Root anatomy of modified root trainer plants of *Hevea Brasiliensis*

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ABSTRACT: *Manipulation of root architecture in a perennial tree crop like Hevea brasiliensis assumes dual significance. Recently, polybag planting materials are being replaced with modern root trainer plants characterised by air pruned tap roots and numerous laterals. Air pruned tap root raises apprehension among the growers on further root development and establishment after field planting. More over, moisture stress and wind susceptibility due to changing climatic conditions are becoming severe in rubber growing areas. In this context attempts to modify the root architecture and detailed investigations on the structural organization of the tissues in the modified roots with respected to polarity was studied. Modification in root architecture could be achieved by allowing the air pruned roots to grow into a root elongation tube (RET) filled with potting medium, and attached tightly to the drainage hole of the root trainer cup. The root core developed in the RET after two months is characterized by 10-12 thick vertical roots, profuse laterals and fine roots. Average root elongation of the vertical roots within the RET was 1.8cm per day. Proportion of various tissues, number of pericycle layers, shape of stele, accumulation of starch and birefringent property of the cellulosic microfibrils in the pith are distinct for vertical and horizontal (lateral) roots, which assume taxonomic significance also.The root architecture thus modified can increase the functionally active surface area for better absorption of soil resources and can ensure better growth and anchorage.*

Key words*: Birefringence, Hevea, Planting material, Root anatomy, Root architecture, Root trainer plants*

Introduction

Shortage of water is a constraint to life that recurs more and more in many regions of the world due to global climate change (IPCC 2007). Increasing drought has an impact on the survival of plants in natural ecosystems, while it also results in reduced yields in crops. The prime factor that determines the ability of the plant to access water and nutrients from the soil is the size and architecture of the root system (Werner et al. 2010). Most studies on organ size control is confined to terrestrial organs (Li et al. 2008), whereas roots are only rarely considered. As the trait is governed by many genes, classical breeding approaches are difficult to achieve the goal (Werner et al. 2010). In this context, understanding and manipulation of root architecture becomes more important.

Hevea brasiliensis, the prime source of natural rubber, is a perennial subtropical tree crop with more than thirty years of economic life span. Among the rubber growing countries India rank first in productivity and fourth in production of natural rubber (NR). As the national production is not adequate to meet the domestic requirement, cultivation is extended to non-conventional rubber growing tract where climatic conditions particularly drought is a serious matter of concern for plant growth. Summer irrigation together with mulching is the way for early plant establishment in these areas (Vijayakumar et al. 2000).

Among the planting materials of rubber, recently introduced root trainer plants have got wide acceptance due to its unique advantages including better root establishment after transplanting (Soman et al. 2013). Budded stumps or germinated seeds *in situ* are planted in a soilless potting media i.e., coir pith or elephant dung (Thomas and Soman 2014; Thomas and George 2014), in plastic containers. In this technique, hardening is a pre-planting cultural operation by which the plant is equipped for facing unexpected stress situations in the open field (Soman et al. 2010). During the course of hardening, tap roots undergoing air pruning near the drainage hole are in a state of starvation. Modification in root architecture could be achieved by allowing it to grow into a root elongation tube (RET) filled with potting medium (Fig. 1 A & B), attached tightly at the drainage hole of the root trainer (Thomas et al. 2015). The plants with modified root architecture is characterized by a lengthy root core comprising of 10-12 thick vertical roots, large number of laterals and fine roots (Fig. 1 C - E), with the expected advantages of mining soil resources from deep 'reservoir-levels' sheltered from evaporation and to provide required anchorage, particularly in non-traditional rubber growing tract of India where drought and cold prevails a major limiting factor for plant growth.

To address the issue of further growth of air pruned roots of root trainer plants, modified root trainer plants (MRTP) were planted in the soil and root samples collected were subjected to anatomical and histochemical studies to explore the tissue orientation of the roots with respect to polarity at different levels of its development.

Materials and Methods

MRTP were prepared following the procedure of Thomas et al. (2015). These plants were planted in the field at HBSS, Paraliar, Tamilnadu. One month after establishment, plants were dig out and both lateral and tap root were collected from three locus viz., the region where the roots originally developed within the root trainer cup, the region within the RET and roots grown into soil. The samples were sectioned with a thickness of 40µm and stained with Toluidine blue O (O'Brien et al. 1964) for general histology. Histochemical stains such as I_2KI and Periodic acid-Schiffs reagent for starch (Johansen 1940), Acid fuchsin (McCully1966; Pramod et al. 2008) and Mercuric chloride- bromophenol blue (Mazia et al. 1953) for protein, Oil red O for lipid (Lillie 1965) and latex vessels (Omman and Reghu 2003) were used and observations under carried out both light and polarization microscopes. Measurements and photomicrographs were taken using a Leica Qwin image analysis system attached to Leitz Diaplan and Leica DM 2500P polarization microscope.

