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# The inclination of laticifers and phloic rays in six *Hevea* Clones

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**Abstract**
*Hevea brasiliensis*, coming under the family Euphorbiaceae is the major contributor of Natural Rubber for commercial utilization. The latex which contains rubber particles is synthesized in the latex vessels present in the secondary phloem. Latex is exploited by the process of controlled wounding called tapping. The commonly followed tapping system is upper left to lower right spiral cut based on the concept that latex vessels are inclined 3-5° towards the right. But anatomically it is proved that latex vessels are inclined to right or left. The present study aims to understand the inclination of latex vessels and phloic rays in six clones of *H.brasiliensis viz.* PB 255, PB 312, PB 314, RRII 417, RRII 422 and RRII 430. The inclination of latex vessels shows a mixed pattern in most of the clones studied. Even though a general trend is not predictable in the present study, clone PB 314 has more tendency towards left and RRII 417 towards rightward angle. The inclination pattern of latex vessels in *Hevea* can be used to design a new angle of tapping after field trials.

**Keywords:** *H. brasiliensis*, bark anatomy, laticifers, phloic rays, inclination

## Introduction
*Hevea brasiliensis* is a major crop utilized for the commercial exploitation of Natural Rubber in the world. Latex vessels or laticiferous system in *Hevea* is a specialized tissue system present in the secondary phloem, which we cut by the process of tapping to exploit latex from *Hevea* (Ridley, 1897; Abraham and Tayler, 1967) [9, 1]. Anatomically laticiferous system in *Hevea* is developed as an articulate anastomose network which is present as rows from the cambium to the outer region of the bark (Omman and Reghu, 2008). The laticifers in *Hevea* are considered to be the tissue system for the synthesis of rubber (Dickerson, 1965; Southorn, 1966; Gomez, 1966) [3, 10].
For the exploitation of latex from *Hevea*, we adopt a spiral cut from upper left to lower right at an angle of 25 in seedling trees and 30 for budded trees. This tapping system is based on the assumption and preliminary anatomical observation on the orientation and inclination of laticifers and other phloic elements in *H. brasiliensis*. The preliminary investigation regarding the orientation of wood elements in 25 trees was made by Petch (1911) [8]. He recommended a left inclined cut, as wood elements inclined to right in 18 trees. De Jong (1916) [2] observed that laticifers were inclined at an average of 3.7° to the right in 93 trees studied and also, he calculated extra yield for the various angle of cut. The extra yield for various angles in *Hevea* was also noticed by Mass (1925) [7] and Dijkman (1951) [4]. Gomez (1967) also noticed the significance of the inclination of laticifers and related it with the tapping and yield of the *Hevea* plant. Omman and Reghu (2008) reported a detailed investigation in 10 *Hevea* clones, that the laticifers and phloic rays have inclination patterns that may be inclined to left, right or even both rightward and leftward inclination in the clone. These investigations and findings put forward an important notion that the inclination of laticifers in *Hevea* is an important anatomical character. Moreover, it also signifies that a detailed investigation has to be taken to ascertain the role of inclination of laticifers with yield. The present investigation was aimed to decipher more information regarding the inclination of laticifers and phloic elements in certain clones, they were not studied earlier. The outcome of the investigation will be highly useful in modelling a change in the pattern of the current tapping angle and for comparison with yield.

## Materials and Methods
For the present investigation, six clones were selected. Trees from each clone as per statistical layout were selected to study the structure of bark. The PB clones *viz.*, , PB 255, PB 312 and PB 314 of *Hevea brasiliensis* were selected from the large scale evaluation trial farm planted in the Rubber Research Institute of India (RRII), Kottayam, Kerala and the RRII clones *viz*., RRII 417, RRII 422 and RRII 430 were selected from the pipeline clones of Botany Division at the Central Experimental Station (CES) of Rubber Research Institute of India, Chethackal, Ranni, Kerala. These trees are planted in a randomized block design (RBD) with three replicates and three trees per plot. The PB clones and RRII clones were at the age of 24 years and 21 years respectively and under regular tapping

