

Morpho-anatomy of Adventitious Roots of Vanilla (*Vanilla planifolia* Jacks. ex Andrews) During Attachment to Support Post

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ABSTRACT

The attachment strategy of vanilla adventitious roots was characterized into three stages according to the size and the extent of adhesion, namely: (1) initial contact; (2) weak to strong attachment and; (3) strong and permanent attachment. The root tip was identified to send signals to produce profuse unicellular elongated root hairs in the velamen on the ventral side of the root facing the substrate which interacted with the surface of the substrate that brought the adhesion. Strong and irreversible attachment was observed between roots and textured substrate.

Keywords: vanilla, adventitious roots, attachment

INTRODUCTION

Vanilla (*Vanilla planifolia* Jacks. ex Andrews) is a hemi-epiphyte, succulent, perennial, tropical monocot vine that belongs to the family Orchidaceae. It is unique in the orchid family, because it is the only climbing plant, and it bears edible fruits. It has a long cylindrical stem that possesses alternate, thick, fleshy, elliptical leaves, and can reach up to a height of 10 to 15 m. Long, whitish adventitious roots are produced opposite the leaves that are used as support for the stem to firmly attach to the support tree. Adventitious roots secrete substance used for adhesion in the climbing substrate that is very effective in securing attachment of the plant to the support. The adhesion of the adventitious roots in the climbing support largely depends on the properties of sticky substance which can provide the strength for the contact.

Research on the attachment mechanism of adventitious roots are still limited and have just began in recent years unlike the extensive studies on the attachment mechanisms of other attachment organs such as tendrils and twining stems that have been done for many years. The presence of gelatinous fibers (G fibers) is responsible for the coiling of the tendrils

while in the growth of twining stem, studies showed that xylary fibers located near the xylem elements, and gelatinous fibers distributed in cortex both increased in amount and are involved in the stiffening of the stem and twining, respectively (Vaughn and Bowling, 2011). While G fibers are significant in the attachment strategy of tendrils and twining stems, Bowling and Vaughn (2009) showed that these fibers were not found in adventitious roots of some species that have been studied, e.g. *Hedera helix* and *Toxicodendron radicans*, and those lax species that are more trailing in nature, e.g. blackberries, bedstraw, and henbit. Thus, they concluded that G fibers are only associated with tissues that are twining or coiling.

In 2009, Melzer *et al.* initiated a systematic approach to analyze the functional morphology and biomechanics of the sticky adventitious roots of English ivy (*Hedera helix* L.). The presented results revealed the four-phase model attachment process: (1) initial contact; (2) chemical adhesion; (3) shape change of the root hairs; and (4) shrinking of the climbing roots, which was based on morphological analysis of adventitious roots. This can be the basis for further studies on morpho-anatomy of adventitious roots in different species of plants, specifically those that are associated

in agricultural production such as vanilla. Hence, this study aimed to investigate morpho-anatomical changes in the adventitious roots of vanilla at different stages of attachment to support structure.

MATERIALS AND METHODS

The field experiment was conducted at Jardin de Hierbas, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), College, Laguna, Philippines from December 2015 to October 2016.

Plant Propagation and Establishment

Vanilla plants were propagated using cuttings with 3 nodes. Cuttings were pre-planted in 8 x 10 cm polyethylene planting bags with garden soil. After 84 days, selected seedlings with new shoots with at least 2 nodes and developed leaves were transplanted to 20 cm x 41 cm clay pots with a mixture of sterilized garden soil and vermicompost (2.5:1 w/w) and were placed in a natural shaded area (*i.e.* under the rubber trees). Corksheet and glass were used as substrates in this experiment. Previous studies of Melzer *et al.* (2009) on adventitious roots of *Hedera helix* L. and Steinbrecher *et al.* (2011) on tendrils with adhesive pads of *Parthenocissus tricuspidata* both have shown that cork board was among the substrates that showed attachment with the adhesive structures. Thus, the cork material available as corksheets was the chosen substrate for this experiment. Glass was also chosen as substrate to clearly see the side of the root facing the substrate.

Light Microscope Analysis

Samplings were collected by carefully cutting a portion of the substrate (for cork only) with attached adventitious root using a sharp blade (Fig. 1). Careful cutting of the samples was done to ensure that the roots were still tightly attached to the substrates upon detachment from the whole plant. On the other hand, ARs were manually detached from glass. Destructive samplings were done wherein AR samples were prepared at different stages of growth and degree of attachment, *i.e.* stage 1 (initial contact), stage 2 (weak to strong attachment) and stage 3 (strong and permanent attachment).

