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# Comparative Histomorphology on Low and High Rubber Yielding Guayule Varieties of India

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**ABSTRACT:** A preliminary comparative histodimensional studies, such as wood to bark ratio, total area of parenchyma cells and bast fibers, height and diameter of vascular rays, pith diameter and phenological parameters, height of the plant, perimeter of the stem, dry weight of the stem and leaves have been investigated for the low and high rubber bearing Guayule plants growing in same and different climatic regions of India. A plausible correlation is found between the percentage of rubber content and anatomical characters in the investigated varieties.

***Key words:*** Guayule, Histodimensional studies, *Parthenium argentatum*, Rubber.

## INTRODUCTION

Guayule-*Parthenium argentatum* Gray is a member of Asteraceae. This is the only species that produces appreciable quantity of rubber. Recently Guayule is being considered the best potential alternative renewable source of energy. Guayule (pronounced as Wy-oo-le)was first discovered in the Mexican boundary survey. Asa Gray (Mehta and Hanson 1983) first gave its systematic citiation authority. The name *Parthenium argentatum* Gray etymologically sounds well, the generic term Parthenium embryologically implies that it is apomictic and the specific epithet *argentatum* owes to its silvery lustrous appearance of leaves.

Unlike Hevea, Guayule rubber occurs in the cells of individual parenchyma cells of cortex, rays of phloem and xylem of stem and root. Guayule rubber does not exude on tapping as in Hevea but its tissue must be macerated thoroughly to free the rubber particles.

Many aspects on Guayule, including stem anatomy of *P. argentatum, P. incanum* and their native Mexican hybrids have been well studied (Mehta and Hanson 1979, Mehta 1982). Knowing the economic importance and commercial prospects of guayule, and the expected crisis of natural rubber from Hevea in future forced India to introduce guayule to utilize waste tract of unused nonconventional land. Cultivation of Hevea is climatelogically restricted to some regions of India. But many areas have to be explored on the lines of Gyayule. Present investigation is aimed at studying the detail on histodimension of stem tissues, the distribution of rubber in the stem tissues of Low Rubber Yielding (LRY) and High Rubber Yielding (HRY) Guayule varieties growing in the same and different agro-climatic regions and also to find out possible correlation if any, between rubber content and anatomical characters. This study is ultimately useful for forecasting the LRY and HRY varieties among the different elites, which are being introduced recently in India.

## MATERIALS AND METHODS

Two Guayule varieties A48118 (LRY-low rubber yielding, 0.61% dry wt.) and 11605 (HRY-high rubber yielding, 2.28 % dry wt.) were collected from the Botanical Garden, Sardar Patel University, Gujarat, India to study the anatomical differences. Plants were collected from the same location to avoid the anatomical differences due to climate or geographical differences.

Samples from 11488 variety collected from National Botanical Research Institute (NBRI) Lucknow, India were utilized to compare anatomical features with the 11605 variety collected from Gujarat (Table 2). This study is to measure the influence of locality factor on the plants growing in different regions of India.

Plants selected were 18 months old and the stem diameter was over 1cm. Samples were fixed in FAA and were brought in ice bag to laboratory to estimate metabolites. Oven dried, stem samples of the collected varieties were sent to Indian Institute of Petroleum (IIP), Dehradun, India to estimate the rubber content.

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Transverse and longitudinal sections were cut at 20-30m thickness from each sample using a sliding microtome. Sections were used to study the distribution of rubber *in situ* (Jayabalan and Shah 1986) and safranin-O stained sections for anatomical features. From each sample dimension of bark, wood, pith, vascular rays height and number of cells, width of vascular rays and cell number, and number of resin ducts in cortex pith region were studied. An average of 100 measurements for each parameter were taken. Drawings of small regions of bark were obtained from the projected image on vesopan to study the total area of parenchyma tissues (principal region for storing rubber) (Mehta *et al.* 1979). The inorganic phryo PP resin and rubber content were estimated from all varieties according to stardmel testing.

## RESULTS AND DISCUSSION

Observation on histomorphological and phenological parameters among the plants growing in same and different agro-climatic regions were tabulated (Tables 1 and 2). In the stem of 18 months old plant, rubber is distributed in cortex- pith parenchyma, rays of phellem and xylem and epithelial cells of resin duct in cortex and secondary phloem. Rubber is absent in phellem cells, phloem tissues including sieve elements, wood parenchyma and in vessels.

