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Morpho-Anatomical Investigation on the Adventitious Rooting of Hard-to-Root Excelsa Coffee (*Coffea excelsa* A. Chev.) Stem Cuttings

Jeff M. Opeña[1,*], Rachel C. Sotto[2], Bong M. Salazar[3] and Calixto M. Protacio[3]

[1]College of Agriculture, Cagayan State University-Gonzaga Campus, Flourishing, Gonzaga, Cagayan, Philippines
[2]Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna, Philippines
[3]Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines

[*]Author for correspondence: jmopena@up.edu.ph, jeffopena122994@gmail.com

Excelsa coffee (*Coffea excelsa* A. Chev.) species is known to be hard-to-root when propagated through stem cuttings. This study sought to examine the morpho-anatomical differences between Excelsa and Robusta coffee stem cuttings in order to identify any physical hindrances to rooting and to trace the origin of adventitious rooting in Excelsa coffee. Rooting of single-node orthotropic Excelsa coffee stem cuttings from water sprouts with and without incisions in the rooting zone applied with auxin plus ferulic acid takes 5 mo and 7 mo under mist, respectively. Morpho-anatomical examinations revealed that Excelsa coffee had thicker stem structures compared to Robusta coffee. However, the most probable anatomical difference why Excelsa coffee is harder to root when compared to Robusta coffee is its narrow, compact and clustered nearly continuous layer of sclerenchyma band as opposed to the discontinuous layer in Robusta coffee. The layer of sclerenchyma physically prevented root initial development which resulted in delayed rooting in Excelsa; this was observed to occur after 4–7 mo compared to Robusta coffee where rooting occurred within 1–2 mo. Restriction of rooting was a result of the physical hindrance on root initial development by the continuous sclerenchyma band rather than by preventing root protrusion or outgrowth. Basal incisions made in the rooting zone physically disrupted the layer of sclerenchyma that enabled the development of root initials which promoted more adventitious roots in the stem cuttings.

Key Words: adventitious rooting, coffee, physical barrier to rooting, sclerenchyma band

Abbreviations: crt – cortex, l – lenticel, per – periderm, pi – pith, pxy – primary xylem, sb – sclerenchyma band, spl – secondary phloem, sxy – secondary xylem, vc – vascular cambium

INTRODUCTION

Asexual propagation through stem cuttings is now commercially practiced in Robusta coffee to multiply clonal selections or cultivars. In contrast, Excelsa coffee lacks a viable clonal propagation system which poses a hindrance to its development as an alternative variety in the coffee industry. Excelsa is now gaining popularity as a specialty coffee and selection of outstanding trees is underway. However, once an outstanding selection is pinpointed, its multiplication will be a problem. This species is mostly propagated through seeds and is known to be hard-to-root when propagated by stem cuttings.

Parreño (2003) and Ramos (2015) have attempted to propagate Excelsa coffee stem cuttings with the use of rooting hormones (IBA) and commercial organic rooting/ potting mix but they observed a very low rooting success (3.33%). Moreover, it took 4 mo before any root emerged. The same difficulty was observed by other students such that they had to shift to other areas of study. A different approach to the rooting problem was clearly needed. A possible direction was suggested by the study of Protacio et al. (1999) in the rooting of stem cuttings of *Mussaenda* 'Lakambini' which is known to be a moderately hard-to-root cultivar. Dipping the cuttings in 100 ppm IBA + 250 μM dopamine solution for 30 min improved rooting. Previously, it was shown that dopamine protects naturally occurring auxins from the action of auxin

oxidase (Protacio et al. 1992; Protacio and Flores 1995) which would qualify it as an auxin synergist.

The list of auxin synergists also includes phenolic compounds which are known to have positive effects in IAA oxidation protection (Volpert et al. 1995; Krylov et al. 1995; De Klerk et al. 1999) and in synergistic action with auxin in adventitious rooting of some plant species (Bose et al.1972; James and Thurbon 1981; Biswas and Roy 1983). Some of the phenolic compounds that reportedly show the auxin synergist action include chlorogenic acid, caffeic acid, and ferulic acid (Kefeli and Kutacek 1977; Stefancic et al. 2007; Krajnc et al. 2013), catechol, gallic acid, epicatechin (Stefancic et al. 2007; Krajnc et al. 2013), sinapic acid, and phloroglucinol (Stefancic et al. 2007).

