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In vitro techniques in *Solanum lycopersicum*, *Solanum tuberosum*, and *Solanum* × *muricatum* anthers

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***Abstract* — In vitro techniques for Solanum transcendental crops were developed, including propagation from tomato seed, micropropagation of potato by tuber sprouts, and sweet pepino anther culture. At all stages of tomato, and potato a completely randomized design (CRD) with or without factorial arrangement was used; and culture media were assessed for sweet pepino anthers. Tomatoes Floradade (V1), and Montego (V2), potatoes Chaucha (CH), Leona Blanca (LB), and Leona Negra (LN), and sweet pepino New Generation (NG) were utilized, as well as two disinfection treatments (TD), different concentrations of gentamicin (50, 68.8, and 75 mg · L^{-1}), fungicides (FF), bacteriostatic and bactericidal (BB). Tomato germination with contamination < 10% was obtained with V1-TD1-68.8ppm = 86.67%, and V2-TD1-68.8ppm = 80.00%; while explant length (mean ± S.E.) ranging from V2-TD1-68.8ppm = 12.63 cm ± 0.49, to V2-TD2-68.8ppm = 3.40 cm ± 1.23 was significantly different; and ex vitro plantlet length was different between V1 = 12.64 cm ± 0.68, and V2 = 17.17 cm ± 0.69. In the micropropagation of potato (contamination 0.0-6.67% and survival 93.33-100%), leaf number was different among CH-FFBB-75ppm = 7.60 ± 0.43 (A), LB-FFBB-75ppm = 4.00 ± 0.58 (B), and LN-FFBB-75ppm = 1.50 ± 0.27 (C), and explant length was similar, with 2.14, 1.79, and 1.96 cm, respectively. Two calluses from sweet pepino anthers were obtained on media with kinetin (0.01-0.1 ppm), auxin, and cytokinin.**

Keywords: **Solanum; gentamicin; clone; callogenesis; microspore.**

***Resumen* — Se desarrollaron técnicas in vitro para cultivos Solanum transcendentales, incluyendo propagación a partir de semilla de tomate; micropropagación de papa por medio de brotes de tubérculo; y cultivo de anteras de pepino dulce. En todas las etapas de tomate y papa se usó un diseño completamente aleatorizado (DCA) con o sin arreglo factorial; y se evaluaron diferentes medios de cultivo para anteras de pepino dulce. Se utilizaron tomates Floradade (V1) y Montego (V2), papas Chaucha (CH), Leona Blanca (LB) y Leona Negra (LN), y pepino dulce Nueva Generación (NG), así como dos tratamientos de desinfección (TD), diferentes concentraciones de gentamicina (50, 68.8 y 75 mg · L^{-1}), fungicidas (FF), bacteriostático y bactericida (BB). La germinación de tomate con contaminación < 10% se obtuvo con V1-TD1-68.8ppm = 86.67%, y V2-TD1-68.8ppm = 80.00%; mientras que, la longitud del explanto (media ± E.E.) que va desde V2-TD1-68.8ppm = 12.63 cm ± 0.49, a V2-TD2-68.8ppm = 3.40 cm ± 1.23 fue significativamente diferente; y la longitud ex vitro de plántula fue diferente entre V1 = 12.64 cm ± 0.68, y V2 = 17.17 cm ± 0.69. En la micropropagación de papa (contaminación 0-6.67% y supervivencia 93.33-100%), el número de hojas fue diferente entre CH-FFBB-75ppm = 7.60 ± 0.43 (A), LB-FFBB-75 ppm = 4.00 ± 0.58 (B), y LN-FFBB-75 ppm = 1.50 ± 0.27 (C), y la longitud del explanto fue similar, con 2.14, 1.79 y 1.96 cm, respectivamente. Se obtuvieron dos callos a partir de anteras de pepino dulce en medios con kinetina (0.01-0.1 ppm), auxina y citoquinina.**

Palabras Clave: **Solanum; gentamicina; clon; callogénesis; microspora.**

I. INTRODUCTION

IN tissue culture of tomato, the shoot is formed in the same basal medium, in one supplemented with higher concentration of cytokinin in relation to auxin, or in another only with cytokinin; while, the callus is induced from several explants, commonly hypocotyl and leaf, in combination with growth regulators [1].

