









Micropropagation of *Vaccinium meridionale* Sw.: Interaction between basal media and cytokinins, physiological quality of shoots, and *ex vitro* rooting

Eyner Huaman^{a,b,*} , Carlos Muñoz^b , Loreto Prat^b , Jegnes Benjamín Meléndez-Mori^a, Raúl Vargas^a , Carmen N. Vigo^a, José Jesús Tejada-Alvarado^c , Ángel Fernando Huaman-Pilco^a , Manuel Oliva-Cruz^{a,**}

^a Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas 01001, Peru

^b Facultad de Ciencias Agronómicas, Universidad de Chile, Santa Rosa 11315, La Pintana, Santiago, Chile

^c Estación Experimental Agraria Amazonas, Dirección de Recursos Genéticos y Biotecnología, Instituto Nacional de Innovación Agraria (INIA), km 3.5 (Carretera Aeropuerto), Chachapoyas 01001, Peru

ARTICLE INFO

Keywords:

Andean blueberry
Andean genetic resources
Auxins
Clonal propagation
Plant tissue culture
Shoot regeneration

ABSTRACT

Vaccinium meridionale is an Andean species of high nutraceutical value whose conventional propagation is limited by its low multiplication rate. In this study, an integrated micropropagation protocol was developed, encompassing *in vitro* establishment through *ex vitro* rooting. During the establishment phase, fungal contaminants were identified, detecting genera such as *Diaporthe*, *Fusarium*, *Colletotrichum* and *Phoma*. In the multiplication phase, the basal media Driver and Kuniyuki (DKW), Woody Plant Medium (WPM) and Murashige and Skoog (MS) were evaluated, supplemented with zeatin (Zea), 2-isopentenyladenine (2iP), meta-topolin (mT) and thidiazuron (TDZ), all applied at equimolar concentrations of 2.5 μ M. Morphogenic parameters, photosynthetic pigment content, SPAD index and elemental composition of regenerated tissues were quantified. DKW medium supplemented with Zea or 2iP promoted the formation of 9–10 shoots, with lengths of 2.5–2.9 cm, SPAD values of 35–36, and chlorophyll content >31 μ g/mL. The accumulation of P, Ca, Mg, Fe, Zn, Cu and Mn in the tissues varied widely among treatments. During rooting, the application of 500 ppm naphthaleneacetic acid (NAA) induced the formation of longer roots, as well as vigorous and elongated shoots with a high number of leaves. This optimized protocol provides a valuable tool for the propagation of *V. meridionale*, with potential applications in conservation, genetic improvement and commercial plant production.

1. Introduction

The Andean blueberry (*Vaccinium meridionale* Swartz) is a shrub of the Ericaceae family endemic to the Andean regions of South America, inhabiting environments ranging from cloud forests to shrublands located between 1,500 and 3,000 m a.s.l. in countries such as Ecuador, Colombia, Venezuela, and Peru^{1–3}. In recent years, several studies have highlighted that fruits of *V. meridionale* contain high levels of anthocyanins, procyanidins, flavonoids, polyphenols, and hydroxycinnamic acids, in addition to exhibiting strong antibacterial and antioxidant

activity^{4–6}. For instance, Garzón et al.² demonstrated that Andean blueberry extracts have strong antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*. Furthermore, these fruits have been reported to reduce the viability and proliferation of human colon cancer cells⁷, display cytotoxic properties against leukemic cell lines^{8,9}, and exert anti-inflammatory and antioxidant effects in overweight women^{10,11}. Other studies revealed the potential of Andean blueberry polyphenols for blood pressure regulation and their adjuvant anti-inflammatory effect when combined with aspirin^{8,11}. Altogether, these benefits have established the Andean blueberry as a nutraceutical food,

* Corresponding author at: Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas 01001, Peru.

** Corresponding author.

E-mail addresses: eyner.huaman@untrm.edu.pe (E. Huaman), carlosmunozschick@uchile.cl (C. Muñoz), mlprat@uchile.cl (L. Prat), jegnes.melendez@untrm.edu.pe (J.B. Meléndez-Mori), raul.vargas.epg@untrm.edu.pe (R. Vargas), canavimes7694@gmail.com (C.N. Vigo), tejada.01634@gmail.com (J.J. Tejada-Alvarado), angel.huaman@untrm.edu.pe (A.F. Huaman-Pilco), manuel.oliva@untrm.edu.pe (M. Oliva-Cruz).

<https://doi.org/10.1016/j.jgeb.2025.100633>

Received 22 September 2025; Accepted 3 December 2025

Available online 22 December 2025

1687-157X/© 2025 The Author(s). Published by Elsevier Inc. on behalf of Academy of Scientific Research and Technology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

both fresh and processed¹².

Due to its multiple properties and uses, demand in both local and international markets has increased considerably. However, meeting market needs poses significant challenges, as fruits are mainly collected from wild populations and small-scale plots with low yields, which limits year-round availability and uniformity in berry size¹³. *V. meridionale* has shown remarkable adaptability to specific cultivation conditions, particularly its ability to thrive in acidic soils (pH 4.4–5.4), making it an ideal crop for Andean regions where fruits are scarce and soils are predominantly acidic^{2,14}.

Expanding production areas necessitates the development of improved propagation methods for the species. Traditionally, *V. meridionale* is propagated through seeds, shoot cuttings, and air layering techniques, which face difficulties such as low germination rates, poor rooting percentages, lack of uniformity, long propagation periods, and a risk of disease dissemination. For example, seed germination rates below 25 %¹⁵ and rooting rates between 18 % and 33 %¹⁶ have been reported, with waiting periods of 1–2 years to obtain plants suitable for field establishment¹⁷. In this context, micropropagation has proven to be a valuable tool for the rapid, efficient, and disease-free production of multiple plantlets^{18–20}. However, the success of this technique is strongly influenced by factors such as genotype/species, culture medium composition, growth regulators, and culture environment²¹. Reports of successful micropropagation for *V. meridionale* have been scarce, especially when nodal segments or explants from adult plants have been used. Studies conducted during the establishment and multiplication phases have shown that WPM (Woody Plant Medium)²² and AND (Anderson Medium) have induced oxidative necrosis of up to 70 % and have promoted the formation of thin, reddish stems and leaves^{23–25}, while MS medium (Murashige and Skoog)²⁶ has resulted in poor elongation²³. Conversely, in *V. arboreum*, a species closely related to *V. meridionale*, the DKW (Driver and Kuniyuki)²⁷ medium has been reported to promote the formation of vigorous shoots with large leaves and rapid elongation²⁵. This highlights the need for research that is species-, variety-, and genotype-specific.

Growth regulators, essential chemicals in micropropagation, also play critical roles¹⁹. For instance, cytokinins regulate a variety of cellular processes involved in shoot induction²⁸, and their effects vary depending on type, classification, and dosage. Regulators such as zeatin, 2iP, and TDZ have been widely used in *Vaccinium* species; for example, 2iP applied at 20 mg L⁻¹ induced small shoots, high callus formation, and hyperhydration in *V. meridionale*²⁹. Similarly, TDZ has been applied to multiply recalcitrant *Vaccinium* species^{24,30}. Other hormones, such as *meta*-topolin (mT), have shown greater stability in some woody species, improving multiplication, maintaining genetic stability, reducing shoot tip necrosis, delaying senescence and hyperhydricity, and alleviating physiological disorders^{31,32}. Thus, the current trend is to optimize protocols using cost-effective cytokinins that enhance shoot regeneration³³.

Knowing that species (genotypes and cultivars) respond differently to the type and concentration of media or growth regulators^{25,34,36}, and that multiplication methods suitable for one cultivar may not be optimal for others³⁵, the present research aims to provide relevant information on the micropropagation process of *V. meridionale*. In this context, the objective of this study was to establish a micropropagation protocol for *V. meridionale*, beginning with establishment, where contaminants were identified, and subsequently evaluating during multiplication the interaction of basal media and types of cytokinins, while also incorporating indicators of physiological development, photosynthetic pigments, nutrient content, and *ex vitro* rooting capacity. Through a sequential approach, both early morphological responses and shoot biomass yield and functional quality were assessed to optimize an integrated micropropagation protocol applicable to this native species of high nutraceutical value.

2. Materials and methods

2.1. Plant material

Shoots of *Vaccinium meridionale* Sw. were obtained from two sources: (i) plants collected directly from their natural habitat, which were taxonomically identified by Tineo et al.³⁶ through morphological and molecular analyses; and (ii) plants obtained by sexual reproduction under controlled greenhouse conditions. From the latter, genotype INDES-002 (Fig. 1) was selected, corresponding to a five-year-old plant in the productive stage, grown in 10 L containers with a slightly acidic substrate (pH 5.34) and 7.69 % organic matter content.

2.2. *In vitro* establishment and identification of contaminants in field explants

Apical shoots of adult plants collected in their natural habitat were subjected to surface disinfection protocols that included immersion in ethanol (70 %, 0.5–2 min), sodium hypochlorite (1.5–3%, 3–15 min), calcium hypochlorite (0.25 %, 3–4 min), and mercuric chloride (0.1 %, 30–60 s). Subsequently, explants were cultured on WPM medium of Lloyd and McCown²² (L449, PhytoTechnology, Shawnee Mission, KS, USA), adjusted to pH 5.2. At 15 and 30 days, their condition was evaluated, and those with fungal contamination were isolated on Potato Dextrose Agar (PDA) medium for molecular identification using the ITS region of ribosomal DNA. Due to the high level of contamination and oxidation, these explants were not used in the subsequent multiplication phases.

2.3. *In vitro* establishment with greenhouse explants

The vigorous and productive mother plant (Fig. 2A) was treated with Protexin® (Carbendazim, 2 mL L⁻¹) every seven days. Young shoots (12 cm) were collected (Fig. 2B), leaves were removed, leaving 2 mm of petiole (Fig. 2C), and the shoots were washed with water and detergent for 30 min. Stems were trimmed to 6 cm and disinfected under a laminar flow hood with 70 % ethanol (1 min), 1 % sodium hypochlorite (5 min), and 0.1 % mercuric chloride (2 min), using Tween 20 as a surfactant. Finally, they were rinsed with sterile deionized water, and the ends were removed.

Explants (3 cm) were transferred to tubes containing 10 mL of WPM medium, supplemented with vitamins, sucrose (30 g L⁻¹), agar (6 g L⁻¹), and ascorbic acid (1.5 mg L⁻¹) (Fig. 2D), until shoot induction was achieved (Fig. 2E)

2.4. *In vitro* multiplication assays and growth conditions

Fifteen treatments resulted from the combination of three basal media: DKW²⁷ (D2470), WPM, and Murashige and Skoog²⁶ (M519), with four cytokinins: zeatin (Zea) (Z125), the isoprenoid 2-isopentenyladenine (2iP) (D525), the aromatic *meta*-topolin (mT) (T841), and the synthetic thiazuron (TDZ) (T888), plus a control without hormones for each medium. All cytokinins were applied at equimolar concentrations of 2.5 µM, determined from preliminary assays with zeatin at different concentrations (0.5, 1.0, 2.0, 2.5, 4.0, 6.0, 8.0, and 18 µM). All reagents were supplied by PhytoTechnology Laboratories, Shawnee Mission, KS, USA.

From a container with hormone-free WPM culture medium (Fig. 2F), 1.5 cm nodal segments with three to five axillary buds were cultured, as shown in (Figs. 2G and 2H) and grow in magenta flasks (Cat. No. 310074, SPL Life Sciences Korea) with 40 mL of medium until development according to the treatments (Fig. 2I). Media were adjusted to pH 5.2 and sterilized (121 °C, 20 min, 105 kPa). Cultures were maintained at 25 ± 1 °C under a 16 h photoperiod (3000 lx). Subcultures were performed monthly maintaining the study treatments.³⁷



Fig. 1. Mother plant of *V. meridionale* (Genotype INDES-002) grown in a greenhouse. A) Front view of the plant, B) Top view, C) Flowering, D) Fruit formation.

