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RESEARCH ARTICLE

An effective disinfection protocol for contamination control *in vitro* establishment of Mortiño (*Vaccinium floribundum* Kunth) and identification of endogenous microbes

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Abstract

The *Vaccinium* genus consists of a variety of berries with high nutritious components consumed worldwide leading to the development of micropropagation protocols to supply the demand. Mortiño, the Andean Blueberry (*Vaccinium floribundum* Kunth) is a wild berry that grows in high-altitude grasslands with nutritious and commercial potential in Ecuador. In this study, the use of PPM™ (Plant Preservative Mixture™) was effective controlling contamination for the *in vitro* establishment of *Vaccinium floribundum* Kunth in contrast to a conventional method using EtOH and Clorox. Stems were defoliated and cut into 1 cm segments, then immersed in liquid MS (Murashige & Skoog) supplemented with 5% v/v PPM™ without pH adjustment for 5 hours under constant shaking. After immersion, segments were transferred to flasks containing WPM (Woody Plant Media) medium supplemented with an additional 2 mlL^{-1} PPM™. Persistent microbial contaminants in the *in vitro* explants were isolated and identified through molecular methods and gene sequences analyzed using the GenBank database resulted in the identification of three bacterial species: *Methylobacterium sp*., *Methylobacterium radiotolerans*, and *Bacillus pumilus*. In addition, three fungal species were also discovered: Xylaria *sp*., *Xylaria feejeensis*, and *Diaporthe lutecens*. Additionally, a multiplication assay was made with the aseptic stems from the sterilization protocol to evaluate four different growth regulators: 2ip, kinetin, zeatin and meta-topolin. kinetin showed very low responses with a mean of 1.2 shoots per stem. The highest number of shoots per stem (9 shoots) was obtained with 5 mg L^{-1} 2ip. The use of zeatin and meta-topolin facilitated shoot proliferation with the following concentrations: 3 mg L^{-1} zeatin + 0.5 mg L^{-1} NAA (1-Naphthaleneacetic Acid) and 3 mg L^{-1} Meta-topolin + 0.5 mg L^{-1} NAA. These findings demonstrate the successful establishment of an *in vitro* disinfection and multiplication protocol for *V. floribundum*. Moreover, the identification of endogenous microbial communities highlights the complex interaction between native endophytes and plant tissues under *in vitro* conditions, offering a foundation for future studies on plant-microbe dynamics and their influence on micropropagation efficiency.

Keywords: *Vaccinium floribundum* Kunth; micropropagation; endogenous contamination; Mortiño; Andean blueberry.

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1. Introduction

Mortiño (*Vaccinium floribundum* Kunth) is a wild plant that belongs to the Ericaceae family. This family in Ecuador has a variety of 22 genus and 221 species, 44% of them are endemic of the country (Pedraza-Peñosa, et al., 2017). Mortiño is commonly referred as the "Andean blueberry" due to its prevalence in high-altitude grasslands across Colombia, Ecuador, Peru, and Venezuela, ranging from 1,600 meters to 4,300 m.a.s.l (Santamaria et al., 2012). It thrives in the Andean region of Ecuador, favoring temperatures between 8 °C to 16 °C and moist well-drained soils (Jørgensen et al., 1999). Mortiño is an evergreen shrub that reaches a maximum height of 2.5 meters

and produces small blue-black berries measuring 5 to 6 millimeters in diameter (Jørgensen et al., 1995). Within the *Vaccinium* genus, consisting of approximately 450 species, *V. floribundum* stands out for its nutritional and antioxidant properties abundant in phenolic compounds, anthocyanins, carotenoids, and ascorbic acid (Aleynova & Kiselev, 2023). Hence, mortiño berries offer potential health benefits, like the prevention of inflammatory disorders and cardiovascular diseases (Skrovankova et al., 2015). An analysis by Vasco et al. (2009) revealed that mortiño berries contain around 17% total carbohydrates, moisture (81%), fat (1%), protein (0.7%), and ash (0.4%). These berries are undomesticated therefore, locals harvest mortiño berries directly from high-altitude grasslands during a short period between October and November because it is as a key ingredient in traditional non-alcoholic beverages, particularly during the "Day of the Dead" celebration on November 2nd in Ecuador (Romero-Benavides et al., 2025). Additionally, mortiño berries are utilized as natural dyes and are processed into jams, ice cream, wine, and medicinal beverages (Santamaria et al., 2012). This Andean blueberry grows at high-altitude grasslands across Colombia, Ecuador, Peru, and Venezuela, where there are unique conditions that makes it special and part of the many super fruits that could be potentially commercialized. The Andean highlands, an important source of ecosystemic resources, are suffering from high temperatures and habitat loss (Cruz & Lasso, 2021). The alteration in climatic conditions due to anthropogenic activities are affecting the wild plant population such as the mortiño and many other undomesticated *Vaccinium* species from South America (Medina et al., 2019).

