

Short Communication

Histological Observations of Adventitious Root Derived from *in vitro* Plantlet and Shoot Bud of *Boesenbergia rotunda* (Zingiberaceae)

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ABSTRACT

Boesenbergia rotunda or locally known as ‘Temu kunci’ is renowned to possess some important bioactive compounds that are promising for pharmacological applications. It is essential to obtain structural information of the inner parts of the *in vitro* roots since growing these roots undertake various anatomical and morphological changes that influence their cells activities and nutrient uptake processes. Although root functions are thought to be significant for the growth of shoot, the morphological and anatomical knowledge of adventitious root for this plant are limited. This particular study aims to investigate and compare anatomical structures of *in vitro* adventitious root derived from *in vitro* plantlet with root derived from shoot bud of *B. rotunda*. Histological sections using resin were done to study the anatomical of adventitious root of *B. rotunda*. The root samples were fixed in a Glutaraldehyde-Paraformaldehyde-Caffeine (GPC) fixative, dehydrated, infiltrated, embedded, cut, stained and mounted with Surgipath mounting medium for observations under light microscope. From the histological observations, adventitious roots of *B. rotunda* had shown the presence of all the three main tissue systems and had the same internal structures containing epidermis, exodermis, suberized sclerenchyma cells, cortex and stele. Both adventitious roots derived from *in vitro* plantlet and shoot bud showed normal growth morphologically, had anatomically same normal cell structures and arrangements.

Keywords: Adventitious root, *Boesenbergia rotunda*, histological, *in vitro*, plantlet, shoot bud

ARTICLE INFO

Article history:

Received: 26 November 2018

Accepted: 23 January 2019

Published: 30 May 2019

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INTRODUCTION

Boesenbergia rotunda locally known as 'Temu kunci' in Malaysia and Indonesia is an herbaceous monocotyledon plant belonging to Zingiberaceae family. This medicinal herb is widely distributed throughout Southeast Asia regions mainly Southern China, Sri Lanka and India. Owing to its aromatic feature, this herb has been regularly used as a condiment in various Asian dishes to stimulate appetite. Traditionally, the rhizomes of *B. rotunda* have been used to treat ailments such as muscle pain, rheumatism, febrifuge, and gout. It is also capable to treat gastrointestinal diseases including stomach ache, flatulence, peptic ulcer, carminative and dyspepsia (Tan et al., 2012). *Boesenbergia rotunda* has been recognized by its valuable bioactive compounds derived from the flavonoids and essential oils. These compounds are known to be the most abundant secondary metabolites found in this plant.

There are three main groups of flavonoids particularly flavanones, flavones and chalcones that have been found in the rhizome of this plant (Chahyadi et al., 2014), which have been reported to have potentials as antimicrobial (Atun et al., 2018; Jitvaropas et al., 2012), anti-human immunodeficiency virus 1 (anti-HIV-1) (Cheenpracha et al., 2006) and showed significant antioxidant activity (Jitvaropas et al., 2012; Tanjung et al., 2013). Panduratin A and 4-hydroxypanduratin A in the rhizome of *B. rotunda* were found to display high inhibition towards dengue-2 virus protease (Eng et al., 2012; Kiat et al., 2006).

In recent years, plant biotechnology approaches including organ, tissue and cell cultures have been widely used to produce various secondary metabolites in medicinal plants that are worth for pharmaceuticals (Baque et al., 2012). Although plant cell culture has been acknowledged to be one of the efficient culture techniques to obtain secondary metabolites, the yields are often too little for commercialization level. Roots of plants are known to be the main location for synthesis and amassing of a wide range of highly potential secondary metabolites (Mahdiah et al., 2015). The adventitious roots induced from the manipulation of suitable plant growth regulators in culture medium under optimum and specific *in vitro* environments have revealed to be efficient in producing higher and stable root biomass and secondary metabolites. This organ culture is also sensitive towards the outer inducements which allow high secondary metabolites excretion into the culture medium. All these advantages have made adventitious roots highly potential and preferable method for future *in vitro* scale up (Ahmad et al., 2015).

