Original research article

Formation of exclusive pattern during accumulation of ligno-suberic material in cell wall of Myrtaceae root tissues including epidermis, exodermis, endodermis and polyderm

Astha Tuladhar, Shizuki Ohtsuka and Naosuke Nii

Faculty of Agriculture, Meijo University, Nagoya 468-8502, Japan
Corresponding author: N. Nii, E-mail: nii@meijo-u.ac.jp, Phone: +81-052-838-2435

Received on January 15, 2014; Accepted on June 16, 2014

Abstract: Details on localized deposition of biopolymers in cell wall have rarely been studied. Anatomical observation is important to understand tissue-specific accumulation of biopolymers and such knowledge can further clarify tissue-function. Myrtaceae roots possess alternating suberized and non-suberized cell layers known as polyderm that exist beyond the endodermis. It is important to understand firstly, where biopolymers accumulate in this tissue and secondly what mechanisms control cell wall modification. This study aims to study areas of biopolymer deposition in Myrtaceae root tissues. Root specimens were sectioned freehand or with an ultramicrotome after embedding in Technovit 7100 resin. Root sections were stained with berberine hemisulfate-aniline blue-safranin O series or just phloroglucinol and observed under a fluorescent or optical microscope.
Different biopolymers accumulated alternatively on opposite sides of the cell wall in polyderm. In non-suberized tissues, lignin accumulation was dominant and its accumulation appeared to be "closed" centripetally resembling the letter "w". In suberized tissues, it resembled the letter "m". This tissue-specific accumulation pattern was common in all five Myrtaceae species.
What factors could control and regulate such patterned and tissue-specific accumulation of biopolymers? Could this accumulation pattern itself be a contributing factor to its protective role? Discovery of this pattern, specific to Myrtaceae root polyderm, triggers more investigations on the effect of biotic and abiotic stress on biopolymer accumulation in it.

Keywords: endodermis-like cells, epidermis, exodermis, polyderm, thick-walled cells

Introduction

In higher plants, the cell wall is the first compartment to be affected by stress signal (Komatsu et al. 2010, Salvador et al. 2013). Lignin and suberin that accumulate in the plant cell wall play an important protective role against fungal diseases and insect attacks (Moerschbacher et al. 1900, Nelson and Wilhelm 1957, Williamson 1984). So far lignin and suberin accumulation has been observed in the bark (Hawkins and Boudet 1996), the fruit (Gong et al. 2013), the leaf (Poovaiah 1974) and roots (Steudle and Peterson 1998) of plants.

Lignin is a polymer of phenol propane units that accumulates within the cellulose strands and toughens the cell wall (Cutter 1970). Lignification has been described as the most important encrusting process in higher plants along with other substances like suberin, tannins etc (Fahn 1982). It has been reported that lignin content in root tissues is affected during acclimation (Soukup et al. 2004) and by environmental stress (Nii et al. 2004, Pan et al. 2006, Song et al. 2011). Under drought stress, *phi*-thickening, a form of lignin accumulation, developed markedly in cortex cells of loquat roots (Nii et al. 2004, Pan et al. 2006). Similarly, in cortex of red bayberry roots, crescent-shaped lignin accumulation increased under drought stress conditions (Song et al. 2011). Suberin, on the other hand, is a polymer with poly (aromatic) and poly (aliphatic) domains that get impregnated in the inter microfibrillar spaces of primary cell walls to form the Casparian strip (Schreiber et al. 1994, Schreiber 1996, Zeier and Schreiber 1997).

From these studies it is evident that accumulating biopolymers such as lignin and suberin in the cell wall is important for plant tissues including roots. Knowing which root tissues accumulate lignin or suberin may provide further insight to understand

Tuladhar A, Ohtsuka S, Nii N 2014 Formation of exclusive pattern during accumulation of ligno-suberic material in cell wall of Myrtaceae root tissues including epidermis, exodermis, endodermis and polyderm. Plant Root 8:24-32. doi:10.3117/plantroot.8.24

their physiological role in plant protection.

Myrtaceae is one of the few plant families (other than Hypericaceae, Onagraceae and Rosaceae) that develop a special type of protective tissue called polyderm in its roots that consist of alternating suberized and non-suberized cell layers (Esau 1970, Fahn, 1982). In our previous paper (Nii et al. 2012) we explained how polyderm layers form due to cell division of the pericycle in feijoa (Myrtaceae) roots. One layer of pericycle divided into three layers. Lignin accumulated in the outermost layer and we called that layer thick-walled cell layer. Suberin accumulated in the middle layer forming the Casparian strip so we called that layer endodermis-like cell layer and the innermost layer retained the pericyclic characters that helped it divide further into a new set of three layers. During this observation, our attention was drawn to biopolymers that accumulated in polyderm tissues.

In this investigation we attempted to locate the accumulation of such phenolic substances (mainly lignin and suberin) in various root tissues such as 1) epidermis (ep), 2) exodermis (ex), 3) cortex (co), 4) endodermis (en), 5) endodermis-like cells (el), and 6) thick-walled cells (twc) in five species belonging to Myrtaceae family with special focus on feijoa. Since chemical analysis of these substances was not conducted, we have called it ligno-suberic material as phrased by Roppolo et al. (2011).

