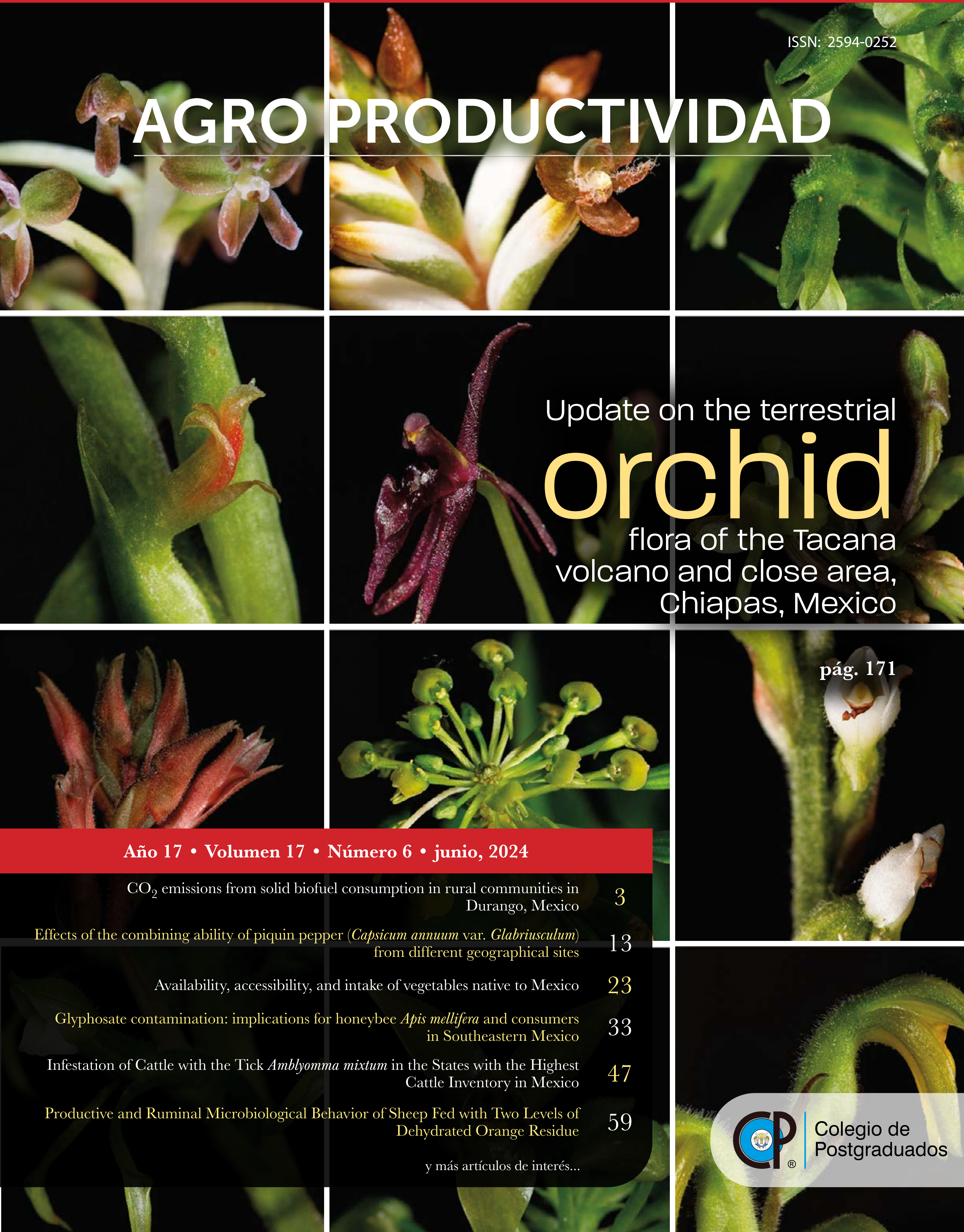

ISSN: 2594-0252

# AGRO PRODUCTIVIDAD

## Update on the terrestrial orchid flora of the Tacana volcano and close area, Chiapas, Mexico

pág. 171

**Año 17 • Volumen 17 • Número 6 • junio, 2024**



Colegio de Postgraduados

# Morphological identification and characterization of the formation of floral primordium in *Vanilla planifolia* (Orchidaceae)

**Pérez-Posadas, Martin[1]; Zavaleta-Mancera, Hilda A.[2]; Delgado-Alvarado, Adriana[1]; Salazar-Rojas, Víctor M.[3]; Herrera-Cabrera, B. Edgar[1*]**

[1] Colegio de Postgraduados, Programa en Estrategias para el Desarrollo Agrícola Regional, Campus Puebla, Boulevard Forjadores de Puebla 205, Santiago Momoxpan, C. P. 72760, Puebla, México.

