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Wood anatomy and ontogeny of interxylary cambium in *Canavalia cathartica* Thouars, *C. gladiata* (Jacq.) DC. and *Pueraria tuberosa* (Willd.) DC. (Fabaceae)

Kishore S. RAJPUT

Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390 002, India. Corresponding author's email: ks.rajput15@yahoo.com

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ABSTRACT: Fabaceae is one of the important families of flowering plants that play a crucial role in the human diet. Besides imperative sources of proteins, folk and traditional medicine, several species are sources of commercially important timbers. However, the wood anatomy of timber trees of the family has been studied extensively but information on climbing members is relatively neglected. The stem anatomy of *Canavalia cathartica* Thouars, and *C. gladiata* (Jacq.) DC. and *Pueraria tuberosa* (Roxb. ex Willd.) DC. was investigated histologically in the present study. In all three species, a single ring of the vascular cambium remained functional throughout the growing period and showed a regular growth pattern like most eudicots. As the plants grew (10-12 mm thick stems), thin-walled xylem parenchyma formed at the beginning of the secondary growth underwent dedifferentiation and formed isolated or group of interxylary sieve elements. Subsequently, more and more adjacent parenchyma produced sieve elements, which resulted in the formation of interxylary phloem islands of various sizes and shapes. In 20–22 mm thick stems of all three species, the non-lignified, thin-walled xylem parenchyma adjacent to these islands divided repeatedly and formed radial files of meristematic cells, referred to as interxylary cambium. These segments of the interxylary cambium had irregular orientation (radial, tangential or diagonal) and exclusively produced phloem elements. The secondary xylem was diffuse-porous with indistinct growth rings and composed of dimorphic vessels, tracheids, and axial and ray parenchyma cells. Small vessels were arranged in clusters while wide vessels were solitary with vasicentric thick-walled lignified parenchyma.

KEY WORDS: Faboideae, interxylary cambia, interxylary phloem, Papilionoideae, perforated rays.

INTRODUCTION

Family Fabaceae (particularly the subfamily Faboideae) is known for its economic importance and several members serve as sources of proteins (various pulses), oil (*Arachis hypogea*, *Glycine max*), various secondary metabolites (*Mucuna*), timbers (*Dalbergia*, *Pterocarpus*) and various herbal medicines of international trade (Devi *et al*., 2021). Fabaceae is thoroughly investigated from different angles, including a global database of legumes (Roskov *et al*., 2006). From a histological point of view, the wood anatomy of commercially important timber trees has been studied extensively by different researchers (Baretta-Kuipers, 1981; Roskov *et al*., 2006; Syofyan *et al*., 2017) but similar studies on climbing members are lacking and remain understudied. Available literature indicates sporadic information on the stem anatomy of different climbing species of Fabaceae (Solereder, 1908; Carlquist, 1985; Patil *et al*., 2011; Moya *et al*., 2018; Nair, 1990, 1993; Rajput, 2003; Rajput *et al*., 2006, 2012, 2023).

Canavalia cathartica Thouars is known for its different medicinal properties and has the potential to be used as a food (Nayak *et al*., 2022) while unripe pods of *C. gladiata* (Jacq.) DC. are used as vegetables in Africa and Asia (Bosch, 2004). In some places, pods and seeds are eaten raw/cooked or fermented (Nayak *et al*., 2022). Due to its higher protein content, it is also under cultivation on a small scale. Similarly, *Pueraria tuberosa* (Roxb. ex Willd.) DC. (Fabaceae) is used in several Ayurvedic preparations as an immune buster, cardiovascular and restorative tonic, antiaging, spermatogenic, fertility disorders, anti-ageing preparations and several more (Maji *et al*., 2014; Bharti *et al*., 2020). Therefore, the former two species have been investigated for their importance as a source of proteins (Semba *et al*., 2021) while the later species is phytochemically studied for its medicinal value. During their lifecycle, the young saplings of all three species are established during monsoon and show vigorous vegetative growth till the end of August-September. Among them, C. *cathartica* and *C. gladiata* initiate flowering in September-October followed by the fruit set, which continues till March-April. In the moist areas, flowering was observed till December and fruit maturation and seed dispersal took place by March-April. During the drier part of the year, plants remain leafless and sprouting of new leaves was observed immediately after the arrival of rains in the next monsoon. *P. tuberosa* shows vegetative growth during monsoon while flowering initiates in February-March followed by fruit maturation and seed dispersal during summer. All three species are well adapted to tropical dry deciduous conditions, which is very well exemplified by *P. tuberosa*

which shows massive flowering followed by fruit setting and ripening when plants are leafless in summer (i.e., March-April). Similar massive flowering is also observed in the other two species of *Canavalia*, in which flowering occurs when the individuals are in full foliage during monsoon (i.e., in August) and continue even in dryer periods till March. However, histological studies on stem anatomy are lacking in all three species.

Previous studies on other members of the Fabaceae showed interesting results such as the formation of successive cambia, formation of interxylary phloem, functionally inverse cambia, and the proliferation of xylem and phloem rays or development of neo-formed vascular cylinders (Rajput *et al*., 2006, 2012, and 2023). In the present study, besides xylem ray cell proliferation and formation of interxylary phloem, all these species (*C. cathartica*, *C. gladiata* and *P. tuberosa*) showed initiation of interxylary cambia, which is reported for the first time in the family. Therefore, the main aim of the present study is to elucidate the ontogeny of interxylary cambium and the formation of interxylary phloem. Moreover, the present study will also help to understand the structural adaptations and growth trajectories of these species to survive under dry conditions.

MATERIALS AND METHODS

Plant material: Samples from the main stems with various diameters measuring 5–35 mm in thickness were collected from the naturally growing population of *Canavalia cathartica*, *C. gladiata* and *Pueraria tuberosa*. To obtain different developmental stages, 50–60 mm long pieces of stems were excised from five individuals at various heights i.e., 30 cm above the ground level and subsequently after every one meter up to 5 mm thickness towards the apical shoots. Individuals of *C. cathartica* and *C. gladiata* were collected from the plants growing on the roadsides in Dangs Forest while *P. tuberosa* were collected from the road cuts at Girnar Taleti, Junagadh, Gujarat state (India). Obtained stem pieces were immediately fixed in Formaldehyde: Acetic acid: Alcohol (Berlyn and Miksche, 1976). After 24–48 hrs of fixation, they were transferred to 70% ethanol for further processing (i.e., sectioning and maceration) and storage.

Histological preparations: Samples of all three species were sectioned directly using a Leica sliding microtome to obtain 15-18 µm thick sections in transverse, tangential and radial longitudinal views without any treatment. These sections were stained with a safranin-Astra blue combination (Srebotnik and Messner, 1994), dehydrated through upgraded ethanol xylene series and mounted in Dibutyl Phthalate Xylene (DPX). Sections were observed using a Leica, (DM 2000) trinocular research microscope and important results were micro-photographed using Leica DFC 295 firewire digital camera fitted on the microscope.

Maceration of the secondary xylem: The secondary xylem adjacent to the cambium from the thickest samples was scrapped with a razor blade and treated with Jeffrey's fluid (Johansen, 1940) for 36-48 hrs at 45°C. Treated samples were gently washed with water and stained with 0.5% aqueous safranin and dimensional details like vessel element length and fibre length and width were obtained from the macerated material. The tangential lumen diameter and height and width of the xylem rays were obtained using transverse and tangential longitudinal sections respectively. Thirty measurements were taken randomly to obtain the mean and standard deviation.

