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DEVELOPING AN EFFICIENT PROTOCOL FOR RAPID *IN VITRO* MULTIPLICATION OF *VANILLA PLANIFOLIA* ANDREWS *(ORCHIDACEAE)* OF MADAGASCAR

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ABSTRACT
Under natural conditions, the lack of endosperm limits the orchid's germination including *Vanilla sp.* Only 1 to 2% of orchids germinate under difficulty after an extensive period of dormancy. This work aims to find out the efficient *in vitro* protocol to speed up *Vanilla planifolia* Andrews (Orchidaceae) strains propagation. Two *in vitro* methods were applied during this work such as the asymbiotic germination using different pods stages development of vanilla *seeds* and the nodal segments propagation using different MS media in combination with various phytohormones. High rate protocorms of 87.28% were induced with mature pods of 9 months using MS basal medium supplemented with 2 mg/ml each of NAA and BAP and that of MS/2 medium supplemented with 1% of Myo-inositol and 0.1% Thiamine-HCl was identified the best to develop 100% of plantlets from protocorms. MS/2 basal medium supplemented with 1 mg/ml of BAP and 1 mg/ml of Kin was the most efficient for nodal culture by developing in 5 days of incubation only the first node and giving liana with 6 nodes at 45 days after cultivation, reaching 77%.These methods could be used and tested for the same species of *V.planifolia* and to others strains for a rapid multiplication and development under *in vitro* conditions.

KEYWORDS: *Vanilla planifolia*, *in vitro* multiplication, asymbiotic germination, nodal culture, growth hormones.

1. INTRODUCTION

Vanilla is a liana plant belonging to the Orchidaceae family, one of the largest families of flowering plants which contains 800 genus and 25 000 species (Cameron *et* Molina, 2006). *Vanilla* genus is the unique orchid that gives a comestible fruit keeping the second most expensive spice after saffron (Ranadive, 2005). Three species including *Vanilla planifolia*, *V.tahitensis*, and *V.pompona* are well-known and among the most cultivated around the world for their aromatic chemical component principles (De Oliveira *et al.*, 2022). Among them, *V.planifolia* is the most commercialized in Madagascar and around the word due to its quality (Harinarivo *et al.*, 2024). The germination of vanilla seeds under natural conditions is very limited due to the absence of endosperm (Salazar-Mercado, 2012; Bhattacharjeeand Md Islam, 2014) that limits also its genetic improvement. Moreover, Philip and Nainar (1988) reported that only 1 to 2% of orchids germinate with difficulty under natural conditions after an extensive period of dormancy and up to date the micro-cutting method is always the way to multiply vanilla. The pool of primary gene is heavily threatened by the increased pressure landed and the destruction of its natural biotope (Duval *etal*., 2006). The climatic risks are also the uncontrollable factors and destructor of vanilla cultivation in the country. In front of this situation and conscious that Madagascar is the famous first vanilla producer in the world, several studies on the strategies of multiplication and conservation of orchid varieties including the *Vanilla spp.* were carried out focusing mainly on varietal improvement and its intensive cultivation (Palama *et al.*, 2010).

Knudson (1922) succeeded in developing the first asymbiotic germination technique for some orchid seeds including *Cattleya*, *Laelia*, *Epidendrum* and also *Vanilla* on agar medium containing sugar. As for the *Vanilla spp*., asymbiotic germination techniques have been developed since 1937 by Bouriquet and Boiteau by using various medium but they obtained a low germination rate after 7 months of seeding (Knudson, 1950). Later, the *in vitro* germination technique has already been applied to several orchid species having ornamental value and medicinal activity (Bhattacharjee and Islam, 2014). Most of the studies carried out for *in vitro* seeds germination of Orchidaceae family including the *Vanilla* genus have shown that the time and the rate of germination depend on the culture medium components, the type and the concentration of growth hormone used and the age of pods (Kaur and Bhutani, 2011). Murashige and Skoog medium (MS) was found among of the best medium for the germination and growth of several orchids species (Ramanampamonjy, 2004), and has shown optimal results (Salazar-Mercado, 2012). In 2009,

In order to contribute to the development of the Madagascar's economy by ensure the continued production of *Vanilla* genera and the conservation of the best quality, this work aims to develop *in vitro* culture techniques able to speed up *V. planifolia* seeds germination, nodal multiplication and its development.