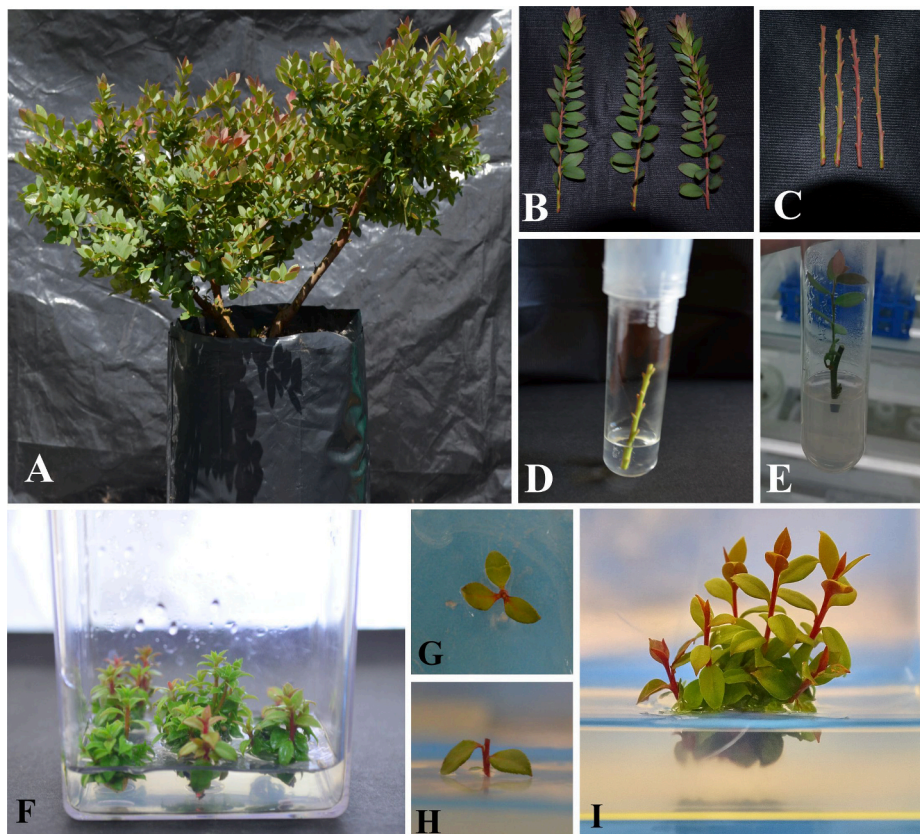


Fig. 2. Aseptic establishment and initial *in vitro* multiplication: A) mother plant, B) collected shoots, C) leaf removal for explant preparation, D) explant placed on culture medium, E) induction of axillary shoots, F) Shoots in hormone-free medium, G) top view of nodal segments, H) front view of nodal segments, I) induction of shoots in the treatments.

2.5. Evaluation of shoot morphology, callus formation, and biomass

Morphological evaluations were carried out at 30 and 60 days of *in vitro* culture. At 30 days, the number of shoots per explant and the percentage of callus formation were recorded. Digital images were taken to measure the length of the longest shoot and the areas occupied by shoots and callus in the culture medium, using ImageJ software^{38,39}, with regions manually delimited using the “freehand” tool. In addition, fresh callus weight was recorded at the time of monthly subculturing.

At 60 days, the total number of shoots per explant and their average length were evaluated. Fresh and dry shoot weights were also determined, with samples dried at 60 °C for 72 h. From these values, dry matter content was calculated using the formula^{40,41}:

$$\text{Dry matter content (\%)} = 100 \times (\text{dry weight} / \text{fresh weight})$$

2.6. Determination of photosynthetic pigments and SPAD index at 60 days

The Soil Plant Analysis Development (SPAD) index, a numerical indicator used to estimate the relative chlorophyll content in leaves, was measured by taking readings from three central leaves using a SPAD-502 Plus meter (Konica Minolta, Japan). To quantify chlorophylls and carotenoids, 0.2 g of fresh tissue was extracted with 80 % acetone, MgCO₃, and centrifuged (2500 rpm, 10 min, 10 °C). Absorbances were measured at 663.2, 646.8, and 470 nm with a spectrophotometer (GENESYS 180, Thermo Fisher Scientific, USA). Concentrations were calculated using the equations of Lichtenthaler and Buschmann⁴²:

$$\text{Chlorophyll } a \text{ (}\mu\text{g}\cdot\text{mL}^{-1}\text{): } 12.25 A_{663,2} - 2.79 A_{646,8}$$

$$\text{Chlorophyll } b \text{ (}\mu\text{g}\cdot\text{mL}^{-1}\text{): } 21.50 A_{646,8} - 5.10 A_{663,2}$$

$$\text{Chlorophyll } a + b \text{ (}\mu\text{g}\cdot\text{mL}^{-1}\text{): Chlorophyll } a + \text{Chlorophyll } b$$

$$\text{Carotenoids (}\mu\text{g}\cdot\text{mL}^{-1}\text{): } (1000 A_{470} - 1.82 \text{ Chl } a - 85.02 \text{ Chl } b) / 198$$

2.7. Quantification of nutrients in *in vitro* explants at 60 days

Mineral nutrient analysis was performed on lyophilized leaf tissue using a 4.5 L FreeZone lyophilizer (Labconco Corp., USA). Samples were finely ground, and 0.2 g was weighed for acid digestion with a nitric acid–perchloric acid mixture in a 6:2 ratio (v/v). After the reaction was complete, the samples were filtered, and the final volume was adjusted to 25 mL with ultrapure water.

The quantification of P, Ca, Mg, Fe, Zn, Cu, and Mn was performed using microwave plasma–atomic emission spectroscopy (MP-AES, model 4100, Agilent Technologies, USA). Measurements were carried out using calibration curves generated from standard solutions within the following ranges: macronutrients (P, Ca, Mg), 0.01–10 ppm; micronutrients (Fe, Cu, Mn, Zn), 0.05–8 ppm.

2.8. *Ex vitro* rooting assay

Uniform shoots (3–5 cm long, with at least two nodes) were selected and transplanted into trays with pre-moistened ARANMIX® #8 substrate. Seven treatments with naphthaleneacetic acid (NAA) (N600, PhytoTechnology Laboratories, Shawnee Mission, KS, USA) were diluted in 1N NaOH at concentrations of 0, 250, 500, 1000, 1500, 2000, and 3000 ppm, by basal immersion of explants for 3 s. The trays were placed in humid chambers under controlled conditions of temperature (25 ± 2 °C) and relative humidity above 80 %.

At 45 days after transplantation, the following variables were evaluated: root length (cm), shoot length (cm), root/shoot ratio, and number of leaves per plant.

2.9. Experimental design and statistical analysis

In the multiplication assays, a completely randomized design (CRD)

with a 3 x 5 factorial structure (15 treatments) was used, with five replications per treatment (magenta jars containing four explants). Generalized Linear Mixed Models (GLMMs) were applied to evaluate the effects of basal media, cytokinins, and their interaction at 30 and 60 days. Analyses were performed using InfoStat v.2020 and R v.4.3.3, with Maximum Likelihood (ML) and Restricted Maximum Likelihood (REML) estimation. Heterogeneous variance structures were adjusted, and jars were considered as random effects. Model selection was based on the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). Hypotheses were tested at $p \leq 0.05$, and Fisher’s LSD test was applied for mean comparisons. The destructive nature of some procedures was taken into account, and priority was given to preserving material for subsequent stages. Percentage variables were transformed using the expression $y = \arcsin(\sqrt{x/100})$ for analysis as parametric data. Results were interpreted on their original percentage scale. For the rooting assays, a completely randomized design (CRD) with seven treatments and five replications was used. Variables were analyzed with General and Mixed Linear Models.

3. Results

3.1. Establishment and identification of contaminants

The assays conducted with apical shoots collected from the field, using different combinations of disinfectants and concentrations, showed 100 % fungal contamination and phenolic oxidation. Eight morphotypes were identified, mainly belonging to the genera *Diaporthe*, *Fusarium*, *Colletotrichum*, and *Phoma*, with identity percentages above 95 % compared with reference strains from GenBank (Table 1). The observed symptoms included white or dark cottony mycelium on the stem, mycelial growth in the culture medium, and pigmented colonies adhering to the container walls.

In contrast, explants obtained from greenhouse-grown plants allowed for more successful *in vitro* establishment, although some losses were recorded during the first weeks. After a long period of four months, the regenerated shoots reached lengths of 5–7 cm (Fig. 2 A–E).

3.2. Morphological response of shoots and callus formation at 30 days

The number of shoots per explant at 30 days varied significantly among treatments (Table 2). The highest values were recorded in WPM with mT (4.45 ± 0.28), DKW with 2iP (4.45 ± 0.21), and WPM with Zea (4.15 ± 0.17). In contrast, treatments with DKW without cytokinins or supplemented with TDZ, as well as MS without cytokinins or supplemented with mT, TDZ, or 2iP, showed the lowest values and were statistically similar to each other.

The longest shoot length at 30 days was observed in DKW with 2iP and Zea, WPM with 2iP, and MS with Zea, which were significantly equal among themselves. The lowest values were DKW with mT and TDZ, WPM with TDZ, and MS supplemented with 2iP, mT, and TDZ. (Table 2).

In terms of sprouting area, the highest values were obtained in DKW with Zea or 2iP and in WPM with Zea or 2iP, treatments that were statistically equal. In contrast, DKW without cytokinins and MS without cytokinins or supplemented with 2iP, mT, or TDZ had the smallest sprouting areas. (Table 2).

Callus induction varied among treatments. The highest percentages were observed in WPM and DKW with TDZ, as well as in treatments with Zea in DKW, WPM, and MS, and in WPM with 2iP. In contrast, no callus formation was observed in the DKW and MS controls, nor in the MS treatment with mT. (Table 2).

In terms of callus surface area in the medium, the highest values were observed in DKW with TDZ (1.02 ± 0.06 cm²) and WPM with TDZ (1.00 ± 0.06 cm²). The greatest callus fresh weight was also obtained with DKW plus TDZ (0.58 ± 0.03 g), followed by WPM with TDZ (0.46 ± 0.03 g) (Table 2).

Table 1
Molecular identification of fungi isolated from *V. meridionale* explants cultured *in vitro*.

Scientific name	Lab code	Host (<i>in vitro</i>)	Observed symptom	Identity (%)	Reference strain (GenBank)	ITS accession number
<i>Diaporthe passiflorae</i>	AFHP102	<i>Vaccinium</i> tissue	White cottony mycelium on the stem.	99.46	KR534744.1	PX658207
<i>Diaporthe</i> sp. CLY-13	AFHP103	<i>Vaccinium</i> tissue	Dark mycelium on the stem.	97.83	AB537342.1	PX658208
<i>Uncultured fungus</i>	AFHP104	<i>Vaccinium</i> tissue	Orange colony attached to the tube.	95.91	MT236569.1	PX658209
<i>Phoma</i> sp. G358	AFHP105	<i>Vaccinium</i> tissue	White-dark mycelium in the medium, beneath the stem.	99.82	KR094449.1	PX658210
<i>Colletotrichum</i> sp.	AFHP106	<i>Vaccinium</i> tissue	Abundant mycelium in the culture medium.	99.78	MN744300.1	PX658211
<i>Fusarium begoniae</i>	AFHP108	<i>Vaccinium</i> tissue	Dark mycelium on the medium and stem.	99.64	KM577645.1	PX658212
<i>Fusarium</i> sp.	AFHP109	<i>Vaccinium</i> tissue	White mycelium in the medium, attached to the stem.	99.82	KX496881.1	PX658213
<i>Diaporthe phaseolorum</i>	AFHP110	<i>Vaccinium</i> tissue	Abundant mycelium in the culture medium, attached to the stem.	98.98	EU272530.1	PX658214

Table 2

Effect of basal media and cytokinin type on morphological variables and growth of shoots and calli during the *in vitro* multiplication phase of *V. meridionale*, 30 days after the beginning of the experiment.

