Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Effects of Elicitation on *Invitro* Regeneration of two Tomato (*Solanum lycopersicum* L.) Cultivars in Tissue Culture

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Received – July 21, 2023; Revision – January 10, 2024; Accepted – January 31, 2024
Available Online – March 15, 2024

DOI: http://dx.doi.org/10.18006/2024.12(1).106.123

KEYWORDS

Concentration

Explant

Growth

Propagation

Tissue culture

ABSTRACT

Exploring alternative avenues, *in vitro* culture emerges as a promising option for potential bioactive compound sources. However, compared to intact plants, only a few cultures demonstrate efficient synthesis of secondary metabolites. Elicitors have gained prominence as stress agents for enhancing *in vitro* micropropagation in specific tissues, organs, and cells. Recent advancements in plant tissue culture involve elicitors, opening new possibilities for *in vitro* production of crucial food crops. This research aimed to investigate the impact of three elicitors (Activane®, Micobiol®, and Stemicol®) on germination and in vitro multiplication of two tomato cultivars explants, employing both direct and indirect *in vitro* organogenesis. Among the texted elicitors, Micobiol® emerged as a successful elicitor, promoting optimal seed germination, survival, and 100% growth compared to the 80% in the control group. Further, Activane® exhibited a favourable induction response and achieved 96%, 95%, and 100% in weight and diameter of callus, yet various elicitor concentrations did not exert significant influence across treatments. In conclusion, an effective disinfection and in vitro implantation of tomato seeds ensured successful germination, promoting seedling survival and growth. Various elicitors positively impacted in vitro organogenesis, particularly in root induction, with higher survival percentages in acclimatized plants. The study guides future research on elicitor treatments for large-scale tomato in vitro propagation, emphasizing the need to identify optimal elicitor concentrations.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Tomato (*Solanum lycopersicum* L.) stands out as a vital perennial crop within the Solanaceae family, holding immense economic and cultural significance globally. Renowned for its nutritional value, it serves as a crucial source of vitamin C, essential minerals like phosphorus and potassium, and carotenoids such as lycopene and β-carotene (SIAP 2020; Aldubai et al. 2022; Liu et al. 2022). Leading tomato-producing nations include China, the United States, India, Turkey, Egypt, Italy, Iran, Spain, Brazil, Mexico, Uzbekistan, and Russia (Peralta and Spooner 2006). In Mexico, the cultivation of tomatoes spans 43,903 hectares, yielding 63,116 tonnes/ha, distributed across various regions. Noteworthy production zones encompass the Northwest (Sinaloa, Sonora, and Baja California), the Pacific coast (Nayarit, Jalisco, Michoacán, Guerrero, and Oaxaca), the Central North Zone (San Luis Potosí and Coahuila), and Las Huastecas (Tamaulipas, Veracruz, San Luis Potosí, and Hidalgo), contributing significantly to meet domestic market demands throughout the year. While traditional open-field and greenhouse cultivation historically dominated tomato production in Mexico, recent trends indicate a growing adoption of innovative farming technologies by Mexican tomato growers (SIAP 2020; Aznar-Sánchez et al. 2020; Maureira et al. 2022).

Today, the genetic transformation of tomatoes requires an efficient micropropagation and regeneration *in vitro* system. Thus, biotechnological methods have played a pivotal role in facilitating the propagation of commercially significant fruit species. These methods enable the selection of elite plant material, mitigate phytosanitary issues, and require minimal space while independent of traditional agricultural inputs (Hussain et al. 2012; Hasnain et al. 2022). Plant tissue culture has emerged as a promising alternative for the sustainable production of various plant species affected by environmental or geographical constraints. Successful application of plant tissue culture relies on establishing efficient culture systems that address key challenges encountered by plants *in vitro*. Direct organogenesis has been widely adopted, a method allowing the regeneration of complete seedlings from plant tissues without dedifferentiation stages like callus or somatic embryo formation. Inducers and elicitors have been employed in this approach to enhance the production of numerous plant species (Hussain et al. 2012; Baenas et al. 2014; Al-Khayri and Naik, 2020; Xu et al., 2023). *In vitro* plant cultures present an appealing and economically viable alternative to traditional methods for producing agriculturally important crops. Notably, they offer the only sustainable and ecological system for obtaining complex chemical structures biosynthesized by rare or endangered plant species resistant to domestication. The *in-vitro* propagation of tomato crops is influenced by various biotic and abiotic conditions (Ramirez-Estrada et al. 2016; Munim et al. 2019).

Moreover, the regeneration of plants through tissue culture techniques is a crucial aspect of biotechnological research and the genetic manipulation of plants. Cultures are meticulously maintained in aseptic environments to ensure the production of disease-free plants and are also free from the risk of reinfection. Achieving high-frequency plant regeneration from in-vitro tissue culture is essential for successfully applying tissue culture techniques in crop improvement. Inducing direct organogenesis in tomato plants is a valuable alternative for the clonal propagation of disease-free plants and is particularly useful for genetic transformation. Therefore, there is an urgent need for technological and biotechnological interventions to enhance the in vitro regeneration of tomato plants (Karim and Kayum 2007; Holmes et al. 2021; Ozyigit et al. 2023).

While regeneration systems for different tomato varieties, organogenesis has been widely documented using various explants such as hypocotyls, leaves, or cotyledons. Evidence of achieving higher in-vitro regeneration using elicitors in tomatoes is limited. Numerous elicitors have been studied for their response in *in-vitro* germination, micropropagation, growth, and crop production in various crops including *S. lycopersicum* (García-Osuna et al. 2015; Murillo-Gómez et al. 2017; Cham et al. 2021; Cham et al. 2022), *Stevia rebaudiana* (Bayraktar et al. 2016), and *Hypericum perforatum* in shoot cultures (Gadzovska et al. 2014). Elicitors are actively being explored as a biotechnological strategy in studying processes related to plant material production, as they enable the rapid generation of healthy crops that can be propagated on a large scale to meet commercial demand. Hence, the objective of this research was to assess the impact of three elicitors (Activane®, Micobiol®, and Stemicol®) on the germination and multiplication of in vitro explants from two tomato cultivars through both direct and indirect in vitro organogenesis.

2 Materials and Methods

2.1 Plant Material and Growth Conditions

The plant materials utilized for the two tomato cultivars were "Semilla Hortaflor Jitomate Bola" and "Semilla Hortaflor Jitomate Saladette." In the case of seeds, they were disinfected using a solution of Sodium Hypochlorite (NaOCl) at concentrations of 15% (v/v Cloralex®). Explants from in vitro plants did not undergo disinfection, as they originated from aseptic plants. The leaf and petiole explants were sourced from in vitro plants of the selected two tomato cultivars. All experiments were conducted during the summer-spring period of 2020-2021 at the Laboratory of Tissue Culture (LCT) within the Faculty of Agronomy at the Autonomous University of Nuevo León (UANL). The laboratory is situated at the Campus of Agricultural Sciences in the municipality of Gral. Escobedo, NL, Mexico.

