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RESEARCH ARTICLE

Relationship between the number of tapping-induced secondary laticifer lines and rubber yield among *Hevea* germplasm

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Abstract The lack of suitable early selection parameters means that traditional rubber breeding for yield is time-consuming and inefficient. Laticifer is a tissue specific for natural rubber biosynthesis and storage in rubber tree. The number of the secondary laticifers in the trunk bark tissues is positively correlated with rubber yield in the matured rubber trees that are regularly tapped. In the present study, the rubber yield from 280 of 4–5 year-old virgin trees from 7 cross combinations was compared with the number of newly differentiated secondary laticifers caused by tapping. Results showed that the number of tapping-induced lines of secondary laticifers varied in different germplasm and was positively related to the rubber yield, indicating this could be used as a suitable parameter for early evaluation of yield potential of rubber trees.

Keywords *Hevea brasiliensis*, rubber yield breeding, early evaluation, tapping, secondary laticifer differentiation

1 Introduction

Rubber trees are the main source of natural rubber in the world. In natural rubber production, latex from the trunk bark of rubber tree is collected by controlled mechanical wounding, i.e., tapping. The rubber yield per year is the sum of the yield per tapping. It is thus difficult to dissect the yield component traits. As a result, selection has to depend on the general yield trait in the traditional yield breeding process. This process is time-consuming and ineffective. It takes more than 40 years to evaluate performance of progenies from hybridization[1]. Therefore, to shorten the breeding period for rubber tree it is essential to develop suitable parameters for early selection of the yield potential. For this purpose, many attempts have been made to establish the relationship between rubber yield potential and factors such as girth[2,3], bark thickness, number of latex vessel rings, latex vessel density[2–4], CO_2 assimilatory capacity[5], physiological characteristics of latex[6], and ATP concentration of the latex[7]. Among the tested parameters, the number of secondary laticifer lines in the trunk bark of mature rubber tree that is regularly tapped is positively related to rubber yield[8]. However, there is no obvious difference in the number of the secondary laticifer lines in different immature virgin trees (i.e., previously untapped trees)[9]. The secondary laticifers are differentiated from vascular cambia. Tapping is effective in enhancing the secondary laticifer differentiation and consequently increases the number of secondary laticifers lines in the trunk bark of rubber tree[10]. In the present study, analysis of the relationship between the number of the tapping-induced secondary laticifers and rubber yield was performed to develop a suitable parameter for early selection in the process of rubber yield breeding.

2 Materials and methods

2.1 Plant materials

In 2008, a total of 280 progeny from seven cross combinations were planted in the rubber breeding base of the Rubber Research Institute of the Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan Province. Of the 280 offsprings, 21 were from the cross of Reken509×Reyan87-4-26, 10 from Reyan7-33-97 × 125, 31 from Reyan7-33-97 × 127, 35 from Reyan78-2 ×

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Reken523, 35 from Reyan78-2×Reken628, 60 from Reyan88-13 × 02, and 88 from Reyan88-13 × 127 (Appendix A, Table S1). The seedlings were planted at 1.5 m spacing in row 3 m apart. Experiments were performed when the seedlings were 4–5 years old (hereafter referred to as virgin trees).

2.2 Treatments

The virgin trees were first tapped ten times at 50 cm above ground with a S/2d/3 tapping system (i.e., the tapping panel was half the stem girth with tapping every three days) in May to June, 2012. Thereafter, the virgin trees were not tapped until September to October when they were tapped ten times again at the first tapped site. The bark tissues were collected from the left side channel near the tapping panel in April, 2013. For yield determination, the virgin trees were tapped ten times in May to June, 2013 and the latex samples from the sixth to tenth tapping were collected.

2.3 Light microscopy

To eliminate tannin-like substances which may be mistaken for the rubber inclusions in laticifer cells, bark samples were fixed in 80% ethanol for 24 h at room temperature, and then treated with iodine and bromine in glacial acetic acid[11], and embedded in paraffin after dehydration. Sections (20 μm thick) were cut with a microtome and stained with fast green (0.5 g in 100 mL of 95% ethanol). The laticifer cells in sections could be traced on the basis of brown colored rubber inclusions under a Leica DMLB microscope (Leica, Wetzlar, Germany). Staining with fast green was carried out to strengthen the image contrast.

2.4 Yield determination

After the latex in the collecting cup had coagulated in the field, the coagulated latex samples were dried at 60°C until the weight was constant. The sum of the dry weights of each latex sample represented the rubber yield per tree.

2.5 Statistics

Data analysis was performed using Microsoft Office 2007 and SPSS statistical software, version 14.

3 Results

3.1 Difference in the number of the tapping-induced secondary laticifer lines and the rubber yield among *Hevea* germplasm

The lines of secondary laticifers that formed under natural conditions before tapping was difficult to count but there was no obvious difference among the 280 trees from different *Hevea* germplasm (Fig. 1). In contrast, the tapping-induced secondary laticifers appeared in a condensed manner and the number of induced secondary laticifer lines was obviously different among the 280 trees (Fig. 1).