Aside from the possible hormonal imbalances that can affect rooting, the idea of a possible physical hindrance to rooting present in the stem structure of the cuttings was explored. Several studies (Ciampi and Gellini 1958; Beakbane 1961; Goodin 1965; Edwards and Thomas 1980) have reported that the poor adventitious rooting ability of the difficult-to-root woody species may be associated with the presence of pericyclic sclerenchyma layer that serves as a physical or mechanical barrier in rooting of the cuttings. Anatomical studies describing adventitious rooting in the stem cuttings of coffee species are limited; the only reports available are those on Robusta (Reaño 1940) and Arabica coffee species (Santos Jesus et al. 2010), hence this study.

This investigation focused on uncovering any anatomical explanation why Excelsa coffee is hard to root by comparing the morpho-anatomical differences between Excelsa and Robusta coffee species. The hypothesis is that there is a physical hindrance to the emergence of root initials. Furthermore, this study was conducted to trace the origin of adventitious rooting in Excelsa coffee.

MATERIALS AND METHODS

Experimental Set-up

Orthotropic watersprouts were gathered from coffee trees planted at the coffee and cacao plantations of the University of the Philippines Los Baños (UPLB). Rooting experiments were conducted employing completely randomized design in three replications. The coffee stem cuttings were allowed to root until 5–7 mo in the propagation beds with mist at the Institute of Crop Science, College of Agriculture and Food Science, UPLB, College, Laguna (latitude: N14°09'58.5"; longitude: E121° 14'22.5") from March to November 2018.

Histological Procedure

Five slides of stem cuttings were examined representing three timelines: at the start, and after 4 and 7 mo in the rooting bins. These were brought to Mt. Zion Scientific Inc. Laboratory, Holy Spirit, Quezon City, Metro Manila, Philippines for the slide preparation of the sample materials. The basal part of the coffee stems was subjected to botanical microtechnique through paraffin method (Johansen 1940) where the plant tissue was fixed and embedded in paraffin, cut thinly using a microtome, and slides were made. Viewing of the stem structures and measuring their thickness were done using the Carl Zeiss ZEN 2® (blue edition) software of the Axioscope GmbH® with the integrated AxioCam® (Axiocam ERc5s Rev. 2.0.), Carl Zeiss Microscopy 37081, Göttingen, Germany at 10x magnification and Euromex®Stereoblue Microscope, Holland at 4x magnification.

Basal Incision as a Method of Breaking the Physical Barrier in the Stem

Single-node stem cuttings without (control) and with incisions in the rooting zone used in rooting experiments were gathered during the month of March 2018 and June 2018 and were allowed to root until September 2018 and November 2018, respectively.

Stem cuttings from the orthotropic watersprouts were cut in 60–80 mm length retaining 10–20 mm above the node that has a leaf and a bud and re-cutting the basal end diagonally (sharp bevel cut). The leaves of the stem cuttings were retained and were cut to ¼ of the original length or about 4–6 cm. The depth of the basal incisions in the stem cuttings was essentially by "feel"; the downward pressure stopped when the blade touched the wood (after the blue arrow in Figure 1) where 4–5 small diagonal cuts (1 cm long) were made in the rooting zone (Opeña 2019). Before rooting, the stem cuttings were surface sterilized by a quick dip in 5 g L^{-1} Funguran OH® solution.

Robusta coffee stem cuttings were dipped in auxin powder (500 ppm IBA + 100 ppm NAA) prior to rooting while the Excelsa coffee stem cuttings were treated with 250 µM of *trans*-ferulic acid and auxin (500 ppm IBA + 100 ppm NAA). The basal end of the stem cuttings was immersed in ferulic acid solution for 30 min prior to dipping in auxin powder. The stem cuttings were allowed to root in propagation beds with mist. The mid-day temperature inside the propagation bed when the mist is on was 28–31°C. During the rooting period, the stem cuttings were sprayed with 3 g L^{-1} Grow More™ Premium Plant Food foliar fertilizer.