2. MATERIALS AND METHODS

2.1. Asymbiotic seeds germination

***Plant material*:** *V.planifolia* green pods of 4 months and mature pods of 9 months were collected at Kianjavato FOFIFA station (*21°22'56"S, 47°52'03"E*, at 64 m above sea level) at Mananjary District in the Vatovavy region of Madagascar.

***Explant sterilization*:** Pods surfaces were washed with liquid detergent, rinsed with tap water, then soaked in 1% of dithane M45 (Dow AgroSciences, France) solution for 1 hour and rinsed again with sterile distilled water. Then, they were disinfected under sterile laminar flow hood by soaking in 15% of calcium hypochlorite (Thermo ScientificAcros, USA) solution added with 3 drops of Tween 20 during 10 minutes, and then rinsed six times in sterile distilled water. Finally, pods were flamed on burner with 96° ethanol.

***Protocorm induction*:** Vanilla seeds were removed from the disinfected pod *via* a longitudinal cut followed by delicate scraping. Seeds were then sown on different culture media for protocorm induction. Seven different media (T_1-T_7) were tested. They were formulated from the Murashige and Skoog basal medium added with 0.2% of activated charcoal, 3% of sugar and solidified with 0.8% of agar. T_1 to T_6 were supplemented with different concentrations of growth hormones and/or biological additives including naphthalene acetic acid (NAA), benzyl aminopurine (BAP) and kinetin (Kin) or furfuryl aminopurine, the combination proportions of each medium are reported in the table 1. T_7 was served as the control medium with hormone and biological additive free. The pH media was adjusted at 5.6 to 5.8. Seeds were sown under a binocular loop and one hundred of mature and immature seeds per Petri dish were poured and plated onto different fresh media (table 1), then Petri dishes were sealed with parafilm, transferred in the room culture and incubated at 25°C for protocorm induction illuminated of 3600 lux under photoperiod 12/24 h condition. One (01) Petri dish per type of culture medium forms one replication, and in total, 10 replications were done for each culture medium.

Table 1: Different culture media component used for protocorm induction of *V. planifolia*.

Media	NAA (mg/ml)	BAP (mg/ml)	Kin (mg/ml)	Coconut water (mg/ml)
T_1	1	1	0	0
T_2	2	2	0	0
T_3	1	0	0,5	0
T_4	1	0	1	0
T_5	1	0	0	200
T_6	0	0	0	200
T_7	0	0	0	0
NAA: naphthalene acetic acid; BAP: benzyl aminopurine; Kin: kinetin				

***Plantlets regeneration*:** After 3 months of incubation, green embryos obtained aged of 15 days after their appearance were transferred onto four different media (R_1, R_2, R_3 and R_4) for seedling regeneration. Those media were made by MS/2 added with 2% of sugar and 0.6% of agar (Ramanampamonjy, 2004). R_1 and R_2 were formulated from hormonal combinations composed of BAP and Kin whose respective concentrations are reported in Table 2. R_3 is hormone free and added with 1% of Myo-inositol and 0.1% of Thiamine-HCl. R_4, hormone and amino acids free was used as a control medium. Twenty embryos per Petri dish were plated and 10 Petri dishes per type of medium were prepared, where one Petri dish represents one replication. Observations were carried out every 5 days.

Table 2: Modification of MS/2 medium used for plantlet regeneration.

Media	BAP (mg/l)	Kin (mg/l)	Myo-inositol (%)	Thiamine-HCl (%)
R_1	1	1	0	0
R_2	2	2	0	0
R_3	0	0	1	0.1
R_4	0	0	0	0
BAP: benzyl aminopurine; Kin: kinetin				

2.2. Nodal segments culture

Explant sterilization*: V.planifolia* nodes were collected from the vanilla culture in the IMRA (InstitutMalgache de RecherchesAppliquées) greenhouse. Nodes were washed with liquid detergent and rinsed with tap water. Then, they were immersed in a 1% of dithane for 1 hour, and rinsed again with tap water. Disinfection was followed by immersing nodes in 400 ml of sterile distilled water, added with 5 drops of Tween 20 for 15 min and soaked in 7% of Calcium hypochlorite solution for 10 min, and then rinsed six times with sterile distilled water.