Basal medium	Cytokinin type	N° of shoots per explant ¹	Length of the longest shoot (cm) ¹	Shoot surface area in the medium (cm ²) ¹	Callus induction (%) ¹	Callus surface area in the medium (cm ²) ²	Fresh callus weight (g) ²
DKW	Control	1.25 ± 0.10 ef	0.64 ± 0.03 bcde	5.86 ± 0.42 gh	Sin Callus	Sin Callus	Sin Callus
	Zea	3.15 ± 0.20b	0.71 ± 0.03 abc	12.11 ± 0.63 a	70.39 ± 0.25 a	0.72 ± 0.06 bc	0.38 ± 0.03c
	2iP	4.45 ± 0.21 a	0.78 ± 0.05 a	11.47 ± 0.59 ab	35.96 ± 0.25c	0.25 ± 0.06 fg	0.23 ± 0.03 d
	mT	2.10 ± 0.24c	0.59 ± 0.04 defg	7.19 ± 0.46 d ef	24.98 ± 0.64c	0.20 ± 0.06 g	0.15 ± 0.03 e
	TDZ	1.15 ± 0.08f	0.54 ± 0.03 fg	6.62 ± 0.48 efg	63.86 ± 0.25 a	1.02 ± 0.06 a	0.58 ± 0.03 a
WPM	Control	1.85 ± 0.18 cd	0.63 ± 0.03 cde	8.18 ± 0.42 d	39.64 ± 0.36 bc	0.46 ± 0.06 d	0.20 ± 0.03 de
	Zea	4.15 ± 0.17 a	0.59 ± 0.03 def	12.01 ± 0.63 a	67.09 ± 0.25 a	0.83 ± 0.06b	0.38 ± 0.03c
	2iP	3.50 ± 0.17b	0.77 ± 0.06 ab	10.69 ± 0.59 ab c	60.72 ± 0.25 ab	0.57 ± 0.06 cd	0.31 ± 0.03c
	mT	4.45 ± 0.28 a	0.65 ± 0.03 bcde	9.78 ± 0.46c	42.19 ± 0.25 bc	0.56 ± 0.06 cd	0.35 ± 0.03c
	TDZ	1.55 ± 0.11 de	0.49 ± 0.02 g	7.40 ± 0.48 d e	88.10 ± 0.25 a	1.00 ± 0.06 a	0.46 ± 0.03b
MS	Control	1.25 ± 0.10 ef	0.28 ± 0.02 h	5.22 ± 0.42 h	Absence of callus	Absence of callus	Absence of callus
	Zea	2.35 ± 0.20c	0.70 ± 0.04 abcd	9.93 ± 0.63 bc	48.92 ± 1.21 abc	0.28 ± 0.06 e fg	0.14 ± 0.03 e
	2iP	1.35 ± 0.15 ef	0.54 ± 0.02 fg	6.40 ± 0.59 efg	24.98 ± 0.64c	0.43 ± 0.06 de	0.17 ± 0.03 de
	mT	1.20 ± 0.09f	0.55 ± 0.05 efg	6.03 ± 0.46 fgh	Sin Callus	Sin Callus	Sin Callus
	TDZ	1.05 ± 0.05f	0.51 ± 0.03 fg	6.26 ± 0.48 efg h	30.22 ± 0.25c	0.41 ± 0.06 d ef	0.15 ± 0.03 e

¹Each value represents the adjusted mean ± standard error (n = 5; pseudoreplicates = 20). Values within the same column followed by different letters are significantly different according to Fisher's LSD mean comparison test ($\alpha = 0.05$).

²Each value in the table represents the adjusted mean ± standard error (n = 5). Mean values followed by different letters within a column are significantly different according to Fisher's LSD test ($p < 0.05$).

3.3. Morphological response and shoot biomass at 60 days

Sprouting capacity varied significantly among the combinations of basal medium and cytokinin at 60 days, with contrasting responses in the number and size of the shoots developed (Figs. 3 and 4). For the number of shoots, the most effective combinations were DKW with Zea (10.50 ± 0.37), WPM with Zea (9.91 ± 0.33), and DKW with 2iP (9.17 ± 0.66), with no statistical differences among them. At the opposite end, the lowest values were observed in the DKW, WPM, and MS media without cytokinins, as well as in MS with mT and WPM with TDZ (Figs. 3 and 4).

Regarding mean shoot length, the treatments with DKW plus Zea (2.93 ± 0.10 cm) and DKW plus 2iP (2.53 ± 0.23 cm) reached the highest values, being significantly superior to the others. In contrast, the smallest shoots were obtained in the WPM, MS, and DKW media supplemented with the cytokinin TDZ, similar to those obtained in MS without cytokinins, all below one centimeter (Figs. 3 and 4).

The analysis of biomass in regenerated shoots reflected similar patterns. The shoots with the highest fresh weight were recorded in DKW with Zea (3334.00 ± 51.54 mg), followed by the combination of DKW

with TDZ (3085.80 ± 83.12 mg), which was determined by callus formation and leaf deformation (Fig. 4). The shoots with the lowest fresh weight corresponded to MS without cytokinins (Table 3).

Regarding shoot dry weight, the combination of DKW with Zea (378.80 ± 18.26 mg) presented the highest values, whereas the MS culture medium without regulators showed the lowest values (0.16 ± 0.13 mg). The percentage of dry matter in the shoots displayed a different pattern, with the highest values obtained in WPM with 2iP ($18.48 \pm 0.01\%$) and DKW with 2iP ($17.63 \pm 0.01\%$), while the lowest percentage was also recorded in MS without regulators ($0.16 \pm 0.04\%$).

3.4. Photosynthetic pigment content and SPAD index at 60 days

The SPAD index was highest in culture media containing DKW combined with 2iP (36.87 ± 0.70) and DKW combined with Zea (35.72 ± 0.70), followed by WPM with 2iP (33.49 ± 0.70) and the WPM control (32.41 ± 0.70). In contrast, the lowest values were recorded in MS culture media with Zea (12.99 ± 0.70) and MS with TDZ (13.65 ± 0.70), with similar values between them (Table 4).

Total chlorophyll content was greatest in DKW with 2iP and DKW

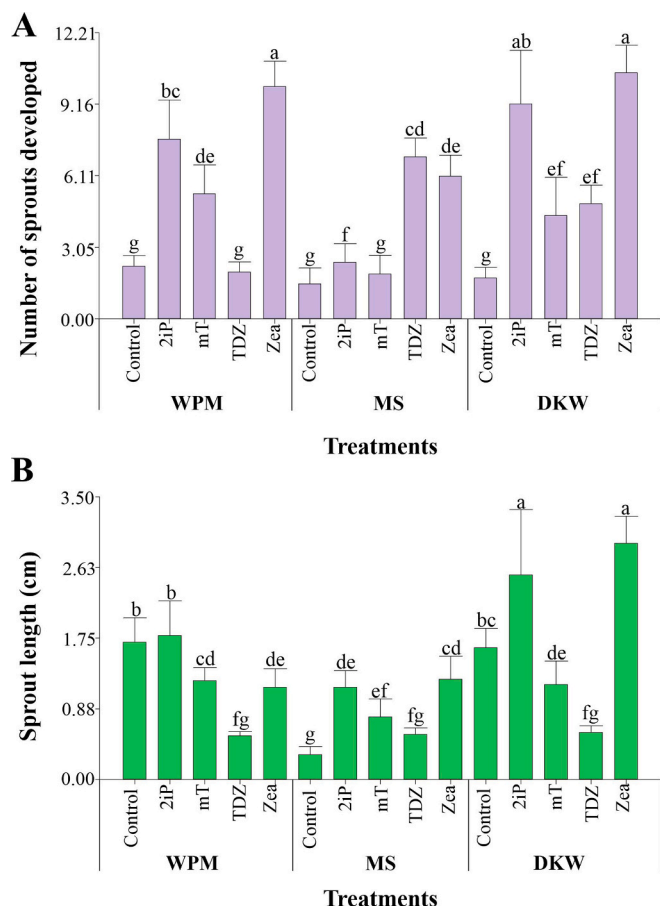


Fig. 3. Effect of basal media and cytokinin types on the *in vitro* multiplication of *V. meridionale* after 60 days of culture. A) Mean number of shoots per explant and B) Mean shoot length. Bars represent adjusted means \pm standard errors ($n = 3$, pseudoreplicate = 12). Different letters above the bars indicate significant differences among treatments according to Fisher's LSD test ($p < 0.05$).

control (both with 40.92 ± 0.04), followed by WPM with 2iP (33.11 ± 0.04). The lowest values were observed in MS with TDZ (8.14 ± 0.04) and WPM with TDZ (11.03 ± 0.04). A similar pattern was found for chlorophyll *a*, where DKW with 2iP and DKW control reached the highest values (27.87 ± 0.03), while MS with TDZ (5.98 ± 0.03) and WPM with TDZ (7.71 ± 0.03) presented the lowest (Table 4).

For chlorophyll *b*, DKW with 2iP and DKW control also recorded the highest values (13.05 ± 0.02), followed by WPM control (10.29 ± 0.02). The lowest values were observed in MS with TDZ (2.15 ± 0.02), WPM with TDZ (3.32 ± 0.02), and MS with mT (4.42 ± 0.02) (Table 4).

The carotenoid content was highest in the WPM control (12.20 ± 0.02), followed by the DKW control and the 2iP treatment (11.65 ± 0.02), while the lowest values were recorded in MS with TDZ (2.03 ± 0.02), WPM with TDZ (2.92 ± 0.02), and MS with Zea (3.78 ± 0.02).

3.5. Nutrient quantification in *in vitro* explants at 60 days

Treatments based on DKW generally showed the highest nutrient contents, whereas treatments in MS medium, particularly with Zea and TDZ, exhibited the lowest levels (Table 5).

Phosphorus (P) content was highest in WPM with 2iP (285.89 ppm) and DKW with TDZ (285.61 ppm), while the lowest value was recorded in MS with Zea (49.90 ppm). For calcium (Ca), the highest levels were observed in DKW with TDZ (1595.41 ppm), followed by DKW with mT (1404.15 ppm). In contrast, MS with Zea showed the lowest value (398.43 ppm) (Table 5).

Regarding magnesium (Mg), DKW with TDZ also reached the highest

concentration (276.73 ppm), followed by DKW with mT, while MS with Zea had the lowest accumulation (56.01 ppm). For iron (Fe), the DKW plus mT treatment stood out with the highest value (57.99 ppm), surpassing all others, while WPM control was the lowest (13.79 ppm) (Table 5).

For zinc (Zn), the highest concentrations were found in DKW with mT (13.25 ppm), while MS with Zea reported the lowest (3.45 ppm). An opposite pattern was observed for copper (Cu), where MS with TDZ and MS with 2iP presented the highest concentrations (3.84 and 3.01 ppm, respectively), in contrast with DKW with TDZ (1.01 ppm) and WPM with 2iP (1.20 ppm) (Table 5).

Finally, the greatest manganese (Mn) accumulation was obtained in DKW with Zea (27.95 ppm), followed by DKW with 2iP (25.95 ppm), whereas MS with Zea (3.51 ppm) and MS with TDZ (4.70 ppm) showed the lowest levels.

3.6. *Ex vitro* rooting induced by NAA

The greatest root development, measured as root length, was observed with the 500 ppm dose, followed by 2500 ppm. The treatments of 1000 to 2000 plus the 3000 ppm dose and the control had the shortest roots (Fig. 5A). In terms of shoot length, the highest values were also recorded with 500 and 2000 ppm, while the lowest was with 3000 ppm.

The root-to-shoot ratio was close to 1 with NAA at 3000 ppm, followed by 500, 1000, and 2500 ppm, with no differences observed between the treatments. The lowest values were observed with 1500 and 2000 ppm (Fig. 5B). The number of leaves varied among the treatments, with the highest values at 500, 1000 ppm, and 1500 ppm, and the lowest at 2500 and 3000 ppm.

