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ORIGINAL ARTICLE

Silver nanoparticles: a positive factor for in vitro flowering and fruiting of purple passion fruit (*Passiflora edulis* Sim f. *edulis*)

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Abstract

In the present study, the positive effects of AgNPs were demonstrated for adventitious shoot formation, in vitro flowering and fruiting of *Passiflora edulis* Sims f. *edulis*—an important commercial crop. The results showed that shoot regeneration from lTCL (87.67%) and oTCL (100%) explants were significantly improved on the medium supplemented AgNPs. The in vitro shoots derived from TCL explants were used for shoot multiplication. The treatment with 1.0 mg/L meta-topolin (mT) and 5 mg/L AgNPs enhanced the shoot multiplication with the highest number of shoots (13.67 shoots/explant), shoot height (4.33 cm), and total chlorophyll content (33.93 nmol/cm^2). For flowering induction, shoot tips cultured in MS medium supplemented with 7 mg/L AgNPs gave the highest of flowering rate (51.67%) and number of flowers per shoot (2.33 flowers) after 60 days of culture. In addition, shoots cultured in medium supplemented with 7 mg/L AgNPs showed significantly lower endogenous hormone of GA_3, ABA, and melatonin levels than the control. In the treatment with 7 mg/L AgNPs, the flower bloom rate was 100% and the flower diameter was the largest (3.43 cm). The in vitro developed flowers self-fertilized and formed fruits. After 90 days of culture, the treatment supplemented with 7 mg/L AgNPs gave the highest fruiting rate (56.67%), number of fruits (1.67 fruits), and fruit diameter (1.13 cm). These findings pave the way for further research into flowering and fruiting mechanisms, as well as improving the efficient breeding process of this plant.

Key message

A first procedure has been established for in vitro flowering and fruiting of purple passion fruit via the application of nanotechnology and the thin cell layer technique. Silver nanoparticles significantly improved shoot formation, in vitro flowering and fruiting of purple passion fruit.

Keywords In vitro flowering · In vitro fruiting · Silver nanoparticles · Thin cell layer · Endogenous hormone

Abbreviations

ABA	Abscisic acid
AgNPs	Silver nanoparticles
BA	Benzyl adenine
GA_3	Gibberellin
mT	Meta-topolin
MS	Murashige and Skoog (1962)
TCL	Thin cell layer

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Introduction synchronization of the flowering time

Knowledge about the flowering of plants provides the theoretical foundation for developing appropriate methods to study plant physiology and selecting suitable hybrids in breeding strategies (Ammar et al. 2012). Understanding the mechanisms and factors affecting flowering contributes to the optimization of flowering time, the number of flowers,

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pollination, and seed production in plants (Haque et al. 2016; Zhu et al. 2022). In addition, accurate control of the flowering time can significantly improve the efficiency of hybridization methods, especially hybridization between distant species (Zulkarnain et al. 2015; Rivero et al. 2021). However, the natural flowering of plants is generally restricted by season and this process is also significantly influenced by many environmental factors (Shen et al. 2020). Under these circumstances, the biotechnology approaches can contribute to overcome these limitations. The in vitro flowering system is considered as a convenient tool to study flower induction, flower senescence and flower organ development (Zulkarnain et al. 2015). This technique facilitates the understanding of flowering and fruiting physiology by controlling the influence of factors such as light, temperature, plant growth regulators, and minerals (Murthy et al. 2012; Sukthavornthum et al. 2018). In addition, in vitro flowering also plays an important role in hybridization in plants, especially in hybridization techniques using pollen from rare cultivars. The barriers of incompatibility in plants can be overcome using in vitro methods and embryo-rescue techniques in distant breeding (van Tuyl et al. 1991; Malakar et al. 2022).

In vitro flowering studies has a great potential in breeding programs for crop improvement based on the advantage of shortening and synchronization of the flowering time (Shen et al. 2020). If the in vitro flowering process is well described, it can serve as a model system for studying flowering mechanisms (Rathore et al. 2013). In vitro flowering was observed mostly in the micropropagation of some ornamental plants and vegetables (Rathore et al. 2013; Sivanesan and Won 2015; Manan et al. 2016; Haque et al. 2018; Sukthavornthum et al. 2018; Sreelekshmi and Siril 2021; Xiong et al. 2021; Liu et al. 2022). The *Passiflora* was the largest genus in the Passifloraceae family, but only purple passion fruit (*P. edulis* Sim.) and yellow passion fruit (*P. edulis* f. *flavicarpa*) have significant commercial value (Yockteng et al. 2011; Taiwe and Kuete 2017). At present, in vitro flowering is not a widespread phenomenon of the genus *Passiflora*, only flowering in *P. suberosa* L. (Scorza and Janick 1980) has been reported under in vitro condition. To the best of the authors knowledge, there is no record of in vitro flowering of *P. edulis* Sime f. *edulis*, one of the significant commercial species in the genus *Passiflora*. Moreover, *P. edulis* Sime f. *edulis* is a self-fertile plant, therefore, the process of in vitro flowering and fruiting play important role in plant breeding such as the production of monoploid clones from in vitro pollen or in vitro hybridization in the process of selecting new varieties.