Samples were immediately transferred to containers with fixative composed of 5% ethanol, 5% glacial acetic acid and 10% formalin. Samples were sent to Micro Biological Laboratory, Inc. (MICROLAB) for processing (plant microtechnique). Prepared slides

were viewed under an Olympus BX53 microscope equipped with an Olympus DP70 camera located at Histology Laboratory, Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute (IRRI). Fresh root samples were also prepared. Cross sectioned roots were stained with Safranin O and were observed under a light microscope using an Olympus CH5 microscope at Morphology Laboratory, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB).

Scanning Electron Microscope (SEM) Analysis

Initial sample preparations were the same as in light microscopy section. Samples were immediately transferred to containers with methanol and were sent to Electron Microscopy Service Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH) for further preparation and coating.

Prepared samples were viewed under a Scanning Electron Microscope (JEOL JSM 6010-LV) at the Micro and Nano Innovations Laboratory (miniLab), Department of Physical Science, College of Science, University of the Philippines Baguio, Governor Park Road, Baguio City.

RESULTS AND DISCUSSION

Morpho-anatomy of Vanilla Adventitious Roots

In this study, the morpho-anatomy of adventitious roots of vanilla during attachment to substrates was observed in different growth stages according to the root length and width and the extent of adhesion. Morpho-anatomically, the roots with cork and glass as substrates were the same except for a slight difference in form. The degree of attachment also varied between the roots attached to the two substrates used.

Stage 1: Initial Contact

The initiation of adventitious roots was noticeable when a bulge was already visible at the ventral side of the node facing the substrate (Fig. 2a). Only one adventitious root was initiated per node but in rare instances, two opposite roots were noted, where the main root developed first and after several days the second root was initiated. Adventitious roots appeared alternately in succeeding nodes in a plant. After 2 days, millimeter-sized, thin and cylindrical roots facing the substrate elongated in the same direction as the leaf in the same node. The attachment of the root to the

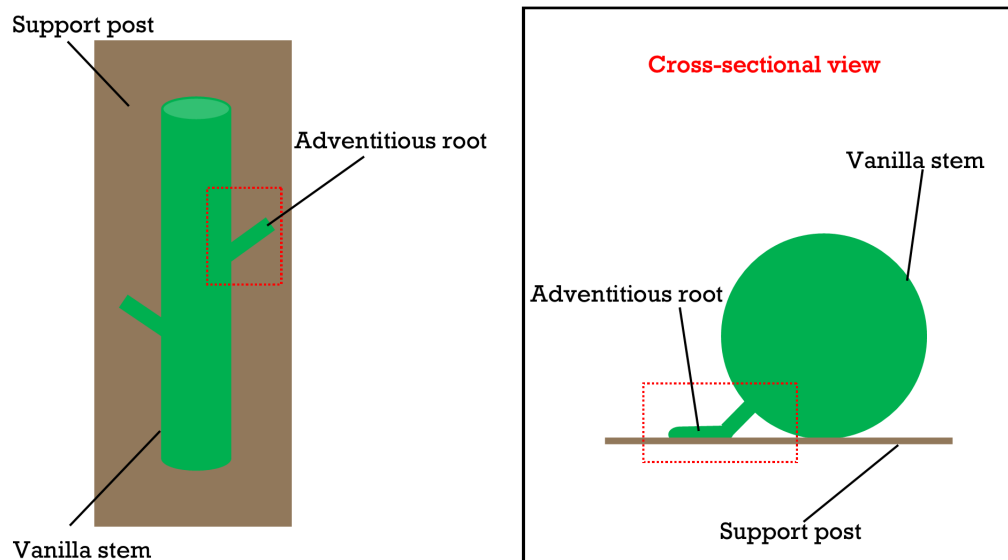


Figure 1. Schematic diagram of the method of sampling for morpho-anatomical analyses. (a) Longitudinal view and (b) cross-sectional view. Broken lines represent sampling area.

