# VARIABILITY IN GROWTH AND DISTRIBUTION OF INTRAXYLARY PHLOEM IN CERTAIN CLONES OF *HEVEA BRASILIENSIS*

**R. Deepthi[1], T. Gireesh and T. Meenakumari**

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

[1]Research Centre, University of Kerala, Thiruvananthapuram-695 034, Kerala, India

Received: 01 December 2021 Accepted: 17 December 2021

Deepthi, R., Gireesh, T. and Meenakumari, T. (2021). Variability in growth and distribution of intraxylary phloem in certain clones of *Hevea brasiliensis*. *Rubber Science,* **34**(3): 257-263.

The presence of Intraxylary Phloem (IP) tissue were quantified and variability assessed in a set of *Hevea brasiliensis* clones. It is expected that the presence of an additional phloem tissue will promote better translocation along with the external phloem. The present work discusses genetic variability of IP in relation to growth and girth increment rate of newly evolved clones in a clonal nursery. Four clones (704, 690, 712, 688) were identified with robust girth, high girth increment rate and high prevalence of IP, in comparison with similar parameters present in the check clone *i.e.*, RRII 429, adapted and recommended for abiotic stress prone non-traditional areas. Results indicated that variability in the number of IP could be an intrinsic anatomical trait useful for screening and shortlisting of clones for non-traditional areas.

**Keywords**: *Hevea brasiliensis*, Intraxylary phloem, Primary phloem, Secondary traits

## INTRODUCTION

The Para rubber tree, *Hevea brasiliensis*, is an important tree species which provides Natural Rubber (NR), an important industrial raw material with multifaceted applications particularly in the automobile and allied industries. *H. brasiliensis* produces high quality latex (poly *cis*-isoprene) in the bark, which stands top in terms of quality. Due to ever growing demand of this material, natural rubber cultivation is expanding to non-conventional areas with extreme climatic factors. In order to circumvent this scenario, development of cultivars with adaptive features is required. Presence of an internal phloem or Intraxylary Phloem (IP) was first reported in members of Cucurbitaceae (Hartig, 1854); later in Solanaceae and Apocynaceae (Vesque, 1875). Most often located at the adaxial end of protoxylem of vascular bundles of many dicots (Gondaliya and Rajput, 2017), it is largely exhibited through primary growth (Evert, 2006). Anatomical studies towards assessing variability of IP in different genotypes/cultivars are scanty. The role of internal phloem in physiological processes associated with drought resistance has been reported by Esau (1974). Presence of this tissue has been reported in crops like Cassava (Graciano-Ribeiro *et al.*, 2009) and Croton (Hayden and Hayden, 1994) and used as an indicator of stress tolerance potential. Literature indicates that

Correspondence : Gireeesh T. (Email: gireesh@rubberboard.org.in)

occurrence of IP is reported in 30 families of dicot (Metcalfe and Chalk, 1983). Recently, presence of internal phloem was reported in *Aquilaria sinensis* (Yang *et al*., 2020). In *Hevea*, Premakumari *et al.* (1985) reported the presence of IP for the first time. It is characterised by the presence of phloem tissues flanking the primary xylem points in stem. It is reported that higher proportion of IP was associated with high girth increment under tapping in *Hevea* (Premakumari *et al*., 1985). Different clones respond differently during tapping stress. In general, tapping stress causes girth retardation across clones. However, there are clones which exhibit low girth retardation on tapping. In this context, identification of intrinsic traits to overcome stress through quantification of IP was examined in different *Hevea* clones. The formation of IP may be an alternative channel for translocating the photosynthates and protection from external injury like physical and insect herbivore (Gondaliya and Rajput, 2017). Hence, in *Hevea* the presence of IP may act as an additional protection against external injury caused due to tapping on bark tissues or any kind of biotic and abiotic stress experienced throughout the productive phase. Under drought stress conditions IP may assist in long distance translocation of photosynthates and growth substances along with the external phloem. Hence, quantification of IP as a secondary trait and assessment of clonal variability for this trait assume significance. The present work discusses genetic variability of IP in relation to growth and girth increment rate of twenty one newly evolved clones.

