Growth and Attachment Strategy of Vanilla (*Vanilla planifolia* Jacks. ex Andrews) Adventitious Roots in Different Types of Support Post

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ABSTRACT

The adventitious roots (ARs) of vanilla (*Vanilla planifolia* Jacks. ex Andrews) are primarily responsible for the firm anchorage and vertical growth and development of this important spice. The effects of different types of supporting structures on the growth and attachment properties of ARs were investigated. The ARs attached to natural support posts (*Gliricidia sepium* [Jacq.] Steud. and *Sphaerobambos philippinensis* [Gamble] S.Dransf.) appeared to be flattened due to the significant increase in root width. In contrast, slender ARs attached to artificial support posts (GI wire [control treatment], polyvinyl chloride (PVC) pipes, concrete and styrofoam) were observed. The appearance of long and slender ARs attached to artificial posts and flattened ARs attached to natural support posts were probably adaptive root traits to increase water retention and improve phosphorus (P) and nitrogen (N) acquisitions. The maximum increment growth in length and width (2.1 mm and 0.25 mm d⁻¹, respectively) of the ARs attached to *G. sepium* was achieved after 4 d. The attachment strength, the force that maintains the permanent attachment of ARs to the support post, was greatest when ARs were attached to *G. sepium* (935.44 \pm 514.60 mN) while the least force was exerted when ARs were attached to *S. philippinensis* (179.70 \pm 148.72 mN) which suggested that the texture of the support post was observed to primarily influence the attachment strengths of ARs.

Keywords: vanilla, adventitious roots, attachment, support post.

INTRODUCTION

Vanilla (Vanilla planifolia Jacks. ex Andrews), a climbing monocot and a member of the orchid family, is the only orchid genus that enters into commercial trade, other than those orchids prized for their ornamental value (Stern and Judd, 1999). Natural vanilla is mainly used for high-quality confectionery, for baking, and it is also increasingly popular in manufacturing ice-cream. (Augstburger et al., 2000). The need for the development of exciting products from natural and organic vanilla that will totally exploit its dietary and medicinal properties will sustain the interest and expansion of the current vanilla market. The development should be coupled with the improvement of cultural management practices in vanilla specifically geared towards sustainable production (De Guzman and Zara, 2012). Vanilla is a hemi-epiphytic orchid that in its cultivation, a tree is crucial to provide physical support, shade and organic material throughout its growing period. Vanilla uses its adventitious roots (ARs) to attach to various substrates for the maintenance of its vertical growth. The adventitious roots secrete a substance used for adhesion in the climbing substrate

that is very effective in securing attachment of the plant to the support.

In order for the adventitious roots to firmly attach to their supporting structures or substrates, there must be a strategy by which these structures are able to grow vertically. A systematic approach to analyze the functional morphology and biomechanics of the sticky adventitious roots of English ivy (Hedera helix L.) was initiated by Melzer et al. (2009). They proposed the fourphase model of attachment process of ARs, namely: (1) initial contact, (2) chemical adhesion, (3) shape change of the root hairs, and (4) shrinking of the climbing roots. Forces of attachment are generated by highly diverse structures on variable host substrates. Attachment forces vary in strength from very light to extremely strong. Root climbers typically attach very tightly to host surfaces by attachment roots and root hairs like English ivy (Rowe and Speck, 2015). The quantification of attachment strengths of attachment pads of Boston ivy (Parthenocissus tricuspidata), attachment roots of English ivy and clustered attachment roots of trumpet creeper (Campsis radicans) was done by Steinbrecher

et al. (2009) through the development of a mobile and adaptable tensile testing machine that worked under natural growth conditions as well as in the laboratory. Attachment roots of English ivy have a tensile strength of 3.4MPa per individual root and a Young's modulus (measured in tension) of 109MPa, which is relatively stiff for non-woody roots. The mean force under uniaxial tension to remove a 2-cm-long internode of English ivy with a typical root cluster from tree bark is 3.8 N. At the finest level of attachment measured, values for detachment and failure of individual roots typically range from 200-400 mN. (Melzer et al., 2010; Steinbrecher et al., 2009).

