Rubber Science, **26**(1): 13-22, 2013

A NEW ETHYLENE RECEPTOR FROM THE BARK TISSUES OF *HEVEA BRASILIENSIS*

S. Sreelatha, M. B. Mohamed Sathik, G. Eric Schaller*, Brenda. P. Hall*, Kristine Hill*, Lisha P. Luke, R. Krishnakumar and James Jacob

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

* Department of Biological Sciences, Dartmouth College, New Hampshire, USA

Received : 17 July 2012 Accepted : 23 January 2013

Sreelatha, S., Sathik, M.B.M., Schaller, G.E., Hall, B.P., Hill, K., Luke, L.P., Krishnakumar, R. and Jacob, J. (2013). A new ethylene receptor from the bark tissues of *Hevea brasiliensis*. *Rubber Science*, **26**(1): 13-22.

Stimulating rubber trees by applying ethephon in the bark is a common practice to increase the latex yield in rubber plantations. The ethylene generated from ethephon induces various physiological and biochemical changes and eventually leads to increased latex production. Ethylene perception and signal transduction is initiated when the ethylene molecules bind with the receptors localized in the cell membranes. Information on ethylene receptors and the molecular mechanism involved in the signal transduction is important to understand the regulation of latex flow in *Hevea* after stimulation. The relative expression of an ethylene receptor gene (*Hb ETR1*) in unstimulated and stimulated trees of *Hevea* clone RRII 105 using relative RT-PCR indicated that the gene was up regulated after stimulation with ethephon. Phylogenetic analysis of the ethylene receptors identified from different plant species and by using a PCR with degenerate primers, a new ethylene receptor was identified in the bark tissues of *Hevea* which was named as *Hb ETR2*. The gene representing this receptor was also found to be up regulated by ethylene stimulation. qRT-PCR also showed up-regulation of these genes immediately after stimulation.

Keywords: Degenerate PCR, Ethylene receptor, Phylogenetic tree

INTRODUCTION

The application of ethephon, a commercially available ethylene stimulant, in the bark of *Hevea brasiliensis* trees increases the volume of latex by delaying the latex vessel plugging and stimulates the latex regeneration mechanism between two tapping (D'Auzac *et al.*, 1993). Physiological and biochemical studies showed that ethylene acts on membrane permeability of lutoid particles (Coupe and Chrestin, 1989; Thomas *et al.*, 1999), increased the activity of invertase resulting in acceleration of glycolysis, increased adenylate pool, changes in polysomes, rRNA contents, enzyme activities, gene expression pattern (Tupy and Primot, 1976; Amalou *et al.*, 1992; Gidrol *et al.*, 1988) and eventually increased the latex yield.

Immediate effects of stimulation on the physiology and metabolism of laticifers under various situations such as reduced tapping frequency with different levels of stimulation, over stimulation, intensive tapping and clonal responses to stimulation have been studied extensively (Chrestin,

Correspondence: S. Sreelatha (Email: sreelatha@rubberboard.org.in)

1989; Gohet *et al.*, 2003; Simon, 2003; Sreelatha, 2003). Cloning of ethylene inducible and laticifer specific promoters from rubber tree has been made (Pujade – Renaud *et al.*, 2001) and two sucrose transporters *Hb SUT1A* and *Hb SUT2A* which are induced by ethylene have been found to be related to increase in sucrose transport into laticifers (Dusotoit-Coucaud *et al.*, 2009). Recently it was reported that stimulation of latex yield by ethylene depends on the expression of sucrose transporter *Hb SUT1B* (Dusotoit-Coucaud *et al.*, 2010). However little is known about the exact mechanism by which ethylene is perceived by its receptors and its signal transduction leading to various metabolic changes in the laticiferous cells of *Hevea*. Studies on the characterization of ethylene receptors and its signal transduction pathways are important to understand the regulation of latex flow in *Hevea* after tapping and stimulation.

Ethylene perception in plant tissues requires membrane- localized receptors and a signal transduction pathway to coordinate the downstream responses (Bleecker and Kende, 2000; Fluhr and Mattoo, 1996). Biochemical and molecular genetic studies on ethylene perception and signal transduction pathway have been studied extensively in *Arabidopsis* and other plant species (Schaller and Bleecker, 1995; Gao *et al.*, 2003).

In general, ethylene signal transduction is initiated by binding the ethylene molecules with receptor proteins and activates a series of reactions involving many transcription factors. In plants, these receptors are predominantly localized in the membranes of endoplasmic reticulum (ER) (Chen *et al.*, 2002). The particular physico-chemical properties of ethylene that allow free diffusion through the membranes and the cytoplasm to reach its receptors in the ER without any active transport system.

