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Evidence of simultaneous occurrence of tylosis formation and fungal interaction in a late Cenozoic angiosperm from the eastern Himalaya

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ABSTRACT

Information on whether tyloses in fossil angiospermic plants form specifically in response to infestation with pathogenic fungi has been lacking so far, and thus the evolutionary history of tylosis formation as a physical restraint strategy against the advancing fungi remains unresolved. Our study addresses this knowledge lacuna. Carbonized woods were recovered from the upper part of the Siwalik succession (Kimin Formation: late Pliocene to early Pleistocene) of Arunachal Pradesh, eastern Himalaya. Samples from different portions of the wood remains were prepared by using standard thin sectioning techniques and studied under transmitted light compound and scanning electron microscopes. The Plio-Pleistocene wood remains are attributed to *Gmelina arborea* Roxb. (Lamiaceae) as *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. Numerous well-preserved tyloses were found mainly in the central heartwood region of the fossil wood. Rare fungal remains indicative of early-stage colonization are characterized by septate fungal hyphae extending through the vascular ray system within the secondary xylem, and fungal spores. As tyloses are present in large numbers and formed before the spread of fungal remains, they served as an effective physical restraint to the spread of the fungi. We conclude that tylosis formation contributed to an antagonistic relationship between pathogenic fungi and *Gmelina* plant in Neogene paleoecosystems of the eastern Himalaya. This report also demonstrates that the development of this histological protrusion has been a significant process in woody plants since Cenozoic.

1. Introduction

Malpighi (1987) initially reported tyloses as “balloon-shaped sacs” in heartwood vessels of angiosperms. Subsequently, he named such structures “Thyllen” (tyloses), derived from the Greek word meaning “small bags” or “containers in vessels”. In the decades that followed, several workers (Esau, 1965; Zimmermann, 1979; Bonsen and Kučera, 1990; Pearce, 1996; Tyree and Zimmermann, 2002; Sun et al., 2008; De Micco et al., 2016) described tyloses as ingrowths of protoplast of adjacent ray or axial paratracheal parenchymatous cells into the lumen of adjacent xylem vessel elements through pits. Although tyloses typically develop in vessels, they also occur occasionally in tracheids (Chrysler, 1908; Gerry, 1914; Esau, 1965; Peters, 1974) and fibers (Gottwald, 1972). They have thick or thin cellulosic walls and clog the vessel lumen partially or completely as a result of their size and number. Development of abundant tyloses in xylem vessels reduces the effective radius available for transport. When no alternate unoccluded pathway for flow exists, tyloses may block the sap flow to distal tissues (Pearce, 1996). The process of tylosis formation is complicated, and includes numerous metabolic changes, as well as the supply of phenolic compounds, lignin and aromatic substances (Mauseth, 2003).

Tyloses in fossil plants were initially reported from Lower Carboniferous progymnosperm (Scheckler and Galtier, 2003) and several ferns (Williamson, 1877, 1880; Weiss, 1906; Phillips and Galtier, 2005, 2011). They have also been documented from Permian silicified conifer stems (Feng et al., 2013); Triassic conifer woods (Jeffrey, 1904; Colani, 1919; Ogura, 1944, 1960; Nishida et al., 1977; Nishida and Oishi, 1982); Early Jurassic permineralized conifer axis (Harper et al., 2012); Jurassic conifer woods (Gothan, 1910; Jordan, 1914; Holden, 1915; Medlyn and Tidwell, 1975); Cretaceous conifer wood remains (Ogura, 1960; Robison, 1977) as well as Cretaceous and Cenozoic permineralized angiosperm woods (Bailey 1924; Bancroft, 1935; Spackman, 1948; Brett, 1960; Manchester, 1983; Nishida et al., 1990; Privé-Gill et al., 1999; Poole and Francis, 1999; Meijer, 2000; Poole and Cantrill, 2001; Takahashi and Suzuki 2003; Terada et al., 2006; Nishida et al., 2006; Castañeda-Posadas et al., 2009; Wheeler and Lehman, 2009; Boonchai et al., 2015).

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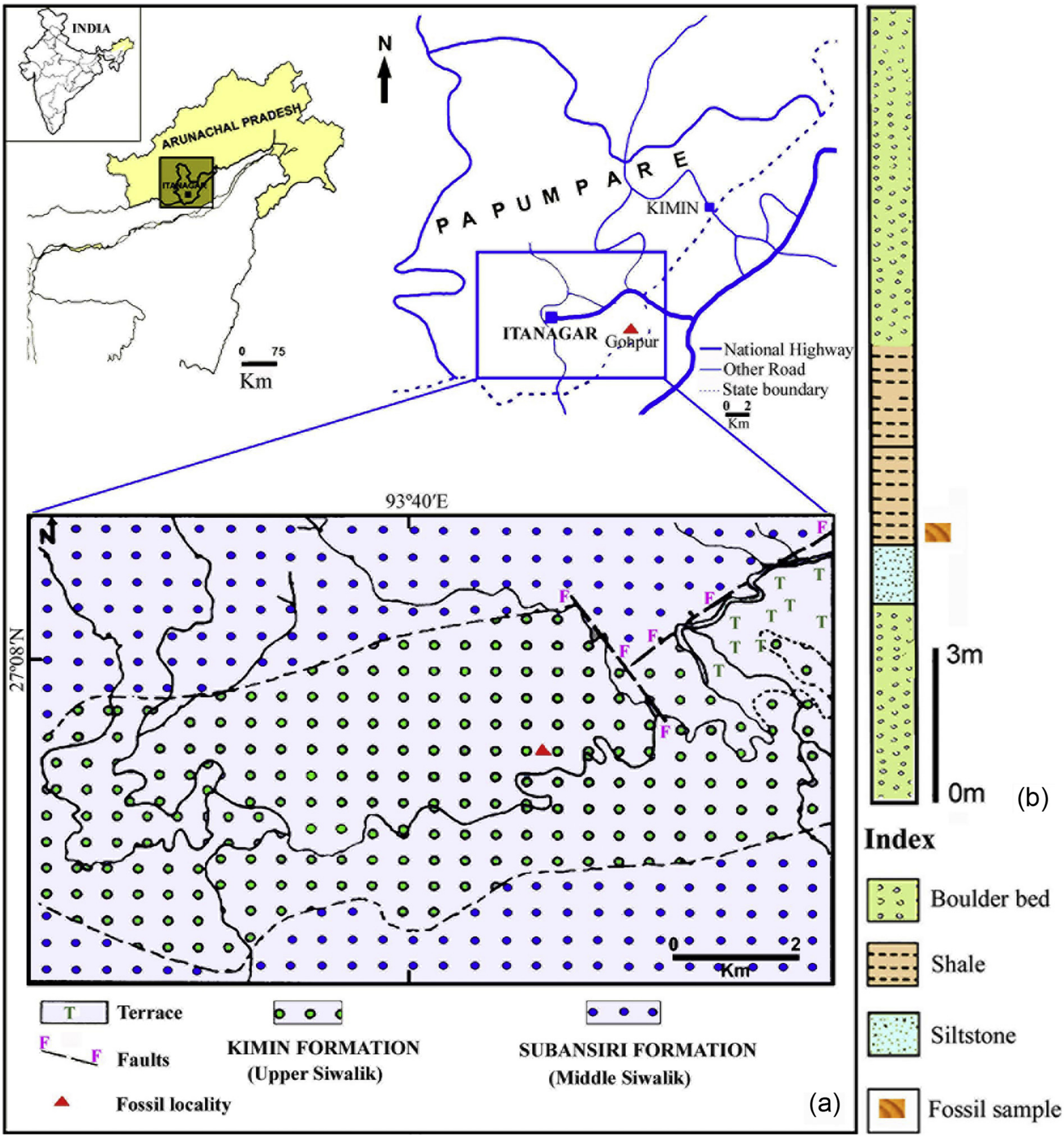

Fig. 1. (a) Location of study area in Papumpare district, Arunachal Pradesh and a geological setting of the area around Chandernagar-Gohpur, triangle indicates the fossil locality; (b) lithological column for part of the exposed road-section along the Chandernagar-Gohpur road in Papumpare district.