A cling strength measuring device was also developed by Gamboa (2016) that can measure the cling strength of climbing vines. He tested this device on *Syngonium* plants that have clusters of clingy adventitious roots per node attached to different host materials: *G. sepium*, bamboo, concrete, and PVC pipes. The experiment showed that *Syngonium* clings strongest in *G. sepium*, followed by concrete while it poorly clings in bamboo and PVC. The maximum forces per root measured were: 2517.14 mN in *G. sepium*, 2446.09 mN in concrete, 1902.67 mN in bamboo and 698.09 mN in PVC.

The underlying mechanism of adventitious roots' attachment of vanilla is significant in providing knowledge on the holistic roles of these structures on vertical growth and development of the plant as affected

by its immediate environment and other external factors. Hence, the study specifically aimed to quantify and compare the growth of adventitious roots of vanilla grown on different types of support post. The knowledge on the attachment strategy of vanilla can be applied in choosing the appropriate supporting structures which is essential in cultural management practices towards sustainable production of this important spice.

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. The vanilla plants were placed outdoors in a natural shaded area (*i.e.* under the rubber trees). After 84 days, selected seedlings with new shoot with at least 2 nodes and developed leaves were transplanted to 20 x 18 cm polyethylene pots containing mixture of sterilized garden soil and vermicompost (4:1 w/w). Sample plants were established using different artificial and natural support posts (Fig. 1). In each support post, one plant was established. Three replications were made and in each replicate,



Figure 1. The transplanted vanilla plants with the support posts (indicated by the red arrows): (a) GI wire (control); (b) *Gliricidia sepium* (Jacq.) Steud.; (c) *Sphaerobambos philippinensis* (Gamble) S. Dransf.; (d) PVC pipe; (e) concrete and; (f) styrofoam.

two plants were established. A space of 1 m x 1 m between and within rows in experimental plants was maintained. The list of artificial and natural support

posts used and their physical nature are shown in Table 1. Recommended cultural management for vanilla was applied throughout the growing period.

Table 1. Characteristics of natural and artificial support posts used

TYPE OF SUPPORT POST	PHYSICAL STRUCTURE
Natural	
1. Gliricidia sepium (Jacq.) Steud.	Stem with smooth bark, without thorns,
(Common name: Kakawate)	whitish to grey in color and dotted all over with lenticels (Kpikpi and Sackey, 2012).
2. Sphaerobambos philippinensis (Gamble)	Culms 4-6 m tall, 5 cm diameter; internodes
S.Dransf. (Common name: Laak)	60-70 cm long, thin-walled, smooth
	(Dransfield, 1989).
Artificial	
1. Galvanized Iron (GI) Wire (control) – to	Size: no. 10; smooth
hold the stem	
2. Polyvinyl chloride (PVC) pipes	Size: no. 2 (5 cm x 3 m); black, smooth surface; rigid; plastic
3. Concrete	Size: 10 cm x 10 cm x 2.2 m.; Rough surface;
	hard
4. Styrofoam	Smooth to rough surface; petroleum-based
-	plastic material; light weight (about 95%
	air); good insulation properties; high
	moisture resistance

Adventitious Root Growth Components

Data gathering was started after the plants have been established with at least one adventitious root attached to the support post. The position of sampled roots was noted by designating the node of the shoot growth from which it was borne (Fig. 2). Roots sampled were from nodes 6 to 11. Three consecutive roots were observed in each sample plant. Collection of data was presented in 4-day intervals. The selected ARs were consistently monitored throughout the experiment with the following parameters: length and width of ARs, elongation with time, lateral expansion with time, internode length (length between two nodes where the sample ARs were found), number of days to attain full length (days until the roots completed maximum length), number of days to attain full width (days until the roots completed maximum width), total number of attached roots (number of permanently attached roots determined from the 6 roots constantly monitored in each plant) and qualitative parameters (color and shape).

Attachment Strength of Adventitious Roots

In this test, another set of firmly attached roots was chosen in each type of support post from the same setup used for AR characterization. Two plants in each type of support post were selected for the determination of attachment strength. In each plant, 3 fully grown and permanently attached ARs were chosen for sampling. Each root tested was considered as one trial. The equipment used in this study was fabricated by Gamboa (2016) (Fig. 3) using low-cost material based on a similar study done by Steinbrecher *et al.* (2011). The calibration setting up were also taken from Gamboa (2016).

