J. Agr. Sci. Tech. (2023) Vol. 25(3): 687-699

Ethephon Stimulation on Trunk Leads to Leaf Physiological Changes in Rubber Tree Seedlings

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ABSTRACT

Ethephon was widely used in rubber tree plantation to stimulate latex production. Numerous studies demonstrated that ethephon treatment prolongs the flow of the latex and promotes latex regeneration in the trunk of mature rubber tree seedlings. However, how rubber tree leaves responded to ethephon treatment on the trunk is still unknown. We used rubber tree seedlings to detect the physiological response of leaves after the trunk treatment with ethephon. The photosynthetic rate, the sugar and starch content, as well as the enzyme activities involved in sugar metabolism were measured after 0, 12, 24, 36 and 48 hours with 0.6% ethephon treatment. The result demonstrated that ethephon treatment increased latex production on the trunk, while the net photosynthetic rate, transpiration rate, and stomatal conductance in leaves were significantly reduced. At the same time, sucrose decreased significantly with concomitant slight increase in glucose and fructose. Also, the enzymatic activities of Sucrose Phosphate Synthase (SPS), Sucrose Synthase (SS) and Neutral/alkaline Invertase (NI) increased significantly after ethephon treatment. Ethephon treatment affected the starch content, but did not change the composition of starch in rubber tree seedlings leaves; the overall starch changing pattern was similar to that of sucrose in leaves. It can be concluded that ethylene-stimulated latex production in rubber tree seedlings is partly due to the alteration of sucrose metabolism in leaves, and ethylene has an adverse physiological effect on rubber trees.

Keywords: *Hevea brasiliensis* Muell. Arg., Enzymes activity, Photosynthesis, Starch, Sucrose.

INTRODUCTION

Rubber tree (*Hevea brasiliensis* Muell. Arg.) originated in the Amazonian Basin and is cultivated in numerous tropical areas (Dusotoit-Coucaud *et al*., 2010). It is the only commercially available source of natural rubber (cis-polyisoprene) (Mooibroek and Cornish, 2000; Beltrano *et al*., 1999; Chrestin, 1989; Rahman *et al*., 2013). All commercially cultivated rubber tree clones were derived from the seeds that were collected by Wickham in 1876 (Souza *et al*., 2015). Although there are slight differences in latex yield in different rubber tree clones, the narrow genetic background of rubber trees makes it difficult to obtain a variety with significant latex production enhancement (Zeng and Huang, 2004).

In order to get more latex from the rubber tree, an alternative way using stimulating chemicals have been applied to rubber trees since the early 20th century (Abraham, 1970), and results indicated that ethylene is the most effective reagent (Chrestin *et al*., 1989). The subsequent research on its stimulating

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[DOI: 10.22034/jast.25.3.687]

mechanism indicates that ethylene not only extends the latex outflow time but also enhances the stability of latex (D'Auzac *et al*., 1997; Sainoi and Sdoodee, 2012). The detailed analysis indicated that with ethephon treatment, latex coagulation activity (Ko *et al*., 2003), rupture index and blockage index were reduced (Wititsuwannakul *et al*., 2008; Chrestin, 1989; Salomez *et al*., 2014). Aquaporins played an important role in ethylene enhanced latex production, after ethephon treatment, and its expression was similar to those using other plant hormones that promoted transient latex production, such as auxin, salicylic acid, and abscisic acid (Tungngoen *et al*., 2009). ERF (Ethylene Response Factors) might also contribute to the increased latex yield. According to Lestari *et al*. (2018), the function of HbERF-IXc5, in contrast with Arabidopsis ERF1, was rubber-specific, as transgenic plants overexpressing HbERF-IXc5 accumulated more starch and had more differentiated latex cells at the histological level. Another study also revealed that *ERF* genes are induced upon laticifer differentiation (Duan *et al*., 2013). Genome sequencing and transcriptome analysis indicated that other physiological changes such as methionine, glutamine synthetase and reactive oxygen species generation were boosted by ethylene.

Sucrose is the main product of photosynthesis and its transportation as well as metabolism provide the precursors for rubber biosynthesis, and recent analysis indicated that it played a vital role in ethylene enhanced natural rubber biosynthesis. Ethylene elevated the invertase activity of latex, thereby accelerating the decomposition of sucrose (Tupý, 1973). The concurrent rapid acceleration of the glycolytic pathway with ethylene treatment provided more precursors for the biosynthesis of Isopentenyl pyrophosphate (IPP) and natural rubber (Liu *et al*., 2016) and subsequently led to the enhanced latex biosynthesis. In addition, The ATPase activity on the protoplasm as well as the expression of sucrose transporter (HbSUT2A, HbSUT2B, HbSUT3, HbSUT1B) genes was improved after ethylene treatment, thereby providing more energy for sucrose transportation (Gidrol *et al*., 1994); on the other hand, it increased the sucrose loading into laticifer cells (Dusotoit-Coucaud *et al*., 2010; Tang *et al*., 2010).