Rooting Parameters and Data Analysis

The number of weeks to first rooting of the coffee stem cuttings was observed from 1–7 mo and rooting success was determined using the formula: % rooting = number of cuttings rooted / total number of cuttings propagated x 100. Roots per stem cutting were counted when at least 2 mm root protrusion is visible. Root intensity rating was determined using the criteria: 0 – no adventitious root, 1 – slightly fibrous adventitious roots, 2 – moderately fibrous adventitious roots, 3 – highly fibrous adventitious roots. Rooting parameters were averaged and the standard error was determined.

RESULTS AND DISCUSSION

Morpho-anatomical Differences of Hard-to-root Excelsa Coffee and Easy-to-root Robusta Coffee Stems

Coffee stem cuttings at the time of collection and after 4 mo in the propagation beds were evaluated and compared based on stem morpho-anatomy. The anatomical structures of Excelsa and Robusta coffee stem cuttings at the time of collection and after 4 mo are presented using photomicrographs. Comparing the cross sections of Excelsa coffee to Robusta coffee through the basal part of the stem showed that newly collected Excelsa coffee stem has thicker cortex (196.631 μm thicker), thicker layer of sclerenchyma band (19.972 μm thicker), thicker phloem (26.126 μm thicker), thicker xylem (56.309 μm thicker), but has relatively thinner vascular cambium (6.232 μm thinner) compared to Robusta coffee. Meanwhile, anatomical structures of stem cuttings after 4 mo in the rooting medium revealed that Excelsa coffee had thicker stem structures than the Robusta coffee stem cutting. The following structures were thicker: cortex (by 154.362 μm), sclerenchyma band (by 15.097 μm), phloem (by 26.060 μm), vascular cambium (by 14.847 μm) and xylem (37.565 μm thicker). The layer of sclerenchyma band in the coffee stem cuttings regardless of species became thinner after 4 mo in the rooting medium (Table 1).

Anatomical findings include the differences in the sclerenchyma band formation of the two coffee species. A discontinuous thick-walled single-layer of sclerenchyma band was observed in the easy-to-root newly collected Robusta coffee while a narrow, clustered, and compact nearly-continuous one to three cell layer of sclerenchyma band was observed in newly collected Excelsa coffee species (Fig. 1). The sclerenchyma band forms a fiber-like structure and is believed to act for protection and support, hence, the clustered and compact nearly-continuous layer of sclerenchyma band which possibly prevented adventitious root initial development in hard-to-root Excelsa coffee.

Some studies have previously shown the involvement of stem structures in preventing rooting. Physical barriers that may be present in stem cuttings of woody stems can be in the form of bands of fibers or sclereids (Lovell and White 1986). Difficult-to-root apple stem cuttings showed differentiation of primary phloem into fibers and sclereids in different cultivars and the level of differentiation was correlated with the difficulty in rooting (Beakbane 1969). In difficult-to-root plant species, a continuous sclerenchyma ring between the phloem and cortex appears as the stem matures or gets older. In easy-to-root species, the said ring is discontinuous or occurs as fewer cell layers (Beakbane 1969). In Arabica coffee, the cells of sclerenchyma tissues occur in non-continuous bands around the vascular tissues (Santos Jesus et al. 2010) which are observed to be the same as in Robusta coffee. It is suspected that this anatomical feature makes the Arabica and Robusta coffee species easy-to-root when propagated asexually.

The Excelsa coffee stem cuttings are capable of rooting, however, it takes time for the root to protrude. It was observed that the development of root initials was delayed in Excelsa coffee stem cuttings without basal incisions. No roots were observed after 4 mo compared to Robusta coffee stem cuttings which have already rooted within 1–2 mo. But eventually, the Excelsa coffee stem cuttings without basal incisions rooted after 5–7 mo. Difficulty in rooting is associated with the nearly continuous layer of sclerenchyma in the cortex which

Table 1. Average stem structure thickness in Excelsa and Robusta coffee stems without basal incision at the time of collection and after 4 mo in the rooting medium.

