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ORIGINAL ARTICLE

Silver nanoparticles enhance the in vitro plant regeneration via thin cell layer culture system in purple passion fruit

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Abstract

The current in vitro plant regeneration of purple passion fruit is mainly based on shoot organogenesis. In particular, thin cell layer (TCL) technology has emerged as a powerful technique for shoot organogenesis from many in vitro explant sources of purple passion fruit. This study further optimized the in vitro plant regeneration from ex vitro explant sources (leaves and internodes) via TCL technology combined with the addition of AgNPs on the culture medium. For leaf TCL explants, the results showed that the optimal shoot induction rate (96.30%) and the highest number of shoots (4.33 shoots/explant) were recorded on MS medium supplemented with 1.0 mg L^{-1} BA and 1.5 mg L^{-1} AgNPs. For internode TCL explants, different internode positions had a significant effect on shoot induction from tTCL explants. After 60 days of culture, the optimal shoot induction rate (70.37%) was observed in the tTCL explants at the 3rd internode from the shoot tip. This study also revealed that the difference in endogenous hormones at different internode positions was one of the significant factors affecting shoot induction from TCL explants. On the other hand, the results indicated that the shoot regeneration coefficient in lTCL was significantly higher than in tTCL. In addition, somatic embryogenesis was recorded for the first time in internode tTCL explants (22.45%). The addition of 3.0 mg L^{-1} AgNPs significantly enhanced the proliferation and maturation of somatic embryos derived from internode tTCL explants. The present study contributes to a significant improvement in the micropropagation efficiency of purple passion fruit.

Key message

Efficient shoot regeneration was observed for thin cell layer explants of ex vitro leaves and internodes.
Differences in endogenous hormones at different internode locations significantly influenced shoot induction.
Somatic embryogenesis was first recorded from internode explants of Passiflora edulis Sims f. edulis and this process was significantly enhanced by silver nanoparticles.

Keywords Endogenous hormones · Internode positions · Passion fruit · Shoot regeneration · Somatic embryogenesis

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
2-iP	N6-isopentenyl adenine
AgNPs	silver nanoparticles
BA	N6-benzyladenine
IAA	Indole-3-acetic acid
lTCL	longitudinal thin cell layer
MS	Murashige and Skoog (1962)
TCL	thin cell layer
tTCL	transverse thin cell layer

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Introduction

The thin cell layer (TCL) culture technique was developed in 1973 (Van 1973a, b) and has become one of the simple and effective propagation methods at present. When using the TCL explant, the surface area of the explant exposed to the medium is relatively larger than in conventional cultures and the transport of the media components is more efficient because they can reach the potential cells of the explant, which allows an easier organogenesis or embryogenesis response compared to conventional cultures (Van 2003). Hence, TCL is used as an explant in the in vitro propagation of many plants, as well as the conservation of endangered plant species (Tung et al. 2022a).

TCL technology has been successfully applied in the micropropagation of more than 20 orchid species (Bhattacharyya et al. 2018; da Silva and Nhut 2003; da Silva et al. 2015; Parthibhan et al. 2018) and many other species of ornamental plants such as *Lilium* (Marinangeli 2016), and *Begonia* × *tubhybrida* Voss (Bao et al. 2022). Several studies using TCL culture technique have been carried out on many medicinal, fruit, and vegetable plant species (Vaidya et al. 2016; Tripathi et al. 2018; Anh et al. 2022; Sabooni and Shekafandeh 2017; Abdolinejad et al. 2020, Hanh et al. 2022). In addition, the TCL explant also offered a viable regenerative potential for tissues that tend to be difficult to induce in vitro regeneration. For example, an efficient in vitro procedure for embryogenic callus from TCL explants derived from immature zygotic embryos of *Pinus patula*, a plant species used for forestation, was generated despite limitations due to in vitro growth incompatibilities (Ramírez-Mosqueda et al. 2019).

For purple passion fruit, in vitro plant propagation was mainly based on shoot regeneration (Pacheco et al. 2016). This regeneration has been successfully performed from several different types of tissues and organs, such as hypocotyl (Dias et al. 2009; Fernando et al. 2007), root (da Silva et al. 2011), leaf (Huh et al. 2017), endosperm (Antoniazzi et al. 2018), and node (Chen et al. 2020). The TCL culture technique plays an essential role in shoot regeneration in purple passion fruit; however, the source of TCL explants was mainly from in vitro leaves and internodes (Hieu et al. 2018a, 2019; Tung et al. 2022a). Hence, in the present study, TCL explants from ex vitro sources were investigated to improve the regeneration efficiency and enhance the initial explant source for micropropagation. Hieu et al. (2018b) also reported that ex vitro internodes induced only callus but not shoots in the in vitro condition. In a recent study, we also successfully regenerated shoots from ex vitro internode longitudinally TCL (lTCL) explants (Phong et al. 2022). In this study, the shoot regeneration was continually investigated for the transverse TCL (tTCL) explants; in addition, shoot regeneration efficiency was compared between tTCL and lTCL explants in the same culture medium.

On the other hand, with the advent and widespread research of nanotechnology, AgNPs are applied in newer aspects to enhance plant growth and development (Mahajan et al. 2022). A significant effect of AgNP has been reported in the sterilization of strawberry and begonias explants (Tung et al. 2021a, b). AgNPs on the culture medium also had a positive effect on shoot multiplication and shoot quality in *Rosa hybrida* L. (Ngan et al. 2020), *Gaillardia pulchella* Foug cv. 'Torch Yellow' (Manokari et al. 2023), and *Swertia chirata* (Saha and Gupta 2018). In addition, nanosilver is also reported to enhance somatic embryogenesis and secondary metabolite production in many plant species (Cuong et al. 2021; Khattab et al. 2022; Mahajan et al. 2022). The underlying mechanisms of these potentials were mainly based on their nanoscale particles, which can be easily transported and penetrated into plant cells (Mahajan et al. 2022). In some instances, AgNPs cause the lengthening of roots and raise chlorophyll, glucose, protein, and photosynthesis rates (Siddiqi and Husen 2022). In addition, the effect of AgNPs on plant growth was suggested to be related to their inhibitory ability of ethylene activity and the increased antioxidant reserves commonly observed in AgNPs-treated plants (Dar et al. 2021; Sarmast and Salehi 2016; Tripathi et al. 2017).

Hence, the use of silver nanoparticles in culture systems, especially thin cell layer culture system, can be a potential research direction to improve the efficiency of in vitro plant production. The objective of this research was to apply AgNPs to enhance shoot regeneration and somatic embryogenesis via TCL explants of leaves and internodes of purple passion fruit.