Results

The modified root trainer plant of rubber has a two part root system i.e., the one developed inside the root trainer cup characterized by a tap root and large number of laterals trained to grow by the vertical ridges present inside the cup, and the second part developed in the root elongation tube (RET). Callus tissue at the wounded area of the tap root of the root trainer plants are under severe stress due to temporary arrest in growth till field planting. Once they are allowed to grow in the RET profuse rooting occurs within the tube. A root trainer plant with a total root length of 70-75cm can be produced before the onset of monsoon so that planting of the MRTP can be done on the same season without delay.

Lateral root anatomy

Cross section of the lateral roots developed in the root trainers, RET and in the soil for one month revealed that they are structurally identical with single layered epiblema ensheathed with a thick and intruded cuticle, a regular cortex constituted of loosely organized parenchyma cells, endodermis, multilayered pericycle and the stele. The epiblema cells protruded out to form fine roots (Fig. 1E) which are functionally active in the uptake of nourishments from the medium (Jessy et al. 2005). Phenolic depositions are observed in some of the cortical cells but are devoid of starch grains. Vascular stele is quadrangular with endodermis, 2-3 layered pericycle, four xylem groups and a quandrangular pith with compactly arranged parenchyma cells is the structural orientation of tissue in the lateral root developed both in root trainers and in RET (Fig. 1 F & G). The vascular stele of the laterals collected from the soil is circular in outline with endodermis, 8-10 layered pericycle and the formation of secondary vascular tissue and a circular pith. Passage cells occur in four locations in the endodermis adjacent to the xylem elements (Fig. 2A).

Lateral roots when viewed under polarization microscopy revealed strong birefringence property for the xylem and pith cells (Fig. 1H & J). In addition, the multilayered pericycle and intruded cuticle over the epiblema also showed birefringence property for the laterals grown in the soil. To understand the relationship between structural organization of tissue and polarity the plants were retained in root trainers for a year. The laterals continued to exhibitd similar anatomical organization of tissue to the recently developed laterals except for the stele where the pericycle is 3-4 layered and an octagonal stele (Fig. 1H - J). Root hairs, octagonal vascular cylinder, primary phloem fibers and pith showed high degree of birefringence (Fig. 1G & I) while it is feeble for phloem cells. Both transverse and radial walls the endodermis with more deposition of suberin, on the latter to form the casparian strip (Fig. 2B). Endodermis possess passage cells in seven sites where it faces to the protoxylem points. Cortical cells are devoid of starch but could localize in some of the pith cells. Lipid materials were found at the apical part of the root hair, as globules attached to the wall of the cortical and phloem cells. Phloem tissue is developed more than that of xylem elements organized in a file. Latex vessels of 10-15 were differentiated more or less in a row in each phloem group (Fig. 2A). Fascicular and interfascicular cambium join to form a continuous ring but the secondary growth is not yet started.

Tap root anatomy

The tap root inside the root trainers developed originally from the radical of the germinating seed. During the course of hardening this root growth is arrested at the drainage hole at the bottom. The callus tissue at the site of injury is instrumental in healing process and the development of new roots in the vertical file into the RET attached to the bottom of the root trainer. It has been noted that the roots originating from the callus tissue retain its polarity in the vertical file.

The vertical roots developed in the root trainer, RET and soil are stiff and structurally identical with a cork zone followed by cortex, secondary vascular tissue and circular pith (Fig. 2C). Starch grains were localized both in the xylem parenchyma and pith cells (Fig. 2 D & E). Within one month of growth in the soil the vertical roots showed quick secondary vascular tissue formation and make the root harder. A strong birefringence property is not noticed for pith and xylem cells of tap root as the case with lateral roots (Fig. 2E).

Irrespective of the growth medium and stages of development before planting, the birefringence property exhibited by the laterals is a unique characteristic and can be used as an anatomical marker for distinguishing laterals from tap root or the roots developed from the callus of the same plant at an early stage (Table 1). Lateral roots are characterized by its polarity (mostly horizontal), single layered epiblema with fine roots, multilayered pericycle, quadra or octangular stele and strong birefringence for xylem and pith cells. Polarity in the vertical file, early development of cork cells and vascular tissue, accumulation of starch in the xylem and pith, circular pith and feeble birefringence for the stelar tissue are the notable features for tap root.