The ethylene receptor gene, *ETR1* and the homologues *ETR2, ERS1, ERS2* and *EIN4* genes have been identified in *Arabidopsis* (Chang and Stadler, 2001; Schaller and Keiber, 2002). Sequence analysis suggests that this receptor family classifies further into two subfamilies. *ETR1* and *ERS1* belong to subfamily-1 having a well conserved histidine-kinase domain, whereas *ETR2, ERS2* and *EIN4* belong to subfamily-2 containing an additional transmembrane segment and a degenerate histidine kinase domain. *ETR1* was the first member of the receptor family identified and characterized in detail (Qu and Schaller, 2001). It contains three predicted transmembrane domains that function as signal input domain based on their ability to bind ethylene. Following this *ETR1*contains a GAF domain and a C-terminal region that consists of a histidine kinase (HK) domain and a receiver domain which is likely involved in signal output (Bleecker, 1999).

Many homologues of ethylene receptor genes also have been identified and their differential expression was studied in other plant species (Lashbrook *et al.*, 1998; Mita *et al.*, 1998; Chang and Shockey, 1999). An ethylene receptor gene *Hb ETR1* (AY 847291) has already been isolated from *Hevea brasiliensis* (Yang *et al.*, 2005). This study was initiated to identify if there are any other members of ethylene receptors in *Hevea* and to study their role in ethylene induced latex production.

MATERIALS AND METHODS

Plant material and ethylene stimulation

Hevea trees (clone RRII 105 planted during 1992) of uniform girth and latex yield under S2 d3 6d/7 tapping system were

1989; Gohet *et al.*, 2003; Simon, 2003; Sreelatha, 2003). Cloning of ethylene inducible and laticifer specific promoters from rubber tree has been made (Pujade – Renaud *et al.*, 2001) and two sucrose transporters *Hb SUT1A* and *Hb SUT2A* which are induced by ethylene have been found to be related to increase in sucrose transport into laticifers (Dusotoit-Coucaud *et al.*, 2009). Recently it was reported that stimulation of latex yield by ethylene depends on the expression of sucrose transporter *Hb SUT1B* (Dusotoit-Coucaud *et al.*, 2010). However little is known about the exact mechanism by which ethylene is perceived by its receptors and its signal transduction leading to various metabolic changes in the laticiferous cells of *Hevea*. Studies on the characterization of ethylene receptors and its signal transduction pathways are important to understand the regulation of latex flow in *Hevea* after tapping and stimulation.

Ethylene perception in plant tissues requires membrane- localized receptors and a signal transduction pathway to coordinate the downstream responses (Bleecker and Kende, 2000; Fluhr and Mattoo, 1996). Biochemical and molecular genetic studies on ethylene perception and signal transduction pathway have been studied extensively in *Arabidopsis* and other plant species (Schaller and Bleecker, 1995; Gao *et al.*, 2003).

In general, ethylene signal transduction is initiated by binding the ethylene molecules with receptor proteins and activates a series of reactions involving many transcription factors. In plants, these receptors are predominantly localized in the membranes of endoplasmic reticulum (ER) (Chen *et al.*, 2002). The particular physico-chemical properties of ethylene that allow free diffusion through the membranes and the cytoplasm to reach its receptors in the ER without any active transport system.

The ethylene receptor gene, *ETR1* and the homologues *ETR2, ERS1, ERS2* and *EIN4* genes have been identified in *Arabidopsis* (Chang and Stadler, 2001; Schaller and Keiber, 2002). Sequence analysis suggests that this receptor family classifies further into two subfamilies. *ETR1* and *ERS1* belong to subfamily-1 having a well conserved histidine-kinase domain, whereas *ETR2, ERS2* and *EIN4* belong to subfamily-2 containing an additional transmembrane segment and a degenerate histidine kinase domain. *ETR1* was the first member of the receptor family identified and characterized in detail (Qu and Schaller, 2001). It contains three predicted transmembrane domains that function as signal input domain based on their ability to bind ethylene. Following this *ETR1*contains a GAF domain and a C-terminal region that consists of a histidine kinase (HK) domain and a receiver domain which is likely involved in signal output (Bleecker, 1999).

Many homologues of ethylene receptor genes also have been identified and their differential expression was studied in other plant species (Lashbrook *et al.*, 1998; Mita *et al.*, 1998; Chang and Shockey, 1999). An ethylene receptor gene *Hb ETR1* (AY 847291) has already been isolated from *Hevea brasiliensis* (Yang *et al.*, 2005). This study was initiated to identify if there are any other members of ethylene receptors in *Hevea* and to study their role in ethylene induced latex production.