Materials and methods

Plant material

In this study, ex vitro leaves and internodes of 6-month-old purple passion fruit plants (*Passiflora edulis* Sims f. *edulis*) in the greenhouse were used as initial materials (Fig. 1). Disinfection steps of the plant samples were based on the procedure of (Hieu et al. 2018b). Following this procedure, the collected samples were washed under running water for 10 min. Next, these samples were pre-sterilized with 70% alcohol (for 30 s) and then washed with three times distilled water. Then, the samples were immersed in a solution containing 0.1% silver nanoparticles (AgNPs) for 15 min. Finally, the samples were washed three times with distilled water and cultured in Murashige and Skoog (MS) medium

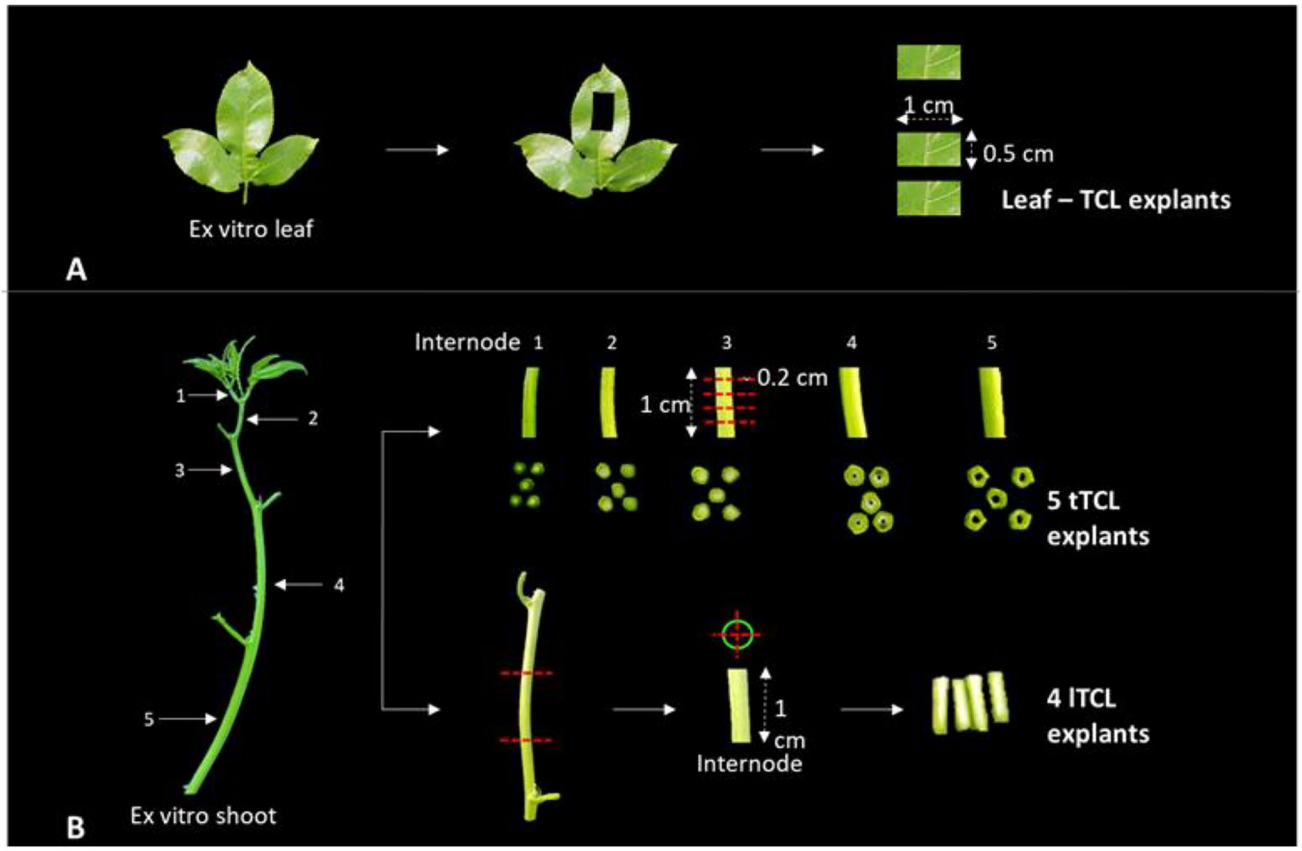

Fig. 1 Establishment of TCL explants derived from ex vitro leaves and internodes of purple passion fruit. **(A)** Leaf explant. **(B)** Internode explant

(Murashige and Skoog 1962) for one week. Clean samples were obtained for the experiments.

Silver nanoparticles solution

Silver nanoparticles (AgNPs) with a size of less than 20 nm were produced by the Institute of Environmental Technology (VAST, Vietnam). The aqueous solution approach was used for the production of AgNPs (Pillai and Kamat 2003). The reaction of AgNPs production was occurring in a homogenous solution of $AgNO_3$ with β-chitosan as a stabilizator. The reducing agent was added successively in drops. The following percentage was used to create the AgNPs solution: The molar ratio between $NaBH_4$ (200 ppm) and $AgNO_3$ (750–1000 ppm) was 1/4, β-chitosan (250–300 ppm), the drip rate of $NaBH_4$ was 10–12 drops/minute (Chau et al. 2008).

Culture media and conditions

MS medium (Murashige and Skoog 1962) containing 30 g L^{-1} sucrose and 8 g L^{-1} agar was used as the basal culture medium in this study. The plant growth regulators or AgNPs were added to the culture medium depending on the research requirements. The culture medium was adjusted to pH = 5.8 before being autoclaved for 30 min at 121 °C (1 atm). For all stages of culture, a room with a temperature of 25 ± 2 °C, a humidity of 55 ± 2%, and a lighting cycle of 16 h per day (using fluorescent lamps with an intensity of 40–45 µmol m^{-2} s^{-1}) was used.

Effect of AgNPs on shoot regeneration from ex vitro leaf TCL explants

The second leaf from the shoot tips of a 6-month-old purple passion fruit plant from the greenhouse was used as the initial material. The central leaf blade containing the leaf veins was cut into 0.5 × 1 cm TCL explants as described in Fig. 1. The explants were cultured on MS medium supplemented with 1.0 mg L^{-1} BA (Sigma-Aldrich®, USA) (Hieu et al. 2018a) and AgNPs at different concentrations (0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}). The indicators of shoot regeneration were recorded after 60 days of culture.

Shoot regeneration from internode TCL explants

Ex vitro internodes at positions 1st to 5th from the shoot tip were cut transversely with a thickness of about 0.2 cm to

create tTCL explants as described in (Fig. 1B). The tTCL explants were cultured on MS medium supplemented with 1.5 mg L^{-1} BA and 1.0 mg L^{-1} NAA (Sigma-Aldrich®, USA) (Hieu et al. 2021) to investigate shoot regeneration. Next, ex vitro internodes (1 cm) at the internode position with the best response were transversely cut into 5 tTCL explants or longitudinally into 4 lTCL explants (Fig. 1B). The explants were cultured on the medium supplemented with 1.5 mg L^{-1} BA, 1.0 mg L^{-1} NAA and with or without 5.0 mg L^{-1} AgNPs (Phong et al. 2022) to compare the shoot induction efficiency. The indicators of Shoot induction rate (%), Average of shoots (taller than 0.5 cm) per explant and Shoot regeneration coefficient were recorded after 60 days of culture.