RESULTS

Structure of the young and mature stems: The young and mature stems of all three species *viz*., *C. cathartica* and *C. gladiata* (5–22 mm thick) and *P. tuberosa* (5–35 mm thick) were oval to circular in outline (Fig. 1A–D). All of them showed regular secondary growth and stem thickness is achieved by a single vascular cambium. In the young stems, the outermost layer i.e., epidermis was composed of oval to elliptic thin-walled cells and it was covered with cuticle (Fig. 1E, F). An epidermis was followed by a 4–8-cells wide cortex that was composed of oval to polygonal thin-walled cells (Fig. 1E, F). As the stem thickness increased, the epidermis was replaced by several cell-wide periderm in all three species (Fig. 2A). As compared to *C. cathartica* and *C. gladiata*, the periderm was thicker in *P. tuberosa* and flecks off periodically in small bits (Fig. 1D). A four to five cell layered pericycle fibres formed a continuous ring, which enclosed the secondary xylem and phloem (Fig. 2A). The central portion of the stem was occupied thin-walled parenchymatous pith (Fig. 2B–D). With the increase in stem diameter, a continuous ring of the pericycle fibres was fragmented and the parenchyma cells within these fibres divided and differentiated into sclerified cells that interconnected the fragmented segments of pericyclic fibres (Fig. 1F).

Structure of the secondary xylem and phloem: During the early part of the secondary growth (when the young saplings or branches were searching for support) in all three species, the secondary xylem was stiff and exclusively composed of thick-walled lignified xylem elements like other self-supporting vascular plants. At this stage, young branches and stems were erect and in search of a supporting host. This xylem was composed of relatively narrow vessels, fibres and lignified, thick-walled parenchyma (Fig. 3A, B). As they found support for climbing, the structure of the secondary xylem was altered drastically and began to produce vessels with large lumen diameter with an abundance of thin-walled, non-lignified axial and ray parenchyma cells (Fig. 3A, B). As the stem grew in thickness, more and more parenchyma formation was observed in all three species (Fig. 2C, D)

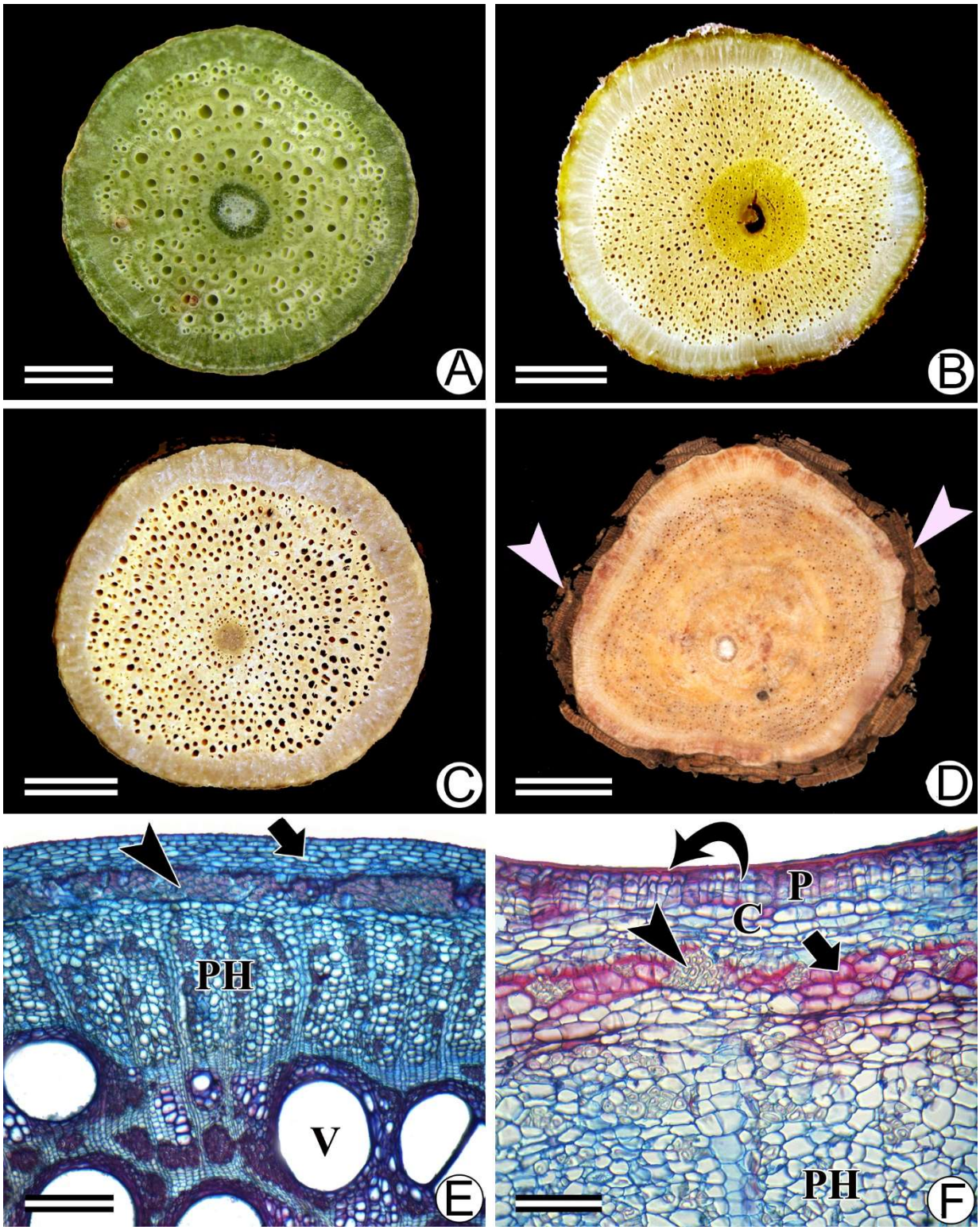

Fig. 1. Macroscopic (A-D) and microscopic (E, F) view of the young and mature stems of ***Canavalia cathartica***, ***C. gladiata*** and ***Pueraria tuberosa***. **A & B.** Young (A) and mature (B) stems of ***C. gladiata*** showing stem outline and its structure and composition. **C.** Macroscopic view of thick stem of ***C. cathartica***. **D.** Thick stem of ***P. tuberosa***. Note the thick periderm (arrowheads). **E.** A 5 mm thick stem of ***C. gladiata*** showing the structure of the epidermis, cortex (arrow), pericyclic fibres (arrowhead), phloem (PH) and xylem. ***Abbreviation***: V = vessel. **F.** A 8-10 mm thick stem of ***C. cathartica*** showing a thick cuticle (curved arrow), differentiating periderm (P), few cells wide cortex with tangentially elongated cortical parenchyma (C). Note the pericyclic fibres (arrowhead) and sclerified parenchyma cells (arrow). ***Scale bars***: A = 1.5 mm; B = 4 mm; C = 5.5 mm; D = 8.7 mm; E = 200 μm; F = 100 μm.