Nodes plating: The 2 parts of the disinfected node were removed away by cutting with a scalpel at an angle and horizontally plated onto the fresh medium in order to facilitate and accelerate the nutrients penetrating into the explant. The segment nodal media tested were made by MS basal with 3% of sugar and 0.8% of agar. The two first media (T_B and T_C) were supplemented with hormones combination where their concentration were reported in table 3, however, the third (T_A) was hormone free and used as medium control. One node was planted in one test tube containing 40 ml of medium. 30 tubes per type of medium were used, and one tube represents one replication. Observations were carried out every 5 days during 50 days.

Table 3: Concentration (mg/m) of the supplemented hormones on the medium used for the nodal segments culture.

Media	BAP	GA_3	Kin
T_A	0	0	0
T_B	1	0,5	0
T_C	1	0	1
BAP: benzyl amino purine; GA_3: Gibberellic acid; Kin: kinetin			

2.3. Statistical analysis

Data were statistically analyzed using Student's *t*-test and the analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) with multiple range test using the SPSS version 20.0. All the differences showing a $p < 0.05$ were accepted as statistically significant. The results were expressed as mean ± standard deviation (SD).

3. RESULTS

3.1. Asymbiotic germination

Protocorm induction: The four months-old pods did not induce any protocorm after 6 months of cultivation on all tested media. While embryogenic protocorm white and friable (Photo 1) were induced onT_1, T_2 and T_4 mediafrom 9 months-old pods after 20 days of cultivation (Figure 1). T_2 was the best medium for asymbiotic seeds germination of Vanilla seeds which has recorded 86.90 ± 7.40 % of induced protocorm, followed by T_4 showing 61.20 ± 8.83 % and the last one is T_1 by keeping 55.70 ± 7.94%. The other media did not respond to the Vanilla seeds germination.

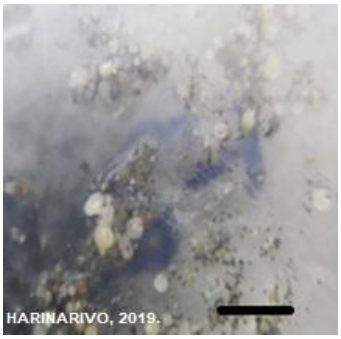

Photo 1: White and friable induced protocorms on medium T2 (bar = 1 cm)

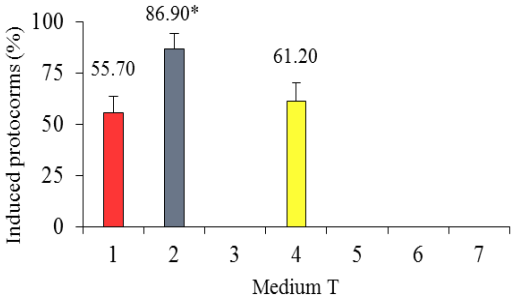

Figure 1. Rate of induced protocorms derived 9-months old-pods in different media (T_1 – T_7) after 20 days of cultivation (*: $p < 0.001$ *vs* T_1 and *vs* T_4)

After 3 months of culture, the embryogenic protocorms on T_1 and T_2 media became green embryos (Photo 2) with 30.40 ± 12.20 % and 67.20 ± 7.07 % transformation respectively (Figure 2). However, no green embryos were obtained with T_4 medium.

Photo 2: Green embryos on T2 medium (bar = 1 cm)

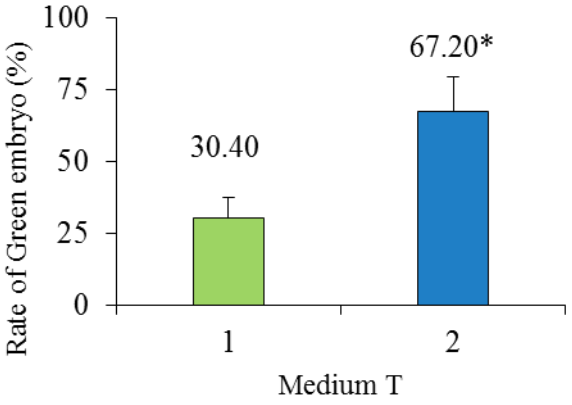

Figure 2: Green embryos rates obtained on T1 and T2 media after 3 months of culture (*: p < 0.001 vs T1),

***Seedling development*:** Only transferred embryos on R_3 have shown roots and leaves formation (Photo 3A) before becoming a small liana after 45 days (Photo 3B). The percentages of plantlet formation after 15, 20 and 25 days of embryos transfer on the R_3 media were given in the Figure 3. The increase of plantlet formation percentages was time-dependent ($p < 0.001$ with ANOVA). The more the time is running, the more the percentage of plantlets formation increases. No seedling development was observed 60 days after transfer on R_1, R_2 and R_4 media.