The Shoot regeneration coefficient based on the growth correction factor formula (da Silva and Dobránszki 2011) used to evaluate the shoot regeneration efficiency of TCL explants: Shoot regeneration coefficient = (Shoot induction rate (%) × Average of shoots/explant (shoots/explant)/100 × Number of TCL explants cut longitudinally or transversally from 1 cm internode fragment (specifically, 5 for tTCL and 4 for lTCL).

Effect of endogenous hormone on shoot induction of tTCL explants from different internode positions

To evaluate the effect of some endogenous hormones on shoot induction, ex vitro internodes from positions 1st to 5th were measured for some endogenous hormones before being added to the same culture medium. The content of endogenous hormones was measured by HPLC according to the following procedure: The internode samples were ground in a solvent solution of $CHCl_3$:MeOH:HCOOH:H_2O with 0.1 g sample per 1 mL of solvent solution. The mixture was extracted at -30 °C for 120 min. Place the sample mixture in a cold centrifuge (4 °C) and collect the supernatant. The remainder was extracted twice in 80% methanol (-4 °C) for 1 h. The solution was loaded on Sep-Pak C18 cartridges which were eluted with methanol solvent. The obtained solution was evaporated at vacuum pressure (50 °C) to remove the solvent and reconstituted with 2 mL of water (pH = 2). The solution injected into the HPLC system was prefiltered through a membrane (0.45 µm). The hormones were isolated by HPLC Thermo-Ultimate 3000 (Thermo Scientific, USA), C18 cartridge (25 cm long, 0.5 µm particle diameter), UV detector at 280 nm. Binary solvent system: (A): acetonitrile, (B): Milli-Q water acidified with 0.5% formic acid. The hormones were quantified using the calibration curves of particular standards.

Somatic embryogenesis from internode tTCL explants

In this experiment, the tTCL from the 3rd internode (Fig. 1B) was used as the explants. The tTCL explants were cultured on MS medium containing 2.0 mg L^{-1} 2,4-D (Phong et al. 2023) for somatic embryogenesis. Internode explant of 1 cm length was used as the control. The somatic embryo induction rate was recorded after 60 days of culture. Next, the somatic embryogenesis from tTCL explants were further investigated on MS medium supplemented with AgNPs at different concentrations (0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}). The indicators of somatic embryogenesis were recorded after 60 days of culture.

Histomorphological analysis

Plant samples were cut into thin tissue slices with a thickness of about 20 µm. Then, the tissue samples were immersed in sodium hypochlorite solution (10%) for 5 min and rinsed with distilled water three times. Next, the samples were immersed in acetic acid solution (10%) for 10 min, followed by three rinses with distilled water. Finally, the tissues were stained with carmine red for 5 min. Sample observations were conducted on an optical microscope at 10 × and 40 × magnification.

Statistical analysis

All experiments were arranged in a completely randomized design. Thirty culture flasks with three explants per flask were used for each treatment. All the experiments were repeated three times. All data were processed by Microsoft Excel 2019 and SPSS Statistics version 26 software. One-way ANOVA analysis followed by Tukey's test was used to determine significant differences at $p < 0.05$. Pearson correlation was analyzed using SPSS Statistics software with significance at the 0.01 and 0.05 levels.

Results and discussions

Effect of AgNPs on shoot regeneration from ex vitro leaf TCL explants

The results showed that the shoot induction rate from leaf TCL explants on the medium supplemented with appropriate AgNPs concentration was significantly higher than that of the control without AgNPs (Fig. 2). The optimal shoot induction rate (88.89–96.30%) was recorded in the treatment supplemented with 1.0 and 1.5 mg L^{-1} AgNPs. The highest number of shoots (4.33 shoots/explant) was

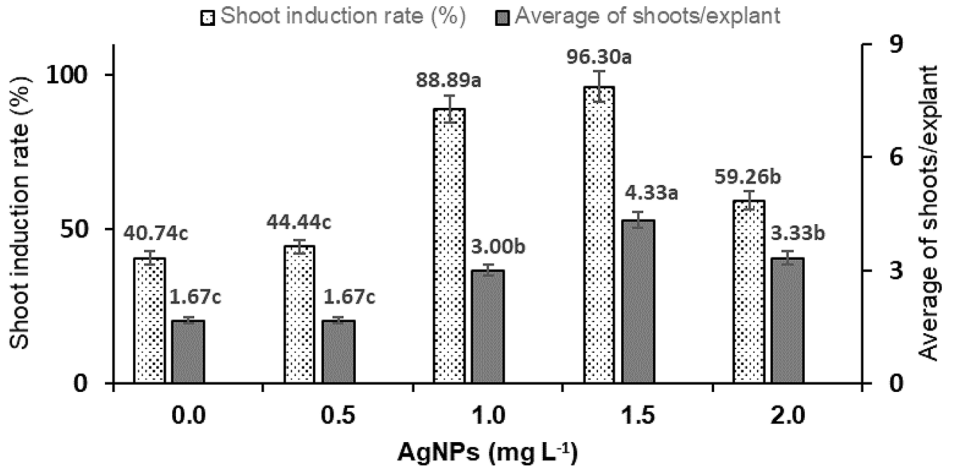

Fig. 2 Effect of AgNPs on direct shoot regeneration from ex vitro leaf TCL explants after 60 days of culture