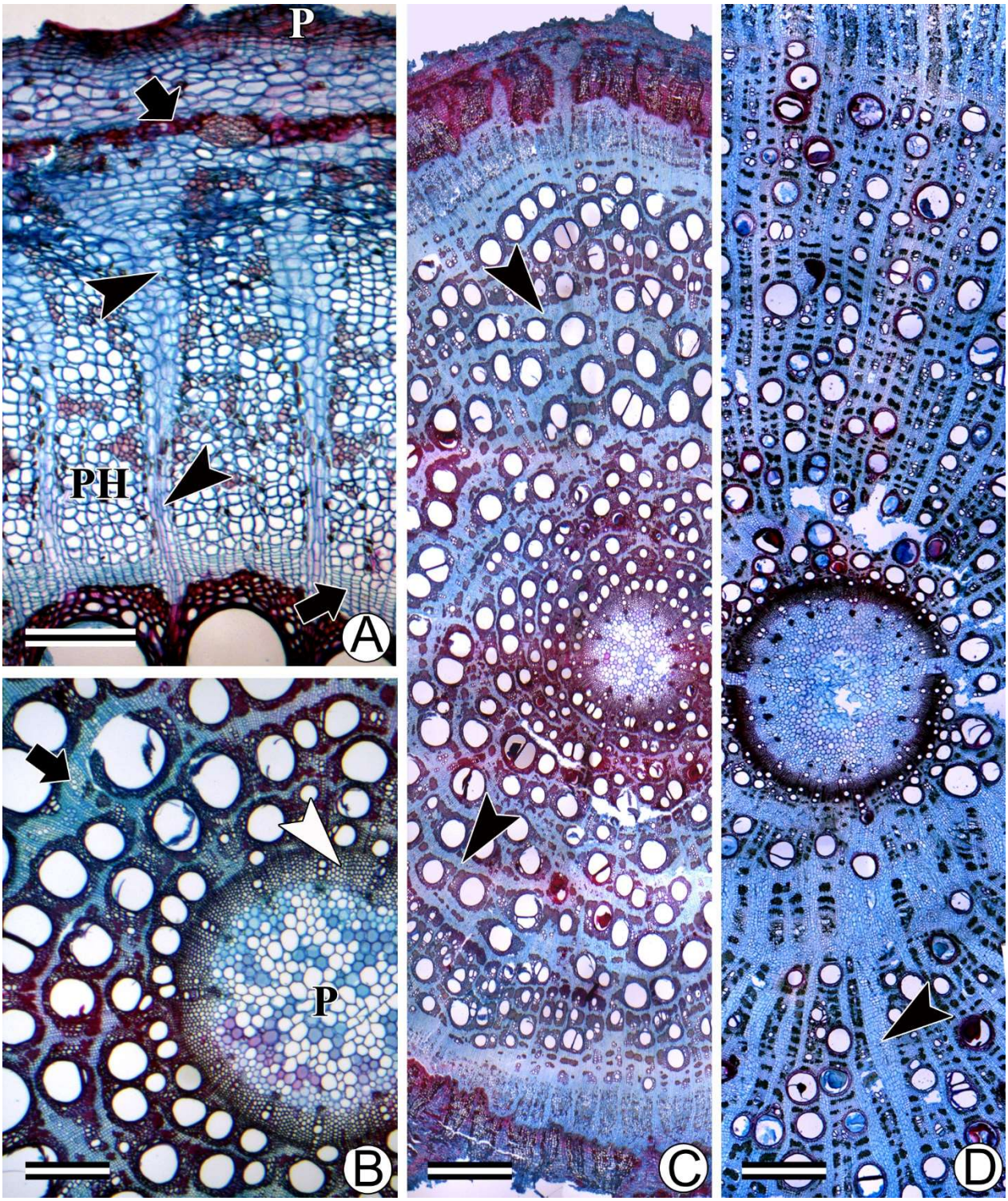

Fig. 2. Transverse view of mature stems of ***C. cathartica*** (A), ***C. gladiata*** (B, C) and ***P. tuberosa*** (D) showing the structure of the secondary xylem and phloem. **A.** A thick stem of **C. *cathartica*** showing the structure and composition of the periderm (P), cortex, pericyclic ring, phloem (PH) and xylem. Note the dilation of phloem rays from the cambium towards the cortex (arrowheads). The upper arrow indicates pericyclic fibres while the lower arrow indicates regular vascular cambium. **B.** Central portion of ***C. gladiata*** stem showing structure of initially formed secondary xylem (arrowhead) and later formed secondary xylem. Note the sudden increase in vessel lumen diameter and formation of tangential bands of unlignified axial parenchyma (arrow). *Abbreviation*. P = pith. **C.** Gross structure of ***C. gladiata*** stem showing structure and composition of the secondary xylem. Arrowheads indicate unlignified parenchyma. **D.** Gross structure of ***P. tuberosa*** stem showing structure and composition of the secondary xylem. The arrowhead indicates ray proliferation. ***Scale bars***. A = 200 µm; B-D = 500 µm.

