Relationships between carbon stock in the rubber tree and latex production

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Abstract

The relationship between tapping systems and the balance between latex production and carbohydrate availability would be very informative. This present work aims to study the effects of tapping systems on enzyme activities involved with Non-structural carbohydrates (NSCs) dynamic. The result indicated that enzyme activities from RRIM600 with upward tapping was higher from that of downward tapping. This could be partly due to the gradient sucrose along the truck as it is more concentrated in higher position than the lower one. Sucrose synthase was proportional to sucrose content in bark of upward tapping. Meanwhile, Sucrose Phosphate Synthase and Amylase were higher in wood than bark. These enzymes were also significantly higher in upward tapping than downward tapping. Using immunolocalization test, amylase was located in both vascular rays of bark and wood. It was noticed that enzyme activities strongly related to sucrose content while the link with starch content could not be clarified yet. To eventually explain the whole picture of NSC balance and latex production, information of latex production yield and enzyme involved in starch synthesis must be taken into account.

Keywords: Non-structural carbohydrates (NSCs); Rubber trees (Hevea brasiliensis Muell. Arg.); Tapping systems; Immunolocalization

INTRODUCTION

Carbohydrates are the primary carbon source used for growth and maintenance in plants. Non-structural carbohydrates (NSCs) are translocated, mainly as sucrose, via the phloem from source (leaves) to sink (importing) tissues. In tapped rubber trees, NSCs, especially sucrose, are used for latex production, in addition to growth and maintenance. As sucrose is both the source of energy and the material to regenerate the latex that is exported by tapping, the balance of sucrose within the latex producing vessels is a key to latex productivity [1]. Latex sucrose can come directly from carbon recently assimilated by photosynthesis, but stored carbohydrate are likely involved too [2] Stored carbohydrates, like starch, have an important role in plant metabolism when supply from photosynthesis is not enough to balance demand. This occurs during bud break and early growth in deciduous species or under environmental stress or pest infestation [3]. As tapping creates an extra-demand for carbohydrate, it was expected that starch reserves would decrease in tapped trees. However, Chantuma *et al.* [3]; U Silpi *et al.* [4] demonstrated that surprisingly latex tapping increased starch reserve whereas trunk radial growth was reduced. This shows that we need to understand better the dynamics of starch and sucrose as related to tapping. Indeed, the dynamics of NSCs during latex tapping depend on enzyme activities that catalyze the biochemical processes. The key enzymes involved in starch and sucrose biosynthetic pathways are sucrose synthase (SuSy), invertases (INVs), sucrose phosphate synthase (SPS), and amylase (AMY). SuSy and INVs are the main enzymes involved with sucrose degradation [5; 6]. SuSy and INV activities were found in the latex [7; 8], bark [9] and seed [10] of rubber

trees. SPS involved in sucrose synthesis is found in leaves and germinating seeds [11-13]. AMY is the one of most important enzyme involved in starch degradation [14]. However, the evolution as related to tapping of the activity of those enzymes in rubber tree trunks remains unknown.

Moreover, most starch reserves are located in wood [3]. To be used for latex regeneration such starch has to be degraded in wood and then be transported to bark. It would be relevant to know better where the related enzymes are located in the wood and the bark of the rubber tree trunk. Immunolocalization is an alternative technique to detect enzymes in biological samples by using the reaction between antigen and antibody probes [15].

Therefore, the present study aims at assessing the activity of the main enzymes involved in NSCs metabolism and at localizing some of them in bark and wood of rubber trees submitted to different tapping systems. Downward tapping is the traditional method for farmers in Thailand [16], using half spiral cut (1/2S) or one third-spiral (1/3S). Upward tapping is an alternative tapping system used once the lower part of the trees has been tapped downward. In this system, the tapping panels are divided in third or fourth-spiral (1/3 or 1/4S) and the bark is cut from down to up. This tapping is supposed to get higher latex yield productivity than downward tapping [17].