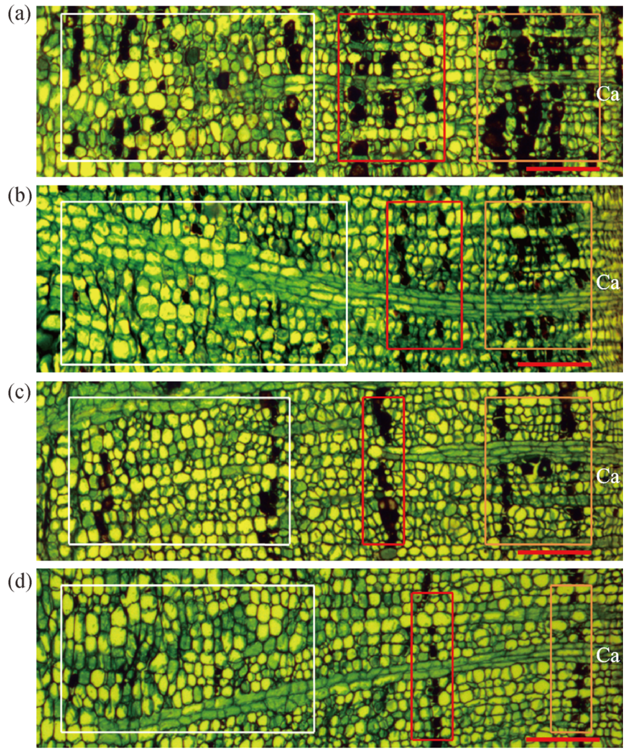

Fig. 1 Light micrographs of bark cross-sections, showing the tapping-induced secondary laticifers. The lines of lacticifers appear dark brown or black in these sections. The number of lines of induced secondary laticifers was > 6 (a), 5–6 (b), 3–4 (c), and < 3 (d). The secondary laticifers lines in the white, red, and orange squares were formed under natural conditions before tapping, in the first half year, and in the second half year after tapping, respectively. Ca, vascular cambia. Scale bars, 200 μm.

In general, two groups of condensed laticifers could be distinguished. These were associated with the ten tappings in the first half year and those in the second half year, respectively. Based on the number of induced secondary laticifer lines, the ability to differentiate secondary laticifers could be roughly divided into four grades: first to fourth grade having < 3, 3–4, 5–6 and > 6 lines, respectively (Fig. 1). Based on these criteria, there were 60, 142, 47, and 31 trees graded one to four, respectively (Fig. 2; Appendix A, Table S1).

The rubber yield varied among the 280 trees, ranging from 2.77 to 344.59 g (Appendix A, Table S1). Yield could be roughly divided into 4 grades: ≤50, > 50–100, > 100–150, > 150 g, respectively. The 280 trees could be divided by yield into 174 grade 1 trees, 57 grade 2, 30 grade 3, and

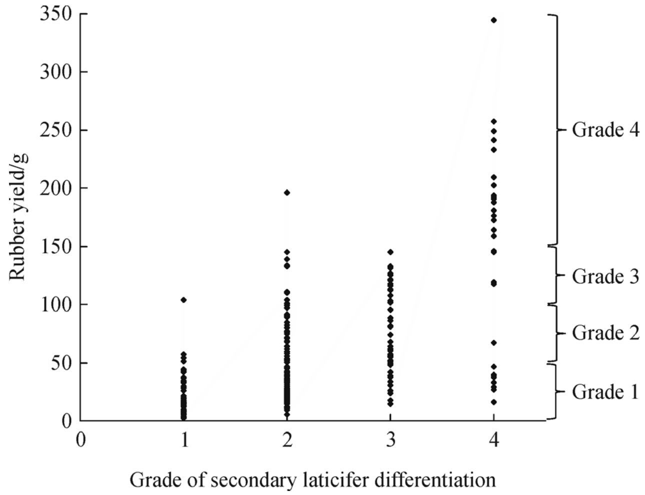

Fig. 2 Difference in the tapping-induced secondary laticifer and rubber yield among *Hevea* germplasm

19 grade four trees (Fig. 2; Appendix A, Table S1).

3.2 Relationship between the grade, number of secondary laticifers differentiated and rubber yield

Of the 19 trees with grade 4 yield, 18 trees were in the fourth secondary laticifers grade, i.e., 94.7% (Fig. 3a). Whereas, the trees with the fourth secondary laticifer grade took percentage of 13.3%, 1.8%, and 4.6% in the trees with grade 3, grade 2, and grade 1 yield, respectively (Fig. 2; Figs. 3b–3d).