[2] Colegio de Postgraduados, Programa de Botánica, Campus Montecillo, Carretera México-Texcoco Km 36.5, Montecillo, Texcoco, C. P. 56264, Estado de México, México.

[3] Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Iztacala, México, C. P. 54090, México.

* Correspondence: behc@colpos.mx

**ABSTRACT**

**Objective**: To morphologically identify and characterize the formation of floral primordium and the individual flower development in *Vanilla planifolia* Jacks Ex. Andrews.

**Design/Methodology/Approach**: Inflorescence primordia and young inflorescences in different development stages were sampled from the stem internodes of the following positions: basal (11-15), middle (6-10), and distal (1-5). Four samples were taken from each stem position from February to May, with five repetitions per sampling date. Observations and characterization were made with a stereo microscope. The study site was located at Rancho Xanathtlan, in Barriles, municipality of Gutiérrez Zamora, Veracruz.

**Results**: The development of the *V. planifolia* raceme is described in seven phases: (I) differentiated meristem of the floral inflorescence; (II) appearance of the third bract; (III) initiation of the racemes formation; (IV) elongation of the floral primordium; (V) development and growth of the individual floral primordium in the acropetal direction, (VI) anthesis of the inflorescence in the acropetal direction; and (VII) complete flowering of the raceme. The development of the buds in the raceme is described in five stages from the appearance of the third bract in the acropetal direction.

**Study Limitations/Implications**: The biochemical processes and the interaction of environmental aspects on the floral development of *V. planifolia* pose questions that remain unanswered.

**Findings/Conclusions**: The first aspects of floral formation within the inflorescence of *Vanilla planifolia* were determined, along with its floral phenology.

**Keywords**: Floral growth, floral phenology, inflorescences, orchids.

**Citation**: Pérez-Posadas, M., Zavaleta-Mancera, H. A., Delgado-Alvarado, A., Salazar-Rojas, V. M., & Herrera-Cabrera, B. E. (2024). Morphological identification and characterization of the formation of floral primordium in *Vanilla planifolia* (Orchidaceae). *Agro Productividad*. https://doi.org/10.32854/agrop.v17i6.2859

**Academic Editor**: Jorge Cadena Iñiguez

**Guest Editor**: Daniel Alejandro Cadena Zamudio

**Received**: March 13, 2024.

**Accepted**: May 16, 2024.

**Published on-line**: July 09, 2024.

*Agro Productividad, 17*(6). June. 2024. pp: 161-169.



## INTRODUCTION

Orchidaceae is one of the largest plant families on the planet, with approximately 28,500 recognized species (Novotná *et al.*, 2023). Given the commercial importance of their flowers, the *Cypripedium*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Miltoniopsis*, *Phalaenopsis*, and *Zygopetalum* are considered valuable genera (López and Runkle, 2005). For their part, the ripe fruits of *Vanilla planifolia* have a high agricultural value (Lima-Morales

*et al.*, 2021) and it is currently the second most expensive spice in the world after saffron (Bramel and Frey, 2021).

According to the FAO (2021), global vanilla production amounted to 8,000 tons until 2019. However, its price has decreased between 60 and 70% compared to the maximum prices obtained from 2017 to 2018 (Bramel and Frey, 2021). In Mexico, the yield of vanilla production has decreased over the past 25 years (Rocha-Flores *et al.*, 2018; Santillán *et al.*, 2018), as a result of its discontinuous annual flowering subject to environmental conditions over the years (Parada-Molina *et al.*, 2022), as well as premature fruit drop (Hernández-Miranda *et al.*, 2020).

*V. planifolia* yield is associated with vegetative and reproductive aspects, such as the number of shoots, flowers, and fruits (Rocha-Flores *et al.*, 2018). In that sense, studying floral phenology is essential, because it plays a substantial role in the production of vanilla fruits.

Studies about the anatomy of orchids are scarce, even if vascular patterns are fundamental for the correct development of the fruit (Gamboa-Gaitán, 2015). Consequently, the shape, development, and growth of inflorescences (floral raceme) usually have a great influence on floral production and crop yields (Ju *et al.*, 2012). For example, genus *Magnolia* has a wide floral morphological variation within the floral primordium in the basal, middle, and distal positions of each plant, as well as in the species that make up the genus (Gutiérrez-Lozano *et al.*, 2021). Likewise, wild orchids, such as *V. planifolia*, experience an irregular flowering between years and between different plant positions (Pfeifer *et al.*, 2006), resulting in low flowering.