Treatment with 500 ppm NAA promoted abundant and well-structured rooting, with fine, branched roots, which facilitated successful acclimatization in substrate, evidenced by vigorous plants with active growth during the nursery phase. (Fig. 6 A–C).

4. Discussion

The *in vitro* establishment of *V. meridionale* from field-derived apical shoots faced a strong limitation due to persistent fungal contamination (100%), even after multiple disinfection protocols⁴³. This level of infection reflects the high load of endophytic microorganisms present in plant tissues from natural environments, which may remain latent and become activated under favorable *in vitro* culture conditions^{44,45}. The molecular identification of eight morphotypes, including *Diaporthe passiflorae*, *Fusarium begoniae*, *Colletotrichum* sp., and *Phoma* sp., confirms that these are common pathogenic genera in blueberries, with high adaptability and resistance to disinfecting agents. Moreover, the presence of pigmented colonies and abundant mycelium suggests elevated metabolic activity in the medium, which may be attributed to the interaction between endogenous phenolic compounds of the explant and opportunistic pathogens, a phenomenon already reported in other *Vaccinium* species⁴⁶. Consequently, it has been proposed that the origin of the explant, rather than the disinfection process itself, is the determining factor for successful culture, with greenhouse-acclimated tissues under strict phytosanitary management being preferable^{44,47,48}. Under these conditions, although initial growth was slow (shoots of 5–7 cm after four months), a more stable and progressive establishment was achieved, highlighting the importance of adjusting not only disinfection conditions but also the physiological and sanitary source of the plant material.

The differential behavior of shoots as a function of basal medium and applied cytokinin reflects a specific interaction between hormonal signaling and the nutritional composition of the medium. Treatments with DKW and WPM combined with 2iP, mT, or zeatin showed superior responses in variables such as shoot number and length, aerial biomass, and SPAD index, suggesting that the concentration and ionic form of nutrients such as calcium, nitrogen, and potassium directly affect cell

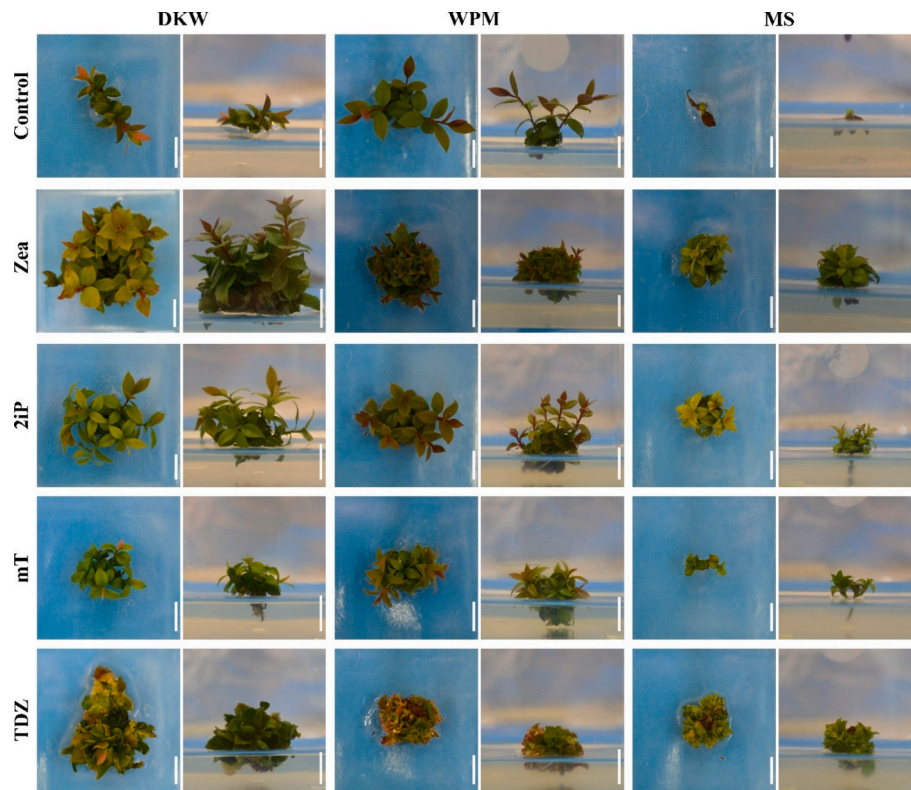


Fig. 4. Top and front views of shoot formation in *V. meridionale* under different combinations of basal media and cytokinins. Representative images of explants cultured on basal media DKW (Driver and Kuniyuki), WPM (Woody Plant Medium), and MS (Murashige and Skoog), supplemented with Zeatin (Zea), 2-isopentenyladenine (2iP), *meta*-topolin (mT), thidiazuron (TDZ) and a control without cytokinin. Vertical (top) and horizontal (bottom) views show morphogenic differences induced by the evaluated combinations. Scale bar = 1 cm.

proliferation and elongation in *V. meridionale*, as has also been reported in other species of the genus^{20,46}. In particular, zeatin remained one of the cytokinins with the most stable response and without adverse physiological symptoms⁴⁹, which is associated with its lower susceptibility to degradation and its compatibility with slightly acidic pH⁵⁰. Cytokinin 2iP, for its part, promoted efficient cell division without inducing hyperhydricity, as previously observed in *V. corymbosum*, *V. ashei*, and *V. arboreum*^{25,46}.

Among the evaluated combinations, the interaction between DKW and 2iP showed the highest performance in shoot number and development, confirming the potential of DKW medium for woody species, as indicated by Rahman⁵¹. This medium, originally formulated for the propagation of walnuts (*Juglans* spp.), contains intermediate levels of ammonium nitrate and lacks potassium nitrate, which could favor a more balanced nitrogen uptake²⁷. Added to this is its higher content of potassium sulfate and magnesium sulfate, which may enhance cell turgor processes and chlorophyll synthesis, key elements in the vigor observed in shoots^{22,26,36}. This response could also be explained by its high calcium concentration, which plays a role in elongation and cell organization in meristematic tissues⁴⁶. Likewise, WPM medium combined with 2iP also showed good results, consistent with previous studies where this medium has been widely recommended for blueberries, especially when 2iP is used as the sole cytokinin source^{49,50,52}. Taken together, the results demonstrate that the physiological response of *V. meridionale* varies depending on the specific combination of basal medium and cytokinin type, including those with zeatin, reinforcing the need to optimize protocols according to the nutritional and hormonal requirements of each species and stage of *in vitro* culture^{24,34}.

The use of TDZ in the *in vitro* multiplication of *V. meridionale* promoted abnormal development, characterized by a marked tendency toward callus formation and disorganized tissue proliferation, which can be attributed to its well-known dual auxin-cytokinin-like activity. This

nonspecific action triggers massive cell divisions without defined meristematic differentiation, a phenomenon widely described in woody species⁴⁴. In our study, the combinations containing TDZ, particularly in MS medium, not only resulted in small shoots with malformations but also produced a substantial increase in callus biomass. The surface area occupied by these calli was considerably greater compared to other treatments, consistent with the observations of Arigundam et al.⁵³ in wild lingonberry clones and of Cappelletti et al.⁵⁴, who also reported extensive callus formation at the base of shoots under TDZ treatments in WPM medium.

Moreover, the behavior observed in MS with TDZ may be influenced by the high total nitrogen concentration and the balance between ammonium and nitrate forms in this medium, which can amplify the already strong effects of TDZ, especially when used at higher concentrations. Although this cytokinin is useful in embryogenic protocols or with recalcitrant genotypes due to its resistance to enzymatic degradation⁵⁵, in vegetative multiplication protocols, it can be counterproductive because of hyperhydricity, fasciation, and reduced shoot elongation⁵⁶. This response was also evident in our preliminary experiments, where replacing zeatin with TDZ in previous treatments produced results similar to those described by Sun et al.⁵⁷ in *V. dunalianum*, with malformed shoots and high callogenesis. Finally, although some treatments without regulators or with mT showed a complete absence of callus, this confirms the need for precise hormonal regulation to avoid undesired responses.

The results obtained after 60 days of culture show that the combination of basal media and cytokinins decisively influences morphogenesis and structural quality of *V. meridionale* shoots. The highest multiplication rates were observed with zeatin and 2iP, especially in DKW medium, suggesting that the interaction between an appropriate hormonal source and a balanced ionic composition favors both shoot induction and elongation⁵⁸. Zeatin, in particular, has been reported as

Table 3

Effects of basal media and cytokinin types on fresh weight, dry weight, and dry matter percentage of explants during *in vitro* multiplication of *V. meridionale* at 60 days after initiation of the trial.

Basal medium	Cytokinin type	Shoot fresh weight (mg) ¹	Shoot dry weight (mg) ¹	Shoot dry matter (%) ¹
DKW	Control	138.00 ± 13.56 i	21.80 ± 2.84 i	15.990 ± 0.4 abcd
	Zea	3334.00 ± 51.54 a	378.80 ± 18.26 a	11.56 ± 0.01 ef
	2iP	570.20 ± 50.13 g	100.20 ± 7.79 f	17.63 ± 0.01 ab
	mT	266.40 ± 41.12 h	41.72 ± 3.72 g h	15.99 ± 0.04 abc
	TDZ	3085.80 ± 83.12 b	320.34 ± 16.54 b	10.24 ± 0.01 f
WPM	Control	324.00 ± 56.49 h	32.72 ± 3.89 h	10.89 ± 0.04 ef
	Zea	828.80 ± 55.30 e	124.68 ± 13.91 ef	15.20 ± 0.01 cd
	2iP	750.60 ± 115.47 ef	138.06 ± 17.25 def	18.48 ± 0.01 a
	mT	1030.20 ± 108.90 de	143.44 ± 11.36 de	14.43 ± 0.04 cde
	TDZ	1253.80 ± 133.33 cd	189.54 ± 24.70 cd	14.43 ± .01 cd
MS	Control	47.80 ± 7.17 j	0.16 ± 0.13 k	0.16 ± 0.04 g
	Zea	377.56 ± 23.32 h	57.85 ± 4.71 g	15.20 ± 0.01 0.01c d
	2iP	403.00 ± 80.72 gh	61.00 ± 9.00 g	15.99 ± 0.01 bcd
	mT	114.00 ± 19.65 i	13.80 ± 1.39 j	12.95 ± 0.04 def
	TDZ	1492.00 ± 177.35 c	227.80 ± 14.67 c	15.99 ± 0.01 bcd

¹Each value in the table is represented as the adjusted mean ± standard error (n = 5). Mean values followed by different letters within a column are significantly different according to Fisher's LSD test (p < 0.05).

an efficient cytokinin for species of the *Vaccinium* genus due to its more physiological action, promoting sustained elongation and structured differentiation⁵⁹. In turn, the use of 2iP generated well-formed and compact shoots, without evidence of excessive callus proliferation, a behavior consistent with previous studies^{60,61}.

Table 4

Effect of basal media and cytokinin types on SPAD index, total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids during the *in vitro* multiplication phase of *V. meridionale* after 60 days of culture.