Feijoa sellowiana is a subtropical species of Myrtaceae family native to South Brazil with secondary dispersion in Uruguay (Thorp and Bieleski 2002). As a newly rising species with ornamental and medicinal properties that also bear edible fruits, feijoa shows great potential in foods, drugs and cosmetics (Hardy and Michael 1970, Kolesnik et al. 1991). In contrast to other plant structures (O' Brien 1994, Canhoto et al. 1996, O' Brien et al. 1996, Kitayama et al.1997, Zhang et al. 2009, Zhang et al. 2011) anatomical studies on root structures of Myrtaceae are very limited, especially those concerning biopolymer deposition at the polyderm.

Materials and Methods

Five species of Myrtaceae family namely: 1) rose apple (*Syzygium jambos)*, 2) strawberry guava (*Psidium cattleianum* Sabine), 3) wax apple (*Syzygium samarangense*), 4) guava (*Psidium Guavaja*), 5) feijoa (*Feijoa sellowiana*) were used for the present investigation. Three-year-old trees were grown in baskets (diameter and depth: 300 mm each, stitch size: 30 mm) covered with non-woven fabric and filled with sandy soil in a greenhouse from spring to summer of 2013 under normal conditions. The plants were watered when the upper surface of the soil became dry. To collect roots, plants were removed from the baskets. New white roots were collected from the root tip to the basal zone. Hand-sections of roots were prepared with a new stainless steel razor blade. Serial transverse sections, commencing at approximately 5 mm from the root tip toward the basal portion of the root, were cut at 10 mm intervals to examine changes in the structural development as roots matured.

Root tissues were examined under fluorescent microscopy (excitation wavelength 365nm) staining with or without several different solutions, including a series of berberine hemisulfate-aniline blue (Brundrett et al. 1988), or phloroglucinol-HCL for lignin (Jensen 1962). Lux et al. (2005) reported a novel method for simultaneously clearing and staining tissue in which the fluorochrome 0.1% berberine hemisulfate was dissolved in a clearing mixture consisting of pure lactic acid saturated with chloral hydrate. We used this method and incubated the root sections in 0.1% berberine hemisulfate solution at 40°C for 30 minutes. The sections were then counterstained with 0.5% aniline blue (dissolved in H_2O) at room temperature for 30 minutes followed by saffranin O for 1 minute. All specimens were then placed on a drop of H_2O on a slide glass and observed under ultraviolet light using fluorescent microscopy.

Sections of the same root samples used for hand sectioning were fixed in 3 % glutaraldehyde (TAAB Laboratories, England, UK) in 0.1 M cacodylate buffer (Sigma Aldrich, Japan, pH 7.2) and stored at 4°C to examine the anatomical features of cell development. The samples were then dehydrated through a graded ethanol series and embedded in Technovit 7100 resin (Heraeus Kulzer GmbH, Wehrheim, Germany). Semi-ultra thin sections (1.5 µm) were cut using a glass knife then stained with methylene blue for histological examination under a BX 60 optical microscope (Olympus, Tokyo, Japan).

Although root age can be determined by surface color (i.e., white roots are young and brown roots are old) and by the distance from the root tip, we considered the degree of xylem differentiation, i.e., the number of xylem vessels per pole as well. When estimating root maturity, taking xylem differentiation into account is considered more accurate than root color or distance from the root tip. For observation, more than twenty roots were sectioned per species but a representative root was selected for report since the basic anatomical features were similar among all root samples. A detailed study of feijoa roots was conducted then similar traits were examined in other four species of Myrtaceae.