Micropropagation enables rapid and massive propagation, circumventing the need for traditional vegetative periods while preserving desired traits (Abdalla et al., 2022). The micropropagation process commences with the *in vitro* establishment of explants, requiring an efficient and thorough disinfection protocol. However, a major challenge arises in determining the optimal disinfection treatment for wild species such as mortiño which is traditionally vegetatively propagated in local nurseries using node cutting techniques. This approach is labor-intensive and strongly relies on the natural vegetative cycles of the plants. Furthermore, nurseries often encounter high mortality rates due to the difficulty in replicating the natural habitat and soil conditions required by mortiño. The establishment of an effective sterilization process for explants involves identifying the optimal and novel disinfectant for eliminating contaminants while promoting explant regeneration. Conventional surface sterilization methods utilize various chemical disinfectants such as commercial bleach, ethyl alcohol, hydrogen peroxide, calcium hypochlorite, silver nitrate, mercuric chloride, and benzalkonium chloride (Leifert et al., 1994). However, the concentration, type, and immersion durations of these disinfectants in a sterilization protocol depend on factors including the tissue type's tolerance to toxicity, the species involved, and the microbial load (Volk et al., 2022). Previous studies suggest that utilizing growing tissue rather than mature tissue is recommended to minimize the presence of endophytes (Park, 2021). While sterilization alternatives such as antibiotics and fungicides exist, concerns regarding phytotoxicity and the development of resistant microbial strains persist (Leifert et al., 1991).

Mortiño plants, harvested directly from their natural habitats, harbor a high microbial load, therefore the development of efficient sterilization protocols for undomesticated crops like mortiño will provide a solution to protect this fruit from excessive wild harvesting, which serves as a principal ingredient in numerous traditional and medical products.

PPM (Plant Preservative Mixture™) is recognized for its biocidal properties, effectively controls microbial contamination in plant tissue culture systems (Plant Cell Technology, 2025). Patent No. 5,750,402 covers its principal components: 5-chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-3(2H)-isothiazolone, along with a mixture of salts, which disrupt fundamental processes in microbial metabolism, including the Krebs cycle and electron transport chain (Compton et al., 2001). The use of PPM offers the advantage of eliminating the need for autoclaving culture media and glassware, thereby reducing labor time and production costs, while also avoiding potential modifications to components due to the high temperatures and pressures involved in autoclaving. Despite its role in media sterilization, PPM demonstrates heat stability, further enhancing its utility in micropropagation studies. Several studies have demonstrated the efficacy of PPM in micropropagation. Joshee et al. (2007) reported a 90% survival rate and successful explant establishment in *Centella asiatica* using a 2% PPM solution for disinfection and media supplementation. Similarly, Ďurkovič et al. (2010) achieved successful sprouting of axillary buds in *Liquidambar styraciflua* using WPM (Woody Plant Media) supplemented with PPM.

After the establishment of an aseptic protocol, the multiplication phase of micropropagation begins, hence the optimization of hormone concentrations to stimulate optimal explant proliferation is required. Hormones such as 6-(γ,γ-dimethylallylamino) purine

(2iP) and Zeatin have shown promising results in promoting shoot formation in Vaccinium species (Schuchovski & Biasi, 2019). Additionally, Meiners et al. (2007) found that Zeatin outperformed Thidiazuron and Meta-topolin in promoting shoot formation in *Vaccinium* species.