MATERIALS AND METHODS

Plant material and ethylene stimulation

Hevea trees (clone RRII 105 planted during 1992) of uniform girth and latex yield under S2 d3 6d/7 tapping system were

stimulated with commercially available ethephon (2.5% in coconut oil) on the bark below the tapping panel. A set of similar trees were kept as control without stimulant application. Bark samples were collected after three days of stimulation (Sreelatha, 2003) from both control and stimulated trees. The soft bark tissue was separated and used for mRNA isolation.

mRNA isolation

The soft bark tissues collected in liquid N_2 were ground and mixed with RNA extraction buffer containing 50 mM Tris (pH 8.0), 150 mM $LiCl_2$, 5 mM EDTA, 5 per cent sodium dodecyl sulphate (SDS), 1 per cent soluble polyvinylpyrrolidone (PVP) and 2 per cent β-mercaptoethanol. After a brief spin to remove the debris, mRNA was isolated from the supernatant by binding with magnetic beads (Dynabeads, Invitrogen) and by capturing on a magnetic column. The quality and quantity of mRNA isolated was confirmed on agarose gel electrophoresis as well as by absorption at 260 and 280 nm (Nanodrop, USA). cDNA was prepared from 25 ng of mRNA in a 10 µl reaction mixture containing using Super Script III First-Strand Synthesis System for RT-PCR (Invitrogen, USA) according to the manufacturer's recommendations.

Semi-quantitative RT-PCR

Five microlitter cDNA, (1:25 and 1:125 dilutions) from both control and stimulated trees were used in semi-quantitative RT-PCR with *Hevea* specific ethylene receptor primers. Ubiquitin gene (primer designed for *Hevea*) was used as internal control. Primer sequences used for ubiquitin (UBQ) and *Hb ETR1* are given in Table 1. PCR was performed according to the standard protocols and the amplified products were visualized on 1 per cent agarose gels.

Sequence comparison and phylogenetic analysis

Amino acid sequences for ethylene receptors from *Arabidopsis*, rice, maize, tomato, peach, beech and poplar were collected from NCBI and poplar genome home page for isolating more members of the receptor gene family. Multiple alignments of complete amino acid sequences were performed using Lasergene-6 (Clustal W) programme and a phylogenetic tree was made using tree view programme. Since the *Hevea* ethylene receptor has close similarity to the known dicot receptors, nucleotide sequences of these dicot receptors were aligned and a phylogenetic tree was again constructed.

Design and use of degenerate primers for PCR and cloning

Degenerate oligonucleotide PCR was performed using primers designed from the highly conserved regions of known ethylene receptors from dicot plants (Table 1). Single stranded cDNA (25 ng) was used as template for PCR using 10 µM each degenerate primer. The PCR reaction products were fractionated on 1 per cent agarose gels and visualized by staining with ethidium bromide. The bands with expected size were recovered and ligated into pCR8/GW/TOPO cloning vector. The ligation mix was introduced into one shot competent cells followed by plating on to agar plates containing 100 µg mL^{-1} spectinomycin and incubating overnight at 37 °C. Single colonies were cultured in LB medium with appropriate antibiotic at 37 °C overnight and cells harboring the right clones were identified using colony PCR. Plasmid DNA from positive clones was purified using Miniprep DNA purification kit followed by sequencing with GW1 primer. Analysis was carried out using Lasergene software package (DNASTAR,

Table 1. **Primer sequences used for both RT-PCR and degenerate PCR**

	Gene	Forward primer	Reverse primer
Hevea specific primers	*Hb ETR1*	5' AGATGGCAGTCTTCAGCTTGACCT 3'	5'AAACCATCAGGCATGCACACATCC3'
	Hb UBQ	5' TTTCCTTCTCAGATCTCCAA3'	5'CCAAACATCTAGACACAACTTGA 3'
Degenerate primers	*ETR*	5'RGARGARTCAATGAGGGCW MGRGAYCT3'	5'ACAAATTGCAAGRCCAAGYCCASTTT 3'
	ETR	5'GRCARTGGCATGYYYATGARCTGG3'	5'TACAAATTGCAAGRCCAAGYCCAS3'
	ETR	5'GRCARTGGCATGYYYATGARTTGG3'	5'TACAAATTGCAAGRCCAAGYCCAS3'
	ETR	5'TGCAAGRCARTGGCATGYYYATGA3'	5'TACAAATTGCAAGRCCAAGYCCAS3'

USA) and the consensus sequence of the new receptor was generated.

Expression analysis by Quantitative PCR

Total RNA and cDNA preparation

Latex samples from trees of clone RRII 105 were collected from unstimulated and stimulated trees (third, fifth and seventh day after stimulation) in lysis solution/2-ME mixture of spectrum plant total RNA kit (Sigma-Aldrich) and was mixed properly. Total RNA was prepared according to the manufacturer's instructions. The quantity and quality of RNA were determined by both spectrophotometrically (Nanodrop, USA) and by agarose gel electrophoresis. cDNA was synthesized by using Superscript III reverse transcriptase (Invitrogen) in a 20 µl reaction after treating 5 µg of total RNA with DNAse (Fermantas, USA). From this cDNA mixture, 1 µl of 1:10 dilution was used as template DNA in the downstream qPCR reactions.