On the basis of anatomical characters, Mehta and Hanson (1983) classified the native Mexican guayule plants at their 5$^{th}$ year, as low or high rubber bearing plants. The plants containing less than 7% of rubber were reported as low rubber bearing plants and classified them as group III Guayule. Some significant anatomical characters of group III Guayule plants, as described by Mehta *et al.* (1979). The group III Guayule plants posses approximate wood: bark ratio = 1: 0.43. The average height / diameter of vascular rays = 542.2m or 22.22 cells/ 49.41m of 4.19 cells, presence of sclerenchymatous cells in vascular and diameter of pith 852.6 nm/18.38 cells across the pith. Group I and II guayule plants which contain more than 7% of rubber have similar anatomical characters but have increased dimensional values, absence or rare occurrence of sclerenchymatous cells in rays, pith, and presence of more number of large sized resin canals (Mehta and Hanson 1983).

Guayule variety 11605 was introduced in India as HRY at its 18 months old stage that had 2.28% of rubber content. It showed anatomical similarities with group II Mexican type, but contained comparatively less amount of rubber. Hence it could be suggested that the guayule variety 11605 introduced in India is basically a LRY variety. Despite notable similarities with group II Mexican type (plants contain more 7.5% and an average of 10% rubber content), the presence of rubber in 11605 variety in the whole plant or in the mature stem alone does not exceed 3% (dry wt).

However quantity of rubber in the stem of 30 months old stage showed 1.1% for A48118 LRY and 4.56% for 11605 (HRY) variety (Srivastava 1985).

Occurrence of less amount of rubber may be due to the physiological or climatic factors as reported in Guayule, Hevea and in other laticifer plants (Shukla and Krishnamurthy 1971; Ostler *et al.* 1980, Braggy and Lacewell 1980, Metha 1982). As 11605 variety, is being introduced recently in India, the new agro climatic conditions may restrict the plant to use the available rubber storing parenchyma tissue to its fullest capacity. The relative

**Table 1.** Comparative histomorphological studies of low and high rubber containing guayule

| S. No. | Parameter studied | LRY (A48118) | HRY (11605) |
|---|---|---|---|
| 1. | Rubber content % | 0.61 | 2.28 |
| 2. | Xylem : Phloem ratio (cortex, phloem) | 1:0.23 | 1:0.38 |
| 3. | No. of growth rings | 2 - 3 | 4 - 6 |
| 4. | Diameter, height and dry wt. of stem | 4.9cm, 58.4 cm, 56.6 g | 6.8cm, 69.8cm, 64.8 g |
| 5. | Dry wt of leaves (50 numbers) | 0.518 g | 0.793 g |
| 6. | Total area of the bark ($mm^2$ ) | 5810.00 | 7836.00 |
| 7. | Area occupied by resin duct, bast fibers and active phloem ($mm^2$) | 2086.00 | 2229.00 |
| 8. | Relative area occupied by resin ducts and bast fibers (%) | 35.90 | 28.00 |
| 9. | Relative area occupied by prenchymatous tissue (%) | 64.00 | 71.00 |
| 10. | Average diameter of pith (m) | 449.87 | 786.43 |
| 11. | Average number of primary vascular rays | 18.34 | 22.64 |
| 12. | Average number of resin duct in bark and pith | 186.77/ 9.92 | 153.48/5.68 |
| 13. | Average height of vascular rays (m) | 356.26 | 538.42 |
| 14. | Average height of vascular rays (cells) | 19.53 | 23.46 |
| 15. | Average width of vascular rays (m) | 24.42 | 33.97 |
| 16. | Average width of vascular ryas (cells) | 2.46 | 4.49 |
| 17. | Sclerenchymatous cells in vascular rays | Present | Rarely present |
| 18. | Sclerenchymatous cells in the pith | Present | Absent |
| 19. | Average No. of cells across pith diameter | 16.68 | 21.23 |

area occupied by parenchyma tissue of bark in HRY is greater (72%) than in LRY (64%). Hence the parenchyma tissue (the storage area for rubber accumulation) is more in HRY and the accumulation of rubber content is also found to be increased. Even though less number of parenchyma cells occur in the LRY, the lignified sclerenchymatous tissue in the pith also additionally restrict the storage area available for rubber content. However, there exists some concrete anatomical difference between the plant containing 0.61% and 2.28% rubber, respectively (Table 1). So, these histological parameters have correlative significance with the respective rubber content.

In the present study an appreciable amount of inorganic pyrophosphate (IPP) is first localised in the epithelial cells of primary resin ducts of young stem.