Although vanilla is a very important species and a product of high culinary value (Borbolla-Pérez *et al.*, 2016; Bramel and Frey, 2021), the formation and development of the inflorescence during the transition to floral shoots within the different positions of the plant have not been described. Nevertheless, some components of its reproductive biology have been analyzed. This study aims to morphologically identify and characterize the formation of the inflorescence primordium and the individual development of the flowers in the inflorescence of *Vanilla planifolia*. The hypothesis was that, if a relationship was found between the morphological and anatomical changes of the *Vanilla planifolia* species, from the differentiated floral bud to the floral opening, the flowering process of the species could be understood.

## MATERIALS AND METHODS

### Experimental site

The study site was located at Rancho Xanathtlan, in the town of Barriles, municipality of Gutiérrez Zamora, Veracruz, Mexico (Figure 1). Table 1 shows the characteristics of the study area.

### Sampling and definition of morphological variables

To morphologically identify and characterize the formation of inflorescence primordium and the individual development of *Vanilla planifolia* flowers, three inflorescences were collected per plant section. Different development stages of the

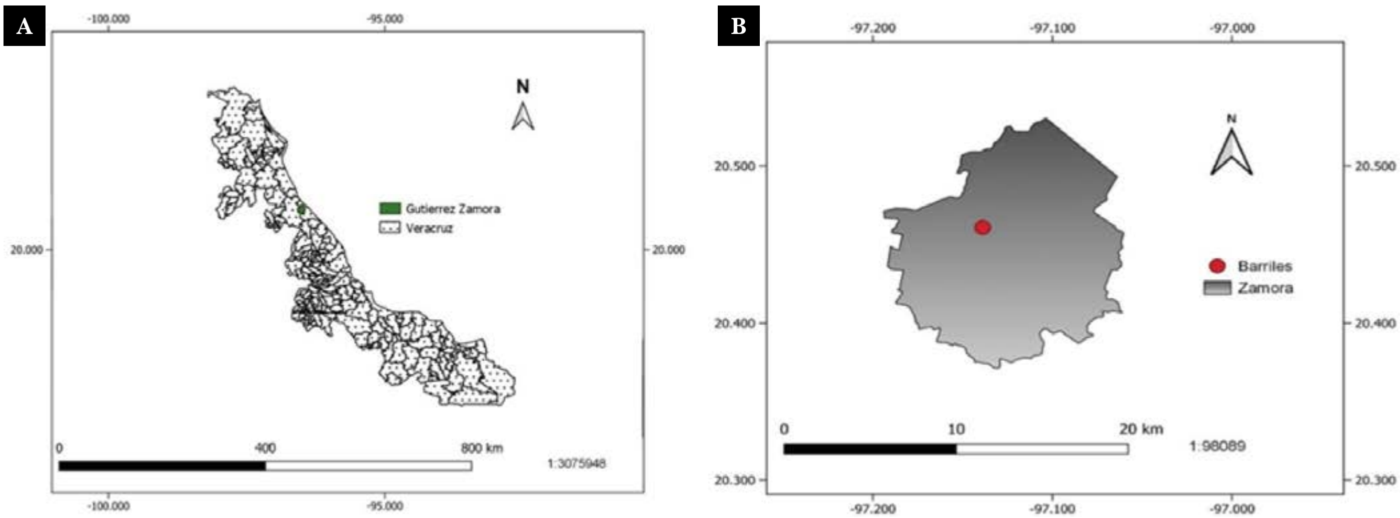


**Figure 1**. Location of the study site. A) Municipality of Gutiérrez Zamora, in Veracruz, Mexico B) Town of Barriles, in the municipality of Gutiérrez Zamora.

**Table 1**. Characterization of the *V. planifolia* agroecosystem in the study site.

| Characteristics | Agroecosystem of the study site |
|---|---|
| Altitude | 20 masl |
| Weather | Cw, temperate with summer rainfall |
| Precipitation | 1200 -1500 mm |
| Soil type | Regosol |
| Predominant vegetation | Medium sub evergreen tropical rainforest |

Source: INEGI (2022).

individual floral primordium were identified and they were classified based on the following morphological traits: appearance of the inflorescence bud, appearance of bracts, elongation of the rachis, and appearance and growth of individual floral primordium and anthesis. Monthly samplings were carried out to identify the morphological formation of the primordium, at the beginning of flowering: February 18, March 18, and April 19. Subsequently, before the floral opening, samples were taken every 15 days (May 4 and May 13, 2022), in order to observe the individual development of the flowers. The plant material was obtained from three different positions of the stem: basal (11-15 internodes), middle (6-10 internodes), and distal (1-5 internodes). A total of 5 repetitions were recorded for each plant section.