Results

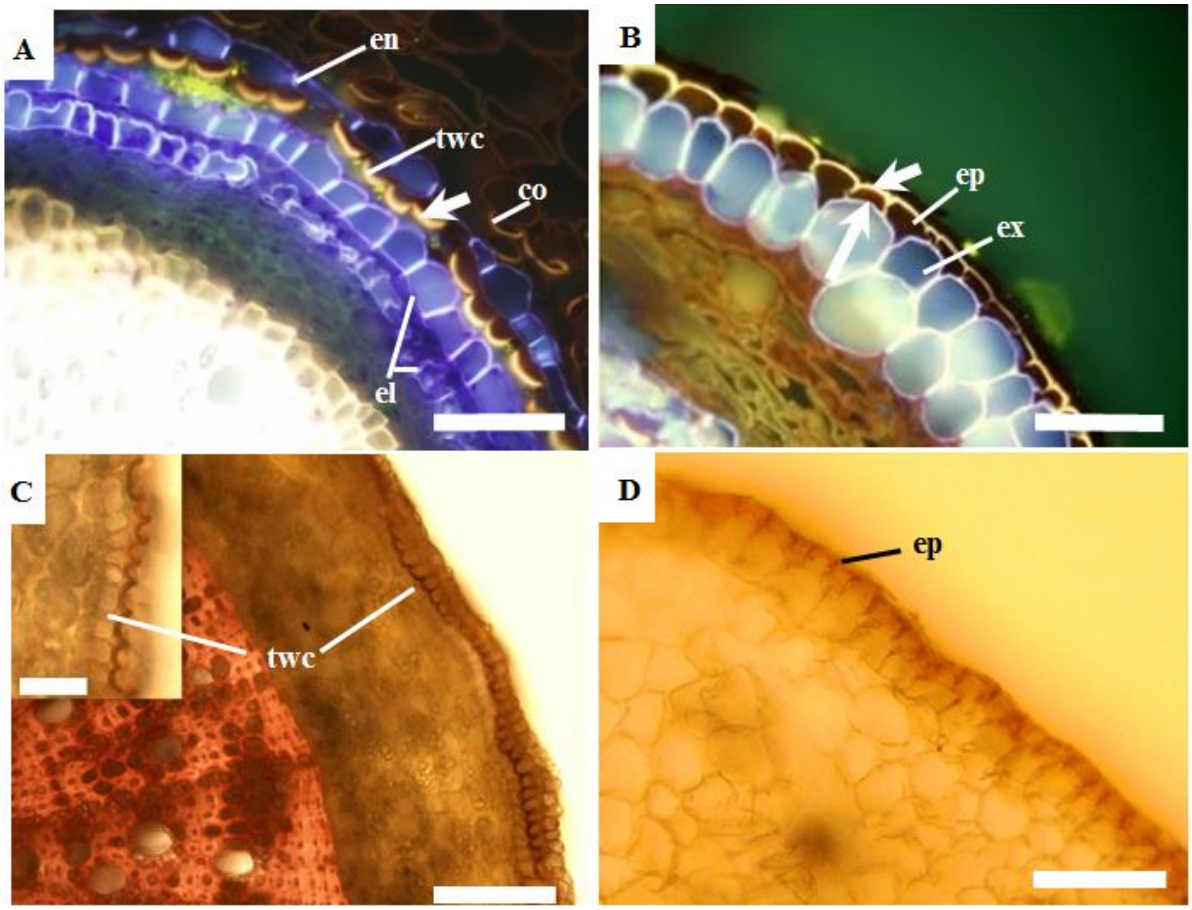

Fig. 1. Difference in lignin accumulating area in the cell wall of root tissues of feijoa roots. Panels A and B show berberine hemisulfate-aniline blue-safranin O stained root samples in which lignin encrusted tissues turned reddish orange under the fluorescent microscope. Panels C and D show phloroglucinol stained root samples in which lignin encrusted tissues turned pinkish-red when observed under optical microscope. In thick-walled cells (twc in Panel A and C) lignin accumulation appeared w-shaped and "closed" centripetally. At the epidermis, (small arrow in Panel B and ep in Panel D) it was m-shaped and "open" centripetally. ep: epidermis; ex: exodermis; en: endodermis; co: cortex; twc: thick-walled cell; el: endodermis-like cell, small arrow: lignin accumulation, large arrow: suberin accumulation. Scale bars = 50 µm (Panels A, B and enlarged section within Panel C), 100 µm (Panels C, D).

Firstly, three root tissues namely the epidermis (Fig. 1B, ep), the cortex cells surrounding the endodermis (Fig. 1A, co) and the thick-walled cells existing beyond the endodermis (Fig. 1A, twc) were examined with or without staining. The term 'thick-walled cell' is used because the cell wall of this cell gets impregnated with phenolic substances on one side.

When stained with berberine hemisulfate - aniline blue - safranin O and observed under a fluorescent microscope, the epidermis (Fig. 1B, ep) and thick-walled cells (Fig. 1A, twc) turned reddish orange color. When stained with phloroglucinol and observed under an optical microscope, the epidermis cells (Fig. 1D, ep) and the thick-walled cells (Fig. 1C, twc) turned pinkish- red showing that lignin was present in these tissues. When unstained root samples were observed under fluorescent microscope, the epidermis (e.g. Fig. 4F, ep) and the thick-walled cells (e.g. Fig. 4D, twc) emitted the bluish-white light indicating that phenolic substances other than lignin could be present in these tissues.

A different accumulation pattern was noted at the epidermis and the thick-walled cells (Figs. 1A and 1B, small arrows). At the epidermis, the lignin accumulation appeared to be "open" centripetally resembling the letter "m" (Figs. 1B and 1D, ep) whereas at the thick-walled cells it appeared to be "closed" centripetally resembling the letter "w" (Figs. 1A and 1C, twc).

When root samples embedded in Technovit 7100 resin were stained with methylene blue and observed under an optical microscope, accumulation of phenolic substances was observed on only one side of the cell wall "closed" centripetally to the central stele (Figs. 2A and 2B, arrowhead). Both cortical cells surrounding the endodermis (Fig. 2A, arrowhead) and the thick-walled cells (Fig. 2B, arrowhead) accumulated, mainly lignin. The thick-walled cells formed at