Table 1 Evaluated treatments with different concentrations of elicitors for the experiments *in vitro*

Treatment	Elicitor	Doses
T1(Cntl)	Control	0.00
T2(Act*)	Activane®	0.20, 0.40, 0.60 mL L^{-1}
T3(Micbl*)	Micobiol®	0.20, 0.40, 0.60 mL L^{-1}
T4(Stml*)	Stemicol®	0.20, 0.40, 0.60 mL L^{-1}

2.2 Preparation of Elicitors

Three elicitors (Activane®, Micobiol®, and Stemicol®) were individually dissolved in 25 mL of distilled water across three required concentrations (0.2, 0.4, & 0.6 mL L^{-1}). Prior to this, stock solutions of Activane®, Micobiol®, and Stemicol® were incorporated into the media (MS) at the desired concentrations after autoclaving at 121°C for 15 minutes. In the current study, the elicitors (Activane®, Micobiol®, and Stemicol®) at three distinct concentrations (0.2, 0.4, & 0.6 mL L^{-1}) were individually added to the media (MS) as elicitors (Table 1).

2.3 Preparation of Culture Medium

The culture medium employed consisted of the fundamental salts of the Murashige-Skoog 1962 (MS) medium, supplemented with 30g L^{-1} sucrose and 4.4g L^{-1} phytagel. The pH was adjusted to 5.8 using NaOH and 1N HCl. This medium was dispensed into Magenta® boxes measuring 7 x 9 cm, each containing 30 mL medium. Subsequently, the boxes were sterilized in an autoclave at 121°C under 15 pounds of pressure for 15 minutes.

2.4 Evaluation of Sterilization Treatments

The plant materials, specifically tomato seeds, underwent surface sterilization through a multi-step process. Initially, they were immersed in 70% ethanol for 60 seconds, followed by three washes with sterile distilled water. Subsequently, the seeds were placed in a solution of Sodium Hypochlorite (NaOCl) at concentrations of 15% and 20% (v/v Cloralex®), supplemented with 0.02% Tween 20, for 15 minutes. This sterilization process was reiterated for thorough disinfection. Following the treatment, the seeds were washed thrice with sterile distilled water, following the protocol outlined by Espinosa (2005).

The sterilized tomato seeds were then introduced into the culture medium (MS) with doses of Sodium Hypochlorite (NaOCl) at 15% and 20% (v/v Cloralex®), with ten repetitions for each treatment. The experimental units were arranged under a photoperiod of 16 hours of light and eight hours of darkness at a temperature of 24 ± 2 °C. The experimental design employed was a completely randomized design with a factorial arrangement (2 X 2), incorporating ten repetitions for each factor: (a) cultivar and (b) doses of the sterilizing agent. Data collection occurred every three days over a month, focusing on evaluating the percentage of germination, contamination, and oxidation at this stage.

2.5 Evaluation of *In Vitro* Seed Germination and Growth

The materials utilized comprised tomato seeds from the two cultivars, namely "Bola and Saladette". The seeds underwent a disinfection process described earlier to eliminate surface contaminants, conducted under a laminar flow hood. Subsequently, the tomato seeds were introduced into the culture medium (MS) with four treatments (Control, Activane®, Micobiol®, and Stemicol®) at three different doses, with 15 repetitions per treatment. After sowing, the experimental units were maintained under photoperiod conditions of 16 hours of light and eight hours of darkness at a temperature of 24 ± 2 °C. The experimental design employed was a completely randomized design with a factorial arrangement (2 x 3 x 3) incorporating fifteen repetitions, considering factor (a) cultivars, factor (b) elicitors, and factor (c) doses of elicitors. Finally, data collection occurred every three days over 8 weeks. At this stage, the evaluation encompassed parameters such as the percentage of germination, oxidation, viability, number of leaves, and plant height.

2.6 Preparation of Explants for *in-vitro* culture

The explants employed in the study were segments of nodes measuring 1.5 to 2.0 cm in length, sourced from both branches and leaves of the mother plants belonging to the tomato cultivars. These mother plants were previously propagated and housed in transparent glass bottles approximately 15-20 cm in length. The propagated plants were incubated at the Laboratory of Tissue Culture (LCT) for 21 days. Under controlled conditions, with a photoperiod of 16 hours of light and eight hours of darkness and at a temperature of 24 ± 2 °C, the explants were allowed to acclimate before being utilized in the experiments (Figure 1).

2.7 Direct and Indirect *in-vitro* Organogenesis or Callogenesis (callus formation)

Two-week-old seedlings (derived from the previous stage) measuring 2.0 cm in height were utilized for the subsequent stage of the experiment. These seedlings were established in a medium (DM) consisting of basic salts, vitamins, 100 mg L^{-1} of myoinositol, and three concentrations of each elicitor: Activane®

Figure 1 The mother plants used as explants for the *in vitro* culture; the explants consist of segments of 1.5 to 2.0 cm in length from branches and leaves of the two tomato cultivars (B) Saladette (Green tag) and Bola (Red tag).

(0.2, 0.4, 0.6 mL L^{-1}), Micobiol® (0.2, 0.4, 0.6 mL L^{-1}), and Stemicol® (0.2, 0.4, 0.6 mL L^{-1}). The medium also included 30 gL^{-1} of sucrose and 4.3 gL^{-1} of phytagel, and four explants were placed per experimental unit. The experiment followed a completely randomized design with a factorial arrangement of (2 x 3 x 3) and incorporated five repetitions for each treatment. The experimental units were subjected to controlled conditions of photoperiod and temperature, comprising 16 hours of light (54 µmol m-2 s^{-1}) and 8 hours of darkness at 24 ± 2 °C. After three months, the following variables were assessed for direct organogenesis: asepsis percentage, growth, oxidation, viability, and the number of leaves. For indirect organogenesis, the flasks were incubated in the dark, and the assessment included asepsis percentage, induction, oxidation, viability, days to first callus formation, callus diameter, and weight.

2.8 Root Induction

In the subsequent phase, regenerated shoots from the previous stage were isolated and transplanted into a new medium containing Murashige-Skoog 1962 (MS) medium. This medium was supplemented with various concentrations of IBA (0 or 1.5 mg L^{-1}), NAA (0, 0.5, 1, or 1.5 mg L^{-1}), putrescine (160 mg L^{-1}), and/or AgNO3 (2, 4, or 6 mg L^{-1}). Additionally, three concentrations of each elicitor were added: Activane® (0.2, 0.4, 0.6 mL L^{-1}), Micobiol® (0.2, 0.4, 0.6 mL L^{-1}), and Stemicol® (0.2, 0.4, 0.6 mL L^{-1}) to induce root development. The experimental units were subjected to controlled photoperiod and temperature conditions, involving 16 hours of light (54 µmol m-2 S^{-1}) and 8 hours of darkness at 24 ± 2 °C. After six weeks, rooting indices were recorded for each treatment, including rooting percentage, root number, and root length. The data recorded encompassed the root initiation response (%), root length (cm), and the number of roots per shoot.

2.9 Acclimatization

The best-rooted plantlets from each treatment were selected and acclimatized in pots containing a mixture of peat moss and vermiculite (1:1 v/v). The agar was thoroughly washed off with distilled water before transplanting. The pots were covered with clear plastic bags with a few holes and were consistently watered to maintain high humidity for three weeks. Subsequently, the hardened plantlets were transferred to a greenhouse set at a day temperature of approximately 25 °C, with a relative humidity of about 85%. Frequent daily watering was continued to keep the soil consistently moist. After 2 weeks in the greenhouse, assessments were made for plant survival, plant height (cm), and the number of leaves.