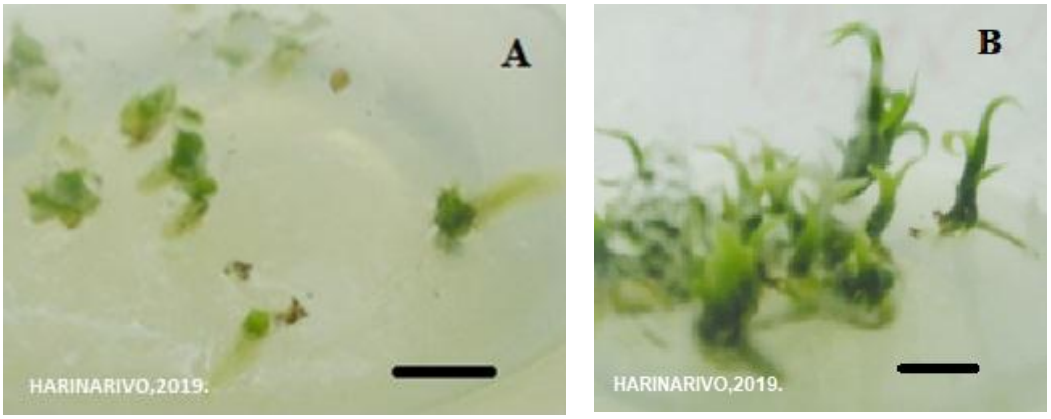

Photo 3: Embryos evolution (A: roots and leaves formation after 15 days; B: young liana with 2 nodes after 6 weeks) after transfer on R_3 medium (bar = 1 cm).

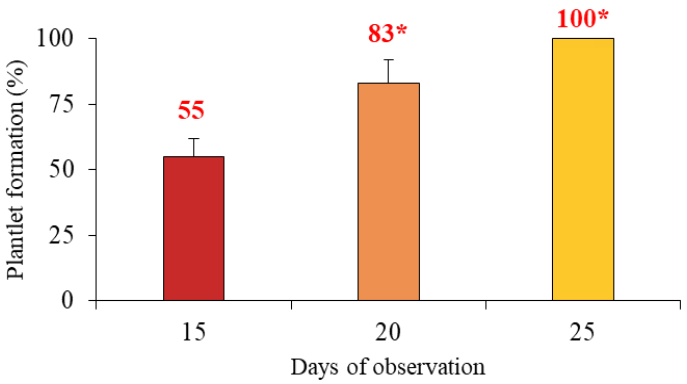

Figure 3: Rate of plantlets formation after 15, 20 and 25 days of embryos transfer on R_3 medium. (*: p < 0.001 *vs* 15 days and *vs* 20 days; p < 0.001)

3.2. Node culture

The first node appearance (Photo 4A) was recorded after only 5 days of culture on T_C medium while, after 10 days for the T_A (control) and T_B media. The 2nd node was appeared after 15 days of culture on T_C medium, while it was 30 and 40 days for the culture on T_B and T_A media respectively. Liana has shown 6 nodes after 45 days of plating on T_C medium (Photo 4B) with a high rate of 77%, compared to that of T_B and T_A media respectively 3 and 2 nodes only. But after 50 days of transfer on T_A, T_B and T_C media, all tested media were able to develop young plantlets of lianas still with 2, 3 and 6 nodes respectively. Results have shown that T_C medium was the most appropriate for nodal culture. The evolution of node number formation on different media was highlighted on figure 4.