## MATERIALS AND METHODS

A preliminary assessment of distribution of IP was attempted in twenty one new clones and its parents (PB 330, RRII 414, RRII 429 and RRII 105). Studies were carried out at the Central Experiment Station of Rubber Research Institute of India, at Chethackal, Pathanamthitta District (Latitude 9°22′N, Longitude 76°5′ E; Altitude 100 m). We studied the stem anatomy of *H. brasiliensis* clones, planted in a clonal nursery with a spacing of 2.5 m x 2.5 m. These clones were under regular tapping and the yield was recorded in two seasons *viz*. peak yielding period (October to January) and lean yielding months (February to May) of each year. S/2 d3 6d/7 system of tapping was followed (Vijayakumar *et al*., 2009) without stimulation. One year old twig samples (two trees per plot) were collected from twelve year old trees of 25 genotypes of *Hevea*. Twig samples were cut into 5-8 cm long pieces and immediately fixed in freshly prepared FAA (Johansen, 1940) and stored for further processing. Thin transverse cross-sections of the middle portion of the brown stem were prepared at 80-100 μm thickness with the help of a sledge microtome. Sections were stained with Toluedene blue O and observations were made using a bright field microscope. The total number of Primary Xylem Points (PXP) and number of IP associated with the PXP were scored and quantified. Growth traits like main trunk girth and girth increment were taken and growth of the trees in the corresponding period in terms of girth increment was estimated. The data was subjected to statistical analysis and variance estimated.

## RESULTS AND DISCUSSION

In the present study, quantification of IP from one year old twig samples was attempted and results are presented in Table 1. Both protoxylem as well as IP tissue recorded

Table 1. **Growth and distribution of intraxylary phloem**

| Clones | Parentage | Girth (cm) | Girth increment (cm) | Diameter (cm) | No. of PXP of twig (±SD) | No. of IP (±SD) |
|---|---|---|---|---|---|---|
| 704 | PB 330 x RRII 414 | 39.4 | 1.0 | 1.1 | 27.9 ± 5.2 | 10.7 ± 1.2 |
| 514 | PB 330 x RRII 414 | 39.8 | 0.9 | 0.9 | 22.6 ± 8.6 | 6.4 ± 2.9 |
| 683 | PB 330 x RRII 414 | 57.5 | 1.3 | 1.1 | 22.6 ± 5.8 | 4.0 ± 1.0 |
| 445 | RRII 429 OP | 45.9 | 1.4 | 1.1 | 21.3 ± 4.5 | 2.8 ± 0.5 |
| 690 | PB 330 x RRII 414 | 48.9 | 1.4 | 1.1 | 19.5 ± 2.4 | 9.8 ± 1.4 |
| 342 | RRII 414 OP | 35.7 | 0.6 | 1.2 | 29.0 ± 7.4 | 18.5 ± 2.5 |
| 575 | RRII 414 OP | 30.2 | 0.6 | 1.1 | 27.8 ± 7.5 | 17.3 ± 3.6 |
| 701 | PB 330 x RRII 414 | 34.4 | 0.9 | 1.0 | 24.2 ± 9.0 | 6.3 ± 0.5 |
| 712 | PB 330 x RRII 414 | 49.9 | 1.3 | 1.1 | 22.8 ± 5.1 | 11.4 ± 2.7 |
| 512 | PB 330 x RRII 414 | 46.1 | 1.4 | 1.2 | 19.2 ± 6.0 | 8.3 ± 1.3 |
| 700 | PB 330 x RRII 414 | 36.2 | 0.9 | 1.1 | 20.1 ± 2.9 | 0.5 ± 0.01 |
| 844 | PB 330 OP | 45.0 | 0.8 | 1.1 | 20.4 ± 9.6 | 6.9 ± 2.7 |
| 477 | RRII 414 x PB 330 | 43.2 | 0.8 | 0.9 | 26.7 ± 6.6 | 9.1 ± 1.2 |
| 822 | PB 330 OP | 43.7 | 0.9 | 1.1 | 21.6 ± 6.1 | 6.2 ± 2.1 |
| 286 | RRII 105 OP | 48.9 | 1.3 | 1.2 | 19.8 ± 6.5 | 0.0 ± 0.0 |
| 335 | RRII 414 OP | 53.5 | 1.3 | 1.1 | 25.5 ± 6.1 | 6.8 ± 3.1 |
| 848 | RRII 429 OP | 19.5 | 0.0 | 1.0 | 23.6 ± 2.8 | 0.0 ± 0.0 |
| 638 | PB 330 x RRII 414 | 45.3 | 0.6 | 1.1 | 16.6 ± 7.3 | 5.4 ± 2.7 |
| 688 | PB 330 x RRII 414 | 54.7 | 1.2 | 1.2 | 24.9 ± 11.2 | 11.7 ± 3.5 |
| 881 | PB 330 x RRII 429 | 38.4 | 0.7 | 1.1 | 21.9 ± 4.2 | 5.6 ± 1.2 |
| 843 | PB 330 OP | 28.8 | 0.6 | 1.1 | 32.2 ± 9.5 | 9.3 ± 2.8 |
| RRII 414 | RRII 414 | 34.6 | 0.6 | 1.0 | 19.0 ± 2.4 | 1.1 ± 0.15 |
| RRII 105 | RRII 105 | 29.5 | 0.3 | 1.0 | 26.2 ± 4.3 | 6.0 ± 1.01 |
| RRII 429 | RRII 429 | 35.5 | 0.5 | 1.1 | 17.9 ± 4.4 | 9.8 ± 3.8 |
| PB 330 | PB 330 | 47.6 | 1.6 | 1.0 | 25.4 ± 7.0 | 4.8 ± 1.0 |
| G. mean | | 41.3 | 0.9 | 1.1 | 23.1 ± 6.3 | 7.1 ± 2.0 |
| CD(P<0.05) | | 12.1 | 0.5 | — | — — | |
| Max. | | 57.5 | 1.6 | 1.2 | 32.2 | 18.5 |
| Min. | | 19.5 | 0.0 | 0.9 | 16.6 | 0.0 |