Basal medium	Cytokinin type	SPAD index ¹	Total chlorophyll (µg/mL) ¹	Chlorophyll a (µg/mL) ¹	Chlorophyll b (µg/mL) ¹	Carotenoid (µg/mL) ¹
DKW	Control	24.87 ± 0.70 d	40.92 ± 0.04 a	27.87 ± 0.03 a	13.05 ± 0.02 a	11.65 ± 0.02 b
	Zea	35.72 ± 0.70 a	31.51 ± 0.04 d	21.24 ± 0.03 d	10.26 ± 0.02 b	9.29 ± 0.02 f
	2iP	36.87 ± 0.70 a	40.92 ± 0.04 a	27.87 ± 0.03 a	13.05 ± 0.02 a	11.65 ± 0.02 b
	mT	25.74 ± 0.70 d	28.92 ± 0.04 f	19.09 ± 0.03 e	9.83 ± 0.02 d	10.72 ± 0.02 c
	TDZ	21.36 ± 0.70 e	18.8 ± 0.04 j	14.01 ± 0.03 i	4.79 ± 0.02 i	4.25 ± 0.02 k
WPM	Control	32.41 ± 0.70 b	31.84 ± 0.04 c	21.55 ± 0.03 c	10.29 ± 0.02 b	12.2 ± 0.02 a
	Zea	28.54 ± 0.70 c	24.43 ± 0.04 h	17.86 ± 0.03 g	6.57 ± 0.02 g	5.79 ± 0.02 h
	2iP	33.49 ± 0.70 b	33.11 ± 0.04 b	22.94 ± 0.03 b	10.17 ± 0.02 c	9.55 ± 0.02 e
	mT	30.32 ± 0.70 c	29.6 ± 0.04 e	21.25 ± 0.03 d	8.35 ± 0.02 f	7.16 ± 0.02 g
	TDZ	18.23 ± 0.70 f	11.03 ± 0.04 m	7.71 ± 0.03 l	3.32 ± 0.02 l	2.92 ± 0.02 m
MS	Control	—	—	—	—	—
	Zea	12.99 ± 0.70 g	15.54 ± 0.04 k	11.4 ± 0.03 j	4.14 ± 0.02 k	3.78 ± 0.02 l
	2iP	24.67 ± 0.70 d	21.48 ± 0.04 i	16.03 ± 0.03 h	5.45 ± 0.02 h	5.59 ± 0.02 i
	mT	16.90 ± 0.70 f	14.3 ± 0.04 l	9.88 ± 0.03 k	4.42 ± 0.02 j	5.16 ± 0.02 j
	TDZ	13.65 ± 0.70 g	8.14 ± 0.04 n	5.98 ± 0.03 m	2.15 ± 0.02 m	2.03 ± 0.02 n

¹Each value corresponds to the adjusted mean ± standard error (n = 3, except SPAD index with n = 5). Values within the same column followed by different letters are significantly different according to Fisher's LSD mean comparison test (α = 0.05).

(-) indicates values not determined due to insufficient biomass for analysis.

In contrast, treatments with TDZ showed high accumulation of fresh biomass without proportional shoot elongation. This behavior is attributable to the hyperproliferation of dedifferentiated tissues such as calli, already described in blueberry, where the auxin-cytokinin dual activity of TDZ induces intense but poorly organized cell division. This response was accompanied by characteristic symptoms of hyperhydricity, such as thickened leaves, rosette-like structures, and loss of tissue organization, a phenomenon that severely compromises the functionality of regenerated shoots⁶². Although an increase in aerial biomass was recorded, this mainly reflected water accumulation and undifferentiated tissues rather than functional growth, as also reported by Stefenon et al.⁶³. This behavior was confirmed by the lower percentage of dry matter in treatments with TDZ and zeatin, whereas 2iP promoted a denser and physiologically competent architecture, with higher dry matter values, reflecting lower water content and reduced incidence of malformations.

TDZ-induced hyperhydricity has been widely documented as a physiological abnormality associated with hormonal and nutritional imbalances in the medium, leading to glassy shoots, soft tissues, and poor differentiation^{64,65}. In addition, the persistence of calli under these conditions contributes to increased weight without representing effective regeneration⁶⁶. Although TDZ can be useful in embryogenesis protocols, its indiscriminate use in vegetative multiplication, especially at doses above 1 µM, may be counterproductive⁶². In this context, the use of zeatin and 2iP, particularly in combination with DKW medium, stands out as a more suitable strategy for obtaining viable, well-formed shoots with potential for subsequent culture phases.

The synthesis of photosynthetic pigments and the functionality of the photosynthetic apparatus are key indicators to assess the physiological quality of *in vitro* shoots. In this study, treatments with DKW medium combined with 2iP or zeatin promoted the highest values of total chlorophyll, chlorophyll a and b, as well as SPAD index, suggesting greater photosynthetic capacity. These results could be explained by the effect of cytokinin on chloroplast biogenesis and cell differentiation, as reported in studies with other woody species⁶⁷. Zeatin, in turn, showed a sustained positive effect, favoring the formation of shoots with higher pigment content, which has been associated with its chemical stability and lower interference in tissue maturation⁶¹.

The SPAD index, an indirect measure of chlorophyll content, was positively correlated with total chlorophyll content and was highest in explants treated with 2iP in DKW, indicating superior photosynthetic functionality. In contrast, treatments with TDZ, especially in

Table 5

Effect of basal media and cytokinin types on the nutritional composition of explants during the *in vitro* multiplication phase of *V. meridionale* at 60 days after initiation of the trial.

Basal medium	Cytokinin type	P (ppm) ¹	Ca (ppm) ¹	Mg (ppm) ¹	Fe (ppm) ¹	Zn (ppm) ¹	Cu (ppm) ¹	Mn (ppm) ¹
DKW	Control	–	–	–	–	–	–	–
	Zea	248.76 ± 6.54b	1020.46 ± 3.34 d	197.76 ± 2.1c	28.59 ± 0.31f	10.35 ± 0.37b	1.42 ± 0.03 fg	27.95 ± 0.06 a
	2iP	219.95 ± 6.54 de	1223.05 ± 3.34c	200.94 ± 2.1c	31.93 ± 0.31c	8.19 ± 0.37c	1.51 ± 0.03f	25.95 ± 0.06b
	mT	244.85 ± 6.54 bc	1404.15 ± 3.34b	229.68 ± 2.1b	57.99 ± 0.31 a	13.25 ± 0.37 a	2.30 ± 0.03c	20.61 ± 0.06c
	TDZ	285.61 ± 6.54 a	1595.41 ± 3.34 a	276.73 ± 2.1 a	32.20 ± 0.31c	10.82 ± 0.37b	1.01 ± 0.03 i	18.41 ± 0.06 d
WPM	Control	238.45 ± 6.54 bcd	525.88 ± 3.34 j	130.57 ± 2.1f	13.79 ± 0.31 k	6.65 ± 0.37 d	1.67 ± 0.03 e	17.42 ± 0.06f
	Zea	217.94 ± 6.54 e	671.26 ± 3.34 g	149.70 ± 2.1 e	29.72 ± 0.31 e	6.54 ± 0.37 d	1.35 ± 0.03 g	16.89 ± 0.06 g
	2iP	285.89 ± 6.54 a	580.72 ± 3.34 h	154.81 ± 2.1 e	16.68 ± 0.31 j	5.31 ± 0.37 e	1.20 ± 0.03 h	18.02 ± 0.06 e
	mT	228.29 ± 6.54 cde	539.60 ± 3.34 i	130.38 ± 2.1f	21.48 ± 0.31 i	5.92 ± 0.37 de	1.23 ± 0.03 h	16.00 ± 0.06 h
	TDZ	167.20 ± 6.54 g	691.43 ± 3.34f	112.02 ± 2.1 g	33.12 ± 0.31b	8.36 ± 0.37c	2.15 ± 0.03 d	14.30 ± 0.06 i
MS	Control	–	–	–	–	–	–	–
	Zea	49.90 ± 6.54 i	398.43 ± 3.34 k	56.01 ± 2.1 i	30.66 ± 0.31 d	3.45 ± 0.37f	2.98 ± 0.03b	3.51 ± 0.06 l
	2iP	191.87 ± 6.54f	839.25 ± 3.34 e	166.66 ± 2.1 d	25.37 ± 0.31 g	3.90 ± 0.37f	3.01 ± 0.03b	12.07 ± 0.06 j
	mT	–	–	–	–	–	–	–
	TDZ	127.33 ± 6.54 h	532.26 ± 3.34 ij	104.26 ± 2.1 h	23.40 ± 0.31 h	4.06 ± 0.37f	3.84 ± 0.03 a	4.70 ± 0.06 k

¹Each value corresponds to the adjusted mean ± standard error (n = 3). Values within the same column followed by different letters are significantly different according to Fisher's LSD mean comparison test ($\alpha = 0.05$).
 (–) indicates values not determined due to insufficient biomass for analysis.

combination with MS, showed significant reductions in both SPAD and photosynthetic pigments, which may be due to callus proliferation and tissue disorganization induced by this cytokinin^{64,68}. These effects of TDZ, despite inducing shoot proliferation, may interfere with the

functional maturation of photosynthetic tissue.

Carotenoids, key pigments for photoprotection and antioxidation, reached their highest levels in treatments with 2iP and in WPM without regulators. Their increase has been linked to defense mechanisms

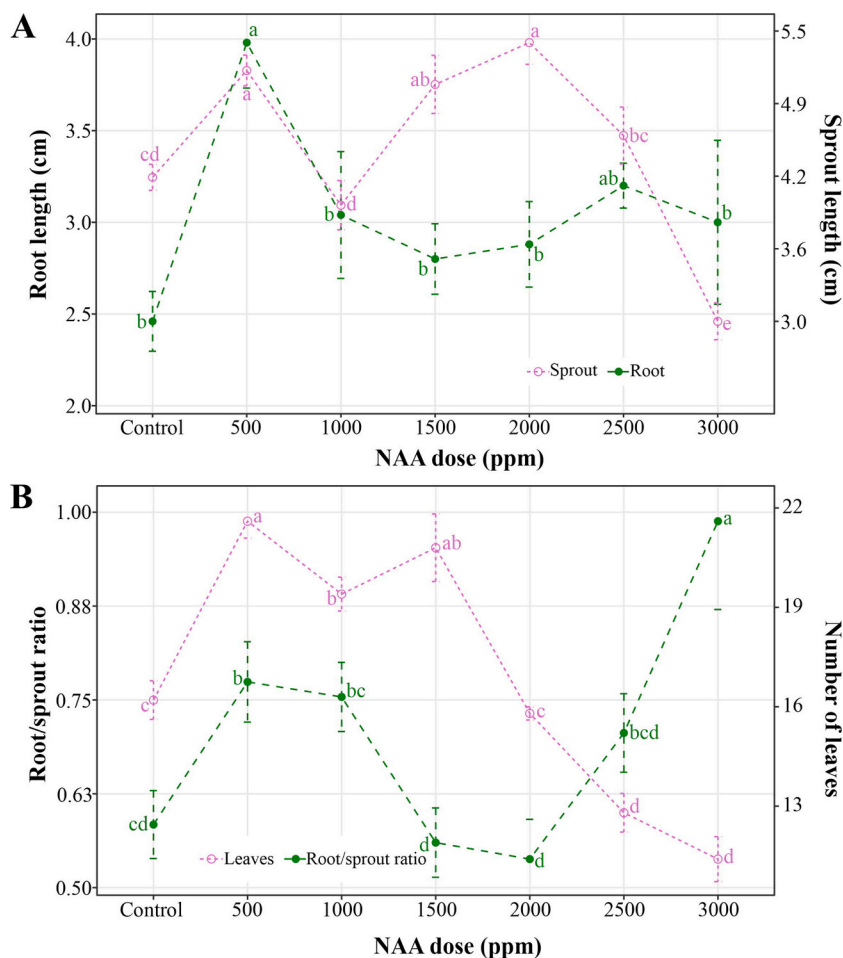


Fig. 5. Effect of different concentrations of NAA (naphthaleneacetic acid) on the *ex vitro* rooting of *V. meridionale*. A) Mean root and shoot length (cm) as a function of NAA doses (ppm), B). Root/shoot ratio and mean number of leaves. Different letters indicate significant differences among treatments for each variable independently ($p < 0.05$).

