Orquídea

Experiences in the propagation of *Vanilla* in Peru

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INIBICO

Revista de la Sociedad Peruana de Orquideas; afiliada a la AOS Nr. 69992. Afiliada Internacional al NGC Nr. 527

Edition: Susi Spittler

October 20

Even though there are areas in Peru with conditions for the commercial cultivation of vanilla, this is a relatively recent activity. The main limitations for the development of vanilla cultivation on a commercial scale in Peru are: The limited availability of plant material (cuttings or seedlings) for the installation of plantations, the limited knowledge of propagation techniques and the limited knowledge of the techniques of cultivation, harvest and curing or benefit (Leon and Delgado, 2019).

Vanilla pompona ssp. grandiflora **(Lindl.) SotoArenas**

Vanilla planifolia **Jacks. Ex Andrews**

Vainilla odorata **C. Presl.**

INTRODUCTION

The species *Vanilla planifolia* Jacks. ex Andrews is a native orchid of Central America and main source of the natural essence of vanilla, spice considered the most expensive in the world after saffron and that it is obtained from the benefited (cured) fruits, being used in the preparation of ice cream, desserts and sweets. Commercial plantations of *V. planifolia* have been established in Madagascar, Mexico, Indonesia, among other countries with tropical or subtropical climates. The other speciescultivated and required by the world market as a source of vanilla they are *Vanilla tahitensis* and *Vanilla pompona*.

OBJECTIVES

Adapt the techniques of sexual and clonal propagation of *Vanilla planifolia* Jacks. ex Andrews under in vitro and nursery culture conditions, for the propagation, improvement and conservation of germplasm.

Specific objectives

- Adapt protocols for the in vitro germination of *Vanilla planifolia.*
- Adapt in vitro clonal propagation protocols using nodal segments.
- Develop methods of vegetative propagation in the nursery.

GERMINATION IN VITRO

For in vitro germination, indehiscent capsules (fruits) of *V. planifolia* were used 6 to 7 months after self-pollination. The protocols for sowing indehiscent orchid capsules were followed (Leon, 1993).

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Procedure

The collected capsule was washed with water and detergent to remove traces of dust or accumulated organic matter that could be a source of contamination.

Disinfection was carried out with a 1% sodium hypochlorite solution for 30 minutes inside the sowing chamber, not requiring rinsing. The capsule is opened by two transverse and longitudinal cuts, exposing the seeds that are extracted with a spatula and inoculated into the culture medium.
The seeded flasks were incubated at 25 ± 2ºC under a photoperiod of 12 hours of light and 12 hours of darkness.

Germination, considered from the formation of whitish or green protocorms, was noticeable 6 months after sowing. The first seedlings were obtained at 12 months. These seedlings were transferred for their growth and development to a modified MS medium to which 10% (v / v) of liquid coconut endosperm was added). Later the plants were transferred to the nursery for acclimatization.