Quantitative Real Time PCR

Suitable primers were designed and synthesized (Table 2) for both *ETR1* and *ETR2* using Primer Express (Applied Biosystems, USA). Quantitative gene expression analysis was eventually carried out by using Light Cycler 480 II, Roche Real Time PCR System. RT-PCR was performed

Table 2. **Primer sequences used for RT-PCR of *Hb ETR 1* and *Hb ETR 2***

Gene	Forward primer	Reverse primer
Semi quantitative primers		
Hb ETR1	5'- CAAGAAACTGGACTGACGCCTGAG- 3'	5'-CAGTTCCAATGATTGATTTTGTGC-3'
Hb ETR1	5'-GCAAGAAACTGGACTGACGCC-3'	5'-CAGTTCCAATGATTGATTTTGTGC-3'
HbETR 2	5'-CAAGAAACTGAGCTTACACCTGAG-3'	5'-TGGTTCCGAAGATTGACTTTGTGC-3'
HbETR 2	5'- GCAAGAAACTGAGCTTACACC-3'	5'-TGGTTCCGAAGATTGACTTTGTGC-3'
qRT Primers		
HbETR1q	5'- GGAAGGACATTGGCATTGGAAG-3'	5'- CGAAGAGTGTAGGAAAGTTGAAGC-3'
HbETR2q	5'- AACCTCCCAGAATACAGCGT-3'	5'- GCCTCATGGTTGAGACAATC-3'

in a 20 µl reaction mixture containing 1 µl from 1/10 dilution of first-strand cDNA reaction, 6.5 nM of each primer and 10 µl of Lightcycler 480 SYBR Green I Master (Roche Diagnostics Gmbh, Germany). PCR cycling was performed by incubating the mix at 95 °C for 7 min, followed by 40 cycles of 95 °C for 20 seconds and 60 °C for 30 seconds. This was followed by a melt curve analysis (95 °C for 20 seconds, 60 °C for one minute and 95 °C for about 5 minutes). Each experiment was repeated two to three times, and each PCR reaction was performed in triplicate with null-template controls. Reaction efficiency of both the target genes and the endogenous control was calculated based on the formula, Efficiency = $10^{(-1/\text{slope})} - 1$. Primers with slope values between -3.2 and -3.5 only were employed for these reactions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous control for the qPCR analysis. The relative quantification (RQ) values were analyzed (using Light Cycler 480 Software; release 1.5.0) and studied the fold change in the expression levels.

Statistical analysis

For each treatment, three biological replications were included in the qPCR analysis. Statistical analysis was performed with the relative quantification data using ANOVA. The ratio with P-value <0.05 was considered as significant for down or up-regulation. The expression values of unstimulated trees were considered as calibrator for calculating the fold change in the test samples.

RESULTS AND DISCUSSION

The expression of *Hevea ETR1* (*Hb ETR1*), an ethylene receptor identified earlier, in control and stimulated trees was studied by performing semi-quantitative RT-PCR using *Hevea* specific primers for *ETR*. *Hevea* ubiquitin (*Hb UBQ*) was used as an internal control. It was found that *Hb ETR1* was up- regulated after ethephon stimulation both in 1:25 and 1:125 dilutions of cDNA and the internal control *Hb UBQ* was same in both control and stimulated trees (Fig. 1).

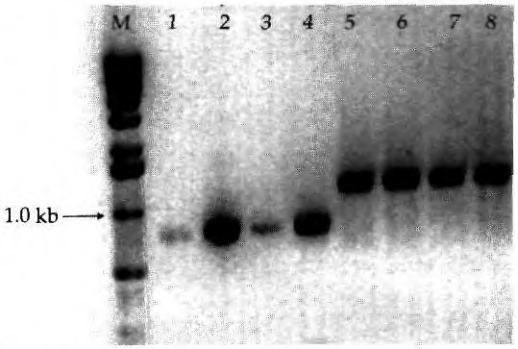

Fig. 1. Semi-quantitative RT-PCR amplification of *Hb ETR1* and ubiquitin (*Hb* UBQ) in bark tissues of *Hevea*. Lanes 1-2: primer *ETR1* (1:25 dilution of cDNA) and 3-4: *ETR1* (1:125 dilution of cDNA); 5-6: ubiquitin primer (1:25 dilution of cDNA) and 7-8: ubiquitin primer (1:125 dilution of cDNA), Lane 1, 3, 5 and 7 were from unstimulated trees; Lane 2, 4, 6 and 8 were from stimulated trees; M-Molecular weight markers

Data from NCBI and poplar genome home page were used for the analysis of aminoacid and nucleotide sequences of ethylene receptors from other plant species. Multiple alignments of complete amino acid sequences were performed and a phylogenetic tree was constructed as shown in Fig. 2. The data indicated that the *Hevea* ethylene receptor has close similarity to subfamily-1 receptors of poplar, peach, *Arabidopsis* and tomato.