Ethylene is a versatile plant hormone widely used in agriculture, and some reports indicated that its application decreased the yield and quality of certain crops. Ethylene response pathway was involved in the induction of dormancy and prevention of flowering in wild chrysanthemum (Sumitomo *et al*., 2008). Exogenous ethylene inhibited the germination of potatoes (Sumitomo *et al*., 2008). Excessive release of ethylene-induced by drought was associated with reduction of wheat grain weight (Beltrano *et al*., 1999; Sumitomo *et al*., 2008), enhanced the production of secondary metabolites such as alkaloids in tobacco (Shoji *et al*., 2010), and increased the yield of taxanes in *Taxus chinensis* cells (Pan *et al*., 2000) and promoted the formation of β-thujaplicin in cypress cells (Zhao *et al*., 2004). In rubber tree seedlings, ethylene was only applied in the bark, but it might disperse to the upper part. Although numerous reports provided detailed latex and bark physiological analysis with trunk treatment of ethephon, how ethylene treatment on bark affected leaf metabolism is still unknown.

In this study, the leaf physiological changes after trunk ethephon treatment was monitored in order to: (1) Know whether ethephon trunk treatment affects leaves and (2) Analyze how ethylene-induced physiological change in leaves that might lead to the enhanced production of latex in the trunk, with special emphasis on leaf sucrose synthesis and decomposition.

MATERIALS AND METHODS

Plant Materials

Eight months old rubber tree seedlings (Reyan 7-33-97) derived from tissue culture were used in this study. The seedlings, about 0.5 cm in stem girth and 50 cm in height, were planted in the experimental plantation

[DOI: 10.22034/jast.25.3.687]

in Chinese Academy of Tropical Agricultural Sciences in Danzhou City, Hainan Province, China.

Ethephon Treatment

Similar seedlings with the trunk diameter of about 0.5 cm were selected. Then, a scalpel was used to remove the leathery layer in the same position by gentle scraping of the bark to avoid injury to the phloem. The scraped cylinders of about 2 cm in length were wrapped with absorbent paper, and 1 mL ethephon with the concentration of of 0, 0.3, 0.6, 0.9 and 1.1% (w/v) was dropped onto the absorbent paper. The treated stems were wrapped with Para film to ensure continuous ethephon stimulation. After 24 hours, the Para films were removed, and 4 longitudinal cuts at the treated positon and the latex flowed from the cuts was collected. After 0, 12, 24, 36, and 48 hours, leaf samples adjacent to the treated sites were collected, froze in liquid nitrogen, and stored at -80℃ until use. The experiment was set up according to the randomized complete block design with five replications.

Chlorophyll Content Determination and Leave Gas Exchange

Fully expanded fresh leaves of 0.5 g were extracted in 95% (v/v) ethanol. Chlorophyll concentrations were determined spectroscopically according to the method of Hu *et al*. (2014). The absorbance of the cleared extract incubated for 48 hours in the dark at 4℃ were measured at 665, 649 and 470 nm for Chlorophyll *a* and Chlorophyll *b*.

The net photosynthetic rate, intercellular CO_2 concentration, transpiration rate, and stomatal conductance were measured with portable photosynthesis system Li-6400 (LiCor Inc., USA). Three fully expanded leaflets were measured in each seedling, and there were 5 replicates per treatment. The measurement was carried out on a sunny day from 9:30 AM to 10:30 AM at the relative humidity of about 70%, leaf temperature of 26±0.5℃, under a controlled CO_2 concentration of 400 μmol m^{-2} $s^{-1}$and light intensity of 800 μmol m^{-2} s^{-1}.

