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Development of xylem plates and stem anatomy of *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae)

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Abstract: *Tinospora cordifolia* (Willd.) Miers belonging to the family Menispermaceae is a large, tropical climbing shrub commonly found throughout India. In Menispermaceae, secondary growth is achieved by formation of multiple cambia or a single cambium. In fully grown plants the stem becomes thick, moderately woody, and parenchymatous with very large and wide rays. In *Tinospora* secondary growth is achieved by single cambium with formation of xylem in plates. *Tinospora* is commonly known for its atypical wood anatomical structure i.e. lignified xylem plates embedded in parenchymatous ray cells. This type of cambial variant is the result of the normal activity of cambium which produces xylem towards the center and phloem towards periphery but is an abnormality in conformation. Unequal activity of the cambium at certain segments results in macro-morphological abnormalities in the stem structure. The conformational changes lead to the development of xylem in plates which were separated by wide rays. Secondary xylem showed vessel dimorphism in both large and narrow vessels, and distinctly wide rays. Formation of ray cambia and its re-differentiation into vessel elements was observed in *Tinospora* which were not reported earlier in this genus. Other anatomical characters such as presence of wide vessels, abundance of parenchyma, and vessel dimorphism favored the scandent habit of the plant, which is discussed in this investigation.

Keywords: *Tinospora cordifolia*, xylem plates, wide rays, cambial variants.

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Introduction

Tinospora cordifolia (Willd.) Miers belongs to the family Menispermaceae. It is a large, deciduous, tropical climbing shrub found throughout India. Most members of this family are woody lianas. The family also comprises shrubs and subshrubs with prominent secondary growth (CARLQUIST 1996). It is commonly known as the "heart-leaved moonseed plant" in English, Guduchi in Sanskrit and Giloy in Hindi (MITTAL et al. 2014). Members of Menispermaceae contain large amounts of alkaloids and terpenoids (SHARMA & PANDEY 2010) and are therefore commonly used in traditional medicine (JIANG 2006). Similarly, the *Tinospora* genus has importance in Ayurveda. Although substantial work has been done on pharmacognosy of *Tinospora cordifolia* (KUMAR & SRIVASTAVA 1995, PRAKASH & RAI 1996, MATHEW & KUTTAN 1997,1999, BAIRY et al. 2000, MANJREKAR et al. 2000, PRINCE & MENON 2000, 2001, SHARMA et al. 2001, PATANI 2000), very few anatomical studies are available. This included METCALFE & CHALK (1950), who gave general features of *Tinospora,* and the comparative study of the general wood anatomy of stem and root of *T. cordifolia* and *T. sinesis* by BONDE & UPADHYE (1989). Most research done so far has been on the stem anatomy of members of Menispermaceae. Most genera of this family show successive cambia formation in the stem (CARLQUIST 1996, RAJPUT & RAO 2003, JACQUES & DE FRANCESCHI 2007, YANG & CHEN 2016). JACQUES & DE FRANCESCHI (2007) observed two secondary growth types within the family, wood with successive cambia and a single cambium.

Cambium in lianas was responsible for flattened stem shape rather than cylindrical, and may be either single or multiple, but in which successive cambia are not involved. In some dicotyledonous wood, fascicular areas were few and were separated by large ray areas that consist of thin-walled parenchyma and constituted a kind of anomaly with respect to cambial activity (PFEIFFER 1926, METCALFE & CHALK 1950). The occurrence of non-lignified xylem is cited by METCALFE & CHALK (1950) as an anomaly in some species of *Breweria, Convolvulus, Exogonium,* and *Ipomoea* (Convolvulaceae). CARLQUIST (1988) was of the opinion that such pattern of cambial activity should not be considered as a cambial anomaly, and perhaps should not be listed as any kind of anomaly at all because cambium is functionally normal i.e. bidirectional. The only difference is related to the formation of lignified and non-lignified tissues. The present investigation was intended to focus on the structure and development of xylem in plates in stem of *Tinospora cordifolia*.

