EFFECT OF ETHEPHON CONCENTRATION ON RUBBER YIELD THROUGH MAGNESIUM ION AND SUCROSE REGULATION IN *Hevea brasiliensis*

A.P. Attanayake*[1], L. Karunanayake[2] and A.H.R.L Nilmini[1]
[1]Rubber Research Institute of Sri Lanka, Ratmalana
[2]Department of Chemistry, University of Sri Jayawardenapura
**Corresponding author (email: anusha_rrisl@yahoo.com)*

Introduction

***Hevea brasiliensis* is currently the world's crucial source of natural rubber (cis-1, 4-polyisoprene). In this species, the rubber is produced in the form of cytoplasmic rubber particles contained within latex produced in laticifers. Natural rubber is made out of isoprene units derived from isopentenyl diphosphate (IPP). Natural rubber is synthesized by rubber transferase which requires an allylic pyrophosphate (APP) to initiate the rubber molecule, isopentenyl pyrophosphate (IPP) as the source of monomer use to elongate the polymer and a divalent cation such as Mg^{2+} or Mn^{2+} as cofactor.**

Biosynthesis of natural rubber, like other secondary metabolites, is affected by various plant hormones. Ethephon stimulation has been widely used in commercial latex production to get high yield with minimum bark consumption rate. Ethephon chemically decomposed to release ethylene and it acts on membrane permeability, leading to prolonged latex flow. On general regenerative metabolism, ethylene treatment increased the activity of invertase resulting in glycolysis acceleration, leading to improving the supply of carbon source such as Acetyl Coenzyme A for rubber biosynthesis (Tupy, 1984).

Sucrose in latex is the primary source of acetate and acetyl CoA essential for biosynthesis of rubber. A high sucrose content in latex may indicate a good loading if latex vessels and active metabolism. It may also indicate low metabolic utilization of sucrose and low productivity. Ethephon stimulation reduced the overall means of the serum sucrose & it converts sucrose to glucose and fructose. Sucrose contents and its metabolism intensity were considered as a limiting factor for rubber biosynthesis.

In vitro, the concentration of Mg^{2+} radically affects the affinity of the *H. brasiliensis* rubber transferase for IPP. Mg, which suggests that the Mg^{2+} concentration may have a regulatory role in rubber biosynthesis (Da Costa *et al*., 2005). The metal ion cofactor concentration also affects the molecular weight of the rubber produced by *H. brasiliensis*.

Here, we characterize the role of ethephon concentration on (a) quality & quantity of rubber produced, (b) Correlation between rubber yield and sucrose concentration, (c) Correlation between rubber yield and magnesium concentration.

Methodology

Five experimental blocks from mono clonal (RRISL 121) mature rubber trees were selected from the Galewatta Division, Dartonfield estate for the experiment. The treatment plots were set up allocating 25 trees to each plot. Trees in each block were stimulated with different concentrations of ethephon ranging from (1-5%). Tapping

frequency was half spiral once in three days. All the experimental tapping blocks were tapped by the same tapper in order to avoid tapper variability. Freshly tapped latex was collected in to vessels immersed in ice and sent to the laboratory immediately for testing without preservation.

Analysis of sucrose content was carried out by using colorimetric method developed by Scolt and Melvin, (1953). Serum was extracted by coagulating 1 g of latex with 2.5% tri-chloro acetic acid (TCA) and brought to 25 mL. Anthrone reagent was prepared by dissolving 0.1 g anthrone in a mixture of conc. H_2SO_4 and water in the ratio of (100:29). 0.1 mL of serum extract was diluted with 0.5 mL of 2.5% TCA and mixed with 3 mL anthrone reagent. Reaction mixture was heated in a boiling water bath for 15-20 min. Samples were cooled to room temperature and optical density was measured at 620 nm. The amount of sucrose present was estimated against a calibration curved prepared using sucrose standard.

Serum magnesium content analysis was carried out by EDTA titrimetric method developed by RRISL. 1g of latex sample was diluted with 100 mL distilled water. 10 mL buffer solution (pH=10) and 4 mL KCN solution was added to diluted field latex sample. It was titrated with 0.01M EDTA solution.