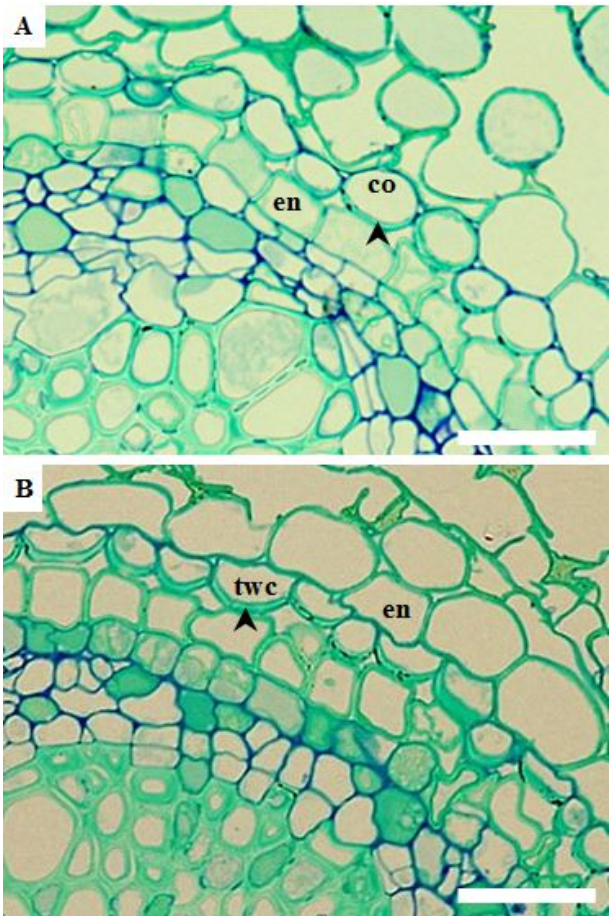

Fig. 2. Observation of accumulation of biopolymers, mostly lignin, in feijoa root sample embedded in Technovit 7100 resin after staining with methylene blue. Cross sections (1.5 µm) were examined under optical microscope. Arrowheads show swelling caused by lignin accumulation in cortex in panel A and in thick-walled cells in panel B. en: endodermis; co: cortex; twc: thick-walled cell. Scale bars = 50 µm.

two areas, initially between the original endodermis and endodermis-like cell layer (Fig. 3A, twc) and successively between every endodermis-like cell layer at the polyderm (Figs. 3B and 3C, twc). It must be highlighted here that the term 'endodermis-like cell' layer is used because it forms beyond the original endodermis and bears the Casparian strip, with suberized cell walls resembling the endodermis. The formation of endodermis-like cell layers is a result of secondary growth of roots.

Secondly, other three root tissues namely the exodermis, the endodermis and the endodermis-like cells were examined. When root samples stained with berberine hemisulfate - aniline blue - safranin O were observed under a fluorescent microscope, the exodermis (Fig. 1B, ex), the endodermis (Fig. 1A, en) and the endodermis-like cells (Fig. 1A, el) existing beyond the endodermis appeared bluish- white. When unstained root samples were observed under fluores-

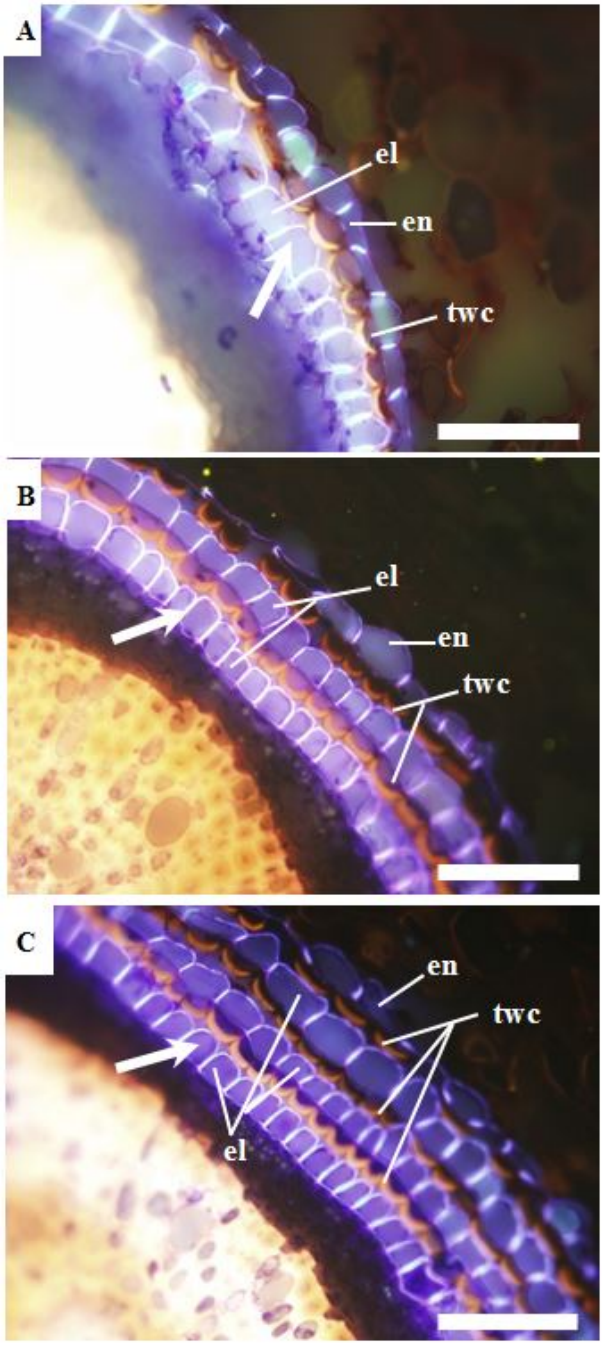

Fig. 3. Increase in polyderm layers beyond the endodermis in cross sections of feijoa roots. Panel A shows lignified thick-walled cells formation between the original endodermis and endodermis-like cell. Panels B and C show lignified thick-walled cells formation between every suberized endodermis-like cell layer with Casparian strip. en: endodermis; el: endodermis-like cell; twc: thick-walled cell. The large arrow shows suberin accumulation which is m-shaped and "open" centripetally. Distance from the root tip is 80 mm (Panel A), 100 mm (Panel B), 120 mm (Panel C). Scale bars = 50 µm.