Interaction between the plant and its associated microorganisms, such as endophytic fungi and endogenous bacteria, plays a crucial role in the plant's growth and development (Aleynova & Kiselev, 2023). These microorganisms colonize the internal tissues of plants without causing harm, contributing to plant health and resilience, enhancing stress tolerance mechanisms by producing antioxidants that mitigate oxidation by UV radiation or synthetizing secondary metabolites that protect plants from hydric stress (Barrera et al., 2020)

Endophytic fungi, residing within the plant tissues without causing harm, have been recognized for their positive impact on plant health and stress tolerance (Rani et al., 2022). Therefore, some species of *Vaccinium*, such as *V. corymbosum*, *V. oldhamii*, and *V. myrtillus*, have been documented to harbor a diverse array of root-associated fungi, particularly ericoid mycorrhizas. These fungal associations play an important role in nitrogen sequestration and mobilization, plant growth, stress tolerance and protections against pathogens (Yang et al., 2002; Baba et al., 2016; White et al., 2019). Likewise, endogenous bacteria residing within the plant contribute to nutrient acquisition and play essential roles in promoting plant growth (Dudeja et al., 2021; Rosenblueth & Martínez-Romero, 2006).

This study investigates the efficacy of Plant Preservative Mixture™ (PPM™) in controlling microbial contamination and eliminating endogenous microorganisms during the *in vitro* establishment of *Vaccinium floribundum* Kunth (mortiño). Additionally, the effects of four cytokinins-2iP, kinetin, zeatin, and meta-topolin- on shoot proliferation are evaluated to optimize micropropagation conditions. The study also aims to identify endophytic microorganisms capable of surviving in PPM™ -supplemented media. Ultimately, this work seeks to establish an effective disinfection protocol for *V.floribundum* while providing insights into its endophytic community under in vitro conditions.

2. Methodology

Plant material and disinfection method

Mortiño plants were obtained from a nursery located in Machachi 170350, Ecuador. They were maintained in the greenhouse at 22 °C with low light intensity for about 40 days irrigated two times per week. Stem segments of 1cm length were cultured in glass vessels containing 25 ml of Woody Plant Medium (McCown & Llyod, 1981) with the addition of 50 mg L^{-1} of Myo inositol, 50 mg L^{-1} ascorbic acid, 30 g L^{-1} sacarose and 3 g L^{-1} Phytagel™, (Sigma Aldrich Inc, St. Louis, MO, USA). Medium pH was adjusted to 5.8 using KOH prior the gelling agent and autoclaving at 121 °C for 18 min. The stem segments were cultured under controlled conditions in a growth room with a temperature of 26 °C with 16 hours of light given by tubular fluorescent lamps and 8 hours of darkness. The contamination and survival rate were registered during the first 30 days and the next 30 days corresponding to a subculture in new media following the protocol.

Stem segments were cut from the different mortiño plants maintained in the greenhouse. All the segments' leaves were removed, and the segments were cut equally into 1cm in length. Two different disinfection protocols were tested. It is important to avoid new plant shoots because they are very susceptible to the disinfection process or the old mature wood stems that are too wide to eliminate endogenous contamination.

Protocol #1 (P1) is the control method that uses EtOH (70%) and Clorox (2%) in the Laminar Flow Hood (LFH). Protocol #2 (P2) involves the use of PPM™ with a few adjustments of the recommendations given by Plant Cell Technology (2025). The details of the two tested protocols are described in Figure 1. The experiment was performed with three replicates of 10 stem segments in each protocol and the whole experiment was repeated three times to ensure result reproducibility.

Isolation, cultivation, and identification of endophytic bacteria/fungi from *in vitro* plants

After a 2-week period following the *in vitro* introduction of *Vaccinium floribundum*, some flasks showed contamination by microorganisms. These contaminants were subsequently isolated and cultured on nutrient agar for bacterial strains and Potato Dextrose Agar (PDA) for fungal species. Genomic DNA extraction was performed utilizing the rapid NaOH method, as described by (Osmundson et al., 2013). Afterwards, DNA were amplified by PCR (Eppendorf thermal cycler, Hamburg) composed of 7.5 µl Green Go Taq DNA polymerase (2x) (Fisherscientific USA), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM) (Invitrogen, USA), nuclease free water up to 15 µl. The 16s gene and the ITS region were amplified for bacteria and fungi, respectively. The resultant products were subjected to genotyping analysis by Macrogen (Seul, Korea).