The tissues were placed in a FAA fixative solution (10% formaldehyde; 50% ethanol; 5% acetic acid; 35% water), and then rinsed and placed in a GAA solution (25% glycerol; 50% ethanol; 25% distilled water) for preservation. The length of individual floral primordia inside the floral cluster was measured with a digital vernier and their morphology was analyzed with a stereo microscope (Leica Microsystem Vertrieb GmbH, Wetzlar, Germany) and a digital camera (Rodríguez-Rojas *et al*., 2021).

### Scanning Electron Microscopy (SEM)

A modified version of the protocols proposed by Ruzin (1999) was used for this procedure. Individual flower buds, fixed and preserved in GAA, were sectioned in the median plane to study their internal structures. The medium fragments were postfixed in osmium tetroxide, 2% aqueous, and subsequently washed with distilled water and dehydrated in graded ethanols (50%, 70%, 96%, 100%, 100%). Subsequently, the tissues were dehydrated with a Samdri®-780A critical point dryer (Tousimis, MD, USA) and coated with gold/palladium with a Desk IV metallizer (Denton Vacuum, NJ, USA). Observations were performed on a JSM 6390 Scanning Electron Microscope (Jeol, Japan) at the Unidad de Microscopía Electrónica of the Colegio de Postgraduados.

### Morphological and anatomical evaluation

According to Feng *et al.* (2021), different phases of floral development can be identified in relation to morphological and anatomical changes of each genus and species. Wei *et al.* (2010) described the development of a single flower of the genus *Phalaenopsis* and identified seven development phases: 1) initial differentiation, 2) differentiation of the inflorescence primordium, 3) differentiation of the flower primordium, 4) differentiation of the sepal primordium, 5) differentiation of the petal primordium, 6) differentiation of the column, and 7) development of the pollinium.

## RESULTS AND DISCUSSION

Based on the morphological measurements and the references described by Feng *et al.* (2021), seven development phases were identified in the floral raceme of *V. planifolia*, from the differentiated floral bud to its floral anthesis: (I) formation of the differentiated inflorescence meristem, (II) appearance of the third bract, (III) initiation of the formation of the floral raceme, (IV) elongation of the floral primordium, (V) development and growth of the floral primordium in an acropetal direction, (VI) beginning of the acropetal floral anthesis, and (VII) complete flowering of the raceme (Figure 2).

From phase IV of the development of the floral raceme, each of the floral primordium (buttons) of the inflorescence (floral raceme) can be clearly identified, due to their acropetal development (from the base to the apex) (Figure 3).

In this floral branch, the floral primordia were classified into five development stages according to their size. In order of appearance within the floral cluster, they were ordered from the youngest (apical) to the most developed (basal) (Figure 3).

In the *Lantana camara* species the inflorescences on the floral branches have an acropetal development. Since they are arranged in a spiral phyllotaxy from the axis, the most developed inflorescences are found in the basal part, while the inflorescences in the apical part are still in bud (Caroprese *et al.*, 2011). This type of acropetal development with spiral phyllotaxy was similar to the one recorded in *V. planifolia*. Ontogenic events occur for all species; therefore, it is important to know where and when certain genes are activated, as well as the self-organized dynamics that are generated from their interactions (Álvarez and Roces 2002).