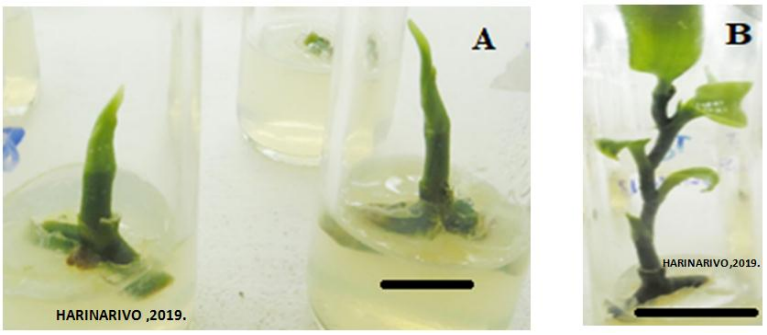

Photo 4: Node evolution of *Vanilla planifolia* strains (A: first new node appears in 5days; B: 6 nodes appeared 45 days after transfer) on T_C medium (bar = 1 cm)

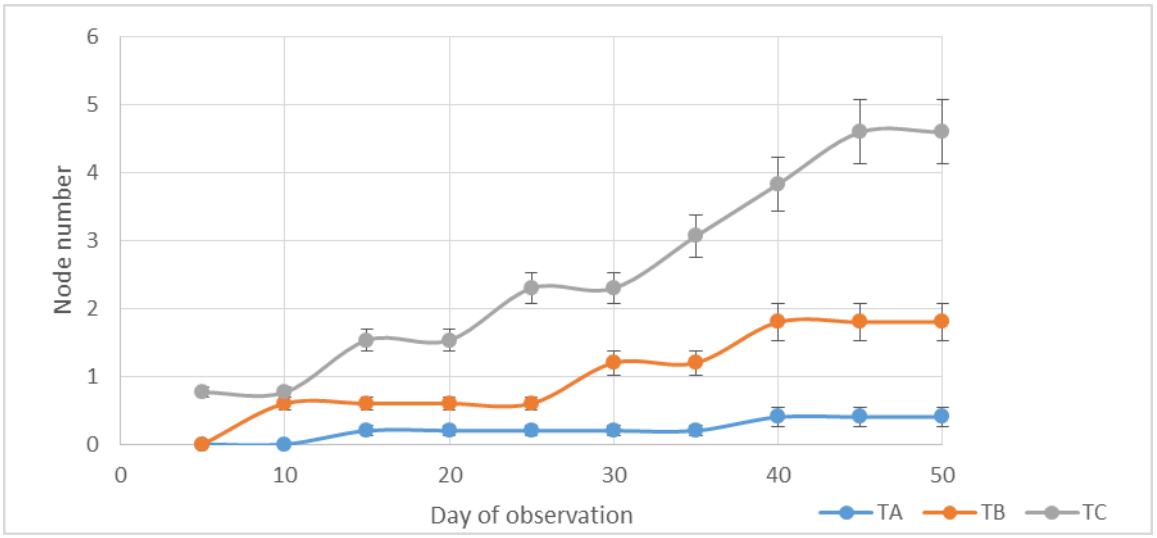

Figure 4: Node number evolution plated on different media (T_A= control).

4. DISCUSSION

Since the first investigation of Knudson and Bernard (1884-1958), germination of orchid seeds has grabbed more attention (Kauth*et al.*, 2008). Asymbiotic germination is an *in vitro* germination technique where seeds are plated onto synthetic medium whithout *Rhizoctonia* fungus inoculation which was always used for symbiosis under natural soil conditions of classic culture (Teixeira da Silva *et al.*, 2015). In this study, no germination was observed for seeds collected from 4-month-old pods, it could be due to its improper stage of embryos which does not reach its maturation. Pods developmental age has played an important role in *Vanilla planifolia* seed germination. This result confirms that reported by Philip and Nainar (1988); Temjensangba and Deb (2006); Kumar *et al.*, (2009); Vasudevan and Van Staden (2010); Thejaswini and Narasimhan (2017) who worked in similar studies on orchid including *V.planifolia*, *Dendrobium ovatum*, *Cleisostomaracem iferum* and *Anseliia Africana*. Kaur and Bhutani (2011) have described that growth regulators must be associated with the basal environment for asymbiotic orchids germination. Our results have demonstrated that the use of NAA at different concentration in T_1, T_2 and T_4 media, have stimulated the protocorm induction of *Vanilla* species, and we can pronounce that NAA is a useful precursor growth regulator of orchids during asymbiotic germination. The more the concentration is higher, the more the high rate of induced protocorm. Our result on protocorm appearance after 20 days of seeding was better than that of Md Islam et *al*, (2014) with asymbiotic germination of *Vanda roxburghii* using the NAA which allowed 24 to 26 days for protocorm formation. This good results of NAA corroborates that mentioned by Kaur and Bhutani, (2011) and that of Cai *et al*, (2022). The combination of NAA with BAP was identified the