Table 1: Distinctive characteristics of lateral and tap roots of root trainer plants

Characters	Lateral root	Tap root
Polarity	Horizontal to vertical	Vertical
Boundary of the root	Single layered epiblema	Cork layer
Fine roots	Present	Absent
Pericycle	Multilayered	Not distinct
Shape of stele	Quadra/octangular- circular	Circular
Secondary tissue formation	Delayed	Early
Starch in the xylem and pith	Absent	Present
Shape of the pith	Quadra or octangular	Circular
Birefringent xylem and pith	Strong	feeble

Secondary xylem and starch grains in the axial and ray parenchyma and in the pith showed birefringence. Xylem rays are multiseriate that become dilated in the secondary phloem in response to the increase in circumference of the axis, by radial anticlinal cell divisions and tangential cell enlargement. Widened connective tissue with tangentially oriented loosely arranged cells traverse through the secondary phloem, and that extends up to the cork layer. These tissues gave differential stainability and may have direct involvement in active translocation. The pericyclic cells undergoing periclinal and anticlinal divisions cause an increase of the number of pericyclic layers in the radial extent.

Early initiation of secondary growth in vertical roots compared to laterals resulting a higher proportion of stelar tissue than cortical cells in the vertical roots (Fig. 3 & 4). In lateral roots proportionately more area is occupied by the cortical cells. Laterals can remain in the root trainers without the initiation of secondary growth for a longer period of time. As soon as the transplanting in the field, laterals started to produce secondary tissue from the continuous cambium formed by the fascicular and inter- fascicular cambium. The area proportion of cortex and stele of laterals and vertical roots in the early stages of its development are different.

Birefringence revealed deposition of radial cellulose microfibril systems on their periclinal walls, as part of polarity, which means specific orientation of plant activity and morphogenesis in space, or the existence of functionally significant asymmetric structures that are formed in response to vectorial cues (Medvedev 2012). The birefringence observed in the cellulose-type cell wall is practically always pure intrinsic birefringence hence, it is important for diagnostic purpose (Ruch 1966). The colour difference observed under polarized light in the present study for the cells of pith, cuticle and root hairs indicate the varied orientation of the microfibril molecules in them. Birefringence is unique for laterals and not observed for tap root or the roots developed from the callus tissue at the wounded end of the budded stump or the air pruned roots developed during the hardening process of the root trainer plants. Lateral roots took its origin from the pericycle and maintains its polarity in the horizontal file while those developed from the callus tissue are always in the vertical file without exhibiting birefringence.

There exists a general belief that the tap root of the rubber plant should not be injured as once damaged, one or two laterals take up the role of tap root and grow vertically downwards. The apprehension is about the anchorage ability of these roots. The cut end of the tap root develop a wound periderm which transforms into a callus from which further root development takes place in the vertical file under conducive conditions. The study conducted by Thomas et al. (2015); Thomas and Soman (2013) revealed that acute stress developed on the tap root is advantageous as the callus tissue developed at the cut end of the tap root/ air pruned roots produce more number of stiff roots that grow vertically downwards into deeper strata in the soil. These vertical roots are however not laterals as they do not exhibit birefringence property under polarization microscopy. The roots thus produced can increase the functionally active surface area for better absorption of soil resources and can ensure better growth and anchorage. Such 'replacement roots' developed following injury to the tap root either by pruning or during planting are common in many plantation and horticultural crops (Esau 1977).

Rubber planting materials with modified root architecture (MRTP) are expected to survive and establish better in regions where drought is a serious limitation for initial field survival of the plant. To meet the domestic demand for NR, rubber cultivation in India is being extended to non-traditional regions faced with adverse climatic conditions. Life saving irrigation and mulching to young rubber plants is the recommended practice for the drought prone north Konkan belt of western India (Vijayakumar et al. 2000). The improved root system of MRTP not only enhances the growth but also can provide substantial savings in irrigation.

Figure 1: (A). Root elongation tube (RET) attached to root trainer plants for the modification of root architecture, (B & C). Initiation and development of roots in the vertical file from the callus tissue of the wounded tap root of root trainer plants, (D). Modified root architecture in the RET, (E). Fine roots on the laterals, (F). Cross section of the lateral roots developed in the RET with large cortex and a quadrangular pith, (G). Both xylem and pith cells showing birefringence property and (H –I). Cross section of the lateral roots collected from one year old root trainer plants showing octagonal stele with birefringence property.