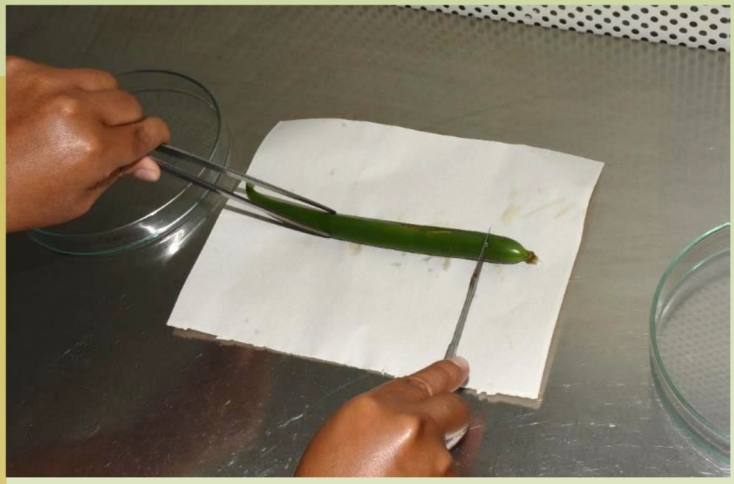
Culture medium

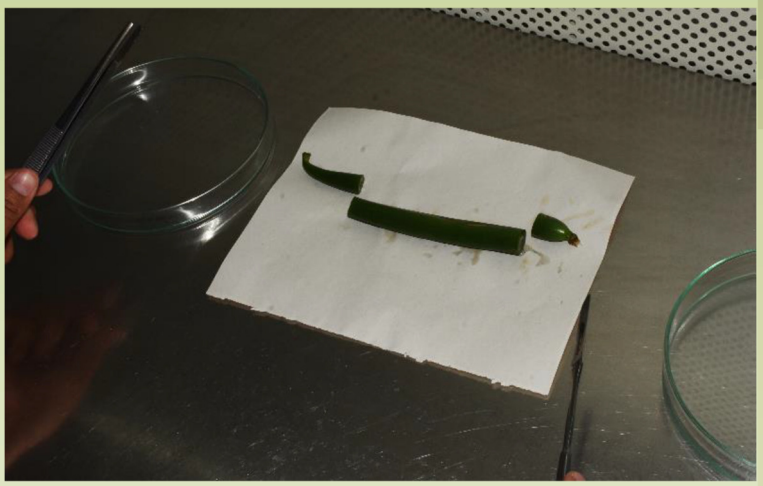
The culture medium for seed germination corresponded to the modified MS formulation (Murashige & Skoog, 1962).

ITEM	Stock	Composicion	Fórmula	Germinación	
				Cant.	Unid
Macro y micro nutrientes					
1	Stock A	Nitrato de Amonio	NH_4NO_3	1.65	g/L
2	Stock B	Nitrato de Potasio	KNO_3	1.90	g/L
3	Stock C	Cloruro de Calcio dihidratado	$CaCl_2.2H_2O$	0.44	g/L
4	Stock D	Fosfato de Potasio monobasico	KH_2PO_4	0.17	g/L
5	Stock E	Molibdato de sodio	$Na_2MoO_2.2H_2O$	0.00025	g/L
		Cloruro de cobalto	$CoCl_2.6H_2O$	0.000025	g/L
		Ioduro de Potasio	KI	0.00083	g/L
		Acido Borico	H_3BO_3	0.0062	g/L
6	Stock F	Sulfato de Manganeso Monohidrato	$MnSO_4 4H_2O$	0.0169	g/L
		Sulfato de Magnesio Heptahidratado	$MgSO_4 . 7H_2O$	0.370	g/L
		Sulfato de Zinc Heptahidratado	$ZnSO_4 . 7H_2O$	0.0086	g/L
		Sulfato de Cobre Pentahidratado	$CuSO_4 . 5H_2O$	0.000025	g/L
7	Stock G	Agente quelante	$Na_2EDTA.2H_2O$	0.0373	g/L
		Sulfato ferroso hepta hidratado	$FeSO_4.7H_2O$	0.0278	g/L
Vitaminas					
8		Tiamina - HCl		0.500	mg/L
9		Acido Nicotinico		0.030	mg/L
Fuentes de carbono					
10		Sacarosa o sucrosa		20.00	g/L
Antioxidantes					
11		Carbon activo		2.00	g/L
Agentes gelantes o solidificantes					
12		Agar Agar		7.0 - 8.0	g/L
		pH:		**5.1 - 5.4**	

* Sales minerales del Medio M&S formulado por Murashige & Skoog (1962)

Orquídea





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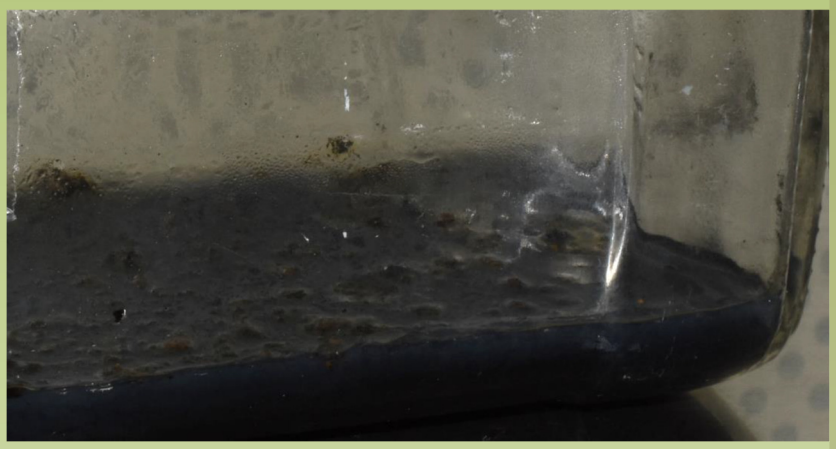
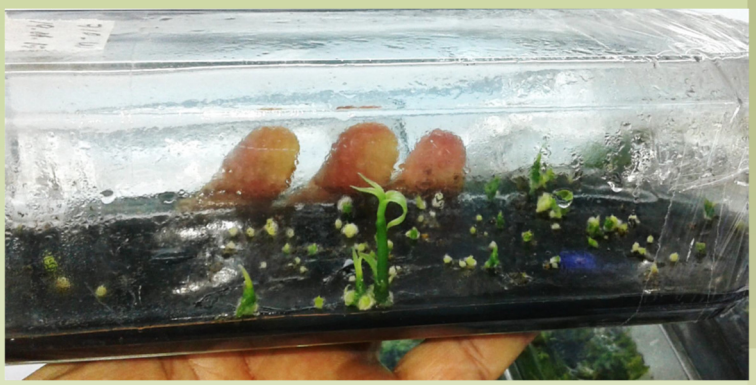