2.10 Statistical analysis

The data obtained from the aforementioned experiments were subjected to statistical analysis using SPSS STATISTIC software version 21.0, a program designed for Windows. The analysis involved Analysis of Variance (ANOVA), and Tukey means multiple comparisons analysis was performed with a significance level set at $P \leq 0.05$.

3 Results and Discussion

3.1 Impact of Various Sterilization Methods on *in-vitro* micropropagation

The aim is to evaluate how various sterilization procedures impact the in vitro germination, contamination, and oxidation of tomato seeds. Sterilization treatments using two concentrations i.e., 15% and 20% (v/v) of sodium hypochlorite (bleach) applied for 15 minutes. The results, based on a thirty-day evaluation of two sterilization treatments using a solution of Sodium Hypochlorite (NaOCl) at 15 and 20% (v/v Cloralex®), indicate superior outcomes for the treatment with a 15% NaOCl solution. This observation holds across all evaluated variables for both tomato cultivars in in-vitro conditions. However, no significant statistical difference was observed between the two treatments (Figure 2).

Sodium hypochlorite is widely employed as a seed and explant sterilization agent in various plant species, with bleach concentration and exposure varying between species. Sterilization

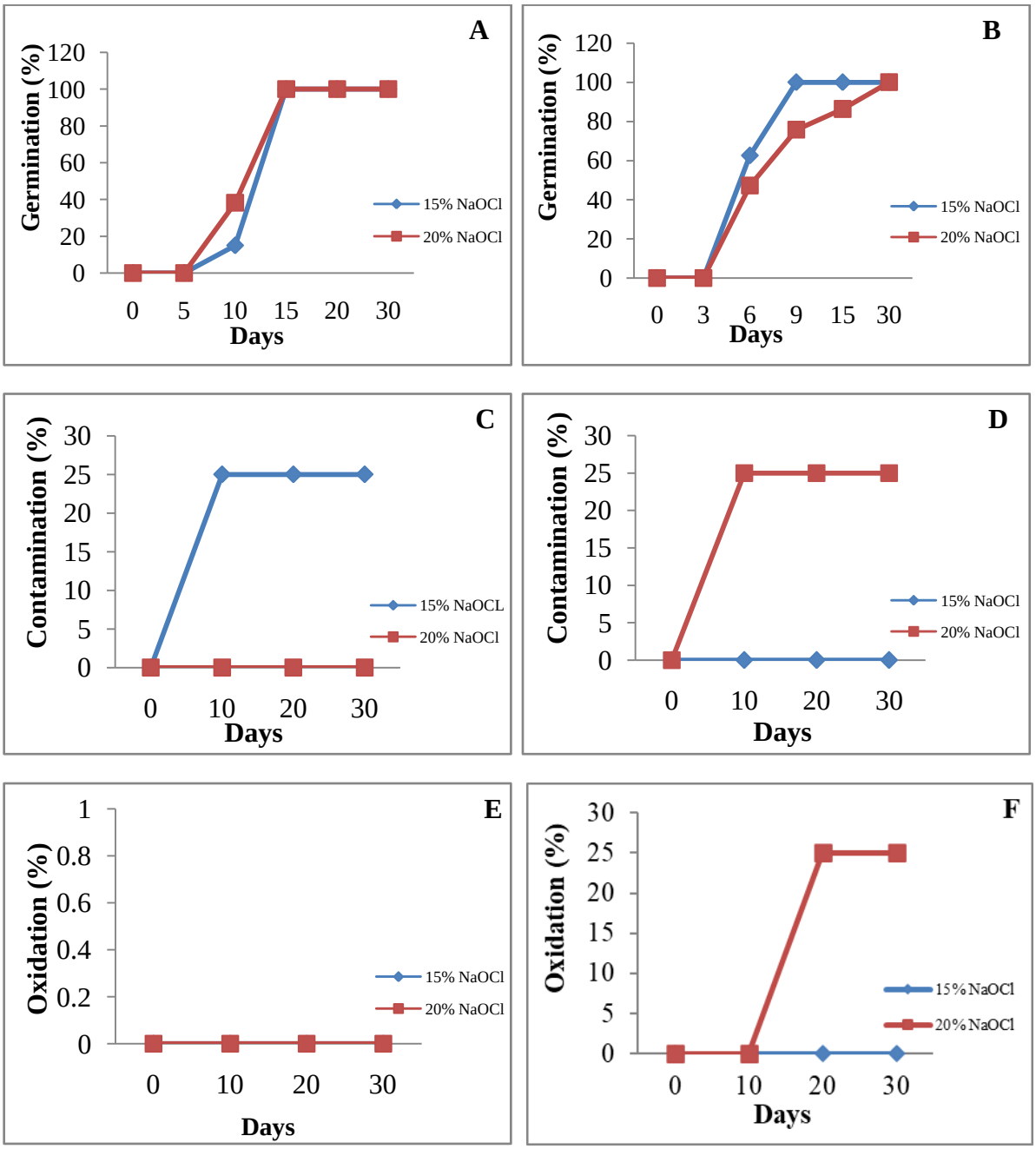

Figure 2 Impact of diverse sterilization methods on germination percentage (A) Saladette experimental materials and (B) Bola experimental materials; (C) contamination in Saladette and (D) contamination in Bola and (E) oxidation of Saladette and (F) oxidation of Bola tomato seeds.

protocols often include a combination of bleach and a 70% ethanol rinse (Espinosa 2005; Barampuram et al. 2014; Calaña-Janeiro et al. 2019). In this study, combined protocols were applied to sterilize seeds of two tomato cultivars using different bleach treatments. The highest germination rates, 80% and 65%, were achieved with Sodium Hypochlorite (NaOCl) concentrations of 15% and 20% (v/v Cloralex®), respectively.

The Saladette cultivar achieved a 75% contamination-free rate with NaOCl at 15%, while the Bola cultivar attained a 75% contamination-free rate with NaOCl at 20%. Additionally, oxidation-free seeds were obtained from the Saladette cultivar, while only 25% oxidation was observed in the treatment with NaOCl at 20% for the Bola cultivar. Across all evaluated variables, NaOCl at 15% proved the most efficient, providing good results

for tomato seeds. This concentration induced greater responses for both cultivars at the experiment's conclusion, aligning with Espinosa's findings (2005).

It is noteworthy that the study's results highlight sodium hypochlorite's efficacy at 15% for surface sterilization of contaminated tomato seeds and promoting their germination. These findings are consistent with prior studies, such as using sodium hypochlorite for surface sterilization of cotton seeds in in vitro culture (Barampuram et al. 2014).

3.2 Effect of Elicitors on *in vitro* Seed Germination and Shoot growth

In this study, the application of elicitors significantly enhanced shoot growth after 4 weeks of *in vitro* culture. Notably, elicitor-treated plants exhibited the highest shoot length, although the various concentrations did not show a discernible effect on the treatments across the two tomato cultivars (Figure 3).

The assessment of in vitro seed germination, height, number of leaves, and viability in the Saladette and Bola cultivars revealed a positive impact of various types and concentrations of elicitors on the studied variables. The statistical analysis conducted under the study conditions indicated significant differences ($P \leq 0.05$) for the number of leaves and height variables in both cultivars. Specifically, the Saladette cultivar exhibited noteworthy variations in the number of leaves and height, with the highest values observed in the T3 treatment (Micobiol®) (Table 2).