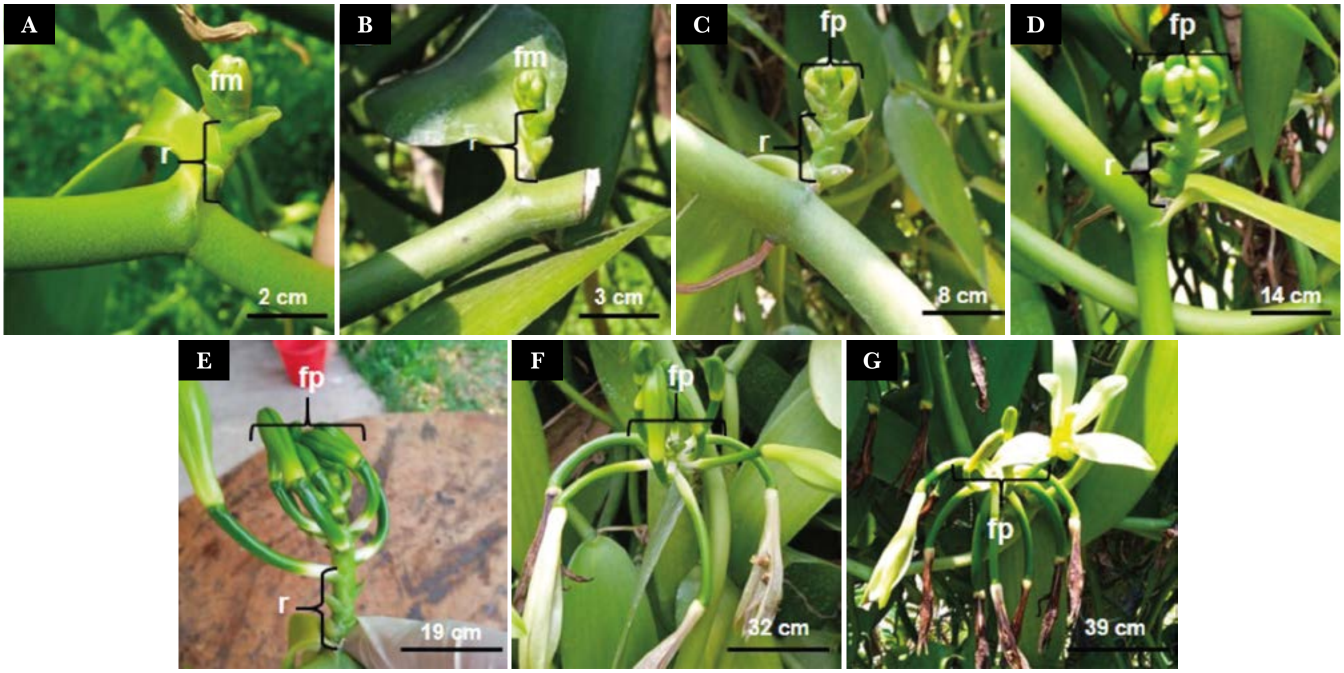


**Figure 2**. Development of the inflorescence (floral raceme) in *Vanilla planifolia*, from the differentiation of the flowering shoot to complete anthesis of the raceme. A. Phase I, recorded on February 18, 2022; B. Phase II, recorded on March 18, 2022; C. Phase III, recorded on March 18, 2022; D. Phase IV, recorded on April 19, 2022; E. Phase V, recorded on May 4, 2022; F. Phase VI, recorded on May 4, 2022; and G. Phase VII, recorded on May 13, 2022. fm=floral meristem; r=rachis; fp=floral primordium.

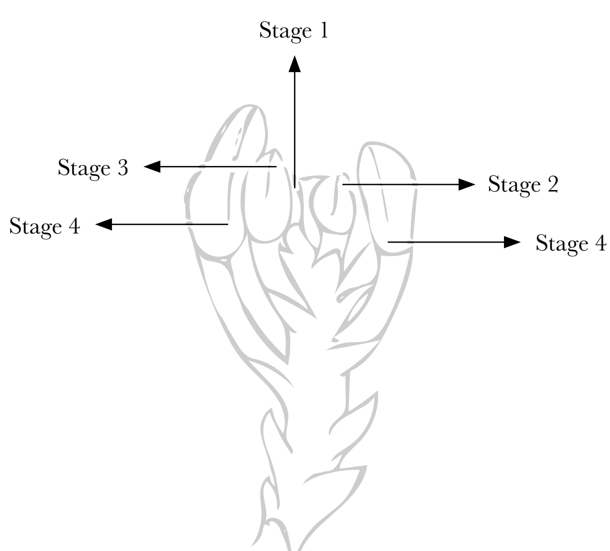


**Figure 3**. Representation of the acropetal formation (from the base to the apex) of floral primordium within the floral raceme of *Vanilla planifolia*.

Figures 4 and 5 describe the developmental stages observed in the first basal bud of *V. planifolia* inflorescence sampled on March 18 and the structural changes occurring over time showing morphological changes and associated dates. Detailed observations of morphological changes were recorded on five key dates, from March 18 to May 13; Stage