The preparation of sampled roots and actual measurements of attachment strength are detailed as follows. First, the length and width of the roots were measured. The positions of the sampled roots were at nodes 19-28. In each type of support post, 6 roots were sampled. Each root sampling was equivalent to 1 trial. Next, a string was tightly knotted around the node where the root was located. It served as the contact between the equipment and the root, the medium during the detachment process. Then, the leaf attached to the node was cut to prevent additional force exertion due to its weight. The internodes above and below the node were also cut to separate this sample portion from the whole plant. Lastly, the equipment was set to start to begin the

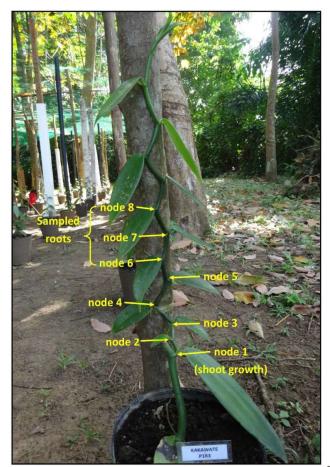


Figure 2. (left) Positions of the sampled roots during data collection.

Figure 3. (below) (a) The setup of the mechanical structure with the equipment used for the determination of attachment strength. (b) Detailed hardware components of the equipment. (c) Schematic diagram of the parts of the equipment (Gamboa, 2016).



detachment process. The weights of the detached roots were measured. The data recorded in the SD card are a series of time (milliseconds) and mass (g). The force exerted by the roots on the attachment was calculated using the formula:

$$F = mg$$

Where:

 $F = force (kg m^{-1}s^{-2} or N)$

m = mass recorded by the equipment (g)

g = standard acceleration due to gravity (9.8 m s⁻²)

Statistical Analysis

The data gathered from the growth comparison of ARs in different types of support post were analyzed using STAR, version 2.0.1 2014, Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, Laguna. Treatment mean comparison was carried out using Least Significance Difference (LSD) at 5 % level of significance. SD was calculated from the data gathered on the determination of attachment strength.

RESULTS AND DISCUSSION

Vanilla Adventitious Roots Growth in Different Types of Support Post

The length and width of adventitious roots of vanilla are presented in Fig. 4. Root length which ranged from 29.5 mm (G. sepium) to 44.4 mm (PVC pipes) was not statistically significant among the different types of support used. On the other hand, root width was significantly different among the support posts used. Roots attached to *S. philippinensis* had the highest width (3.9 mm) which was comparable to the width of the roots attached to *G. sepium* (3.5 mm). Results revealed that natural support posts greatly affected the lateral expansion of the roots as compared to those attached to artificial posts. Hanging adventitious roots in the GI wire (control) only showed slight lateral expansion (1.6 mm).

Fig. 5 shows the elongation with time of the adventitious roots in different support posts. During this time, roots hanging in GI wire, attached to PVC pipes and styrofoam showed high increment growth (8.3, 8.3 and 8.5 mm, respectively) compared to the increment growth of the roots attached to *G. sepium*, *S. philippinensis* and concrete (6.7, 7.8 and 6.8 mm, respectively). Roots were elongating for about 1.7-2.1 mm d-1. Roots continued to elongate until the 8th day. Gradual increase in the length of the roots was observed until 12 d. Moreover, roots attached to *G. sepium* and GI wire reached maximum length on the 12th day. On 16th day, slight increase in the length of the roots attached to *S. philippinensis*, PVC

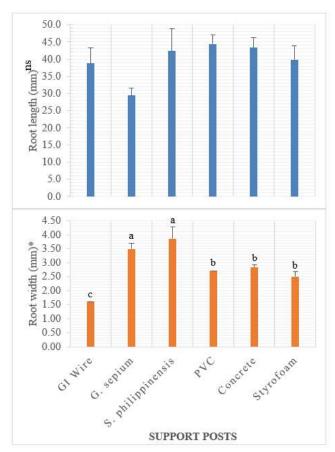


Figure 4. Length and width (mm) of adventitious roots of vanilla attached to different types of support post. Legend: ns, * - not significant & significant, respectively, using LSD at 5% level. Means with the same letter are not significantly different; error bars represent SE of the mean (n=18).