While the studied elicitors demonstrated a positive impact on the variables of leaf number, growth, as well as the germination and viability percentages of explants, the varied concentrations of elicitors did not yield a significant effect on the outcomes. The germination percentage of seeds in both tomato cultivars showed a slight influence from the elicitors, increasing explant viability. Simply, the application of elicitors in this study led to substantially higher values in treated plants than those without elicitors.

These findings align with previous reports on *Stevia rebaudiana*, *Physalis peruviana*, *Fagonia indica*, and *Ajuga bracteosa*. In these instances, the in vitro use of elicitors, including salicylic acid (SA), chitosan (CHI), and methyl jasmonate (Me-J), had a positive impact on suppressing germination and plant growth in cultured tissues (Bayraktar et al. 2016; Ali et al. 2018; Khan et al. 2018).

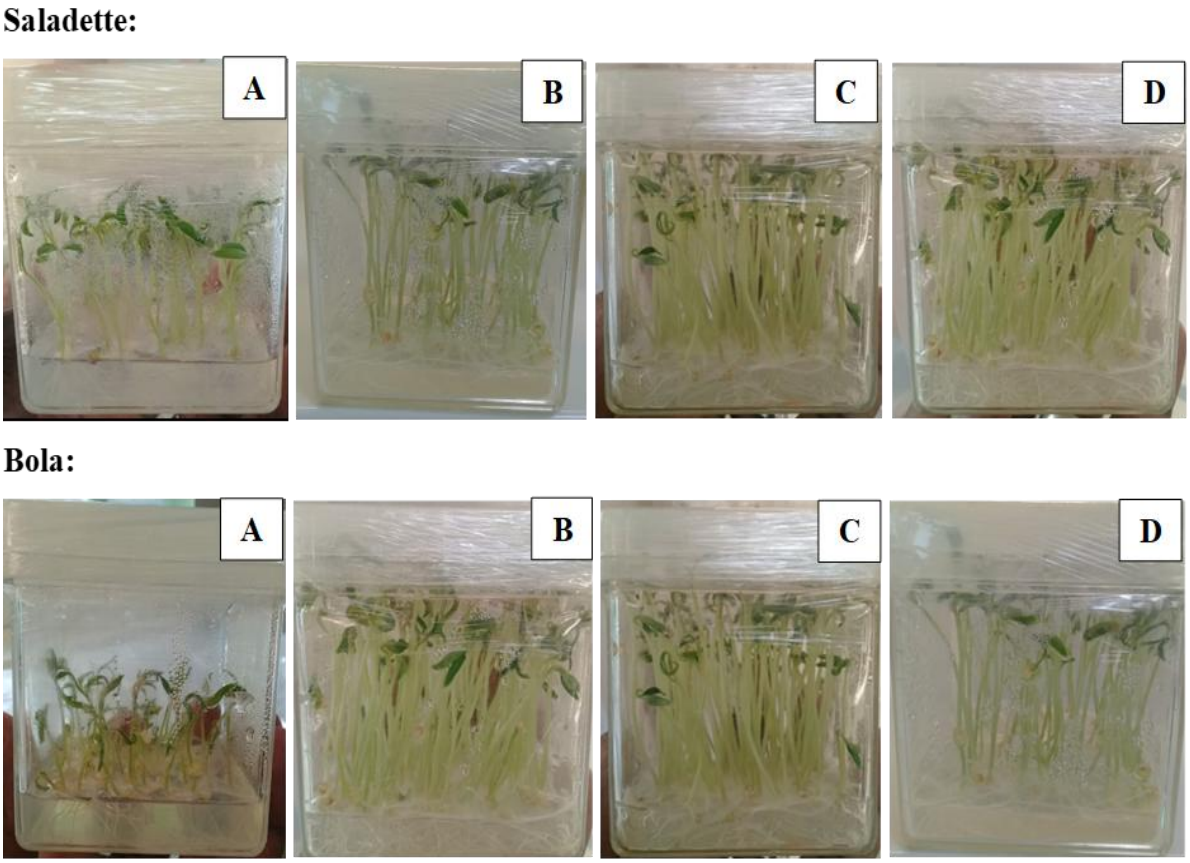

Figure 3 Effect of different types of elicitors on *in vitro* shoot growth and percentage of germination from the seeds of the two tomato cultivars after 4 weeks of culture, the treatments were (A) T_1-Control, (B) T_2-Activane®, (C) T_3-Micobiol® and (D) T_4-Stemicol®.

Table 2 Impact of various types and concentrations of elicitors on the number of leaves, growth, germination percentage, and *in vitro* viability of two cultivars of *S. lycopersicum* L. after an 8-week culture period

Concentration of Elicitors (mL/L⁻¹)	Number of Leaves	Height (cm)	Germination (%)	Viability (%)
Tomato Saladette:				
Cntl				
D = 0.00	5.13^{ab}	5.67^{b}	80	80
Act®				
D_1 = 0.20	5.33^{a}	7.67^{a}	100	90
D_2 = 0.40	5.47^{a}	7.57^{a}	80	85
D_3 = 0.60	5.60^{a}	7.44^{a}	100	100
Micbl®				
D_1 = 0.20	5.60^{a}	7.77^{a}	100	85
D_2 = 0.40	6.00^{a}	7.47^{a}	100	100
D_3 = 0.60	6.00^{a}	7.23^{a}	100	100
Stml®				
D_1 = 0.20	6.00^{a}	7.83^{a}	80	100
D_2 = 0.40	5.60^{a}	7.73^{a}	100	100
D_3 = 0.60	5.60^{a}	7.50^{a}	100	80
Tomato Bola:				
Cntl				
D = 0.00	4.23^{ab}	4.10^{b}	70	90
Act®				
D_1 = 0.20	5.37^{a}	7.30^{a}	80	88
D_2 = 0.40	5.10^{a}	6.20^{ab}	80	98
D_3 = 0.60	5.30^{a}	6.93^{a}	80	95
Micbl®				
D_1 = 0.20	5.50^{a}	6.90^{a}	80	100
D_2 = 0.40	5.63^{a}	7.30^{a}	80	100
D_3 = 0.60	5.57^{a}	7.30^{a}	80	96
Stml®				
D_1 = 0.20	5.37^{a}	7.23^{a}	100	95
D_2 = 0.40	4.13^{b}	7.77^{a}	100	90
D_3 = 0.60	5.43^{a}	6.27^{ab}	80	100

Doses one (1) = D_1, Doses two (2) = D_2 and Doses three (3) = D_3, the different letters are significantly different ($P \leq 0.05$) according to Tukey's multiple range test.

3.3 Effect of Elicitors on *in vitro* Direct Organogenesis

The explants cultivated in the preceding stage displayed uniform growth, exhibiting similar patterns across the two tomato cultivars. The overall health of the explants was notably robust, owing to the optimal environmental conditions maintained during the two-week preparation period. This ensured their suitability for the subsequent developmental stage. Direct and callus induction initiation involved using a medium incorporating elicitation treatments. Following explant induction, direct organogenesis became evident. The elongation of shoots and subsequent leaf development accompanied shoot regeneration. Additionally, leaves exhibited the generation of new shoots originating from the base of the petiole, while stems demonstrated elongation and the emergence of new leaves from the axillary bud (Figure 4).