Otherwise, in tissue culture of potato visible bacterial contaminants *Bacillus infantis* (Gram +), *Enterobacter cloacae* sub. sp. *dissolvens* (Gram -), and *B. alcalophilus* (Gram +) susceptible to gentamicin and rifampicin were identified by molecular, and biochemical methods, respectively [2], and others including *Pseudomonas* sp., *Staphylococcus* sp., *Klebsiella* sp., *Corynebacterium* sp., *Proteus* sp. and *Bacillus* sp. susceptible to 50 mg · L^{-1} of gentamicin were identified by morphological and biochemical characteristics [3]; while exogenous fungi contaminants *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Fusarium* sp. and *Rhizopus* sp. susceptible to carbendazim (0.15%) have been isolated from potato cultures [3]; carbendazim is a systemic fungicide that belongs to benzimidazole family, a group of organic fungicides extensively used in agriculture

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[4], [5]. Also, latent infection caused by endogenous microorganisms is an aspect of serious concern; small changes in the culture medium conditions cause a rapid proliferation of pathogens [6], representing a threat to the vitality and growth of the explant [7]. Both types of contamination, could be controlled with antibiotics, but in a large extent antibiotics can affect explant survival and regeneration [8]. Gentamicin, which belongs to the aminoglycosides group, inhibits protein synthesis, and rifampicin from the rifampicin group inhibits RNA synthesis, and consequently bacterial growth [9].

In contrast, the development of microspores of sweet pepino through anther culture involves gametophytic advantages compared to other alternative methods for breeding purposes [10].

II. MATERIAL AND METHODS

The research was carried out during November 2023-2024, at GENNBIO laboratory, Latacunga, Cotopaxi, Ecuador.

A. In vitro propagation of tomato

The first superficial disinfection treatment (TD1) for seeds consisted in alcohol 30% (v/v) for 10 min, followed by sodium hypochlorite (NaClO) 30% (v/v) for 10 min, hydrogen peroxide (H_2O_2) 7% (v/v) for 8 min, and washes with sterile distilled water after each disinfecting agent [11]. The second treatment (TD2) consisted in ethyl alcohol 70% (v/v) for 2 s, followed by NaClO 0.6% (v/v) with 0.5 mL of surfactant for 15 min, and washes with sterile double-distilled water three consecutive times [12]. Basal culture medium was used for *in vitro* introduction (stage 1) [13], supplemented with sucrose, activated charcoal, systemic, and protective fungicide, adjusted to pH 5.7 ± 0.1, and previously autoclaved [11], [12], [14] (Table 1).

A completely randomized design (CRD) with factorial arrangement 2A × 2B × 3C was used, along with two varieties of tomato (V1 and V2), two seed disinfection treatments (TD1 and TD2), and three concentrations of gentamicin (50, 68.8, and 75 mg · L^{-1}) (n = 30; N = 360). The experimental unit consisted of a culture capsule containing two seeds, each seed represents one sample unit. The Shapiro-Wilk test, as well as the Bartlett's test were used. The Analysis of variance (ANOVA) includes Tukey-Kramer's procedure for unequal sample sizes [15], while Welch's ANOVA for unequal variances includes pairwise comparisons using the Games-Howell method recommended when the sample sizes are greater than five, otherwise it could be too liberal when sample sizes are small, [16]. After sealing the culture capsules with transparent plastic, these were placed in the incubation room at constant temperature of 25-26 °C with 16-hour photoperiod [12], for five weeks. The evaluated variables were: germination percentage, contamination percentage, number of leaves, and explant length.

Ex vitro proliferation and rooting of tomato (stage 2) was executed by the addition of 0.5 mg · L^{-1} of 6-benzyl amino purine (6-BAP), 0.5 mg · L^{-1} of indole acetic acid (IAA), macro and micronutrients, and indole butyric acid (IBA) as a rooter in peat [11]. A CRD with factorial arrangement 2A × 2B was used, along with two varieties (V1 and V2), and two concentrations of IBA (0.0 and 2.0 mg · L^{-1}) (n = 12; N = 48). The experimental unit consisted of a culture capsule containing one seedling as the sample unit. The Shapiro-Wilk test, as well as the Bartlett's test were used. ANOVA includes Duncan's test which is more powerful, and hence less conservative than Tukey's HSD procedure [17], to protect adequately type I error rate [18]. After sealing the transparent plastic culture capsules, they were placed in the pre-acclimation room at 24-26 °C [12], and natural photoperiod conditions for six weeks. The evaluated variable was: plantlet length. Statistical analysis was performed using Minitab 16, Minitab 17, Infostat 2020I, and R 4.3.2. package agricolae [19].