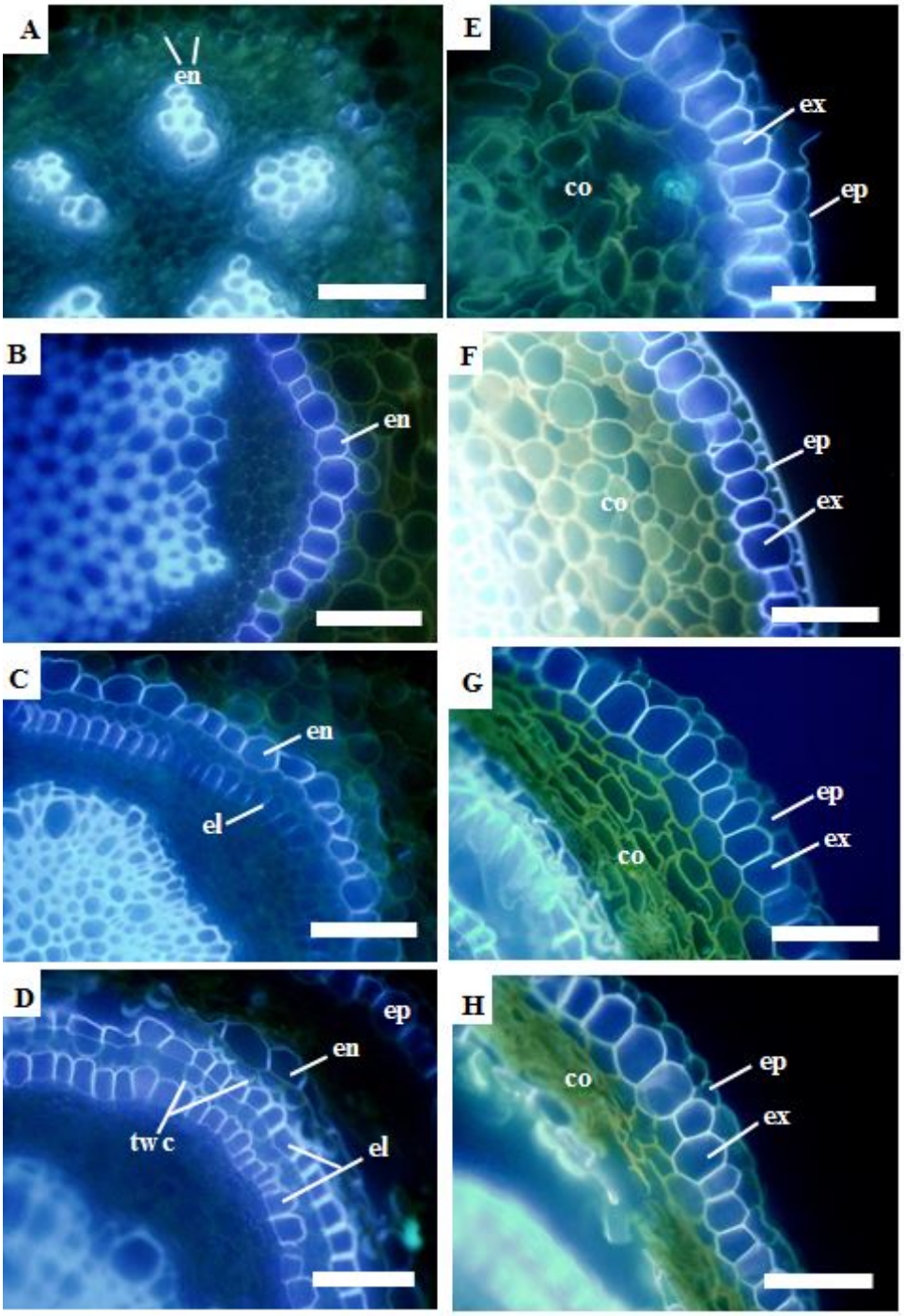

Fig. 4. Suberin accumulation in feijoa root tissues according to distance from the root tip. Unstained cross sections were observed under fluorescent microscope. Distance from the root tip is 10 mm (Panels A, E), 40 mm (Panels B, F), 60 mm (Panels C, G), 80 mm (Panels D, H). Left panels show suberization with Casparian strip at endodermis (en) and endodermis-like cells (el). Right panels show suberization with Casparian strip at exodermis (ex). The bluish white autofluorescence in lignin encrusted tissues such as the epidermis (ep) and the thick-walled cells (twc) indicating that these tissues accumulate substances other than lignin. The compression in cortex (co) layers can be observed in Panels E-H. Scale bars = 50 µm.

cent microscope, these tissues still emitted the same bluish-white color (Fig. 4) implying phenolic substances most likely to be suberin accumulated in these tissues.

Suberization and the Casparian strip formation in the endodermis and endodermis-like cells occurred as roots matured. In younger roots with about 8-10 vessels at each xylem pole, suberin-like accumulation was observed on the entire cell wall at the exodermis (Fig. 4E, ex) whereas at the endodermis, Casparian strips appeared to be small line-like (Fig. 4A, en) initially then biopolymers, including suberin, accumulated in the entire cell wall of all endodermis cells (Fig. 4B, en). In the endodermis-like layers, suberic substances accumulated on only one side of the cell wall opposite to the stele appearing to be "open" centripetally resembling the letter "m" (Fig. 3C, large arrow; Fig. 4D).