**Table 1:** Details of the materials selected

| Sl. No. | Wickham Clones | Age (in years) | Origin / Parentage |
|---|---|---|---|
| 1 | PB 255 | 24 | Hybrid clone (PB 5/51 x PB 32/36) evolved by Prang Besar Estate, Malaysia |
| 2 | PB 312 | 24 | Hybrid clone (RRIM 600 x PB 235) evolved by Prang Besar Estate, Malaysia |
| 3 | PB 314 | 24 | Hybrid clone (RRIM 600 x PB 235) evolved by Prang Besar Estate, Malaysia |

| | | | |
|---|---|---|---|
| 4 | RRII 417 | 21 | Hybrid clone (RRII 105 x RRIC 100) evolved by the Rubber Research Institute of India |
| 5 | RRII 422 | 21 | Hybrid clone (RRII 105 x RRIC 100) evolved by the Rubber Research Institute of India |
| 6 | RRII 430 | 21 | Hybrid clone (RRII 105 x RRIC 100) evolved by the Rubber Research Institute of India |

To study the orientation and inclination of laticifers / phloic elements, virgin bark samples were collected from the selected trees at 150 cm height The sampling method reported by Gomez (1967) with modifications suggested by Omman and Reghu (2003) was adopted for the present investigation. The samples collected were fixed in formalin-acetic -alcohol (FAA) and were sectioned at 30 – 60 µm thickness at different planes viz. cross-sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) plane, using Reichert Jung sledge microtome. Sections were stained with Oil Red O (Omman and Reghu, 2003) and mounted in 50% glycerin and the micro slides were prepared by maintaining the orientation of the tissues as in the tree.

The bark sections were observed under the Leitz Aristoplan Research microscope attached to Leica Q 5000 I W Image Analysis System. The images of the bark sections documented in the Image Analysis System were used to measure the inclination of laticifers / phloic rays using Leica Q Win V.2.1 Image analysis software. The TLS of the bark was used to measure the inclination of laticifers and phloic rays. For each anatomical parameter, observations from ten microscopic fields were taken per plant.

**Result and Discussion**

The bark tissue is found as the outer tissue system formed from the cambium. This secondary phloem consists of sieve tubes, companion cells, phloem parenchyma consisting of ray and axial parenchyma, phloem fibres and a network of latex vessels. Based on the position of tissues in the bark three regions are present, the innermost region exists as a continuation of the cambium termed as soft bark (SB), the middle region consisting of most of the functional latex vessels termed as inner hard bark (IHB) region and the outer hard bark zone, marked by the presence of sclerified stone cells and disrupted latex vessels termed as outer hard bark (OHB) zone. The nature of the latex vessels in the SB and IHB is anatomically noticeable to measure the inclination and any other anatomical parameters of the latex vessels. Similarly, the inclination features of phloic rays in these two regions were also measured for the investigation.

**The angle of inclination of latex vessels in the soft bark**

Latex vessels show a mixed pattern of inclination among the trees in PB 255, five trees show an inclination to the left with an average value of 0.83° and four trees recorded 0.44° rightward inclination (Table 1). PB 312 exhibited a slight leftward inclination of 0.93° in three plants and six plants made a rightward inclination with an average value of 3.02°. PB 314 also recorded a mixed pattern within the clone. In seven trees latex vessels were inclined to the left with an average value of 1.1° and two plants made a rightward inclination with the value of 0.57°. In RRII 417, the clone depicted a rightward pattern of 1.87° in 8 plants and only one plant showed a deviation to left with a value of 1.95°. In RRII 422, three plants show a leftward inclination of 1.22° and six plants made a rightward inclination of 1.27° (Fig.3 a).

The clone RRII 430 also recorded inclination to both sides, six plants made leftward inclination with a value of 1.05° and in three plants latex vessels inclined to rightward direction with a value of 1.02° (Fig.3d).