Following direct organogenesis in the two tomato cultivars, the applied elicitors positively impacted bud growth while concurrently reducing phenolic oxidation. This contributed to the overall enhancement of explant viability. The findings presented in Table 3 indicate that concerning the number of buds and bud growth variables, no significant effects were observed for the concentration of elicitors or the different types of elicitors individually. However, a noteworthy outcome emerged as the interaction between both variables proved significant ($P \leq 0.05$).

In the case of leaf explants, the comparison of mean tests revealed significant differences ($P \leq 0.05$) in the response of elicitors, with the highest average observed in the treated explants for the variable total growth. Specifically, elicitor T3-Micobiol® induced greater responses across all three concentrations (D1=21.45, D2=21.50, and D3=22.50 mL L -1). Interestingly, the three concentrations of the elicitors evaluated did not exhibit any significant differences ($P \leq 0.05$) across all treatments. These results suggest that the application of elicitors not only influences the growth of shoots/buds but also enhances the percentage of oxidation, minimizes contamination, and improves the in vitro viability of the explants.

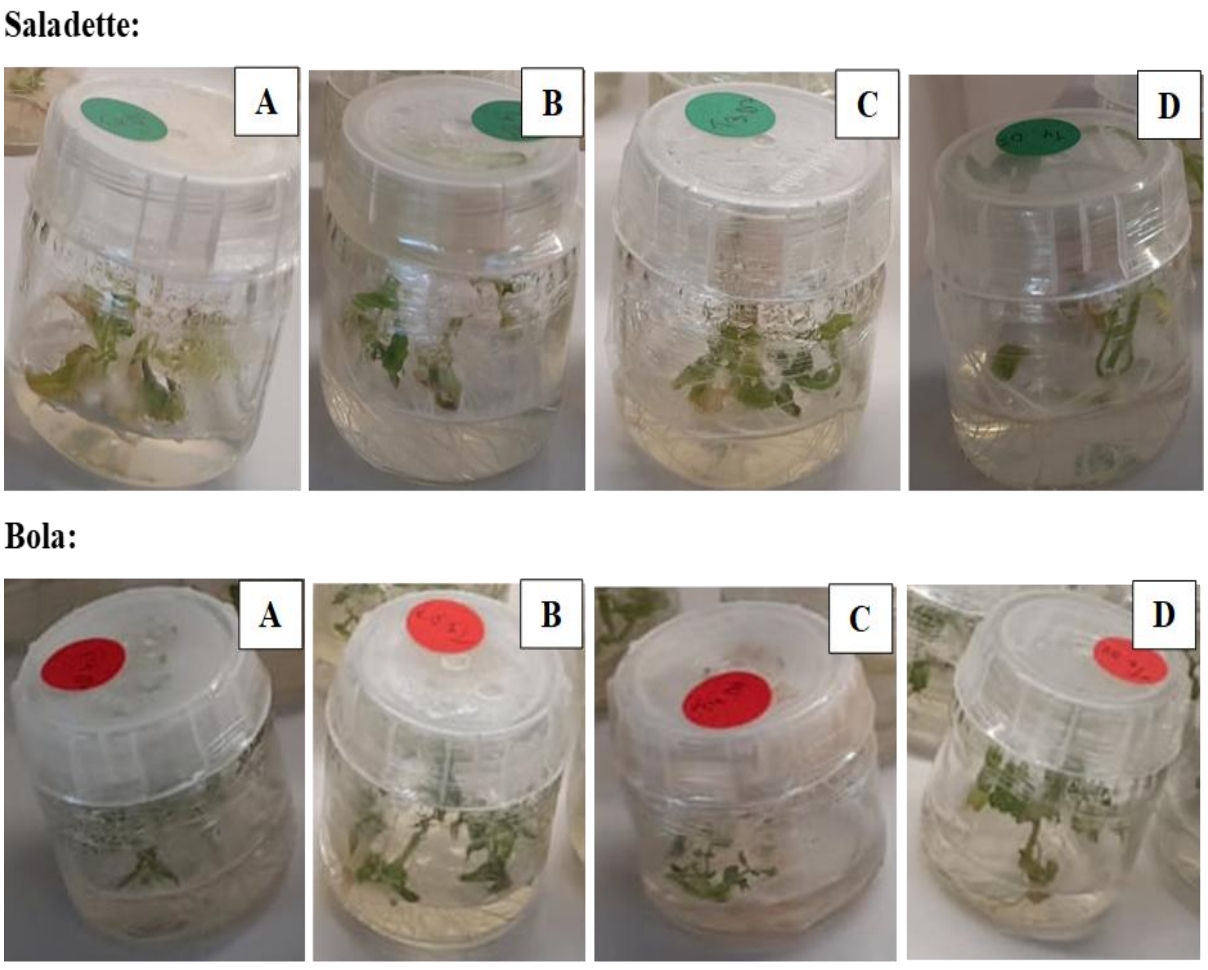

Figure 4 Effect of different types of elicitors on direct *in vitro* shoot organogenesis of the two tomato cultivars after 4 weeks of culture, the treatments were (A) T1-Control, (B) T2-Activane®, (C) T3-Micobiol® and (D) T4-Stemicol®.

Table 3 Impact of different types and concentrations of elicitors on bud number, growth, oxidation percentage, and *in vitro* viability in two cultivars of *S. lycopersicum* L. over a 12-week culture period

Concentration of Elicitors (mL/L^{-1})	Number of Buds	Bud Growth	Length/Total Growth	Viability (%)	Oxidatión (%)
Tomato Saladette					
Cntl					
$D_{=}$ 0.00	3.25	5.00	18.50^{b}	83	50
Act®					
$D_{1=}$ 0.20	3.00	5.50	16.50^{c}	100	100
$D_{2=}$ 0.40	3.00	6.00	18.50^{b}	89	100
$D_{3=}$ 0.60	3.00	6.00	18.00^{b}	100	100
Micbl®					
$D_{1=}$ 0.20	3.00	6.00	18.00^{b}	100	100
$D_{2=}$ 0.40	4.00	5.66	22.32^{a}	100	100
$D_{3=}$ 0.60	3.50	5.83	20.16^{ab}	98	100
Stml®					
$D_{1=}$ 0.20	4.75	4.62	20.90^{ab}	99	100
$D_{2=}$ 0.40	3.50	5.42	18.82^{b}	97	100
$D_{3=}$ 0.60	3.50	5.62	19.87^{b}	100	100
Tomato Bola					
Cntl					
$D_{=}$ 0.00	3.20	4.25	14.12^{b}	100	100
Act®					
$D_{1=}$ 0.20	3.50	5.08	17.83^{ab}	100	100
$D_{2=}$ 0.40	3.25	5.12	16.50^{ab}	100	100
$D_{3=}$ 0.60	3.10	5.37	16.12^{ab}	100	100
Micbl®					
$D_{1=}$ 0.20	4.00	5.32	21.45^{a}	100	100
$D_{2=}$ 0.40	3.75	5.75	21.50^{a}	100	100
$D_{3=}$ 0.60	3.75	6.00	22.50^{a}	100	100
Stml®					
$D_{1=}$ 0.20	3.00	4.62	13.87^{ab}	100	100
$D_{2=}$ 0.40	3.25	5.37	17.37^{ab}	100	100
$D_{3=}$ 0.60	3.50	5.75	20.00^{ab}	100	100

Doses one (1) = $D_{1,}$ Doses two (2) = D_2 and Doses three (3) = D_3; the different letters are significantly different ($P \leq 0.05$) according to Tukey's multiple range test.