MATERIAL AND METHOD

Plant materials and experimental parameters

The experiment was done in 2014 at the Chachoengsao Rubber Research Center, Rubber Authority of Thailand (CRRC-RAOT), Eastern Thailand with rubber trees (*Hevea brasiliensis)* clone RRIM600, 25 year old. Trees were open in May, 2002. We used 2 tapping systems (downward tapping and upward tapping) compared to untapped trees (control), in May (beginning of the tapping season) and October (peak of the tapping season). Only the results in May are presented here. We took bark and wood samples in tapped panels (Pa1) and untapped panels (Pa2). In controls, although the trees were not tapped, the side corresponding to the tapped panel of tapped treatments was called 'Tapped panel' and similarly for the 'Untapped panel'.

Sampling procedure

The samples of bark and wood were collected on the rubber tree trunk positions shown in Figure 1.

Samples for NSCs assays: For downward tapping, bark and wood were collected below tapped panel (Pa1) and above tapped panel on the opposite side (untapped panel, Pa2) by increment borer. Previous research showed that the opposite panel above tapping has high sucrose and phosphorus contents [4]. That is why this position was selected for analysis. For upward tapping, bark and wood were collected above tapped panel (Pa1) and at the same level on the opposite side (untapped panel, Pa2) by increment borer. Samples were 0.5 cm diameter and 5 cm long cores (about 1cm of bark and 4 cm of wood).

Samples for enzyme activity assays: Bark was collected by auger. Samples were 3 cm diameter and 1 cm long cores. Then, wood was collected by increment borer in the same location (0.5 cm diameter and 4 cm long cores).

Samples for Immunolocalization: Bark and wood were collected at one point in the same position as samples for assays in downward tapping. They were fixed in 4% paraformaldehyde and one drop of Tween 20 and were dehydrated by ethanol. Then, samples were cut by Cryotome at speed 30 mm/s, frequency 70 Hz, amplitude 0.8 mm and thickness 60 µl.

Biochemical analysis

Non-structural carbohydrates (NSCs): For soluble sugar extraction, the fine powder of sample was dried in the oven at 65 ^{o}C for 2 h. They were extracted from 20 mg sample with 80 % ethanol two times for 30 min and 50 % ethanol one time for 30 min. All supernatants were pooled and were filtered through mini-columns containing polyvinyl polypyrrolidone (PVPP) and activated charcoal. Ethanol was evaporated by the oven at 65 ^{o}C for 12 h. For

starch extraction, the pellet was dried at 65 °C for 2 h and then it was hydrolyzed with 0.02 N NaOH at 90 °C for 90 min. The hydrolyzed pellet was degraded by α-amyloglucosidase at 50 °C for 60 min to produce glucose. Soluble sugars and starch were estimated by the reaction of Hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PDH) and phosphoglucose isomerase (PGI). The product of NADPH obtained from enzymatic reaction was followed by spectrophotometer at 340 nm.