There were 153 trees in the first and second secondary laticifer grades, representing 87.9% of the 174 trees with grade 1 yield (Fig. 3; Appendix A, Table S1). There were 38 trees with the first and second secondary laticifer grades with the grade 2 yield, i.e., 66.7%. This percentage decreased to 33.3% and 5.3% in the trees with grade 3 and 4 yield, respectively. These changes in the percentage demonstrated that the number of the tapping-caused secondary laticifer lines was positively related to the rubber yield. It is important to note that there were 8 trees in the fourth secondary laticifer grade of the 174 trees that yielded less than 50 g (Fig. 3; Appendix A, Table S1).

4 Discussion

Secondary laticifers are specific for natural rubber biosynthesis and storage in rubber trees. The number of lines of secondary laticifers in the trunk bark is one of the key factors that determine rubber yield[12]. The secondary laticifers is differentiated from the vascular cambium[12]. Factors such as degree of latex exploitation[10], mechanical wounding[13–15], exogenous jasmonic acid[12] and coronatine[16], an active jasmonate homolog are effective in inducing secondary laticifer differentiation. The mechanical wounding-induced secondary laticifer differentiation is associated with a burst of endogenous jasmonate[17] and latex exploitation (tapping) causes a change in laticifer turgor, which may cause a rapid and transient increase in

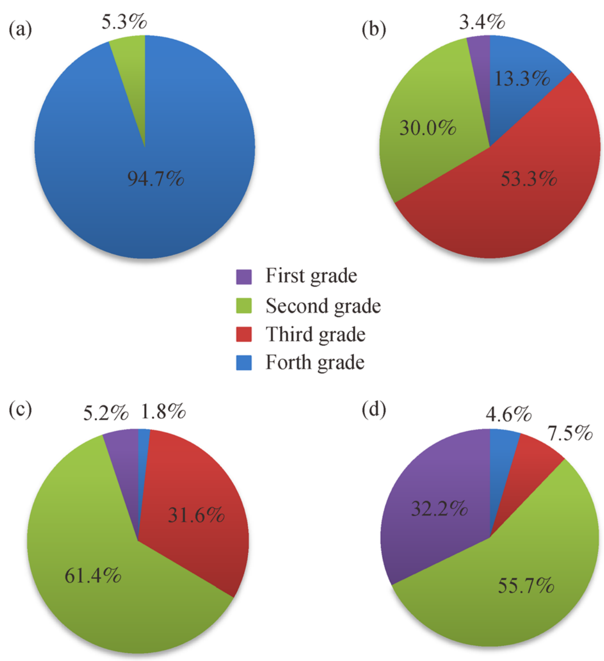

Fig. 3 Relationship between the ability to differentiate secondary laticifers and rubber yield. (a) Trees with rubber yield > 150 g; (b) trees with rubber yield > 100 –150 g; (c) trees with rubber yield > 50–100 g; (d) trees with rubber yield ≤ 50 g.

the level of endogenous jasmonate[18]. In the present study, 10 tapping times may have resulted in a pulsed production of endogenous jasmonates. The difference in the number of tapping-induced lines of secondary laticifers may be related to difference in the responsive ability of vascular cambia to the pulsed stimuli of the endogenous jasmonates. Although the number of the secondary laticifer lines in the trunk bark of regularly tapped rubber trees is positively related to the rubber yield[8], there is no obvious difference between the virgin trees[9]. Moreover, it is difficult to accurately count the secondary laticifer cells and the secondary laticifer lines in the secondary phloem. First, as the secondary laticifer cells in the same line link each other as a result of the fusion of the transverse convexity at side walls, it is difficult to distinguish the laticifer cells from the transverse convexity in the cross sections. Second, it is also difficult to discern the lines of secondary laticifers in non-functional secondary phloem because of their irregular arrangement[19]. By contrast, the number of the tapping-induced lines of secondary laticifers is easy to count.

In the present study, the ability to differentiate the secondary laticifers after ten times tapping varied among the *Hevea* germplasm assessed. Most of the virgin trees with high rubber yield had high ability to differentiate the secondary laticifers, and vice versa. However, as small number of trees with rubber yield less than 50 g also had a high number of secondary laticifer. This phenotype may be ascribed as obstructed latex flow as is the case for rubber tree clone PR107 which shows obstructive latex flow and has high numbers of secondary laticifer. The rubber yield-based selection always eliminates this phenotype from *Hevea* germplasm. This is why no selected rubber tree clones in the world have this phenotype.

5 Conclusions

Given that the number of the tapping-caused secondary laticifer lines is easy to count and positively related to the rubber yield, it may be a suitable parameter for early selection of the rubber yield potential in breeding for enhanced rubber yield.

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Supplementary materials The online version of this article at http://dx.doi.org/10.15302/J-FASE-2016122 contains supplementary materials (Appendix A).

Compliance with ethics guidelines Yueyi Chen, Xinsheng Gao, Xiaofei Zhang, and Weimin Tian declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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