"Common tyloses" in vessels are present in 17% of world woods, with much higher percentages in India and Australia, and very low values in the Mediterranean basin, temperate Europe and New Zealand (InsideWood 2004-onwards; Wheeler, 2011; De Micco et al., 2016). Tyloses commonly occur in the heartwood of dicotyledonous angiosperms as a response to physical damage or pathogenic stimulants (Chattaway, 1949; Meyer, 1967; Panshin and DeZeeuw, 1980; Parameswaran et al., 1985; Wilson and White, 1986; Biggs, 1987; Pearce, 1990; Saitoh et al., 1993). Although the occurrence of tyloses is a common feature of heartwood, these organic occlusions can also occur in sapwood both normally and as a result of wounding (Gerry, 1914; Murmanis, 1975). Relatively few investigations on tyloses occurrence in other vascular plant (non-dicotyledonous) groups have been conducted (Tomlinson et al., 2011).

Tylosis formation, one of the main processes in the compartmentalization of decay in tree plants, serves to slow down or prevent the spread of pathogens (Shigo, 1984; Agrios 1997; Barry et al., 2001; Mehrotra, 2005; Pallardy, 2008). In addition, tyloses can form around wounds to prevent water loss, even in the absence of decay, in non-functional xylem (Harper et al., 2012). They also have been reported as a possible mechanism of plant resistance to certain vascular diseases in a number of woody plants (De Micco et al., 2016). These resistance structures are also formed in xylem vessels of most plants under various conditions of stress and during invasion by most xylem-invading pathogens.

Fungi, an integral part of virtually all modern ecosystems, are the primary pathogens responsible for the delignification and degradation of wood in extant forest ecosystems (Dighton et al., 2005). Some wood-infecting fungi may also be effective as parasites and causal agents of mild to severe infections. The anatomical particularities of our fossil materials from Arunachal sub-Himalaya provide a rare opportunity to assess how woody plants responded or adapted to pathogenic fungi during the late Neogene.

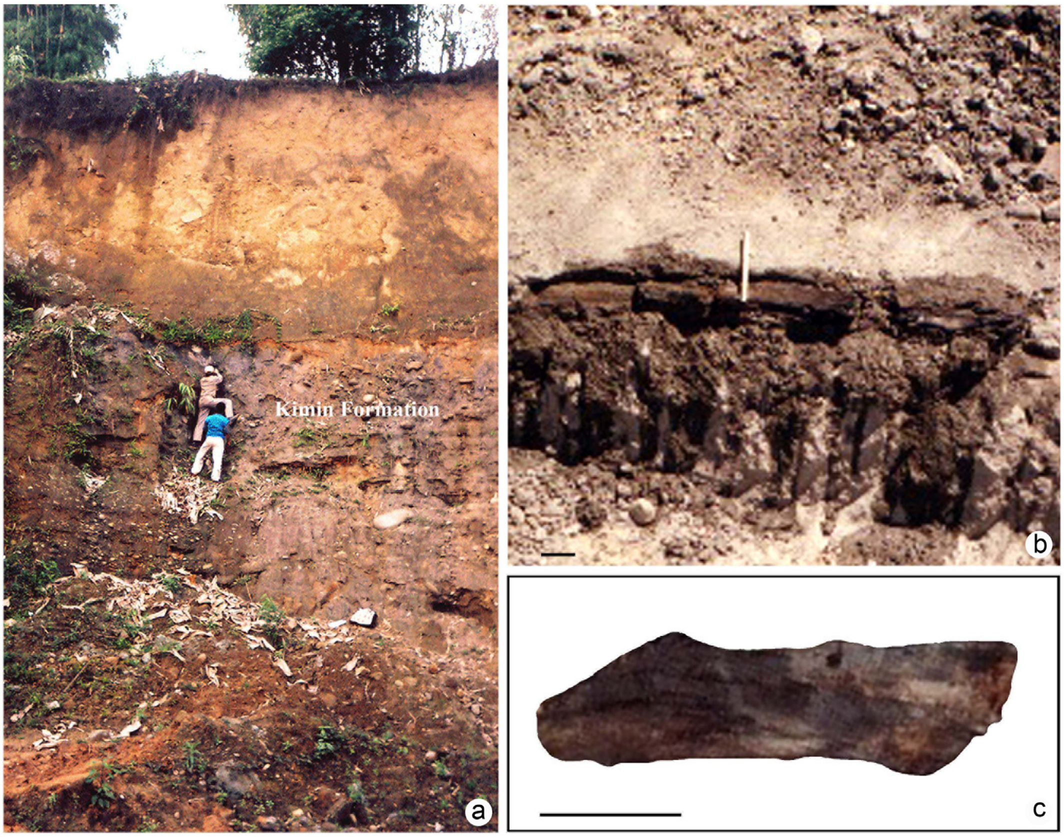

Fig. 2. (a) Image of the fossil locality in the Papumpare district, Arunachal sub-Himalaya; (b, c) collected carbonized woods (Scale = 1 cm).

In this contribution, we have studied carbonized fossil woods recovered from the late Pliocene-early Pleistocene Siwalik sediments of Arunachal sub-Himalaya and described as a new species *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. having affinity with modern *Gmelina areborea* Roxb. of family Lamiaceae. The most significant attribute of this new fossil wood is the numerous well-preserved tyloses co-occurring with rare fungal remains. This observation leads us to suggest that tylosis formation in the *Gmelina* plant might have occurred as a direct, non-specific response to the initial stage of fungal infestation.

2. Material and methods

The carbonized woods (four small well-preserved pieces of secondary xylem) described in this study were collected from the upper Siwalik strata (Kimin Formation, late Pliocene to early Pleistocene; Kumar, 1997) exposed along the Chandernagar-Gohpur road in Papumpare district (latitude 27.06589° N, longitude 93.64561° E) of Arunachal Pradesh (situated between 26.46444° N and 29.498333° N and 91.497222° E and 97.415556° E), eastern Himalaya (Figs. 1, 2). These sediments include alternating layers of soft grey to orange-colored, loosely packed very coarse to fine grained, grey sandstones, silty-clay and gravelly beds and interbeds of pebbly conglomerate. The sandstones are pebbly in places and are intercalated with claystones and shales with wood remains (Fig. 2a, b). Recently, we have reported a variety of angiosperm taxa from the same Siwalik Formation (Khan et al. 2011, 2014, 2016, 2017a, b). Magnetostratigraphic data suggests that the Siwalik Formation of Arunachal Pradesh was deposited between 13 and 2.5 Ma (Chirouze et al., 2012). The transition between the lower and middle Siwaliks is dated at about 10.5 Ma and the middle to upper Siwaliks transition is dated at 2.6 Ma.

The samples from different portions of the fossil wood remains were prepared by using the standard thin sectioning technique (Figueiral, 1999; Hass and Rowe, 1999) along three planes (transverse, tangential longitudinal and radial longitudinal). Photomicrographs showing structural details of the fossil wood were made using a transmitted light compound microscope with a photographic attachment (Zeiss Axioskop 2) and an SEM (S-440, Leica Leo, Cambridge, UK). Composite images were stitched together using Adobe Photoshop (Adobe, San Jose, CA, USA) software. Relationships to extant plants were assessed by reference to the Inside Wood website (http: insidewood.lib.nesu.edu/search), followed by examination of the published xylotomical databases (Metcalfe and Chalk, 1950; Cutlar and Gregory, 1998), images on various web sites, and microscope reference slides of extant woods. Terminologies for wood anatomical characters follow the recommendations of the IAWA list (IAWA Committee, 1989).

3. Results

3.1. Systematics of host plant

Order: Lamiales
Family: Lamiaceae
Genus: ***Gmelina*** Roxb
Species: *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. (Plates I, II).

Species diagnosis: Wood diffuse porous; vessels circular to elliptical, small to large solitary or in radial multiples of 2–3, inter-vessel pits bordered, alternate, axial parenchyma paratracheal, vasicentric, 1–2 seriate sheath around the vessels; xylem rays 1–4 seriate, predominantly biseriate; ray tissue weakly heterocellular, made up of mostly procumbent cells, and upright cells; fibers thick-walled, semi-libriform and septate.

Holotype: CUH/PPL/C_3/44.

Type locality: Road cuttings along the Chander Nagar-Gohpur road in Papumpare district, Arunachal Pradesh (situated between 26.46444° N and 29.498333° N and 91.497222° E and 97.415556° E).