In tissues closer to the soil surface, such as epidermis and exodermis, accumulation of biopolymers were aligned (Fig. 1B, small arrow and large arrow). In other words, both appeared to be "open" centripetally resembling the letter "m". In tissues beyond the endodermis, biopolymers mostly suberin, accumulation in the endodermis-like cells appeared to be "open" centripetally resembling the letter "m" whereas accumulation in the thick-walled cells appeared to be "closed" centripetally resembling the letter "w." (Figs. 3B and 3C, twc, el). Like feijoa, this pattern of biopolymer accumulation was common in all four species of Myrtaceae (Fig. 5).

Discussion

From the present study, a common pattern in biopolymer accumulation was detected in cross sections of all examined Myrtaceae roots (Fig. 5). Biopolymer accumulation was observed in specific area of the cell wall in specific tissues. Since chemical analysis was not conducted in this study, it was not possible to distinguish whether biopolymers accumulating in tissues were only lignin or suberin and since their chemical composition differs their physiological role may vary. Bernards (2002) highlighted that suberized tissues possess a specialized cell wall modification consisting of poly (aliphatic) and poly (phenolic) domains which are spatially distinct but coexist in suberized cells. He further mentioned that the phenolic monomer profiles for lignified and suberized tissues are different and tissue specific.

In the examined roots, we concluded that biopolymer accumulating in epidermis and thick-walled cells was mostly lignin because these tissues turned red when stained with phloroglucinol (Figs. 1C and 1D). Furthermore, in roots embedded in Technovit, noticeable "w" shaped cell wall thickenings were observed (Fig. 2).

Earlier studies on biopolymers have examined the amount of suberin and lignin accumulation in root tissues. According to Bernards (2002), in general there is enough evidence supporting the hypothesis that suberized tissues contain a significant amount of hydroxycinnamic acids and a relatively smaller amount of monolignols than expected for a lignified tissue. He summarized the results of these analyses as follows: as one moves from the stele to the epidermis of roots, there is an increasing amount of hydroxycinnamic acid in the poly (phenolic) components of the cell walls, ranging from essentially 100% monolignol in the stele (as expected for lignified walls of the vascular tissues) to a mixture of monolignol and hydroxycinnamic acid in the epidermis. However, the actual area of accumulation in the cell wall of root tissues has not gained much attention.

This anatomical study has its limitations because it is not sufficient to determine the chemical nature of substances accumulating in the polyderm. Esau (1970) has defined polyderm as a tissue composed of alternating layers of suberized and non-suberized cells. What substances accumulate in the non-suberized cells of polyderm is not mentioned. This is the layer that is termed as thick-walled cell layer in this paper, specifically because accumulation of what seems to be dominantly lignin, caused noticeable "w" shaped swelling/ thickening in its cell wall. This study points out that the actual area of the cell wall where biopolymers get accumulated may be different according to root tissues.

Biopolymers impregnate plant cell wall to establish a paracellular diffusion barrier. However, the mechanism of generating this tight junction remains unclear in molecular terms (Naseer et al. 2012). Perhaps, positioning of biopolymer in the cell wall is crucial for selective uptake of nutrients and pathogen exclusion because it could affect apoplastic pathways of water.

The present study alone is not enough to explain why ligno-suberic materials accumulate on opposite sides of the cell wall in polyderm tissues. Nevertheless, it has provided details of a distinct lay out pattern of phenolic substances deposited in polyderm, a tissue unique to roots belonging to only a small number of plant families including Myrtaceae. Although the significance of such accumulation pattern is unknown, it is clear that certain factors regulate the deposit of biopolymers in this manner and it is beneficial to the plant.

The presence of lignin in thick-walled cells between every endodermis-like cell layer and the existence of multiple layers of Casparian strips in the