3.4 Effect of Elicitors on *in vitro* Indirect Organogenesis (Callus Formation)

To stimulate *in vitro* indirect organogenesis in two cultivars of *S. lycopersicum*, the impact of three elicitors was assessed concerning callus formation and induction in the explants (Figure 5). In both cultivars, the explants exhibited the highest induction rates in elicitor-treated callus, with values reaching 97% and 95.66% for treatments T2-Activane® and T3-Micobiol®, respectively. Notably, a superior induction rate was observed in T2-Activane®, with no significant differences identified among the various concentrations of the elicitors even 12 weeks after the explant inoculation (Table 4).

The intensity of callus formation and induction in explants changed under the influence of elicitation. Leaf explants from two tomato cultivars were subjected to incubation in MS nutrient medium containing various elicitors concentrations, and the callus formation processes were subsequently assessed. The treatment with T2-Activane® yielded the highest average callus-forming densities (97), closely followed by T3-Micobiol® (95.66). In most elicitor treatments, the first signs of callus were observed by day 6. Explants in the T1-Control treatment exhibited low-density callus, attributed to a high presence of oxidation and reduced viability. Despite viability being compromised by elevated contamination levels, some explants demonstrated lower averages of callus formation. Earlier studies have shown that employing a two-stage culture system with a combined treatment of mannitol (2mM) and JA (40µM) led to the optimal accumulation of resveratrol in the callus biomass of *Vitis vinifera* (Raluca et al., 2011).

3.5 Root Induction

Root induction and development on regenerated shoots were observed after three weeks into the culture in MS medium supplemented with elicitors (Figure 6). The highest percentage of root initiation responses was observed in explants treated with the elicitors. Our findings also revealed that the highest average root length was observed in treatments T2-Activane® (2.0 cm) and T4-Stemicol® (2.4 cm) for the Saladette cultivar. Similarly, for the Bola cultivar, the highest average root lengths were observed in T2-Activane® (2.2 cm) and T3-Micobiol® (2.3 cm) (Table 5).

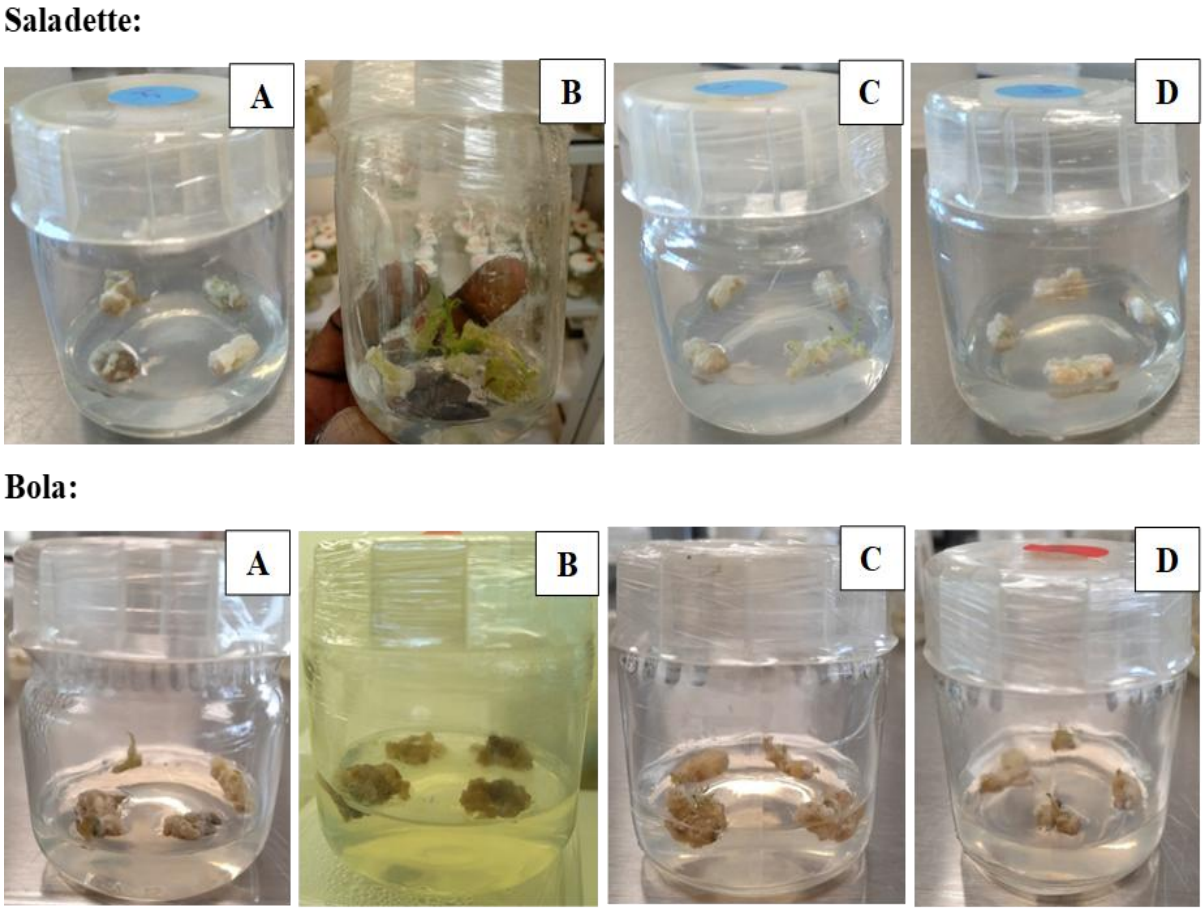

Figure 5 Effect of different types of elicitors on indirect *in vitro* organogenesis on the explants callus formation and induction of the two tomato cultivars after 12 weeks of culture; the treatments were (A) T_1-Control, (B) T_2-Activane®, (C) T_3-Micobiol® and (D) T_4-Stemicol®

Table 4 Impact of varied types and concentrations of elicitors on callus presence, initial callus appearance, callus induction, oxidation, and in vitro viability in two cultivars of *S. lycopersicum* L. over a 12-week culture period

Concentration of Elicitors (mL/L^{-1})	First Callus Formation (day)	Callus Weight (gram)	Callus Diameter (mm)	Callus Induction (%)	Viability (%)	Oxidation (%)
Tomato Saladette						
Cntl						
$D_{=}$ 0.00	7	0.4^{d}	2.20^{e}	90^{b}	83	50
Act®						
$D_{1=}$ 0.20	7	0.84^{b}	8.30^{a}	96^{a}	100	100
$D_{2=}$ 0.40	6	1.06^{a}	7.20^{ab}	95^{a}	89	100
$D_{3=}$ 0.60	7	0.67^{c}	5.10^{c}	100^{a}	100	100
Micbl®						
$D_{1=}$ 0.20	6	0.42^{d}	6.35^{b}	97^{a}	100	100
$D_{2=}$ 0.40	6	0.15^{e}	1.60^{e}	90^{b}	100	100
$D_{3=}$ 0.60	6	0.41^{d}	4.35^{cd}	100^{a}	98	100
Stml®						
$D_{1=}$ 0.20	7	0.21^{e}	3.55^{d}	88^{b}	99	100
$D_{2=}$ 0.40	7	0.54^{d}	4.90^{c}	87^{c}	97	100
$D_{3=}$ 0.60	6	0.20^{e}	2.15^{e}	96^{a}	100	100
Tomato Bola						
Cntl						
$D_{=}$ 0.00	8	0.40^{e}	4.85^{f}	88^{bc}	90	80
Act®						
$D_{1=}$ 0.20	7	1.00^{cd}	9.10^{bc}	91^{b}	99	90
$D_{2=}$ 0.40	7	1.07^{c}	7.55^{de}	93^{b}	95	95
$D_{3=}$ 0.60	7	1.32^{b}	9.45^{abc}	96^{ab}	90	100
Micbl®						
$D_{1=}$ 0.20	7	0.92^{d}	7.00^{e}	98^{a}	100	100
$D_{2=}$ 0.40	8	1.37^{b}	8.35^{cd}	91^{b}	99	95
$D_{3=}$ 0.60	7	0.49^{e}	5.45^{f}	98^{a}	97	100
Stml®						
$D_{1=}$ 0.20	8	1.58^{a}	10.7^{a}	90^{b}	100	98
$D_{2=}$ 0.40	7	1.62^{a}	10.2^{ab}	95^{ab}	95	100
$D_{3=}$ 0.60	6	1.26^{b}	9.85^{ab}	94^{ab}	99	95