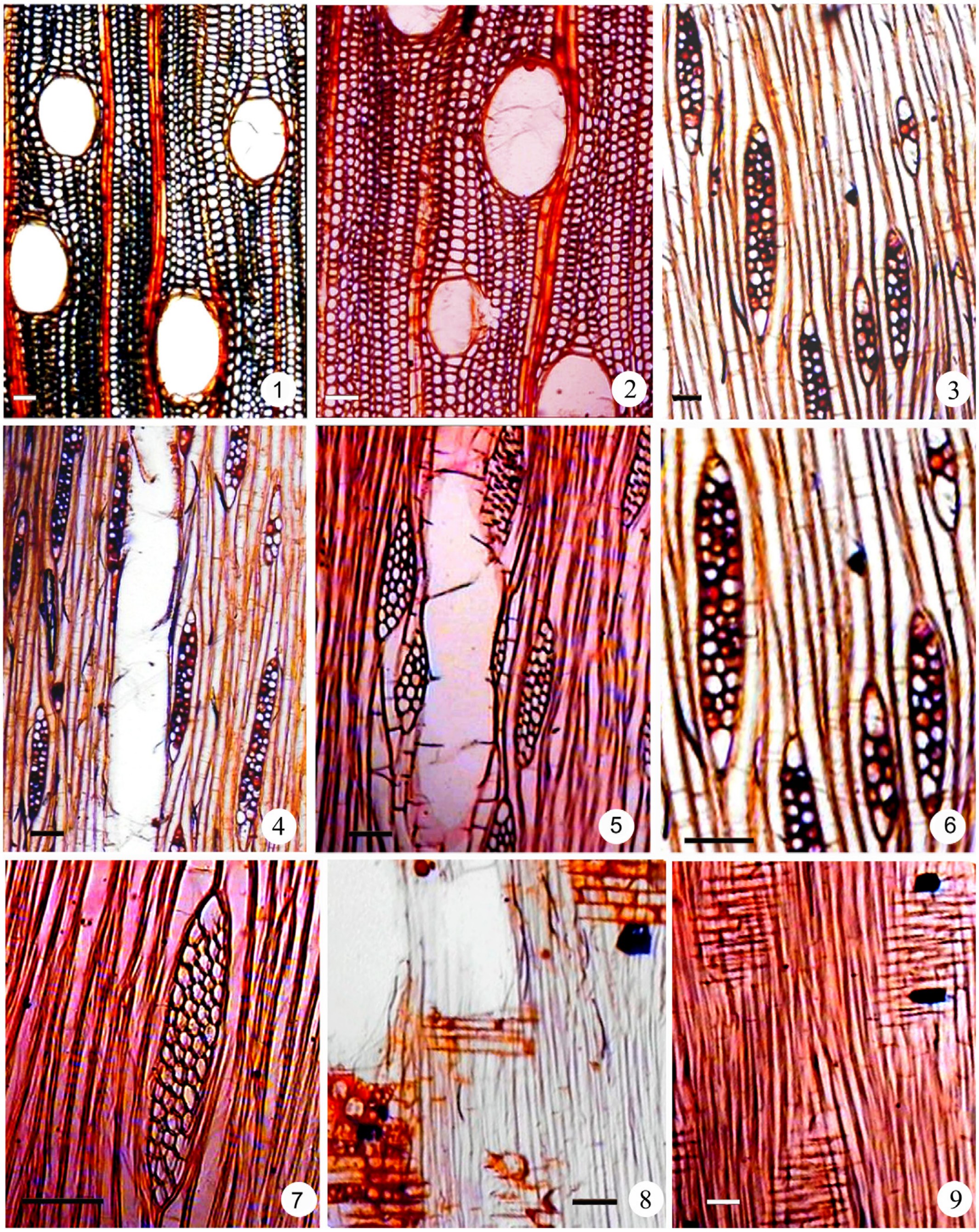

Plate I. Light micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1) Transverse section of the secondary xylem showing diffused vessel and paratracheal parenchyma; (2) Transverse section of the secondary xylem of modern *Gmelina arborea* Roxb. showing diffused vessels and paratracheal parenchyma; (3, 4, 6) Tangential longitudinal sections of the secondary xylem showing 2–3 seriate ray cells; (5, 7) Tangential longitudinal sections of the secondary xylem of modern wood of *Gmelina arborea* showing 2–3 seriate ray cells; (8) Radial longitudinal section of the secondary xylem showing heterocellular condition of ray tissue; (9) Radial longitudinal section of the secondary xylem of modern *Gmelina arborea* showing heterocellular condition of ray tissue (Scale Bar = 50 μm).

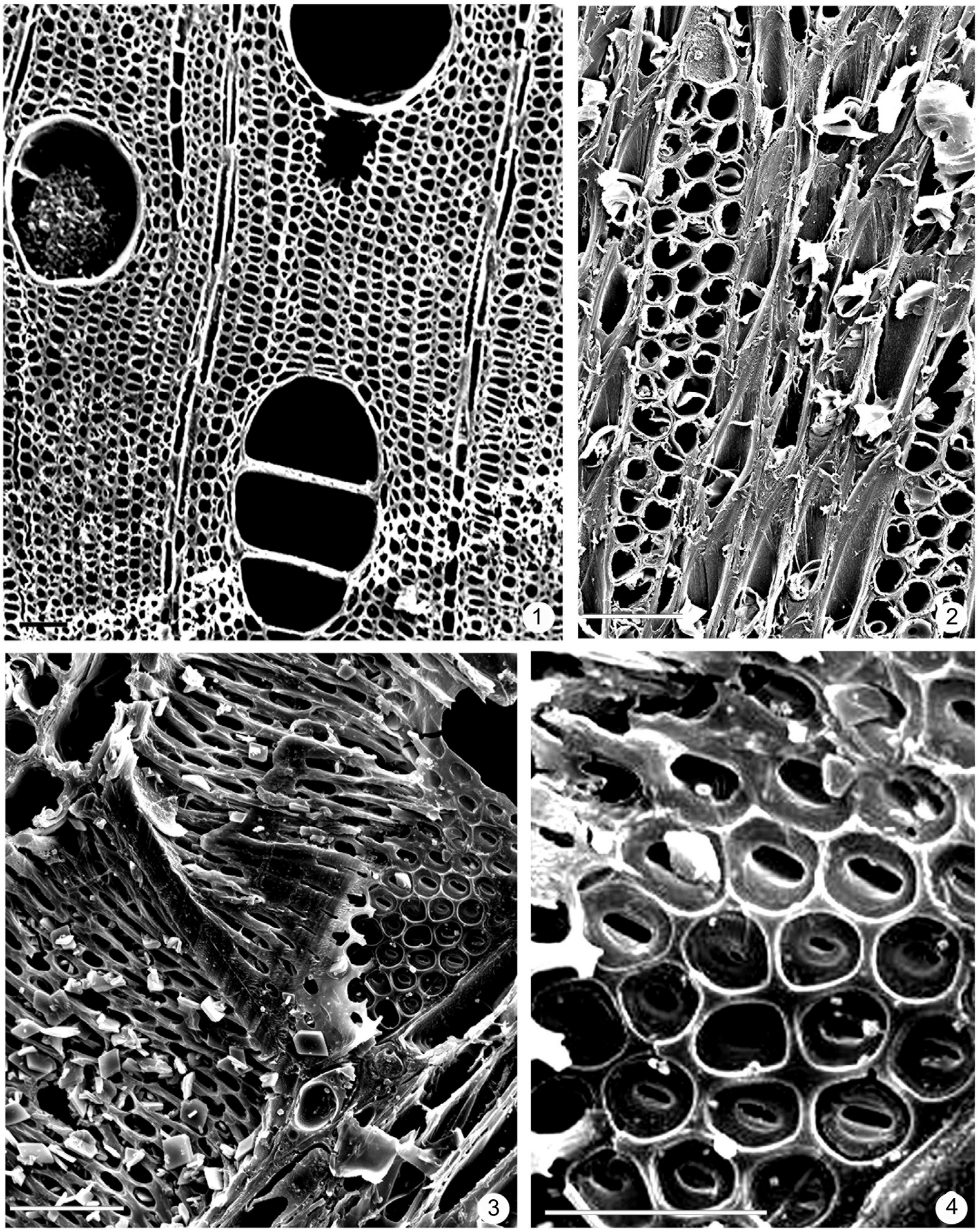

Plate II. Scanning Electron micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1) Transverse section of the secondary xylem showing diffused vessels and paratracheal parenchyma; (2–4) Tangential longitudinal sections of the secondary xylem: (2) ray cells; (3) Ray cells and vessel with bordered pits (Scale Bar = 50 μm); (4) Enlarged view of vessel with bordered pits (Scale Bar = 25 μm).

