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Physiological and anatomical changes in the lacticiferous tissues of *Hevea* under ethylene stimulation

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INTRODUCTION

Stimulating rubber trees with ethylene compounds is a common plantation practice for increasing crop productivity. Commercially available yield stimulant such as ethephon (2-chloroethyl-phosphonic acid) is widely used for this purpose. Ethephon application can induce several stress compounds which may cause metabolic changes in the lacticiferous tissues. It include increase in turgour pressure, initial flow rate and decrease in plugging index that are leading to prolonged latex flow and increased latex yield. Ethylene, the active ingredient in ethephon, is a gaseous plant hormone involving various growth and development activities in plants. Frequent stimulation of rubber trees with ethephon is reported to induce several physiological disorders (Paranjothy *et al.*, 1979). Exogenous application of ethylene is known to enhance endogenous ethylene production autocatalytically (Kevin *et al.*, 2002). In stimulated trees, the high ethylene level in the bark tissues can eventually lead to oxidative stress. This may ultimately lead to inhibition of rubber biosynthesis and reduced crop productivity. Hence, a study has been carried out to analyze the long term effect of stimulation in virgin and renewed bark tissues of *Hevea* trees.

MATERIALS AND METHODS

A group of trees with renewed panel (B1-2) under regular tapping (S/2 d3) with uniform latex yield were selected from a mature population of the clone RRII 105. Another group of trees of same age and clone under tapping in virgin panel (BO-1) were also used for comparison. Both groups of trees were stimulated with 5% ethephon through bark application, three times in a year (September, November and April). Another set of unstimulated trees from both the groups were maintained as control. Bark samples were collected from the tapping panel of stimulated and unstimulated trees periodically (one month after stimulation) for analyzing the biochemical components like Cyanide (CN), Hydrogen peroxide (H_2O_2) Malondialdehyde (MDA), β-cyanolalanine synthase (β-CAS) and Peroxidase (PX) as per the standard protocols. Latex yield, dry rubber content (DRC) and tapping panel dryness (TPD) were also analyzed at monthly intervals. Bark tissues from the tapping panel were collected simultaneously for anatomical studies. Microtome sections at 60µm thickness were taken and stained with Oil red for microscopic observations.

RESULTS AND CONCLUSIONS

Increased latex yield was noticed in both the groups of stimulated trees. However, trees with virgin panel showed yield enhancement for longer duration than the trees with renewed panel. The trees stimulated on

Table 1. Biochemical Components in the Virgin Panel (BO-1)

Treatments		Components analysed				
		H_2O_2 (µmol/ mgtissue)	Px (unit/min/ mg protein)	CN (mg/g tissue)	β-CAS (nmol H_2s/min/ mg protein)	MDA µmol/g (fractional wt
I[st] stimulation	Stimulated	0.21 ± 0.01	0.16 ± 0.06	7.21 ± 0.30	0.45 ± 0.03	6.38 ± 0.47
	Control	0.15 ±0.02	0.36 ±0.16	5.48 ±0.65	1.39 ± 0.19	4.96 ± 0.49
2[nd] stimulation	Stimulated	0.17 ± 0.01	0.31 ± 0.13	7.30 ± 0.39	1.44 ±0.17	6.67 ± 0.45
	Control	0.14 ± 0.01	0.74 ± 0.09	5.82 ±0.27	1.73 ± 0.18	3.35± 0.33
3[rd] stimulation	Stimulated	0.19± 0.03	1.37 ±0.44	8.62 ± 0.71	0.04±0.01	8.08 ± 0.37
	Control	0.12±0.028	3.66 ± 0.50	6.08 ± 0.26	0.16±0.03	5.58 ± 0.53

Table 2. Biochemical Components in the Renewed Panel (B1-2)

Treatments		Components analysed				
		H_2O_2 (µmol/mgtissue)	Px (unit/min/ mg protein)	CN (mg/g tissue)	β-CAS (nmol H_2s/min/ mg protein)	MDA (µmol/g fractional wt)
I[st] stimulation	Stimulated	0.2 ± 0.02	0.56 ± 0.21	8.43 ± 0.68	0.04±0.02	6.36 ± 0.37
	Control	0.15±0.01	1.61 ± 0.58	6.36 ±0.52	1.09 ± 0.06	3.80 ±0.59
2[nd] stimulation	Stimulated	0.14±0.01	1.04 ± 0.22	7.91 ± 0.58	0.79±0.10	7.19±0.97
	Control	0.12 ±0.02	1.55 ± 0.33	5.53 ±0.40	1.03±0.09	3.34 ± 0.49
3[rd] stimulation	Stimulated	0.18 ±0.01	0.76 ± 0.32	8.30±0.62	0.07±0.02	7.76 ± 0.52
	Control	0.13±0.02	5.10 ± 0.32	5.88 ± 0.25	0.12±0.03	4.47 ± 0.38

virgin and renewed panels showed oxidative stress as evidenced by the appearance of stress components (Table 1 and 2). H_2O_2 showed significant increase in the stimulated trees irrespective of the age of the panel. Though the peroxidase activity showed seasonal variations, the stimulated trees showed decreased peroxidase enzyme activity compared to unstimulated controls. The accumulation of H_2O_2 in the bark tissues of stimulated trees may be due to the decreased level of the scavenging enzyme, peroxidase. The stimulated trees also showed high malondialdehyde (MDA) contents as a result of the enhanced lipid peroxidation. Cyanide, a co-product of ethylene biosynthesis, increased in the tissues of stimulated trees irrespective of the age of the panel. However, the maximum accumulation of cyanide was noticed in trees stimulated on renewed panel. On the contrary, β-cyanolalanine synthase (β-CAS) activity was decreased in stimulated bark. β-CAS activity was significantly high in the unstimulated trees with a low CN content. This indicate that the production of cyanide molecules at levels much higher than what can be scavenged by the detoxifying enzyme (β-CAS) in stimulated trees can cause oxidative stress.

Bark anatomical studies showed an increase in the bark thickness in trees stimulated on virgin panel. But, no significant change was observed in the bark thickness of trees stimulated on renewed panel. The percentage of functional and disorganized lacticiferous vessels were more in trees stimulated on virgin panel compared to unstimulated trees. This variation in the lacticiferous vessels was not noticed in trees stimulated on renewed

panel. The anatomical observations showed that ethephon has a major role in accelerating lacticiferous tissue differentiation in *Hevea*. More TPD incidence was noticed in trees stimulated on virgin panel compared to renewed panel.

Ethephon application could induce changes in lacticiferous tissue disorganization and oxidative stress in *Hevea* trees. Therefore, it is advisable to use ethylene compounds judicially in rubber plantations.

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