Material and methods

The plant samples of *T. cordifolia* were collected from the campus and botanical garden of S.S. Ayurved Mahavidyalaya, Hadapsar, Pune and from M.J. College campus, Jalgaon. Dried materials were submitted to Botanical Survey of India (BSI) Herbarium, Pune for authentication; the corresponding voucher number was VP-9. Mature leaves and stem materials were also collected and fixed in FAA (JOHANSEN 1940) for further use. Suitably trimmed samples were directly embedded in Leica OCT solution. Serial transverse, tangential and radial longitudinal sections of 15–25 µm thickness were obtained with the help of a Cryostat microtome (Leica CM1520). Sections were stained with Safranin–fast green combination (JOHANSEN 1940), dehydrated through ethanol-xylene series and subsequently mounted in DPX.

For scanning electron microscopic analysis, stem samples were observed using the SEM (JEOL JSM-6360A, Japan) at different magnifications ranging from 100x to 10000x. Small pieces of the stem were macerated with Jeffery's solution (BERLYN & MIKSCHE 1976) at 55 ºC to 60ºC for 24-38 hours and stained with 0.5% aqueous Safranin to obtain the length and width of vessel elements and fibers and to study their morphology. One hundred readings were taken to obtain the mean and standard deviation. Important results were micro-photographed with a Leica DM 3000 LED research microscope.