pipes, concrete and styrofoam was observed. On the succeeding days, roots in all the support posts already showed steady state growth. Although roots attached to concrete and styrofoam were noticed to have minimal increment growth, this did not affect the overall length of the roots The root length of the adventitious roots in all the support posts sharply increased after 4 d. The lateral expansion of the adventitious roots with time differed among the support posts used (Fig. 6). Similar to the elongation of the roots in all support posts, lateral expansion markedly increased after 4 d. Noticeably, roots attached to G. sepium had the highest expansion growth while the roots hanging in the GI wire and roots attached to styrofoam had the minimal expansion growth among the treatments during the 4-day interval. During this time, the maximum increase in width based on the results was 0.25 mm per day as observed in the roots attached to G. sepium. On the 8th day, roots in the control already reached optimum width while roots attached to solid support posts continued to expand. The increment growth of roots attached to S. philippinensis was also

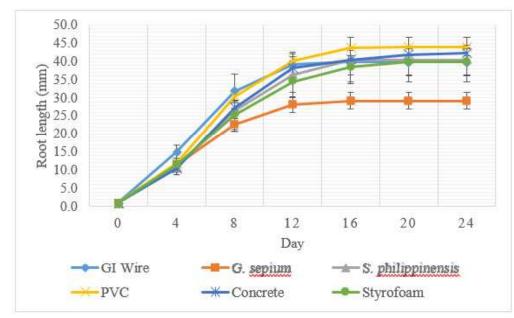


Figure 5. Elongation (mm) with time (day) of adventitious roots of vanilla attached to different types of support post. Error bars represent SE of the mean (n=18).

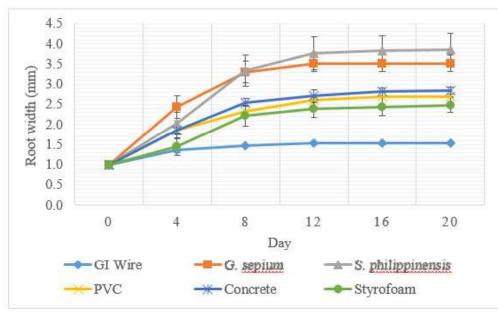


Figure 6. Lateral expansion (mm) with time (day) of adventitious roots of vanilla attached to different types of support post. Error bars represent SE of the mean (n=18).

higher than the other treatments. This contributed to the increase root width even reaching the same width of the roots attached to *G. sepium*. After 12 d, ARs markedly reached the optimum width. The roots attached to *S. philippinensis* had the highest root width (3.9 mm) compared to the roots attached to the other support posts. An increment growth of 0.1-0.2 mm was still measurable on this day until 20th day of observation, however this probably has minimal or no effect at all on overall expansion growth.

The number of days to attain full length ranged from 14.7-18.1 d, however, this did not vary with the treatments (Fig. 7). Shorter growth duration (7.1-13.3 d) was noted for root width compared to the number of days to attain full length. Moreover, the number of days to attain full width was extended for about five

days when adventitious roots were attached to a solid support post unlike when it was just hanging as observed in the control.

The number of attached roots was counted from the roots that were constantly monitored per type of support post (Fig. 8a). Only the number of roots attached to solid support posts were analyzed since it was expected that no roots will be attached in the control, however, in some instances, it was observed that the roots in the control were capable of adhering to the stem and leaves. Fig. 8a shows that all the sampled adventitious roots were firmly attached to *G. sepium*. The highest number of adventitious roots (6) attached to *G. sepium* was comparable to the number of adventitious roots attached to other support posts except for concrete.