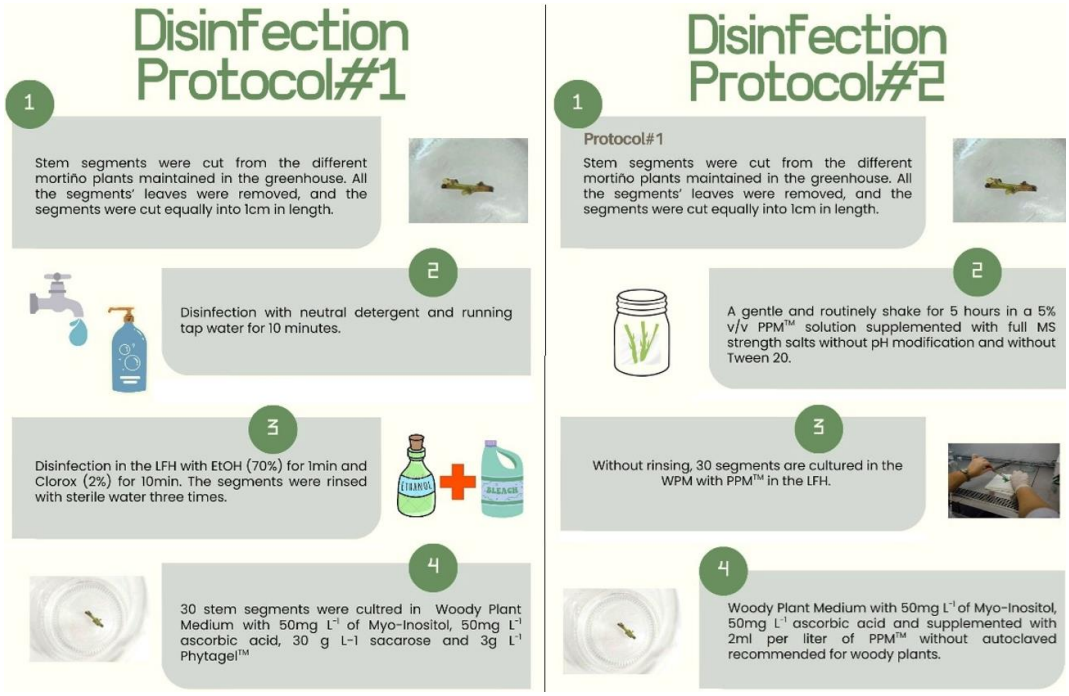

Figure 1. Disinfection protocol description.

Multiplication trial of mortiño stem segments

The aseptic stem segments obtained from the results of the sterilization experiment were transferred to different multiplication media. The stem segments were cultured for 16 weeks and subcultured every 4 weeks. The number of shoots was registered at the end of each 16 weeks period due to the slow growth of mortiño segments following the method by (Cobo et al., 2018). The composition of the culture media is described in Table 1. A second stage of this multiplication trial was performed to evaluate the effect of two concentrations of Metatopolin and Zeatin in the number of shoots in mortiño aseptic segments as shown in Table 2. The results from the first multiplication trial were subjected to variance analysis (ANOVA) and Tukey test to determine statistical significance between the different treatments using R (version 4.2.2; R Core Team, 2022) program. The results from the second multiplication trial were subjected to a two tailed T test using R (version 4.2.2; R Core Team, 2022) program.

Table 1
Treatments of the first multiplication trial

Treatment	Media composition
T1	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**5 mg L^{-1} 2ip**
T2	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**3 mg L^{-1} 2ip + 0.5 mg L^{-1} NAA**
T3	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**1 mg L^{-1} Kinetin**
T4	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**3 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA**

Table 2
Treatments of the second multiplication trial

Treatment	Media composition
T1	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**3 mg L^{-1} Meta-topolin + 0.5 mg L^{-1} NAA**
T2	WPM + 50 mg L^{-1} Myoinositol + 50 mg L^{-1} ascorbic acid +**3 mg L^{-1} Zeatin + 0.5 mg L^{-1} NAA**

3. Results and discussion

Sterilization assay

In tissue culture studies it is vital to establish an efficient disinfection protocol to reduce time, production costs and optimize resources in every experiment. Besides there are a several disinfectant reagents and each explant species/genotype responses differently to them. The first challenge of working with wild crops is to eliminate endogenous contamination. In this study the use of PPM for surface and media sterilization was evaluated and compared to the traditional disinfection protocol which includes EtOH and Clorox immersions. This study followed the concentration suggested by the company (Plant Cell Technology, 2025) for the surface sterilization which is 5% v/v PPM in constant shaking for 4 - 12 hours however, in terms of logistics we chose 5 hours and the addition of 0.2% PPM in culture media following the recommendation for woody plants.