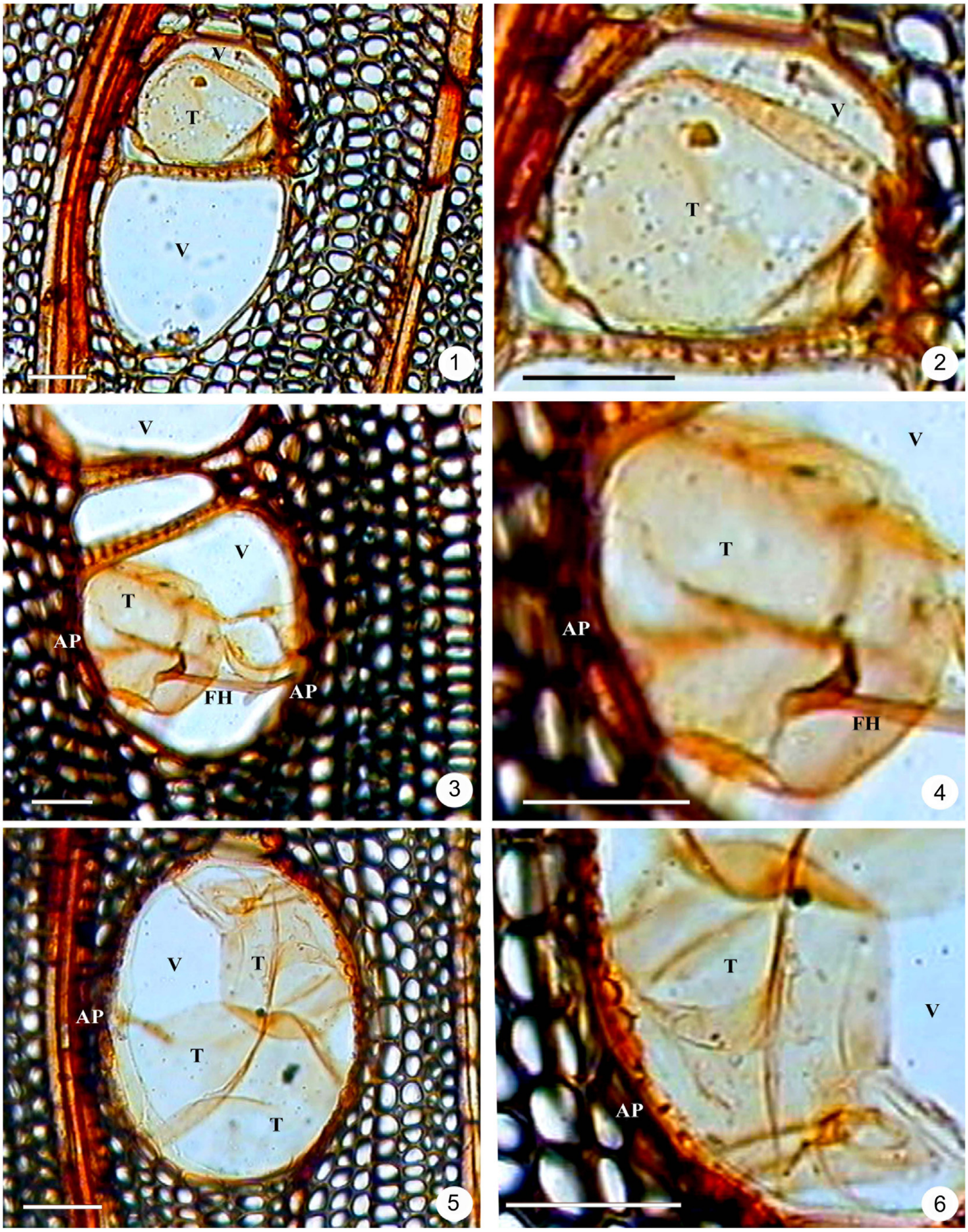

Plate III. Light micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1–6) Transverse sections of the secondary xylem: (1) Xylem vessel with tylosis; (2) Close-up of a tylosis in a vessel; (3) A fungal hypha penetrating into the tylosis in vessel, where the hypha branches before extending into the adjacent axial parenchyma cells, possibly suggesting some chemical defense mechanism from the tylosis; (4) Detail of a fungal hypha penetrating into the tylosis in vessel; (5) Vessel with closely spaced three tyloses; (6) Close-up of Fig. 5 (Scale Bar = 50 μm). Abbreviations: AP, Axial parenchyma cell; FH, Fungal hypha; T, Tylosis; V, vessel.

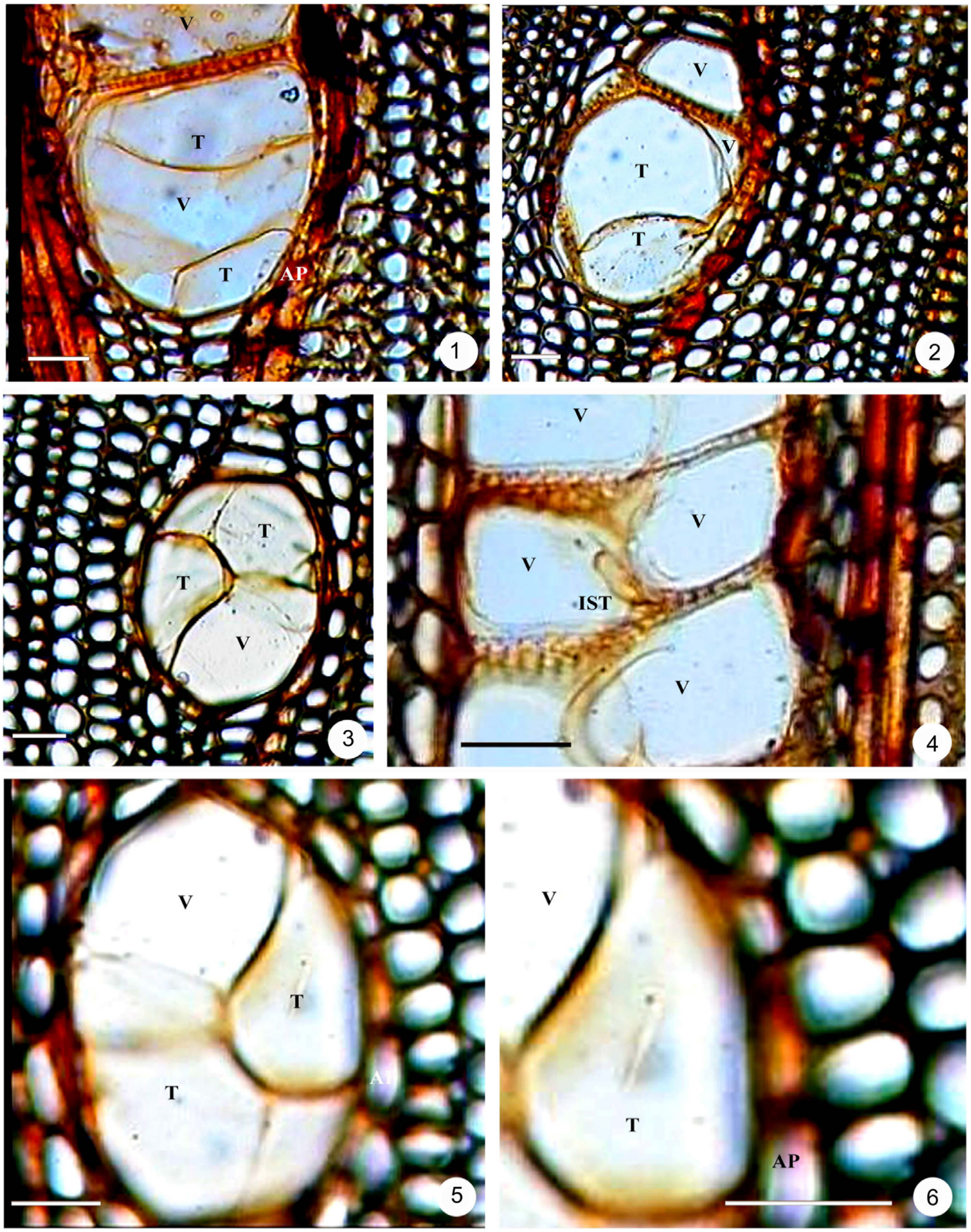

Plate IV. Light micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1–6) Transverse sections of the secondary xylem: (1, 2) Xylem vessel with two septa-like tyloses; (3) Xylem vessel with two tyloses; (4) Initial stage of development of tylosis started with small, bulbous protrusion; (5) Vessel with tyloses; (6) Close-up of Fig. 5 (Scale Bar = 50 μm). Abbreviations: AP, Axial parenchyma cell; IST, Initial stage of development of tylosis; T, Tylosis; V, vessel.