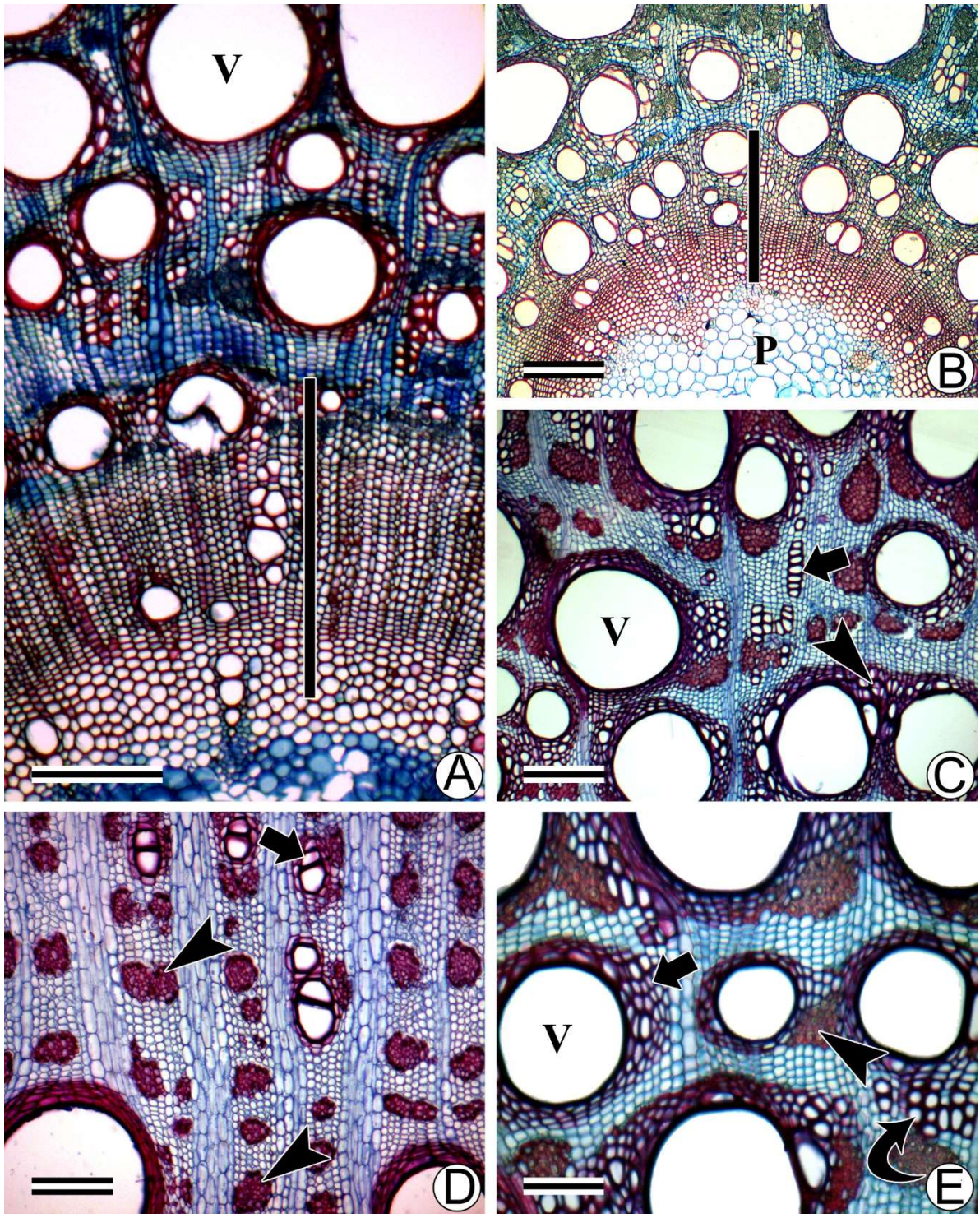

Fig. 3. Transverse (A-E) view of the mature stems of ***Canavalia*** and ***Pueraria***. **A.** A 10 mm thick stem ***P. tuberosa*** showing stiff xylem formed before the climbing habit while the xylem formed after the initiation of climbing is composed of an abundance of non-lignified parenchyma. **B.** A 10 mm thick stem of ***C. gladiata*** showing the structure and composition of the secondary xylem. Note the variation in the xylem structure formed before (vertical bar) and after the initiation of climbing. **C.** Structure of secondary xylem of ***C. cathartica*** showing nonlignified axial parenchyma, wide vessels (V) and narrow vessels in radial multiples (arrow). Note vessel-associated lignified parenchyma, narrow vessels (arrowhead) and fibres that form a sheath around wide vessels. **D.** Structure of the secondary xylem of ***P. tuberosa*** showing vasicentric parenchyma that forms a sheath around the vessels. Note the narrow vessels in radial multiples (arrow) while xylem fibres (arrowheads) are embedded within the nonlignified parenchyma background of the secondary xylem. **E.** Structure of secondary xylem of **C. *gladiata***. Note the vasicentric parenchyma (arrow), fibres (arrowhead) and narrow vessels (curved arrow). ***Scale bars***. A-D = 200 μm; E = 100 μm.

Table 1. Dimensional details of vessel elements (both wide and narrow), fibres, fibre tracheids and xylem rays. Values in parentheses indicates range.

Species	Wide vessel element		Narrow vessel element		Xylem Ray		Libriform Fibre	Tracheid
name	Length (µm)	Diameter (µm)	Length (µm)	Diameter (µm)	Height (µm)	Width (µm)	length (µm)	length (µm)
Canavalia gladiata	170 ± 58.72 (119 – 267)	286 ± 44.34 (208 – 386)	219 ± 11.90 (129 – 277)	69 ± 5.98 (40 – 99)	249 ± 29.15 (159 – 348)	89 ± 8.30 (12-171)	1188 ± 74.34 (901 – 1559)	401 ± 56.25 (327 – 515)
Canavalia cathartica	194 ± 48.92 (183 – 211)	223 ± 39.53 (182 – 259)	244 ± 39.85 (218 – 264)	47 ± 4.90 (38-61)	253 ± 36.05 (146-356)	78 ± 21.23 (13 – 147)	1175 ± 61.39 (950 – 1544)	365 ± 48.61 (248 – 481)
Pueraria tuberosa	327 ± 59.90 (207 – 475)	275 ± 8.72 (208 – 327)	228 ± 42.23 (158 – 317)	71 ± 4.30 (49 – 99)	318 ± 30.86 (248 – 354)	282 ± 14.74 (447 – 514)	3270 ± 98.97 (2812 – 4138)	687 ± 31.98 (465 – 831)

while lignified elements like vessels, fibres and thick-walled lignified parenchyma formed small pockets embedded within nonlignified parenchymatous background (Fig. 3C–E). The secondary xylem in all investigated species was diffuse-porous with indistinct growth rings (Fig. 2C, D). It was composed of vessels, tracheids, fibres, and an abundance of non-lignified axial and ray parenchyma cells (Fig. 3C–E). Vasicentric axial parenchyma thick-walled, lignified and formed a partial or complete sheath around the vessel elements (Fig. 3C–E). In both species of *Canavalia*, these vasicentric parenchyma cells were often intermixed with xylem fibres and narrow vessels (Fig. 3C, E). In *P. tuberosa* non-lignified parenchyma were more as compared to both species of *Canavalia* while thick-walled vasicentric axial parenchyma formed a narrow sheath (Fig. 3D). In all three species, the non-lignified and lignified parenchyma cells were storied, septate, chambered and showed the presence of prismatic crystals (Fig. 4A-C). Large diameter vessels mostly solitary and oval to circular in outline (Fig. 3A) while narrow vessels were arranged either in radial files of 2–3 vessel elements (Fig. 3C) or they may be arranged in clusters (Fig. 4C). The xylem rays tri-seriate, hetero-cellular with vertically elongated marginal sheath cells (Fig. 4A, D).

The length of the wide vessel elements was maximal (327 ± 59.90 µm) in *P. tuberosa* while it was minimal (170 ± 58.72 µm) in *C. gladiata.* The lumen diameter was maximal (286 ± 44.34 µm) in *C. gladiata* and minimal (223 ± 39.53 µm) in *C. cathartica*. In contrast, the length of narrow vessel elements was maximal in *C. gladiata* and minimal in *P. tuberosa* (Table 1). Xylem fibres non-septate with simple pits, their length was measured maximal (3270 ± 98.97) in *P. tuberosa* and minimal (1175 ± 61.39 µm) in *C. cathartica*. Xylem rays were tall, bi-multiseriate, non-storied and hetero-cellular with the presence of marginal sheath cells (Fig. 4A). The height and width of the xylem rays differed within species (Table 1) and it was measured highest (318 ± 30.86 µm) in *P. tuberosa* while it was lowest in (249 ± 29.15 µm) in *C. gladiata*. Similarly, the length of the tracheids differed within the species studied (Table 1).