**The angle of inclination of latex vessels in the inner hard bark region (IHB)**

The pattern of inclination of latex vessels in the inner hard bark region exhibited some slight change in comparison to that in the soft bark region Table 1). PB 255 it shows a pattern among the number of trees compared to that in the SB region. Five trees made leftward inclination with an average value of 0.84° and four trees showed leftward inclination with an average value of 0.78° (Fig.3 b). Similarly, the clone PB 312 depicted an average of 1.98° towards the left in three plants and six plants made an average rightward inclination of 4.15° (Fig.3 c). All the plants exhibited an inclination to left with an average value of 0.82° in PB 314. Similarly, all the plants had a rightward inclination of 1.09 in RRII 417. In RRII 422, two plants showed leftward inclination with 0.60°, while all the other 7 plants made rightward inclination 0.64°. In RRII 430, five plants have recorded a leftward inclination of 1.02° while the other four plants made a rightward inclination of 1.49° (Figure 1).

**The angle of inclination of phloic rays in soft bark (SB)**

Phloic rays exhibited an inclination pattern almost the same as the pattern shown by the latex vessels (Table 2). In the clone, PB 255 four plants showed phloic rays inclination towards left with an average value of 0.89° and in five plants it turned to the right with 0.91°. In PB 312, phloic rays' inclination was the same in the number of trees to left and right as that by latex vessels in SB. Here, three showed leftward with an average value of 1.07° and six trees turned to right having the value of 0.41°. PB 314, showed leftward inclination in eight trees with a value of 1.79° and only one tree to the right side with 1.24°. All the trees have depicted rightward inclination with an average value of 1.60° in RRII 417. In RRII 422, three trees made leftward inclination with 0.81° and six trees made rightward inclination with 1.06. Clone RRII 430 also exhibited an inclination to both directions. In seven plants phloic rays inclined to left with a value of 0.79° and in two plants inclination was to right with a value of 0.86° (Figure 2).

**The angle of inclination of phloic rays in the inner hard bark (IHB)**

In the inner hard bark region of PB 255, two plants showed an inclination towards the left with the value of 0.99° and seven plants showed an average of 1.81° (Table 2). PB 312 showed leftward inclination in two plants with an average of 0.77° and seven plants showed it to the right side with the highest value of 4.16°. In clone PB 314, six trees inclined towards the left with 1.02° and three plants inclined towards the right with a value of 1.21°. RRII 417 exhibited an inclination to right in all the nine trees with a value of 2.16°. Clone RRII 422 depicted leftward inclination in one plant with 0.79° and rightward inclination in eight trees with an average of 1.76°. RRII 430 made leftward inclination in 5 trees towards left with the value of 2.09° and four trees to right with a value of 0.81° Figure 2).