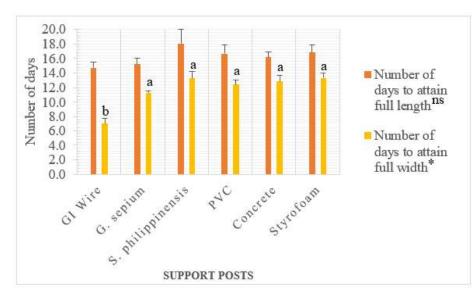


Figure 7. The number of days to attain full length and full width of vanilla adventitious roots attached to different types of support post. Legend: ns, * - not significant & significant, respectively, using LSD at 5% level. Means with the same letter are not significantly different; error bars represent SE of the mean (n=18).

The internode length was also measured in this experiment to determine if the growth of the stem in different support posts would affect the growth of the adventitious roots (Fig. 8b). Internode length, which ranged from 40.4-49.2 mm, was not affected by the treatments.

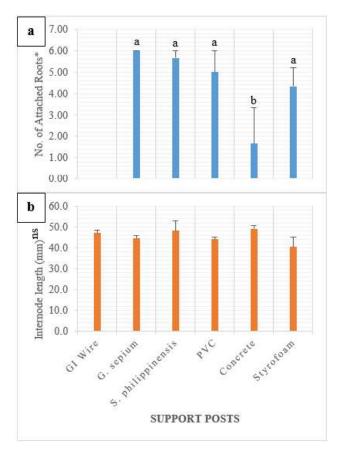


Figure 8. (a) Number of attached roots and (b) internode length of adventitious roots of vanilla attached to different support posts. Legend: ns, * - not significant & significant, respectively, using LSD at 5% level. Means with the same letter are not significantly different; error bars represent SE of the mean (n=18).

The quantitative parameters presented reflected the variations in the morphology of ARs attached to different support posts (Fig. 9). Vanilla plants without solid support posts had cylindrical and thinner ARs (Fig. 9a). ARs attached to natural support posts appeared bigger and flattened (Fig. 9b-c) than ARs attached to artificial support posts (Fig. 9d-f). Moreover, root hairs developed on the side of the ARs where attachment occurred whereas ARs in the GI wire had no root hairs.

At different stages of growth, changes in the color of the adventitious roots were also monitored, which were observed to be the same in all the support posts. Fig. 10 shows the change in color of an adventitious root attached to G. sepium. The initiation of roots was detected when a bulge just proximal to the node was noticed (Fig. 10a). The root tip touched the surface of the post. Light green bulge continued to elongate on the succeeding days. Whitish to light green roots rapidly elongated, accompanied by an increase in width for about a week (Fig. 10b). Gradual decline in the elongation and expansion of the roots on the following week also showed a decrease in the brightness of the roots (Fig. 10c). This continued until the roots attained full length and full width (Fig. 10c). Fully grown roots exhibited dark green appearance (Fig. 10d). The green-colored ARs of vanilla was due to its chlorophyll content which is capable of slight light transmission but showed no net photosynthesis because of its primary role in anchorage and absorption (Aschan and Pfanz, 2003).

The differential growth of the adventitious roots as influenced by the type of support posts is clearly revealed in this study. The thin, long and cylindrical roots observed hanging in GI wire probably resulted in 'not finding' the substrate of the roots due to size limitation of the material. On the other hand, flattened roots attached to solid support post were observed



Figure 9. Vanilla adventitious roots (ARs) attached to different types of support post: (a) GI wire; (b) *Gliricidia sepium* (Jacq.) Steud.; (c) *Sphaerobambos philippinensis* (Gamble) S.Dransf.; (d) PVC; (e) concrete; and (f) styrofoam.



Figure 10. Changes in the color of the adventitious roots (ARs) of vanilla at different stages of growth and development. (a) Visible light green bulge on the 1st day of observation. (b) Elongated and expanded light green root after 6 d. (c) Green root at full length and permanently attached to *Gliricidia sepium* (Jacq.) Steud. after 17 d. (d) Root turned dark green after 21 d.

indicating a root-substrate contact. In similar studies, it was demonstrated that the growth of plant attachment structures was certainly influenced by the type of substrates. Steinbrecher et al. (2011) reported that the attachment pads of *P. tricuspidata* were highly efficient structures that secrete an adhesive fluid and were found to attach to a wide variety of substrates, both organic and inorganic. Moreover, it was shown that the mean projected attachment pad area was found to be dependent on the substrate. The mean projected attachment pad area is comparable to the root width of the adventitious roots in this study. Upon successful contact to the substrate, the roots began to expand, doubling its width after 4 d as exhibited with G. sepium and S. philippinensis as support posts. The roots attached to G. sepium was also observed to simultaneously slow down the rate of elongation and increased the lateral expansion rate while the roots attached to other support posts continuously elongated. On the other hand, roots attached to S. philippinensis showed continued elongation and expansion simultaneously, even exceeding the value of the width of the roots attached to G. sepium.