7 months



9 months

12 months

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PROPAGATION BY NODAL SEGMENTS

Its objective was to develop a protocol for the cloning of V. planifolia. Node segments obtained from vegetative shoots in active growth were used. The culture medium used is a modified MS (Sánchez et al., 2016).
The shoots were cut to obtain 5 cm long knots with a vegetative bud, washed with water and detergent and sterilized with a 0.5% sodium hypochlorite solution for 20 minutes. Three rinses were carried out inside the sowing chamber, with sterile distilled water.

The ends of the sterilized nodal segments were cut to eliminate the parts burned by the disinfectant and obtain an ex-plant that is easy to plant in a test tube. The nodal segments that produced vegetative shoots were transferred to a growth and development medium constituted by a modified DM supplemented with 10% of liquid coconut endosperm and continued to propagate from the new nodal segments obtained in vitro.
The seedlings obtained were transferred to the nursery for acclimatization.

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FORMULATION OF THE GROWING MEDIUM FOR THE PROPAGATION OF THE CLONE OF VANILLA PLANIFOLIA

ITEM	Stock	Composicion	Fórmula	Inducción	
				Cant.	Unid
1	Stock A	Nitrato de Amonio	NH_4NO_3	1.65	g/L
2	Stock B	Nitrato de Potasio	KNO_3	1.90	g/L
3	Stock C	Cloruro de Calcio dihidratado	$CaCl_2.2H_2O$	0.44	g/L
4	Stock D	Fosfato de Potasio monobasico	KH_2PO_4	0.17	g/L
5	Stock E	Molibdato de sodio	$Na_2MoO_2.2H_2O$	0.00025	g/L
		Cloruro de cobalto	$CoCl_2.6H_2O$	0.000025	g/L
		Ioduro de Potasio	KI	0.00083	g/L
		Acido Borico	H_3BO_3	0.0062	g/L
6	Stock F	Sulfato de Manganeso Monohidrato	$MnSO_4 4H_2O$	0.0169	g/L
		Sulfato de Magnesio Heptahidratado	$MgSO_4 . 7H_2O$	0.370	g/L
		Sulfato de Zinc Heptahidratado	$ZnSO_4 . 7H_2O$	0.0086	g/L
		Sulfato de Cobre Pentahidratado	$CuSO_4 . 5H_2O$	0.000025	g/L
7	Stock G	Agente quelante	$Na_2EDTA.2H_2O$	0.0373	g/L
		Sulfato ferroso hepta hidratado	$FeSO_4.7H_2O$	0.0278	g/L
8		Tiamina - HCl		0.500	mg/L
9		Acido Nicotinico		0.030	mg/L
10		Sacarosa o sucrosa		20.00	g/L
11		BAP		2.00	mg/L
12		ANA		1.00	mg/L
13		Carbon activo		2.00	g/L
14		Agar Agar		7.0 - 8.0	g/L
15		Agua de coco		50.00	ml/L
16		Vitrofural		0.11	mg/L
		pH:		5.1 - 5.4	

* Sales minerales del Medio M&S formulado por Murashige & Skoog (1962)