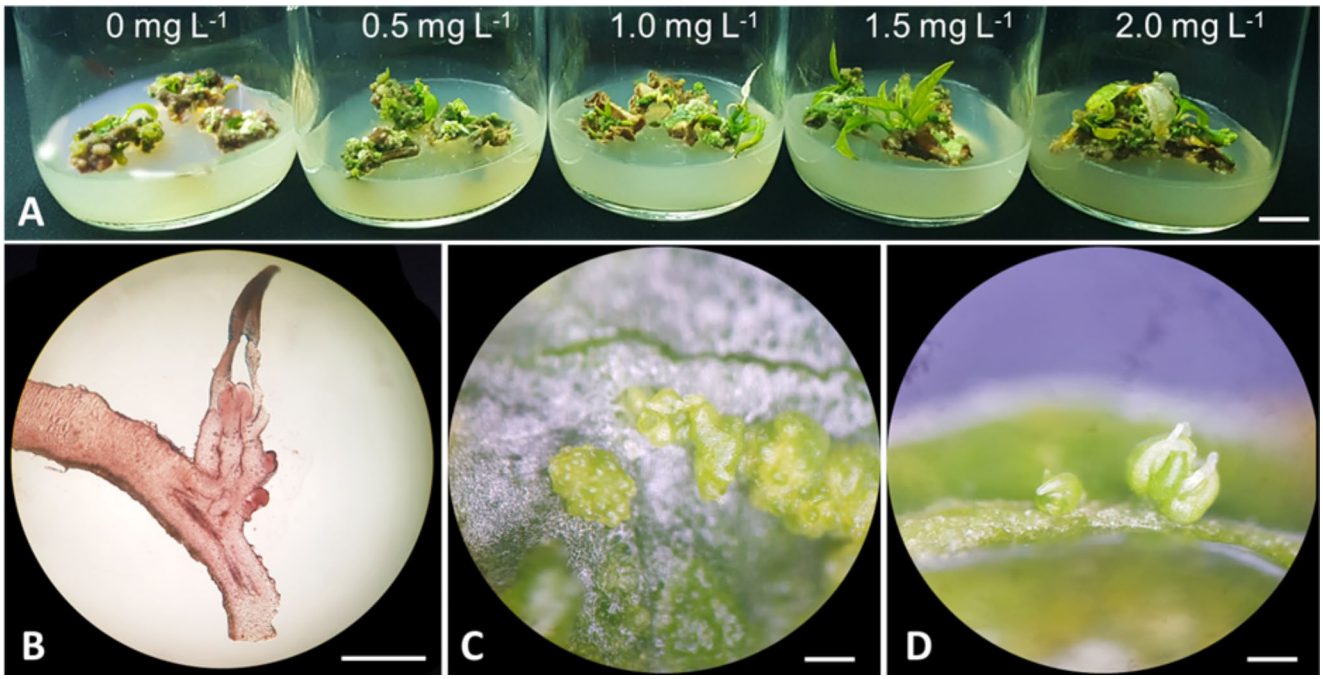

Fig. 3 Effect of AgNPs on shoot regeneration from ex vitro leaf TCL explants. **(A)** Shoot induction on medium supplemented with different concentrations of AgNPs after 60 days of culture (Bar 1 cm). **(B)** Direct shoot regeneration from leaf TCL explant (Bar 40 µm). **(C)** and **(D)** Shoot regeneration in lamina and midrib after 30 days of culture (Bars 1 mm)

recorded for explants on medium supplemented with 1.5 mg L^{-1} AgNPs. However, both the shoot induction rate and the number of shoots per explant were significantly reduced on the medium supplemented with 2.0 mg L^{-1} AgNPs (Figs. 2 and 3 A). In addition, direct shoot formation was observed in both lamellar tissue and leaf veins (Fig. 3B-D).

For leaf explants, shoot regeneration through TCL explants was reported for in vitro leaves (Hieu et al. 2018a; Tung et al. 2022a). In this study, shoot regeneration was further investigated for ex vitro leaves to increase the source of initial material for in vitro regeneration. This study showed that ex vitro leaf explants could give high regeneration rates on medium supplemented with AgNPs. Similarly, enhancement of shoot regeneration on medium supplemented with AgNPs was also observed in *Swertia chirata* (Saha and Gupta 2018) and *Lavandula angustifolia* (Jadczak et al. 2019). On the other hand, the application of AgNPs in shoot regeneration also offers advantages for subsequent stages of micropropagation such as in rooting or acclimatization in the greenhouse (Cuong et al. 2021; Tung et al. 2021a). However, this effect needs to be investigated in the micropropagation stages of purple passion fruit in further studies.

Nevertheless, several growth inhibitions in some plant species due to excessive accumulation of AgNPs have also

been reported (Farrokhzad et al. 2022; Phong et al. 2022). The excessive concentration of AgNPs in plant tissues could induce many toxic effects due to the increased production of reactive oxygen species (ROS) or form barriers in the cell wall (Tripathi et al. 2017). The energy production was stimulated by AgNPs, thus releasing more energy and accelerating the physiological metabolic responses against stress while subsequently reducing the cell vitality. ROS enhances cell survival at low levels but can induce apoptosis and necrosis at high levels via control of endoplasmic reticulum signaling pathways. In addition, the nanoparticles also form a barrier on the cell wall by blocking the cell wall pores and altering the permeability to eventually reach the plasma membrane (Kumar et al. 2023). In this study, AgNPs at high concentrations also significantly decreased the shoot regeneration from ex vitro leaves of purple passion fruit.

Effect of internode position on shoot induction from tTCL explants

The internode tTCL explants at positions from 1 to 5 from the shoot tip were cultured to investigate the possibility of shoot formation. The results showed that different internode locations had a significant effect on morphogenesis from tTCL explants (Figs. 4A and 5). After 30 days of culture, most of the tTCL explants recorded callus formation (Fig. 5A). After 60 days of culture, the optimal rate of shoot induction (66.67–70.37%) was observed in the explants at positions 3 and 4. The shoots of explants at the 5th internode were more obvious than that of positions 3 and 4; however, the rate of shoot induction was significantly lower (40.74%) (Figs. 4A and 5B-D).

The results also showed that the content of auxin (AUX) and cytokine (CK) was significantly different among the internodes at different positions. The highest AUX content was recorded in the 5th internode (97,772 µg g^{-1}). In comparison, the highest CK content (20,492 µg g^{-1}) was recorded in the 2nd internode and tended to decrease gradually for the posterior internodes. The AUX/CK ratio ranged from 1 to 9.34. The ratio of AUX/CK in internodes at positions 3, 4, and 5 was higher than in positions 1 and 2 (Fig. 4B). The results of the correlation analysis showed that the shoot induction rate was positively correlated with the position of the internodes (with a correlation coefficient of 0.655). Similarly, IAA content and AUX/CK ratio positively correlated with increasing internode position. In contrast, the content of CK groups was negatively correlated with increasing internode positions. On the other hand, the results also indicated that the shoot induction rate was negatively correlated with the Zeatin content with a correlation coefficient of 0.935 (Fig. 4C).

Hieu et al. (2018b) reported that ex vitro internodes induced only callus but not shoots in the in vitro condition. In a recent study, we also successfully regenerated shoots from ex vitro internode longitudinally TCL explants (68.00%) (Phong et al. 2022). In this study, shoot regeneration continued to be recorded for internode tTCL explants (66.67–70.37%). Thereby, it can be seen that the application of TCL technique on ex vitro stem explants has successfully produced regenerated shoots compared with conventional explants. Therefore, the application of TCL technique can improve and enhance the regeneration of stubborn explant sources.

The identification of optimal shoot induction internodes contributes to increase shoot regeneration efficiency. In fact, internodes away from the apex are usually longer; using explants in the 3rd internode instead of the usual 1st and 2nd internodes increases the source of initial material for in vitro regeneration. In addition, the study also indicated that the difference in endogenous hormones in the initial internode was one of the factors affecting shoot induction from TCL explants of purple passion fruit in the same culture medium. However, other factors such as the different responses with exogenous plant growth regulators or differences in diameter and the contact surface of different internode explants need to be considered and clarified in further studies.