TABLE I
CULTURE MEDIA FOR *IN VITRO* TECHNIQUES IN *S. LYCOPERSICUM*, *S. TUBEROSUM*, AND *S.* × *MURICATUM*

Component	Basic	Proliferation	Anther
GENNBIO Basal	25 mL · L^{-1}	_	_
GENNBIO ¼	_	25 mL · L^{-1}	25 mL · L^{-1}
Fungicide mixture: carboxin, thiram	100 mg · L^{-1}	_	_
Antibiotic: gentamicin	68.8 mg · L^{-1}	75 mg · L^{-1}	68.8 mg · L^{-1}
Fungicide: metalaxyl	_	222.32 mg · L^{-1}	222.32 mg · L^{-1}
Fungicide: thiophanate-methyl (benzimidazole)	_	275.2 mg · L^{-1}	275.2 mg · L^{-1}
6-BAP	_	0.5 mg · L^{-1}	0.5 mg · L^{-1}
IAA	_	0.5 mg · L^{-1}	0.5 mg · L^{-1}
KN	_	_	0.01-0.1 mg · L^{-1}
Bacteriostatic: chloramphenicol	_	10 mg · L^{-1}	10 mg · L^{-1}
Bactericidal: rifampicin	_	100 mg · L^{-1}	100 mg · L^{-1}
Sucrose	3.0% (w/v)	2.5% (w/v)	3.0% (w/v)
Agar-agar	8.5 g · L^{-1}	8.0 g · L^{-1}	7.2 g · L^{-1}

B. Clonal micropropagation of potato using tuber sprouts

Asepsis starts by washing the tuber sprouts (BT) of potato with running tap water for 5 min. The first treatment for disinfection (TD) of explants consisted in ethanol 70% (v/v) for 1 min, followed by NaClO 20% (v/v) for 20 min, and rinsed them with sterile distilled water [20]. The treatment for decontamination (FF) consisted in the same TD, and the addition of combined metalaxyl (222.32 mg · L^{-1}) [21], and thiophanate-methyl (275.2 mg · L^{-1}) to the proliferation medium (Table 1). The effect of gentamicin was evaluated at 68.8, 75, and 100 mg · L^{-1} [9], [22]; the medium also contained 6-BAP [23], and IAA [11]. Each explant was cultured individually.

A CRD design with factorial arrangement 2A × 3B was used for the variety Chaucha (CH), along with two disinfection or decontamination treatments (TD, and FF), and three concentrations of gentamicin (68.8, 75, and 100 mg · L^{-1}) (n = 40; N = 240). The experimental unit consisted of a culture tube containing one tuber sprout as the sample unit. Levene's test which is powerful and robust to non-normality [24], and Bartlett's test were used. Culture tubes remained for 21 days in incubation

room [9], [22], with 16 h of light and 8 h of darkness, and 23 ± 2 °C [9]. The evaluated variables were: contamination percentage, survival percentage, number of leaves, explant length, and root formation.

Tuber sprouts from Chaucha (CH), Leona Blanca (LB), and Leona Negra (LN) varieties were used in a subsequent experiment, the superficial disinfection treatment (TD) was preserved, followed by decontamination with fungicides (FF) added to the culture medium, with 75 mg · L^{-1} of gentamicin (Table 1); plus growth regulators [11], [23], chloramphenicol (10.0 mg · L^{-1}), and rifampicin (100 mg · L^{-1}) [22]. A CRD with three treatments (CH-FF-BB-75ppm, LB-FF-BB-75ppm, and LN-FF-BB-75ppm) was used (n = 15; N = 45). The experimental unit consisted of a tube culture containing a tuber sprout as a sampling unit. Welch's ANOVA was used [16]. Also, Levene's [24], and Tukey's tests were used for the ANOVA. Culture tubes remained for 21 days in incubation room with 16-hour photoperiod, and 23 ± 2 °C. The evaluated variables were: contamination percentage, survival percentage, number of leaves, explant length, number of nodes, and root formation. Statistical analysis was performed using Minitab 16, Infostat 2020I, and R 4.3.2. package agricolae [19].