Development of interxylary phloem and interxylary cambium: As the stem grew in thickness (in 12–15 mm thick stems), in both species of *Canavalia*, the non-lignified parenchyma formed at the beginning of the secondary growth underwent dedifferentiation (Fig. 4E) and directly redifferentiated into isolated (Fig. 4E) or axial parenchyma may divide and one of the cells may produce interxylary sieve elements (Fig. 4F). Most often divisions in these axial parenchyma cells were more frequent and formed groups of sieve elements (Fig. 4G). These sieve elements were like external regular sieve elements in their length and width. They were characterised by the presence of simple sieve plates oriented transversely with a single companion cell associated with them. Subsequently, more and more interxylary sieve elements differentiated and formed islands of interxylary sieve elements. As the growth progressed further, the parenchyma cells adjacent to the newly differentiated sieve elements divided repeatedly and became meristematic to form radial files of meristematic cells like vascular cambium (Fig. 5A–D). These segments of the interxylary cambia were formed on either side of the phloem islands (Fig. 5C, D) and sometimes completely encircled the phloem islands (Fig. 5B). Gradually formation of such meristematic cells extended tangentially and formed continuous segments of the interxylary cambium that were interrupted by the xylem rays (Fig. 5A–D). Initially, these cambial segments were unidirectional and exclusively produced interxylary sieve elements. Over time, these cambial segments were bidirectional and began to form the xylem elements particularly fibres and narrow vessel elements (Fig. 5C). In thick stems of all three species, marginal ray cells also became meristematic and formed interxylary ray cambium (Fig. 5B, D, E) and differentiated into perforated ray cells (Figs. 5F, 6A). These perforated ray cells were similar to the ray cells in shape and size except they were lignified and possessed simple perforation plates at both ends and bear alternate bordered pits on their lateral walls like vessel elements (Fig. 6A).

The formation of interxylary phloem in *P. tuberosa* differed from the other two species of *Canavalia.* Interxylary phloem in *P. tuberosa*, always differentiated from the interxylary cambium while its direct differentiation from the non-lignified xylem parenchyma was observed occasionally. Most often, the formation of interxylary phloem in this species was observed only after the initiation of interxylary cambium (Fig. 6B, C). In 15–18 mm thick stems, the non-lignified xylem parenchyma situated away from the cambium underwent repeated

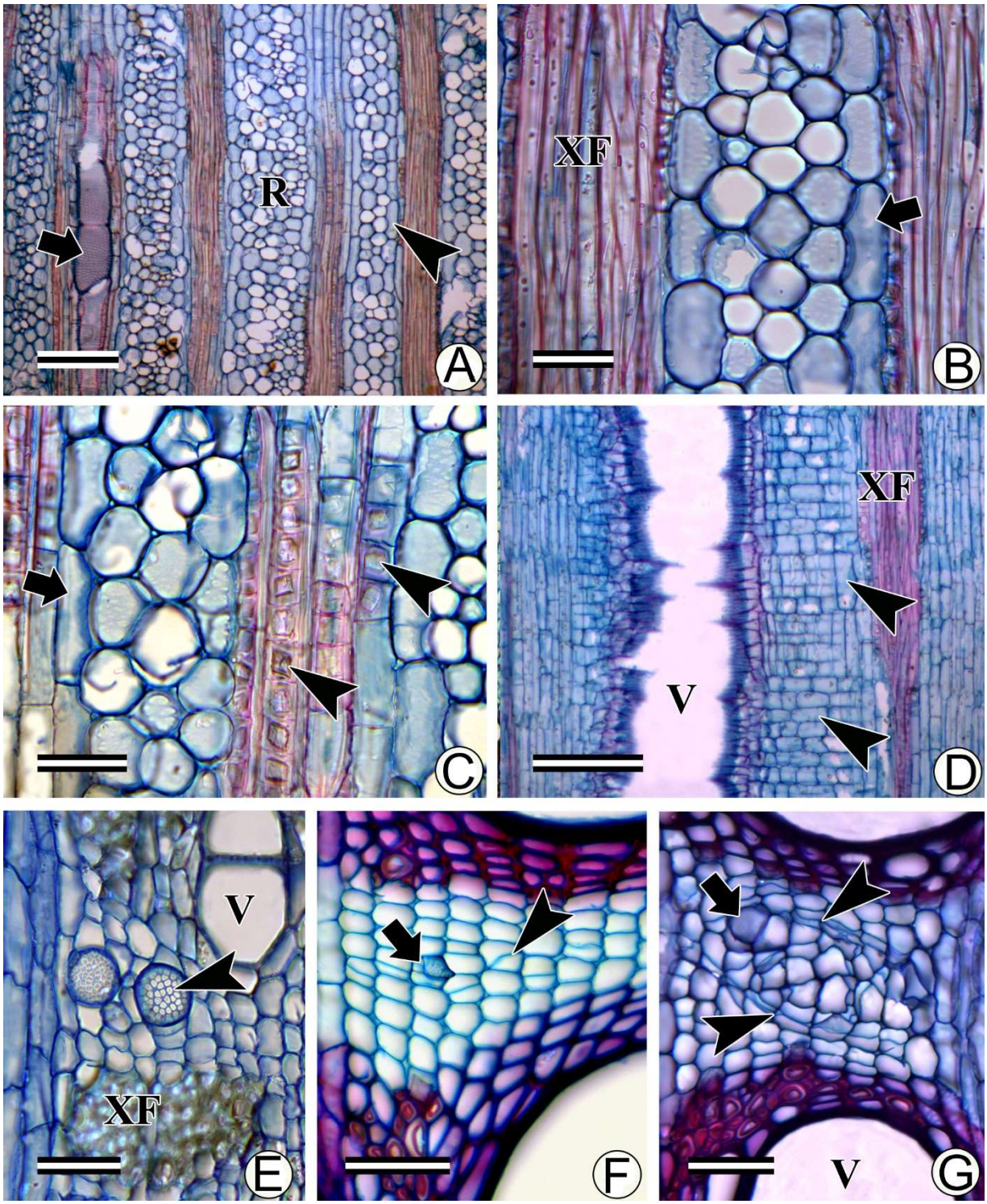

Fig. 4. Tangential (A-C), radial (D) longitudinal and transverse view (E-G) of secondary xylem of ***Canavalia*** and ***Pueraria***. **A.** Tangential longitudinal view of secondary xylem of ***C. cathartica*** showing non-storied rays (R), vessels (arrow) and vertically upright marginal ray cell (arrowhead). **B.** Enlarged view of ***P. tuberosa*** xylem showing structure and composition of ray. Note the shape and size (arrow) of the marginal ray cell. *Abbreviation*. XF = xylem fibres. **C.** Secondary xylem of ***C. gladiata*** showing marginal sheath cells (arrow), and chambered crystalliferous parenchyma with rhomboidal crystals (arrowheads). **D.** Radial longitudinal view of the secondary xylem of ***P. tuberosa***. Arrowheads indicate the arrangement of ray cells. ***Abbreviations***. XF = xylem fibres, V = vessel. **E.** Interxylary sieve element (arrowhead) showing simple sieve plates in ***C. gladiata***. *Abbreviations*. XF = xylem fibres. **F.** Initiation of interxylary cambium (arrowhead) in ***C. cathartica***. Note the differentiating sieve element (arrow). **G.** Newly initiated interxylary cambium (arrowheads) in ***C. gladiata***. The arrow indicates the sieve tube element. ***Scale bars***. A, D = 200 μm, B, C, E-G = 50 μm.