Thus the nucleotide sequences of these known dicot receptors were aligned and a

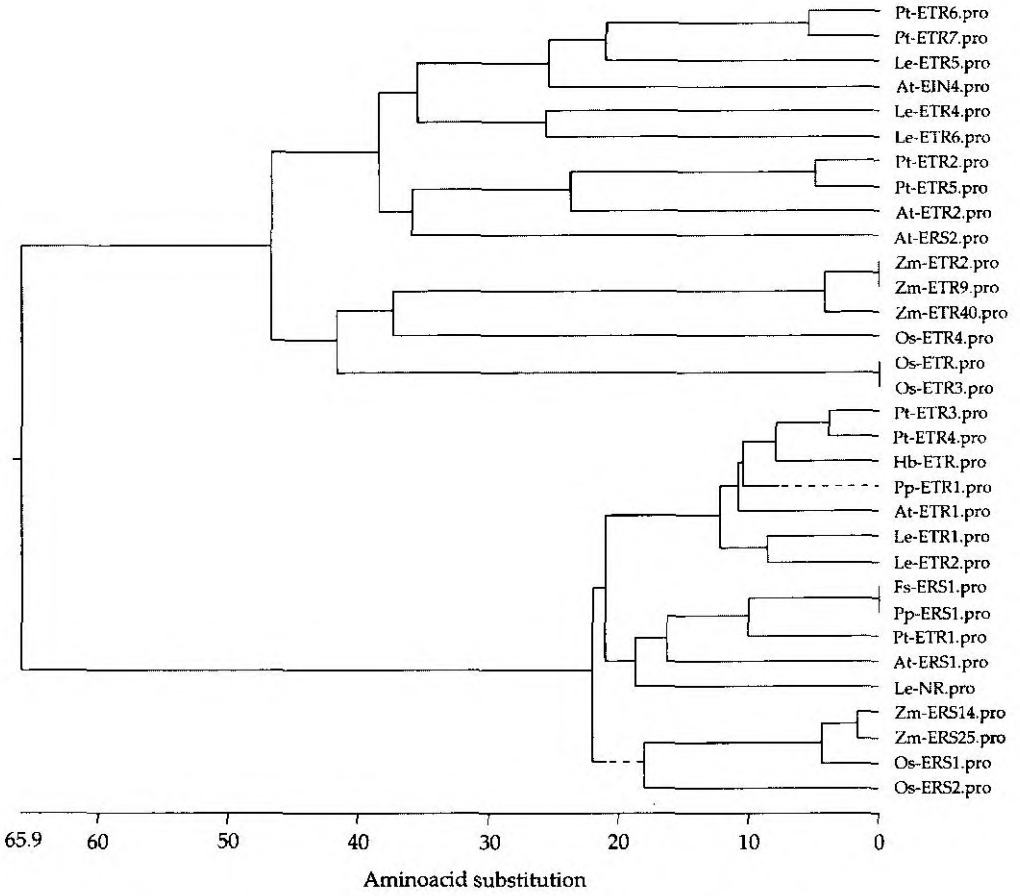

Fig. 2. Phylogenetic tree of different plant ethylene receptors. Plant species and receptor types compared are: *Arabidopsis*, poplar, peach, beech, maize, rice, *Hevea* and tomato. Amino acid substitutions are shown on the X-axis

phylogenetic tree was made (Fig. 3). Degenerate RT-PCR with primers designed from the more conserved regions of dicot receptors yielded amplicons of expected sizes with primers 1, 3 and 4 (Fig. 4). Cloning and transformation of the reaction product and sequencing of the clones resulted in the identification of a new receptor, based on its homology to known ethylene receptors in the database. Out of the 54 clones sequenced, 22 sequences were 100 per cent homologous with database *Hb ETR1* sequences and others represented a new *ETR* sequence. Alignment of the new sequence was carried out and the consensus sequence was generated (Fig. 5) based on the multiple clones we isolated and this new *ETR* sequenced is designated as *Hb ETR2*.

To find out if this new gene is ethylene induced or not, two forward and one reverse