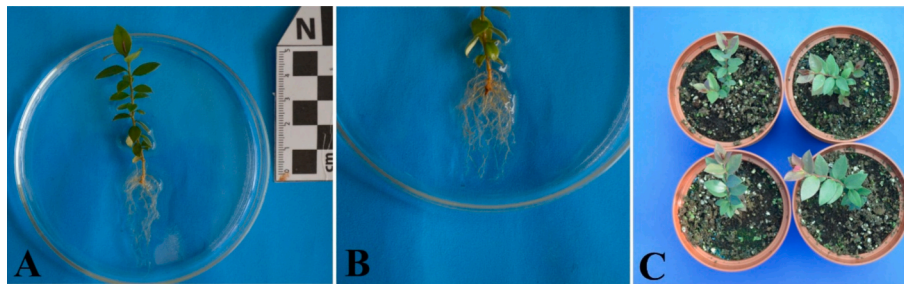


Fig. 6. Stages of seedling development. A) *In vitro* seedling with well-defined root system and shoot, B) Details of the root system after the rooting phase, and C) Seedlings in pots during *ex vitro* acclimatization.

against light, oxidative, and saline stress characteristic of *in vitro* cultures⁶⁹. These compounds participate in dissipating excess energy, preventing the formation of reactive oxygen species such as singlet oxygen, thereby protecting chloroplast integrity and favoring successful subsequent acclimatization⁷⁰.

In contrast, the use of TDZ resulted in marked reductions in chlorophyll and carotenoid levels, suggesting a generalized dysfunction of the photosynthetic apparatus, possibly induced by oxidative stress and poor tissue organization^{62,71}. This functional loss may compromise not only photosynthesis but also shoot viability in *ex vitro* phases. Taken together, the results highlight that the type of cytokinin and basal medium affect not only morphogenesis but also the physiological quality of shoots, with 2iP and zeatin in DKW being particularly efficient combinations for maintaining both structure and functionality during the multiplication stage.

The nutritional profile of explants cultivated *in vitro* does not depend solely on the availability of nutrients in the basal medium but also on the capacity of developing tissues to actively absorb and assimilate them. This process is influenced by factors such as the mineral and hormonal composition of the culture medium, which regulates regeneration and growth^{72,73}, and in turn conditions the absorption of nitrates, phosphates, and cations⁷⁴, as well as the physiological state of donor plants providing explants^{75,76}.

In the present study, treatments supplemented with DKW medium exhibited a greater accumulation of nutrients, including Ca, Mg, P, and Mn. This result is consistent with reports highlighting the suitability of DKW medium, given its formulation with higher salt concentrations enriched in Ca and NO₃⁻, which favors both cell expansion and physiological stability of shoots^{25,27}. On the other hand, MS medium showed notably lower nutritional values, consistent with reports evidencing physiological deficiencies and low photosynthetic efficiency in *Vaccinium* shoots cultured in this medium^{26,77}. Shoots regenerated in MS often exhibit hyperhydricity and lower survival compared to those in WPM or DKW^{78,79}, as well as a significant reduction in photosynthesis, stomatal conductance, and chlorophyll content⁸⁰. These limitations have been associated with nutritional imbalance and the accumulation of toxic compounds, which generate oxidative stress and cellular damage⁸¹. This explains the lower nutrient content in explants maintained in MS compared with those cultured in WPM or DKW, where higher photosynthetic efficiency and more vigorous growth are observed.

In our study, a high accumulation of Ca and Mg was observed in treatments with TDZ. There is evidence that TDZ may enhance the availability and translocation of essential minerals. Ca and Mg play critical roles in membrane stability, enzymatic activity, and structural integrity of plant tissues^{82,83}. Ca accumulation showed a differential pattern, reaching significantly higher values in TDZ treatments with DKW, whereas levels in WPM and MS were notably lower. The study shows that the initial availability of Ca in the medium alone is not sufficient to explain the final accumulation in tissues; instead, the interaction with TDZ modulates the transport and partitioning of this element. TDZ has been reported as a regulator that alters carbohydrate and nitrogen metabolism as well as ion transporter activity, which could

explain this synergistic effect^{84,85}. Likewise, TDZ application is associated with increased absorption and accumulation of Ca and Mg under abiotic stress contexts, by modulating antioxidant enzymes and improving hormonal balance⁸⁶.

The micronutrient profile was also significantly influenced by the interaction between cytokinins and the basal medium. Higher levels of Zn and Fe were detected in treatments with mT and TDZ in DKW, while Mn tended to accumulate in the presence of zeatin. Studies have shown that zeatin contributes to restoring hormonal balance under stress conditions and enhances nitrogen metabolism by inducing nitrate reductase activity⁸⁷, suggesting that this regulator promotes a more integrated adjustment in micronutrient availability and redistribution. Consequently, the differential response observed may be associated not only with a direct effect of cytokinin on ion uptake but also with its ability to modulate metabolic and hormonal signaling networks related to mineral nutrition⁸⁸.

The morphogenetic response obtained with NAA in *V. meridionale* showed a dose-dependent behavior. Intermediate doses (500 and 2000 ppm) of NAA promoted greater root development and shoot elongation. This result is consistent with reports in other wild *Vaccinium* species, such as *V. macrocarpon* (100 %) and *V. floribundum* (70 %), where the use of NAA as a root inducer favored root formation and development^{89,90}.

The root/shoot ratio suggests that, in addition to root growth, hormonal balance was also modulated toward balanced vegetative development, a response expected when the applied auxin does not exceed phytotoxicity thresholds. The root/shoot ratio is closely related to the balance between auxin and cytokinin^{91,92}. Variable responses in leaf number could be associated with a negative interaction between high NAA levels and the availability of endogenous cytokinins. Elevated auxin levels can suppress cytokinin biosynthesis, while cytokinins can induce auxin biosynthesis, ensuring a balance between both growth regulators⁹³. High auxin doses can be toxic, leading to excessive callus proliferation and even necrosis in shoots^{94,95}. These reports align with our observations, where intermediate NAA concentrations proved to be the most effective in most of the variables evaluated. In contrast, high NAA doses caused a reduction in both shoot and root length.

5. Conclusion

The present study established a comprehensive protocol for the micropropagation of *V. meridionale*, covering the process from *in vitro* establishment to the *ex vitro* rooting stage. In the establishment with apical shoots from the field, high phenolic oxidation and significant fungal contamination by *Diaporthe*, *Fusarium*, *Colletotrichum*, and *Phoma* species were observed, compromising their viability. In contrast, the use of plant material from plants kept in greenhouses allowed for a more successful establishment, ensuring asepsis and initial regeneration.

During the multiplication phase, DKW medium in combination with zeatin or 2iP promoted a greater number and length of shoots, improved photosynthetic parameters, and more efficient accumulation of essential nutrients compared with MS and WPM. On the other hand, although TDZ increased fresh weight and callus development, it did not support

organized growth or physiological functionality of explants. Finally, *ex vitro* rooting was most efficient with the application of 500 ppm NAA, producing fine, abundant, and functional roots that facilitated an effective transition to non-sterile conditions.

Overall, the results support recommending an efficient protocol for the propagation of *V. meridionale*, based on the use of greenhouse-derived shoots, DKW medium with zeatin or 2iP for multiplication, and *ex vitro* rooting induced by NAA. This approach contributes to improving the availability of high-quality genetic and physiological material for conservation programs and commercial production.

CRedit authorship contribution statement

Eyner Huaman: Conceptualization, Investigation, Methodology, Writing – original draft. **Carlos Muñoz:** Conceptualization, Validation, Writing – original draft. **María Loreto Prat:** Conceptualization, Supervision, Writing – original draft. **Jegnes Benjamín Meléndez-Mori:** Investigation, Methodology, Writing – original draft. **Raul Vargas:** Data curation, Methodology, Writing – original draft. **Carmen N. Vigo:** Formal analysis, Visualization, Writing – original draft. **José Jesús Tejada-Alvarado:** Conceptualization, Investigation, Writing – review & editing. **Angel Fernando Huaman-Pilco:** Formal analysis, Methodology, Writing – review & editing. **Manuel Oliva-Cruz:** Conceptualization, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the National Program of Scholarships and Educational Credit (PRONABEC) of Peru for the support provided through the Bicentennial Generation Scholarship in the training of the first author, and the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, INDES-CES. This work was supported by the SNIP Project N° 312252 “Creación del Servicio de un Laboratorio de Fisiología y Biotecnología Vegetal de la Universidad Nacional Toribio Rodríguez de Mendoza, región Amazonas”-FISIOBVEG. The vice chancellor’s office of research at the National University Toribio Rodríguez de Mendoza of Amazonas funded the APC.