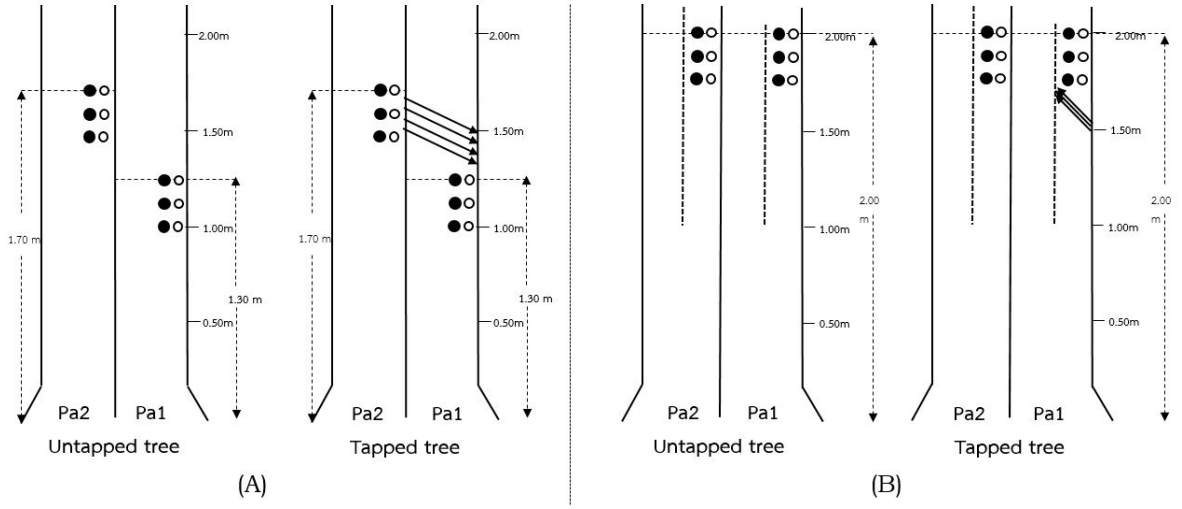

Figure 1 The collecting position on the rubber tree trunk for downward tapping (A) and upward tapping (B) in tapped panels (Pa1) and untapped panels (Pa2) for NSCs assay (o) and enzyme assay (●)

Enzyme activities: The fine powder of sample was mixed with PVPP that the ratio of sample and PVPP was 1:1. The mixed sample was blended with 10 ml of extraction buffer (50mM Hepes, 5mM MgCl, 1mM EDTA, 5mM DTT, protease inhibitor cocktail, pH 7.0) for 5 min. The extracted sample was centrifuged at 10,000 rpm, 4 °C for 10 min. The pellet was re-extracted as described above. The supernatant obtained from the first and second extraction was pooled. Enzymes in the supernatant was concentrated with 20% and 80% $(NH4)_2SO_4$ precipitation. The concentrated enzymes were estimated by modification of enzyme activities method of sucrose synthase [18], Invertase [19], sucrose phosphate synthase [20] and α-amylase [21]. Total protein was analyzed by Bio-Rad protein assay.

Immunolacalization: Cross sections of sample were blocked in blocking buffer at 4 °C for 3 h. Primary antibody (Ac1) of Rabbit anti-amylase was added in the sections and incubated at 4 °C for 24 h. Then, secondary antibody (Ac2) of alkaline phosphatase anti-rabbit IgG was added in the sections and incubated at 4 °C for 1 h. The observation of amylase was performed by Light microscope.

Data analysis: All statistical analyses were performed using XLSTAT Version 2014.5.03 (Addinsoft SARL, France). The significance of the effects of tapping system (downward and upward tapping) on NSCs (sucrose and starch) and enzyme activities was assessed by analysis of variance (ANOVA) with 3 replications (trees) per treatment. A critical value of Fisher's LSD test at $\alpha = 0.05$ was used for the tests of significance.

RESULT AND DISCUSSION

NSCs content in bark and wood of rubber trees

Similarly to previous studies [3], sucrose content was higher in bark than wood. In contrast, starch content was higher in wood than bark.

The effect of tapping systems and sample location on trunk NSCs content

In bark: sucrose content was higher in higher positions, in accordance to the increasing bottom-up sucrose gradient usually found in trees [22] (**Figure 2 A1,A2**). It was also higher in the tapped trees than in the corresponding untapped control under upward tapping, but not under downward tapping. Starch content did not show significant trends. There was an unexplained difference between the starch content of the two sides (supposed to be similar) of the control of the downward tapping system. (**Figure 2 B1,B2**).

In wood: sucrose content was also higher in higher positions. There was no differences between trees tapped downward and control, but sucrose was significantly lower on both panel of trees tapped upward than in control (**Figure 3 A1,A2**). Starch content was higher in lower positions, consistently with the decreasing bottom-up gradient usually found in trees [23]. Starch increased slightly with downward tapping in the tapped panel similarly to previous results [3; 4], but it did not change significantly with upward tapping (**Figure 3 B1,B2**).