As a monocotyledonous crop, ginger plant does not allow plentiful of explants range for micropropagation process. Rhizome and shoot bud were often used as source of responsive explants (Lincy & Sasikumar, 2010). Adventitious roots can be simply understood as roots that emerged from unusual plant parts (non-root tissues) other than the radicle including leaves, twigs, branches underground stems and aerial stems. It can also grow from old

roots or even as branches of secondary roots from the primary root itself (Steffens & Rasmussen, 2016). The development of a single root has always been illustrated through orthodox route involving division, elongation, and finally maturation phase (Alarcón et al., 2014). Consistently, the formation of the root initials is highly influenced by parenchyma cells. These cells are usually competence to revert themselves to meristematic activity that eventually will start to divide into real root as stated by Hassan and Dodd (1989) and Srikanth et al. (2016). This work has been done particularly to observe the morphological growth and compare the cell and tissue arrangements of *in vitro* adventitious root derived from *in vitro* plantlet and shoot bud of *B. rotunda*.

MATERIALS AND METHODS

Stock Plant

For the *in vitro* plantlet, micropropagation medium of *B. rotunda* was established by Yusuf et al. (2011), which was Murashige and Skoog (MS) medium supplemented with plant growth regulators; 2.0 mg/L 6-Benzylaminopurine (BAP) and 0.5 mg/L 1-Naphthaleneacetic acid (NAA). The medium had been used to culture and continuously maintained the *in vitro* plantlets of *B. rotunda* for subsequent uses. The cultures were placed in a controlled *in vitro* environment of culture room. This included constant lighting exposure from white fluorescent cylinder tube with the light intensity of 30 - 35 $\mu\text{E m}^{-2}\text{s}^{-1}$. Adventitious roots derived from shoot bud of *B. rotunda*

were cultured onto the half strength of MS medium supplemented with combination of plant growth regulator and cytokinin; 0.5 mg/L 1-Naphthaleneacetic acid (NAA) and 0.1 mg/L Kinetin (KIN) (Azhar et al., 2018). The roots (three replicates of roots per category) used in this study were obtained from the *in vitro* plantlet and shoot bud of *B. rotunda* of four subculture cycles. The adventitious root cultures were maintained in total darkness at temperature of $25 \pm 2^\circ\text{C}$.

Microscopy

Both adventitious root samples were collected and cut into small pieces consist of longitudinal and transverse sections in 2 cm. The samples were fixed according to Jalil et al. (2008) in a Glutaraldehyde-Paraformaldehyde-Caffeine (GPC) (Sigma Chemical Co., USA) fixative (50 ml 0.2 M, pH 7.2 phosphate buffer; 20 ml 10% (v/v) paraformaldehyde; 4 ml 25% (v/v) glutaraldehyde; 1 g caffeine and distilled water to a total volume of 100 ml) for 24-48 hours at room temperature. The samples were then dehydrated at different concentrations of ethanol (EtOH); 30%, 30 min; 50%, 45 min; 70%, 45 min; 80%, 60 min; 90%, 60 min; 95%, 60 min and lastly twice for 60 min in pure EtOH. The tissues were infiltrated using Technovit 7100 resin for 24 hours at 4°C. The specimens were embedded in a mould and left for 24 hours to solidify at room temperature before sectioning. Semi-thin sections of 3.5 μm were cut using a microtome. The slides were soaked in 100% EtOH. The sections were placed in distilled water in a glass

container with black paper underneath to facilitate picking up of the sections. The sections were arranged in clean dry slide and each section was stained with 0.5% (w/v) toluidine blue stain (Sigma, USA) to check tissues. Selected sections were double-stained with 1% (w/v) periodic acid for 5 min, then rinsed four times in distilled water (pH 4.5). Then they were soaked in Schiff's reaction in the dark for 20 min and were rinsed again in distilled water (pH 4.5) four times. Lastly, the slides were stained with naphthol blue black (Sigma, USA) at 60°C for 5 min and were rinsed well with distilled water. The slides were mounted with Surgipath mounting medium and were left dried for 24 hours. Photomicrographs were taken with a Leica camera on a Leica (LEITZ DMRB) light microscope (x20/0.5; x40/0.7; and x100/1.3). Observations of longitudinal and transverse sections with magnification in a range of 200× to 800× were made to determine the presence of three main components in tissue systems; epidermis, cortex, and the vascular bundle in the adventitious root of *B. rotunda*.

RESULTS AND DISCUSSION

Based on morphological observations, we found that both adventitious roots of *B. rotunda* derived from *in vitro* plantlet and shoot bud mutually showed a similar growth pattern physically when cultured on their own specific medium formulation. Generally, the development of a single primary root can be studied by observing and defining the arrangement of its tissues through longitudinal and transverse sections

which will be viewed under the light microscope. The arrangement of tissues in plant roots commonly made up of three main tissue systems which include the epidermis, cortex and vascular cylinder (Alarcón et al., 2014). During staining procedure, it is very crucial to select the good quality of targeted tissues. Consequently, for any recalcitrant tissues, an extensive dehydration method could be carried out using 100% (v/v) butanol for three times with minimum 24 hours for each treatment to soften the tissue (Schwendiman et al., 1988). Periodic acid Schiff particularly stains polysaccharides (starch reserves and walls) while blue black naphthol particularly stains reserve protein blue-black or soluble (Fisher, 1968).