In recent years, the application of silver nanoparticles (AgNPs) in plant biotechnology and agriculture has received considerable attention from scientists (Mahajan et al. 2022). In particular, the positive influence of AgNPs in plant micropropagation has been reported in many aspects such as explant disinfection, somatic embryo formation and proliferation, increased shoot quality and secondary compound accumulation (Tung et al. 2021; Cuong et al. 2021; Khattab et al. 2022; Mahajan et al. 2022). On the other hand, adding silver to the culture medium was suggested to have an impact on the in vitro flowering of plants. Several studies reported the in vitro flowering and setting fruits of *Capsicum* spp. (Haque et al. 2016), *Solanum americanum* and *Solanum villosum* (Haque et al. 2018) in medium supplemented with silver nitrate ($AgNO_3$) or silver thiosulfate ($Ag_2O_3S_2$). The in vitro flower longevity of *Dianthus chinensis* was improved in medium supplemented with $AgNO_3$ (Sreelekshmi and Siril 2021). Therefore, the use of silver in the form of nanoparticles could be a new and potential research direction for in vitro flowering of plants (Salachna et al. 2019; Ngan et al. 2019).

In this study, the shoot regeneration through the thin cell layers of ex-vitro internode was enhanced in medium containing AgNPs. In addition, in vitro flowering and fruiting from shoot tips of *P. edulis* Sime f. *edulis* were first observed. This study also demonstrated the positive effects of AgNPs on in vitro flowering and initial fruiting. The present results may facilitate rapid breeding and expedite research on the flowering and fruiting mechanisms of this plant.

Materials and methods

Plant materials

In this study, the third internodes from the top of 6-months-old *P. edulis* Sim f. *edulis* seedling were used as initial material. The internodes were disinfected according to the procedure of Hieu et al. (2018). Briefly, the samples were collected from healthy plants and washed under running water for 10 min. Next, these samples were pre-sterilized with 70% alcohol for 30 s and then rinsed with distilled water 3 times. Then, it was disinfected with 0.1% AgNPs solution for 15 min. Finally, the samples were rinsed 3 times with distilled water and cultured in MS medium (Murashige and Skoog 1962) for 7 days. Clean explants were obtained and used for shoot regeneration experiments. For in vitro flowering induction, in vitro 60-days-old shoot tips derived from internode-TCLs were used. The type materials used for the specific experiments were shown in Fig. 1.

Silver nanoparticles solution

The Institute of Environmental Technology (VAST) investigated and developed silver nanoparticles (AgNPs) with sizes less than 20 nm. According to information provided by the manufacturer, the nanoparticles were produced by

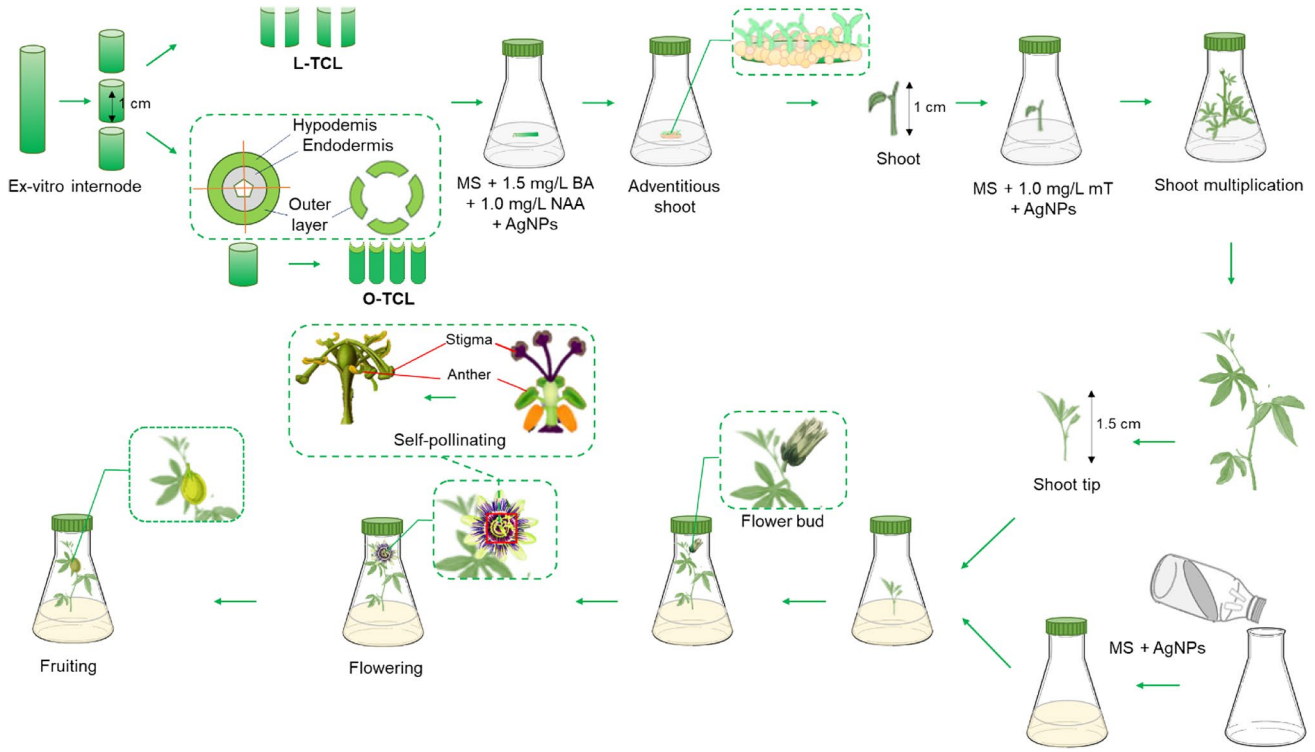

Fig. 1 The schematic diagram of the experimental design

the method described by Chau et al. (2008). The following ratio was used to make AgNPs solution: $AgNO_3$ at 750–1000 ppm, $NaBH_4$ at 200 ppm, the molar ratio between $AgNO_3$ and $NaBH_4$ was 4/1, the dripping rate of $NaBH_4$ was 10–12 drops/min, β-chitozan at 250–300 ppm.