Stem Structure	Thickness (μm) at Collection		Thickness (μm) after 4 mo	
	Excelsa	Robusta	Excelsa	Robusta
Cortex	422.676 ± 1.94	226.045 ± 1.18	365.306 ± 7.05	210.944 ± 3.11
Sclerenchyma band	46.133 ± 3.39	26.161 ± 1.80	37.335 ± 1.44	22.238 ± 2.19
Phloem	116.896 ± 2.03	90.770 ± 2.72	126.894 ± 1.44	100.834 ± 2.30
Vascular cambium	30.369 ± 1.30	36.601 ± 2.92	48.837 ± 3.76	33.990 ± 2.14
Xylem	418.087 ± 7.25	361.778 ± 0.82	226.944 ± 1.19	189.379 ± 2.64

n= 5, mean ± SE

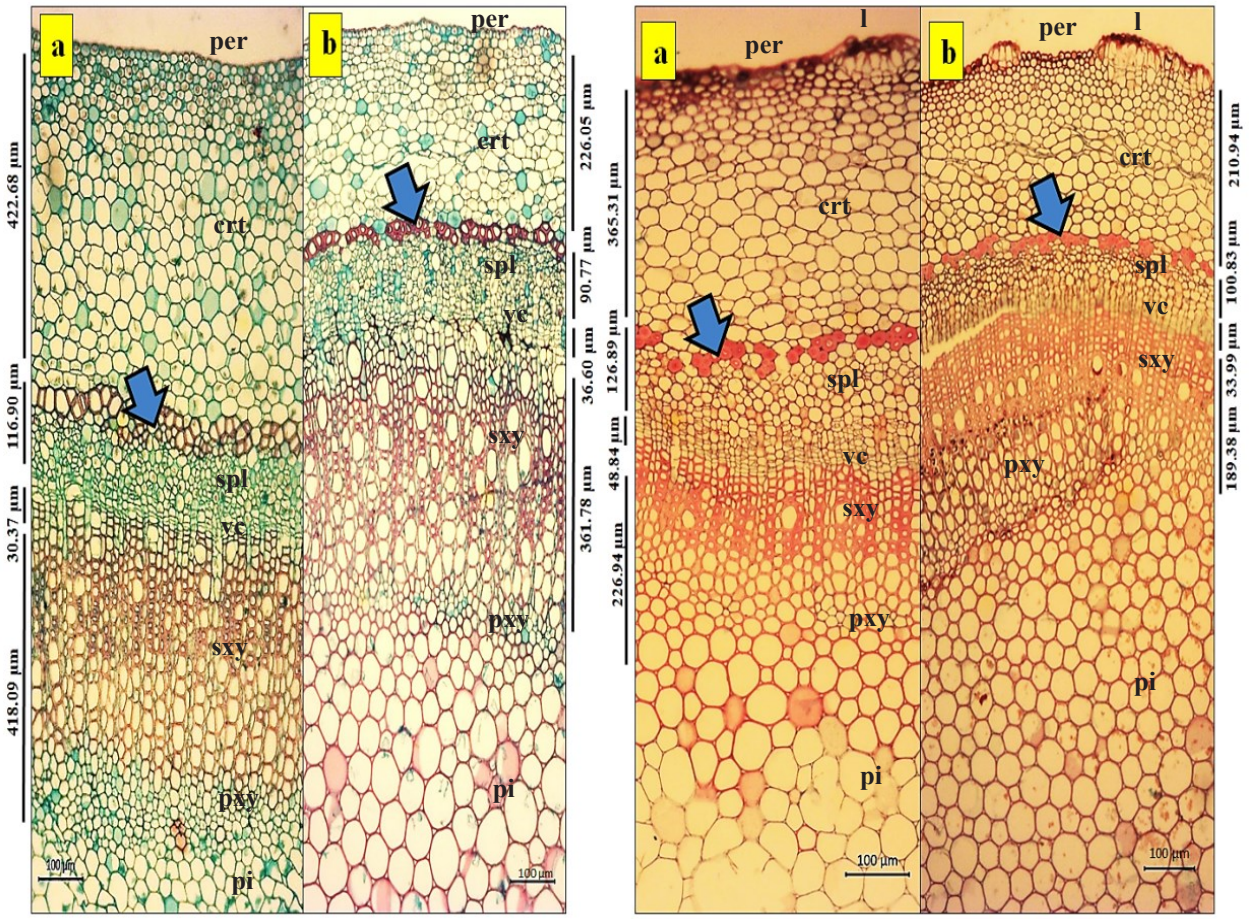

Fig. 1. A portion of the cross section of Excelsa coffee (*a*) and Robusta coffee (*b*) stem cuttings at the time collection (*left photo*) and after 4 mo in the rooting bins (*right photo*). The layer of sclerenchyma band that lies in the cortex is shown by an arrow. Stem structures: periderm (*per*), lenticel (*l*), cortex (*crt*), secondary phloem (*spl*), vascular cambium (*vc*), secondary xylem (*sxy*), primary xylem (*pxy*), pith (*pi*). The photographs were taken using Axioscope GmbH® with the integrated AxioCam (Axiocam ERc5s Rev. 2.0®), Carl Zeiss 37081, Göttingen, Germany at 10x magnification. Bar in the photomicrographs indicates a measurement of 100 µm.

acted as a physical hindrance on root initial development, resulting in the late adventitious rooting in Excelsa coffee species. This observation was also reported by Lovell and White (1986).

The continuous layer of sclerenchyma band, however, breaks down with time. Our observation is that in the stem cuttings that have been in the rooting bins after 4 mo, the layer of sclerenchyma band in Excelsa coffee becomes discontinuous (Fig. 1). The appearance of the discontinuous band is similar to that observed in Robusta coffee stem cuttings.

With prolonged rooting observation until 4 mo, the layer of the sclerenchyma band in Excelsa coffee produced gaps. When the Excelsa coffee stem cuttings were rooted for an extended period (5–7 mo), rooting occurred due to the discontinuity of the sclerenchyma band which gave way for possible root initiation. The formation of root initials in Robusta coffee did not become a problem since it has a natural discontinuity in the sclerenchyma band prior to rooting. Upon making basal incisions in the Excelsa coffee stem cuttings which created gaps in the sclerenchyma band, several roots protruded due to the breakage of the band prior to rooting.