C. Development of sweet pepino microspores through anther culture

Once anthers were extracted, the explants were disinfected in ethanol 96% (v/v) for 2 min [25], followed by NaClO 20% (v/v) for 15 min, and washed with sterile double-distilled water [10]; after that, the cultures passed to the incubation room in darkness at 28-35 °C for 8 days in callus culture medium [10], [25], supplemented with 0.01 mg · L^{-1} of KN (Table 1), followed by incubation with 16 h of light and 8 h of darkness for more than four days at 25 °C [10], [26]; and transferred to the proliferation medium containing 0.1 mg · L^{-1} of KN [25], [26], with or without 6-BAP and IAA [11], and 16-hour photoperiod for twelve days at 25 °C [10].

The anther counting and evaluation were performed in different culture media (Table 1), two for callus with KN (0.0 and 0.01 mg · L^{-1}), and three for proliferation with KN and other growth regulators (0.0 mg · L^{-1} KN, 0.1 mg · L^{-1} KN, and 0.1 mg · L^{-1} KN with 0.5 mg · L^{-1} 6-BAP and 0.5 mg · L^{-1} IAA) (n = 100; N = 600). The experimental unit consisted of a culture plate containing 10 anthers, each anther represents one sample unit. The evaluated variables were: induction of callogenesis, transfer of decontaminated explants, and callus obtained.

III. RESULTS AND DISCUSSION

A. In vitro propagation of tomato

In stage 1, germination percentage of tomato seeds in V2-TD1-50ppm = 96.67%, followed by V2-TD1-75ppm = 86.67%, V1-TD1-68.8ppm = 86.67%, and V2-TD1-68.8ppm = 80.00%; the germination in V2-TD1-50ppm is higher than 92% reported for *S. caripense* using the same treatment (TD1) [11]. The contamination percentage in V1-TD1-68.8ppm = 6.67%, V1-TD2-68.8ppm = 6.67%, V2-TD1-68.8ppm = 0.00%, and V2-TD2-68.8ppm = 0.00%, was less than 10% [27].

The explant length (mean ± S.E.) for germinated seeds of tomato at day 35 of the stage 1 is statistically different in CRD and factorial arrangement 2A × 2B × 3C, with p-value = 0.04388 for the interaction variety × disinfection × gentamicin (Table 2); and coefficient of variation = 31.07%. Treatment V2-TD1-68.8ppm = 12.63 cm ± 0.49 (E), followed by V2-TD1-50ppm = 9.27 cm ± 0.48 (D), V2-TD1-75ppm = 8.21 cm ± 0.49 (CD), mainly; while the shortest length was obtained in V2-TD2-68.8ppm = 3.40 cm ± 1.23 (A). Normality in the residual errors was checked by the Shapiro-Wilk test with p-value = 0.4697 and W = 0.99301; nevertheless, there was no homogeneity of variances by Bartlett's test with p-value = 0.01673; and the conservative Tukey-Kramer's test (alpha = 0.05) was used [15] (Fig. 1), due to Tukey's Honestly Significance Difference (HSD) procedure is probably the most used test for controlling type I error in multiple pairwise comparisons [28]. By Welch's ANOVA, it was demonstrated that means of the treatments are statistically different with p-value = 0.000; it includes pairwise comparisons using the Games-Howell method (95% confidence) [16] (Table 3). Note that the explant length in varieties V1-TD1-68.8ppm = 7.25 cm, and V2-TD1-68.8ppm = 12.63 cm, was significantly different through both statistical procedures, considering its germination (86.67-80.00%), and contamination (6.67-0.00%) percentages; while the explant length in V2-TD1-68.8ppm is significantly higher than that of V2-TD1-50ppm through both statistical procedures; and the explant length in V2-TD1-75ppm is similar to V2-TD1-50ppm, and shorter than V2-TD1-68.8ppm.

