Industrial Crops and Products 50 (2013) 803–808

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Ultrasound-assisted tapping of latex from Para rubber tree *Hevea brasiliensis*

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ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 22 August 2013
Accepted 22 August 2013

Keywords:
Ultrasound stimulation
Rubber yield
Latex
Physiological parameters
Rubber tree
Hevea brasiliensis

ABSTRACT

Para rubber tree, *Hevea brasiliensis*, is a major commercial source of natural rubber that has been extensively used to manufacture high quality rubber products. It is important to develop a safe and efficient latex harvest technique associated with suitable stimulation for high and sustained rubber yields. This work investigates an innovative rubber yield stimulation technology, using ultrasound as a pre-treatment on the tapping cut surface of the rubber trees. The field trial results demonstrate that ultrasound treatment of 4 min can increase latex and dry rubber yields by 23% and 14%, respectively, on an average of 50 replications which is similar to Ethrel stimulation. Unlike the Ethrel stimulation which increases the latex fluidity through dilution, ultrasound treatment may have also enabled higher supply and uptakes of sucrose for more active rubber biosynthesis, as evidenced from the sonication induced changes in sucrose and inorganic phosphorus contents. However, a decrease in thiols content in ultrasound treatment is notable. This ultrasound technology may also be useful for the stimulation of other latex, juice, and paint producing plants.

1. Introduction

Natural rubber (cis-1,4-polyisoprence) is a kind of high molecular weight polymeric substance. The Para rubber tree, *Hevea brasiliensis*, a member of the spurge family Euphorbiaceae, is a major commercial source of natural rubber latex whose main constitute is rubber particles (Dusotoit-Coucaud et al., 2010). Natural rubber particles are synthesized in the specialized articulated laticifer network embedded in the inner soft bark tissues of *H. brasiliensis*. Upon bark tapping, the laticifer vessels are opened and their milky phloem cytoplasm, i.e., latex, is expelled due to the liber tissue turgor pressure of the laticifer vessels until their extremity is plugged from coagulation (d'Auzac and Jacob, 1989).

Even though the demand for natural rubber materials has been markedly increased over the last decades, the cultivation region of *H. brasiliensis* is limited climatically. Therefore, increasing the rubber yield of *H. brasiliensis* trees is very important to meet the demands for high quality rubber materials. Substantial efforts have been made to understanding yield-limiting factors and developing new technologies to increase rubber yield (Kongsawadworakul et al., 2009; Priyadarshan, 2011; Tungngoen et al., 2009, 2011). While the underlying mechanisms governing rubber production and regeneration are not fully understood yet, it has been revealed that the rubber yield is primarily determined by two factors (Priyadarshan, 2011):

1. the latex flow which is mainly affected by the aggregation–coagulation of rubber particles in the latex within the laticifer vessels and governs the quantity of latex exudation after tapping; and
2. the latex regeneration ability which controls the reconstitution of rubber particles and the latex exudation potential between two tappings.

Up to now, various chemical treatments have been applied to increase latex yield of every tapping (Priyadarshan, 2011) while genetic modifications or chemical stimulations have been applied to enhance metabolites of rubber trees (Tungngoen et al., 2009, 2011). Among them, the chemical treatment with Ethrel (2-chloroethylphosphonic acid), an ethylene releaser upon plant metabolism, on the tapping cut surface of the rubber tree barks is the most widely used one which can increase latex yield up to 1.5–2.0 folds and has now been used as a routine stimulant in rubber tree plantations worldwide (Zhu and Zhang, 2009). However,

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0926-6690/$ – see front matter
http://dx.doi.org/10.1016/j.indcrop.2013.08.065

the overuse of Ethrel could cause deleterious effects during the economic life of rubber trees and thus eventually reduce yield (Jetro and Simon, 2010). In addition, Ethrel has been classified as hazardous, environmentally deleterious and harmful to human being either by inhalation or in contact with skin. Therefore a green technology such as environmental friendly physical and mechanical stimulations will be very useful.

Ultrasound is a form of mechanical energy which can vibrate and move particles within a specific medium and has extensively been used as an effective method to obtain stable dispersions of mineral or organic solid compounds in liquid medium in chemical, biological or materials laboratories (Filgueiras et al., 2001; Ma and Zhang, 2004; Miller, 1983; Rokhina et al., 2009; Starchevsky et al., 2012; Tomsa et al., 2008). More recently, ultrasound assisted technology has been applied to modern wastewater treatment (Ayyildiz et al., 2011; Pilli et al., 2011; Sanchez-Prado et al., 2008), and food processing, preservation and extraction (Albu et al., 2004; Boonkird et al., 2008; Cravotto et al., 2008; Eh and Teoh, 2012; Lima and Andrade, 2011; Sun et al., 2011). For instance, ultrasonic treatment has been successfully applied to control the viscosity of starch (polysaccharide) solutions and extract active compounds from various plants (Ebringerova and Hromadkova, 2010; Iida et al., 2008; Vinatoru et al., 1997). Ultrasonication was also employed as a suitable technique to improve kasturi lime (*Citrus microcarpa*) juice quality by retaining its antioxidant and bioactive compounds and improving its safety and quality standards (Bhat et al., 2011). Major advantages of using ultrasound treatment include more effective mixing, faster energy and mass transfer, reduced concentration gradients, selective extraction, faster response to process extraction control, increased production and elimination of process steps (Chemat et al., 2011).