Values are means of observations (n=24); PXP: Primary xylem points; IP: Intraxylary phloem

clonal variability among the 25 clones. Number of protoxylem points ranged from 16.6 (clone 638) to 32.2 (clone 843), whereas, the range in IP varied from 0 (clone 848) to 18.5 (clone 342). The highest number of IP was observed in clone 342 with moderate level of girth increment (0.6 cm). Clone 690 possessed high girth increment as well as high number of IP while the diameter of twig ranged from 0.9 to 1.2 cm.

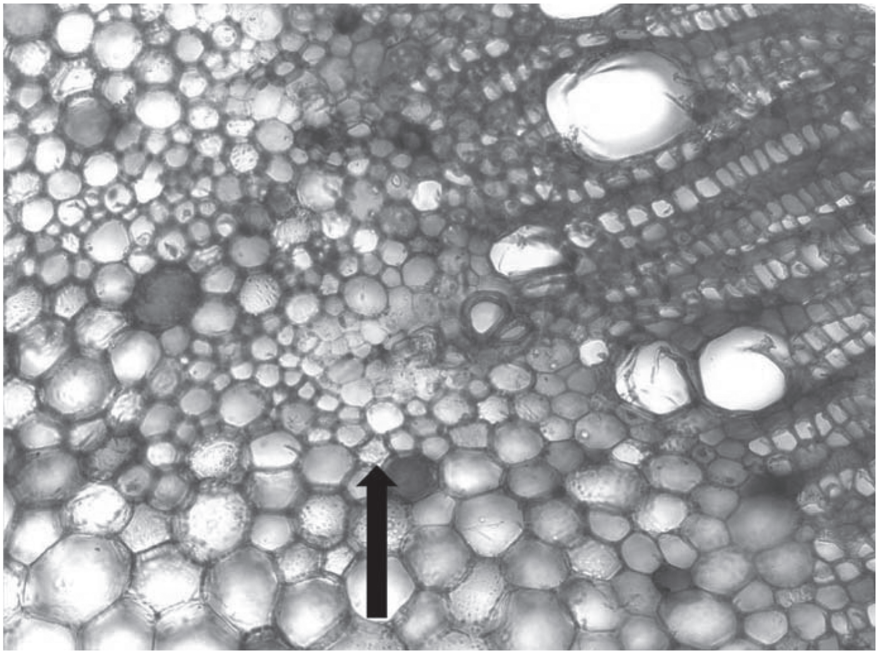

Fig. 1. Intraxylary phloem of RRII 429 in the pericentral region stained with Toluidine Blue O (magnification 25 x under light microscope)