Shoot regeneration from ex vitro internode tTCL and lTCL explants

The results of the previous experiment showed that the tTCL explant from the 3rd internode was suitable for shoot induction. In this experiment, the 3rd internode was used for shoot regeneration through tTCL and lTCL explants. The results showed that the rate of shoot induction in tTCL and lTCL explants was not significantly different (69.22% and 67.78%, respectively). However, the number of shoots in lTCL explants (3.33 shoots/explant) was significantly higher than in tTCL explants (1.33 shoots/explant) after 60 days of culture (Fig. 6A and B). In addition, the shoot regeneration coefficient (based on shoot induction rate, average number of shoots per explant and number of TCL explants from a 1 cm internode fragment) in lTCL explants (9.04) was significantly higher than in tTCL explants (4.61) (Fig. 6B). In the other hand, the shoot regeneration was successful from ex vitro internode lTCL explants and was enhanced on MS medium supplemented with 5.0 mg L^{-1} AgNPs in a recent study (Phong et al. 2022). In the present study, the shoot regeneration efficiency between tTCL and lTCL explants was compared in the similar culture medium. The results showed that the number of shoots (11.67 shoots/explant) and the shoot regeneration coefficient (40.86) in lTCL explants were significantly higher than that of tTCL

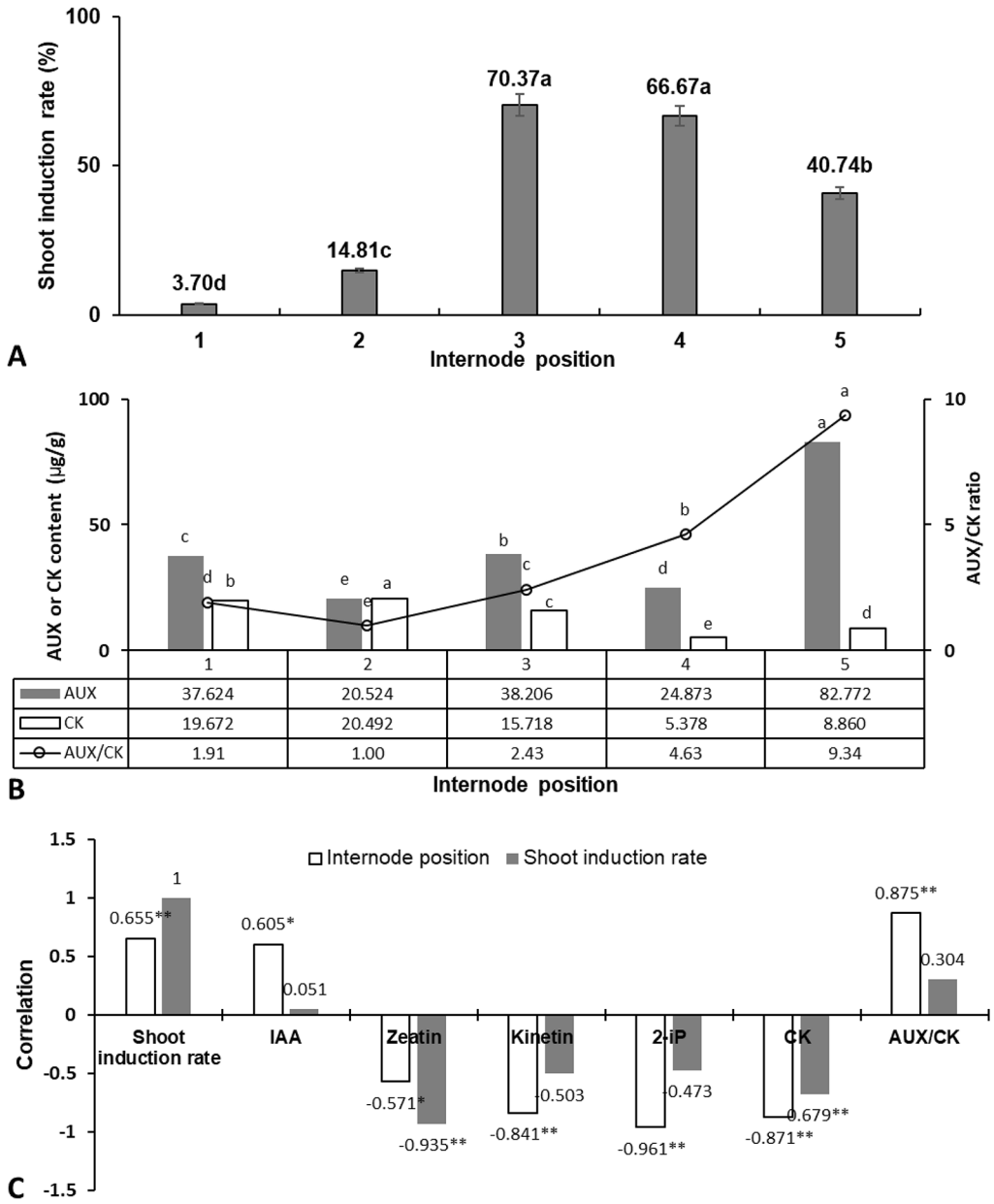

Fig. 4 Effect of internode position on shoot induction from tTCL explants. **(A)** The shoot induction rate of ex vitro internode tTCL explants at positions 1 to 5 after 60 days of culture. **(B)** Auxin – AUX (IAA) content, Cytokinine – CK (total kinetin, zeatin, and 2-iP) content, and AUX/CK ratio of internode explant at different positions. **(C)** Correlation between endogenous hormones and shoot induction ability of internode tTCL explants (**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level)

explants (7.00 shoots/explant and 29.13, respectively) on medium containing 5.0 mg L^{-1} AgNPs after 60 days of culture (Fig. 6C and D).

In this study, based on the identification of the third internode for the high rate of shoot induction, the shoot regeneration efficiency between tTCL and lTCL explants at this internode was compared in the same culture medium. The results showed that the lTCL explants had a higher regeneration efficiency than the tTCL explants derived from ex vitro internode of purple passion fruit both on the medium with or without the addition of AgNPs. Higher shoot regeneration efficiency of lTCL explants compared with tTCL explants was also observed in some studies for some other plant species. For example, Hieu et al. (2021) reported that tTCL explant (1 mm in thickness) from the stem segments of *P. edulis* 'Monte Alegre' did not react to the culture medium