Although the sclerenchyma band may have acted as a barrier to root initial development or could have prevented the necessary expansion and proliferation of parenchymatous cells needed for root initials to form (Amissah et al. 2008), other possibilities for the Excelsa coffee's being hard-to-root may be the lack of root initiation sites or the failure of parenchymatous cells to dedifferentiate into cells forming the root primordia (Davies and Hartmann 1988). In olive varieties, ease-of-rooting appears to be related to the ease-of-formation of

root initials rather than mechanical restriction of sclerenchyma. Moreover, hard-to-root varieties form fewer and slower developing root initials compared to easy-to-root types which relates to the ability of the cell of the root-initiating tissues to proliferate and organize root primordia (Sachs et al. 1964).

Adventitious Rooting in Excelsa Coffee Stem Cuttings

Excelsa coffee stem cuttings without basal incisions were observed until 7 mo under mist. Our study looked into the initiation and origin of adventitious root in Excelsa coffee stem cuttings and it was observed that the origin of root initial development was in the secondary xylem-vascular cambium region (Fig. 2). It makes sense that the newly emerged adventitious root has a direct attachment to the secondary xylem since it is the tissue for the transport of water and mineral elements.

Initial root development in Excelsa coffee was due to the dedifferentiation of parenchymatous cells. The dedifferentiation of the xylem parenchyma cells leads to the formation and elongation of root primordium of Excelsa coffee. As the cells proliferate and elongate, the newly-formed adventitious root protrudes through the phloem and cortex and pushes out of the periderm. At that time, the layer of sclerenchyma band has already totally broken or ruptured. Once root formation takes place, the root primordia penetrates the band discontinuities and emerges through the cortex as has also been observed by Amissah et al. (2008). The sclerenchyma band broke as the parenchyma cells grow and differentiate to form the root primordium in the course of root development. The sclerenchyma band was not seen as a physical barrier to root emergence or outgrowth.

Basal Incision as a Method of Breaking the Physical Barrier in the Stem

Basal incisions were made around the rooting zone of the stem cuttings to facilitate breaking of the hypothesized physical hindrance present in the stem prior to rooting. The technique also promoted rooting in *Ilex aquifolium* cuttings where Edwards and Thomas (1980) surmised that rooting was aided by wounding which disrupted the sclerenchyma band. In this study, basal incisions promoted more root initiation (3.25 roots per cutting) in