Both the roughness of *G. sepium* bark due to the presence of numerous lenticels and the smoothness of *S. philippinensis* culm have shown significant contributions to the expansion growth. The textures of the substrates were also considered in the growth of mature attachment pads in *Parthenocissus quinquefolia* and *P. tricuspidata*. Steinbrecher *et al.* (2009) reported that fully grown attachment pads were observed in rough organic substrates like wood and for smooth inorganic substrates like ceramic tiles. Except for ceramic tiles, all inorganic substrates (Mylar foil, PVC, aluminum, glass and steel) showed a tendency for small, stunted or secondary detached attachment structures, which were denoted as rudimentary. Furthermore, attachment pad showed lignification filling the vessel elements of

the wood substrate, thereby creating the perfect form closure with the substrate (Steinbrecher *et al.* 2011). However, it was also emphasized that substrate surface roughness cannot be the only important stimulus for the development of an attachment pad due to large variation even within one tested plant specimen in their study. Moreover, it was stated that the growing site with all its biotic and abiotic environmental conditions plays a key role in the development of a plant and its organs.

Attachment Strength of Vanilla Adventitious Roots on Different Types of Support Posts

Results show that the greatest force was exhibited by the roots attached to G. sepium (935.44 \pm 514.60 mN) while the lowest force was observed in the roots attached to S. philippinensis (179.70 \pm 148.72 mN) (Fig. 11). Moreover, roots attached to PVC and concrete had relative forces that were contrary to the reported cling strength of Syngonium roots (Gamboa, 2016). Vanilla ARs attached to PVC had higher attachment strength (791.76 mN) than Syngonium roots (698.09 mN) attached to the same type of post. Whereas, the attachment strength of vanilla ARs attached to concrete was lower (414.06 mN) than the Syngonium roots (2446.09 mN). Presumably, the reason for the high force exhibited by the vanilla ARs attached to PVC was the alteration of its surface probably due to erratic weather condition in the natural environment. The smooth-surfaced PVC pipes became slightly rough due to soil particles and other dust particles that were continuously splashed to the surface by rain and wind. The compact concrete slowly disintegrated through time and was observed to produced dusty particles which could have affected the adhesion of the roots. These circumstances were probably the causes of contrasting results compared to the previous study in Syngonium. In the case of roots attached to G. sepium and styrofoam, surface contact was formed at the earlier stage of root

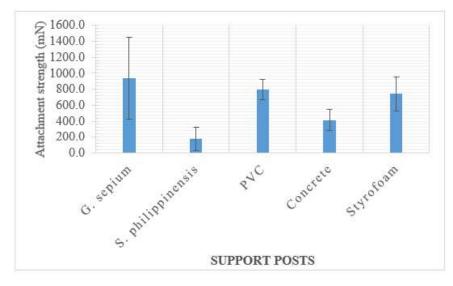


Figure 11. Attachment strength of adventitious roots of vanilla attached to different types of support post. Error bars represent SD (n=29).

growth between the root hairs and the substrates due to the action of root adhesives that can be supported by the mechanical interlock between irregularities of the contact surfaces. Increased surface roughness usually results in an increased strength of the adhesive joint (Gorb, 2008). Hence, strong and permanent adhesion was already achieved. In contrast, the root hairs of the ARs attached to S. philippinensis culm could have lost its capability to form an interlock with the surface due to the substrate's smoothness. Rowe and Speck (2015) emphasized that attachment mechanisms are often geometrically complex, thus measurements can be difficult to replicate under typical natural conditions. Furthermore, mechanical properties often change during ontogeny and so measurements depend on the maturity and the length of time the attachment has been active.