The present investigation gives the conformity that the two tissue systems, phloic rays and laticifers are aligned in the same orientation within the bark of *Hevea*. So that the inclination values recorded were almost the same for both phloic rays and laticifers. From this anatomical pattern, it can be assumed that the inclination of phloic elements may be an anatomical characteristic having a genetic link and this requires further detailed study. The present data also confirmed that both laticifers and phloic rays in five clones viz. PB 235, PB 255, PB 312, RRII 422 and RRII 430, were inclined to right and left, even though slight variation is existing soft bark and inner hard bark region. Excluding slight variation, the clone PB 314 and RRII 417 can be considered as pattern inclination to left and right respectively. The numerical difference in the laticifer inclination between soft bark and inner hard bark was irrelevant. This may be due to the influence of phloic rays' inclination, as the majority of the latex vessels are weaving around the phloic rays, within the bark. In this context, it is pertinent to correlate the inclination of laticifers with tapping systems adopted in *Hevea* in terms of latex yield. The slope of tapping cut from upper left to lower right and vice versa was a subject of debate during the early evolution of the Tapping system in *H. brasiliensis*. Petch (1911) [8] described an increase in yield in *Hevea* when the slope of cut was given from upper left to lower right. De Jong (1916) [2] measured the angle of inclination of latex vessels in 93 trees from unspecified clones and reported the average laticifer inclination towards the right as 3.7°. Mass (1925) [7] attempted to modify the slope of tapping cut in certain seedling trees and budded trees to get maximum latex yield. Considering the economic significance of latex yield and labour of tapping, Dijkman (1951) [4] suggested that the inclination of laticifers from vertical was the most important parameter related to the yield increase. Gomez and Chen (1967) considered different aspects of alignment of bark tissue and slope of tapping cut. He noticed from the recommended practice of giving 30°- 45° tapping slope (upper left to lower right) for budded trees, with the concept of 3-4° rightward inclination of laticifers obtained a yield increase of 2-3%, but the length of the cut to be tapped is increased by 22%. Presently, a spiral cut from upper left to lower right, slopes at an angle of 25° for seedling trees and 30° for budded trees are followed. The present study revealed that the inclination of laticifers varied from clone to clone towards left or right with a range of 1.95° to 0.83° and 3.02° to 0.44°, respectively, among the six clones under investigation. In this context, the suggestions made by Gomez and Chen (1967) assume significance. According to them, if more than half of the trees consistently displayed leftward orientation of laticifers, and then a right-hand half spiral cut might be recommended. Hence, it is suggested that the tapping practice being followed at present, needs further refinement, based on the inclination of laticifers in each clone.

## Conclusion

The present study revealed that in *Hevea*, the phloic rays and latex vessels were closely associated with the orientation and inclination. The alignment and inclination of phloic rays and latex vessels were found to be almost the same. This study reiterates the fact that the inclination of latex vessels in the *Hevea* should not be always rightward, it can turn left also. Even certain clones have an affinity towards leftward inclination. Hence a serious understanding and further field verification of latex vessel inclination and yield are essential to propose a tapping pattern in *Hevea*.

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**Table 1:** The angle of inclination of latex vessels in soft bark (SB) and inner hard bark (IHB)

| | SB | | | IHB | | |
|---|---|---|---|---|---|---|
| **Clones** | **No. of trees** | **Left/Right** | **Mean (degrees)** | **No. of trees** | **Left/Right** | **Mean (degrees)** |
| PB 255 | 5 | LEFT | 0.83 | 5 | LEFT | 0.84 |
| | 4 | RIGHT | 0.44 | 4 | RIGHT | 0.78 |
| PB 312 | 3 | LEFT | 0.93 | 3 | LEFT | 1.98 |
| | 6 | RIGHT | 3.02 | 6 | RIGHT | 4.15 |
| PB 314 | 7 | LEFT | 1.1 | 9 | LEFT | 0.82 |
| | 2 | RIGHT | 0.57 | - | - | - |
| RRII 417 | 1 | LEFT | 1.95 | - | - | - |
| | 8 | RIGHT | 1.87 | 9 | RIGHT | 1.09 |
| RRII 422 | 3 | LEFT | 1.22 | 2 | LEFT | 0.60 |
| | 6 | RIGHT | 1.27 | 7 | RIGHT | 0.64 |
| RRII 430 | 6 | LEFT | 1.05 | 5 | LEFT | 1.02 |
| | 3 | RIGHT | 1.02 | 4 | RIGHT | 1.49 |