Culture media and conditions

MS medium (Murashige and Skoog 1962) containing 30 g/L sucrose and 8 g/L agar was used as the basal culture medium in this study. This medium was supplemented with plant growth regulators or AgNPs depending on the stage of study (Fig. 1). The culture medium was adjusted to pH 5.8 before being autoclaved for 30 min at 121 °C and 1 atm. The culture room with a temperature of 25 ± 2 °C, a humidity of 55–60%, the lighting time of 16 h per day (using fluorescent light with an intensity of 40–45 µmol m^{-2} s^{-1}) was used for all the cultures.

Shoot induction from internode-TCL explants

The ex-vitro internodes were cut according to two methods described in detail in Fig. 1. First, the internode segments with length of 1 cm and diameter of 0.4 cm were cut longitudinally into 4 pieces (lTCL). Second, the explants were cut similarly to the lTCL explants, but the inner part of internode was removed and only the outer cell layer (thickness approx 0.1 cm) were kept (oTCL). Then, the undersides (wound section) of the lTCL and oTCL explants were placed in exposing with the surface of the culture media. The explants were cultured in MS medium supplemented with 0.5 mg/L BA, 1.0 mg/L NAA, 30 g/L sucrose, 8 g/L agar (Hieu et al. 2022), and AgNPs at concentrations of 0, 1, 3, 5, and 7 mg/L to investigate the shoot regeneration. Each treatment was conducted with 30 cultured flasks with 3 explants per flask. The indicators of shoot regeneration were collected after 60 days of culture.

Shoot multiplication

The shoots regenerated from the internode-TCL explants at the optimal treatment in the previous experiment were used for shoot multiplication. The shoots with a length of 1 cm were excised and cultured in MS medium supplemented with 1.0 mg/L meta-topolin (mT) (Chen et al. 2020) and AgNPs at concentrations of 1, 3, 5, and 7 mg/L. The treatment without AgNPs was used as control. The 30 cultured flasks (1 shoot per flask) were used for each treatment. Some indicators of the number of shoots (shoots/explant), shoot height (cm), total chlorophyll content (nmol/cm^2) were recorded after 60 days of culture.

In vitro flowering and fruiting

To investigate the effect of AgNPs on the in vitro flowering and fruiting, in vitro healthy shoots multiplied in the medium supplemented with AgNPs at the optimal concentration after 60 days of culture in the previous experiment were used as explants. Shoot tips approximately 1.5 cm in length with 3 leaves were excised and cultured in MS medium supplemented with AgNPs at different concentrations (1, 3, 5, 7, and 9 mg/L). The treatment without AgNPs was used as control. Each treatment was conducted with 30 flasks with a single shoot per flask. The contents of gibberellin (GA_3), abscisic acid (ABA), and melatonin (µg/g) of shoots were determined by High—performance liquid chromatography (HPLC) analysis after 60 days of culture. The indicators related to flowering and fruiting were observed and recorded at every 3-day interval during the culture process.

High performance liquid chromatography (HPLC) analysis

The plant 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. Take the sample mixture and centrifuge cold (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 (Thermi 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 with gradient running mode as follows: 0–10 min, A:B = 75:25; 11–17 min, A:B = 50:50; 18–25 min, A:B = 75:25. In this study, the endogenous hormones measured were Gibberellin (GA3), Abscisic acid (ABA) and melatonin. The hormones were quantified using the calibration curves of particular standards.

Plant anatomy

The sliced samples were bleached in Sodium hypochlorite solution (15%) for 5 min. Next, the sample was immersed in Acetic acid solution (10%) for 10 min. The tissue was stained by carmine red (or violet purple) for 5 min. Finally, they were rinsed with distilled water, placed on a glass slide, and covered with a coverslip. Observation of the sample was conducted on an optical microscope at ×10 and ×40 magnification. The TCL explants were anatomized every 3 days of culture.

Statistical analysis

In the present study, all treatments were repeated three times. All data were processed by Microsoft Excel 2019 and SPSS 26.0 statistical analysis software basing on *Tukey's test* method with $p < 0.05$. Comparison of endogenous hormone differences between AgNPs treatment and control treatment by *t-test* method.

Results and discussion

Effect of AgNPs on shoot induction from lTCL and oTCL explants

The results showed that the TCL explants induced shoot after 9 days of culture; however, shoots regenerated from oTCL explants showed clearly leaf primordia than from lTCL explants (Fig. 2A). Moreover, regenerated shoots could be observed on the oTCL explant surface after 30 days of culture (Fig. 2B). After 60 days of culture, the shoot regeneration rate of oTCL explants was 78.33%, while for lTCL explants it was 68.00% (Table 1). The treatments supplemented with AgNPs gave a significantly higher shoot regeneration compared to the control. For lTCL explants, the treatment with 5 mg/L AgNPs gave the highest shoot regeneration rate (87.67%). Moreover, the highest number of shoots (11.33 shoots/explant) and shoot height (2.53 cm) were also recorded in the treatment with 5 mg/L AgNPs (Table 1, Fig. 2C). For oTCL explants, the highest shoot regeneration rate (100%), number of shoots (15.33 shoots/explant) and shoot height (2.61 cm) were also recorded in the treatment with 3 mg/L AgNPs. Enhanced shoot regeneration was also observed in the shoots exposed to AgNPs supplemented in culture medium of *Swertia chirata* (Saha and Gupta 2018) and *Lavandula angustifolia* (Jadczak et al. 2019). In addition, in this study, roots formed from the explants were observed in the medium supplemented with 5 or 7 mg/L AgNPs (Fig. 2C). Similarly, root formation when supplemented with AgNPs at high concentrations was also observed in shoot regeneration of strawberry; accordingly, number of shoots decreased at high concentrations of AgNPs, while the number of roots and root length increased (Tung et al. 2021). This result indicated that AgNPs not only improved shoot regeneration but also affected the rooting of explant. The present results also revealed that the shoot regeneration rate and the number of shoots tended to decrease when the AgNPs concentration was increased to 7 mg/L (Table 1).