As a whole, the trends indicated a higher mobilization of NSCs towards latex producing tissues (bark) under upward tapping. This was in accordance with the higher latex yield expected with such tapping system.

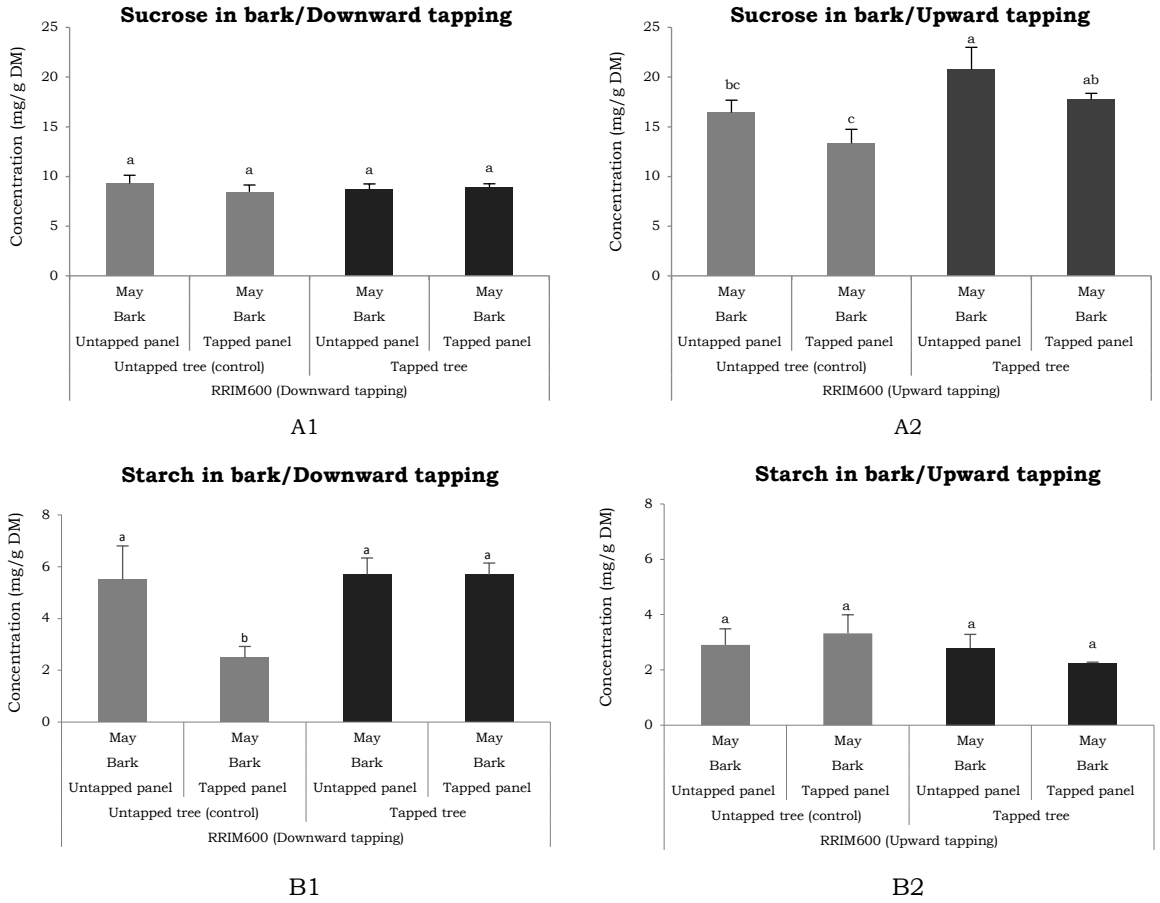

Figure 2 The effect of tapping methods on sucrose and starch concentration (mg/g DM) in bark of May, average for 3 replications in 2014. Sucrose (■) and starch (■) contents in bark of clone RRIM600 tapped with Downward (A1,B1) and Upward (A2,B2) methods during May (Control, Tapped tree). Untapped panel', 'Tapped panel' in control, as there is actually no tapping. The error bar shows the standard error (N=3). Different letters indicate significant differences of Fisher's LSD test at $\alpha = 0.05$.