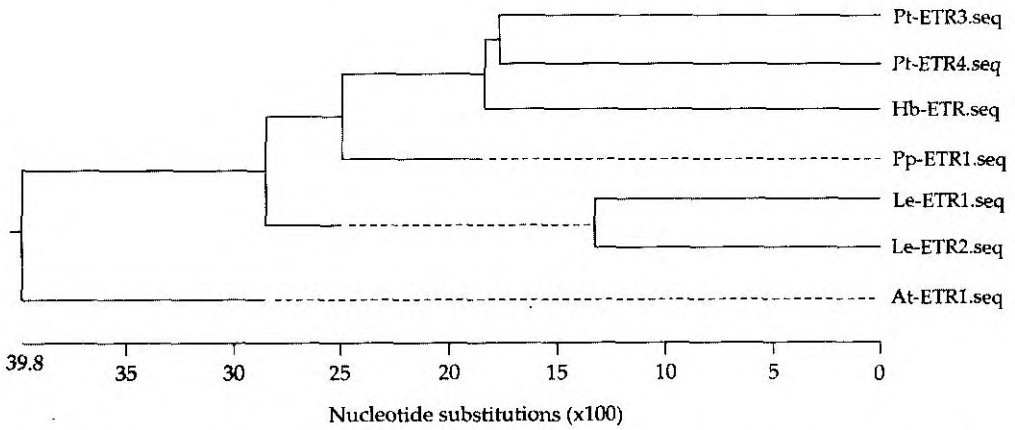

Fig. 3. Phylogenetic tree of dicot receptors most closely related to *Hb ETR1* constructed based on nucleotide sequence data. Plant species included are *ETR's* of *Arabidopsis*, poplar, peach, *Hevea* and tomato

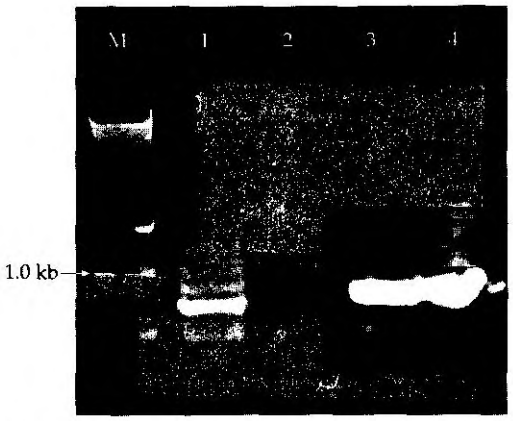

Fig. 4. *Degenerate PCR amplification of Hevea* ethylene receptors.Lane-M: molecular marker; Lane 1-degenerate primer-1; Lane 2-degenerate primer-2; Lane 3-degenerate primer-3; Lane 4-degenerate primer-4

primers were designed (Table 2) based on the nucleotide sequences of the *Hb ETR1* and *Hb ETR2* genes (from RRII 105) to perform relative RT-PCR. The gel electrophoresis analysis revealed that both *Hb ETR1* and the new *Hb ETR2* were up-regulated after ethylene stimulation (Fig. 6). Upon finding this up regulation, relative quantification of *ETR1 and ETR2 was carried out at different* tapping days after stimulation and was compared with unstimulated control trees (Table 3). Significant up regulation of *ETR1* (about 1.5 and 1.6 fold on the third and 7th day respectively) with a considerable dip on the 5th day (to the levels of unstimulated trees) was noticed. However *ETR2* level went up only on the third day (to 1.4 fold) followed by significant reduction to a level of about 0.6 fold. The preliminary data on its expression during the first three alternate tapping days after stimulation indicate that both *ETR1* and *ETR2* were up-regulated immediately after stimulation followed by a significant reduction in its levels in the subsequent tapping days.

The action of ethylene is based on two types of responses such as its cellular concentration and change in the sensitivity of tissues to ethylene. Genetic and biochemical evidences indicate that ethylene receptors act as negative regulators of downstream responses by suppressing the

TGTACACACAAATAAAAGTGACTATCACTTGGCACTGGGA 40
AAAAATCAGGAAACCGAGGATCTCTCAATGATTCTGGTTT 80
AGCAACAAAAGCAGTGATTGAGATGTTCCCTTCTTTAGAA 120
AACTTCACAGCATTACCAACAACATTTAAAATGGTTTGCA 160
TAAGGCGTTTCTGATCACCAATGGCATATTCCGGCAAATC 200
TGGAGCCAAATTTAATGTAACAGGCAACTTTTTAACAGAT 240
GCAATAGGCTTGATCAAGTTAAGAACCTCCCAGAATACAG 280
CGTGAAGATTAAAAGTTCCTAGGTCAAGTTGAAGGCTGCC 320
ATCTTCAAGCCTTGAAAGGTCTAATACATCATTTATTAGA 360
GTAGCCAAGAGGTTACTACTCTTAAGGATTGTCTCAACCA 400
TGAGGCGCTGCTCAGGTGTAAGCTCAGTTTCTTGCAGTAA 440
GGAAGAAAGTGCAATAATTGCATGCATGGGAGTTCTCATT 480
TCATGGTTCATGACAGCTAAGAAATCATTACGAGCACGGA 520
TAGCTGTTTCTGCTTCTCTCCTTGCAAGATCAAGTGCAAC 560
ATTCTGCTCCATAAGAAGATCCCTTGCCCTCATCGACTCT 600
TCTAAGATAGCAGCATGTGATAGAGCAACAGCC 633

Fig. 5. Sequence of *Hb ETR2* clone of *Hevea*.