Determination of Sugar and Starch Content in Leaves

The sucrose, fructose, and glucose content in the leaves were extracted and assayed using the HPLC-ELSD method according to Wang *et al.* (2007). In brief, leaf samples were ground in liquid nitrogen. Approximately 0.15 g powder was transferred into a 2 mL centrifuge tube, and 1 mL of hot distilled water was immediately added to the powder. The tubes were boiled at 100℃ for 15 minutes, and cooled to room temperature, then, centrifuged at 10,000×g for 10 minutes. The supernatant was transferred to a clean tube. The pellet was extracted twice with 0.4 mL hot distilled water. The supernatants were combined and dried at 80℃. Their residue was dissolved in 75% (v/v) acetonitrile to a final volume of 0.5 mL, and stored at 4℃ until use. The samples were filtered with 0.22 μm membrane before loading into the HPLC column. HPLC system equipped with PrevailTM Carbohydrate ES HPLC Columns (250×4.6 mm, 5 μm) and AllTech-ELSD 2000 evaporative light scattering detector was used to quantify the sugar content. The flow rate of the mobile phase (75% acetonitrile, v/v) was set at 1.0 mL min^{-1} with the pressure of 1,030 PSI at room temperature. The flow rate of the carrier gas (nitrogen) and the drift tube temperature was set at 2.0 L min^{-1} and 85℃, respectively. Sugar content was calculated according to the standard curve using the corresponding standard chemicals.

The content of amylose and amylopectin content was measured as described previously (Li *et al*., 2016). The leaves were firstly immersed in anhydrous ethanol to remove the interfering substance. Then, they

[DOI: 10.22034/jast.25.3.687]

were dried and pulverized with the mortar. The resulting tissue powder was then sieved via a 60-mesh sieve and 20 mg powder was added to 8 mL of 2M KOH, after water bath at 70°C for 15 minutes. The mixture was adjusted to pH 3.0 with 2 M HCl, and brought to 25 mL with double distilled water. An aliquot of 4 mL reacted with 1 mL 1% KI-0.1% I2 solution at room temperature for 15 minutes. The amylose content was estimated based on optical density at 620 nm against the standard curve established with standard amylose. Similarly, the amylopectin content was estimated based on the optical density at 550 nm value. The total content of the starch was the sum of amylose and amylopectin.

Enzymes Activity Assay

Invertase activity and sucrose synthase cleavage activity were determined following the method described previously (Miller, 1959; Ttxpy and Peimot, 1976). Sucrose synthase activity and sucrose phosphate synthase activity were measured with the protocol of Huber (Huber, 1981). The protein content were determined according to the method of Bradford (1976) using Bovine Serum Albumin (BSA) as a standard.

Statistical Analysis

All data were analyzed using SPSS Statistic 17.0 software by one-way significant variance analysis (ANOVA) followed by Duncan's post hoc test ($P< 0.05$). Data calculation using Microsoft Excel and graphing using GraphPad Prism 6.

RESULTS

Effects of Ethephon on Latex Yield of Rubber Tree Seedlings

In this study, eight months old rubber tree seedlings were used as experimental material and their trunk were treated with ethephon. Our results demonstrated that ethephon concentration ranging from 0.3 to 1.1% (w/v) increased latex yield after 24 hours trunk treatment, and significant differences in latex yield were observed after different doses of ethephon treatment (Figure 1). The rubber tree seedling treated with 0.6% ethephon had the highest latex yield, amounting to 0.92 g tapping^{-1}, which is about 4 times higher than that of the

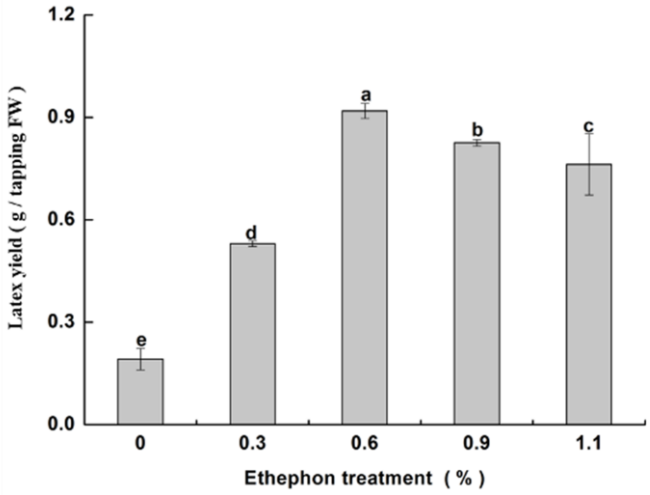

Figure 1. Effects of ethephon on latex yield of rubber tree seedlings. Different letters indicated a significant difference between treatment and control ($P< 0.05$). The values were means of six biological replicates.

[DOI: 10.22034/jast.25.3.687]

control. Latex yield increased gradually as ethephon concentration increased from 0 to 0.6%, and then decreased with higher doses. Therefore, in the following study, 0.6% ethephon was chosen to treat the trunk of rubber tree seedlings.