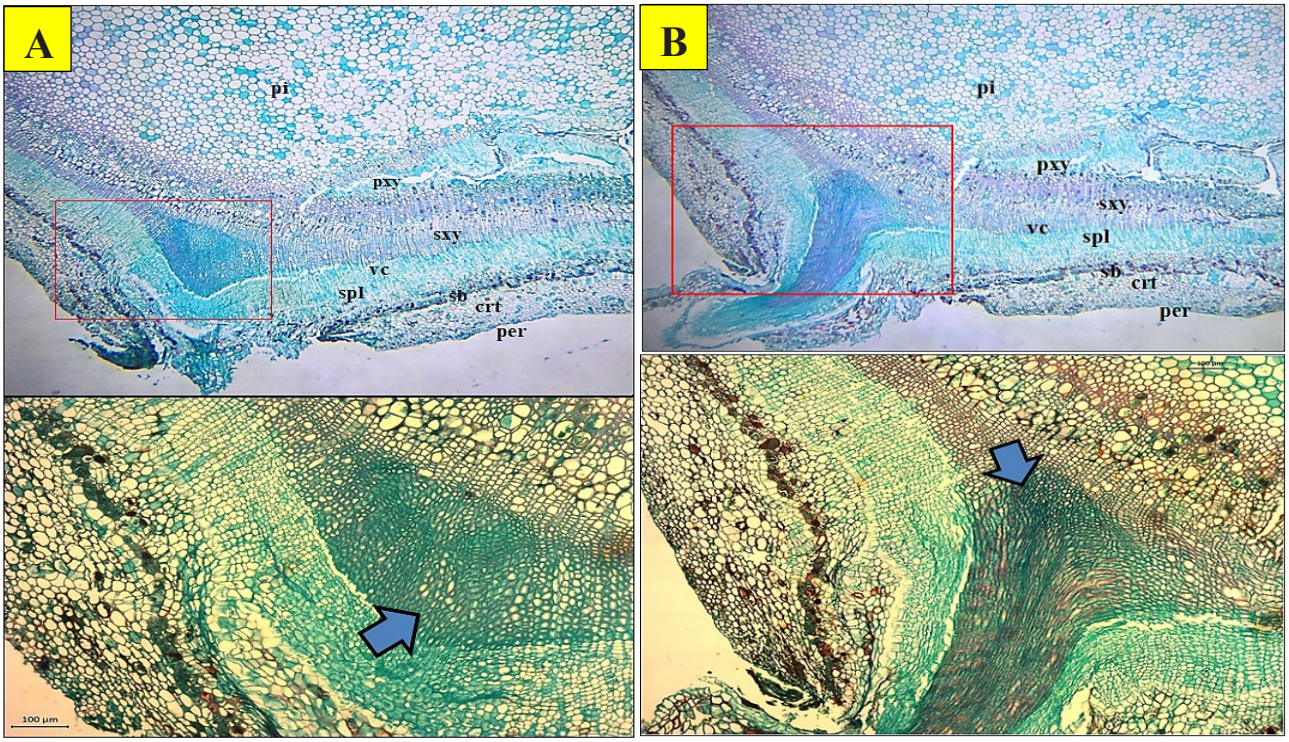

Fig. 2. The development and initiation (*A*) and protrusion (*B*) of a developed adventitious root primordia in stem cuttings of Excelsa coffee after 7 mo in the rooting bins. The origin of adventitious root in the stem cutting is shown by the arrow. The upper photos with a box were taken using Euromex®Stereoblue Microscope, Holland at 4x magnification while the lower photos with an arrow were taken using Axioscope GmbH® with the integrated AxioCam (Axiocam ERc5s Rev. 2.0®), Carl Zeiss 37081, Göttingen, Germany at 10x magnification. Stem structures: periderm (*per*), cortex (*crt*), sclerenchyma band (*sb*), secondary phloem (*spl*) vascular cambium (*vc*), xylem (*xy*), secondary xylem (*sxy*), primary xylem (*pxy*), pith (*pi*). Bar in the photomicrographs indicates a measurement of 100 µm.

Fig. 3. Rooted single-node orthotropic Excelsa coffee stem cuttings with basal incisions. Bar indicates a measurement of 10 mm.

the stem cuttings (Table 3, Fig. 3).