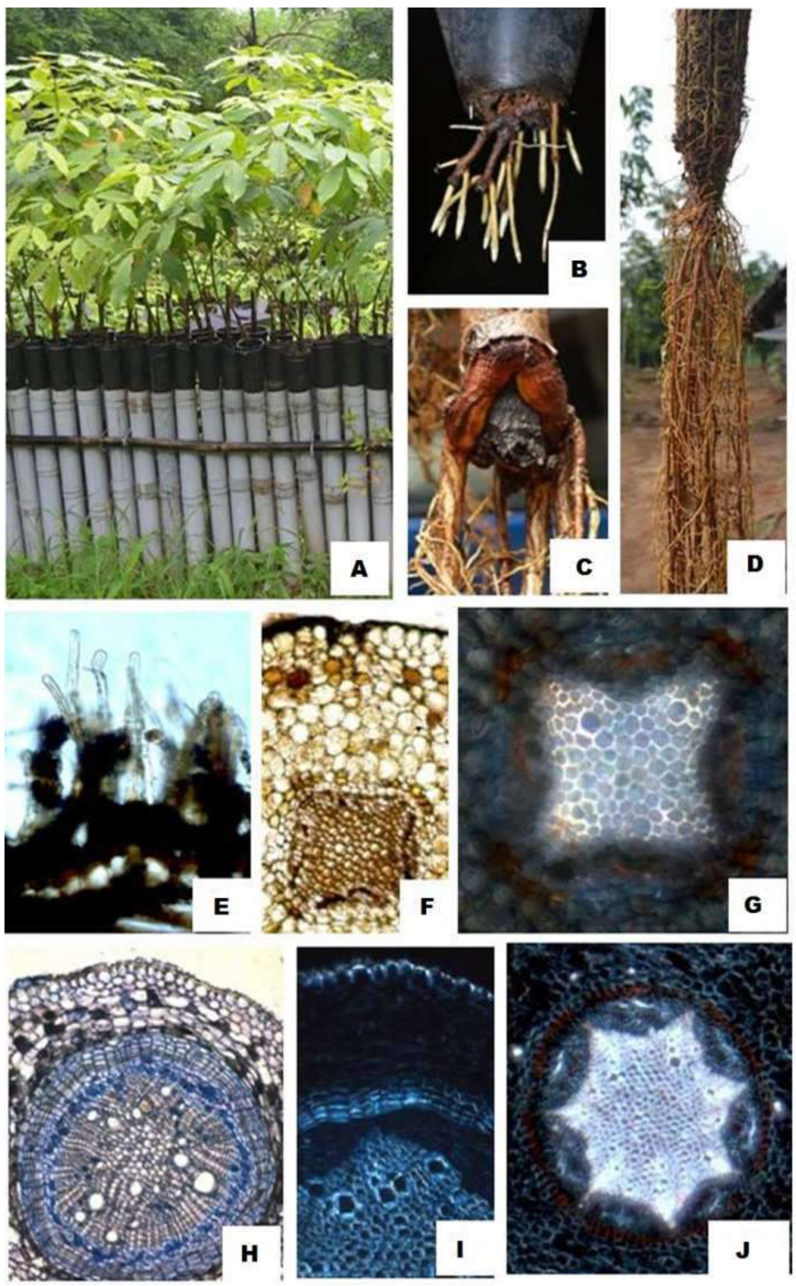


Figure 2: (A). Passage cell in the endodermis of laterals under polarization microscopy. Latex vessels are interspersed with phloem cells, (B). Casparian strip of endodermis and (C-E). Cross section of vertical root showing secondary tissues, starch accumulation in the xylem parenchyma and feeble birefringence for the pith cells.