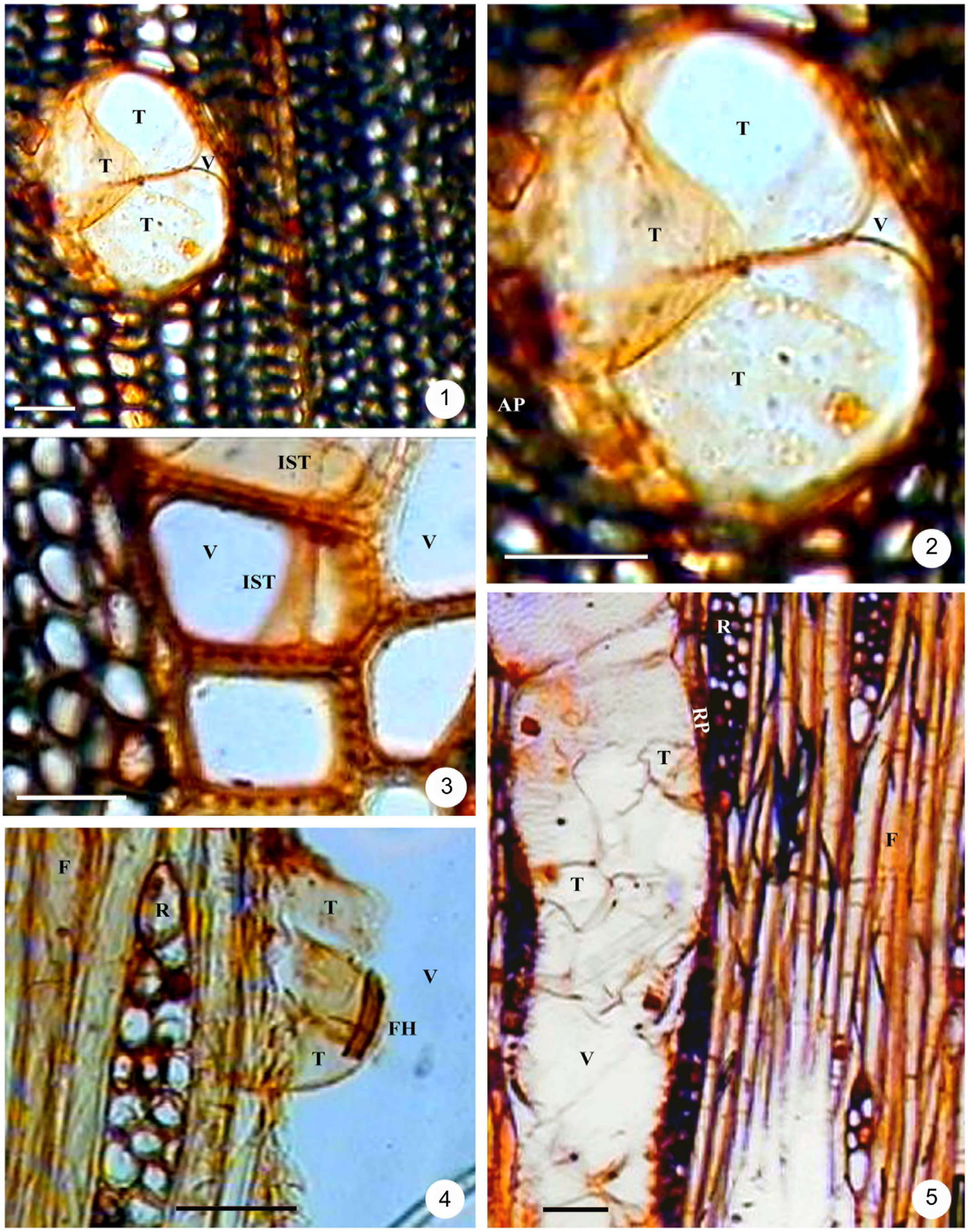

Plate V. Light micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1–3) Transverse sections of the secondary xylem: (1) Three closely-placed tyloses almost completely sealing off the lumen of the vessel; (2) Close-up of Fig. 1; (3) Initial stage of development of tyloses starting with small, bulbous protrusions. (4, 5) Tangential longitudinal sections of the secondary xylem: (4) Vessel with two tyloses and one incomplete, septate fungal hypha penetrating into the one tylosis; (5) Xylem vessel with some tyloses developing from the ray parenchyma cells (Scale Bar = 50 μm). Abbreviations: AP, Axial parenchyma cell; F, Fiber; FH, Fungal hypha; RP, Ray parenchyma cell; R, Ray cell; IST, Initial stage of development of tylosis; T, Tylosis; V, vessel.

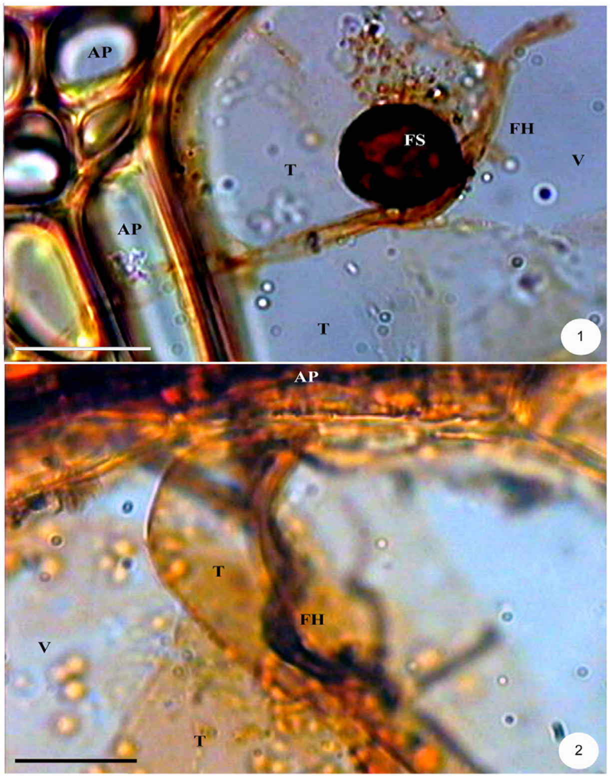

Plate VI. Light micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1, 2) Fungal remains (hyphae and spore) penetrating into the tyloses in vessels of secondary xylem, where the hyphae branch before extending into the adjacent axial parenchyma cells. Abbreviations: AP, Axial parenchyma cell; FH, Fungal hypha; FS, Fungal spore; T, Tylosis; V, vessel.

Type horizon: Upper part of the Siwalik succession of sediments (Kimin Formation: late Pliocene to early Pleistocene).

Etymology: The specific epithet "*siwalika*" recognizes Siwalik sediments.

Collectors: Mahasin Ali Khan and Subir Bera.

Repository: Herbarium and Museum, Department of Botany, University of Calcutta, India (CUH).

Description: Wood diffuse porous (Plates I, 1; II, 1); growth rings indistinct; vessels circular to elliptical, small to large (Plates I, 1; II, 1), t.d. 110–175 μm, r.d. 120–230 μm, solitary or in radial multiples of 2–3, 7–14 per sq. mm; perforations simple; tyloses many (Plates III, IV, V, VI, VII, VIII), well-developed; axial parenchyma paratracheal, vasicentric, 1–2 seriate sheath around the vessels (Plates I, 1; II, 1); rays non-storied, 7–10 per mm, 1–4 seriate (Plates I, 3, 4, 6; II, 2), predominantly bi-seriate, inter-vessel pits bordered, alternate (Plate II, 3, 4), circular with lenticular apertures, 8–11 μm in diameter; ray cells thick-walled, ray tissue weakly heterocellular (Plate I, 8), made up of mostly procumbent cells, 20–30 μm in tangential height and 30–40 μm in radial length, upright cells 40–60 μm in tangential height and 20–30 μm in radial length; fibers polygonal in cross-section (Plates I, 1; II, 1), thick-walled, 14–20 μm in diameter, 240–450 μm in length, semi-libriform, septate (Plates I, 3, 4, 6; II, 2).