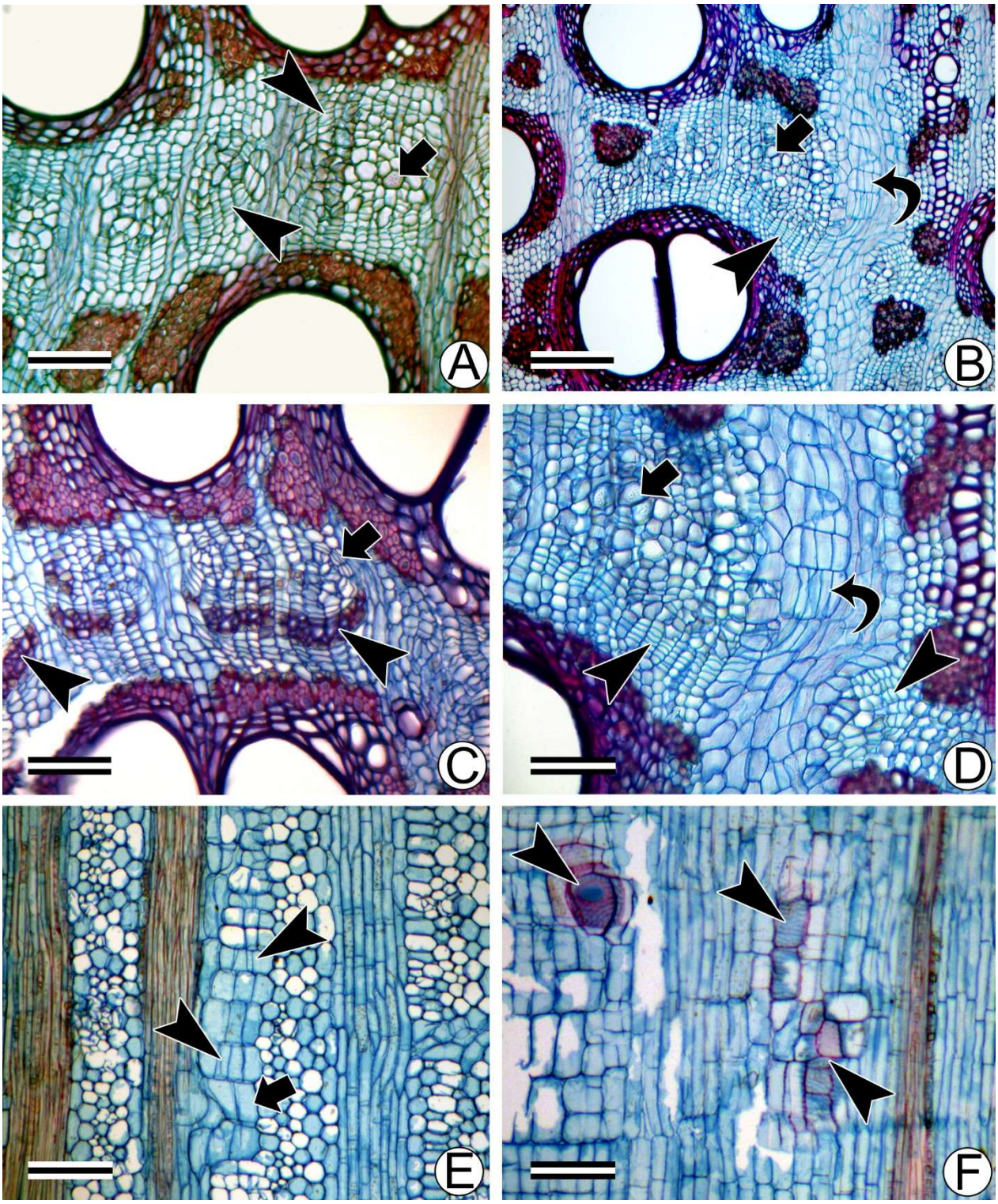

Fig. 5. Transverse (A-D), tangential (E) and radial (F) longitudinal view of the mature stems of ***Canavalia***. **A & B.** Well-established interxylary cambium (arrowheads) in ***C. gladiata*** and ***C. cathartica*** respectively. Arrow(s) indicates a group of interxylary sieve elements formed from the interxylary cambium. Note the tangentially oriented interxylary cambium formed at the ray margin (curved arrow). **C.** Bidirectional activity of interxylary cambium (arrow) showing recently differentiated xylem derivatives (arrowheads) in ***C. gladiata***. **D.** Enlarged view of Figure 5B showing interxylary cambium (arrowheads) in the stem of ***C. cathartica***. Note the sieve elements (arrow) deposited by the interxylary cambium while the curved arrow shows interxylary ray cambium. **E.** Initiation of interxylary ray cambium (arrowheads) in ***P. tuberosa***. The arrow indicates an expansion of the ray cell before division. **F.** Differentiation of vessels (arrowheads) in xylem rays of ***P. tuberosa***. ***Scale bars***. A, C-F = 100 µm; B = 200 µm