best for inducing *Vanill*a protocorms and the rate was the mixture concentration dependent. This combination could be recommended for orchid seeds asymbiotic germination and for Vanilla shoots multiplication, where it has shown positive results by giving green embryos in T_1 and T_2 media and the highest rate was observed after 3 months of seeding in T_2 medium (Figure 2). Therefore, asymbiotic germination of *V. planifolia* seeds are BAP-dependent. This observation is similar to that of Kauth *et al.*, (2008) and Erawati *et al.*, (2020) who observed that the combination of NAA and BAP has shown positive results for *Vanilla* asymbiotic germination and to other orchid species including *Dendrobium ovatum* (Thejaswini and Narasimhan, 2017), *Acampepremorsa*, *Agrostophylum khasianum* and *Phalaenopsis cornorerris* three native species of Bangladesh (Bhattacharjee and Islam, 2014), and *Cymbidium aloifolium* (Pradhan et *al*, 2013). Coconut water was not able to promote vanilla seeds germination even supplemented with NAA. MS/2 medium supplemented with 1% of Myo-inositol and 0.1% of Thiamine-HCl in R_3 medium has allowed 100% of protocorm development to plantlets at 25 days after embryos transfer (Figure 3). This type of medium has already been used by Ramanampamonjy (2004) and showed promising results for protocorm development of other genera *Aeranthes grandiflora* (Orchidaceae Family). Murashige and Skoog medium diluted by half is often used in plant cell and tissue culture thanks to its significant quantity in macro elements compared to other culture media (Nartop, 2018). Besides, the culture media rich in nitrogen, potassium and phosphorus promote buds neoformation as mentioned by Pasqua *et al.*, (2002). Additionally, an advantage was taken for rooting and leaf formation, which were developed simultaneously through the use of Myo-inositol and Thiamine-HCl combination associated with MS/2 medium hormone-free.

Nodal culture is widely used among the rapid multiplication techniques thanks to the ease plant and materials preparation. In addition, the node is the place where leaves, branches and aerial roots develop from the stems (Matkowsky, 2008). The presence of BAP in T_B and T_C media has significantly increased the nodes development ($p < 0.05$ *vs* T_A). According to Abebe *et al.*, 2009), the first node appeared 9 days after cultivation, where MS/2 was associated with the combination of GA_3 + BAP (T_B), however, for T_C medium associated with the BAP + Kin, it was observed in only 5 days. Therefore, its presence in the culture media enhances node development to plantlets by giving liana with 6 nodes in 45 days. Plantlets development was increased with all tested media ($p < 0.001$, Figure 4). The obtained results are found better and advantageous, does not consume more media than that of previous studies on *V. planifolia* nodal culture where Abebe et *al*, (2009) used 4 types of culture media to develop plantlets regeneration and the culture required acclimatized environment; and to that of De Oliveira *et al.* (2013) and Zuraida *et al.* (2013), who needed to add supplement subculture media every 30 days to the initial culture media.

5. CONCLUSION

Two simple, rapid and effective *in vitro* mass propagation techniques of *V. Planifolia* strains were developed by testing different concentrations of growth regulators and nutrient conditions. The supplemented MS medium with NAA (2 mg/ml) and BAP (2mg/ml) was the most efficient for asymbiotic germination of Vanilla seeds while that of Myo-inositol (1%) and Thiamine-HCl (0.1%) using MS/2 medium was found the best for plantlets regeneration showing 100% of seedling development,which does not require hormonal treatment for rooting. For nodal culture, the MS/2 medium supplemented with BAP (1 mg/ml) and Kin (1 mg/ml) was the most effective for node development. *In vitro* asymbiotic germination could be well applied for all Vanilla strains propagation and for its improvement since *V. Planifolia* is pollinated manually. On the other hand, the double beveled excision close to the nodes of both ends of the cutting, horizontally plated onto the medium could be a great of success, boosting the nutrients penetrating into the explant vessel by developing in a few days the first node, and accelerating new strains cloning which is much faster than micro cuttings.