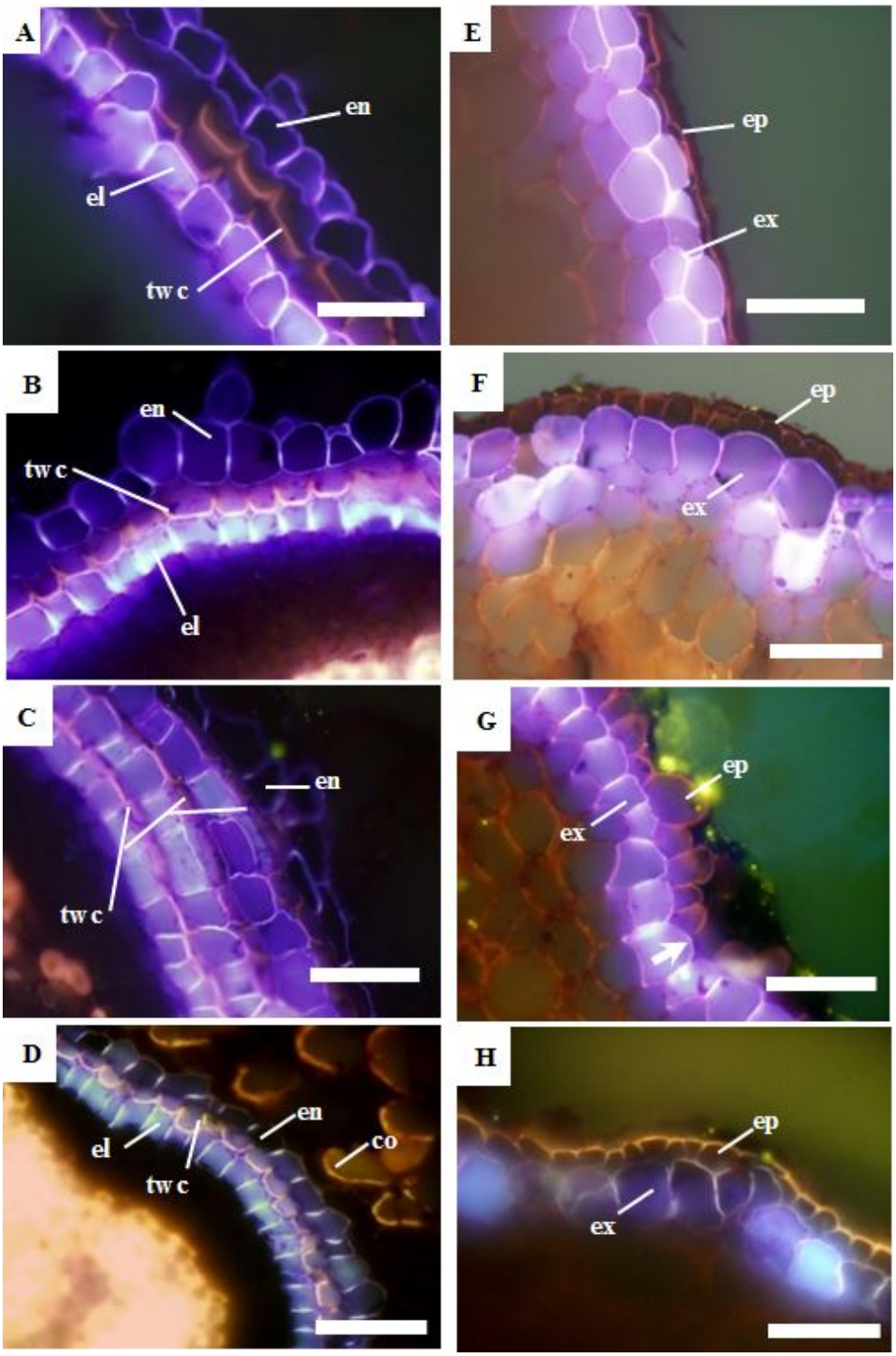

Fig. 5. Common traits of lignin and suberin accumulation found in stained cross-sections of four Myrtaceae species (A, E: *Syzygium jambos*, B, F: *Psidium cattleianum*, C, G: *Syzygium Samarangense*, D, H: *Psidium Guavaja*). Left panels (A-D) show w-shaped, lignified thick-walled cells (twc) forming between suberized endodermis-like layers (el) in the polyderm. Right panels (E-H) show m-shaped lignin and suberin accumulation in epidermis (ep) and exodermis (ex). Lignified tissues were appeared reddish orange and suberized tissues appeared bluish white when observed under fluorescent microscope. co: lignin accumulation in cortex cell wall. Scale bars = 50 µm.

endodermis-like layers, in addition to the original endodermis, could be functioning as multiple filters to enhance root function even under unfavorable growth conditions. Could the biopolymer accumulation in the polyderm be affected by stress conditions? Our previous results have shown that in wax apple (*Syzygium samarangense*) roots belonging to Myrtaceae, the number of suberized endodermis-like cell layers increased up to 5 layers and thick-walled cells with lignin increased up to 10 layers at some areas of the root circumference under drought stress conditions (unpublished data).

Lastly, this anatomical study has broadened our knowledge concerning polyderm bearing roots of Myrtaceae at cell level. It is interesting to question - what factors might limit biopolymers from accumulating in the entire cell walls of polyderm and how plants can benefit from this alternating pattern of biopolymer deposition.

References

Bernards MA 2002 Demystifying suberin. Can. J. Bot. 80: 227-240.

Brundrett MC, Enstone DE, Peterson CA 1988 A berberine-aniline blue fluorescent staining procedure for suberin, lignin and callose in plant tissue. Protoplasma 146: 133-142.

Canhoto JM, Mesquita JF, Cruz GS 1996 Ultrastructural changes in cotyledons of pineapple guava (Myrtaceae) during somatic embryogenesis. Ann. Bot. 78: 513-521.

Cutter EG 1970 Plant Anatomy: Experiment and Interpretation. Edward Arnold, London. p. 39.

Esau K 1970 Anatomy of Seed Plants 2nd Edition. John Wiley and Sons, New York. p. 185.

Fahn A 1982 Plant anatomy 4th Edition. Pergamon Press, Oxford. p. 33, 368.

Gong DQ, Zhu SJ, Gu H, Zhang LB, Hong KQ, Xie J H 2013 Disease resistance of 'Zill' and 'Keitt' mango fruit to anthracnose in relation to defence enzyme activities and the content of anti-fungal substances. J. Hort. Sci. Biotech. 88: 243-250.

Hardy PJ, Michael BJ 1970 Volatile components of feijoa fruits. Phytochem. 9: 1355-1357.

Hawkins S, Boudet A 1996 Wound-induced lignin and suberin deposition in a woody angiosperm (*Eucalyptus gunii* Hook.) histochemistry of early changes in young plants. Protoplasma 191: 96-104.

Jensen WA 1962 Botanical Histochemistry - principles and practice. University of Californnia, Berkeley. W.H. Freeman and Company, p. 205.