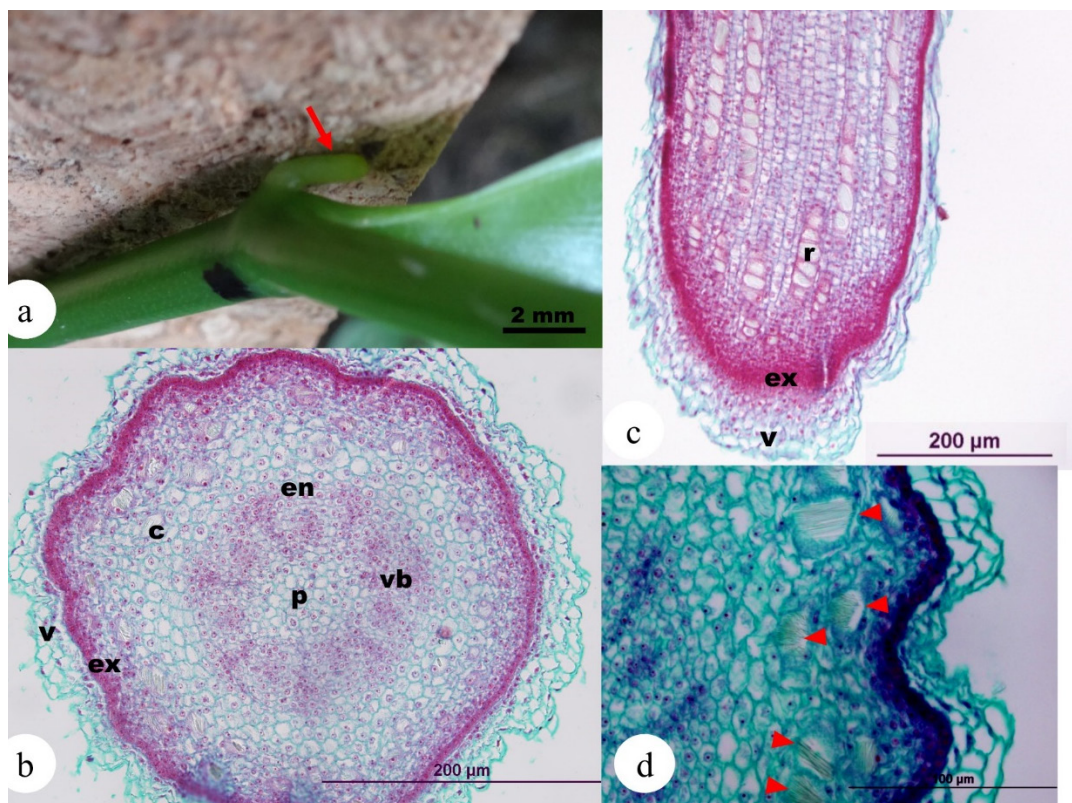


Figure 2. Morpho-anatomy of vanilla adventitious root (AR) at initiation stage. (a) Millimeter-sized AR touching the surface of the cork. (b) Cross-section of AR (200x). (c) Longitudinal section of the AR tip showing abundant raphide crystal idioblasts (100x). (d) Enlarged image of raphide crystal idioblasts in the cortex (indicated by the red arrowheads) (400x). (Legend: v-velamen; ex-exodermis; c-cortex; en-endodermis; p-pith; vb-vascular bundles; r-raphide crystals idioblasts).

substrate was observed after 5 days. The attachment was weak but perceptible since the roots were easy to detach. The attached mid-region of the roots slightly expanded, and scarce whitish root hairs were visible to the naked eye and were observed except at the tip. This finding does not agree with the early reports of Stern and Judd (1999) that the root tip was clothed with root hairs. Based on their findings, root hairs of *V. pompona* were only found on the surface of the roots facing the support post. Furthermore, this study was in agreement with the reports of Lenaghan and Zhang (2012) that the root tip is the key structure responsible for finding the contact which provided the signal to induce formation and elongation of the root hairs that were responsible for the adhesion. The cross-section and longitudinal sections of the roots at initiation stage are presented in Fig. 2b–c. Sample roots used for this stage were not yet attached to the substrates, thus expanded roots and root hairs were not observed in the following anatomical characterization. A pronounced characteristic of the Orchidaceae is having multiple epidermis i.e. velamen, which is certainly discernible in the adventitious roots of vanilla, even at early stage of growth. The velamen is composed of 3–4 strata of irregularly-shaped parenchyma cells separated from the inner cortex by highly lignified and compacted layer of exodermal cells. The cortex is composed of thin-walled parenchyma cells. Raphide

crystal idioblasts composed of calcium oxalate are prominently distributed throughout the cortex (Kausch and Horner, 1983) (Fig. 2c–d). Calcium oxalate functions include calcium regulation, plant protection, detoxification (e.g. heavy metals or oxalic acid), ion balance, tissue support/plant rigidity, and even light gathering and reflection (Franceschi and Nakata, 2005). The arrangement of vascular tissues is indistinct; although, it is assumed that the small vacuolated cells radially arranged inner to the endodermis will be developed conspicuously as the phloem and xylem cells. A pith is distinct at the center of the root composed of parenchyma cells.