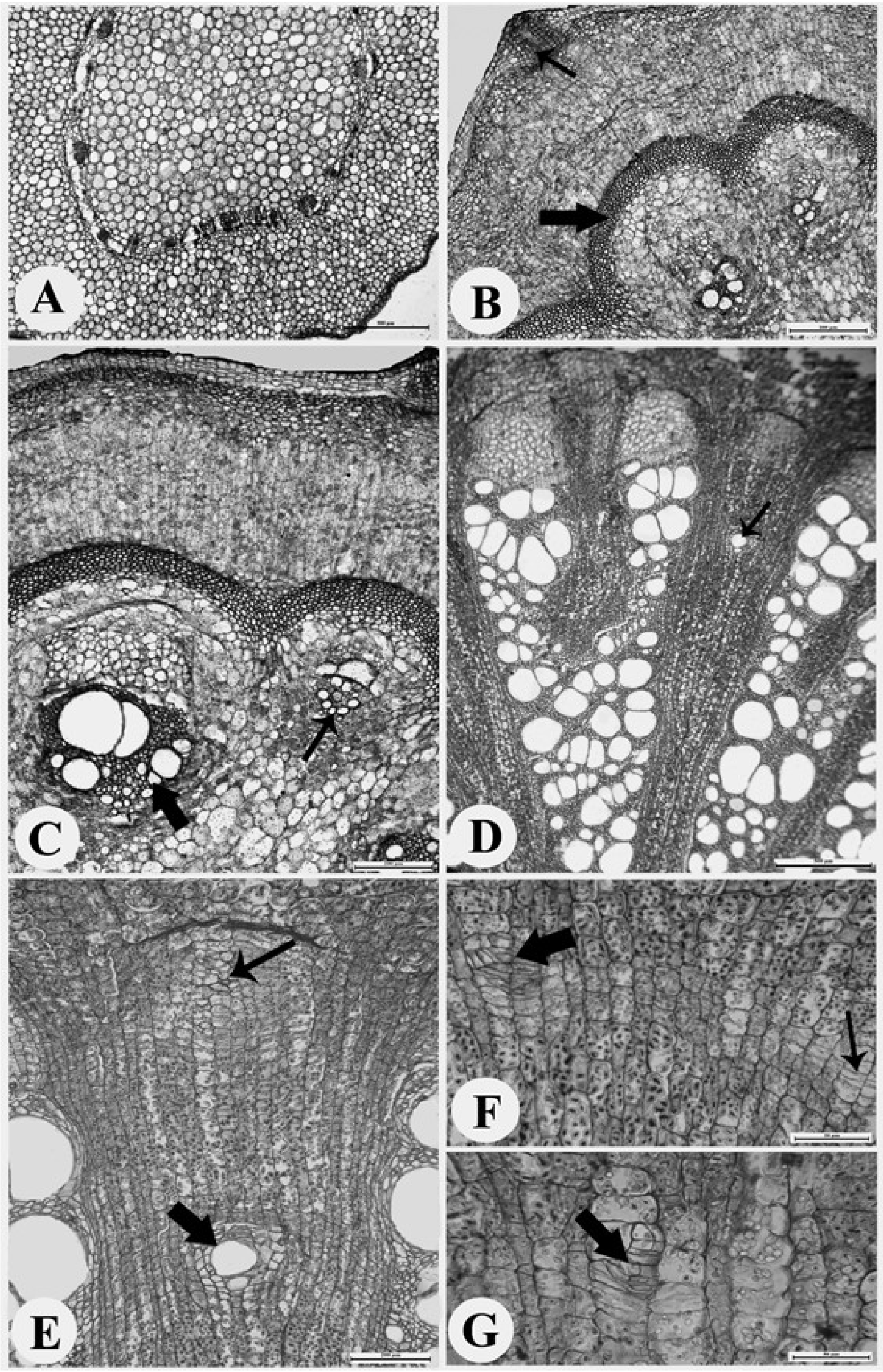
Results

Anatomy of young stem

In young stems, thin cuticle covered the epidermis. Lenticels protruded out and broke the cuticle at several places. Epidermis consisted of a single compact layer of isodiametric and thin walled parenchyma cells (Fig. 1A). A 2-3 cell layered hypodermis composed of parenchyma cells differentiated beneath it while the cortex was composed of thin-walled parenchyma cells. Cortical cells showed heavy accumulation of oval to circular starch grains. Cortex was followed by pericycle and was composed of thick-walled cells which covered the vascular bundle forming a cap and was called pericyclic parenchyma (Figs. 1B, C). These fibrous caps formed a circular outline with adjacent parenchymatous cells. Eight to ten vascular bundles were joined by interfascicular cambium forming a ring. In the fifth or sixth node of the stem, 14 to 15 vascular bundles were observed which were formed by the action of fascicular and interfascicular cambium. Cambial rays were large, heterocellular and compound with procumbent cells (Figs. 1B, C). These rays later formed wider rays which separated the secondary xylem in mature stem and the stem appeared like xylem plates separated by parenchymatous wide rays. Pith was relatively small and consisted of parenchymatous cells showing heavy deposition of starch grains.

Structure secondary xylem and xylem in plates

Thick stem of around 1-1.5cm were studied. The outer periderm layer surrounding the stem periodically peeled off. Secondary growth in the main stem was achieved by a single ring of vascular cambium which remained functional throughout the life of the plant. The fascicular and interfascicular cambium differentiated into xylem inside and phloem outside resulting in the increase of stem size. The cambium was functionally normal and formed secondary xylem centripetally and secondary phloem centrifugally. Secondary xylem had large vessels and wide rays (Figs. 1D, E; 2C-E). The wood was diffuse porous. In xylem, small and round to large and oval shaped vessels were observed mostly solitary, sometimes in pairs and rarely in groups of 3-4, and were evenly distributed (Fig. 1D, 2A, B). Tyloses were common and filled with starch grains (Fig. 2E).