Remarks: The diagnostic anatomical characters of the Siwalik fossil wood such as vessels with perforation plates and bordered pits, paratracheal, vasicentric parenchyma; 1–4 seriate, heterocellular rays and thick-walled, septate fibers, clearly indicate its affinities to the modern wood of family Lamiaceae (Plate I, 2, 5, 7, 9; Pearson and Brown, 1932; Metcalfe and Chalk, 1950). In order to compare and find the nearest generic affinity of the present fossil wood, six available modern genera of Lamiaceae were examined (FFPRI Wood Database; InsideWood, 2004-onwards). These modern genera are *Callicarpa* L., *Leucosceptum* Sm., *Premna* L., *Clerodendrum* L., *Vitex* L. and *Gmelina* L. showing anatomical similarities with the present fossil wood. Both *Callicarpa* and *Premna* differ in having predominantly multi-seriate ray cells as compared to the predominately bi-seriate ray cells in our fossil specimen. They also differ by having ray tissue with procumbent, upright and square cells. Our fossil specimen possesses ray tissue without square cells. *Vitex* differs in having solitary and paired vessels and multiseriate (3–5 seriate) ray cells. It also differs from our Siwalik fossil in having homocellular ray tissue with only procumbent cells. However, our fossil specimen possesses heterocellular ray tissue. *Clerodendrum* differs in having vasicentric to confluent as compared to the vasicentric axial parenchyma and thick-walled fibers in our fossil specimen. *Leucosceptum* lacks inter-vessel bordered pits differentiating it from our fossil specimen. Out of the above mentioned taxa, anatomical characters of *Gmelina* specially *G. arborea* show closest resemblance to the present fossil wood as both have vessels with simple perforations and alternate bordered pits, paratracheal, vasicentric parenchyma; 1–4 seriate, weakly heterocellular rays and thick-walled, septate fibers. The size and shape of vessels and ray cells closely resemble in both. However, the present fossil specimen differs from *G. arborea* in having a greater frequency of the vessels with well developed tyloses.

So far, only one fossil wood, *Gmelina tertiara* Bande (1986), having affinity with modern wood of *Gmelina* has been reported from the Deccan Intertrappean beds of India. Our Siwalik fossil differs in having paratracheal vasicentric parenchyma and circular inter-vessel pits as compared to the paratracheal vasicentric to aliform to aliform-confluent parenchyma and polygonal inter-vessel pits in *Gmelina tertiara*. Our fossil specimen is also distinct from Bonde's specimen in having comparatively more thick-walled fibers and ray cells, and more closely spaced axial parenchyma bands. As the Siwalik fossil wood is anatomically dissimilar to *Gmelina teriara* in the shape and size of intervessel pit-pairs, the distribution of parenchyma, and structure of the rays and fibers, it is regarded as a different species, namely *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov.

3.2. Tylosis

Profuse, well developed tyloses are found along parenchyma cells within the secondary xylem of our specimen (Plates III–VIII). They originate from both axial (Plates III; IV; V, 1, 2; VI, 1, 2, 3; VII, 1, 2, 4; VIII) and ray parenchyma cells (Plates V, 4, 5; VII, 3) and balloon out through axial and ray parenchyma pits into the adjacent vessels (Plates III–VIII). Tyloses with different morphologies occur in different size ranges (20–95 μm in diameter). Development of the tyloses started initially with small, bulbous protrusions (Plates IV, 4; V, 3). Intermediate stages are morphologically more or less similar to these small protrusions but larger in size (Plate V, 4, 5). Fully developed and mature are large, globose structures with undistinguishable bases (Plates III, 1, 2, 3, 4; V, 1, 2). They are frequently empty and more or less translucent (Plate IV, 5, 6). However, most of the tylosis encloses dark amorphous or granular contents (Plates VII, 4; VIII, 1). In our specimen secondary metabolites (i.e. gums, resins and tannin-like substances) are not found deposited along the adjacent parenchyma cells. Some septa-like tyloses are also present in the secondary xylem (Plate IV, 1, 2).

3.3. Pathogen

Fungal hyphae are frequently present endogenously inside the fibers, ray cells and vessels of the secondary xylem (Plates VI, IX) and are grayish yellow to brownish orange to dark brown, well-preserved, septate and relatively uniform in size, ranging from 10 to 16 μm in diameter (Plate IX). The hyphal walls are 2–3 μm thick, and the lumen 7–13 μm in width. Some hyphae possess seemingly knob-like outgrowths (Plate IX, 8). The hyphal septae are irregularly spaced, at right angles to the hyphal wall, and are commonly

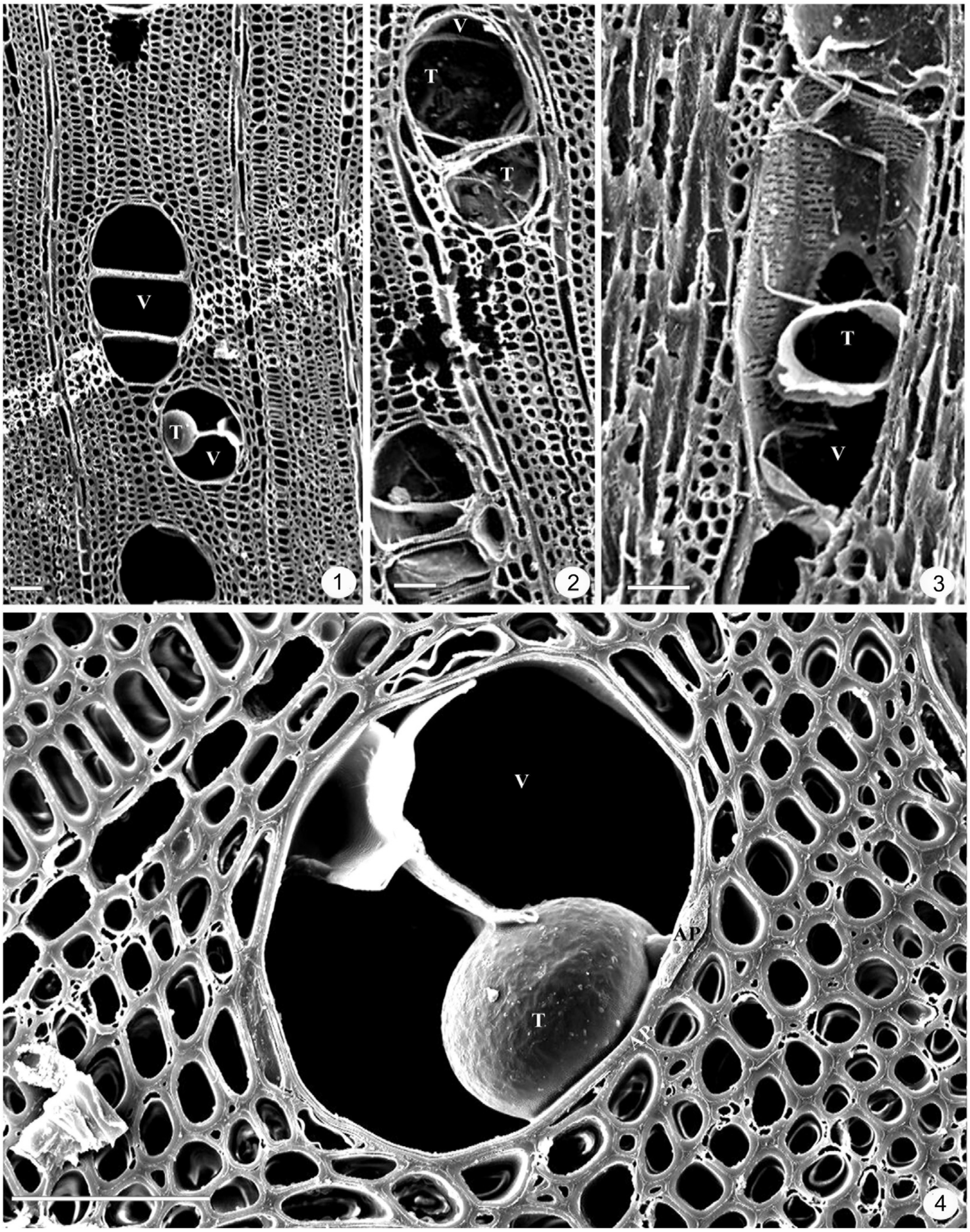

Plate VII. Scanning Electron micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1, 2) Transverse sections of the secondary xylem showing vessels with tyloses; (3) Tangential longitudinal section of the secondary xylem showing vessel with tylosis; (4) Transverse section of the secondary xylem showing vessel with a prominent tylosis (Scale Bar = 50 μm). Abbreviations: AP, Axial parenchyma cell; T, Tylosis; V, vessel.