Stage 2: Weak to Strong Attachment

The length and width of sample roots used at this stage ranged from 20–30 mm and 2.0–2.5 mm, respectively, after 8 days (Fig. 3a). Scanning electron micrographs (Fig. 3c–d) showed profuse root hairs distributed on the ventral side facing the substrate interacting with the surface that brought attachment except for the root tip having no root hairs and was not in contact with the surface. Anatomically, root tissues were already well developed (Fig. 3b). Distinct arrangement of tissues was observed. The velamen was composed of bistratified cells, wherein the outer layer was composed of compacted and smaller cell which was

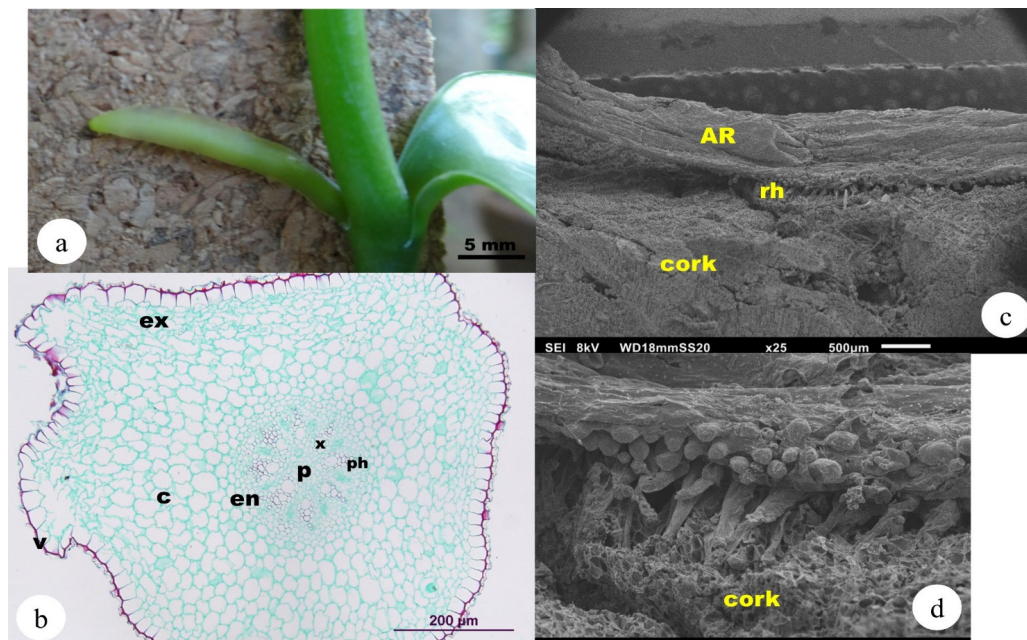


Figure 3. Morpho-anatomy of vanilla adventitious root (AR) exhibiting weak to strong attachment. (a) Flattened AR attached to cork. (b) Cross-section of AR showing well-developed tissues (100x). (c) SEM image of AR displaying the attachment between the root hairs and surface of the cork substrate (25x). (d) Enlarged SEM image of root hair-cork substrate surface contact (150x). (Legend: v-velamen; ex-exodermis; c-cortex; en-endodermis; p-pith; ph-phloem; x-xylem).



Figure 4. Morpho-anatomy of vanilla adventitious root (AR) exhibiting strong and permanent attachment. (a) Mature AR behind the glass with profuse dried root hairs (indicated by the red arrow). (b) Cross-section of permanently attached AR (100x). (c) Cross section (400x) and (d) SEM image of root hairs (150x). (Legend: v-velamen; c-cortex; vb-vascular bundles; p-pith; rh-root hairs).

separated from the compacted, cuboidal and bigger cells by thin was deposit layer. The cortex occupied the greatest area in the root. The stele is classified as eustele. Vascular bundles were arranged radially, wherein xylem cells were located inner to the phloem cell. Small and parenchymatous cells constituted the pith in the center of the root. Syncytial raphide crystal idioblasts were still present.

Stage 3: Strong and Permanent Attachment

Roots exhibiting permanent and strong attachment already achieved the optimum size after 12 days. Root length ranged from 35.0–45.0 mm while root width ranged from 3.0–4.0 mm. Morphologically, whole roots attached to cork were dark green and flattened but the roots attached to glass were slender (Fig. 4a). Profuse dried root hairs were observed both in roots with cork and glass as substrates (Fig. 4c–d). However, the degree of attachment differed between the roots attached to glass and cork, as mentioned earlier. Although root hairs were present throughout the whole growth of the roots attached to glass, weak

root-substrate attachment was observed unlike the strong and irreversible root-substrate attachment formed in the roots attached to cork. Thus, this shows that the texture of the substrate greatly influenced the ‘closure’ that formed between the roots and substrate, more specifically the bond between the root hairs and the surface of the substrate. Anatomically, cross-sections clearly showed the flattened shape of the roots wherein root hairs were located on the underside of the whole root attached to the substrate (Fig. 4c–d). Elongated unicellular root hairs originated from the outer layer stratum of the velamen. Only few raphide crystal idioblasts were found in the cortex.

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