The diffusivity of water and dry matter in apple (Granny Smith var.) immersed in a sucrose solution could be sped up significantly under ultrasound treatment when the acoustic intensity was above 10.8 W/cm^2 (Cárcel et al., 2007) as ultrasound irradiation will result in cavitation phenomena and enhancement in mass transfer (Rooze et al., 2013). Ultrasound can influence plant physiologies, such as respiration intensity, germination rate, rooting, and some metabolic pathways (Ma and Zhang, 2004). It can play an important role in the growth and secondary metabolite biosynthesis of cultured *Panax ginseng* cells (Lin et al., 2001) since ultrasound stimulation is beneficial not only to inducing the defence responses of ginseng cells but also to the secondary metabolites synthesis without affecting the biomass yield of ginseng cell cultures.

This study aims to investigate the potential of using ultrasound-assisted tapping technology to increase rubber yield from the Para rubber tree *H. brasiliensis*. The influence of ultrasound treatment time (0, 1–4 min, respectively) on latex yield, dry rubber content and relative rubber yield was evaluated and compared with untreated trees. The impact of sonication on the biochemical compositions of the latex was also examined as they provide very useful information on the status of the laticiferous system.

2. Materials and methods

2.1. Planting materials

The Para rubber tree *H. brasiliensis*, was employed as a model plant to study and understand the effects of ultrasound stimulation. To eliminate the impacts of cultivation environment, plant materials and tapping history on rubber yield, 30 budded rubber trees with similar girth, latex yield and dry rubber content and without any previous stimulation were selected from the same experimental tapping task which was established in the same year (2002) with uniform cultivar (CATAS 7-33-97) at the trial field of Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan, China. The planting density was 3 m × 7 m. The rubber trees in the tapping task were 8 years old and subjected to 2 years regular tapping under the tapping system of s/2d/3 (tapping every 3 days with half spiral) at the experiment time of June–July 2010. The 30 selected trees were randomly divided into six groups, each having five trees: 1 control group (CK) without any physical and chemical treatments, 4 ultrasound groups treated with a treatment time of 1, 2, 3, and 4 min, respectively (i.e., UT1, UT2, UT3 and UT4), and an Ethrel group (ET) treated with 1 g of 0.5% Ethrel every three tappings on the tapping cut.

2.2. Ultrasound and Ethrel stimulations

Considering the threshold of ultrasound to vascular system cavitation, latex enzyme activity, and also its applicability to ultrasound assisted tapping and market availability, a sonic brush (Qianbaimei Electrical Co. Ltd., Ningbo, China) with an output power of 3 W at 5 V and ultrasound frequency of 32 kHz was used in this preliminary study.

The electrical acoustic intensity dissipated from the ultrasonic brush and on the surface line of the tapping cuts could be calculated according to the following formula:

$$I = \frac{P}{A} \tag{1}$$

where P is the input power, A is the area of the sonic brush. Therefore, it generated an ultrasonic power density of 2.14 W/cm^2 at the scanning area of 2.0 × 0.7 cm^2 of the brush.

In the experiment, the five trees in the control group were not treated with any stimulation. For the trees treated with ultrasound, ultrasound was applied to a respective period at roughly 10 h before next tapping for rubber latex. Typically, a small amount of water, as an efficient ultrasound transmission medium, was supplied with a sprayer during the ultrasound treatment. Simultaneously, the brush scans forth and backwards on the tapping cut surface of laticiferous network in the rubber tree barks for 1, 2, 3, and 4 min, respectively. For the Ethrel stimulation trees, 1 g of 0.5% (v/v) Ethrel in carboxyl methyl cellulose (w/w, 1%) was applied on the tapping cut 24 h before the tapping as the conventional Ethrel stimulation tapping system. Then the trees were subjected to tapping three times and stimulated with Ethrel again.

2.3. Latex tapping

In the present study, all of the rubber trees were consecutively tapped 10 times under the same conditions, i.e., same depth, height, direction, and frequency of tapping (i.e., every three days) in a half spiral pattern (S/2).

Ten to 24 h after each ultrasound and Ethrel treatment, the rubber trees were tapped at the same time and latex samples were collected from each tree and kept in a sterilized, air tight container at 4 °C for further analysis. All sample preparations, yield and tests were carried out from ten tappings at each tree. The results are expressed as mean ± standard deviation.

2.4. Rubber yield

In order to calculate relative rubber yield, latex yield and dry rubber content were firstly determined.

2.4.1. Latex yield

To determine latex yield, latex was collected into a pre-weighed cup and then transported to laboratory after the cessation of latex exudation. The net weight of latex was thus determined and the latex was subsequently prepared for other measurements.