Table 3 shows the results obtained from the sterilization essay using the two protocols and the replications.

Table 3
Sterilization Assay Results

Protocol	Contamination Percentage	Number of death segments	Number of days	Trial
P1	36.67%	4	60 days included one subculture	Experiment#1
P2	3.33%	4		
P1	70%	0	60 days included one subculture	Experiment#2
P2	6.67%	1		
P1	53.33%	0	60 days included one subculture	Experiment#3
P2	3.33%	0		

The results from using PPM as biocide were effective to obtain 95.56% of aseptic stem segments in contrast with 46.67% utilizing EtOH and Clorox. There are few studies published of micropropagation of *Vaccinium floribundum* Kunth, one of them reported a 40% of contamination of the explants using EtOH (70%) and Clorox (2.5%) with 3 - 4 drops of Tween 20 (Torres et al., 2010). Another study obtained 100% decontamination with the use of a fungicide Carbendazim (0.05%) in MS (Murashige & Skoog, 1962) media with a previous immersion of ClO_2 (2%) for 10 minutes without mortality of the explants (Llivisaca et al., 2020). Huh et al. (2015) reported that the addition of 1 mg L^{-1} PPM in culture media was effective to control bacteria and fungi growth in blueberries (*Vaccinium corymbosum*). A similar evaluation of the use of PPM was made by Mahmoud et al. (2016), where the best surface sterilization treatment for nodal segments of *Cestrum nocturnum L.* was a constant shaking of 4 hours in a range solution 4-6% PPM reporting a total decontamination and 70% survival. Although, they only tested surface sterilization in the previous study, another study reported 97% of efficiency in microbe control in *A. confusa* adding 2 ml L^{-1} PPM into culture media (Ho et al., 2022).

The presence of fungi growth was the principal problem following protocol#1, on the other hand, this type of contamination appeared using PPM only if necrosed segments were used. Nevertheless, the aseptic stem segments obtained from using both protocols presented normal growth characteristics. These results indicate that the use of PPM following protocol#2 does not affect plantlet regeneration in mortiño stem segments.

Multiplication trial

A variety of micropropagation protocols have been established for commercial *Vaccinium* species in which analyze the effect of different types of cytokinins on shoot proliferation. Zeatin and 2ip are cytokinins reported with better responses in shoot proliferation on many *Vaccinium* species nevertheless there are no studies reporting the effect of kinetin on multiplication of mortiño.

The results registered after 16 weeks presented a mean of 9.83 shoots per stem segment adding 5 mg L^{-1} of 2ip. This result is similar to a mean of 9 shoots per bud obtained by (Cobo et al., 2018) in micropropagation of mortiño with 5mg L^{-1} of 2ip combined with 0.1 mg L^{-1} NAA. On the other hand, in this study a mean of 1.4 shoots per segment adding 3 mgL^{-1} 2ip + 0.5 mg L^{-1} NAA to media differs to the results reported by (Cobo et al., 2018) obtaining 4.76 shoots using 3 mg L^{-1} 2ip without NAA. The micropropagation of European lingonberry cultivars (ssp. *vitis-idaea*) and Canadian wild clones (ssp. *minus*) was efficient with media containing 2.5 mgL^{-1} 2iP or 1.25 mg L^{-1} zeatin (Debnath & McRae, 2001). Another study reported a multiplication rate of 6.73 at the fifth subculture of highbush blueberry (*Vaccinium corymbosum* L.) 'Toro' cultivar with media containing 3 mg L^{-1} zeatin and 2 mg L^{-1} 2iP (Georgieva et al., 2016). This could suggest the combination of these two cytokinins for a future study in multiplication of mortiño. The lowest number of shoots were obtained using Kinetin (Table 4).