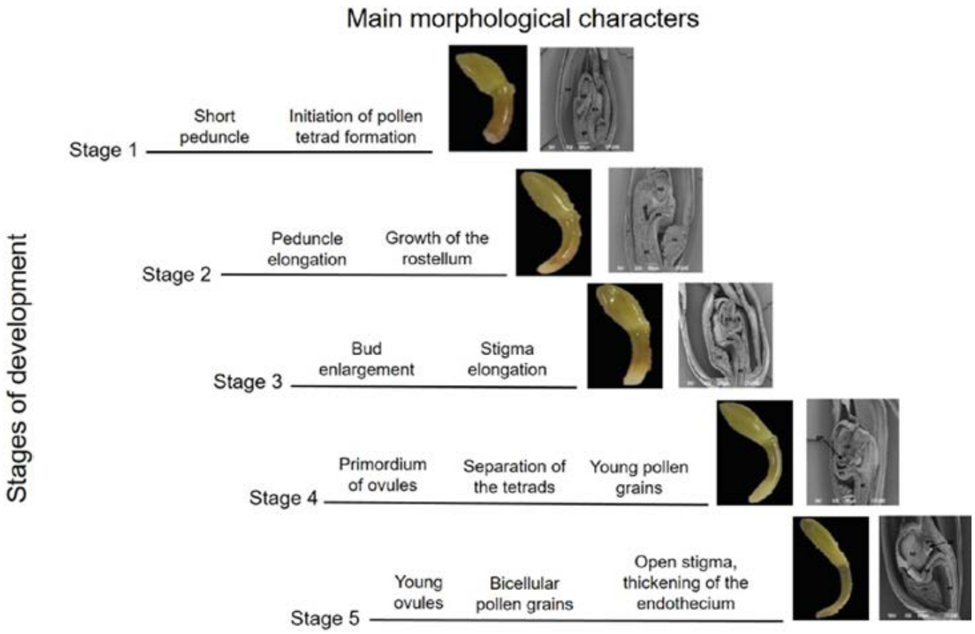


**Figure 4**. Flower bud development from the appearance of the first bract on the inflorescence of *V. planifolia*. Structural characters that characterize five stages of development. Stage 1 corresponds to the first flower bud of Phase III with dimensions of 6 mm long by 5.3 mm wide.

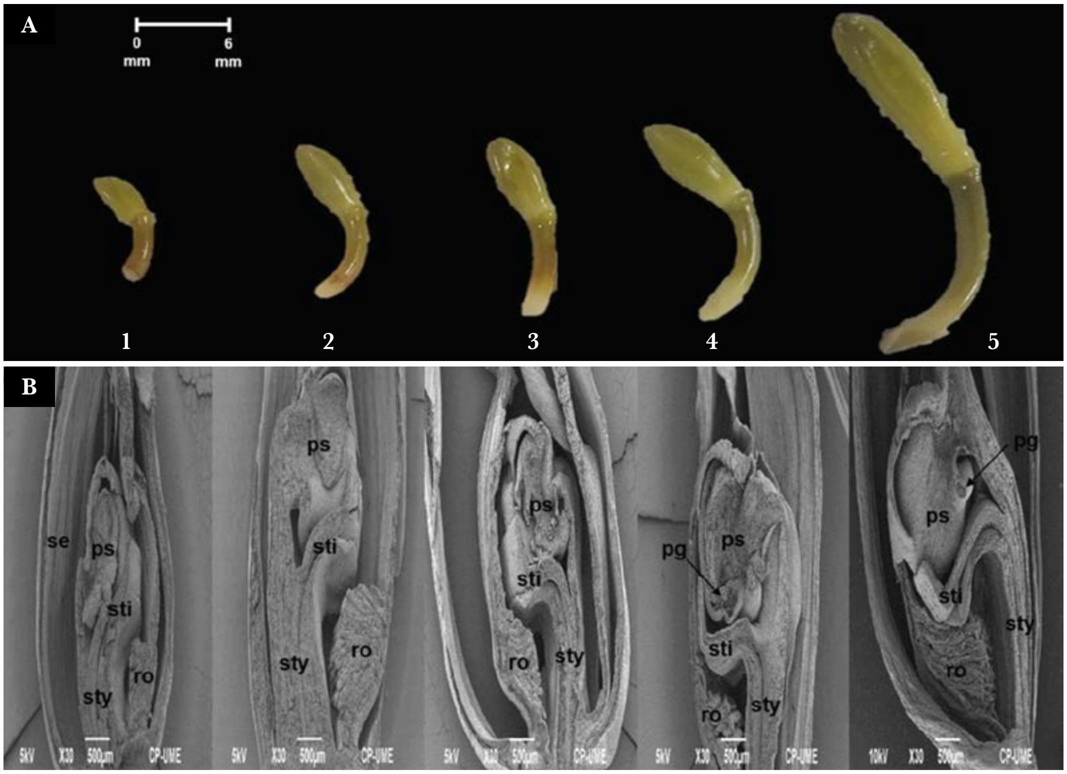


**Figure 5**. Morphology and structure of the floral buds at different stages of development in the raceme of *V. planifolia*. A. External morphology of the 5 stages of development: 1. Stage 1, 3.51-6.50 mm; 2. Stage 2, 6.51-9.50 mm; 3. Stage 3, 9.51-12.50 mm; 4. Stage 4, 12.51-16.50 mm; 5. Stage 5, 16.51-20.50 mm. B. Scanning microscopy of the microstructure of the development stages described in Figure 4. sti=stigma; sty=style; pg=pollen grains; ro=rostelo; se=sepal; ps=pollen sac.