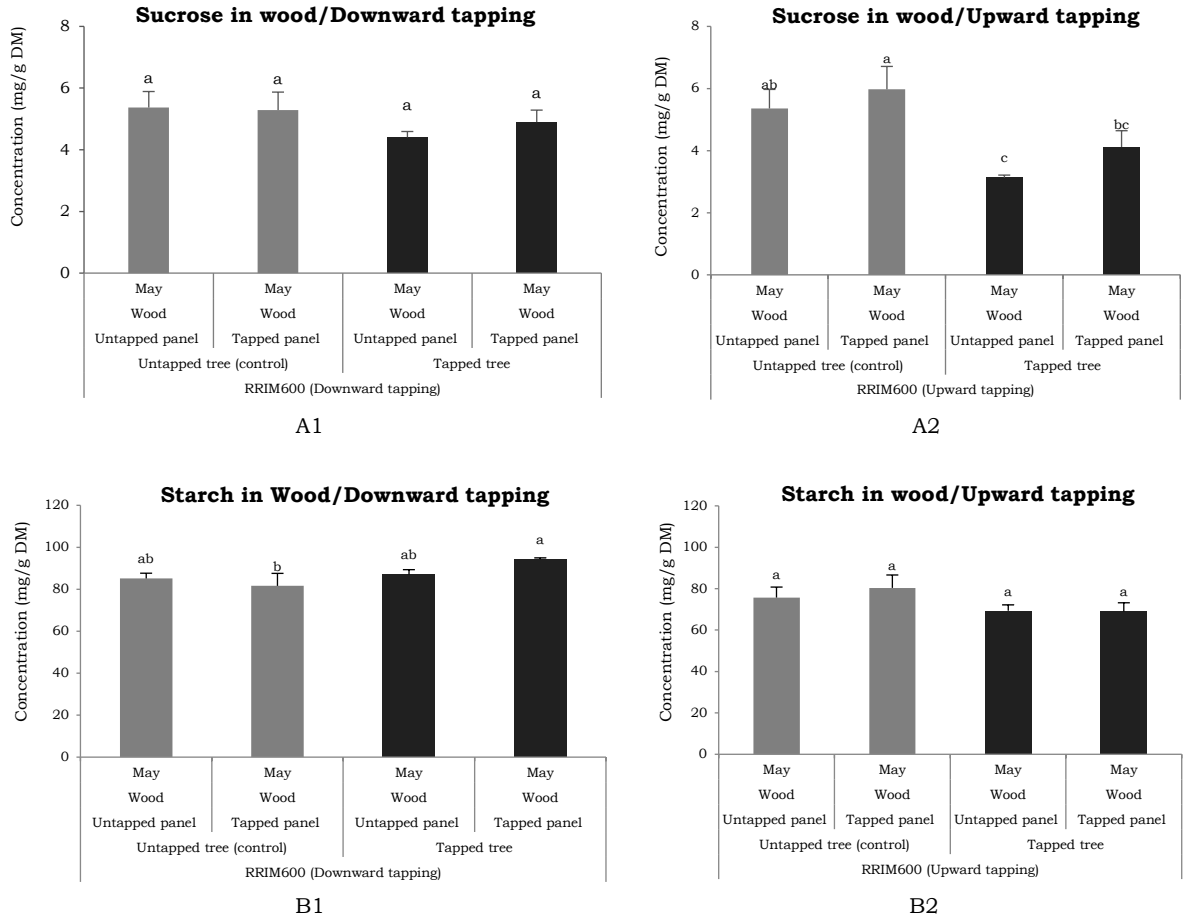

Figure 3 The effect of tapping methods on sucrose and starch concentration (mg/g DM) in wood of May, average for 3 replications in 2014. Sucrose (▒) and starch (■) contents in bark of clone RRIM600 tapped with Downward (A1,B1) and Upward (A2,B2) methods during May (Control, Tapped tree). Untapped panel', 'Tapped panel' in control, as there is actually no tapping. The error bar shows the standard error (N=3). Different letters indicate significant differences of Fisher's LSD test at $\alpha = 0.05$.