Table 4
Results from the first multiplication trial

Treatment	Hormone	Number of shoots (mean)	p-value
T1	5 mgL^{-1} 2ip	9.83	0.000358
T2	3 mgL^{-1} 2ip + 0.5 mg L^{-1} NAA	1.40	
T3	1 mg L^{-1} Kinetin	1.20	
T4	3 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA	1.20	

These results have similarity to the ones reported by Schuchovski & Biasi (2019) where BAP and Kinetin added to media of *in vitro* multiplication of 'Delite' Rabbiteye Blueberry microshoots showed very low responses. The number of shoots per treatment was evaluated with a one-way ANOVA analysis to determine statistical differences between treatments. A Tukey test was performed indicating T1 as the treatment with a statistical difference between each treatment as shown in Figure 2.

A second multiplication trial was performed to determine the effect of zeatin and meta-topolin in mortiño shoot proliferation. meta-topolin belongs to a group of aromatic cytokinins, that could be

considered as an alternative to other common hormones used for *in vitro* multiplication such as trans-zeatin, 2ip and kinetin (San José et al., 2021). Shoot proliferation in cranberries (*Vaccinium macrocarpon* Ait.) was obtained with media supplemented with low concentrations of zeatin (0.5 - 0.9 mg L^{-1}) (Debnath, 2008). In the case of blueberries (*Vaccinium corymbosum* L.'Duke'), (Cappelletti & Mezzetti, 2014) reported a high axillary bud proliferation adding 2 mgL^{-1} of zeatin. Meneses et al. (2022) reported better results in multiplication and growth of mortiño shoots using the cytokinins Trans-zeatin and zeatin in low doses. An evaluation made in blueberry (*Vaccinium corymbosum*) and lingonberry (*Vaccinium vitis-idaea)* cultivars by Meiners et al. (2007) determined that zeatin (4.38 mg L^{-1}) as superior to TDZ and meta-topolin in promoting adventitious shoot formation.

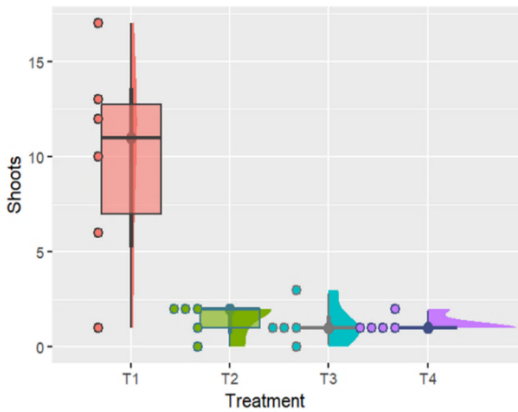

Figure 2. Violin plot of first multiplication trial.

The results of the number of shoots obtained were analyzed with two sample T-test. The two treatments presented similar means without statistical significance difference (Table5). These results could be useful for future studies using meta-topolin. Figure 3 shows the distribution shape of the data obtained from the second multiplication trial.

Table 5
Second multiplication trial results

Treatment	Concentration	Number of Shoots (mean)	p-value
Meta-topolin	3 mg L^{-1} Meta-topolin +0.5 mg L^{-1} NAA	2.48	0.9244
Zeatin	3 mg L^{-1} Zeatin + 0.5 mg L^{-1} NAA	2.53	

Isolation, cultivation, and identification of endophytic bacteria/fungi from *in vitro* plants results

Analysis of the sequenced genes revealed a limited diversity of microorganisms capable of growing in WPM medium supplemented with PPM at 5 ml L^{-1}.

