JP Li, NN Wang & MC Shih (2011). The AP2/ERF transcription factor AtERF73/HRE1 modulates ethylene responses during hypoxia in *Arabidopsis*. *Plant Physiol,* 156**,** 202-12.

Yang SF & NE Hoffman (1984). Ethylene Biosynthesis and its Regulation in Higher Plants. *Annual Review of Plant Physiology,* 35**,** 155-189.

Yang Z, L Tian, M Latoszek-Green, D Brown & K Wu (2005). *Arabidopsis* ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. *Plant Mol Biol,* 58**,** 585-96.

Zarei A, AP Korbes, P Younessi, G Montiel, A Champion & J Memelink (2011). Two GCC boxes and AP2/ERF-domain transcription factor ORA59 in jasmonate/ethylene-mediated activation of the PDF1.2 promoter in *Arabidopsis*. *Plant Mol Biol,* 75**,** 321-31.

Zhang L, Z Li, R Quan, G Li, R Wang & R Huang (2011). An AP2 domain-containing gene, ESE1, targeted by the ethylene signaling component EIN3 is important for the salt response in *Arabidopsis*. *Plant Physiol,* 157**,** 854-65.