The explant length in V2-TD1-68.8ppm is higher than TM0 = 11.08 cm ± 1.24 without growth regulators at proliferation stage of *S. caripense* [11]; and the number of leaves from V1 (4.27), and V2 (4.86) treated with 68.8 mg · L^{-1} of gentamicin was lower than TM0 = 10.25 without growth regulators at proliferation stage of *S. caripense*, after 35 days of subculture [11].

Plantlet length (mean ± S.E.) from V1-0.0ppm, V1-2.0ppm, V2-0.0ppm, and V2-2.0ppm, at day 42 of stage 2, was statistically similar in CRD and factorial arrangement 2A × 2B, with p-value = 0.7345; and coefficient of variation = 22.62%. Therefore, the variety × rooter interaction was sent to the experimental error; and the analysis continued with its main effects. Subsequently, the plantlet length was significantly different for the variety factor, with p-value < 0.0001, and coefficient of variation = 22.39%. Normality in the residual errors was checked by the Shapiro-Wilk test with p-value = 0.7295, and W = 0.98326; as well as, homogeneity of variances through Bartlett's test with p-value = 0.1315. V1 = 12.64 cm ± 0.68 (A) was significantly different from V2 = 17.17 cm ± 0.69 (B) using Duncan's test (alpha = 0.05) [17], [18].

TABLE II
ANOVA FOR EXPLANT LENGTH IN TOMATO (*S. LYCOPERSICUM*) VARIETIES AT *IN VITRO* INTRODUCTORY STAGE

S.V.	Adjus. S.S.	D.F.	Adjust. M.S.	F	p-value
Model	939.98	11	85.45	14.21	< 0.0001
Var	102.96	1	102.96	17.12	0.0001
TD	107.34	1	107.34	17.85	< 0.0001
Antib	2.28	2	1.14	0.19	0.8272
Var × TD	189.06	1	189.06	31.44	< 0.0001
Var × Antib	1.60	2	0.80	0.13	0.8752
TD × Antib	220.67	2	110.34	18.35	< 0.0001
Var × TD × Antib	38.23	2	19.12	3.18	0.0439
Error	1118.32	186	6.01	-	-
Total	2058.30	197	-	-	-

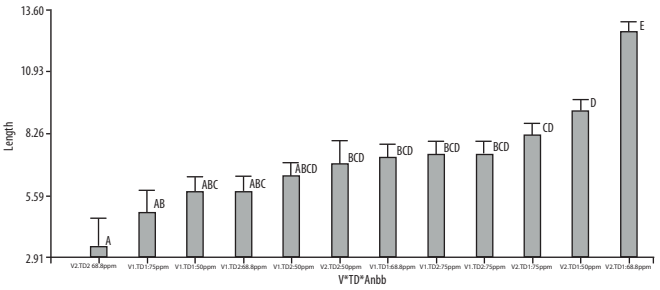

Fig. 1. Length of germinated tomato (*S. lycopersicum*) seeds at *in vitro* introductory stage.

TABLE III
THE GAMES-HOWELL METHOD FOR EXPLANT LENGTH IN TOMATO (*S. LYCOPERSICUM*) VARIETIES AT *IN VITRO* INTRODUCTORY STAGE

Treatments	n	Mean (cm)	St. Dev.	Grouping
V2-TD1-68.8ppm	25	12.628	3.572	A
V2-TD1-50ppm	26	9.265	2.163	B
V2-TD1-75ppm	25	8.212	1.931	B C
V2-TD2-75ppm	22	7.400	2.857	B C D
V1-TD2-75ppm	18	7.361	1.801	B C D
V1-TD1-68.8ppm	19	7.253	2.676	B C D
V2-TD2-50ppm	6	6.967	1.821	B C D
V1-TD2-50ppm	17	6.429	2.572	C D
V1-TD2-68.8ppm	15	5.820	1.594	D
V1-TD1-50ppm	15	5.773	2.316	C D
V1-TD1-75ppm	6	4.850	1.394	D
V2-TD2-68.8ppm	4	3.400	1.577	D

Plantlet length in V2-2.0ppm = 17.82 cm ± 0.97, and V1-2.0ppm = 13.61 cm ± 0.97 is higher than those of plantlets without IBA. A concentration higher than 2.0 mg · L^{-1} of IBA decreases growth in stem length of *S. caripense*; the length in V2-2.0ppm, and V1-2.0ppm after 42 days of subculture is shorter than TE3 = 34.17 cm with 2.0 mg · L^{-1} of IBA in *S. caripense* [11].