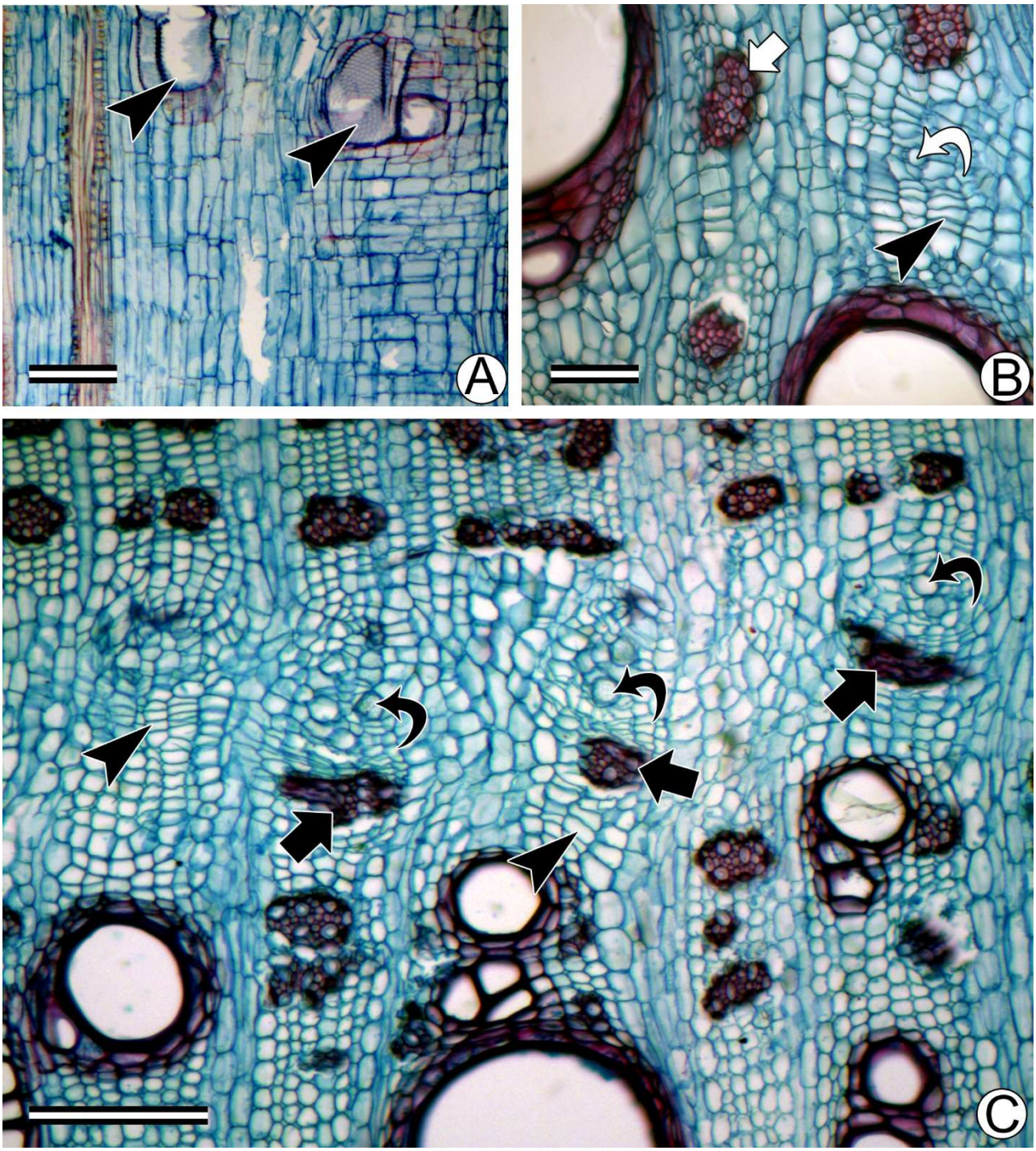

Fig. 6. Radial (A) and transverse (B-C) view of the secondary xylem of ***Pueraria tuberosa***. **A.** Differentiation of vessels (arrowheads) from the xylem ray cells. **B.** Initiation of interxylary cambium (arrowhead) due to periclinal divisions in non-lignified xylem parenchyma. The curved arrow shows a recently differentiated sieve tube element while the arrow indicates patches of xylem fibres. **C.** Small segments of interxylary cambium (arrowhead) in ***P. tuberosa*** showing recently formed sieve elements (curved arrows) and xylem elements (arrows). ***Scale bars***. A = 100 μm; B, C = 200 μm

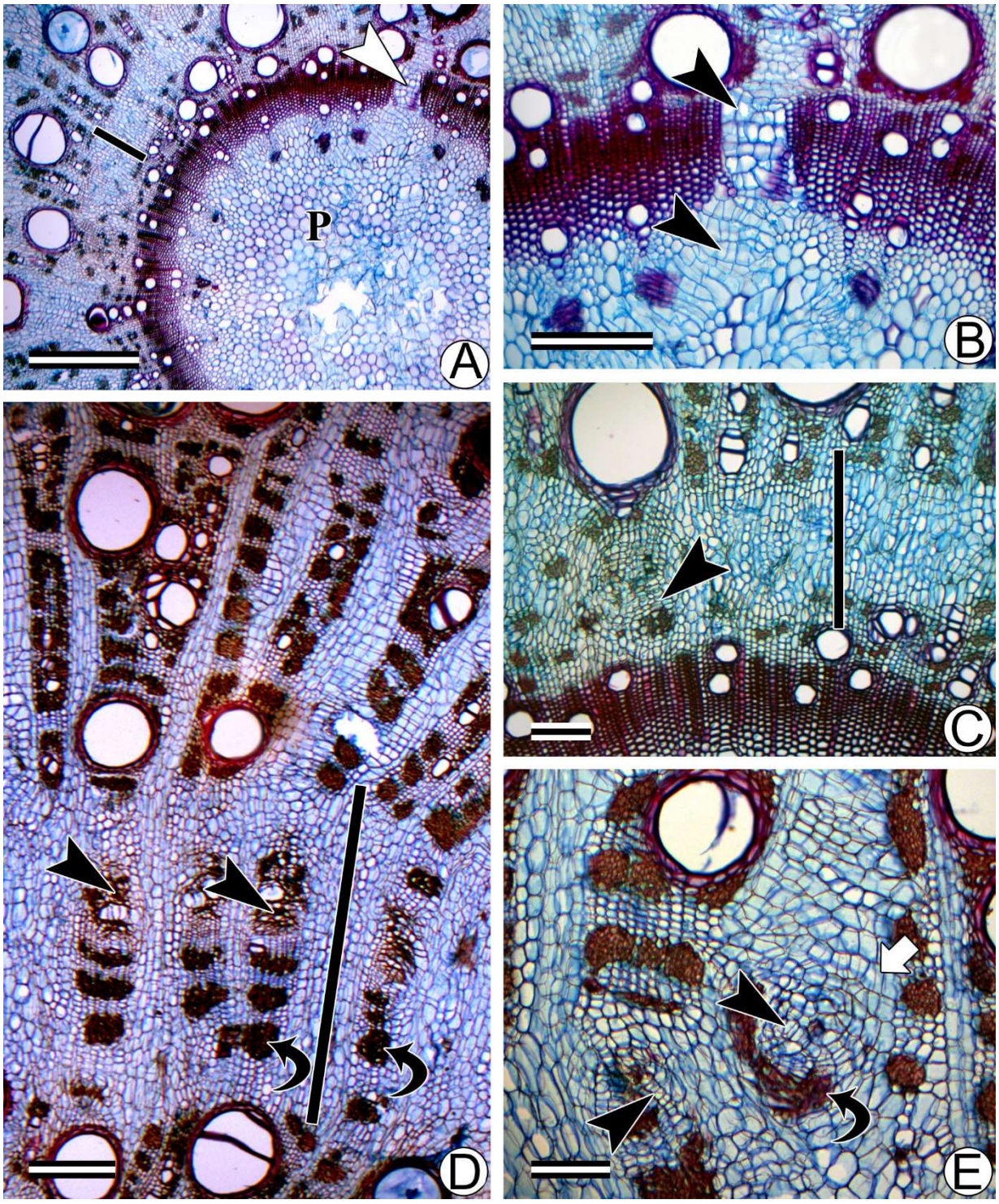

Fig. 7. Transverse view of the mature stems of ***Pueraria tuberosa*** showing proliferation of non-lignified xylem parenchyma. **A.** Proliferation of the non-lignified xylem parenchyma (vertical bar) formed at the beginning of the secondary growth. The proliferation of marginal pith cells (white arrowhead) leads to the rupture of the initially formed xylem cylinder. **B.** Enlarged view of Figure 7A showing proliferation of pith cells (lower arrowhead) and rupture of xylem cylinder (upper arrowhead) formed at the self-supporting stage. **C.** Enlarged view of Figure 7A showing proliferation of axial parenchyma (vertical bar) formed immediately after the initiation of the climbing habit. The arrowhead indicates meristematic centres and differentiation of the xylem. **D.** Relatively advanced stage of parenchyma proliferation (vertical bar) in the thick stems. Note the increased width of proliferating cells and differentiation of phloem (arrowheads) and xylem (curved arrow). **E.** Formation of meristematic centres (arrowheads) in nonlignified axial parenchyma of the secondary xylem. Note the expansion and dividing axial parenchyma (white arrow) and differentiation of lignified elements (curved arrow). ***Scale bars***. A, B = 500 µm; C, E = 250 µm; D = 200 µm.

periclinal divisions and formed small segments of meristematic cells arranged in radial files (i.e., interxylary cambium). In which the central cells of the interxylary cambium showed the differentiation of the interxylary sieve elements (Fig. 6B). As the plants grew further, the interxylary cambium became functionally bidirectional and showed differentiation of the lignified elements like fibres and narrow vessels (Fig. 6C). Gradually, several such small segments of the interxylary cambia initiated throughout the stem. In 20–25 mm thick stems, the thin-walled, non-lignified xylem parenchyma formed at the beginning of the secondary growth (i.e., close to the pith) divide repeatedly into various planes and showed proliferation of these cells (Fig. 7A). Gradually, the proliferation of parenchyma cells extended towards the centre of the stem leading to the fracture of the xylem cylinder and protruded into the pith region (Fig. 7A). Besides proliferation, some of the proliferating cells located at the pith margin also showed differentiation of lignified elements (Fig. 7B).