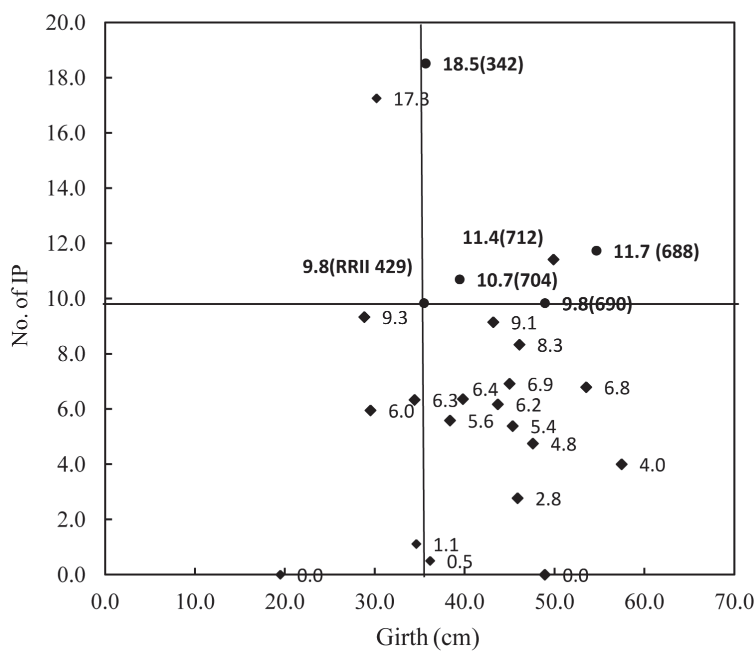


Fig. 2. Clonal variation in growth: Girth and IP

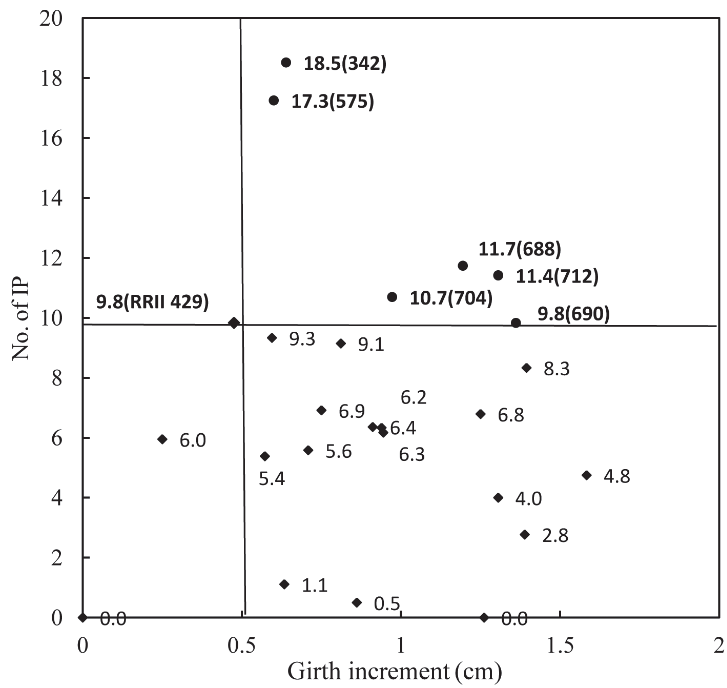


Fig. 3. Clonal variation in growth: Girth increment and IP

Among the check clones, RRII 429 recorded the highest IP (9.8). Fifty five per cent of the protoxylem of RRII 429 showed IP strands (Fig. 1) attached to it. Biplots were constructed for growth as well as girth increment rate *vis-a-vis* number of IP points (Fig. 2 and 3). Four clones were identified (704, 690, 712, 688) with high girth, girth increment and IP, which imparted superior performance than RRII 429. Two clones, 342 and 575 which recorded high IP showed poor growth and girth increment rate. All the four clones were descendants of the cross combination PB 330 x RRII 414. Clone PB 330 has been proven for its high growth vigour (Deepthi and Gireesh, 2021). Moreover, the performance of these clones was better than that of RRII 429, the second vigorous check clone. RRII 429 is proven for abiotic stress tolerance both in the cold-prone NE India (Dey *et al.*, 2019) as well as drought-prone eastern India region (Meenakumari *et al.*, 2018).

In rubber, harvesting latex through tapping is a process of controlled wounding which induces stress and therefore girth increment rate of trees on tapping has a major role in sustaining yield in the long run. Premakumari *et al.* (1988) reported a positive correlation of the presence of IP with girth increment on tapping. According to Gondaliya and Rajput (2017), IP may be an adaptive feature to prevent the sieve elements to become non-conducting during summer when the temperature is much higher. Mercy *et al.* (2017) observed significant clonal variability for IP in terms of number of strands and quantity of phloem tissue among a set of wild accessions grown in a severely drought-prone region of Maharashtra. Rubber cultivation is sprawling not only in the non-traditional region but also within the traditional region. Abiotic stresses and rising atmospheric temperature are major threats for rubber cultivation in the years to come. The indications from the present study offer scope for selecting adaptive structural features for sustained growth and yield under abiotic stress.

## ACKNOWLEDGEMENTS

The authors are thankful to the University Grants Commission (UGC), Government of India for providing financial support. The technical support received from Mr. P. Aneesh, Asst. Statistician and Dr. Vinoth Thomas, Principal Scientist is gratefully acknowledged.