**Table 2:** The angle of inclination of phloic rays in soft bark (SB) and inner hard bark (IHB)

| | SB | | | IHB | | |
|---|---|---|---|---|---|---|
| **Clones** | **No. of trees** | **Left/Right** | **Mean (degrees)** | **No. of trees** | **Left/Right** | **Mean (degrees)** |
| PB 255 | 4 | LEFT | 0.89 | 2 | LEFT | 0.99 |
| | 5 | RIGHT | 0.91 | 7 | RIGHT | 1.81 |
| PB 312 | 3 | LEFT | 1.07 | 2 | LEFT | 0.77 |
| | 6 | RIGHT | 0.41 | 7 | RIGHT | 4.16 |
| PB 314 | 8 | LEFT | 1.79 | 6 | LEFT | 1.02 |
| | 1 | RIGHT | 1.24 | 3 | RIGHT | 1.21 |
| RRII 417 | | | | | | |
| | 9 | RIGHT | 1.60 | 9 | RIGHT | 2.16 |
| RRII 422 | 3 | LEFT | 0.81 | 1 | LEFT | 0.79 |
| | 6 | RIGHT | 1.06 | 8 | RIGHT | 1.76 |
| RRII 430 | 7 | LEFT | 0.79 | 5 | LEFT | 2.09 |
| | 2 | RIGHT | 0.86 | 4 | RIGHT | 0.81 |

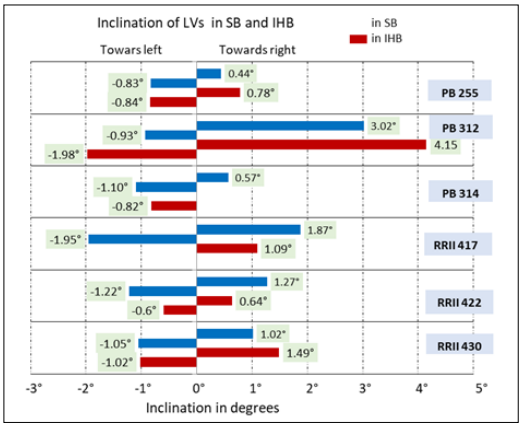


**Fig 1:** Graphical representation of Inclination of latex vessels in the SB and IHB

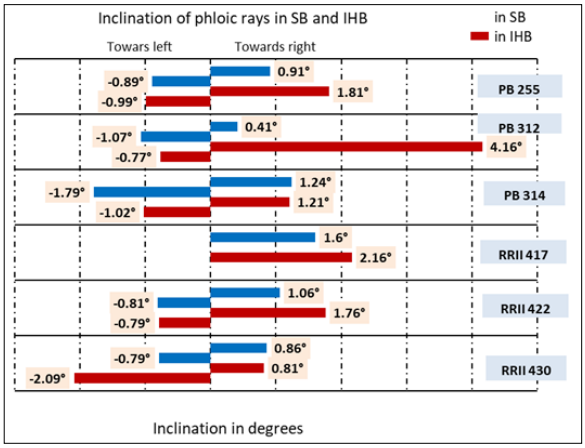


**Fig 2:** Graphical representation of Inclination of phloic rays in the SB and IHB

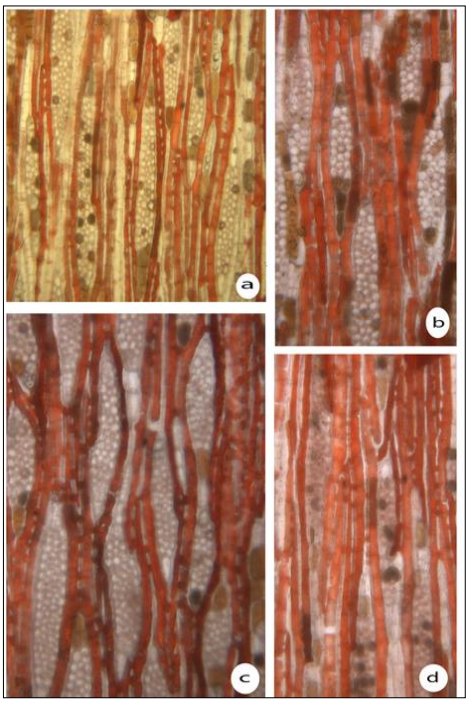


**Fig 3:** a-d. TLS of brak showing inclination of laticifers and phloic rays in the bark tissue. a- latex vessels and pholic rays inclined to right in the SB in rall 422. b- the inclination of latex vessels and phloic rays towards the left in the IHB in PB 255. c- laticifers and phloic rays show both right and leftward inclination in the IHB d.- latex vessels and phloic rays inclined to left in the SB in rall 430.

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