Physiology of Adventitious Root-Host Surface Association

The marked differences in the growth and development of ARs, significantly in terms of lateral expansion, attached to different types of support post have been discussed in the previous section. It was also described in the methodology section that the support posts used in this study have different physical properties that can influence the lateral expansion of the ARs. Results have shown that the width of ARs attached to natural support posts (*G. sepium* and *S. philippinensis*) was higher than the width of ARs attached to artificial posts (PVC, concrete and styrofoam). Moreover, ARs in the control (GI wire) only had slight increase in width throughout the growth period.

The attachment mechanism of vanilla ARs is comparable to the description of the four-phase attachment process in English ivy (Melzer et al., 2011). In this study, it was observed that the root tip had no root hairs which was assumed to be the site of perception. This is in agreement with Lenaghan and Zhang (2012) who also reported that the root tip acts as the pressure sensor that provides the signal to attach. The root exhibited thigmomorphogenic behavior, in which development and adhesion take place in response to the touch stimulus. Thigmomorphogenesis was also observed in P. quinquefolia which caused its epidermal cells to become papillated that may produce adhesive because of modification and remobilization of wall components of these cells after a touch stimulus (Isnard and Silk, 2009). In the same way, the vanilla root tip is primarily responsible for producing the touch stimuli after its contact with the substrate. AR responded to the touch stimulus by producing root hairs in the velamen that are eventually accountable for the attachment in solid supports. The initial contact of the root is also the stage

of structural adaptation of the root system with the topology of the climbing substrate accompanied by the first reaction which is enlargement of the contact between the attachment root and the climbing substrate, thereby bringing closure between the root hairs and substrate (Melzer *et al.*, 2011), rapid increase in the size of the root complements, and the successful enlargement of contact between the root and the substrate.

The continuous growth of ARs is also dependent on the acquisition of water and nutrients from its immediate surrounding (i.e. the substrates) which probably caused the differences in the length and width of vanilla ARs attached to different types of support post in this study. Likewise, studies on the terrestrial roots of wheat have shown that the development of the roots is altered based on the soil conditions. Increased rooting depth and production of greater length of roots in the deeper soil layers in response to soil drying in surface layers have been reported as an important drought-adaptive root trait in many crop species including wheat (Manschadi et al., 2013). Also, these roots have tendency for vertical growth to improve water acquisition. This is comparable to the growth of long vanilla ARs attached to relatively dry surfaces of S. philippinensis, PVC, concrete and styrofoam, which is an adaptive mechanism to increase the root surface area for higher water retention. On the other hand, the noticeable increase in the lateral expansion of vanilla ARs attached to G. sepium and S. philippinensis could be attributed to the roots' adaptive trait for acquiring nutrients. A study on common bean and soybean showed that improved phosphorus (P) uptake caused greater dispersion of lateral roots which enable foraging in the topsoil where P availability is high. Also, a study on wheat revealed that root diameter is also an important determinant of P acquisition efficiency. Furthermore, early and more extensive horizontal growth of the roots in the top 70 cm of the soil profile was responsible for the superior nitrogen (N) acquisition by vigorous wheat breeding lines (Manschadi et al., 2013).

CONCLUSION

The results of this study revealed that the type of material used as support posts for growing vanilla (*Vanilla planifolia* Jacks. ex Andrews) had an influence on the growth and attachment properties of the ARs which are the main structures responsible for the ascending growth of this spice. ARs attached firmer on natural/organic substrates like *Gliricidia sepium* than on artificial substrates because of high attachment strength and increase in width exhibited by the ARs that maintain permanent attachment to the substrate and improve foraging of mineral and nutrients, respectively.

ACKNOWLEDGEMENTS

The main author would like to thank Department of Science and Technology-Science Education Institute (DOST-SEI) for granting her the Accelerated Science and Technology Human Resource Development Program (ASTHRDP) that helped her pursued graduate studies and funded this research.

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Received: 5 March 2021 Accepted: 31 January 2022 Published: 28 March 2022 Published in Print: 30 June 2022