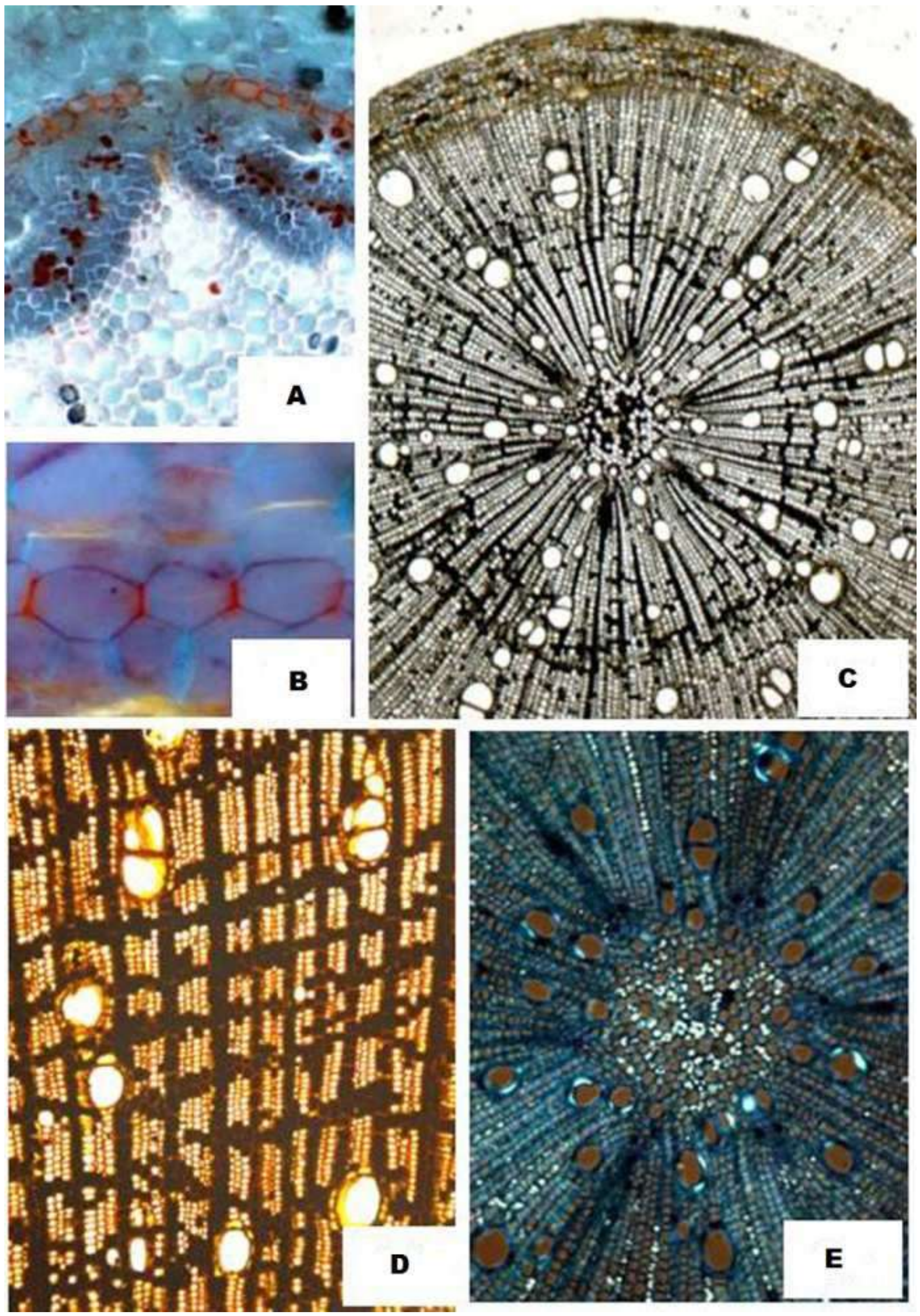


Figure 3: Proportion of different tissues in the lateral root

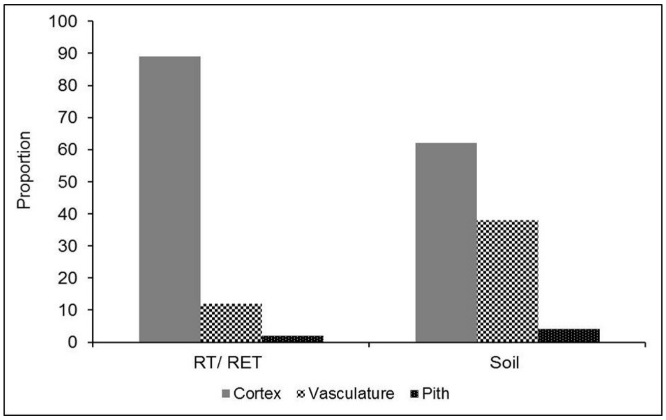
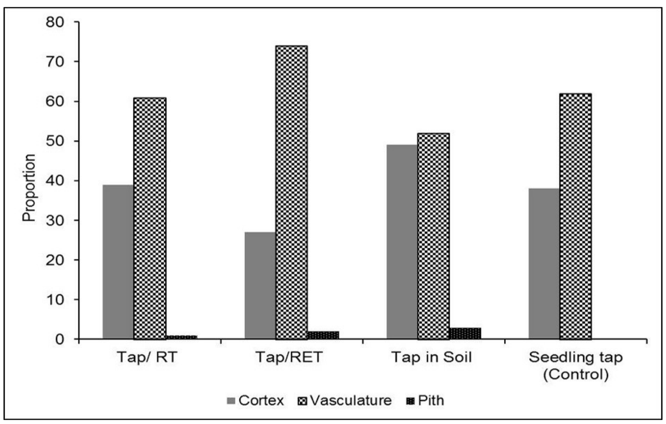


Figure 4: Proportion of different tissues in the tap root



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