Results and Discussion

As shown in Figure 1, a transient decrease in serum sucrose concentration was observed up to 3% ethephon concentration, followed by continuous increment afterwards. Yield response to ethephon greatly depends on sucrose availability in latex vessels determining the possibilities for an enhancement of invertase activity after treatment. There is a negative correlation between yield and sucrose level as shown in Figure 2. Sucrose tends to accumulate in the latex serum when in situ regeneration is complete or latex metabolism slows down.

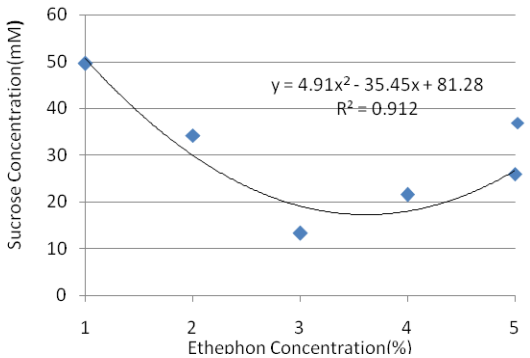

Figure 1. Serum sucrose concentration dependence on ethephon concentration in RRISL 121 clone,S 1/2 d/3 tapping system. Sucrose level change with polynomial pattern with minimum sucrose at 3% ethephon.

Da Costa *et al* (2005) suggested that, cytosolic magnesium concentration as a regulatory mechanism for rubber bio synthesis and molecular weight *in vivo*. The increase in latex yield which coincided with the decrease in magnesium of latex serum soon after

stimulation lends some support to this postulation which, however, requires further investigation.

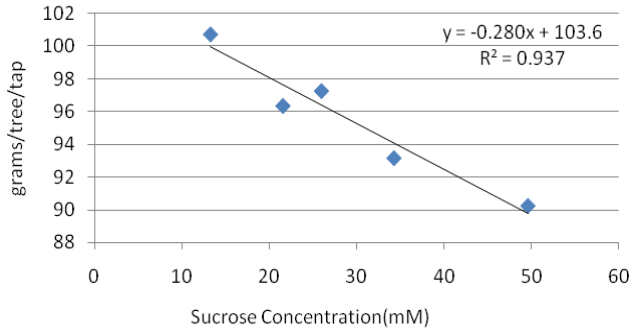

Figure 2. Correlation between serum sucrose level and yield (grams/tree/day) in RRISL 121 Clone,S 1/2 d/3 tapping system with ethephon treatment

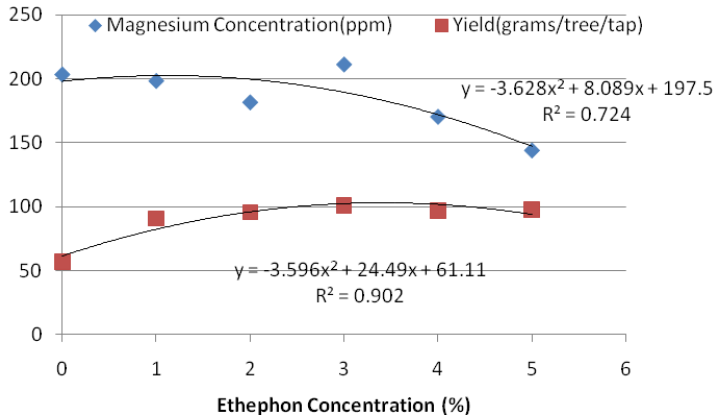

Figure 3. Dependence of ethephon concentration for serum magnesium level and yield in RRISL 121 clone, S/2 d/3 tapping system.

Conclusions and Recommendations

Our study indicates that serum sucrose level gradually reduced up to 3% while serum magnesium level and rubber yield increased up to 3% ethephon concentration. There is a negative correlation between rubber yield with serum sucrose levels and serum magnesium level. This might be due to high utilization rate of both sucrose and magnesium for latex regeneration. It can be concluded that the both magnesium and sucrose levels can be regulated by ethephon concentration which has a regulatory effect on rubber biosynthesis.

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