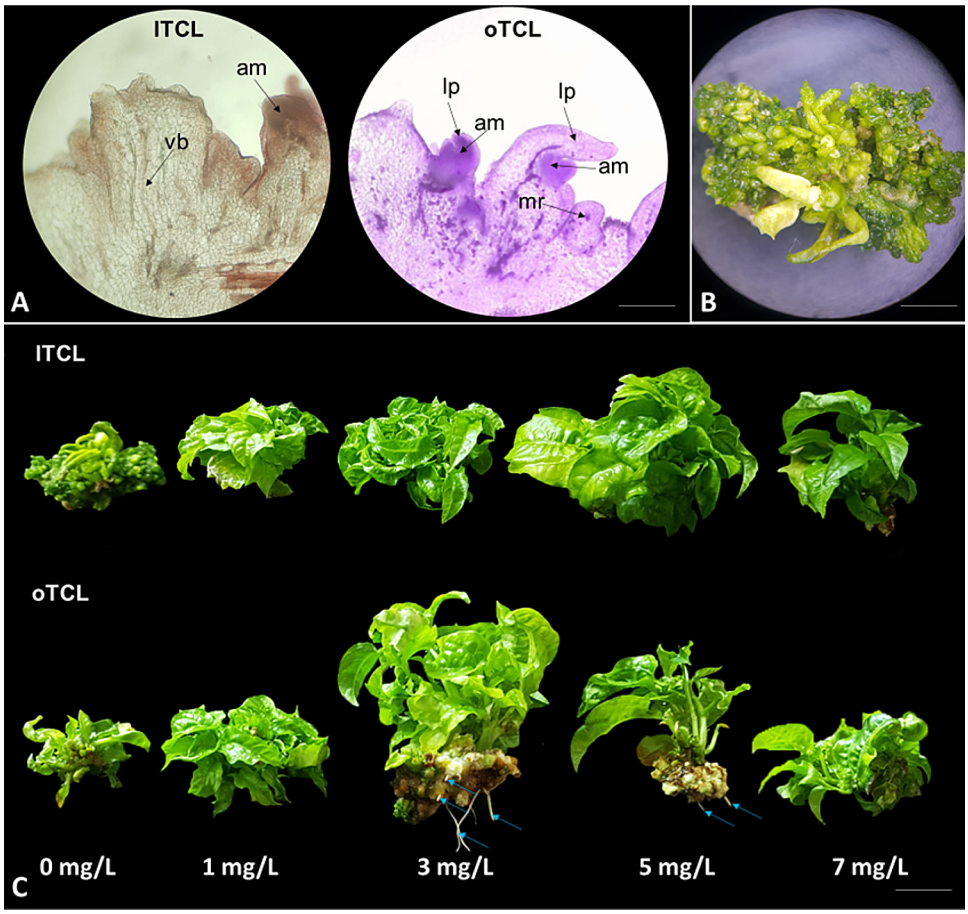

Fig. 2 Shoot regeneration from lTCL and oTCL explants in medium supplemented with AgNPs. **A** Shoots regeneration from lTCL and oTCL explants after 9 days of culture (bar 40 µm). **B** Shoots formed from oTCL explant surface after 30 days of culture (bar 1 cm). **C** The shoot regeneration from lTCL and oTCL explants in medium containing AgNPs at different concentrations after 60 days of culture (bar 1 cm). The blue arrows indicate the roots formed from the explant. Apical meristem (am), meristematic region (mr), lp (leaf primordia), vascular bundle (vb)

Table 1 Effect of AgNPs on shoot regeneration from lTCL and oTCL explants after 60 days of culture

AgNPs (mg/L)	Shoot regeneration (%)		No. of shoots/explant		Shoot height (cm)	
	lTCL	oTCL	lTCL	oTCL	lTCL	oTCL
0	68.00^{d*}	78.33^{c}	3.00^{d}	3.33^{d}	0.93^{c}	1.05^{d}
1	69.33^{d}	87.67^{b}	5.00^{c}	10.67^{b}	1.23^{bc}	1.54^{c}
3	72.67^{c}	100.00^{a}	7.33^{bc}	15.33^{a}	1.37^{b}	2.61^{a}
5	87.67^{a}	87.33^{b}	11.33^{a}	7.33^{c}	2.53^{a}	2.50^{b}
7	80.00^{bc}	82.33^{bc}	7.67^{b}	7.00^{c}	2.50^{a}	1.47^{cd}

*Different letters (a, b,…) in the same column represent statistically significant differences at $p < 0.05$ (Tukey's test)

Hieu et al. (2022) also reported high shoot regeneration (71.67%) for the lTCLs of in vitro *P. edulis* 'Monte Alegre' stem. In our study, high shoot regeneration (87.67%) was also observed from lTCLs of ex-vitro *P. edulis* Sim f. *edulis* internode. Moreover, a higher rate of shoot regeneration (100%) was observed with oTCL explants compared with lTCL explants. This may be due to the elimination of regions of slow division and the direct exposure of regions of strongly dividing cells to the culture medium of the oTCL explants. The different responses of cell regions and excision patterns to plant growth regulators have also been reported and partly elucidated in several previous studies (da Silva and Nhut 2003; Tripathi et al. 2018; Gorelova et al. 2021; Tung et al. 2022; Hanh et al. 2022).