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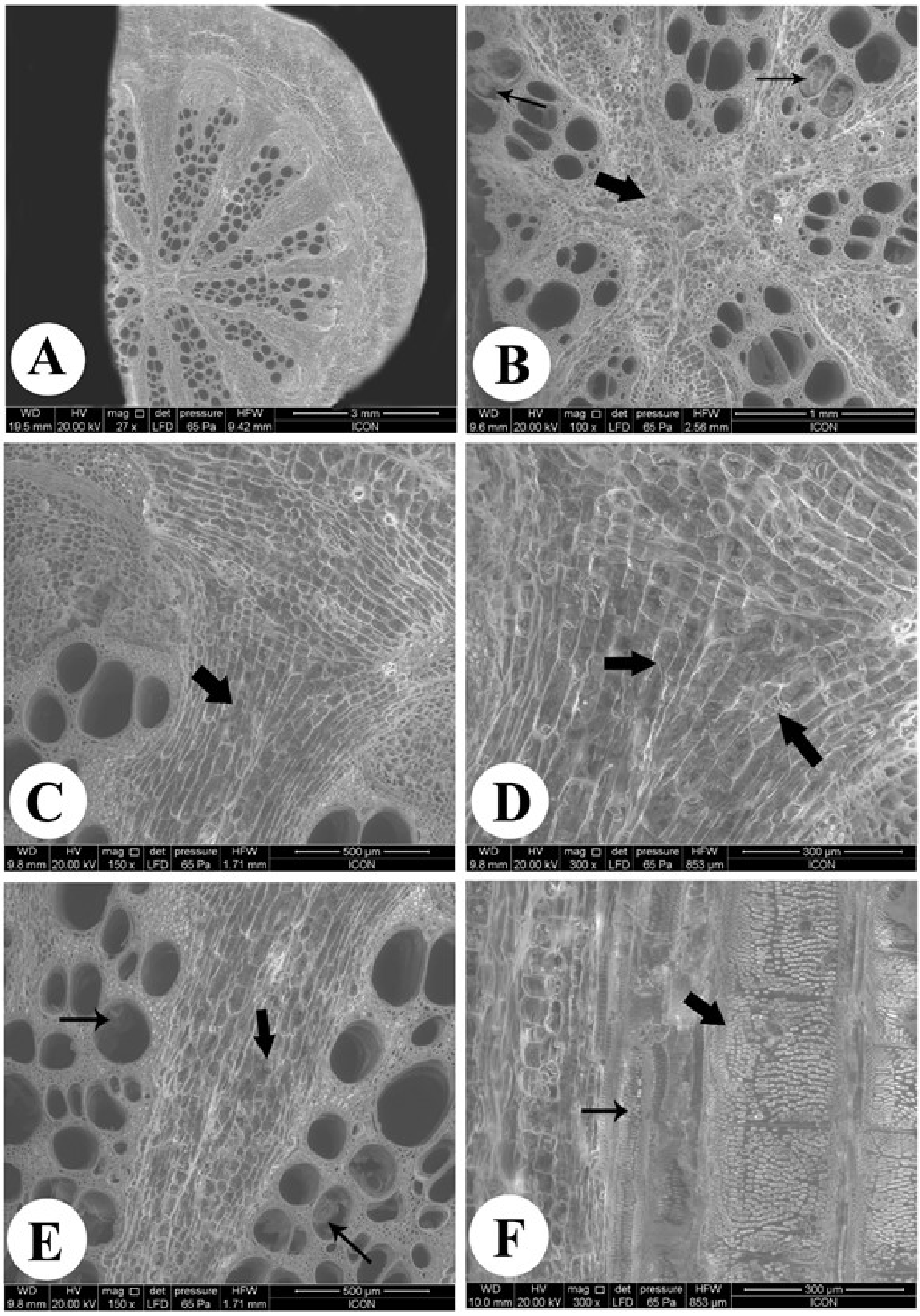
← Fig.1. Transverse view (A-F). (A) Young stem (3rd node) of *Tinospora cordifolia* showing young vascular bundles arranged in a ring. (B) Young stem (7th node) of *T. cordifolia* showing vascular bundles arranged in ring, arrow showing thick walled pericycle (thick arrow) and periderm (thin arrow). (C) Young stem (9th node) of *T. cordifolia* showing fascicular (thick arrow) and interfascicular (thin arrow) vascular bundles arranged in ring, covered with thick walled pericycle. (D) Matured stem of *T. cordifolia*, showing xylem in plates separated by wide parenchymatous rays and newly formed ray vessel (arrow). (E) Wide ray region showing differentiating ray parenchyma cells (thin arrow) and newly developed vessel element in ray region (thick arrow). (F, G) Ray regions enlarged, showing ray parenchyma cells differentiation (thick arrows) and periclinal division in ray parenchyma cells (thin arrow).