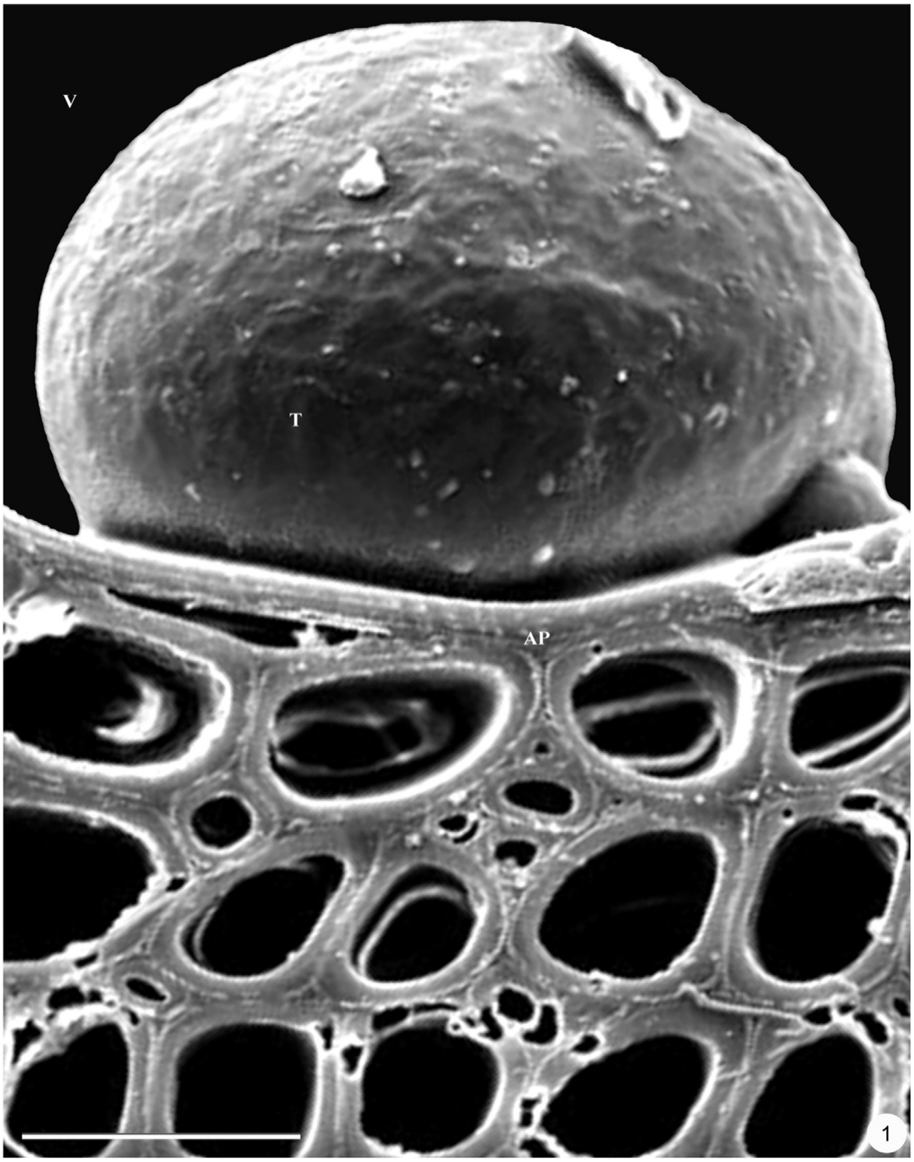

Plate VIII. Scanning Electron micrograph of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1) Transverse section of the secondary xylem showing a vessel with a prominent well-developed tylosis originating from the axial parenchyma cell. (Scale Bar = 25 μm). Abbreviations: AP, Axial parenchyma cell; T, Tylosis; V, vessel.

associated with a slight constriction of the hypha. Hyphae usually occur in a relatively straight or slightly curving course (Plate IX) and in places where fungal hyphae change direction, they may branch to form inverted Y-shaped (Plate IX, 1) and H-shaped dichotomies (Plate IX, 7). Some fungal spores are also present endogenously inside the vessels (Plates VI, 1; IX, 5) and fibers of the secondary xylem are dark brown in color, globose to oval in shape, and 15–20 × 25–30 μm in size. The systematic affinities of the fungal morphs could not be determined with certainty due to lack of significant morphological features, including those of reproductive structures.

4. Discussion

Although fossil evidence reveals that tylosis formation has commonly existed in woody plants since at least the Carboniferous Period (Scheckler and Galtier, 2003), descriptions and functional significances of tyloses have been obtained almost entirely from modern dicots (Zürcher et al., 1985). Among modern plants, tyloses are often found more frequently in infected than in healthy plants (Mehrotra, 2005). However, while massive development of tyloses limits transpiration flows, they also reduce or assist in restricting the movement of pathogenic fungi through the xylem vessels by transported propagules (Barry et al., 2001). Tyloses in susceptible plants develop more slowly than in resistant plants (Elgersma, 1973; Beckman and Talboys, 1981; Agrios 1997; Beeckman, 2016). If tyloses are formed rapidly well before the spread of fungi, they will confer resistance to the host, but if they are formed after extensive fungal colonization they will themselves be instrumental in bringing about more wilt symptoms (Elgersma, 1973; Agrios 1997). In elm (*Ulmus*) trees, rapid tylosis formation within xylem vessels is correlated with increased resistance to Dutch elm disease caused by the fungus *Ophiostoma novo-ulmi* (Elgersma, 1982). It is possible that the rapid formation of tyloses in advance of the fungi effectively seals off the vessels, limiting or retarding further spreading of wood-invading pathogenic fungi (Chrysler, 1908; Yamada, 2001). It has been also shown that once a fungal pathogen has invaded the wood, tyloses can be produced in areas of the wood that have not yet been infected (Talboys, 1964).

In the present study, a noticeable feature from our new species of Plio-Pleistocene wood, i.e. *Gmelina siwalika* sp. nov., is the occurrence