References

- Ligarreto GA. Phenotypic plasticity of *Vaccinium meridionale* (Ericaceae) in wild populations of mountain forests in Colombia. *Rev Biol Trop*. 2011;59(2):569–583.
- Garzón GA, Soto CY, López-R M, Riedl KM, Browmiller CR, Howard L. Phenolic profile, *in vitro* antimicrobial activity and antioxidant capacity of *Vaccinium meridionale* Swartz pomace. *Heliyon*. 2020;6(5), e03845. <https://doi.org/10.1016/j.heliyon.2020.e03845>.
- Medina-Cano CI, Ligarreto-Moreno GA, Vargas-Arcila MO. Proposal of descriptors to study the variability of *Vaccinium meridionale* Swartz. *Rev Fac Nac Agron Medellin*. 2023;76(3):10445–10455. <https://doi.org/10.15446/rfnam.v76n3.102634>.
- Shen M, Li K, Jing H, Zheng L. In vivo therapeutic effect of *Vaccinium meridionale* Swartz in ischemia-reperfusion induced male albino rats. *J Food Sci*. 2018;83(1): 221–228. <https://doi.org/10.1111/1750-3841.13986>.
- Agudelo CD, Luzardo-Ocampo I, Campos-Vega R, Loarca-Piña G, Maldonado-Celis ME. Bioaccessibility during *in vitro* digestion and antiproliferative effect of bioactive compounds from yean berry (*Vaccinium meridionale* Swartz) juice. *J Agric Food Chem*. 2018;66(28):7358–7366. <https://doi.org/10.1021/acs.jafc.8b01604>.
- Arango-Varela SS, Luzardo-Ocampo I, Maldonado-Celis ME, Campos-Vega R. Yean berry (*Vaccinium meridionale* Swartz) juice in combination with Aspirin modulated anti-inflammatory markers on LPS-stimulated RAW 264.7 macrophages. *Food Res Int*. 2020;137, 109541. <https://doi.org/10.1016/j.foodres.2020.109541>.
- Agudelo-Quintero ML, Luzardo-Ocampo I, Lopera-Rodríguez JA, Maldonado-Celis ME, Arango-Varela SS. Bioactive compounds from yean berry (*Vaccinium meridionale* Swartz) juice inhibited cell viability and proliferation from SW480 and SW620 human colon adenocarcinoma cells. *Biol Life Sci Forum*. 2022;18(1):17. <https://doi.org/10.3390/Foods2022-12984>.
- González M, Samudio I, Sequeda-Castañeda LG, Celis C, Iglesias J, Morales L. Cytotoxic and antioxidant capacity of extracts from *Vaccinium meridionale* Swartz (Ericaceae) in transformed leukemic cell lines. *J Appl Pharm Sci*. 2017;7(3):24–30. <https://doi.org/10.7324/JAPS.2017.70305>.
- Agudelo CD, Ceballos N, Gómez-García A, Maldonado-Celis ME. Yean berry (*Vaccinium meridionale* Swartz) juice improves plasma antioxidant capacity and IL-6 levels in healthy people with dietary risk factors for colorectal cancer. *J Berry Res*. 2018;8(4):251–261. <https://doi.org/10.3233/JBR-180312>.
- Marín-Echeverri C, Blesso CN, Fernández ML, Galvis-Pérez Y, Ciro-Gómez G. Effect of agraz (*Vaccinium meridionale* Swartz) on high-density lipoprotein function and inflammation in women with metabolic syndrome. *Antioxidants*. 2018;7(12):185. <https://doi.org/10.3390/antiox7120185>.
- Arango-Varela SS, Luzardo-Ocampo I, Reyes-Dieck C, Yahia EM, Maldonado-Celis ME. Antiproliferative potential of yean berry (*Vaccinium meridionale* Swartz) juice in combination with Aspirin in human SW480 colon adenocarcinoma cells. *J Food Biochem*. 2021;45(6), e13760. <https://doi.org/10.1111/jfbc.13760>.
- Celis ME, Franco Tobón YN, Agudelo C, Arango SS, Rojano B. Yean berry (*Vaccinium meridionale* Swartz). In: Yahia EM, ed. *Fruit and Vegetable Phytochemicals*. Chichester, UK: John Wiley & Sons; 2017:869–882.
- Cobo MM, Gutiérrez B, de Lourdes TM. Regeneration of mortiño (*Vaccinium floribundum* Kunth) plants through axillary bud culture. *In Vitro Cell Dev Biol Plant*. 2018;54:112–116. <https://doi.org/10.1007/s11627-018-9884-3>.
- Ligarreto-Moreno GA, Torres-Aponte WS, Ariza-Castillo CA. Propagation of the neotropical fruit *Vaccinium meridionale* Swartz by air layering. *Agron Colomb*. 2013; 31(2):169–175.
- Suárez-Ballesteros CI, Calderón-Hernández M, Mancipe-Murillo C. Propagación sexual y tolerancia a la desecación del agraz (*Vaccinium meridionale* Sw.) de tres fuentes semilleras localizadas en Ráquira, San Miguel de Sema (Boyacá) y Gachetá (Cundinamarca). *Rev Acad Colomb Cienc Exactas Fis Nat*. 2018;42(163):207. <https://doi.org/10.18257/raccefyfyn.614>.
- Cuchumbe-Avirama KJ, Ruiz-Erazo XA, Morales-Velasco S, López-Quintero SL, Martínez-Mamián CA. Evaluation of different strategies regarding the asexual propagation/spreading of the *Vaccinium meridionale*. *Sylvan*. 2020;164(8):11.
- Medina-Cano CI, Lobo Arias M, Castaño Colorado AA, Cardona LE. Análisis del desarrollo de plantas de mortiño (*Vaccinium meridionale* Swartz) bajo dos sistemas de propagación: clonal y sexual. *Cienc Tecnol Agropecu*. 2015;16(1):65–77. https://doi.org/10.21930/rcta.vol16_num1_art:390.
- Gupta S, Singh A, Yadav K, Pyey N, Kumar S. Micropropagation for multiplication of disease-free and genetically uniform sugarcane plantlets. In: Rai AC, Kumar A, Modi A, Singh M, eds. *Advances in Plant Tissue Culture*. Cambridge, UK: Academic Press; 2022:31–49.
- Sudheer WN, Praveen N, Al-Khayri JM, Jain SM. Role of plant tissue culture medium components. In: Rai AC, Kumar A, Modi A, Singh M, eds. *Advances in Plant Tissue Culture*. Cambridge, UK: Academic Press; 2022:51–83.
- Clapa D, Nemes SA, Ranga F, Hărta M, Vodnar D-C, Călinoiu L-F. Micropropagation of *Vaccinium corymbosum* L.: an alternative procedure for the production of secondary metabolites. *Horticulturae*. 2022;8(6):480. <https://doi.org/10.3390/horticulturae8060480>.
- Haque MI, Singh PK, Ghuge S, et al. A general introduction to the background of plant tissue culture: past, current, and future aspects. In: Rai AC, Kumar A, Modi A, Singh M, eds. *Advances in Plant Tissue Culture*. Cambridge, UK: Academic Press; 2022. <https://doi.org/10.1016/B978-0-323-90795-8.00019-9>.
- Lloyd G, McCown B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Comb Proc Int Plant Propag Soc*. 1980;30: 421–427.
- Rache-Cardenal LY, Pacheco-Maldonado JC. Propagación *in vitro* de plantas adultas de *Vaccinium meridionale* (Ericaceae). *Acta Bot Bras*. 2010;24(4):1086–1095. <https://doi.org/10.1590/S0102-33062010000400024>.
- Litwińczuk W. Micropropagation of *Vaccinium* sp. by *in vitro* axillary shoot proliferation. In: Lambardi M, Ozudogru EA, Jain SM, eds. *Protocols for Micropropagation of Selected Economically-Important Horticultural Plants*. New York, NY: Humana Press; 2012:63–76.
- Li Q, Yu P, Lai J, Gu M. Micropropagation of the potential blueberry rootstock *Vaccinium arboreum* through axillary shoot proliferation. *Sci Hortic*. 2021;280, 109908. <https://doi.org/10.1016/j.scienta.2021.109908>.
- Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant*. 1962;15(3):473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>.
- Driver JA, Kuniyuki AH. *In vitro* propagation of paradox walnut rootstock. *HortScience*. 1984;19(4):507–509. <https://doi.org/10.21273/HORTSCI.19.4.507>.
- Hnatuszko-Konka K, Gerszberg A, Weremczuk-Jeżyna I, Grzegorzczak-Karolak I. Cytokinin signaling and de novo shoot organogenesis. *Genes*. 2021;12(2):265. <https://doi.org/10.3390/genes12020265>.
- Castro-Restrepo D, Álvarez-Guzmán JA. Micropropagación clonal de tres genotipos mortiño, *Vaccinium meridionale* Sw., por proliferación de yemas axilares. *Actual Biol*. 2013;35(99):135–144. <https://doi.org/10.17533/udea.acbi.329114>.
- Anwar F, Sharmila P, Saradhi PP. No more recalcitrant: chickpea regeneration and genetic transformation. *Afr J Biotechnol*. 2010;9(6):782–797. <https://doi.org/10.5897/AJB2010.000-3009>.
- Aremu AO, Bairu MW, Szűčová L, Finnie JF, Van Staden J. The role of meta-topolins on the photosynthetic pigment profiles and foliar structures of micropropagated ‘Williams’ bananas. *J Plant Physiol*. 2012;169(15):1530–1541. <https://doi.org/10.1016/j.jplph.2012.06.006>.
- Gantait S, Mitra M. Role of meta-topolin on *in vitro* shoot regeneration: an insight. In: Ahmad N, Strnad M, eds. *Meta-Topolin: A Growth Regulator for Plant Biotechnology*