1; the flower bud has a short peduncle (ovary+accessory tissue) and the pollen tetrads begin to form on the pollinium. Stage 2; the elongation of the peduncle and the growth of the rostellum were the crucial indicators of this stage. Stage 3; the enlargement of the floral bud and the elongation of the stigma were highlighted. Stage 4; it is characterized by the beginning of the ovule emergence from the placenta, and the disappearance of the callose and the separation of the tetrads, leaving the young pollen grains free, contained in an average floral bud of 12.51 mm. Stage 5; this is the stage prior to anthesis, characterized by a pronounced elongation of the peduncle containing the ovary, the ovules are still young, but the pollen grains are bicellular and the endothecium shows thickened walls. The thickening of the endothecium is the preparation for flower anthesis. Also at this stage the stigma is split in two, showing glandular cells at the internal epidermis. It is evident that the development of the ovules is late with respect to the development of the pollen grains, which mature first (Figure 5A-B). But these observations provide the basis for future studies of the ovule and embryo sac.

The orchid *Laelia anceps* has similar developmental sequence characteristics to those reported in this research; therefore, the growth and floral development variables were similar to each other with regard to the section within the plant (Sánchez-Vidaña *et al*., 2018). As mentioned by Gutiérrez-Lozano *et al.* (2021), this phenomenon may be caused, to a large extent, by the external factors found in the milieu of the species; therefore, as long as the plant has the necessary conditions to carry out the floral transition within all plant positions, a similar growth and development of floral meristem within the plant will be recorded (Aguilar-Delgado *et al*., 2018).

Thus, during flowering, *V. planifolia* shows that the growth and development of the floral meristems is more intense in the distal section of the stem, since in this position the meristems are more exposed to external factors, such as light and temperature (Sánchez-Vidaña *et al*., 2018).

However, many questions remained unanswered. Although this study is very similar to the work of Caroprese *et al.* (2011), Aguilar-Delgado *et al.* (2018), and Gutiérrez-Lozano *et al.* (2021), most of the documented information corresponds to plants of specific growth. Therefore, species of indeterminate growth (*e.g.*, planifolia and the genus *Vanilla*) may have similar floral characteristics to species of determinate growth and fruit trees.

## CONCLUSIONS

This research shows the first aspects of the development of floral buds within the floral raceme of *Vanilla planifolia*. Important characteristics about the flowering of the species were observed in each development stage. This is a major discovery in the otherwise scarcely studied flowering of the Orchidaceae family.

## ACKNOWLEDGEMENTS

We thank Cesar Emir Arellano and Maria de Lourdes Garcia de Arellano of Rancho Xanathtlan for donating the plant material used in the study.

## REFERENCES


Aguilar-Delgado MJ, Acosta-García G, Espitia-Rangel E, González-Chavira MM, Lozano-Sotomayor P, De Folter S, Barrales-López L, Guevara-Olvera L. 2018. Crecimiento indeterminado y determinado en *Amaranthus hypochondriacus*: ontogenia del meristemo apical y efecto sobre el peso de semilla. *Agrociencia*, 52: 695-711.

Álvarez E, Roces B. 2002. La diversidad de las formas vegetales. Variaciones sobre un mismo tema. *Ciencias*, 65: 19-28.

Borbolla-Pérez V, Iglesias-Andreu LG, Herrera-Cabrera BE, Vovides-Papalouka A. 2016. Aborción prematura de frutos de *Vanilla planifolia* Jacks. ex Andrews. *Agroproductividad, 9*(1): 11-12.

Bramel P, Frey F. 2021. Global strategy for the conservation and use of Vanilla genetic resources. *Global Crop Diversity Trust. Bonn, Germany*. 3-59. https://doi.org/10.5281/zenodo.7544770

Caroprese JF, Parra MI, Arrieta D, Stashenko E. 2011. Anatomía microscopía y metabolitos secundarios volátiles en tres estadios del desarrollo de las inflorescencias de *Latana cámara* (Verbenaceae). *Revista de Biología Tropical, 59*(1): 473-486.

Feng JQ, Xiao Q, Zhang FP, Wang JH, Zhang SB. 2021. Is seasonal flowering time of Paphiopedilum specis caused by differences in initial time of floral bud differentiation? *AoBPlantas, 13*(5): 1-10. https://doi.org/10.1093/aobpla/plab053

Gamboa-Gaitán MA. 2015. Estudios morfoanatómicos en *Cattleya trianae* (Orchidaceae). I. Etapas tempranas del desarrollo del fruto. *Mutis, 5*(1): 6-13.