Effect of AgNPs on shoot multiplication

Chen et al. (2020) reported that replacing BA with mT (1 mg/L) significantly improved shoot quality during the micropropagation of 'Tainung No.1' Passion Fruit (*Passiflora edulis* Sims). In the present study, the combination of mT and AgNPs at appropriate concentrations showed significant improvement in shoot multiplication of *P. edulis* Sims f. *edulis*. Specifically, in vitro shoots regenerated from TCL

explants were excised and cultured in medium supplemented with 1 mg/L mT and AgNPs at different concentrations for shoot multiplication. The results showed that the addition of AgNPs in a cultured medium significantly enhanced the shoot multiplication. The treatment of 5 mg/L AgNPs gave the highest of shoot numbers (13.67 shoots/explant) and shoot height (4.33 cm). Moreover, the addition AgNPs in the culture medium also significantly increased the chlorophyll content in leaves (33.93 nmol/cm^2) compared with the control (22.18 nmol/cm^2) (Fig. 3).

Many studies have also highlighted the positive effects of AgNPs on in vitro plant growth (Mahajan et al. 2022). Several reports suggested antioxidant enzymes triggered when explants were exposed to AgNPs had an effect on shoot proliferation and thereby improved the production of the number of shoots per explant (Sarmast et al. 2015; Saha and Gupta 2018). The addition of AgNPs in the culture medium also increased shoot formation and shoot fresh weight in *Lavandula angustifolia* (Jadczak et al. 2019). Ngan et al. (2020) reported that shoots of *Rosa hybrida* when cultured in MS medium supplemented with AgNPs (2 mg/L) showed high chlorophyll levels and shoot height increase. However, the addition of AgNPs was not completely favorable for shoot multiplication in plants. For example, the shoot growth in *Phalaenopsis amabilis* were significantly reduced when using AgNPs at high concentrations (Farrokhzad et al. 2022). A similar trend was observed in our results, the addition of 7 mg/L AgNPs in the culture medium reduced the number of shoots per explant and the total chlorophyll content in leaves. Overall, in this experiment, the positive effects of AgNPs were again demonstrated in shoot multiplication from the in vitro shoot tips of *P. edulis* Sim f. *edulis*.

Effects of AgNPs on in vitro flowering

In this study, the 1.5 cm shoot tips were cultured on MS medium supplemented with AgNPs to study the flowering induction. After 60 days of culture, shoot height in all treatments supplemented with AgNPs was significantly higher than that of the control. The highest shoot height (7.50 cm) was recorded at the concentration of 7 mg/L AgNPs treatment. Flowering was observed in shoots cultured in a medium supplemented from 3 to 9 mg/L AgNPs with flowering rates ranging from 11.67 to 51.67% after 60 days of culture. In which, shoots cultured in medium supplemented with 7 mg/L AgNPs gave the highest of flowering rate (51.67%) and the number of flower buds (2.33 buds/shoot) (Table 2, Fig. 4A). The results also showed the flower bloom rate was observed in the treatments supplemented with AgNPs (23.33–100%) after 70 days of shoot

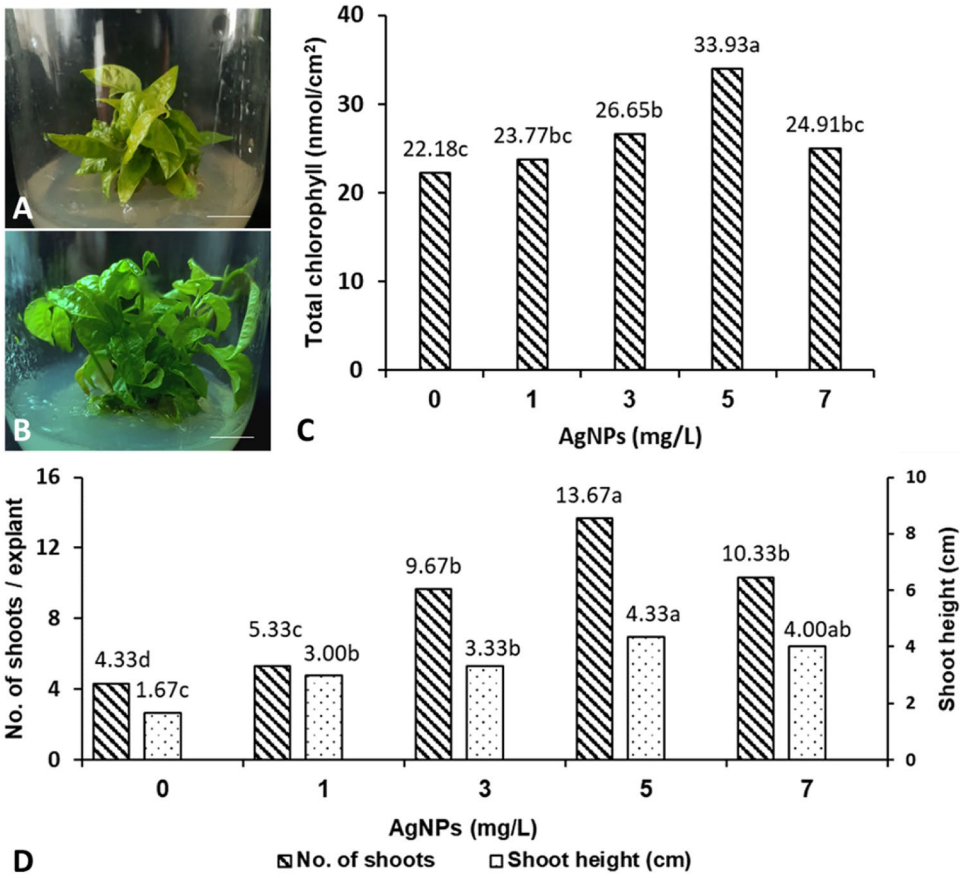

Fig. 3 Effect of AgNPs on the shoot multiplication efficiency in medium containing 1.0 mg/L mT after 60 days of culture. **A** Control treatment (bar 1 cm). **B** Treatment supplemented with 5 mg/L AgNPs (bar 1 cm). **C** Effect of AgNPs on total chlorophyll content of leaf. **D** Effect of AgNPs on number of shoots per explant and shoot height