The effect of tapping methods on enzyme activities

In bark (**Figure 4 A1,A2**): under upward tapping, SuSy, the enzyme involved in sucrose degradation, had a higher activity in tapped panels as compared to untapped trees or untapped panels (0.16 vs 0.04 U/mg protein in control). The trend was less clear under downward tapping (0.04 U/mg protein in tapped panel vs 0.02 in untapped panel). INV activity was not different in downward and upward tapping when compared with control tree. The enzymes involved in starch degradation and sucrose synthesis, AMY and SPS, had higher activities in higher positions (particularly SPS) for both tapped and untapped trees. AMY had a higher activity in tapped panel than untapped panel under upward tapping (**Figure 4 B1,B2**). These trends were consistent with the higher sucrose and lower starch content found at high positions and also with the higher sucrose content in bark of trees tapped upward.

In wood: SuSy activity was very low at lower position (less than 0.008 U/mg protein), without effect of tapping, whereas it was higher in higher position with a clear increase under upward tapping (0.072 U/mg protein) but no effect of tree side (panel). INV activity was not detectable (**Figure 5 A1,A2**). Upward tapping also had a strong increasing effect on the activity of enzymes involved in starch degradation and sucrose synthesis (AMY and SPS). SPS and AMY were high at higher position on both untapped and tapped panels as compared to untapped trees under upward tapping but they increased slightly with downward tapping (**Figure 5 B1,B2**). Despite this higher activity, sucrose content was lower in wood of trees

tapped upward. Our hypothesis is that the sucrose synthesized from starch hydrolysis was exported towards latex producing tissues.