Doses one (1) = $D_{1,}$ Doses two (2) = D_{2} and Doses three (3) = $D_{3,}$ the different letters are significantly different ($P \leq 0.05$) according to Tukey's multiple range test.

Table 5 Impact of different types and concentrations of elicitors on the number of roots, root length, contamination, oxidation, and in vitro viability of two cultivar of *Solanum lycopersicum*

Concentration of elicitors (mL/L^{-1})	Number of roots	Root length (cm)	Contamination Free (%)	Viability (%)	Oxidation (%)
Tomato Saladette					
Cntl					
D = 0.00	8	1.68^{c}	92	90	68
Act®					
D_1 = 0.20	11	2.09^{b}	98	99	100
D_2 = 0.40	12	2.01^{b}	100	100	100
D_3 = 0.60	11	2.0^{b}	100	100	100
Micbl®					
D_1 = 0.30	12	2.02	99	100	100
D_2 = 0.50	11	1.78^{c}	100	100	98
D_3 = 0.70	11	2.0^{b}	100	100	100
Stml®					
D_1 = 0.60	10	2.40^{a}	100	99	100
D_2 = 0.80	13	2.38^{a}	99	96	100
D_3 = 0.100	11	2.29^{a}	100	100	99
Tomato Bola					
Cntl					
D = 0.00	7	1.78^{d}	88	98	88
Act®					
D_1 = 0.20	11	2.28^{b}	100	100	100
D_2 = 0.40	10	2.29^{b}	100	100	100
D_3 = 0.60	11	2.36^{a}	100	100	100
Micbl®					
D_1 = 0.30	10	2.28^{b}	100	100	100
D_2 = 0.50	10	2.29^{b}	98	100	100
D_3 = 0.70	11	2.36^{a}	100	100	100
Stml®					
D_1 = 0.60	11	2.10^{c}	98	100	100
D_2 = 0.80	10	2.38^{a}	100	100	100
D_3 = 0.100	11	2.10^{c}	100	100	100

Doses one (1) = D_1, Doses two (2) = D_2 and Doses three (3) = D_3; **The different letters are significantly different ($P \leq 0.05$) according to Tukey's multiple range test

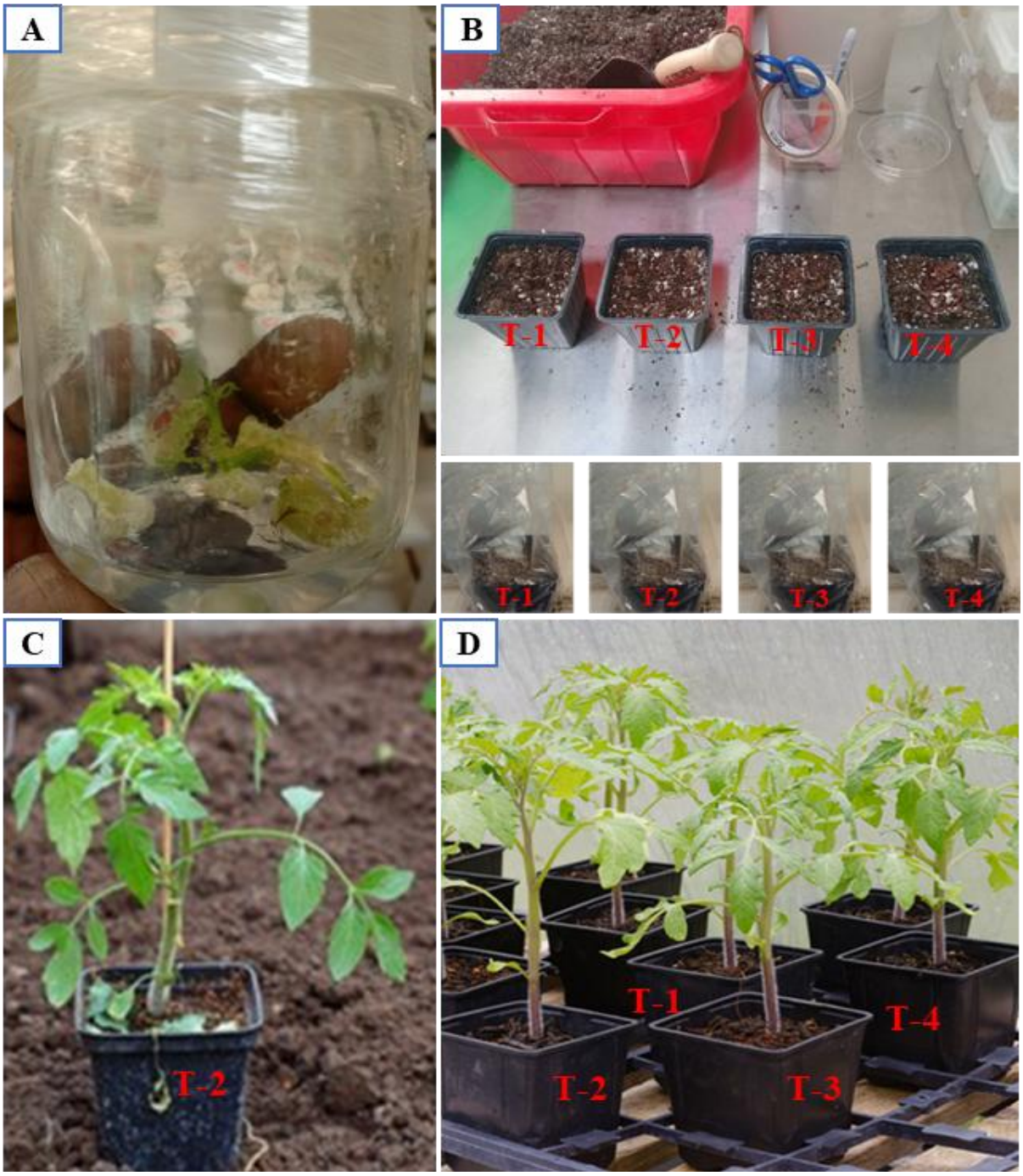

Figure 6 *In vitro* callus formation, root induction and acclimatization in the greenhouse of the two tomato cultivars (A) Callus, root and shoot induction, (B) Pots containing peat moss and vermiculite for the acclimatization, (C) Fully developed plantlets under adaptation in the greenhouse and (D) Developed individual plant continuing growth.