Effects of Trunk Ethephon Treatment on Leaf Gas Exchange

Ethephon treatment on the trunk rapidly decreased the leaf net photosynthetic rate. The net photosynthetic rate of the control was about 6.6 μmol m^{-2} s^{-1}, while it was only 0.33 μmol m^{-2} s^{-1} after 48 hours' ethephon treatment (Figure 2-A). In contrast, the intercellular carbon dioxide concentration significantly increased after 12 hours ethephon treatment and reached 199.67 μmol mol^{-1} (Figure 2-B), which is more than 2 times the concentration of the control, indicating that, although the available carbon dioxide was abundant, only a small proportion was utilized. Similar to the response of net photosynthesis, transpiration rate, and stomatal conductance decreased rapidly and gradually during 48 hours ethephon treatment (Figures 2-C and -D). Our results also showed that all the controls at different times (0, 12, 24, 36 and 48 hours) were almost the same, so, in the subsequent experiments, treatment at 0 hour was used as control. The content of chlorophyll a and chlorophyll b showed no significant difference between control and the treatments, indicating that although photosynthesis was greatly affected by ethephon, the photosynthetic apparatus had not been severely damaged (Table 1).

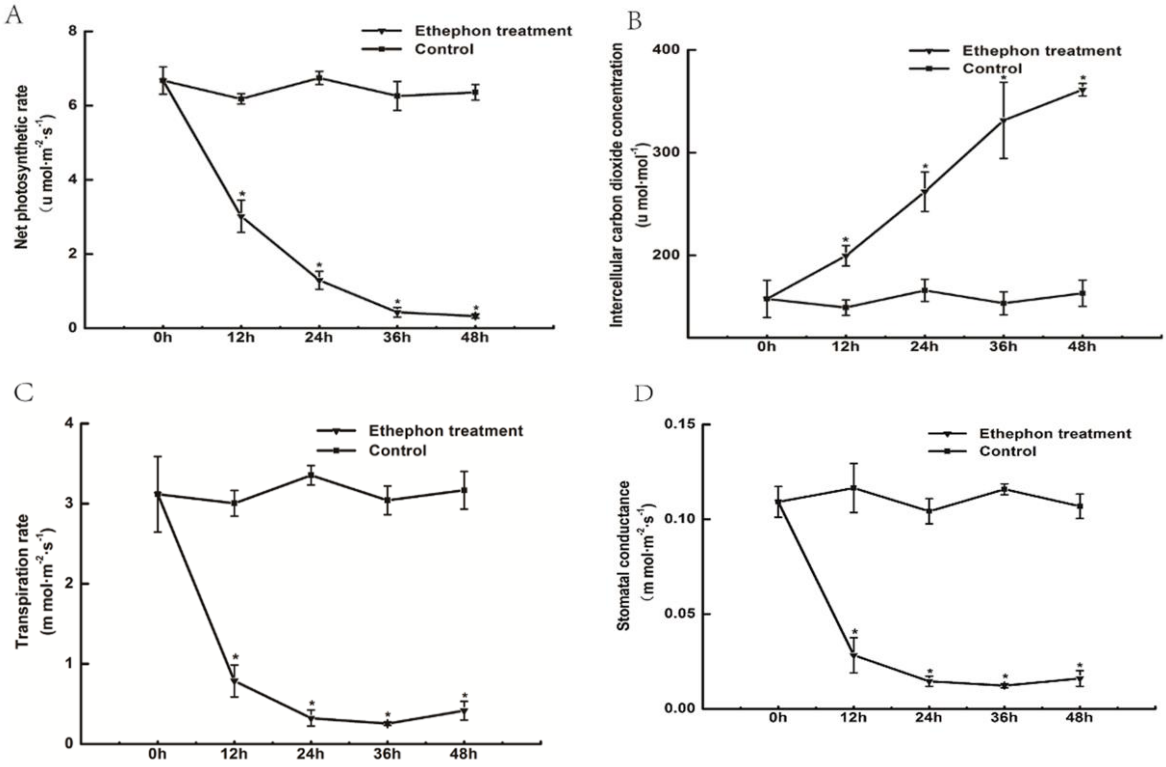

Figure 2. Effect of ethephon (0.6%) treatment on photosynthesis (A), intercellular carbon dioxide concentration (B), transpiration rate (C), and stomatal conductance (D). Data were means±SD of five biological replicates. *Indicated significant difference at 0.05 level ($P< 0.05$).

[DOI: 10.22034/jast.25.3.687]

Table 1. Effect of 0.6% ethephon on leaf chlorophyll content.