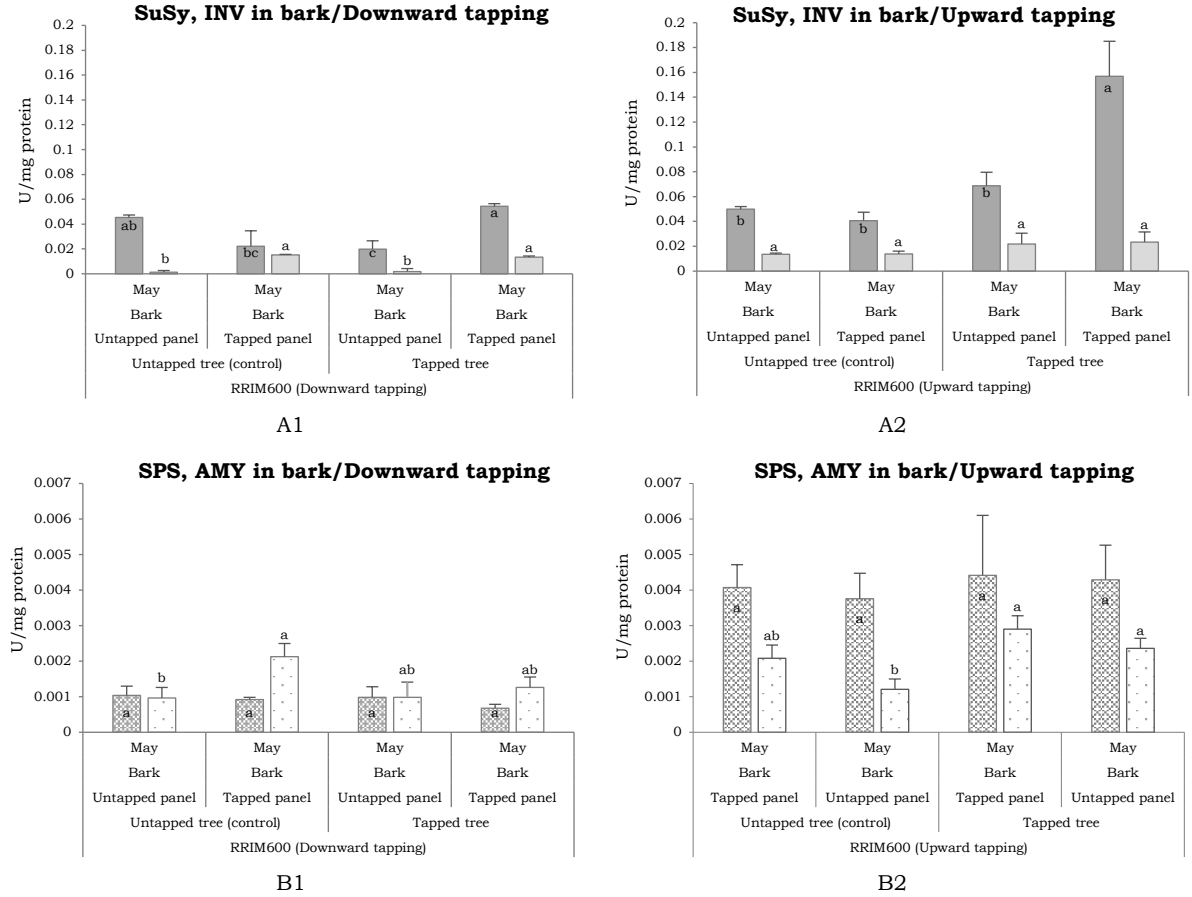

Figure 4 The effect of tapping methods on ■ SuSy, □ INV activities (A1,A2) and ▩ SPS, ▫ AMY activities (B1,B2) in bark of May, average for 3 replications in with Downward (A1,B1) and Upward (A2,B2) methods during May (Control, Tapped tree). Untapped panel', 'Tapped panel' in control, as there is actually no tapping. The error bar shows the standard error (N=3). Different letters indicate significant differences of Fisher's LSD test at $\alpha = 0.05$.

Immunolocalization: alternative method for enzyme detection

Amylase was detected (not shown) in both bark and wood, especially in the vascular rays (VR) which constitute the horizontal system of secondary tissues for translocation of carbohydrates between bark and wood. Moreover, amylase was localized with higher density in tapped trees than in untapped trees (control). Tapping seemed to induce a higher amylase content in both bark and wood to hydrolyze starch for latex regeneration in latex vessel (LV) of bark.