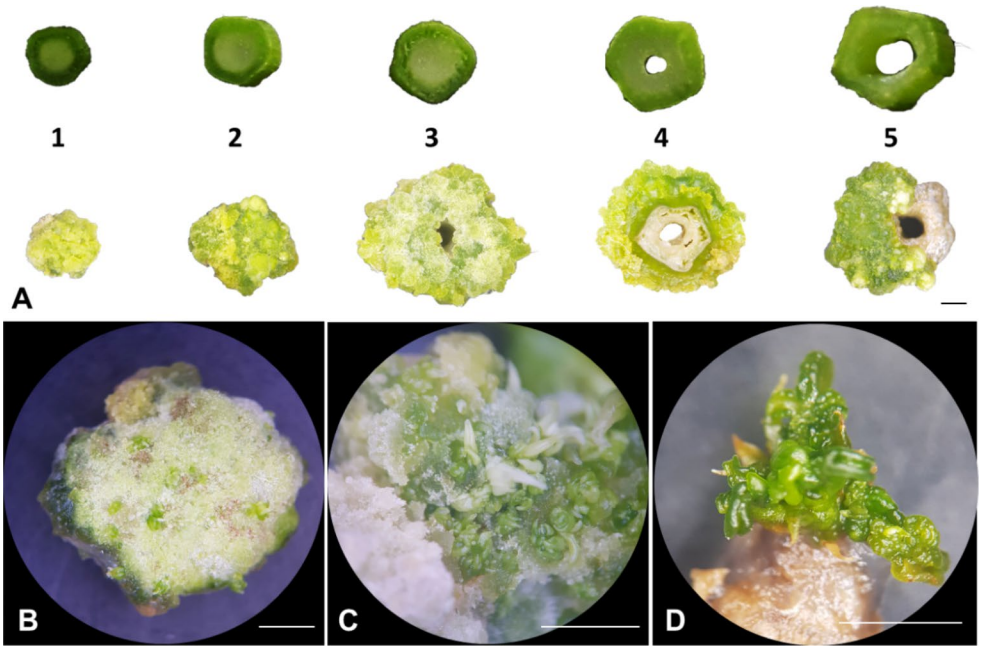

Fig. 5 Shoot induction from ex vitro internode tTCL explants. **A.** The callus induction from tTCL explants in internode positions from 1 to 5 after 30 days of culture. **B**, **C** and **D.** Shoots regeneration from tTCL explants of 2nd, 3rd and 5th internodes after 60 days of culture. (Bars 1 mm)

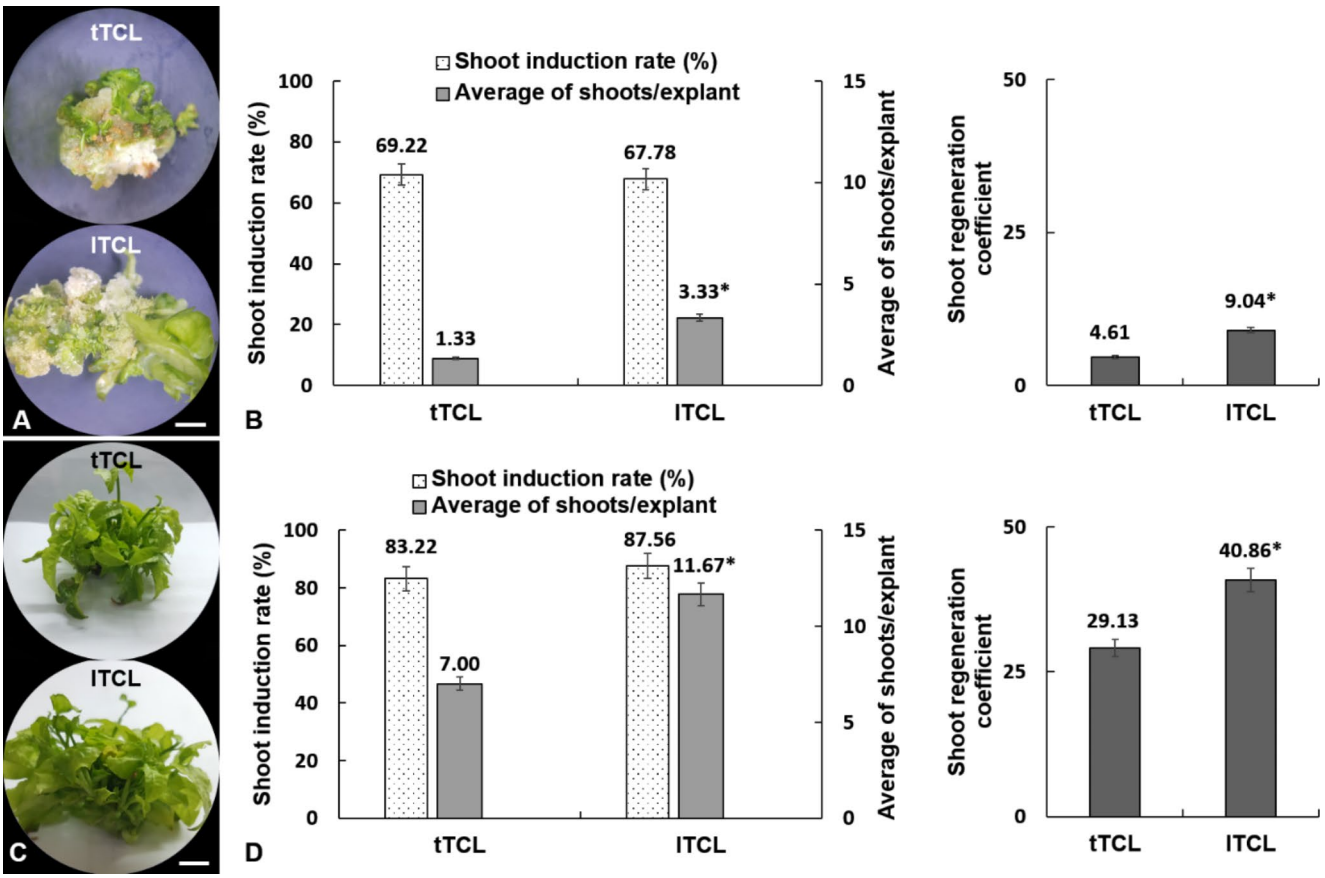

Fig. 6 Shoot regeneration of TCL explants derived from ex vitro 3rd internode after 60 days of culture. **(A)** Shoots regeneration from tTCL and lTCL explants on medium without AgNPs. **(B)** Comparison of shoot induction rate, the average number of shoots, and shoot regeneration coefficient between tTCL and lTCL explants on medium without AgNPs. **(C)** Shoots regeneration from tTCL and lTCL explants on medium containing 5.0 mg L^{-1} AgNPs. **(D)** Comparison of shoot induction rate, the average number of shoots, and shoot regeneration coefficient between tTCL and lTCL explants on medium containing 5.0 mg L^{-1} AgNPs. (Bars 2 mm)

and browned after 60 days; while the lTCL explant induces shoot regeneration. In contrast, tTCL explants showed a better response to the formation of protocorm-like bodies in *Cymbidium* and *Dendranthema grandiflora* than in lTCL explants (da Silva and Dobránszki 2014). Hence, the selection of the appropriate TCL explant depends on the purpose of regeneration, and the differences in tissue type and plant species. In the case of lTCL, the explant consists of only one or two types of tissue, such as epidermal and subepidermal tissues, while tTCL can have many different tissues (Nhut et al. 2003). The selection of explants is important for the results of in vitro organogenesis. It was an important indicator to be optimized when conducting plant tissue culture processes (Bhattacharyya et al. 2018; da Silva et al. 2015; Ramírez-Mosqueda et al. 2019).