Table 2 Effects of AgNPs on in vitro flowering after 60 days of culture

AgNPs (mg/L)	Shoot height (cm)	No. of leaves /shoot	Total chlorophyll (nmol/cm^2)	Flowering rate (%)	No. of flower buds/shoot
0	2.07^{e*}	6.67^{e}	27.12^{c}	0.00^{e}	0.00^{c}
1	3.50^{d}	10.67^{d}	28.40^{b}	0.00^{e}	0.00^{c}
3	7.23^{ab}	13.33^{b}	30.12^{a}	11.67^{d}	0.67^{bc}
5	6.27^{c}	10.67^{d}	25.68^{d}	23.33^{c}	1.00^{b}
7	7.50^{a}	12.00^{c}	24.73^{e}	51.67^{a}	2.33^{a}
9	6.77^{bc}	14.67^{a}	24.00^{f}	38.33^{b}	1.33^{b}

*Different letters (a, b,…) in the same column represent statistically significant differences at $p < 0.05$ (Tukey's test)

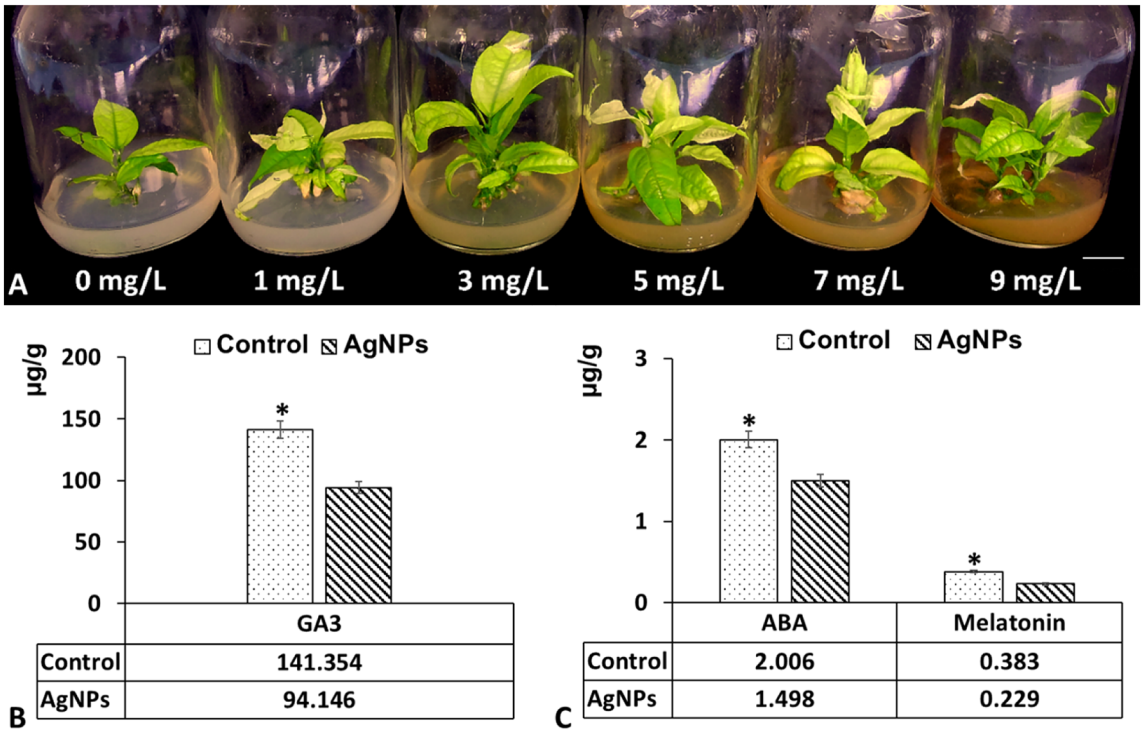

Fig. 4 Effect of AgNPs on in vitro flowering and endogenous hormone changes of shoot after 60 days of culture. **A** In vitro flowering in medium supplemented with AgNPs at different concentrations (bar 1 cm). **B** and **C** The contents of GA_3, ABA and melatonin of shoots cultured in medium supplemented with 7 mg/L AgNPs and the control. (*) Represent statistically significant differences based on a two-sample t-test

culture. The highest bloom rate was recorded in the treatment supplemented with 7 mg/L AgNPs (100%), however, when the concentration of AgNPs was increased to 9 mg/L, the bloom rate decreased sharply (63.33%). In addition, the largest flowers were also observed in the treatment supplemented with 7 mg/L AgNPs (Figs. 5 and 6A, B).