In guayule, the total production of xylem and phloem at a particular time depends on the availability of water, day-night temperatures and environmental factors (Lloyd 1911, Archwager 1945). Two plants investigated containing 0.61% and 2.28% rubber content which were under the same climatic conditions (20 °C -40°C and 170-200 mm rain fall) and received the same amount of irrigation water stress etc., produced different amount of xylem and phloem (i.e xylem bark ratio) (Table 2) and variable phenological characters viz., height of the plant circumference (perimeter) of the stem, dry weight of the stem and leaves. Therefore, it is suggested that besides environmental factors, the deciding factor for the production of any type of tissue might be due to genetic making (hormonal or gene) of the plant which is evinced by the present study on two plants containing different amounts of rubber.

Variations observed in the total height of the plant, circumference and diameter of the stem in LRY (0-61% rubber content) and HRY 2.28% rubber content) is due to the difference in the number of cells and their sizes. The sizes of the cell in pith diameter of high or low rubber bearing plant is also due to the difference in the genetic make up of the plant (Addicott and Pankhurst 1944).

Studies in guayule and in other plants indicate that usually in normal plants the production and the earlier report it is suggested that the high rubber bearing plant has more, large sized resin ducts than the low rubber bearing plant (Mehta and Hanson 1983, Mehta 1982). However the present study shows that the plants contain less amount of rubber (0-61%) have also numerous large sized ducts and less number of resin ducts in the plants containing more rubber (2-28%).

Number of epithetical cells, resin ducts and the amount of resin production are more important determining factors in rubber biosynthesis. It is obvious that the amount of resin (an oxidative hydrocarbanaceous acetone soluble terpene compounds) production depends on the number of resin ducts and the amount of precursors inorganic phyrophosphate. The high yielding variety contains more amount of IPP, less amount of resin and more amount of rubber (2.28%) while the low yielding is found to contain comparatively less amount of IPP, and less amount of IPP, more amount of resin and less amount of rubber (0.61%) which are evinced by biochemical quantification.

Biosynthesis of resin starts long before the biosynthesis of rubber in guayule and it is reported that rubber biosynthesis takes place first in the epithelial cells of resin duct. So it is presumed here that the ducts utilized more amount of IPP an inorganic phosphate, which acts as a common precursor for the synthesis of resin and rubber (Arreguin *et al.*1951, Archer 1980, McIntyre *et al.* 1980). Proportion of phloem in relation to xylem (xylem: bark ratio) remain constant (Waisel and Fahn 1965, Waisel *et al.* 1966, Mehta and Hanson 1983). Hence comparative study

**Table 2.** Comparative histomorphological studies on 11488 and 11605 guayule varieties

| S. No. | Parameters studied | 11488 Varieties | 11605 Varieties |
|---|---|---|---|
| 1. | Rubber content(%) | 1.98 | 2.28 |
| 2. | Xylem: Phloem ratio | 1:0.37 | 1:0.38 |
| 3. | Total area of the bark ($mm^2$) | 7118.00 | 7836.00 |
| 4. | Area occupied by resin duct, bast fiber, active phloem ($mm^2$) | 2239.00 | 2229.00 |
| 5. | Relative area occupied by resin duct and bad fibers (%) | 31.45 | 28.00 |
| 6. | Relative area occupied by parenchymatous tissue (%) | 68.55 | 72.00 |
| 7. | Average diameter of pith cells | 713.33 | 786.43 |
| 8. | Average No. of primary vascular rays cells | 20.47 | 22.64 |
| 9. | Average No. of resin duct in bast and in pith | 159.35, 6.53 | 153.48, 5.68 |
| 10. | Average height of vascular rays m | 507.62 | 538.42 |
| 11. | Average height of vascular ray cells | 21.66 | 23.46 |
| 12. | Average width of vascular rays (m) | 31.78 | 33.97 |
| 13. | Average width of vascular rays (m) | 31.78 | 33.97 |
| 14. | Average width of vascular rays (cells) | 3.97 | 4.49 |
| 15. | Schlerenchymatous cells in vascular rays | Rarely present | Rarely present |
| 16. | Schlerenchymatous cells in pith | Rarely present | Absent |
| 17. | Average number of cells across pith diameter | 19.98 | 21.23 |

made on two different varieties having almost the amount of rubber content, but collected from different locations i.e., guayule variety 11488 collected from NBRI, Lucknow and the 11605 variety collected from Botanical Garden Sardar Patel University have shown similarity in xylem. Bark ratio (1:0.37 and 1:0.38 respectively) and other anatomical characters (Table, 2). Though these two plants were collected from different locations they contain appreciable amount of parenchymatous tissues (68% and 72%). This shows that the production of tissues in plants growing in different locations of India is not affected by climatic conditions prevailing in India, but less accumulation of rubber is due to genetic make up of the plant or soil factors.

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