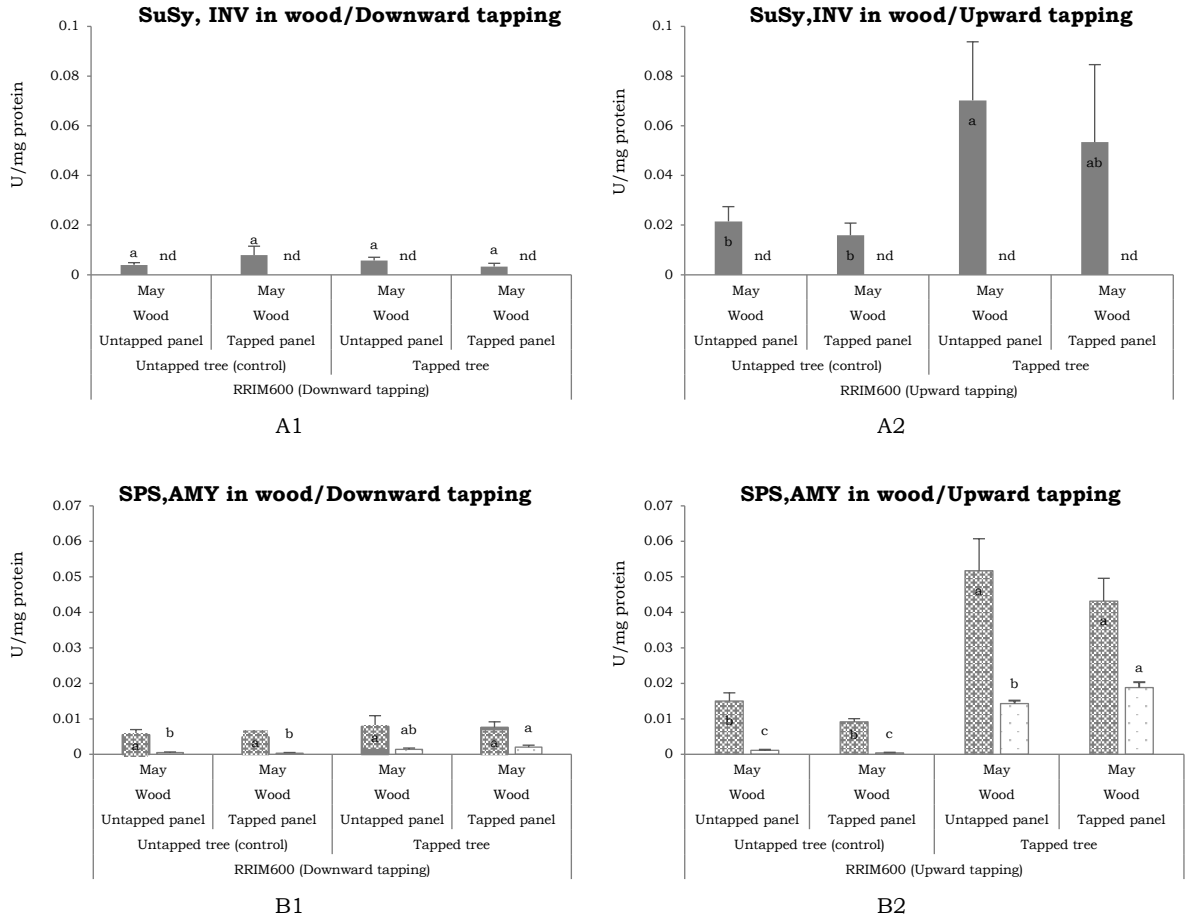

Figure 5 The effect of tapping methods on ■ SuSy, □ INV activities (A1,A2) and ▩ SPS, ▫ AMY activities (B1,B2) in wood of May, average for 3 replications in with Downward (A1,B1) and Upward (A2,B2) methods during May (Control, Tapped tree). Untapped panel', 'Tapped panel' in control, as there is actually no tapping. The error bar shows the standard error (N=3). Different letters indicate significant differences of Fisher's LSD test at $\alpha = 0.05$.

CONCLUSION

From this study on clone RRIM600, upward tapping had a higher effect to induce the mobilization of NSCs via a higher activity of the involved enzymes than downward tapping. This effect was in accordance with the higher latex yield potential of upward tapping. The dynamics of sucrose and starch depended on SuSy, SPS and AMY activities. All enzymes were active in bark at higher position under upward tapping, inducing sucrose synthesis and starch degradation. Moreover, upward tapping increased SPS and AMY activities in wood whereas sucrose content was low in this tissue. This showed that we need to integrate the functioning of the enzymes with the transport of NSCs between wood, bark and latex producing vessels. The observation of the location of the enzymes by immunolocalization method (started with amylase) will help unravelling this complex system.

ACKNOWLEDGEMENT

This work was funded by Kasetsart University Research and Development Institute (KURDI), project number 9.59 and Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO), project number AG-BIO/59-001-007. We thank Chachoengsao Rubber Research Center, Rubber Authority of Thailand (CRRC-RAOT) for samples and field work. Grateful thanks to Laboratory of Biochemistry and Technology of Natural Rubber (LBTNR) for laboratory work.

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