In addition, shoots cultured in medium supplemented with 7 mg/L AgNPs showed significantly lower levels of GA_3 and ABA than the control after 90 days of culture (Fig. 4B). Yan et al. (2019) also reported a significant decrease in GA_3 and ABA content of flowering plants compared with nonflowering plants of *Glycyrrhiza uralensis*, in addition, high levels of GA and ABA were also suggested to be proportional to the rate of flower and fruit drop. Furthermore, in this study, the melatonin content in shoots cultured in the treatment supplemented with 7 mg/L AgNPs (0.229 μg/g) was also significantly lower than in the control (0.383 μg/g) (Fig. 4C). In many plants, melatonin is primarily involved in stressful situations, but it is also involved in germination, plant growth, root formation, and as a protective agent that improves critical plant functions (Arnao and Hernández-Ruiz 2020). Melatonin content also has been reported to affect different stages of flower development (Shi et al. 2016; Arnao and Hernández-Ruiz 2020). For the flowering induction phase,

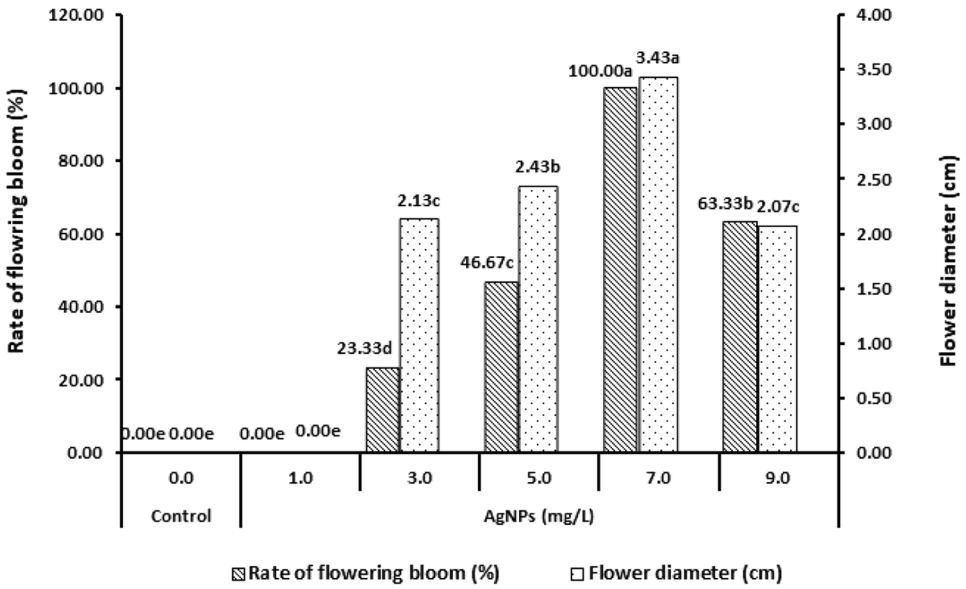

Fig. 5 Effect of AgNPs on in vitro flower bloom and flower diameter after 70 days of culture

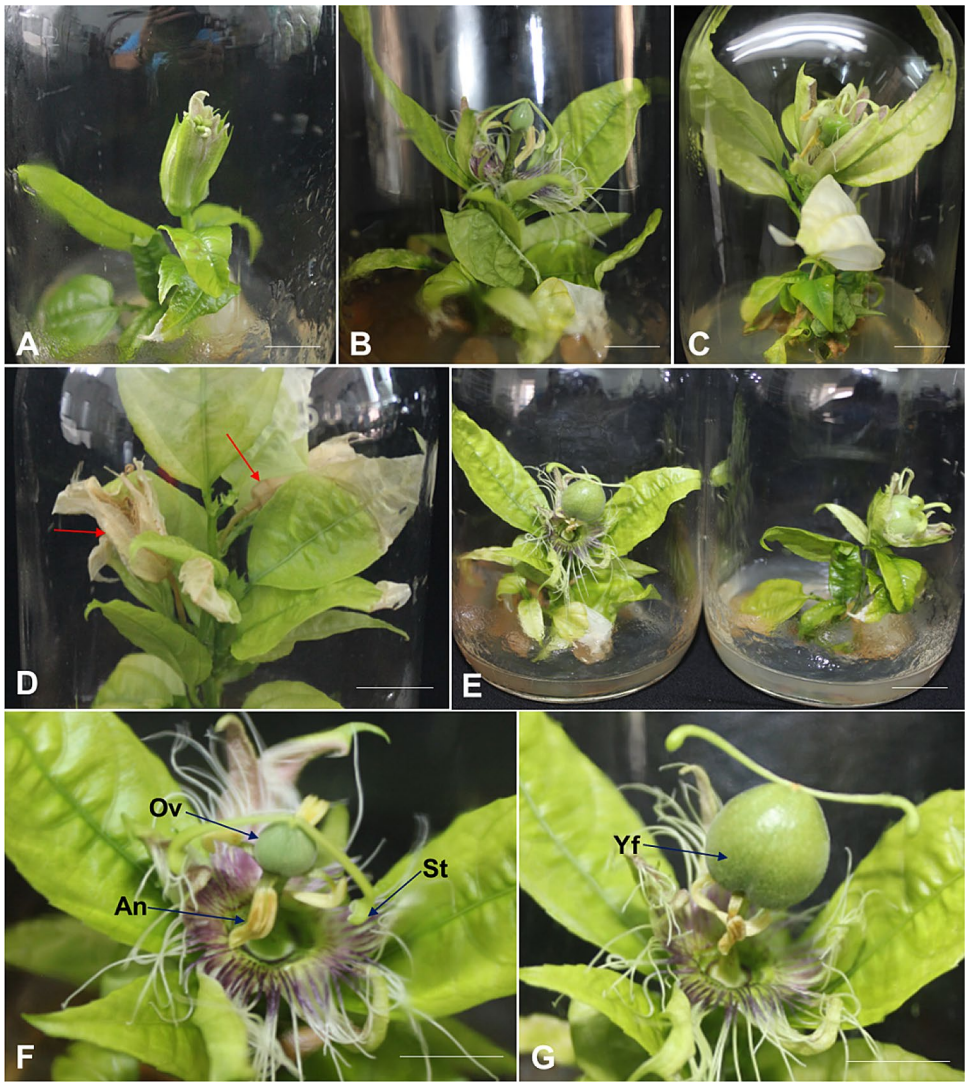

Fig. 6 The development stages of in vitro flower in medium containing 7 mg/L AgNPs. **A** In vitro flower bud after 60 days of culture. **B** and **F** Flower bloom after the next 70 days of culture. **C** Fruit setting after 10 days from the flower bloom. **D**. Flowers that do not produce fruit wilted after 10 days of blooming (red arrows indicate the location of the flowers). **E** and **G** Young fruit was formed after 90 days of shoot culture (bars 1 cm). Ovary (Ov), stigma (St), anther (An), young fruit (Yf)