Chlorophyll category	Chlorophyll content (mg g^{-1} DW)				
	0 hour	12 hours	24 hours	36 hours	48 hours
Chlorophyll a	4.82±0.40 ab	5.30±0.32 a	5.51±0.95 a	4.67±0.99 ab	4.05±0.49 b
Chlorophyll b	1.72±0.15 ab	1.81±0.20 ab	1.95±0.32 a	1.75±0.37 ab	1.49±0.33 b
Chlorophyll (a+b)	6.54±0.54 ab	7.11±0.40 a	7.45±1.27 a	6.41±1.36 ab	5.53±0.77 b

a-b Different letters indicated significant difference at 0.05 level (P< 0.05), the data were derived from five replicates.

Effect of Trunk Ethephon Treatment on Leaf Sugar Content

Previous results indicated that sucrose transport played an important role in ethephon induced latex production in the trunk of the rubber trees. In this work, we measured the leaf sugar content after ethephon treatment on the trunk. Sucrose, fructose, and glucose were detected in leaves via a High-Performance Liquid Chromatograph with Evaporative Light Scattering Detector (HPLC-ELSD). The content of sucrose in rubber tree seedlings leaves was higher than that of fructose and glucose. Twelve hours after ethephon treatment, the highest content of sucrose was detected in rubber tree leaves, reaching 66.22 mg g^{-1}, then, it decreased gradually. After 48 hours, less than one-third of sucrose content was detected in leaves, which was significantly different from the control. By contrast, ethephon treatment increased the fructose and glucose content. The fructose content was about 11 mg g^{-1} after 36 and 48 hours' ethephon stimulation, which was much higher than the control, so did the glucose content, which also increased significantly by ethephon treatment (Figure 3).

Effect of Trunk Ethephon Treatment on Activity of Leaf Enzyme Related to Sucrose Metabolism

Our results demonstrated that Sucrose Phosphate Synthase (SPS) activity gradually increased with the duration of ethephon treatment, and reached its maximum 36

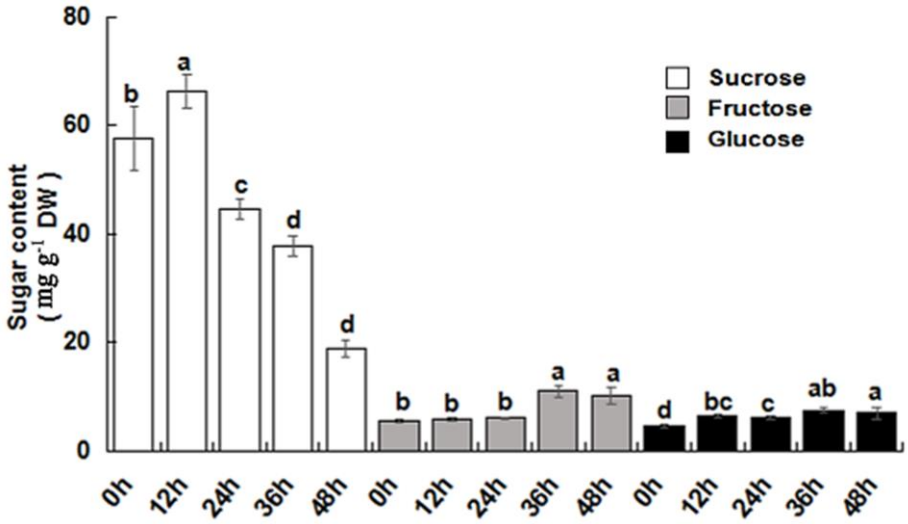

Figure 3. The change of leaf sugar content after ethephon (0.6%) treatment. Data of sucrose, fructose, and glucose were compared with the corresponding control, respectively; different letters indicate significant difference at 0.05 level (P< 0.05). Data are means±SD of five biological replicates.

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hours after treatment, then decreased. Its activity 36 and 48 hours after ethephon treatment was 0.33 and 0.21 μmol h^{-1} mg^{-1} protein, respectively (Figure 4). At the same time, Sucrose Synthase (SS) activity also gradually increased after ethephon treatment, but it reached its peak after 48 hours, which was slightly different from that of SPS (Figure 5**)**. Neutral/alkaline Invertase (NI) activity was significantly enhanced 24, 36, and 48 hours after ethephon treatment compared with that of the control. The Sucrose Synthase (SS) cleavage activity was not significantly different from that of control after 48 hours' ethephon treatment. The Vacuolar Invertase (VI) activity and Cell Wall Invertase (CWI) activity had no significant differences when compared with the control (Table 2).