Fiber tracheids were 600-700 µm in length and 25-30 µm in width. Vessels were mostly solitary, however radial or tangential multiples of 3-5 vessels were also observed infrequently. Vessels were mostly oval to oblong in cross section, and often obstructed by numerous tyloses which sometimes completely blocked the lumen (Fig. 2E). Such tyloses often showed deposition of prominent starch granules. On the basis of their diameter, vessels may be categorized into wide or narrow vessel elements. The length of the wider vessel elements was less than its diameter. They were 165-300 µm in length and 170-425 µm in diameter, while the narrower vessel elements were longer than their diameter, i.e. 237-300 µm in length and 62-100 µm in width. Vessels possessed simple perforation plate on their slightly oblique to transverse end walls with alternate bordered pits (Fig. 2F). They were oval to oblong, sometimes approaching rhomboidal shape with a narrowly oval aperture 6-8 µm wide and 7-10 µm in vertical diameter.

In transverse section, axial elements of secondary xylem in plates were frequently separated by large rays (Figs. 1D, 2A-E). These rays often developed meristematic centers which is a small group of ray cells de-differentiate into meristematic cells at several places (Figs. 1F, G). Meristematic centers are also known as polycentric rays.

These cells were differentiated into xylem cells and in later stage vessels ran tangentially in the ray (Figs. 1D, E). At some places vessels ran horizontally from lateral side of xylem plate into the rays which can also be observed in radial tangential section (Figs. 3 A-D). The rays were measured at around 1450-2300 µm in height and 300-390 µm in width. Xylem rays were of two types, one was multiseriate separating the xylem forming xylem plates, and the other was uniseriate which were present in xylem at the margins of xylem plates. In tangential view, the rays were heterocellular and most were procumbent, while square and upright cells were found only on the ray periphery. Ray cells were 32-50 µm diameter. No uniseriate rays were observed.


A
3 mm
B
1 mm
C
500 µm
D
300 µm
E
500 µm
F
300 µm

← Fig. 2. Scanning electron microscopy images, transverse view (A-E) and TLS of *Tinospora cordifolia* mature stem. (A) Matured stem of *T. cordifolia* showing xylem plates separated by wide parenchymatous rays. (B) Center of the stem which showing pith region filled with starch grains (thick arrow) and thin arrows showing tyloses in vessels. (C, D, E) SEM images of enlarged region of transverse region of stem showing xylem plates and wide ray region where thick arrows showing ray parenchyma cells filled with starch grains and thin arrows showing vessels with tyloses. (F) SEM image of tangential view of mature stem, showing big and narrow vessels (thick and thin arrow) with fiber tracheids.

Discussion

Tinospora cordifolia is a perennial climber. In the fully-grown plant, the stem becomes thick, moderately woody, and parenchymatous with very large and wide rays. A similar structure is also observed in some members of Menispermaceae, for example in genera *Coscinium*, *Borismene*, *Fibraurea*, *Tinomiscium*, *Parabaena*, *Penianthus* and *Tinospora* (JACQUES & DE FRANCESCHI 2007). Other family members like *Aristolochia* are commonly known for their atypical wood anatomical structure, i.e. lignified xylem plates embedded in parenchymatous ray cells. SCHENCK (1893) first named such anomalies as "*Aristolochia*-Typus". Family Menispermaceae is comprised of woody lianas and show a cambial variant in almost all members of the family (TAMAIO et al. 2010). It is known for having successive cambia but normal secondary growth is also prominently observed in some members (RAJPUT & RAO 2003, JACQUES & DE FRANCESCHI 2007, TAMAIO et al. 2009, 2010). Members where secondary growth develops from a single ring of vascular cambium always show high and wide rays (JACQUES & DE FRANCESCHI 2007).