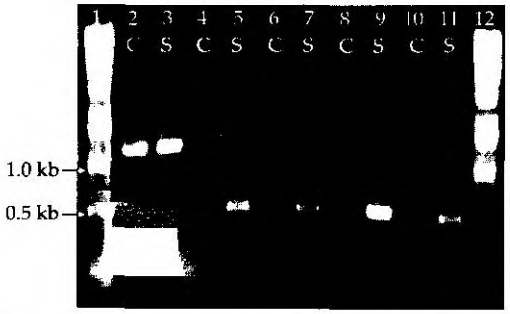

Fig. 6. RT-PCR amplification of *Hb ETR1* and *Hb ETR2*, Lane-1&12- molecular markers; Lane - 2&3 ubiquitin; Lane 4&5- *Hb ETR1* with primer-1; Lane 6&7- *Hb ETR1* with primer-2; Lane 8&9- *Hb ETR2* with primer-1; Lane 10&11- *Hb ETR2* with primer-2; C-Control S-Stimulated

expression of ethylene responsive genes. A receptor in the absence of ethylene actively suppresses the expression of ethylene inducible genes. Once a receptor has bound ethylene, the suppression is relieved and thus activating the downstream responses (Hua and Meyerowitz, 1998). Only way that a tissue can turn off the ethylene response is to synthesize additional receptors (Klee, 2002).

Regulation of each receptor gene by ethylene is different and the variations in expression levels of genes coding for the receptors might be reflected in the intensity of ethylene sensitivity. Investigations on the

Table 3. **Relative quantification of *ETR1* and *ETR2* in ethylene stimulated samples of the *Hevea* clone RRII 105 compared to unstimulated control**

Treatment	*ETR1*	*ETR2*
Unstimulated (control)	1	1
Third day after stimulation	1.54	1.43
Fifth day after stimulation	0.99	0.63
Seventh day after stimulation	1.62	0.62
CD (P<0.05)	0.230	0.065

relationship between changes in ethylene production and the levels of all members of receptor gene family is required to study the function of each ethylene receptor in inducing the responses. The transcriptional induction of these two receptors by ethylene in *Hevea* may likely to play an important role in mediating the immediate ethylene response and increased latex flow after stimulation.

CONCLUSION

Using a degenerate PCR approach, a new ethylene receptor (*Hb ETR2*) in the bark tissue was identified and cloned. Both *Hb ETR1* and *Hb ETR2* were found to be up-regulated immediately after ethephon stimulation.

ACKNOWLEDGEMENTS

This work was supported by Rubber Board, Ministry of Commerce, Govt. of India and the research was performed at Dartmouth College, USA with support from US Department of Energy. The suggestions given by Dr. K. Annamalainathan, Deputy Director, RRII and the help rendered by Ms. Nasnim Ebrahim, Junior Research Fellow, RRII are gratefully acknowledged.

REFERENCES

Amalou, Z., Bangatz, J. and Chrestin, H. (1992). Ethrel (Ethylene releaser) induced increase in adenylate pool and trans tonoplast ΔpH within latex cells. *Plant Physiology*, **98**:1270-1276.

Bleecker, A.B. and Kende, H. (2000). Ethylene: a gaseous signal molecule in plants. *Annual Review of Cell and Developmental Biology*, **16**: 1-18.

Bleecker, A. B. (1999). Ethylene perception and signaling: an evolutionary perspective. *Trends in Plant Science*, **4** : 269-274.

Chang, C. and Shockey, J.A. (1999). The ethylene response pathway: Signal perception and gene regulation. *Current Opinion in Plant Biology*,**2**: 352-358.

Chen, Y.F., Randlett, M.D., Findell, J.L. and Schaller, G.E. (2002). Localisation of the ethylene receptor *ETR1* to the endoplasmic reticulam of *Arabidopsis*. *Journal of Biological Chemistry*, **277**: 19861-19866.

Chang, C. and Stadler, R. (2001). Ethylene hormone receptor action in *Arabidopsis*. *Bioassays*, **23**: 619-627.

Chrestin, H. (1989). Biochemical aspects of bark dryness induced by over stimulation of rubber tree with Ethrel. In: *Physiology of Rubber Tree Latex*, (Eds. J.D'Auzac, J.L.Jacob and H. Chrestin). *CRC Press, Boca Raton*, pp. 431-441.

Coupe, M. and Chrestin, H. (1989). The hormonal stimulation of latex yield: physico-chemical and biochemical mechanism of hormonal (ethylene) stimulation. In: *Physiology of Rubber Tree Latex*, (Eds. J.D'Auzac, J.L.Jacob and H. Chrestin). *CRC Press, Boca Raton*, pp. 295-319.