Effect of Trunk Ethephon Treatment on Leaf Starch Content

Starch is the major carbon reserve in leaves, which can be either synthesized or utilized after the accumulation or depletion of sugar. In this study, the dynamic changes of starch in rubber tree leaves were also measured. The ratio of amylose to amylopectin is almost the same (about four) in all treated leaves, indicating that ethephon treatment did not change the composition of starch in rubber tree leaves. Twelve hours after ethephon treatment, the highest content of amylose and amylopectin were detected

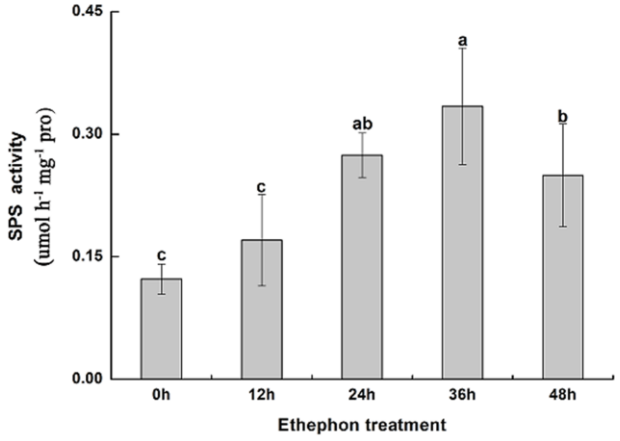

Figure 4. Effect of ethephon (0.6%) treatment on leaf sucrose phosphate synthase activity. Different letters indicate significant difference at 0.05 level (P< 0.05). Data are means±SD of five biological replicates.

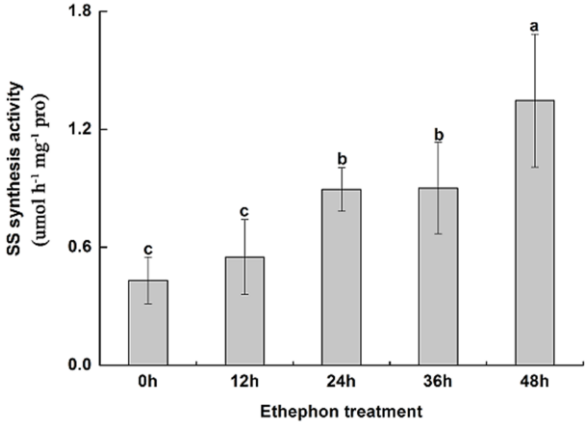

Figure 5. Effect of ethephon (0.6%) treatment on sucrose synthase activity in leaf. Different letters indicate significant difference at 0.05 level (P< 0.05). Data are means±SD of five biological replicates.

[DOI: 10.22034/jast.25.3.687]

Table 2: Effect of ethephon (0.6%) treatment on leaf enzyme activity related to sucrose decomposition.

Time after treatment	Neutral/Alkaline invertase activity (μ mol h^{-1} mg^{-1} Pro)	Vacuolar invertase activity (μ mol h^{-1} mg^{-1} Pro)	Cell wall invertase activity (μ mol h^{-1} mg^{-1} Pro)	SS cleavage activity (μ mol h^{-1} mg^{-1} Pro)
0 hour	33.04±1.56 d	24.91±10.59 a	48.53±10.72 a	43.64±3.65 b
12 hour	33.33±1.46 cd	29.27±15.65 a	49.60±15.18 a	56.11±4.46 ab
24 hour	35.40±0.94 ab	31.71±8.05 a	48.65±9.25 a	64.55±16.61 ab
36 hour	34.90±0.75 bc	31.79±10.89 a	54.84±20.97 a	65.41±16.98 ab
48 hour	36.98±1.59 a	34.76±14.05 a	65.75±15.84 a	78.44±30.09 a

a-d Different letters indicate significant difference between rows (P< 0.05), the data are derived from five replicates.

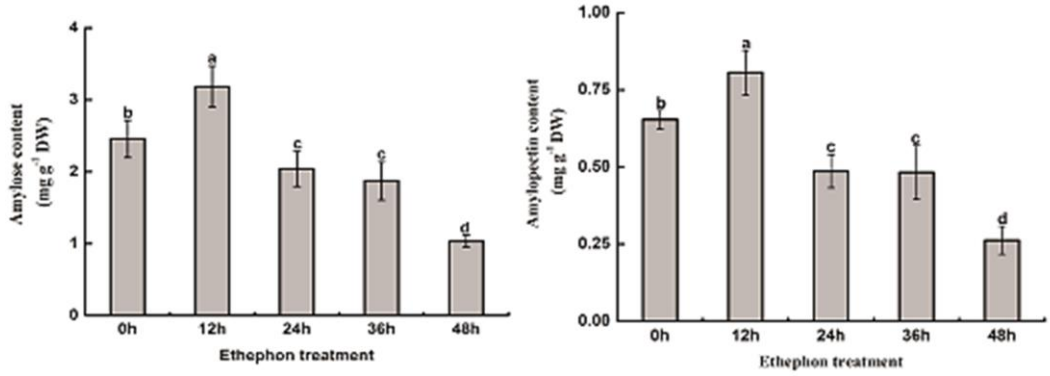

Figure 6. The change of leaf starch content after ethephon (0.6%) treatment. Data of amylose and amylopectin were compared with the corresponding control, respectively. Different letters indicate significant difference at 0.05 level (P< 0.05). Data are means±SD of five biological replicates.