Gutiérrez-Lozano M, Vázquez-García JA, Reyes-Ortiz JL, Aguilar O, Galván-Hernández DM, Sánchez-González A. 2021. Variación en la morfología foliar, floral y frutal de Magnolia Mexicana (DC.) G. Don (Sección Talauma, Magnoliaceae) en México. *Botanical Sciences, 99*(4): 955-975. https://doi.org/10.17129/botsci.2800

Hernández-Miranda OA, Reyes-Mateos DA, Campos JE, Sandoval-Zapotitla E, Herrera-Cabrera BE, Salazar-Rojas VM. 2020. Expresión diferencial del complejo ARF8-IAA25-like-TIR1 durante el síndrome post-polinización en dos genotipos de vainilla. *Revista Fitotecnia Mexicana 43*(4-A): 575-582. ISSN 01877380, https://doi.org/10.35196/rfm.2020.4-A.575

INEGI. 2022. Aspectos geográficos (Veracruz) 2021. Instituto Nacional de Estadística y Geografía, México. 50.

Ju PS, Jiang K, Schatz M, Lippman ZB. 2012. Rate of meristem maturation determines inflorescence architecture in tomato. *Proceedings of the National Academy of Sciences*, 109: 639-644. https://doi.org/10.1073/pnas.1114963109

Lima-Morales M., Herrera-Cabrera B.E. & A., Delgado-Alvarado. 2021. Intraspecific variation of *Vanilla planifolia* (Orchidaceae) in the Huasteca region, San Luis Potosí, Mexico: morphometry of floral labellum. *Plant Syst Evol* 307, 40:1-11 (2021). https://doi.org/10.1007/s00606-021-01761-4

López RG, Runkle ES. 2005. Environmental physiology of growth and flowering of orchids. *HortScience*, 40: 1969-1973. http//doi.org/10.19303/j.issn.1008-0384.2019.02.005

Novotná A, Mennicken S, De Paula CCP, Vogt-Schilb H, Kotilínek M, Těšitelová T, Šmilauer P, Jersáková J. 2023. Variability in nutrient use by orchid mycorrhizal fungi in two medium types. *Journal of Fungi, 9*(1): 88. https://doi.org/10.3390/jof9010088

Parada-Molina, P.C., Pérez-Silva, A., Cerdán-Cabrera, C.R., Soto-Enrique, A. (2022). Climatic and microclimatic conditions of vanilla (*Vanilla planifolia* Jacks. ex Andrews) production systems in México. *Agronomía Mesoamericana. 33*(2): 48682. http://dx.doi.org/10.15517/am.v33i2.48682

Pfeifer M, Heinrich W, Jetschke G. 2006. Climate, size and flowering history determine flowering pattern of an orchid. *Botanical Journal of Linnean Society*, 151: 511-526. https://doi.org/10.1111/j.1095-8339.2006.00539.x

Rocha-Flores RG, Herrera-Cabrera BE, Velasco-Velasco J, Salazar-Rojas VM, Delgado-Alvarado, A, Mendoza-Castillo, MC. 2018. Determinación preliminar de componentes de rendimiento para el cultivo de vainilla (*Vanilla planifolia* Jacks. ex Andrews) en la región totonacapan, México. *Agroproductividad, 11*(3): 9-14.

Rodríguez-Rojas, T. de J., Andrade-Rodríguez, M., Castillo-Gutiérrez, A., y Villegas-Torres O.G. 2021. Fenología floral de nueve variedades de nochebuena (*Euphorbia pulcherrima* Willd Ex Klotzsch). *Polibotánica*, 51: 123-139. https://doi.org/10.18387/polibotanica.51.8.

Ruzin SE. 1999. Plant microtechnique and microscopy. 322 pp. Oxford, New York: Oxford University Press

Sánchez-Vildaña MR, Tejada-Sartorius O, Hernandez-Anguiano AM, Trejo-Téllez LI, Soto-Hernández RM, Gaytán-Acuña EA. 2018. Ambiente y antecedentes de floración en el crecimiento, Inducción y desarrollo floral de *Laelia anceps* subesp. Anceps (Orchidaceae). *Agrociencia*, 52: 35-54.

Santillán FA, Salas ZA, Vásquez, BN. 2018. La productividad de la vainilla (*Vanilla planifolia* Jacks. ex Andrews) en México de 2003 a 2014. *Revista Mexicana de Ciencias Forestales, 9*(47): 50-69. https://doi.org/10.29298/rmcf.v9i47.165

Wei L, Peng FR, Wang SB, Tan PP. 2010. Morphology and changes of several metabolites content during flower bud differentiation in *Phalaenopsis*. *Acta hortícola Sinica*, 37:1303-1310. https//doi.org/10.17660/ActaHortic.2003.624.20