D'Auzac, J., Crestin, H., Bouteau. F., Clement, A., Jacob, J.L., Lacrotte, R., Prevot, J.C., Pujade-Renaud, V. and Rona, J.P. (1993). Stress ethylene in *Hevea brasiliensis*: physiological, cellular and molecular aspects. In: *Current Plant Science and Biotechnology in Agriculture*, (Eds. J.C. Pech, A. Latche, and Balague, C.). vol.**16.**

Dosotoit-Coucaud, A., Brunel, N., Kongsawadworakul, P., Viboonjun, U., Lacointe, A., Julien, J.L. Chrestin, H. and Sakr, S. (2009). Sucrose importation into laticifers of *Hevea brasiliensis*, in relation to ethylene stimulation of latex production. *Annals of Botany*, **104**: 635-647.

Dosotoit-Coucaud, A., Kongsawadworakul, P., Maurousset, L., Viboonjun, U., Brunel, N., Pujade-Renaud, V., Chrestin, H. and Sakr, S. (2010). Ethylene stimulation of latex yield depends on the expression of a sucrose

transporter (*Hb SUT1B*) in rubber tree (*Hevea brasiliensis*). *Tree Physiology*, **30**:1586-1598.

Fluhr, R. and Mattoo, A.K. (1996). Ethylene-biosynthesis and perception. *Critical Reviews in Plant Sciences*, **15**: 479-523.

Gao, Z., Chen, Y.F., Randlette, M.D., Zhao, X.C., Findell, J.L., Kieber, J.J. and Shaller, G.E. (2003). Localisation of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. *Journal of biological chemistry*, **278:** 34725-34732.

Gidrol, X., Chrestin, H., Mounoury, G. and D'Auzac, J. (1988). Early activation by ethylene of the tonoplast H^+ pumping ATPase in the latex from *Hevea brasiliensis*. *Plant Physiology*, **86**: 899-903.

Gohet, E., Chantuma, R., Lacrotte, R., Obouayeba, Dian, K., Clement Demange, A., Kurnia, D. and Eschbach, J.M. (2003). Latex clonal typology of *Hevea brasiliensis*: Physiological modeling of yield potential and clonal response to ethephon stimulation. *International workshop on Exploitation Technology*, 15-18 December, 2003, pp. 199-217.

Klee, H.J. (2002). Control of ethylene mediated processes in tomato at the level of receptors. *Journal of Experimental Botany*, **53** (377): 2057-2063.

Hua, J. and Meyerowitz, E.M. (1998). Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell*, **94**: 261-274.

Lashbrook, C.C., Tieman, D.M. and Klee. H.J. (1998). Differential regulation of the tomato ETR gene family throughout plant development. *Plant Journal*, **15**: 243-252.

Mita, S., Kawamura, S., Yamawaki, K., Nakamura, K. and Hyodo, H. (1998). Differential expression of genes involved in the biosynthesis and perception of ethylene during ripening of passion fruit. *Plant Cell Physiology*, **39**: 1209-1217.

Pujade-Renaud, V., Montoro, P., Sanier, C., Kangsawadworakul, P. and Chrestin, H. (2001). Specific promoters for genetic engineering of rubber tree. *Proceedings of the IRRDB Symposium Biotechnology and Rubber Tree*, Montpellier, France, pp. 125-132.

Qu, X. and Schaller, G.E. (2004). Requirement of the histidine kinase domain for signal transduction by the ethylene receptor ETR1. *Plant Physiology*, **136**: 2961-2970.

Schaller, G.E. and Bleecker, A.B. (1995). Ethylene binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. *Science*, **270**:1809-1811.

Schaller, G.E. and Keiber. J.J. (2002). Ethylene. In: *The Arabidopsis book*, (Eds. C.Somerville and E.Meyerowitz), http://www.aspb.org/publications/Arabidopsis/DOI/10.1199/TAB.0071, American Society of Plant Biologists, Rockville, MD.

Simon, S.P. (2003). *Carbohydrate metabolism and ionic balance in the latex production mechanism in* Hevea brasiliensis. Ph. D. Thesis, Mahatma Gandhi University, Kottayam, India, 187p.

Sreelatha, S. (2003). *Biochemical factors influencing latex flow during stress, tapping frequency and stimulation in Hevea brasiliensis*. Ph. D. Thesis, Mahatma Gandhi University, Kottayam, India, 204p.

Thomas, M., Nair, D.B., Gopalakrishnan, J., Sreelatha,.S. and Jacob, J. (1999). Ethephon application increases the rubber yield from *Hevea brasiliensis* by altering the water relations of latex. *Journal of Plant Biology*, **26** (3): 191-198.

Tupy, J. and Primot, L. (1976). Control of carbohydrate metabolism by ethylene in latex vessels of *Hevea brasiliensis* Mull. Arg. in relation to rubber production. *Biologia Plantarum*, **18**: 373-383.

Yang, X., Jin, Z., Zhang, G., Xu, B., Zhang, J. and Feng, R. (2005). Cloning of the ethylene receptor gene *ETR1* of *Hevea brasiliensis* (Gen Bank submission AY847291).