in rubber tree leaves, reaching 3.18 and 0.81 mg g^{-1}, respectively, then, both kinds of starch reduced gradually (Figure 6). The overall changing tendency was similar to the sucrose change in leaves.

DISCUSSION

Adverse Effect of Ethylene on Rubber Tree

Previous studies demonstrated that the application of ethephon increased latex production (Dusotoit-Coucaud *et al*., 2010), and ethephon application is a popular practice to increase the latex yield in the field (Auzae, 1969; Dusotoit-Coucaud *et al*., 2010). Usually, the 1.5% ethephon was used to treat the trunk of a mature tree. In our work, ethephon concentration ranging from 0.3-1.1% on the rubber tree seedlings also increased the latex production, similar to the result obtained from mature rubber trees, while the decrease of the latex production at higher concentrations (0.9 and 1.1%) indicates that ethylene may also have an adverse effect. Similarly, the physiological responses including thickening, flaking or browning the bark along with the ethephon overstimulation was observed in mature rubber trees (Liu *et al*., 2015). But, the effect of trunk ethephon treatment on leaves has not been shown, probably because (1) the Long distance between treatment site on the trunk and the leaf; (2) The small amount of ethephon treatment on the trunk site may be greatly diluted due to the large volume of the mature rubber tree. Most works on ethylene-induced latex production focused on latex from the trunk, and the results indicated that various physiological parameters such as the Total Solid Content (TSC), Reduced thiol (R-SH), bursting index, plugging index and sucrose contents changed significantly (Eschbach *et al*., 1984;

[DOI: 10.22034/jast.25.3.687]

Milford *et al*., 1969). In this study, we used rubber tree seedlings, focusing on the physiological changes of ethephon on leaves when the seedling stem was treated with ethephon. Our results showed that although 0.6% can increase the latex production temporally (Figure 1), it greatly affected the leaves of the rubber tree, as demonstrated by the decrease of photosynthesis rate, transpiration rate, stomatal conductance, and the increase in intercellular CO_2 concentration (Figure 2). It has been reported that Tapping Panel Dryness (TPD) is a physiological disorder resulting from ethylene overstimulation (Chrestin, 1989; Jacob *et al*., 1994; Wu *et al*., 2008). Our work in rubber tree seedlings indicated that overdose of ethephon inhibited the leaf photosynthesis, thus, it will reduce the photosynthetic product prerequisite for latex production. The long-term lack of photosynthetic product might be one of the possible reasons for the TPD, but this conclusion needs verification. Nevertheless, our results showed that ethephon treatment adversely affected rubber trees because it significantly changed the physiological and functional role of the leaves.

Sucrose is the primary product of photosynthesis and its metabolites provide the precursor for nature rubber biosynthesis. Sucrose has long been recognized as one of the four main parameters to monitor the latex physiological status of rubber trees (Jacob *et al*., 1986; Jacob *et al*., 1989). Many studies showed that sucrose metabolism in trunk laticifers played an important role in ethephon induced latex production (Souza *et al*., 2015; Duan *et al*., 2013; Mesquita *et al*., 2006). In this study, we found that leaf sucrose content increased 12 h after ethephon treatment, and then, it gradually decreased during the treatment (Figure 3). It is possible that after ethephon treatment, sucrose will be mobilized to synthesize more latex, leading to the starch breakdown in leaves, which caused the over accumulation of sucrose, but due to the inhibited photosynthesis, the sucrose could not be regenerated in time and, finally, leading to the decreased content of sugar in leaves. Previous studies (Ttxpy and Peimot, 1976) showed that the content of sucrose in latex decreased after ethephon stimulation, consistent with the change of sucrose content in leaves in this study. It is speculated that sucrose may be decomposed and transformed to increase latex biosynthesis in the leaf. Similar to the dynamic sucrose changes in latex from the trunk after ethephon stimulation (Ttxpy and Peimot, 1976), in mature rubber trees, the sucrose content increased after ethephon treatment and reached the maximum value after 2 weeks, then, it gradually decreased. Fructose and glucose are the decomposition product of sucrose, and the content of both sugars increased gradually after ethephon treatment (Figure 3). At the same time, starch (amylose and amylopectin) content in leaf increased firstly, then, decreased gradually, which is similar to that of sucrose (Figure 6). In addition, the activities of enzymes involved in sucrose cleavage also slightly or significantly increased after ethephon treatment (Table 2). These dynamic physiological changes showed that ethephon treatment led to leaf sucrose utilization, similar to the changes of latex after ethephon treatment. It is known that carbohydrates, such as sucrose, glucose, fructose and starch in rubber trees ultimately come from carbon fixation (the Calvin Cycle) or from stored photosynthetic products (Liu, 2016).