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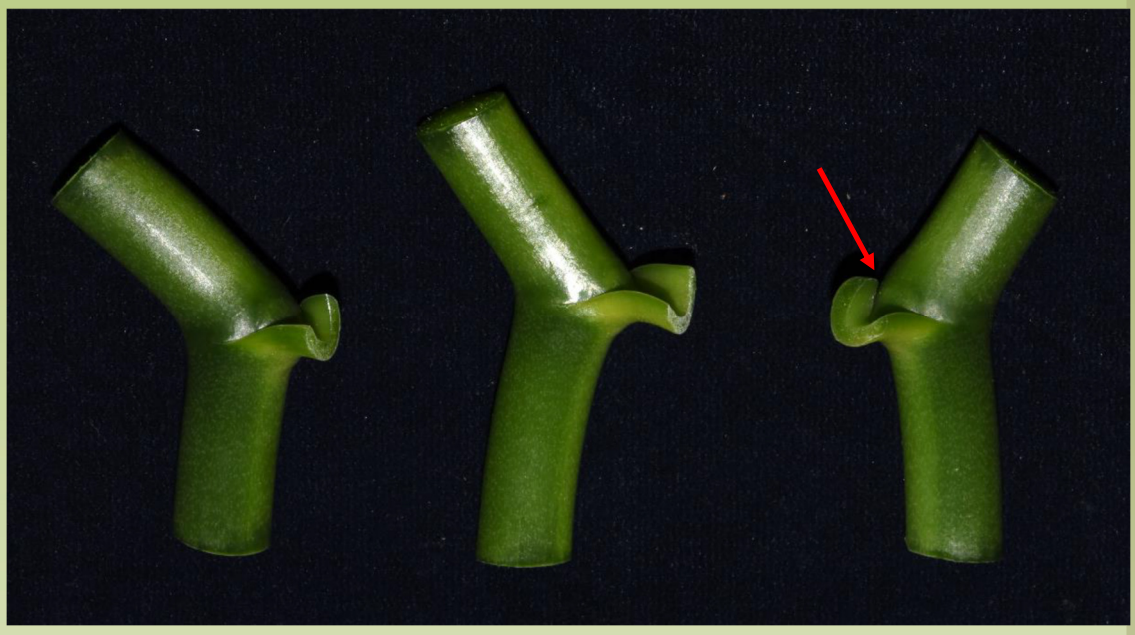

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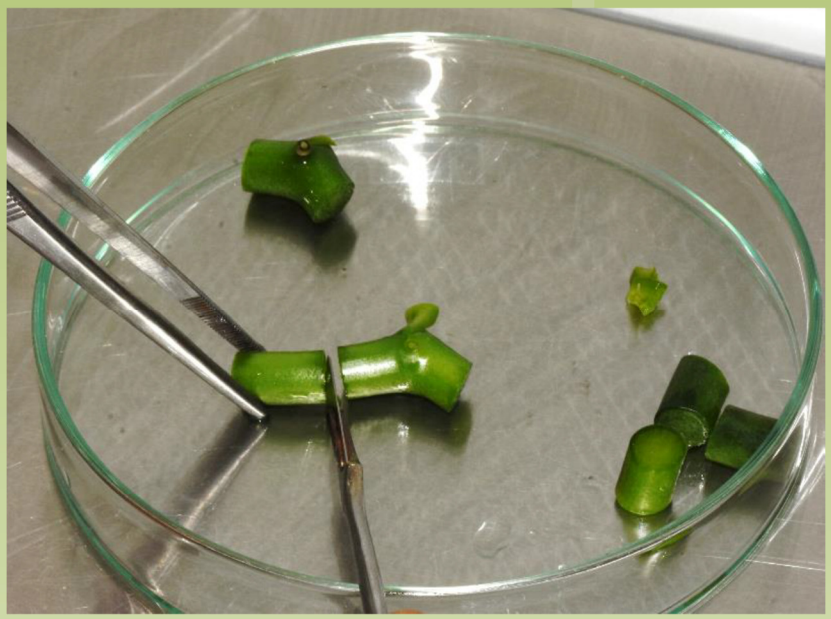
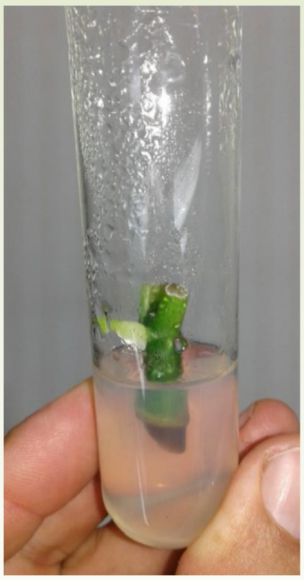