- and Agriculture. Singapore: Springer; 2021. https://doi.org/10.1007/978-981-15-9046-7_12.
33. Peixe A, Raposo A, Lourenço R, Cardoso H, Macedo E. Coconut water and BAP successfully replaced zeatin in micropropagation of olive (*Olea europaea* L.). *Sci Hortic*. 2007;113(1):1–7. <https://doi.org/10.1016/j.scienta.2007.01.011>.
 34. Scalzo J, Donno D, Miller S, et al. Effect of genotype, medium and light on *in vitro* plant proliferation of *Vaccinium* spp. *N Z J Crop Hortic Sci*. 2016;44(4):231–246. <https://doi.org/10.1080/01140671.2016.1206946>.
 35. Liu C, Callow P, Rowly LJ, Hancock JF, Song G. Adventitious shoot regeneration from leaf explants of southern highbush blueberry cultivars. *Plant Cell Tiss Organ Cult*. 2010;103(1):137–144. <https://doi.org/10.1007/s11240-010-9755-z>.
 36. Tineo D, Bustamante DE, Calderon MS, Huaman E. Exploring the diversity of year berries from northern Peru based on molecular analyses. *Heliyon*. 2022;8(2), e08839. <https://doi.org/10.1016/j.heliyon.2022.e08839>.
 37. Phillips GC, Garda M. Plant tissue culture media and practices: an overview. *In Vitro Cell Dev Biol Plant*. 2019;55(3):242–257. <https://doi.org/10.1007/s11627-019-09983-5>.
 38. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671–675. <https://doi.org/10.1038/nmeth.2089>.
 39. Rueden CT, Schindelin J, Hiner MC, et al. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*. 2017;18:529. <https://doi.org/10.1186/s12859-017-1934-z>.
 40. Shipley B, Vu T. Dry matter content as a measure of dry matter concentration in plants and their parts. *New Phytol*. 2002;153(2):359–364. <https://doi.org/10.1046/j.0028-646x.2001.00320.x>.
 41. Saura-Mas S, Lloret F. Leaf and shoot water content and leaf dry matter content of Mediterranean woody species with different post-fire regenerative strategies. *Ann Bot*. 2007;99(3):545–554. <https://doi.org/10.1093/aob/mcl284>.
 42. Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Curr Protoc Food Anal Chem*. 2001;1(1):F4.3.1-F4.3.8. <https://doi.org/10.1002/0471142913.faf0403s01>.
 43. Romadanova NV, Tolegen AB, Kushnarenko SV, Zholdybayeva EV, Bettoni JC. Effect of plant preservative mixture™ on endophytic contaminants in apple tissue culture: cultivar-specific responses. *Plants*. 2022;11(19):2624. <https://doi.org/10.3390/plants11192624>.
 44. Moreno-Peña L, Hidalgo-Escobar K, Cevallos-Cevallos JM, Sanchez-Timm E. An effective disinfection protocol for contamination control in *in vitro* establishment of mortiño (*Vaccinium floribundum* Kunth) and identification of endogenous microbes. *Sci Agropecu*. 2025;16(3):375–384. <https://doi.org/10.17268/sci.agropecu.2025.028>.
 45. Fan M, Chen X, Luo X, et al. Diversity of endophytic fungi from the leaves of *Vaccinium dunalianum*. *Lett Appl Microbiol*. 2020;71(5):479–489. <https://doi.org/10.1111/lam.13345>.
 46. Correia S, Matos M, Leal F. Advances in blueberry (*Vaccinium* spp.) *in vitro* culture: a review. *Horticulturae*. 2024;10:533. <https://doi.org/10.3390/horticulturae10060533>.
 47. Coronel NT, Jácome GA, Martínez R, Hernández F. *In Vitro* techniques to domesticate Mortiño (*Vaccinium floribundum* Kunth) and other vaccinium species: a review. *Plants*. 2025;14:1596. <https://doi.org/10.3390/plants14111596>.
 48. Brenes-Angulo I, Madrigal-Carballo S, Rojas-Chaves M. Micropropagación del arándano (*Vaccinium corymbosum* L.) en Costa Rica. *Agron Costarricense*. 2015;39(1):47–55.
 49. Borsari O, Hârta M, Pamfil D, Clapa D. The effect of cytokinins on micropropagation success of highbush blueberry (*Vaccinium corymbosum* L.). *Agric Rev Stiinta Pract Agr*. 2019;28(3/4):93–100. <https://doi.org/10.15835/agrisp.v11i13-4.13546>.
 50. Kharel P, Creech MR, Nguyen CD, Vendrame WA, Muñoz PR. Effect of explant type, culture medium, and BAP concentration on *in vitro* shoot development in highbush blueberry (*Vaccinium corymbosum* L.) cultivars. *In Vitro Cell Dev Biol Plant*. 2022;58(6):1057–1065. <https://doi.org/10.1007/s11627-022-10299-0>.
 51. Rahman SS. DKW emerges as a superior media factor in *in vitro* plant regeneration. *J Agri*. 2018;1(1):3–4.
 52. Fan S, Jian D, Wei X, et al. Micropropagation of blueberry 'Bluejay' and 'Pink Lemonade' through *in vitro* shoot culture. *Sci Hortic*. 2017;226:277–284. <https://doi.org/10.1016/j.scienta.2017.08.052>.
 53. Arigundam U, Varyath AM, Siow YL, Marshall D, Debnath SC. Liquid culture for efficient *in vitro* propagation of adventitious shoots in wild *Vaccinium vitis-idaea* ssp. *minus* (lingonberry) using temporary immersion and stationary bioreactors. *Sci Hortic*. 2020;264, 109199. <https://doi.org/10.1016/j.scienta.2020.109199>.
 54. Cappelletti R, Sabbadini S, Mezzetti B. The use of TDZ for the efficient *in vitro* regeneration and organogenesis of strawberry and blueberry cultivars. *Sci Hortic*. 2016;207:117–124. <https://doi.org/10.1016/j.scienta.2016.05.016>.
 55. Huetteman CA, Preece JE. Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell Tiss Organ Cult*. 1993;33:105–119. <https://doi.org/10.1007/BF01983223>.
 56. Schuchovski C, Sant'Anna-Santos BF, Marra RC, Biasi LA. Morphological and anatomical insights into de novo shoot organogenesis of *in vitro* 'Delite' rabbiteye blueberries. *Heliyon*. 2020;6(11), e05468. <https://doi.org/10.1016/j.heliyon.2020.e05468>.
 57. Sun D, Wang M, Zhao P, Ding Y, Huang B, Tang J. Evaluation of the role of different types of cytokinins as zeatin replacement in *in vitro* proliferation of *Vaccinium dunalianum*. *Pak J Bot*. 2023;55(1). [https://doi.org/10.30848/PJB2023-1\(25\)](https://doi.org/10.30848/PJB2023-1(25)).
 58. Raspor M, Motyka V, Kaleri AR, et al. Integrating the roles of cytokinin and auxin in de novo shoot organogenesis: from hormone uptake to signaling outputs. *Int J Mol Sci*. 2020;22(16):8554. <https://doi.org/10.3390/ijms22168554>.
 59. Arista-Bustamante JP, Leiva-Espinoza ST, Guerrero-Abad JC, Collazos-Silva R. Effect of cytokinins on the *in vitro* multiplication of four varieties of *Vaccinium corymbosum*. *Rev Cient UNTRM Cienc Nat Ing*. 2020;2(2):55–62. <https://doi.org/10.25127/ucni.v2i2.520>.
 60. Teixeira MG, da Silva MRL, Carvalho M, et al. Direct organogenesis and callogenesis in zygotic embryos of bacupari (*Garcinia brasiliensis* Mart.). *Plant Cell Tiss Organ Cult*. 2024;159:66. <https://doi.org/10.1007/s11240-024-02928-w>.
 61. Nowakowska K, Pińkowska A, Siedlecka E, Pacholczak A. The effect of cytokinins on shoot proliferation, biochemical changes and genetic stability of *Rhododendron* 'Kazimierz Odnowiciel' in the *in vitro* cultures. *Plant Cell Tiss Organ Cult*. 2022;149:675–684. <https://doi.org/10.1007/s11240-021-02206-z>.
 62. Hazarika BN. Morpho-physiological disorders in *in vitro* culture of plants. *Sci Hortic*. 2006;108(2):105–120. <https://doi.org/10.1016/j.scienta.2006.01.038>.
 63. Stefenon VM, Marques MV, Guerra MP, Ribeiro de Freitas F, Boing da Silva VJ, Viera P, dos Santos DD. *In vitro* callogenesis for the induction of somatic embryogenesis and antioxidant production in *Eugenia uniflora*. *Biotechnol Veg*. 2020;20(2):135–146.
 64. Cortleven A, Leuendorf JE, Frank M, Pezzetta D, Bolt S, Schmülling T. Cytokinin action in response to abiotic and biotic stresses in plants. *Plant Cell Environ*. 2019;42(3):998–1018. <https://doi.org/10.1111/pce.13494>.
 65. Duan J, Yu H, Yuan K, et al. Strigolactone promotes cytokinin degradation through transcriptional activation of cytokinin oxidase/dehydrogenase 9 in rice. *Proc Natl Acad Sci USA*. 2019;116(28):14319–14324. <https://doi.org/10.1073/pnas.1810980116>.
 66. Karunadasa SS, Kurepa J, Shull TE, Smalle JA. Cytokinin-induced protein synthesis suppresses growth and osmotic stress tolerance. *New Phytol*. 2020;227(1):50–64. <https://doi.org/10.1111/nph.16519>.
 67. Liu L, Lin N, Liu X, Yang S, Wang W, Wan X. From chloroplast biogenesis to chlorophyll accumulation: the interplay of light and hormones on gene expression in *Camellia sinensis* cv. *Shuchazao* Leaves. *Front Plant Sci*. 2020;11, 500333. <https://doi.org/10.3389/fpls.2020.00256>.
 68. Wang Y, Zhang X, Jiang Z, et al. Establishment and optimization of micropropagation system for southern highbush blueberry. *Horticulturae*. 2023;9(8):893. <https://doi.org/10.3390/horticulturae9080893>.
 69. Bakhshpour M, Mafakheri M, Kordrostami M, et al. *In vitro* multiplication, genetic fidelity and phytochemical potentials of *Vaccinium arctostaphylos* L.: an endangered medicinal plant. *Ind Crops Prod*. 2019;141, 111812. <https://doi.org/10.1016/j.indcrop.2019.111812>.
 70. Tian Y, Garcia G, Bian Q, et al. Mitochondrial stress induces chromatin reorganization to promote longevity and UPRmt. *Cell*. 2016;165(5):1197–1208. <https://doi.org/10.1016/j.cell.2016.04.011>.
 71. Debnath SC. Propagation of *Vaccinium* *in vitro*. *Int J Fruit Sci*. 2007;6(2):47–71. https://doi.org/10.1300/J492v06n02_04.
 72. Leifert C, Murphy KP, Lumsden PJ. Mineral and carbohydrate nutrition of plant cell and tissue cultures. *Crit Rev Plant Sci*. 1995;14(2):83–109. <https://doi.org/10.1080/07352689509701923>.
 73. Ramage CM, Williams RR. Mineral nutrition and plant morphogenesis. *In Vitro Cell Dev Biol Plant*. 2002;38(2):116–124. <https://doi.org/10.1079/IVP2001269>.
 74. Ibrahim IA, Nasr MI, Mohammed BR, El-Zefzafi MM. Nutrient factors affecting *in vitro* cultivation of *Stevia rebaudiana*. *Sugar Tech*. 2008;10(3):248–253. <https://doi.org/10.1007/s12355-008-0044-7>.
 75. Minutolo M, Chiaiese P, Di Matteo A, Errico A, Corrado G. Accumulation of ascorbic acid in tomato cell culture: influence of the genotype, source explant and time of *in vitro* cultivation. *Antioxidants*. 2020;9(3):222. <https://doi.org/10.3390/antiox9030222>.
 76. Andreu P, Marin JA. Micropropagation enhances *in vitro* establishment and multiplication of new cultures from field grown plants of Adesoto 101 (*Prunus insititia*) rootstock. In: I International Symposium on Rootstocks for Deciduous Fruit Tree Species. *Acta Hortic*. 2004;658:605–609. <https://doi.org/10.17660/ActaHortic.2004.658.91>.
 77. Barney DL, Lopez OA, King E. Micropropagation of cascade huckleberry, mountain huckleberry, and oval-leaf bilberry using woody plant medium and Murashige and Skoog medium formulations. *HortTechnology*. 2007;17(3):279–284. <https://doi.org/10.21273/HORTTECH.17.3.279>.
 78. Wang G, Liu M, Liu G, Bao Z, Ma F. Establishment and optimization of agrobacterium-mediated transformation in blueberry (*Vaccinium* species). *Sci Hortic*. 2022;304, 111258. <https://doi.org/10.1016/j.scienta.2022.111258>.
 79. Tetsumura T, Matsumoto Y, Sato M, et al. Evaluation of basal media for micropropagation of four highbush blueberry cultivars. *Sci Hortic*. 2008;119(1):72–74. <https://doi.org/10.1016/j.scienta.2008.06.028>.
 80. Jeranyama P, DeMoranville CJ. Gas exchange and chlorophyll content of cranberry under salt stress. In: IX International *Vaccinium* Symposium. *Acta Hortic*. 2009;810:753–758. <https://doi.org/10.17660/ActaHortic.2009.810.100>.
 81. Sen A, Alikamanoglu S. Antioxidant enzyme activities, malondialdehyde, and total phenolic content of PEG-induced hyperhydric leaves in sugar beet tissue culture. *In Vitro Cell Dev Biol Plant*. 2013;49(4):396–404. <https://doi.org/10.1007/s11627-013-9511-2>.
 82. Clark RB. Physiological aspects of calcium, magnesium, and molybdenum deficiencies in plants. In: Soil Acidity and Liming. 2nd ed. *Agron Monogr*. 1984;12:99–170. <https://doi.org/10.2134/agronmonogr12.2ed.c3>.
 83. Karley AJ, White PJ. Moving cationic minerals to edible tissues: potassium, magnesium, calcium. *Curr Opin Plant Biol*. 2009;12(3):291–298. <https://doi.org/10.1016/j.pbi.2009.04.013>.
 84. Guo B, Abbasi BH, Zeb A, Xu LL, Wei YH. Thidiazuron: a multi-dimensional plant growth regulator. *Afr J Biotechnol*. 2011;10(45):8984–9000.
 85. Ahmad Z, Shahzad A. Thidiazuron influenced morphogenesis in some medicinal plants. In: Ahmad N, Faisal M, eds. *Thidiazuron: from Urea Derivative to Plant Growth*

- Regulator*. Singapore: Springer; 2018. https://doi.org/10.1007/978-981-10-8004-3_11.
86. Olatunji OA, Okunlola GO, Rufai AB, et al. Foliar application of thidiazuron alleviates Cd toxicity by modulating stress enzyme activities and stimulating pigment biosynthesis in *Cajanus cajan* (L.). *J Plant Growth Regul.* 2024;1–17 <https://doi.org/10.1007/s00344-024-11591-6>.
 87. Dłuzniewska P, Gessler A, Kopriva S, et al. Exogenous supply of glutamine and active cytokinin to the roots reduces NO₃⁻ uptake rates in poplar. *Plant Cell Environ.* 2006;29(7):1284–1297. <https://doi.org/10.1111/j.1365-3040.2006.01507.x>.
 88. Ghosh P, Roychoudhury A. Phytohormone signaling in the cross talk of micro- and macro-nutrient deficiency stress responses in plants. In: *Essential Minerals in Plant-Soil Systems*. Amsterdam, Netherlands: Elsevier; 2024. <https://doi.org/10.1016/B978-0-443-16082-0.00022-9>.
 89. Sedlák J, Paprstein F. Micropropagation of cranberry (*Vaccinium macrocarpon*) through shoot tip cultures. *Hortic Sci.* 2011;38(4):159–162. <https://doi.org/10.17221/115/2010-HORTSCI>.
 90. Meneses LS, Morillo LE, Vásquez-Castillo W. *In vitro* propagation of *Vaccinium floribundum* Kunth from seeds: promissory technology for mortiño accelerated production. *Can J Plant Sci.* 2022;102(1):216–224. <https://doi.org/10.1139/cjps-2020-0290>.
 91. Kurepa J, Smalle JA. Auxin/cytokinin antagonistic control of the shoot/root growth ratio and its relevance for adaptation to drought and nutrient deficiency stresses. *Int J Mol Sci.* 2022;23(4):1933. <https://doi.org/10.3390/ijms23041933>.
 92. Kurepa J, Shull TE, Smalle JA. Antagonistic activity of auxin and cytokinin in shoot and root organs. *Plant Direct.* 2019;3(2), e00121. <https://doi.org/10.1002/pld3.121>.
 93. Jones B, Gunnerås SA, Petersson SV, et al. Cytokinin regulation of auxin synthesis in *Arabidopsis* involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *Plant Cell.* 2010;22(9):2956–2969. <https://doi.org/10.1105/tpc.110.074856>.
 94. Yun A, Kim TD, Kim JA, Lee NN, Cheong EJ, Kim YW. Plant regeneration through multiple-shoot induction and ex vitro rooting in *Vaccinium oldhamii* Miq. *J Plant Biotechnol.* 2022;49(1):82–89. <https://doi.org/10.5010/JPB.2022.49.1.082>.
 95. Cuce M, Bektaş E, Sokmen A. Micropropagation of *Vaccinium arctostaphylos* L. via lateral-bud culture. *Turk J Agric for.* 2013;37(1):40–44. <https://doi.org/10.3906/tar-1204-31>.