Wood features of *Tinospora* suggest scandent habit which are characterized by wide and long vessels, but they also occasionally possess narrow vessels referred to as fibriform vessels. A significant characteristic shown by the plant studied in the present investigation was the presence of vessel dimorphism. Vessel dimorphism can refer to the presence of wide as well as narrow vessel elements (CARLQUIST 1981, CARLQUIST & HANSON 1991). Narrow vessel elements are considered to be at least as effective as tracheids in resisting the formation of air embolism in vessels, because air embolism form far less commonly in narrow vessels than in wide ones (ELLMORE & EWERS 1985, CARLQUIST & HANSON 1991). Thus, narrow vessel elements, which are common in *Tinospora*, can form a subsidiary conductive system with degree of conductive safety virtually as high as that provided by tracheids (AYENSU & STERN 1964, CARLQUIST 1991).

Secondary xylem of *Tinospora* was characterized by the presence of abundant parenchyma. The occurrence of abundant parenchyma in stems of lianas and vine has long been noted (CARLQUIST 1988, FISHER & EWERS 1992, LOPES et al. 2008, ISNARD & SILK 2009). Various functions have been ascribed to the abundance of parenchyma: they provide greater flexibility (SCHENCK 1893,

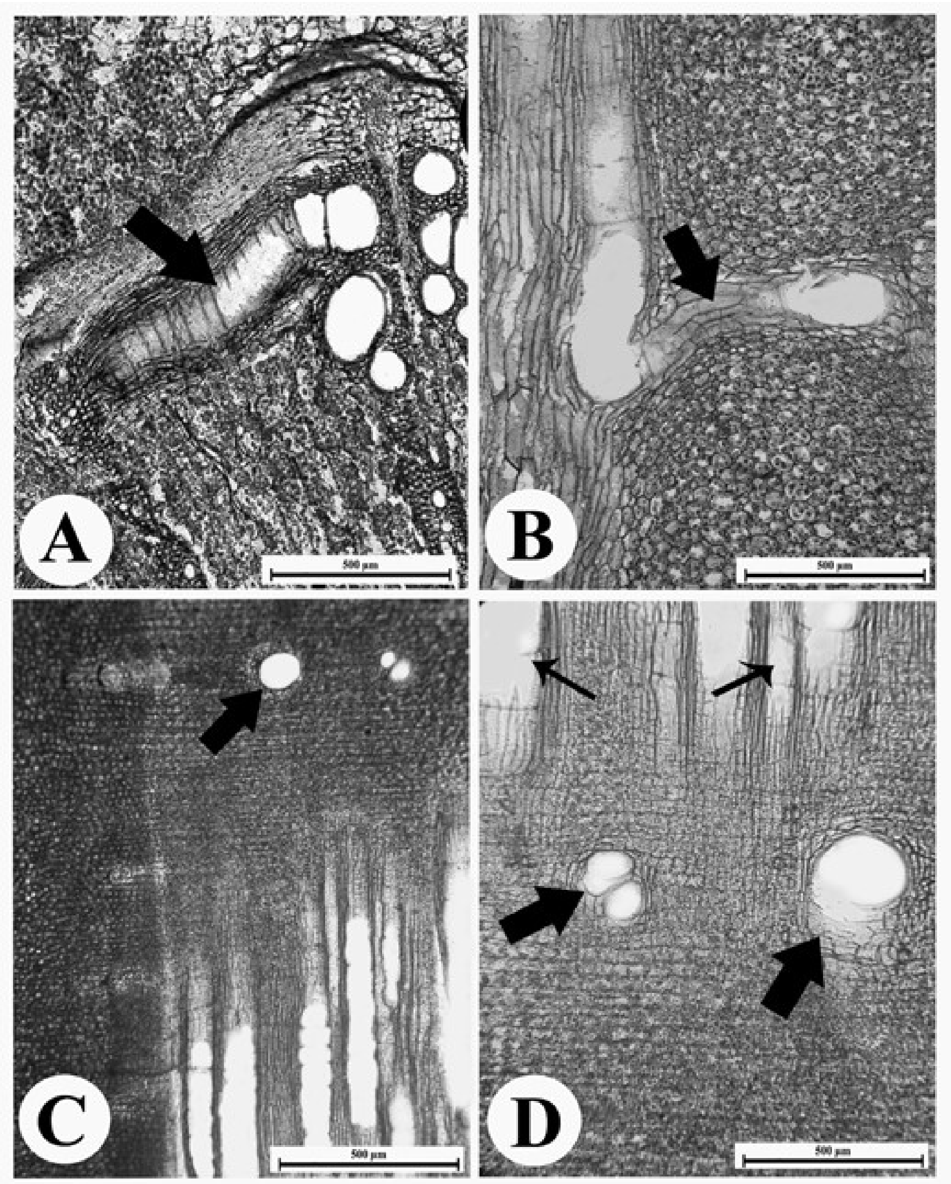


Fig. 3. Transverse (A) and tangential view (B- D) of *Tinospora cordifolia* mature stem; (A) Matured stem of *T. cordifolia* arrow showing radially running vessels through parenchymatous rays; (B) Radially conducting vessels directing from tangential vessels toward rays; (C) Tangential section of mature stem showing radial vessels developed in rays (D) Enlarge view of radially developing vessels (thick arrows) and thin arrow showing vertically directing vessels.