As the stem grew in thickness, the zone of non-lignified parenchyma formed in response to proliferation was increased significantly in width and subsequently showed the differentiation of phloem elements (Fig. 7C). With time, more and more parenchyma underwent proliferation and also showed differentiation of conducting and elements of the xylem and phloem (Fig. 7D). In 25–30 mm thick stems, xylem parenchyma proliferation and formation of the meristematic centre was observed frequently in the earlier formed xylem. Before the cell division, the axial parenchyma expanded in all directions followed by differentiation of interxylary phloem and lignified elements was observed frequently in the axial and ray parenchyma cells of the secondary xylem (Fig. 7E).

DISCUSSION

Family Fabaceae is one of the largest families, comprising herbs, shrubs, trees, vines, and woody climbers. Several climbing members of the family are characterised by the presence of unique cambial variants like successive cambia, functionally inverse cambia, interxylary phloem, flattening of stems due to initiation of successive cambia, only on two opposite lateral sides of the stem, and the formation of external vascular cylinders (Wagner, 1946; Basson and Bierhorst, 1967; Caballe, 1993; Nair and Mohan Ram, 1990; Nair, 1993; Rajput *et al*., 2006, 2012, 2023). For both species of *Canavalia* (i.e., *C. cathartica* and *C. gladiata*) and *P. tuberosa* the secondary xylem showed vessels dimorphism (wide and narrow vessels), an abundance of non-lignified, thin-walled axial parenchyma, large and wide rays and the presence of cambial variant, which are considered as a typical characteristic of the climbing habit (Fisher and Ewers, 1991; Angyalossy *et al*., 2015; Rajput *et al*., 2023). In all three species, the groundmass of the secondary xylem was composed of non-lignified, thin-walled axial and ray parenchyma while thick-walled axial parenchyma and fibres formed a sheath around the vessels. Vessels dimorphic (i.e., wide and narrow vessels), tracheids vasicentric, rays thin-walled, wide and huge/tall, while xylem fibres either formed tangential narrow bands or small pockets embedded within the non-lignified parenchymatous background.

In climbing plants, an abundance of thin-walled, non-lignified parenchyma is correlated with increased stem flexibility and healing of internal injury (Carlquist, 1985, 2001; Fisher and Ewers, 1991; Angyalossy *et al*. 2015). Besides these, thin-walled parenchyma plays an important role in the storage of carbohydrates that can be used when the plants are leafless and the sprouting of new leaves occurs before the arrival of rains or massive flowering and fruit set takes place. All three species shed their leaves in December-January and remain leafless during the drier part of the year till the arrival of rains in June. Moreover, flowering and fruit setting in *P. tuberosa* occurs during peak summer. Another possible reason for the abundance of non-lignified parenchyma cells is their ability to divide and differentiate into new vascular elements (Angyalossy *et al*., 2015). Similarly, the occurrence of vessel dimorphism and their higher frequency as compared to self-supporting plants is ascribed to be associated with the supply of a required quantity of water through the narrow stem (Carlquist, 1991, 2001; McCulloh *et al*., 2010; Isnard and Field, 2015; Angyalossy *et al*., 2015). However, wide vessels are susceptible to failure due to embolism (Zimmermann and Jeje, 1980; Ellmore and Ewers, 1985; Ewers *et al*., 1990, 1997; Lens *et al*., 2022). In contrast, narrow vessels are more resistant to embolism (Ellmore and Ewers, 1985; Carlquist, 1991, 2001) and that could be the reason that narrow vessels were often found associated with wide vessels to overcome this problem of embolism (Angyalossy *et al*., 2015). The presence of narrow and wide vessels creates hydraulic bridges between narrow and wide vessels (Carlquist, 1985; Brodersen *et al*., 2013). Moreover, lignified parenchyma form a sheath around the vessels that also has a role during vessel failure due to embolism (Carlquist, 2001) while a sheath formed by the vessel-associated lignified parenchyma along with fibres helps the vessels from internal damage during the stem torsion.

The stem anatomy and development of interxylary phloem in both species (i.e., *C. cathartica* and *C. gladiata*) and *P. tuberosa* are investigated for the first time. As mentioned above, thin-walled parenchyma retains the ability of cell division and differentiation (Angyalossy *et al*., 2015), in the present study also all three species showed dedifferentiation of thin-walled parenchyma and the formation of interxylary phloem. A similar development of interxylary phloem in *Canavalia*

ensiformis has been investigated earlier by Rajput (2003). Formation of interxylary phloem is frequently reported in other members like *Mucuna* (Carlquist, 2001), *M. pruriens* (Patil, 2011), *Dolichos lablab* (Rajput *et al*., 2006), and *Phaseolus lunatus* (Rajput *et al*., 2023) of the family Fabaceae. All the above-mentioned members also possess the secondary xylem composed of an abundance of non-lignified parenchyma that undergoes dedifferentiation and forms interxylary sieve elements (Carlquist, 2001; Patil, 2011; Rajput *et al*., 2006, 2023). The non-lignified, thin-walled parenchyma in the thick stems positioned on either side of the phloem islands forms interxylary cambium.

The formation of interxylary cambium is a rare feature and reported in a few members of eudicots (Patil *et al*., 2011; Rajput *et al*., 2014; Pace *et al*., 2018; Rajput *et al*., 2023). These small segments of the interxylary cambium may be functionally unidirectional and exclusively produce sieve elements and may become bidirectional (Patil *et al*., 2011; Rajput *et al*., 2022, 2023). In both species of *Canavalia*, initially, the interxylary cambium was unidirectional but subsequently, it became bidirectional and produced fibres and narrow vessels. Carlquist (1982, 2013), correlated the formation of interxylary phloem with sudden and massive flowering, which is an energy-consuming process. As per our field observation, the initiation of interxylary phloem development in both species of *Canavalia* coincides with the flowering season (i.e., the reproductive phase of the lifecycle). Interxylary phloem is said to play an important role in the rapid translocation of photosynthate (Carlquist, 1982, 2001; Rajput *et al*., 2021; Thacker *et al*., 2024) and the coincidence of its development during the flowering season is evidence of its role in the supply of photosynthate for fruit development. A similar correlation has also been established in our previous studies on members of other families (Rajput *et al*., 2022; Thacker *et al*., 2024).

CONCLUSION

Both species of *Canavalia* and *P. tuberosa* increase their stem thickness through regular secondary growth and share typical features of lianescent habit. An abundance of thin-walled, non-lignified parenchyma plays an important role in the formation of interxylary phloem and interxylary cambium. The formation of interxylary phloem takes place by the dedifferentiation of non-lignified parenchyma into the interxylary cambium. Formation of interxylary phloem may be associated with more phloem production for the rapid translocation of photosynthates from the source to sink to fulfil the demand *vs.* supply within the narrow stem as compared to self-supporting plants. Besides, storage tissue, an abundance of non-lignified parenchyma may provide cushion to protect the vessels from damage while the formation of the sheath around the vessel by the thick-walled vasicentric parenchyma. Vessel dimorphism and wide and tall xylem rays are correlated with the climbing habit.

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