On the other hand, the culture of TCL explants on media supplemented with AgNPs significantly enhanced the shoot regeneration efficiency. In the present study, a positive effect of AgNPs was again demonstrated on shoot regeneration in both tTCL and lTCL explants derived from the internodes of purple passion fruit. Hieu et al. (2021) also reported that the number of shoots doubled on the culture medium supplemented with 5.0 mg L^{-1} AgNPs compared with the medium without AgNPs in yellow passion fruit. Several studies reported that AgNPs affect morphogenesis processes via alterations of endogenous hormones such as auxin, cytokinin, abscisic acid (Cuong et al. 2023), and antioxidant enzyme activity (Tung et al. 2022b) of explants. This effect can be enhanced by the advantage of a large surface area in contact with the culture medium of TCL explants. However, the interactions need to be studied in further research.

Effect of AgNPs on somatic embryogenesis from internode tTCL explants

In this experiment, the internode tTCL explants were cultured on MS medium containing 2.0 mg L^{-1} 2,4-D (Phong et al. 2023) for somatic embryogenesis. The results showed that internode tTCL explants were capable of somatic embryogenesis after 60 days of culture (22.45%), while the control explants only formed calli (Fig. 7). Then, the explants were transferred to MS medium containing different concentrations of AgNPs. The results showed that the indirect embryogenesis through the callus was significantly enhanced on the medium supplemented with AgNPs after 60 days of culture (Table 1). The addition of AgNPs at all experimental concentrations gave a higher number of embryos per explant than the control. The culture medium supplemented with 3.0 mg L^{-1} AgNPs gave the highest number of embryos (33.00 embryos/explant). However, when increasing AgNPs concentration to 5.0 mg L^{-1}, the number of embryos per explant decreased significantly (26.33 embryos/explant) (Table 1).

In addition, the maturation and transformation of somatic embryos were significantly improved on AgNPs-supplemented medium after 60 days of culture (Table 1; Fig. 8). When treated with 2.0–5.0 mg L^{-1} AgNPs, the number of embryos at the heart-shaped, torpedo, and cotyledon stages was significantly improved. The addition of 3.0 mg L^{-1}

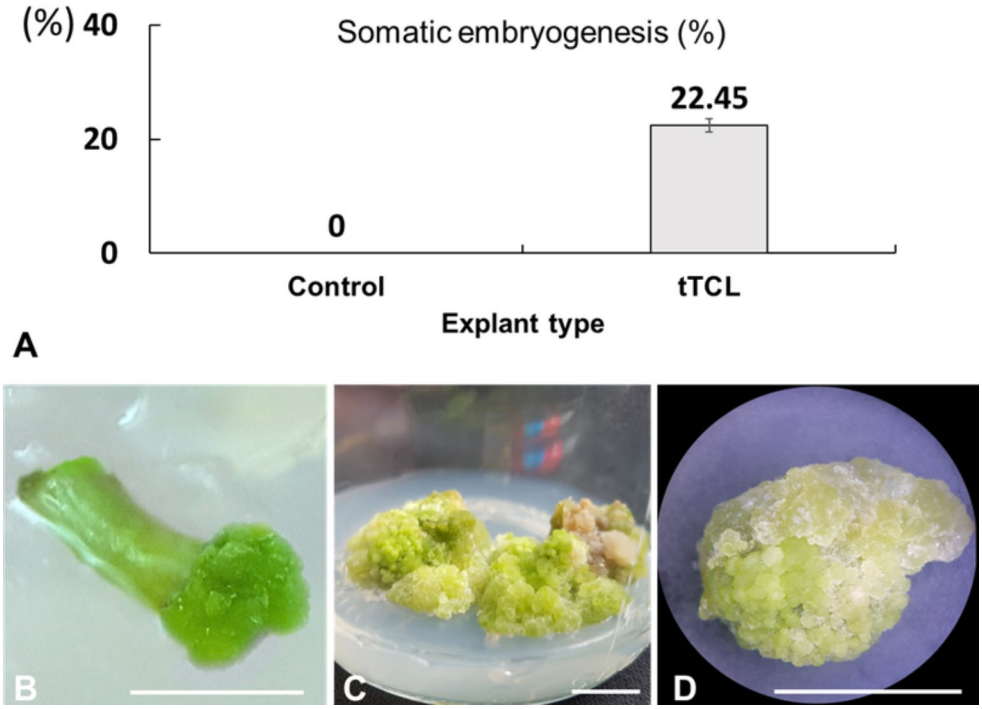

Fig. 7 Somatic embryogenesis via callus from ex vitro internode tTCL explants on MS medium supplemented with 2.0 mg L^{-1} 2,4-D after 60 days of culture. **(A)** Internode explant of 1 cm length (control). **(B)** and **(C)** Internode tTCL explants. (Bars 1 cm)

Table 1 Effect of AgNPs on somatic embryogenesis derived from internode tTCL explants after 60 days of culture

*Different letters (a, b,…) in the same column represent statistically significant differences at $p < 0.05$ (*Tukey's test*). Values are mean ± Standard Errors of three independent experiments

AgNPs (mg L^{-1})	Total no. of embryos /explant	No. of globular embryos /explant	No. of heart-shaped embryos /explant	No. of torpedo embryos /explant	No. of cotyledonary embryos /explant
0.0	$9.67 \pm 0.33^{e*}$	6.33 ± 0.33^{b}	2.33 ± 0.33^{d}	0.67 ± 0.33^{c}	0.33 ± 0.33^{c}
1.0	14.67 ± 0.67^{d}	7.33 ± 0.33^{ab}	3.00 ± 0.00^{d}	2.33 ± 0.33^{c}	2.00 ± 0.00^{c}
2.0	25.67 ± 0.88^{c}	8.00 ± 0.58^{ab}	7.00 ± 0.58^{bc}	5.67 ± 0.33^{b}	5.00 ± 0.58^{b}
3.0	33.00 ± 1.00^{a}	8.33 ± 0.33^{ab}	9.33 ± 0.33^{a}	8.00 ± 0.58^{a}	7.33 ± 0.33^{a}
4.0	29.67 ± 0.88^{ab}	9.33 ± 0.88^{a}	8.67 ± 0.33^{ab}	6.00 ± 0.00^{b}	5.67 ± 0.33^{ab}
5.0	26.33 ± 0.33^{bc}	9.00 ± 0.58^{a}	6.33 ± 0.33^{c}	5.67 ± 0.33^{b}	5.33 ± 0.33^{b}