In the present study, diminishing of the photosynthesis (as shown in Figure 2) indicated that the carbohydrate pool of the leaf will be reduced, but fructose and glucose, and possibly the other metabolites involved in latex biosynthesis, increased; as the result, the stored carbohydrate pool will be utilized, as can be seen by the gradual decrease of starch and sucrose (Figures 3 and 6). Since the mature leaf is the source organ, ethephon treatment increased latex production (Figure 1) and *SUTs* genes (HbSUT1A, HbSUT2A and HbSUT3) expression (Gidrol *et al*., 1994; Dusotoit-coucaud *et al*., 2009), we could infer that

[DOI: 10.22034/jast.25.3.687]

ethylene stimulated latex yield via sucrose import into laticifers and the sucrose is partly derived from the leaf source tissue directly.

CONCLUSIONS

Our results show that ethephon treatment increased latex production in the stem in rubber tree seedling, while it also significantly reduced the net photosynthetic rate, transpiration rate, and stomatal conductance in leaves. The sucrose decreased significantly concomitant with slight increase in glucose and fructose. The enzymatic activities of Sucrose Phosphate Synthase (SPS), Sucrose Synthase (SS) and Neutral/alkaline Invertase (NI) also significantly increased after ethephon treatment.

It can be concluded that ethylene stimulated latex production in rubber tree seedling, partly due to the sucrose metabolism in leaves. However, ethylene also had adverse effect on rubber tree leaves.

ACKNOWLEDGEMENTS

We would like to thank Chinese Academy of Tropical Agricultural Sciences for material support.

Conflicts of Interest

The authors declare no conflict of interest.

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[DOI: 10.22034/jast.25.3.687]

تحریک اتفون (Ethephon) در تنه درخت لاستیک باعث تغییرات فیزیولوژیکی برگ در نهال می شود

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چکیده

برای تحریک تولید لاتکس، اتفون به طور گسترده در کاشت درخت لاستیک استفاده می شود. مطالعات پرشمار نشان داده است که تیمار اتفون جریان لاتکس را طولانی تر می‌کند و بازسازی لاتکس را در تنه نهال های درخت لاستیک بالغ افزایش می‌دهد. با این حال، چگونگی واکنش برگ‌های درخت لاستیکی به تیمار اتفون روی تنه هنوز ناشناخته است. در این پژوهش، ما از نهال درخت لاستیک برای تعیین پاسخ فیزیولوژیکی برگها پس از تیمار تنه با اتفون استفاده کردیم.نرخ فتوسنتز، محتوای قند و نشاسته و همچنین فعالیت آنزیمی درگیر در متابولیسم قند پس از ۰، ۱۲، ۲۴، ۳۶ و ۴۸ ساعت با تیمار اتفون ۰.۶ % اندازه‌گیری شد. نتایج نشان داد که تیمار اتفون باعث افزایش تولید لاتکس روی تنه شد، در حالی که سرعت خالص فتوسنتز، سرعت تعرق و هدایت روزنه‌ای در برگ‌ها به طور قابل‌توجهی کاهش یافت. همچنین، ساکارز با کاهش معنادار ولی گلوکز و فروکتوز به طور ملایمی افزایش یافت. نیز، فعالیت آنزیمی ساکارز فسفات سنتاز (SPS)، ساکارز سنتاز (SS) و اینورتاز خنثی/ قلیایی (NI) پس از تیمار اتفون به طور معناداری افزایش یافت. تیمار اتفون بر محتوای نشاسته تأثیر گذاشت، اما ترکیب نشاسته را در برگ‌های نهال درخت لاستیک تغییر نداد. الگوی کلی تغییر نشاسته شبیه به ساکارز در برگ بود. می توان نتیجه گرفت که تولید لاتکس تحریک شده با اتیلن (ethylene) در نهال درخت لاستیک تا حدودی به دلیل تغییر متابولیسم ساکارز در برگ است و اتیلن اثر فیزیولوژیکی نامطلوبی بر لاستیک دارد.

[DOI: 10.22034/jast.25.3.687]