B. Micropropagation of potato using tuber sprouts

Very high contamination was observed in Chaucha variety, ranging from BT-TD-100ppm = 77.5% to BT-FF-75ppm = 90.0%. In contrast, after the first proposed disinfection treatment (TD) in purple flesh potatoes, 20.34% (very low) of contamination was obtained [20]; whereas, the survival percentage was low, ranging from BT-TD-100ppm = 5.0% to BT-TD-75ppm = 35.0%, and it is lower than 79.99% of survival reported for purple flesh potatoes [20].

The number of leaves (mean ± S.E.) in Chaucha variety at day 21 of micropropagation was different for the disinfection × gentamicin interaction with p-value = 0.0480; ranging from BT-FF-100ppm = 4.33 ± 1.97 (A) to BT-TD-100ppm = 13.00 ± 2.79 (B), using Tukey's test (alpha = 0.05). There was no normality in the residual errors using the Shapiro-Wilk test with p-value = 0.01534; therefore, homogeneity of variances was checked by Levene's test, with a p-value = 0.070 [24].

The explant length (mean ± S.E.) in Chaucha variety was similar for all treatments in the disinfection × gentamicin interaction with p-value = 0.6332; ranging from BT-FF-100ppm = 1.57 cm ± 0.34 to BT-TD-100ppm = 2.20 cm ± 0.48, using Tukey's test (alpha = 0.05). There was normality in the residual errors using the Shapiro-Wilk test with p-value = 0.3633, and there was homogeneity of variances using Bartlett's test with p-value = 0.7234. Nevertheless, the explant length in BT-TD-100ppm is shorter than the average length of other potato shoots using gentamicin at 100 mg · L^{-1} (5.07 cm), and similar to L. Delikates (2.15 cm) after three weeks of cultivation [22].

Low percentage of contamination was obtained with CH-FF-BB-75ppm = 6.67%, LB-FFBB-75ppm = 0.00%, and LN-FFBB-75ppm = 6.67%, and high survival percentage corresponding to 100%, 100%, and 93.33%, respectively (Table 4). In contrast, in purple flesh potatoes the contamination percentage of 48.89% with survival of 51.11%, is considered low in abundance of contamination, and 20.34% a very low percentage of contamination [20]. Root formation was observed in CH-FF-B-75ppm (100%), LB-FFBB-75ppm (13.33%), and LN-FFBB-75ppm (6.67%). In previous research, the number of roots from tuber sprouts with 10 mg · L^{-1} of gentamicin was similar to the control without the antibiotic, whereas with increasing gentamicin concentration the number of roots decreased [9]; on the other hand, 20, 50 and 100 mg · L^{-1} of chloramphenicol are not recommended due to the strong detrimental effect on tissue regeneration [22].

The number of leaves (mean ± S.E.) among CH-FF-BB-75ppm = 7.60 ± 0.43 (A), LB-FFBB-75ppm = 4.00 ± 0.58 (B), and LN-FFBB-75ppm = 1.50 ± 0.27 (C) was significantly different; normality in the residual errors checked by the Shapiro-Wilk test with p-value = 0.05386 and W = 0.94975; and continued by Welch's ANOVA with p-value = 0.000 [16]. The number of nodes (mean ± S.E.) among CH-FF-BB-75ppm = 6.53 ± 0.44 (A), LB-FFBB-75ppm = 3.53 ± 0.39 (B), and LN-FFBB-75ppm = 3.00 ± 0.47 (B) was significantly different by ANOVA with p-value = 0.000, using Levene's test with p-value = 0.846 [24], and Tukey's test (alpha = 0.05). While, the length of explant was statistically similar through normality in the residual errors checked by the Shapiro-Wilk test with p-value = 0.3896 and W = 0.97317; and followed by Welch's ANOVA with p-value = 0.193 [16].