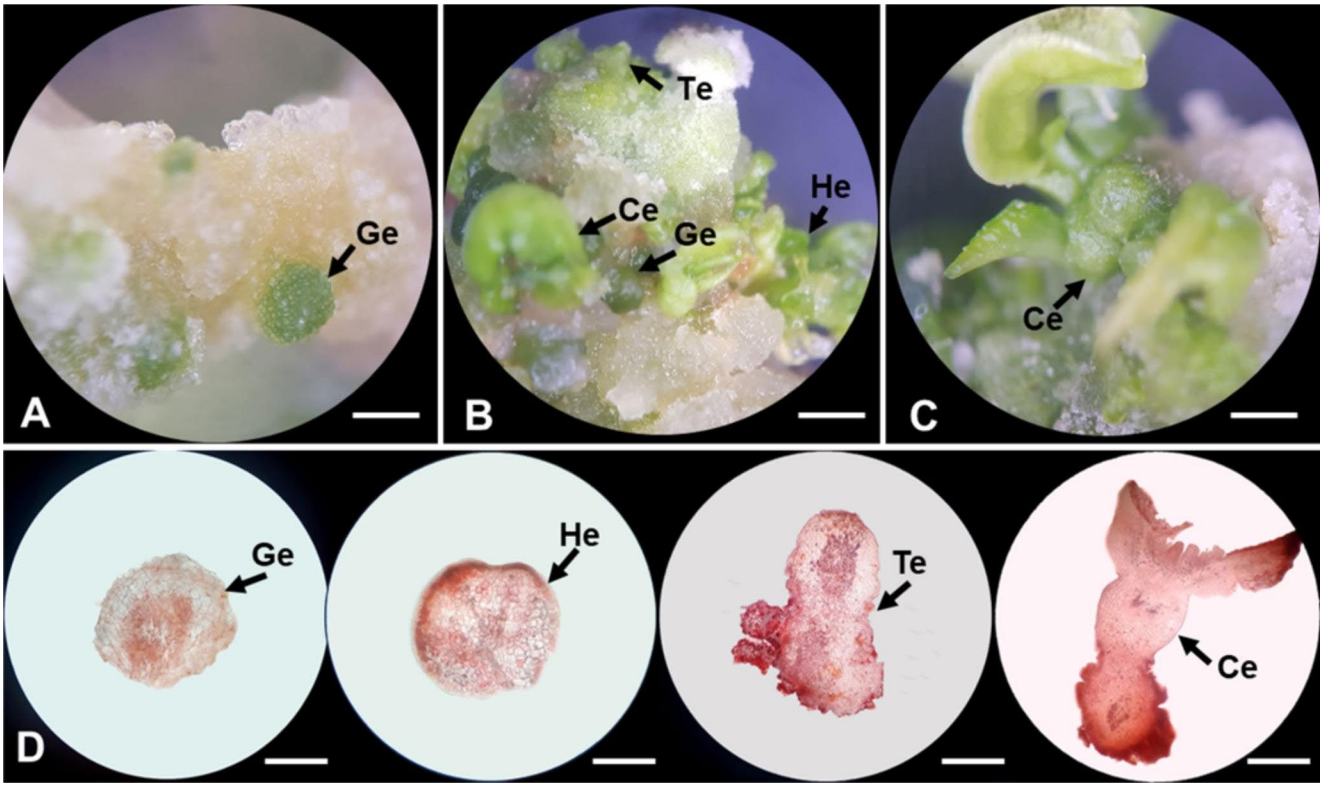

Fig. 8 Effect of AgNPs on indirect somatic embryogenesis from ex vitro internode tTCL explants after 60 days of culture. **(A)** Somatic embryogenesis on hormone-free MS medium (Bar 1 mm). **(B)** and **(C)** Somatic embryogenesis on MS medium supplemented with 3.0 mg L^{-1} AgNPs (Bars 1 mm). **(D)** Indirect somatic embryos at distinctive stages of development were observed on MS medium supplemented with AgNPs (Bars 100 µm). Globular embryo (Ge), Heart-shaped embryo (He), Torpedo embryo (Te), Cotyledonary embryo (Ce)

AgNPs gave the highest number of heart-shaped embryos (9.33 embryos/explant), torpedo embryos (8.00 embryos/explant), and cotyledon embryos (7.33 embryos/explant) (Table 1). The results of anatomical observation indicated that indirect somatic embryogenesis through callus derived from internode tTCL has the ability to fully develop stages including globular, heart-shaped, torpedo-shaped, and cotyledonary stages after about 60 days of culture (Fig. 8D).

In vitro plant regeneration via somatic embryogenesis was highly desirable to enhance the homogeneous growth of plantlets (Nhut et al. 2015). In addition, somatic embryogenesis plays an important role in mass propagation (Guan et al. 2016), germplasm conservation (Pacheco et al. 2016), and plant breeding (de Almeida et al. 2022) of many plant species. For purple passion fruit, the somatic embryogenesis was successful on seed tissues (Cruz et al. 2022; Pinto et al. 2011; Rocha et al. 2015) and leaf tissues (Huh et al. 2017; Phong et al. 2023). Therefore, the potential of other tissues for somatic embryogenesis in this plant remains largely unknown. In the present study, indirect somatic embryogenesis was recorded for the first time from internode tTCL explant.

On the other hand, in this study, the number of somatic embryos of tTCL explant was significantly enhanced on medium supplemented with AgNPs. Positive effects of AgNPs were also reported in somatic embryogenesis in

several plant species such as *Gloriosa superba* (Mahendran et al. 2018), *Panax vietnamensis* (Cuong et al. 2021), *Begonia tuberous* (Tung et al. 2021b). Furthermore, a better maturation response of somatic embryos was also obtained on medium supplemented with AgNPs. These effects may be related to the change of ethylene content in plant tissues under the influence of AgNPs which has been observed in the previous reports (Manickavasagam et al. 2019; Phong et al. 2022). On the other hand, Cruz et al. (2022) showed that the increase in endogenous polyamines under the influence of polyethylene glycol positively affected the maturation of somatic embryos from mature zygote embryos in purple passion fruit. Thus, the development of somatic embryos in the present study may be related to the ability to enhance the polyamine synthesis of AgNPs which has been observed in previous studies (Bais et al. 2000; Rakesh et al. 2021). A similar effect was also observed in somatic embryogenesis from purple passion fruit leaf explant on medium containing AgNPs (Phong et al. 2023). Hence, the ability of AgNPs to regulate embryo maturation presents a possible pathway for improving the efficiency of somatic embryogenesis in purple passion fruit and can be researched for use in a variety of other plant species.

Conclusion

In this study, shoot regeneration from ex vitro leaf and internode TCL explants was significantly enhanced on medium containing AgNPs. In addition, somatic embryogenesis was observed for the first time from internode tTCL explants. Furthermore, the addition of AgNPs on the culture medium had positive effects on both shoot organogenesis and somatic embryogenesis from TCL explants. The present study contributes to a significant improvement in the micropropagation efficiency of purple passion fruit.

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Author contributions THP and DTN designed the study. THP conducted experiments. THP, TH, HTT, NTNM, HDK, DMC, VQL, and NBN participated interpretation of data and revision of intellectual content. All authors discussed the results and wrote manuscripts.

Declarations

The authors have no conflict of interest.

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