Anatomical investigation showed the differences in the structure of sclerenchyma band of the two coffee species. Basal incisions in Excelsa coffee prior to rooting of stem cutting create artificial discontinuities in the sclerenchyma band whereas a natural discontinuous sclerenchyma band was already present in Robusta coffee prior to rooting. Through basal incisions in the stem cuttings, faster rooting was observed in Excelsa coffee which took about 4 mo for the first rooting to occur. However, a later root initial development was noted in the stem cuttings compared to Robusta coffee which already rooted within 1–2 mo. Time of root initial development was affected by the structure of the sclerenchyma band (see Fig. 1). Although basal incisions promoted faster rooting in Excelsa coffee than in Robusta coffee, the latter species rooted earlier. Robusta coffee rooted earlier than the basal-incised Excelsa coffee because the energy utilization of the former species focused solely on the development of root initials while the energy utilization of the latter species was focused on the repair of the damaged cells caused by wounding prior to energy utilization for root initial development.

It took 7 mo for single-node orthotropic Excelsa coffee stem cuttings to root without basal incisions, where a 37.50% rooting success was observed. However, when incisions were made at the base of the stem cutting, rooting was observed after only 5 mo although rooting success was lower at 13.33% as more cuttings were still expected to root had the observation time been extended. Slightly fibrous secondary roots were observed in the Excelsa coffee stem cuttings (Tables 2 and 3). Adventitious rooting in Excelsa coffee stem cuttings occur with the application of auxin + *trans*-ferulic acid. In this study, ferulic acid acted as a crop protection agent which was reported to have antimicrobial activities (Kwon et al. 1997; Jeong et al. 2000; Ou and Kwok 2004; Borges et al. 2013).

CONCLUSION

It is possible to propagate Excelsa coffee by single-node stem cuttings with the application of auxin and *trans*-ferulic acid but rooting takes up to 6–7 mo. The difficulty

Table 2. Rooting in single-node orthotropic Excelsa and Robusta coffee stem cuttings without basal incisions after 7 mo in the rooting bins.

Rooting Treatment	Rooting Parameters			
	Weeks to First Rooting	Rooting Success (%)	No. of Roots	Root Intensity*
Control species (Robusta coffee)	>4	70.83	2.29 ± 0.21	2.53 ± 0.15
Control treatment (Excelsa coffee)	-	-	-	-
Auxin (500 ppm IBA + 100 ppm NAA)	-	-	-	-
Auxin (500 ppm IBA + 100 ppm NAA) + 250 µM *trans*-ferulic acid	>20	37.50	1.27 ± 0.24	1.27 ± 0.30

n=30, mean ±SE
*0 – no root, 1 – slightly fibrous adventitious roots, 2 – moderately fibrous adventitious roots, 3 – highly fibrous adventitious roots
(-) died
(0) alive but no rooting

Table 3. Rooting in single-node orthotropic Excelsa coffee stem cuttings with basal incisions after 5 mo in the rooting bins.

Rooting Treatment	Rooting Parameters			
	Weeks to First Rooting	Rooting Success (%)	No. of Roots	Root Intensity*
Control treatment	0	0	0	0
Auxin (500 ppm IBA + 100 ppm NAA)	0	0	0	0
Auxin (500 ppm IBA + 100 ppm NAA) + 250 µM *trans*-ferulic acid	>16	13.33	3.25 ± 0.63	1.25 ± 0.25

n=30, mean ±SE
*0 – no root, 1 – slightly fibrous adventitious roots, 2 – moderately fibrous adventitious roots, 3 – highly fibrous adventitious roots
(0) alive but no rooting

in rooting the stem cuttings was associated with the presence of narrow, clustered, compact and nearly continuous band of sclerenchyma cells three layers thick which may have mechanically prevented the development of any root initials. Adventitious rooting in Excelsa coffee was found to originate in the secondary xylem-vascular cambium region and these cells were constricted by the continuous band of sclerenchyma from multiplying and growing to form root primordia.

The sclerenchyma band was observed to break down naturally after 4 mo, forming numerous wide gaps through which root initials can grow and pass through in 5–6 mo. Basal incisions made in the rooting zone of Excelsa coffee stem cuttings at the onset of propagation resulted in visible roots at 4 mo, suggesting that root initiation occurred earlier probably as a result of artificially breaking the continuous sclerenchyma band. However, histological evidence for the artificially created gaps still needs to be established by future studies.

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