Development medium



Replant

Replanting to the developement medium

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Development medium

FORMULACION DEL MEDIO DE CULTIVO PARA DESARROLLO DE PLANTULAS DE *VANILLA PLANIFOLIA*

ITEM	Stock	Composicion	Fórmula	Desarrollo	
				Cant.	Unid
1	Stock A	Nitrato de Amonio	NH_4NO_3	1.65	g/L
2	Stock B	Nitrato de Potasio	KNO_3	1.90	g/L
3	Stock C	Cloruro de Calcio dihidratado	$CaCl_2.2H_2O$	0.44	g/L
4	Stock D	Fosfato de Potasio monobasico	KH_2PO_4	0.17	g/L
5	Stock E	Molibdato de sodio	$Na_2MoO_2.2H_2O$	0.00025	g/L
		Cloruro de cobalto	$CoCl_2.6H_2O$	0.000025	g/L
		Ioduro de Potasio	KI	0.00083	g/L
		Acido Borico	H_3BO_3	0.0062	g/L
6	Stock F	Sulfato de Manganeso Monohidrato	$MnSO_4 4H_2O$	0.0169	g/L
		Sulfato de Magnesio Heptahidratado	$MgSO_4 . 7H_2O$	0.370	g/L
		Sulfato de Zinc Heptahidratado	$ZnSO_4 . 7H_2O$	0.0086	g/L
		Sulfato de Cobre Pentahidratado	$CuSO_4 . 5H_2O$	0.000025	g/L
7	Stock G	Agente quelante	$Na_2EDTA.2H_2O$	0.0373	g/L
		Sulfato ferroso hepta hidratado	$FeSO_4.7H_2O$	0.0278	g/L
8		Tiamina - HCl		0.500	mg/L
9		Acido Nicotinico		0.030	mg/L
10		Sacarosa o sucrosa		30.00	g/L
11		Carbon activo		2.00	g/L
12		Agar Agar		7.0 - 8.0	g/L
13		Agua de coco		100.00	ml/L
		pH:		5.1 - 5.4	

*** Sales minerales del Medio M&S formulado por Murashige & Skoog (1962)**

Seedlings of *V. planifolia,* obtained by clonal propagation, ready to be acclimatized.

Acclimatization

The rooted seedlings, with 3 to 5 nodes, were acclimated in community pots and individual pots. In both cases, white moss (*Sphagnum magellanicum*) was used as substrate. The seedlings planted in pots were kept in plastic bags to maintain high humidity and facilitate their gradual adaptation to the growing conditions in the nursery.

10 weeks after acclimatization, a fertilizer solution with 190 ppm of N, 35ppm of P and 210ppm of K and with micronutrients (Fetrilon Combi - Compo Expert BASF) was applied every 15 days

- **Substrate: Sphagnum**

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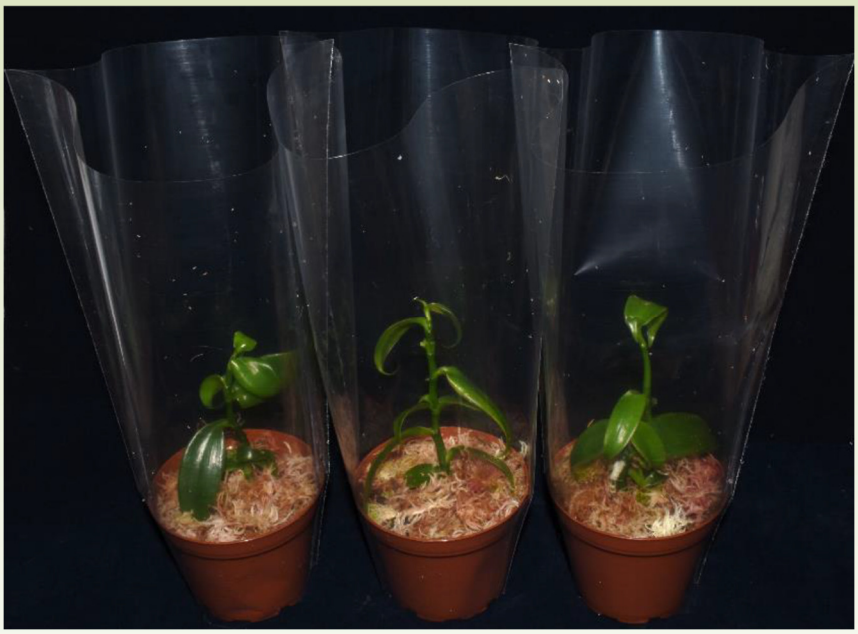

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10 weeks: fertilizer solution with 190 ppm of N, 35ppm of P and 210ppm of K and with micronutrients (FetrilonCombi –CompoExpertBASF) every 15 days

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Nursery propagation -Cuttings

The entire procedure was performed in a nursery, no in vitro propagation laboratory is required. Segments of three nodes obtained from mature and healthy stems were used.