TABLE IV
MICROPROPAGATION OF POTATO
(*S. TUBEROSUM*) BY TUBER SPROUTS

Treatment	Contamination (%)	Survival (%)	Num. leaves (mean ± S.E.)	Num. nodes (mean ± S.E.)	Explant length (cm ± S.E.)	Root formation (%)
CH-FFBB-75ppm	6.67	100	7.60 ± 0.43 (A)	6.53 ± 0.44 (A)	2.14 ± 0.11 (A)	100
LB-FFBB-75ppm	0.00	100	4.00 ± 0.58 (B)	3.53 ± 0.39 (B)	1.79 ± 0.16 (A)	13.33
LN-FFBB-75ppm	6.67	93.33	1.50 ± 0.27 (C)	3.00 ± 0.47 (B)	1.96 ± 0.22 (A)	6.67

Note. CH = Chaucha; LB = Leona Blanca; LN = Leona Negra; FF = fungicides; BB = bacteriostatic and bactericidal; ppm (gentamicin).

C. Development of sweet pepino microspores through anther culture

The anthers swelled and became necrotic (induction of callogenesis) in M1.2. = 43% and M2.2. = 7%; while microspores proliferated within the pollen sac [26], or inside the anthers, as obtained with the media M1.2. = 2 calluses (Table 5).

Embryogenesis (androgenesis) has been reported for pepper with up to 2.23% of anthers grown in combination of 6-furfurylaminopurine (KN) with 2-4 dichlorophenoxyacetic acid (2,4-D) (1.0 mg · L^{-1} of both), or IAA with KN (0.1 mg · L^{-1} of both) [25]; however, in uchuva, anther viability was 70% post disinfection, with callus formation between 90-20%, and plant regeneration of 95-10% in B5 and LS culture media [29]; nevertheless, a mixture of MS salts is preferably in *Solanaceae* species [30].

TABLE V
EVALUATION OF CULTURE MEDIA USED FOR SWEET PEPINO
(*S.* × *MURICATUM*) ANTHER CULTURE

Callus medium	Proliferation medium	Callogenesis induction (%)	Transfer of decontaminated explants (%)	Callus obtained
M1 = 0.01 mg · L^{-1} KN	M1.0 = 0.0 mg · L^{-1} KN	0	70	0
M1 = 0.01 mg · L^{-1} KN	M1.1 = 0.1 mg · L^{-1} KN	1	60	0
M1 = 0.01 mg · L^{-1} KN	M1.2 = 0.1 mg · L^{-1} KN, 0.5 mg · L^{-1} 6-BAP, and 0.5 mg · L^{-1} IAA	43	80	2
M2 = 0.0 mg · L^{-1} KN	M2.0 = 0.0 mg · L^{-1} KN	0	40	0
M2 = 0.0 mg · L^{-1} KN	M2.1 = 0.1 mg · L^{-1} KN	0	70	0
M2 = 0.0 mg · L^{-1} KN	M2.2 = 0.1 mg · L^{-1} KN, 0.5 mg · L^{-1} 6-BAP, and 0.5 mg · L^{-1} IAA	7	70	0

Note. M1.0. = M1 and 0.0 mg · L^{-1} KN; M1.1. = M1 and 0.1 mg · L^{-1} KN; M1.2. = M1 and 0.1 mg · L^{-1} KN with auxin and cytokinin; M2.0. = M2 and 0.0 mg · L^{-1} KN; M2.1. = M2 and 0.1 mg · L^{-1} KN; M2.2. = M2 and 0.1 mg · L^{-1} KN with auxin and cytokinin.

IV. CONCLUSIONS

The *in vitro* propagation of tomato with contamination < 10% was achieved by the germination in V1-TD1-68.8ppm = 86.67%, and V2-TD1-68.8ppm = 80.00%, and the differences in *ex vitro* plantlet length between V1 = 12.64 cm, and V2 = 17.17 cm. The micropropagation of potato with 0.0-6.67% of contamination, and 93.33-100% of survival, demonstrated differences in leaf number among CH-FFBB-75ppm = 7.60 (A), LB-FFBB-75ppm = 4.00 (B), and LN-FFBB-75ppm = 1.50 (C); and similar explant length with 2.14, 1.79, and 1.96 cm, respectively. Two calluses from sweet pepino anthers were obtained with media M1.2.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ARTIFICIAL INTELLIGENT STATEMENT

The authors declare that no generative artificial intelligence tools were used in the preparation of this manuscript.

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