CARLQUIST 1975, 1985, 1988, ROWE et al. 2004), site for starch storage (SAUTER 1966a, b, BRAUN 1970, 1983, 1984, CARLQUIST 1985, 1988), help in wound healing process (DOBBINS & FISHER 1986, FISHER & EWERS 1989, CARLQUIST 1988) and also act as background tissue (CARLQUIST 1988). In the stem of *Tinospora*, presence of thin-walled parenchyma cells mixed with thick-walled xylem tissues presumably provide higher flexibility to climbing habit (ROWE et al. 2004, ISNARD & SILK 2009), and may be facilitating twisting of the stem without damaging the xylem (CARLQUIST 1988). Alternatively, these structures might also play a role in photosynthate storage (CARLQUIST 1988) and wound repair (DOBBINS & FISHER 1986). Apart from abundant axial parenchyma observed in *Tinospora*, non-lignified, very high and wide rays were also observed. Tall rays were generally present in lianas (DOBBINS & FISHER 1986, FISHER & EWERS 1992, CARLQUIST 2001). Lignified and non-lignified rays are present in Menispermaceae and many climber species of other families (TAMAIO et al. 2010). Similarly, present investigation showed two types of rays. First, uniseriate lignified ray present on the lateral periphery and inside the xylem, and second, multiseriate parenchymatous wide ray which separated xylem plates. According to the works of MENNEGA (1982), CARLQUIST (1996b) and TAMAIO et al. (2009, 2010), lignified rays were more common in plant species showing successive cambia. Formation of ray cambium is also common in lianas having parenchymatous wide rays and the cell division activity of the cambium is greater in bundles compared to rays and greater in ray margins than in central ray regions (JACQUES & DE FRANCESCHI 2007, PATIL et al. 2011). Vessels were running horizontally in the wide rays from the edges of the xylem plate. This interesting feature were not reported earlier. This unusual structure may assist in radial translocation. Parenchymatous wide rays present between wood segments provide great flexibility and permits lianas to adapt to twisting around supporting trees. This allows for torsion of the stems with minimum damage to vessels and sieve tubes (SIEBER & KUCERA 1980, CARLQUIST 2001, ROWE & SPECK 2005, MASRAHI 2014). Similarly, our observations supported their findings.

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