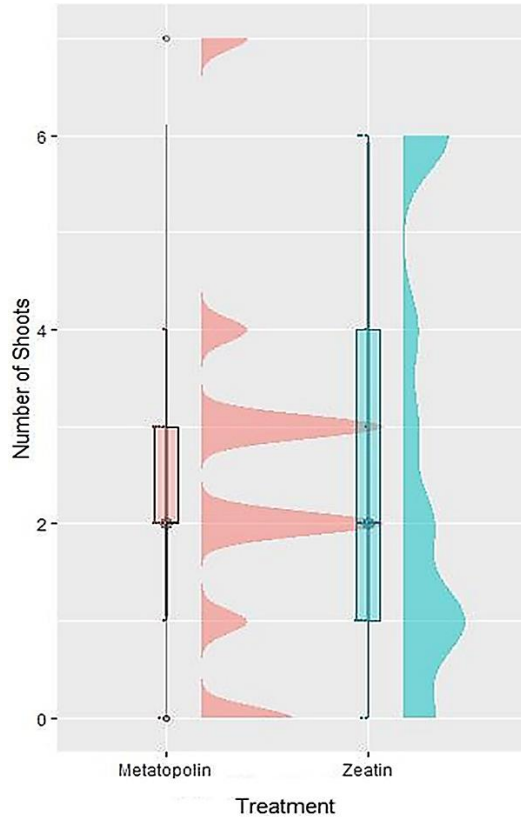

Figure 3. Violin plot of second multiplication trial.

The most abundant genera identified were *Methylobacterium* among bacteria and *Xylaria* among fungi (Figure 4). Gene sequence analysis using the GenBank database led to the identification of three bacterial species: *Methylobacterium sp.*, *Methylobacterium radiotolerans*, and *Bacillus pumilus*. Additionally, three fungal species were identified: *Xylaria sp.*, *Xylaria feejeensis*, and *Diaporthe lutecens*. The genus *Methylobacterium* is well known for its ubiquitous distribution across diverse environments, including soil, water, and air (Cordovana et al., 2019). It also constitutes an essential component of the plant-associated microbiome (Palberg et al., 2022). Notably, the pink-colored colonies characteristic of *Methylobacterium* have been reported to enhance plant metabolism and improve UV radiation absorption. This suggests that secondary metabolites, produced by *Methylobacterium* may contribute to the radiotolerance of plants growing in the Andean highlands (Yoshida et al., 2017).

Furthermore, studies by Gholizadeh (2012) and Gamit et al. (2023) highlight a direct correlation between the presence of *Methylobacterium* and increased flavonoid and carotenoid content in plant tissues, further emphasizing its role in plant health and resilience.