From the histological examinations, adventitious roots derived from *in vitro* plantlet and shoot bud of *B. rotunda* have showed the presence of all three main tissue systems which particularly similar in roots from monocot plants. The arrangement of tissues that form the body of the primary root can be studied through the longitudinal sections (Rost, 2011). Based on the histological examinations in Figure 1, both single segment of the adventitious roots (Figures 1a and 1b) showed physically cylindrical shaped roots. Both adventitious roots also displayed series of distinctive sequential root growth regions that consisted of root meristem, root cap, distal elongation zone and elongation zone. Root meristem and root cap could be seen clearly at the end of the distal elongation zone. This finding was supported by Jones and Dolan (2012), which had stated that a root cap was derived to protect the root apical meristem (RAM).

The RAM is important in continuously generating new cells as foundation to set up different targeted tissues in plant roots. RAM is made up of undifferentiated meristematic cells which are constantly and actively dividing to ensure continuance of root growth (Rost, 2011).

The root system of monocots which comprises of primary root is often temporary and only plays significant role at the initial stage of seedling growth while adventitious roots and seminal roots are considered more prominent. Both roots (adventitious and lateral) were capable in producing lateral roots which help in providing better support and sufficient nutrient uptakes for plants (Bell & Bryan, 2008). Based on Figure 2, there are no distinguishable differences in anatomical structures of adventitious roots derived from *in vitro* plantlet and shoot bud of *B. rotunda*. From the histological observations, there were presence of root hairs detected from both adventitious roots (Figures 2a and 2b) which indicated as good signs for root growth. Root hairs are

normally grown as side extensions from the cells in the epidermis layer. Root hairs assist to increase the surface areas of both adventitious roots which allow the root system to boost up the water and nutrients uptake from the culture media (Leitner et al., 2010).

Like any other organs in plant, root also comprises three main specific tissue arrangements that include cortex, vascular bundle and epidermis. Both of the transverse sections of adventitious roots from *B. rotunda in vitro* plantlet and shoot bud (Figures 3a and 3b) showed the presence of important root anatomic structures including epidermis, endodermis, pericycle, phloem, cortex, late metaxylem and protoxylem pole. All these specifications are crucial for root growth in *in vitro* or *ex vitro* environments. The root cortex is a region which basically acts as storages for important organic products such as carbohydrates or other materials including essential oils and tannins, latex or resins. The cortical cells at the inmost layer of cortex will undergo cells

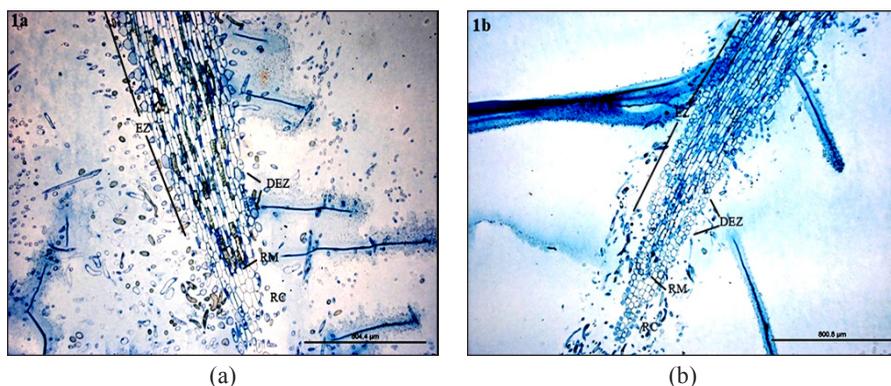


Figure 1. Longitudinal cross section of adventitious roots segments of *B. rotunda* derived from (1a) *in vitro* plantlet. (1b) shoot bud. The root sections of both (1a) and (1b) displayed the cell structures and development that include RM root meristem, EZ elongation zone, DEZ distal elongation zone, and RC root cap, Magnification: $\times 800$