After cutting, the cuttings are kept in the shade to facilitate the healing of the cuts and achieve a slight dehydration of the stems. This will facilitate handling during planting, reducing the incidence of diseases.

The sowing was carried out in 1 liter plastic pots, using pure white moss (*Sphagnum magellanicum)* as substrate. The cuttings are bent so that at least one of the nodes remains within the substrate. Four weeks after planting, the first roots are observed, applying a fertilizer solution with 190 ppm of N, 35ppm of P and 210ppm of K and with micronutrients (Fetrilon Combi - Compo Expert BASF) every 15 days.

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The long vegetative shoots were tutored to continue their growth and facilitate the sowing work in the definitive field or in intensive cultivation beds.

The new growths can later be used to obtain segments to restart the propagation cycle.

4 weeks: fertilizer solution with 190 ppm of N, 35ppm of P and 210ppm of K and with micronutrients (FetrilonCombi –CompoExpertBASF) every 15 days

- From week 12: sowing in the final field.
- This method increases the propagation rates of *V. planifolia* under conditions of scarce initial material for propagation.
- The new growths can later be used to obtain segments to restart the propagation cycle.

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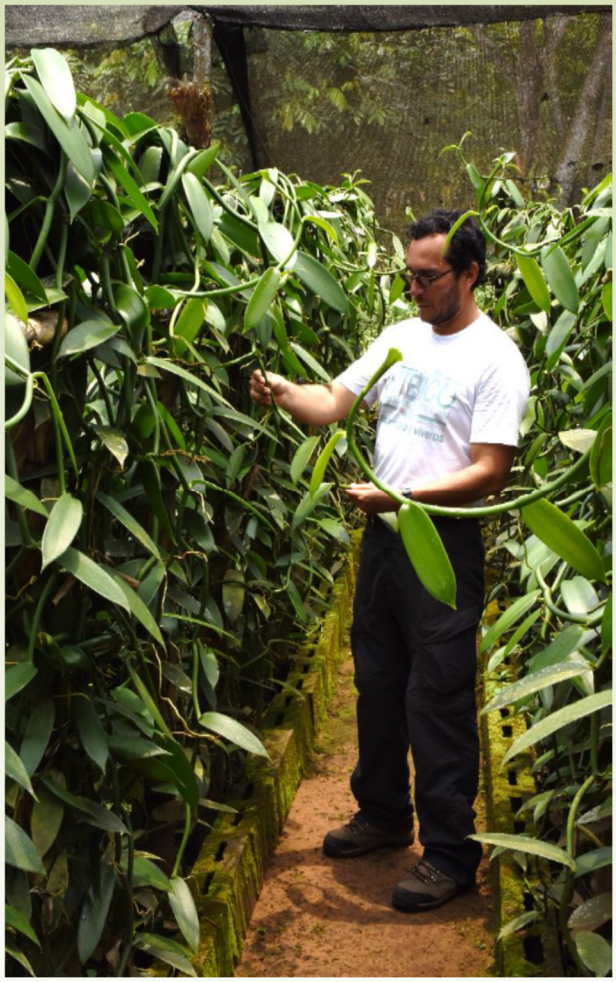

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CONCLUSIONS

The in vitro propagation developed technologies can be applied in obtaining interspecific hybrids for breeding purposes, in the production of plants for the installation of cultivation areas, in the conservation of species of the genus *Vanilla* native to Peru, as well as in the development of new propagation technologies such as somatic embryogenesis, artificial seed and the use of bioreactors.

The nursery propagation method using three-knot cuttings increases the propagation rates of *V. planifolia* under conditions of scarce initial material for propagation and when there are no facilities for in vitro propagation.

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GRATITUDE

We thank the

Biologist Marco Leon

For sharing with us his studies on his

EXPERIENCES IN THE PROPAGATION OF *VANILLA* IN PERU

AboutOrquídea, the newsletter of the Peruvian Orchid Society.

We hope you have enjoyed reading Orquidea, now in its 89th edition. Our goal is to keep our friends around the world informed about the enormous diversity of orchids, their cultivation and reproduction, and the activities of our society.

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