3.6 Acclimatization

The acclimatization of the plantlets was successfully achieved by providing a favourable environment across all treatments. *In vitro* rooted plantlets from each treatment were subsequently transplanted into a natural and well-maintained greenhouse condition. The gathered data revealed a 100% survival rate for all treatments in the Saladette cultivar. In the case of the Bola cultivar, treatments with elicitors exhibited an average survival rate of 99.7%, compared to 96% for the control (Table 6).

Table 6 Effect of elicitors on the acclimatization of the plantlets for the two tomato cultivars

Concentration of Elicitors (mL/L⁻¹)	Plant height (cm)	Number of Leaves	Survival Rate (%)
Tomato Saladette:			
Cntl			
$D_{=}$ 0.00	18.6	14 ^{b}	100
Act®			
$D_{1=}$ 0.20	19.4	15 ^{ab}	100
$D_{2=}$ 0.40	20.3	17 ^{a}	100
$D_{3=}$ 0.60	21.5	17 ^{a}	100
Micbl®			
$D_{1=}$ 0.20	20.4	16 ^{a}	100
$D_{2=}$ 0.40	20.0	16 ^{a}	100
$D_{3=}$ 0.60	19.8	14 ^{b}	100
Stml®			
$D_{1=}$ 0.20	21.1	16 ^{a}	100
$D_{2=}$ 0.40	18.9	13 ^{bc}	100
$D_{3=}$ 0.60	19.0	13 ^{bc}	100
Tomato Bola:			
Cntl			
$D_{=}$ 0.00	17.8	11 ^{c}	96
Act®			
$D_{1=}$ 0.20	19.2	14 ^{ab}	100
$D_{2=}$ 0.40	18.0	13 ^{b}	98
$D_{3=}$ 0.60	18.3	13 ^{b}	100
Micbl®			
$D_{1=}$ 0.20	19.5	15 ^{a}	100
$D_{2=}$ 0.40	19.0	15 ^{a}	100
$D_{3=}$ 0.60	19.0	15 ^{a}	100
Stml®			
$D_{1=}$ 0.20	17.9	11 ^{c}	100
$D_{2=}$ 0.40	18.3	13 ^{b}	100
$D_{3=}$ 0.60	18.6	12 ^{bc}	100

Doses one (1) = $D_{1,}$ Doses two (2) = D_2 and Doses three (3) = D_3; **The different letters are significantly different ($P \leq 0.05$) according to Tukey's multiple range test

Contrastingly, the outcomes indicated significant differences in the number of leaves per plant among the two tomato cultivars originating from different explant sources and subjected to varying concentrations of elicitors. However, no significant difference was observed in the plant height (cm) across the treatments. The plants exhibited robust and sturdy growth, achieving full rigidity after 2 weeks in the greenhouse, and continued their subsequent growth and development.

In the current study, the sterilization protocols utilizing 15% Sodium Hypochlorite (NaOCl) in Cloralex® have demonstrated effectiveness in achieving oxidation and contamination-free seeds, leading to 100% seed germination within two weeks in the tomato cultivars (Espinosa 2005; Barampuram et al. 2014; Calaña-Janeiro et al. 2019). The investigation delved into the effects of elicitation on *in vitro* seed germination, as well as direct and indirect *in vitro* organogenesis in the explants. These elicitors are commonly employed as potential additives alongside plant growth regulators during different in vitro plant propagation stages, aiming to enhance plant quality, regeneration rates, and overall process efficiency. Elicitation stands out as one of the most effective methods for promoting plant growth and elevating the production of *in vitro* secondary metabolites (Zhao et al. 2005).

Biotic elicitors, derived from living organisms, are recognized by specific receptors bound to the cell membrane. This stimulus is then transmitted to the cell through a signal transduction system, leading to changes that ultimately result in the formation of phytoalexins (Baenas et al. 2014). On the other hand, abiotic elicitors are substances of non-biological origin, often inorganic compounds like salts or physical factors. These can be utilized to produce bioactive compounds by modifying plant secondary metabolism across various plant species in culture systems (Verpoorte et al. 2002; Namdeo 2007; Gorelick and Bernstein 2014; Ramirez-Estrada et al. 2016). In a study by Bayraktar et al. (2018), all the elicitors (MeJA, SA, and CHI) demonstrated a positive impact, resulting in an increase in the number of leaves, shoot growth, and biomass accumulation when compared to untreated in vitro plantlets.

In this study, plants treated with elicitors exhibited the highest levels of callus formation, weight, diameter, and callus induction in the two *S. lycopersicum* cultivars. However, varying concentrations of elicitors did not yield significant differences in the treatments. Elicitor treatments also enhanced shoot growth after 4 weeks of *in vitro* culture, with the highest shoot length achieved in plants treated with elicitors. Elicitors have been reported to be effective in various plant developmental processes, including seed germination and seedling growth (Bayraktar et al. 2016; Ali et al. 2018; Khan et al. 2018; Li et al. 2018).

Among various treatments applied to callus cultures of *Fagonia indica*, AlCl3 (0.1 mM concentration) was reported to improve biomass accumulation (fresh weight) to the highest extent compared to the control (Khan et al. 2021). As abiotic elicitors, heavy metals have been widely used in several plant species to enhance growth, biomass, phytochemical accumulation, and antioxidant potential (Kurz et al. 1987; Khan et al. 2021). Various salts of cadmium (Cd2+) and aluminium chloride (AlCl3) have been reported to increase the production of compounds in callus cultures of *Rauvolfia serpentina*, Melissa officinalis L., and cell cultures of *Vitis vinifera* (Cai et al. 2013; Urdová et al. 2015; Zafar et al. 2017).

Previously, elicitors were also employed to improve plant propagation in various *in-vitro* cultures, including callus and adventitious root cultures of *F. indica* (Saeed et al. 2017), shoot growth and development, callus, and cell suspension cultures in *Hypericum perforatum* L. explants (Gadzovska et al. 2013). Elicitation in callus cultures of *Stevia rebaudiana* stimulated callus induction response and callus physiology, resulting in good callus texture at lower concentrations (Javed et al. 2017).

Conclusions

An effective disinfection methodology was employed for the in vitro implantation of tomato seeds in the two cultivars, ensuring successful seed germination (explants) and promoting the adequate survival and growth of the seedlings. The application of various elicitors in direct and indirect in vitro organogenesis on the explants positively affected the evaluated variables. However, different concentrations of elicitors did not yield significant differences in the treatments. Root induction was successful, with a greater response in root initiation and higher survival percentages observed after acclimatization in plants treated with elicitors. The results obtained from this study can guide future research endeavours aimed at expanding the use of elicitor treatments in large-scale in vitro propagation of tomatoes, focusing on identifying the most effective concentrations for optimal results.

Acknowledgements

The authors express their gratitude for the support provided by the National Council of Science and Technology (CONACyT), the Faculty of Agronomy of the Autonomous University of Nuevo Leon (FAUANL), and Lida de México SA DE CV.

Compliance with Ethical Standards

The authors declare no conflicts of interest associated with this publication. Additionally, there are no ethical issues infringed upon in this study. The authors have adhered to the principles of ethical and professional conduct. No funding was secured for this research, and no humans or animals were involved.

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