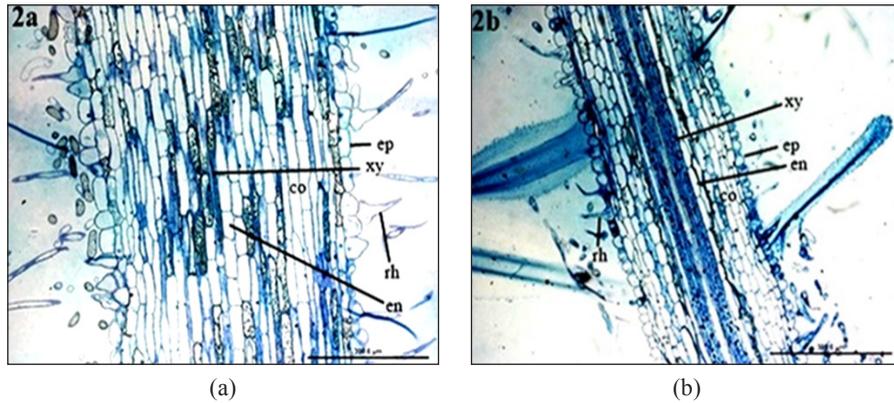


Figure 2. Longitudinal cross section of column pericycle of *B. rotunda* adventitious roots derived from (2a) *in vitro* plantlet. (2b) shoot bud. Stained cross section showed **rh** root hair, **co** cortex, **ep** epidermis, **en** endodermis, **xy** xylem. Magnification: $\times 300$

divisions to form endodermis. Endodermis is essential to ensure a one-way mode of water transport into the plant, whereas pericycle is usually where the new lateral roots initiated (Seago & Fernando, 2013).

Besides, the presences of vascular tissues were also highlighted in this histological inspection. Vascular tissues in roots specifically xylem and phloem are vital for conducting transportation of water, nourishments and yields from

photosynthesis process to all over the parts of the plant. As an addition, these tissues help plants to adjust in unfamiliar and different environments together with the ability to act as aiding tool for mechanical support to build up the plant strength for continuous root growth (Bellini et al., 2014; Nanda & Melnyk, 2018). The cell and tissues arrangements from longitudinal and transverse sections of ten replicates each in this study were consistent.

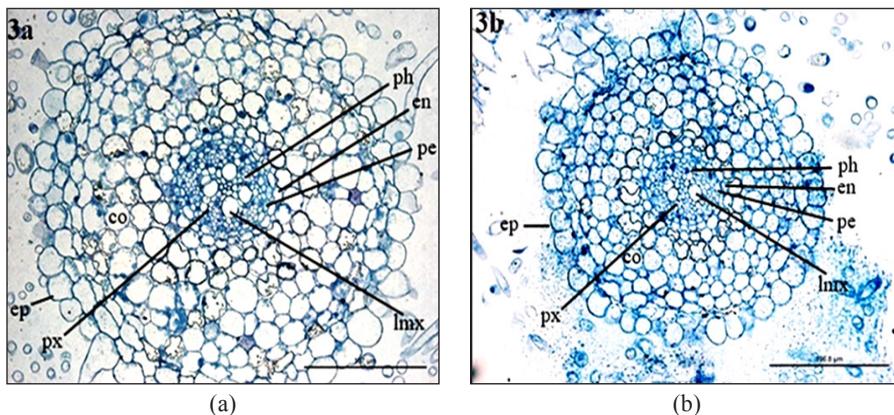


Figure 3. Transverse section of adventitious roots of *B. rotunda* segments for (3a) *in vitro* plantlet. (3b) shoot bud. Stained cross section showed, **co** cortex, **ep** epidermis, **en** endodermis, **ph** phloem, **lmx** late metaxylem, **pe** pericycle, **px** protoxylem pole. Magnification: $\times 200$

CONCLUSIONS

This gives confirmation that the adventitious roots derived directly from shoot bud explants are comparable with the adventitious roots derived from *in vitro* plantlets in their morphological growth and they have same normal cell structures and tissue arrangements. Although the growth and morphological characteristics of adventitious roots derived from shoot bud explants were seen as proficient as the roots from *in vitro* plantlets, histological analysis had to be done to further identify and clarify the root growth at cellular level. This study gives a validation that the adventitious roots of *B. rotunda* offer many interesting perspectives which can be useful for future studies.

ACKNOWLEDGEMENTS

We thanked Universiti Teknologi MARA (UiTM) for financially supporting this particular study through a research grant No. 600-IRMI/Dana KCM 5/3/GIP (090/2017) UiTM Excellence Fund.

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