Kitayama K, Pattison R, Cordell S, Webb D, Dombois DM 1997 Ecological and genetic implications of foliar polymorphism in *Metrosideros polymorpha* Gaud. (Myrtaceae) in a habitat matrix on Mauna Loa, Hawaii. Ann. Bot. 80: 491-497.

Kolesnik AA, Golubev VN, Gadzhieva AA 1991 Lipids of the fruit of *Feijoa sellowiana*. Chem. Nat. Compd. 27: 404-407.

Komatsu S, Kobayashi Y, Nishizawa K, Nanjo Y, Furukawa, K 2010 Comparative proteomics analysis of differentially expressed proteins in soybean cell wall during flooding stress. Amino Acids 39: 1435-1449.

Lux A, Morita S, Abe J, Ito K 2005 An improved method for clearing and staining free-hand sections and whole-mount samples. Ann. Bot. 96: 986-996.

Moerschbacher BM, Noll U, Gorrichon L, Reisener HJ 1990 Specific inhibition of lignification breaks hypersensitive resistance of wheat to stem rust. Plant Physiol. 93: 465-470.

Naseer S, Lee Y, Lapierre C, Franke R, Nawrath C, Geldner N 2012 Casparian strip diffusion barrier in *Arabidopsis* is made of a lignin polymer without suberin. Proc. Natl. Acad. Sci. USA 109: 10101-10106.

Nelson PE, Wilhelm S 1957 Some aspects of strawberry root. Hilgardia 26: 631-642.

Nii N, Ohtsuka S, Ye L, Song Y 2012 Formation of endodermis-like cells with Casparian strip and thick wall cells derived from pericycle in the roots of *Feijoa sellowiana* (Myrtaceae). J. Japan. Soc. Hort. Sci. 81: 314-319.

Nii N, Pan CX, Ogawa Y, Cui SM 2004 Anatomical features of the cell in-growth in the cortical cells outside the endodermis and the developpment of the Casparian strip in loquat trees. J. Japan. Soc. Hort. Sci. 73: 411-414.

O'Brien SP 1994 Pistil structure and pollen tube pathways in *Leptospermum myrsinoides* and *L. continentale* (Myrtaceae). Ann. Bot. 73: 225-230.

O'Brien SP, Loveys BR, Grant WJR 1996 Ultrastructure and function of floral nectaries of *Chamelaucium uncinatum* (Myrtaceae). Ann. Bot. 78: 189-196.

Pan CX, Nakao Y, Nii N 2006 Anatomical development of *phi* thickening and the Casparian strip in loquat roots. J. Japan. Soc. Hort. Sci. 75: 445-449.

Poovaiah BW 1974 Formation of callose and lignin during leaf abscission. Amer. J. Bot. 61: 829-834.

Roppolo D, Rybel BD, Tendon VD, Pfister A, Alassimone J, Vermeer J, Yamazaki M, Stierhof Y, Beeckman T, Geldner N 2011 A novel protein family mediates Casparian strip formation in the endodermis. Nature 473: 380-383.

Salvador VH, Lima RB, Dos Santos WD, Soares AR, Bohm PA, Marchiosi R, Maria LF, Osvaldo FF 2013 Cinnamic acid increases lignin production and inhibits soybean root growth. PLoS ONE 8: e69105 10.1371/ journal.pone.0069105.

Schreiber L 1996 Chemical composition of Casparian strips isolated from *Clivia miniata* Reg. roots: Evidence for lignin. Planta 199: 596-601.

Schreiber L, Breiner H, Reiderer M, Duggelin M, Guggenheim R 1994 The Casparian strip of *Clivia miniata* Reg. roots: Isolation, fine structure and chemical nature. Bot. Acta 107: 353-361.

Song Y, Ye L, Nii N 2011 Effects of soil water availability on development of suberin lamellae in the endodermis and exodermis and on cortical cell wall thickening in red bayberry (*Myrica rubra* Sieb. et Zucc.) tree roots. Sci. Hort. 129: 554-560.

Soukup A, Mala J, Hrubcova M, Kalal J, Votrubova O, Cvikrova M 2004 Differences in anatomical structure and lignin content of roots of pedunculate oak and wild cherry-tree plantlets during acclimation. Bio. Planta. 48: 481-489.

Steudle E, Peterson CA 1998 How does water get through roots? J. Exp. Bot. 49: 775-788.

Thorp G, Bieleski R 2002 Feijoas: Origins, Cultivation and Uses, Hort Research, New Zealand, p. 87.

Williamson B 1984 Polyderm, a barrier to infection of red

raspberry buds by *Didymella applanata* and *Botrytis cinerea*. Ann. Bot. 53: 83-89.

Zeier J, Schreiber L 1997 Chemical composition of hypodermal and endodermal cell walls and xylem vessels isolated from *Clivia miniata*: Identification of biopolymers lignin and suberin. Plant Physiol. 113: 1223-1231.

Zhang M, Tang HR, Wang D, Ren SX, Liu RD 2009 A study of rooting characteristics and anatomical structure of feijoa cuttings. Agri. Jour. 4: 86-90.

Zhang M, Tang HR, Wang D, Ren SX, Fan LZ 2011 Study on growth characteristics of young feijoa trees in different propagation methods. Bot. Res. J. 4: 26-28.

Ms. Astha Tuladhar's research interest is focused on anatomical studies of fruit tree roots under different abiotic stress conditions like drought and water-logging.