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BIBLIOGRAPHY

1. Abebe, Z., Mengesha A., Teressa, A.,Tefera W., Efficient *in vitro* multiplication protocol for *Vanilla planifolia*using nodal explants in Ethiopia. *African Journal of Biotechnology*, 2009; 8(24): 6817-6821.
2. Bhattacharjee, B., Islam, S.M.S., Development of an efficient protocol for *in vitro* germination and enhancing protocorm-like body development in three indigenous orchid species in Bangladesh. *Asian Pacific Journal of Molecular Biology and Biotechnology*, 2014; 22(3): 209-218.
3. Cai, J., Chen, B., Li, W., Xu, P., Di, Y., Xu, H., Li, K., Transcriptome analysis reveals the regulatory mode by which NAA promotes the growth of *Armillariagallica*. *Plos one*, 2022; 17(11): e0277701. DOI: 10.1371/journal.pone.0277701
4. Cameron, K.M., Molina, M.C., Photosystem II gene sequences of psbB and psbC clarify the phylogenetic position of *Vanilla* (*Vanilloideae*, *Orchidaceae*). *Cladistics*, 2006; 22(3): 239-248. DOI: 10.1111/j.1096-0031.2006.00102.
5. De Oliveira S.O.D., Sayd R.M., BalzonT.A. and Scherwinski-Pereira J.E., A new procedure for *in vitro* propagation of vanilla (*Vanilla planifolia*) using a double-phase culture system. *Scientia Horticulturae*, 2013; 161: 204-209. DOI: 10.1016/j.scienta.2013.06.039
6. De Oliveira, R.T., Da Silva Oliveira, J.P., Macedo,

A.F., *Vanilla* beyond *Vanilla planifolia*and *Vanilla*× *tahitensis*: Taxonomy and Historical Notes, Reproductive Biology, and Metabolites. *Plants*, 2022; 11(23): 3311. DOI: 10.3390/plants11233311