## REFERENCES

Deepthi, R. and Gireesh, T. (2021). Recombination breeding for growth and stem straightness using certain indigenous and exotic clones of *Hevea brasiliensis.* In: *Proceedings of NHPS International Conference on New Horizons in Plant Science,* 4-9 January 2021, (Ed. T.S. Swapna), Department of Botany, University of Kerala, Thiruvananthapuram, India, pp. 176-178.

Dey, S.K., Antony, P.D., Das, G., Meenakumari, T., Mandal, D., Singh, R., Reju, M.J., Mydin, K.K. and Jacob, J. (2019). Performance of RRII 429 in North East India- A Report. Rubber Research Institute of India, Kottayam, India, 30p.

Esau, K. (1974). O Caule: Estagioprimario de crescimento. In: *Anatomia das Plantas com Sementes* (Ed. E. Blucher). Edgard Blucher Ltda., Sao Paulo, pp. 160-185.

Evert, R.F. (2006). *Esau's Plant Anatomy*. Ed.3, John Wiley & Sons, Inc., New Jersey. 601p.

Gondaliya, A.D., Rajput, K.S. (2017). Stem anatomy and development of inter- and intraxylary phloem in *Leptadenia pyrotechnica* (Forssk.) Decne. (Asclepiadaceae). *Plant Biosystems*, **151**(5): 855-865. doi: org/10.1080/11263504.2016.1218968.

Graciano-Ribeiro, D., Hashimoto, D.Y.C., Nogueira, L.C., Teodoro, D., Miranda, S.F. and Nassar, N.M.A. (2009). Internal phloem in an interspecific hybrid of cassava, an indicator of breeding value for drought resistance. *Genetics and Molecular Research*, **8**(3): 1139-1146.

Hartig, T. (1854). Über die Querscheidewändezwischen den einzelnen Gliedern der Siebröhrenin *Cucurbita pepo*. *Bot Zeitung*, **12**: 52-54.

Hayden, S. M. and Hayden, W. J. (1994). Stem development, medullary bundles and wood anatomy of *Croton glandulosus* var. Septentrionalis (Euphorbiaceae). *International Association of Wood Anatomists Journal*, **15**: 51-63.

Johansen, D. A. (1940). *Plant Microtechnique*. Mc-Graw Hill, New York. 523 p.

Meenakumari, T., Meenattoor, J. R., Thirunavoukkarasu, M., Vinod, K.K., Krishan, B., Gireesh, T., Thomas, V., Mydin, K.K. and Jacob, J. (2018). Dynamics of long-term adaptive responses in growth and rubber yield among *Hevea brasiliensis* genotypes introduced to a dry sub-humid climate of Eastern India. *Industrial Crops and Products*, **119**: 294-303.

Mercy, M.A., Nair, D.B., Meenakumari, T., Singh, M. and Mydin, K.K. (2017). Performance of wild Amazonian accessions and hybrid *Hevea* clones of Wickham origin in a drought stressed environment. *Proceedings of National Seminar on Natural Resource Management for Horticultural Crops under Changing Climatic Conditions*, NRMHCCC-2017, 16-17 March 2017, Centre for Water Resources Development and Management, Calicut, India, Abstracts, pp. 81-82.

Metcalfe, C.R. and Chalk, L. (1983). *Anatomy of the Dicotyledons*. Ed. 2. Clarendon Press, Oxford.

Premakumari, D., Panikkar, A.O.N. and Shankar, S. (1985). Occurrence of intraxylary phloem in *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. *Annals of Botany*, **55**(2): 275-277.

Premakumari, D. and Panikkar, A.O.N. (1988). Studies on the intraxylary phloem and its association with certain growth characters in *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell.

Arg. *Indian Journal of Natural Rubber Research*, **1**(2): 41-44.

Vesque, J. (1875). Mémoiresurl' anatomie comparée de l'écorce. *Annales des Sciences Naturelles Botanique,* **2**: 82-198.

Vijayakumar, K.R., Eric Gohet, Thomas, K.U., Wei Xiaodi, Sumarmadji Lakshman Rodrigo, Do Kimthanh Pichit Sopchoke, Karunaichamy, K. and Mohd Akbar Md. Said. (2009). Revised International Notation For Latex Harvest technology, p. 1-19.

Yang, S.Z., Chen, P.H. and Chen, C.F. (2020). Cambial variants combine successive cambia and intraxylary phloem in *Convolvulaceae* in Taiwan. *American Journal of Plant Sciences*, **11**(03): 437-453. https://doi.org/10.4236/ajps.2020.113032.