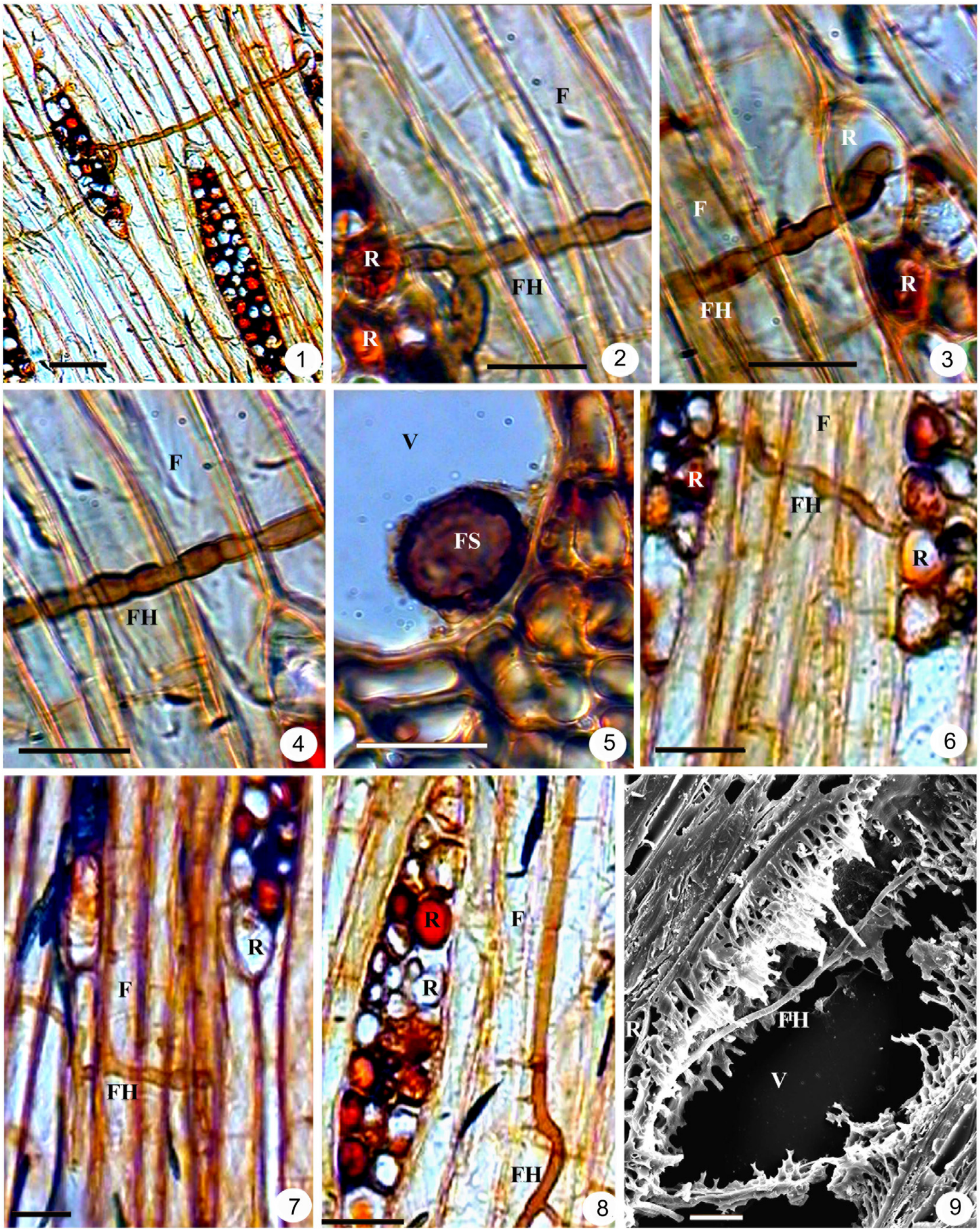

Plate IX. Light and Scanning Electron micrographs of *Gmelina siwalika* Khan, Bera M et Bera S, sp. nov. from the Kimin Formation (late Pliocene to early Pleistocene), Arunachal Pradesh, eastern Himalaya. (1–4; 6–9) Tangential longitudinal section of the secondary xylem: (1) An endogenous branched fungal hypha inside the both fibers and ray cells of the secondary xylem; (2–4)) Close-up of Fig. 1; (5) Transverse section of the secondary xylem showing a fungal spore inside the vessel; (6) Light micrograph of septate fungal hyphae penetrating into the adjacent ray cells of secondary xylem; (7) Light micrograph of a branched fungal hypha forming H-shaped dichotomies inside the fibers; (8) light Micrograph of fungal hyphae occurring inside the fibers of the secondary xylem; (9) Scanning Electron micrograph of branched fungal hyphae occurring inside the vessels of secondary xylem. Abbreviations: F, Fiber; FH, Fungal hypha; FS, Fungal spore; R, Ray cell; V, Vessel.

of a large number of prominent tyloses in the vessels within the secondary xylem. However, fungal remains, i.e. hyphae, are not extensive and their colonization within the secondary xylem is very limited suggesting that coloniozation was only at the initial stage. The presence of some fungal spores within the secondary xylem, but the absence of reproductive structures, confirms the existence of fungal infection in the *Gmelina* plant but that infection had not proceeded to the stage where reproductive structures were being produced. Therefore, it is reasonable to suggest that the interaction between the *Gmelina* tree and pathogenic fungi during Neogene time was at the very initial stage of fungal colonization and tyloses were formed rapidly well before the spread of the fungi. Because of its co-occurrence with fungal hyphae, it seems possible that abundant tylosis formation within the secondary xylem of the *Gmelina* plant was a direct response to the infection of wood-infecting fungi, and served to build up structural barriers against the advancing hyphae.

The Siwalik plant–fungal interaction contributes to a better understanding of the antagonistic relationships that existed between pathogenic fungi and angiospermic *Gmelina* plant in the Neogene (Plio-Pleistocene) paleoecosystems of Arunachal sub-Himalaya, as well as providing evidence of interactions between fungi and tyloses in Cenozoic wood. The relationship between tylosis formation and fungal

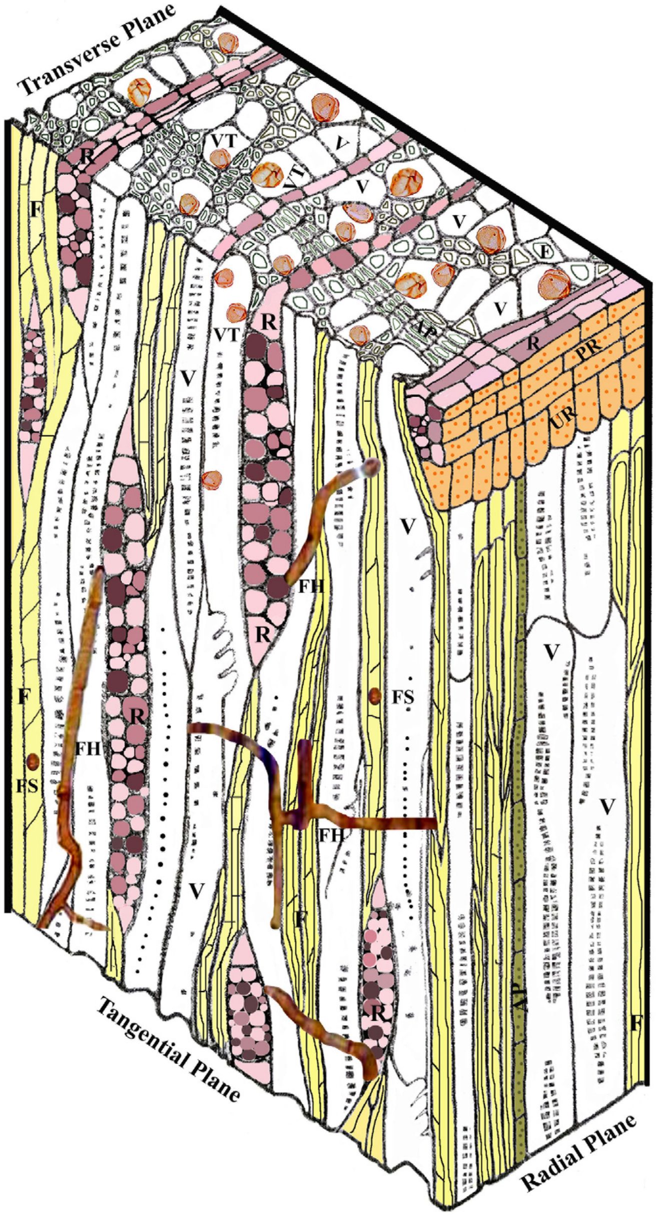

Fig. 3. Diagrammatic representation of the relationship between tylosis formation and fungal interactions in a three-dimensional block diagram of the secondary xylem presented in this study. Abbreviations: AP, Axial parenchyma cell; F, Fiber; FH, Fungal hypha; FS, Fungal spore; PR, Procumbent ray cell; R, Ray cell; UR, Upright ray cell; V, Vessel; VT, Vessel with tylosis.

interactions in a late Cenozoic angiosperm is summarized pictorially in Fig. 3. Due to the lack of evidence of any wound healing or wood traumatic events but the extensive formation of tyloses within the secondary xylem, we believe that tylosis formation was not induced by mechanical injury.

Throughout the secondary xylem there is some evidence of fungal remains penetrating into the tyloses (Plates III, 3, 4; V, 4; VI, 1, 2) but then some hyphae branch before extending into the adjacent axial parenchyma cells (Plates III, 3, 4; VI, 1). Certain fungi are known to produce enzymes that break down plant suberin (Fernando et al., 1984; Ofong and Pearce, 1994; Dickison, 2000). In this respect, the presence of short irregular branches of fungal hyphae occurring inside or in close proximity to a tylosis is of particular significance (Harper et al., 2012). This may indicate that this fungus was capable of producing such enzymes capable of breaking down the wall of the tyloses and thus surmount the resistance mechanism of the plant. Knotted fungal hyphae (Plates III, 3, 4; VI, 1, 2) within and around the tyloses suggest that the fungi have some ablity to overcome the tylosic barriers.

As a modern tropical forest element, *Gmelina* trees must have encountered a diverse suite of microorganisms, including fungi, and developed several mechanisms for resistance, including the production of abundant tyloses to deter the spread of pathogenic fungi. The aforementioned anatomical peculiarities of this woody plant might suggest that tyloses formed abundantly and quickly ahead of the pathogenic fungi, while the fungi were still in the young stage, and blocked further advance of the fungal remains. This report also demonstrates that the production of this histological protrusion in woody plants was in place in Himalayan ecosystems since at least Plio-Pleistocene time.

5. Conclusions

In many previous studies, the understanding of tylosis formation has not been the main research focus, and different approaches and methods have been used sometimes leading to contrasting opinions. The present study describes a massive development of tylosis formation within the secondary xylem of a Cenozoic angiosperm and postulates that tyloses might have been effective as a restraint to the spread of advancing fungi. Our results build upon current understanding of the relationships in paleoecosystems and the co-evolutionary processes that have developed between trees and external biotic agents through geologic time. However, further studies on fossil woods are necessary to prove the significance of our current assumptions and to show the interrelatedness of different plant biological disciplines such as ecology, physiology, pathology, and developmental anatomy over evolutionary timescales.

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