7. Duval, M.F., Bory, S., Andrzejewski, S., Grisoni, M., Besse, P., Causse, S., Charon, C., Dron, M., Odoux, E., Wong, M., Diversité génétique des vanilliers dans leurs zones de dispersion secondaire. *Les Actes du BRG*, 2006; 181-196.
8. Erawati, D.N., Wardati, I., Humaida, S., Mawadah, Y., Ikanafi'ah, A., Ryana, W.M., Shoots multiplication of vanilla (*Vanilla planifolia*) with benzyl amino purine and kinetin modification. In IOP conference series: Earth and environmental science, 2021; 672(1): 012007. IOP Publishing. DOI: 10.1088/1755-1315/672/1/012007
9. Harinarivo, H.L., Tombozara, N., Rabemanantsoa, C., Randriamampionona, D., Lebret, D., Rasoarahona, J., Raonizafinimanana, B., Rasoarahona, F., Raherimandimby, M., Andrianjara, C., El-Jaziri, M., Quality of *Vanilla spp.* from Madagascar: evolution of the compound fingerprints from the green beans to the vanilla pods' preparation. *Biotechnology, Agronomy, Society and Environment*, 2024; 28(1): 28-36. DOI: 10.25518/1780-4507.20622.
10. Kaur, S., Bhutani, K.K., *In vitro* Propagation of *Dendrobiumchrysotoxum*(Lindl.).In: Floriculture and Ornamental Biotechnology; *Global Science Books*, 2011; 50-56.
11. Kauth, P.J., Dutra, D., Johnson, T.R., Stewart, S.L., Kane, M.E., Vendrame, W., Techniques and applications of *in vitro* orchid seed germination. In: Teixeira da Silva JA (ed) Floriculture, Ornamental and Plant Biotechnology: advances and topical issues. 1st edn. vol V., *Global Science Books*, Ltd., Isleworth, 2008; 45: 375-391.
12. Knudson, L., Nonsymbioticgermination of orchidseeds. *Botanical Gazette*, 1922; 73(1): 1-25. DOI: 10.1086/332956
13. Knudson, L., Germination of seeds of *Vanilla*. *American Journal of Botany*, 1950; 37(3): 241-247. DOI: 10.2307/2437909
14. Kumar, P.T., Stephen, F. andAlex, S., Studies on *invitro* seed culture *inVanilla*. *Indian Journal Horticulture*, 2009; 66(4): 547-548.
15. Matkowski A., Plant *in vitro* culture for the production of antioxydants. A review. In Biotechnology advances, 2008; 26: 548-560. Elsevier. DOI:10.1016/j.biotechadv.2008.07.001
16. Md. Islam R., Md.KabirK. R., Md. Hossain S., Md. Hossain F. et Md. Khalil I., Efficient *in vitro* Cultural Techniques for Seeds Germination of *Vanda roxburghii*, *in* World Journal of Agricultural Sciences, 2014; 10(4) *in* Bangladesh, 163-168. DOI: 10.5829/idosi.wjas.2014.10.4.1819
17. Nartop, P., Engineering of biomass accumulation and secondary metabolite production in plant cell and tissue cultures. *In* Plant metabolites and regulation under environmental stress, 2018; 169-194. *Academic press*. DOI: 10.1016/B978-0-12-812689-9.00009-1
18. Palama, T.L., Menard, P., Fock, I., Choi, Y.H., Bourdon, E., Govinden-Soulange, J., Bahut, M., Payet, B., Verpoorte, R., Kodja, H., Shoot differenciation from protocorm protocorm cultures of *Vanilla planifolia* (Orchidaceae): proteomic and metabolic responses at early stage. *BMC Plant Biology*, 2010; 10(1): 1-18. DOI: 10.1186/1471-2229-10-82
19. Pasqua, G., Manes, F., Monacelli, B., Natale, L., Anselmi, S., Effects of the culture medium pH and ion uptake in *in vitro* vegetative organogenesis in thin cell layers of tobacco. *Plant Science*, 2002; 162(6): 947-955. DOI: 10.1016/S0168-9452(02)00048-1
20. Philip, V.J., Nainar, S.A.Z., Structural Changes During the *in vitro* Germination of *Vanilla planifolia* (Orchidaceae). *Annals of Botany,* 1988; 61(2): 139-145. DOI: 10.1093/oxfordjournals.aob.a087536.
21. Pradhan, S., Regmi, T., Parmar, G., Pant, B., Effect of Different Media on *in vitro* Seed Germination and Seedling Development of *Cymbidium aloifolium* (L.) Sw. *Nepal Journal of Science and Technology,* 2013; 14(1): 51-56.
22. Ramanampamonjy, N.E., *Conservation des ressources génétiques d'Orchidées*.Ph D Thesis, Faculty of Sciences, University of Antananarivo, 2004; 134.
23. Ranadive, A.S., Vanilla cultivation. *In: Vanilla:The first international congress. Allured Publishing Corporation*, 2005; 25-32.
24. Salazar-Mercado, S.A., Asymbiotic seed germination and *in vitro* seedling formation of *CattleyamendeliiDombrain* (Orchidaceae). *Actaagronomica*, 2012; 61(1): 67-76.
25. Teixeira da Silva, J.A., Tsavkelova, E.A., Ng, T.B., Parthibhan, S., Dobránszki, J., Cardoso, J.C., Rao, M.V., Zeng, S., Asymbiotic*in vitro* seed propagation of *Dendrobium*. *Plant cell reports*, 2015; 34: 1685-1706. DOI: 10.1007/s00299-015-1829-2
26. Temjensangba, Deb, C.R., Effect of different factors on non-symbiotic seed germination, formation of protocorm-like bodies and plantlet morphology of *Cleisostomaracemiferum* (Lindl.) Garay. *Indian Journal of Biotechnology*, 2006; 5: 223-228.
27. Thejaswini, R., and Narasimhan, S., Undefined Organic Additives Stimulates *in Vitro* Seed Germination of *Dendrobiumovatum*(Willd.) Kraenzl, a Medicinal Orchid, January 2017. *International Journal of Pharma Medicine and Biological Sciences*, 2017; 6(1): 29-31. DOI: 10.18178/ijpmbs.6.1.29-31
28. Vasudevan, R., Van Staden, J., *In vitro*asymbiotic seed germination and seedling growth of *Anselliaafricana*Lindl. *ScientiaHorticulturae*, 2010; 123(4): 496-504. DOI: 10.1016/j.scienta.2009.11.010
29. Zuraida, A.R., Izzati, K.H.F.L., Nazreena, O.A., Zaliha,W.S.W., Radziah, C.M.Z.C., Zamri, Z.,

Sreeramanan, S., A simple and efficient protocol for the mass propagation of *Vanilla planifolia*. *American Journal of Plant Sciences*, 2013; 4: 1685-1692. DOI: 10.4236/ajps.2013.49205