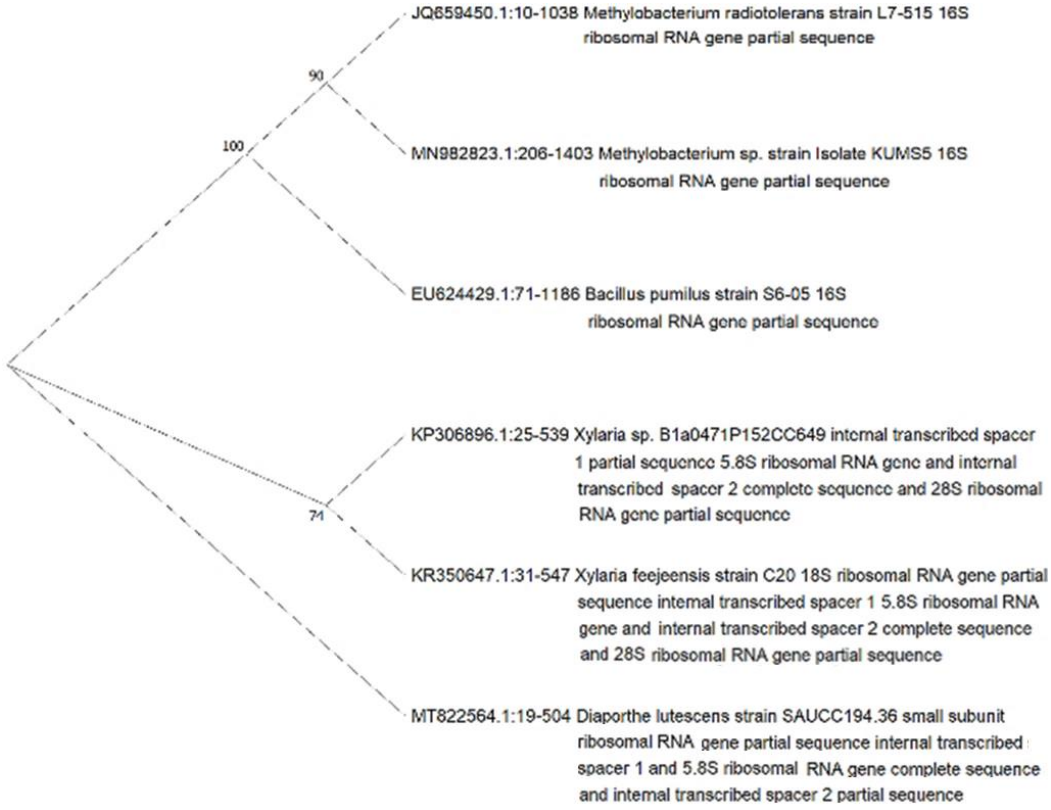

Figure 4. Phylogeny of sequences retrieved from microorganisms isolated from *in vitro* micropropagation via organogenesis of mortiño (*Vaccinum florubumdun* Kunth). Full reference gene sequences were selected from the NCBI nucleotide database. The tree was drawn in Mega11 using the neighbor-joining method. Scale bar indicates 0.02 substitutions per site.

It has been reported that *Bacillus pumilus* exhibits biocontrol properties against phytopathogens, attributed to its production of lipopeptides, enzymes and organic volatile compounds. Additionally, it promotes plant growth and protection, further enhancing its potential as a beneficial microorganism (Dobrzyński et al., 2023).

Xylaria is an endophytic genus associated with seed plants that produce secondary metabolites that control other pathogenic fungi species such as *F. oxysporum*, with a reduced disease severity (31.71%) when compared to untreated tomato seeds (57.13%). Furthermore, soil treated with 10% *X. feejeensis* mycelium showed 55.55% severity of fusarium wilt in tomato plants when compared with 91.66% in un-treated soil (Macías-Rubalcava & Sánchez-Fernández, 2017; Brooks et al., 2022). The genus *Diaporthe* has been reported as a pathogens, endophyte and saprophyte fungi that produces secondary metabolites (Hilário & Gonçalves, 2022).

4. Conclusions

The establishment of an effective disinfection protocol is the starting point to a successful micropropagation protocol. In this study, an efficient disinfection protocol for the *in vitro* establishment of *Vaccinium floribundum* Kunth was developed using PPM^{TM}. The protocol involves a surface sterilization of a constant shake for 5 hours in a 5% v/v PPM^{TM} solution supplemented with full MS strength salts without pH modification plus the addition of 2mlL^{-1} in culture medium recommended by Plant Cell Technology without affecting growth characteristics. A multiplication trial was conducted evaluating the effect of four cytokinins in which a concentration of 5 mg L^{-1} 2ip showed the highest shoot proliferation with a mean of 9.83 shoots per segment. Nevertheless, zeatin has been proved to be successful in multiplication of many commercial *Vaccinium* berries but in very low doses, in this case 3 mg L^{-1} zeatin + 0.5 mg L^{-1} NAA presented a mean of 2.53. Future studies of the use of meta-topolin in *Vaccinium* species, specially mortiño are required, focusing on different concentrations of the hormone as an alternative to other common cytokinins.

The study of endophytic bacteria and fungi present in *Vaccinium floribundum* Kunth and their implications in its *in vitro* cultures demonstrated the intricate relationship between plants and their microbial symbionts. Through a comprehensive analysis of endophytic microorganisms emerged after the explant disinfection process with PPM, it

becomes evident that *Vaccinium floribundum* Kunth harbors a moderate diverse array of bacterial and fungal species within its tissues, potentially contributing to the host's health and resilience in its natural habitat.

The establishment of *in vitro* cultures of *Vaccinium floribundum* Kunth presents a valuable platform for exploring the dynamics of endophyte-plant interactions under controlled conditions. Besides, it is important to acknowledge the complexities associated with *in vitro* systems, including potential shifts in microbial composition and functionality compared to their native environments. As such, future studies should aim to characterize the stability and functionality of endophytic communities in *in vitro* cultures over successive subcultures and explore strategies to optimize their beneficial effects on plant growth and performance. Further research efforts in this area are warranted to unlock the full potential of endophytic microorganisms in supporting the growth and resilience of *Vaccinium floribundum* Kunth to its harsh native environmental conditions.

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Authors contribution

L. Moreno-Peña and **K. Hidalgo-Escobar** wrote the original draft, carried out research/investigation, and data analysis. **J. M. Cevallos-Cevallos** and **E. Sánchez-Timm** were involved in the Funding acquisition, experimental design, supervision, writing, review and editing.

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