high melatonin level was also reported to inhibit flowering in *Chenopodium rubrum* (Kolar et al. 2003), *Arabidopsis thaliana* (Arnao and Hernández-Ruiz 2020).

In vitro flowering can be a crucial tool for studying flowering and optimizing the commercial production of specific compounds from flower organs (Ammar et al. 2012; Zeng et al. 2013). It's also important for the recombination of hereditary material in fertilization between non-hybrid lines in selective hybridization. Under in vitro conditions, flower induction is season-independent, occurs year-round, and shortens the flowering time. In vitro flowering has been reported in several plant species such as *Cleome viscosa* (Rathore et al. 2013), *Scrophularia takesimensis* (Jeong and Sivanesan 2015), *Withania somnifera* (Sivanesan and Won 2015), *Ocimum basilicum* (Manan et al. 2016), *Exacum affine* (Sukthavornthum et al. 2018), *Dianthus chinensis* (Sreelekshmi and Siril 2021), *Portulaca pilosa* (Xiong et al. 2021), *Torenia fournieri* (Nhut et al. 2022), *Ananas bracteatus* (Liu et al. 2022). In our study, in vitro flowering of *P. edulis* Sim f. *edulis* was first recorded from shoot tips.

One of the most crucial stages in the lifecycle of plant is the transition from vegetative to reproductive (Amasino et al. 2017). This process is influenced by various factors including plant growth regulators, sucrose, light, temperature, and others acting as morphological genetic triggers for the transitions (Murthy et al. 2012; Manan et al. 2016). Several studies also showed that the silver element has positive effects on in vitro flowering in some plants such as *Cichorium intybus* (Bais et al. 2000), *Solanum nigrum* (Geetha et al. 2016), *Solanum americanum* and *Solanum villosum* (Haque et al. 2018), *Dianthus chinensis* (Sreelekshmi and Siril 2021). Silver in the type of ions or nanoparticles has been known to be an effective inhibitor of ethylene activity and involved in flower-inducing reactions (Bais et al. 2000; Sharma et al. 2008; Dar et al. 2021; Naing et al. 2021). In addition, blocking receptors involved in ethylene biosynthesis can lead to the polyamine synthesis due to the availability of S-adenosyl methionine (Rakesh et al. 2021); Therefore, the addition of the silver element can promote the synthesis of polyamine, an important factor in flowering in many plant species (Bais et al. 2000; Sreelekshmi and Siril 2021). In this study, the influence of AgNPs on ethylene activity and polyamine synthesis could be the important factors promoting the in vitro flowering of purple passion fruit (Fig. 7). In plant tissue culture, silver was commonly used as silver nitrates or silver thiosulphate, but in plants, silver thiosulphate is much more mobile than silver nitrates (Würschum 2015). In *Capsicum*, silver thiosulphate proved more effective than silver nitrate in enhancing in vitro flowering (Haque et al. 2016). On the other hand, AgNPs with sizes ranging from 1 to 100 nm with high mobility and exhibit unique physical, chemical and biological properties unlike their bulk counterparts (Mahajan et al. 2022) have been expected with high potential in the plant growth as well as the plant flowering processes. Ngan et al. (2019) reported on in vitro flowering of *Rosa hybridal* in medium supplemented with 5 ppm AgNPs. Our results also revealed culture medium added AgNPs positively affected the in vitro flowering of *P. edulis* Sim f. *edulis*.

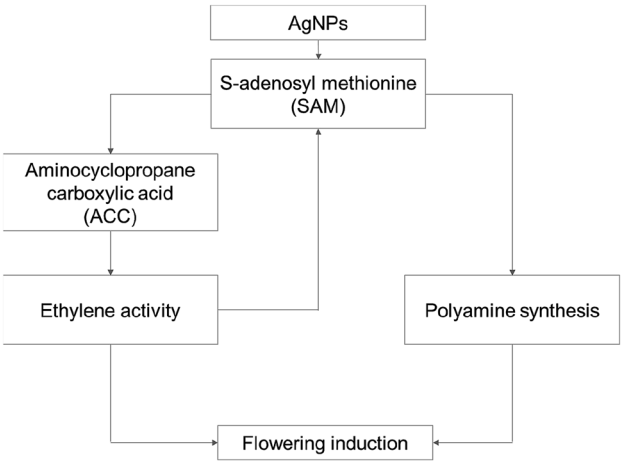

Fig. 7 The probable mechanism of action of AgNPs in promoting flowering induction

Table 3 Effects of AgNPs on in vitro fruiting after 90 days of culture

AgNPs (mg/L)	Fruiting rate (%)	No. of fruits/shoot	Diameter of young fruit (cm)
0	0.00^{d*}	0.00^{c}	0.00^{d}
1	0.00^{d}	0.00^{c}	0.00^{d}
3	20.00^{c}	1.00^{b}	0.07^{c}
5	36.33^{b}	1.00^{b}	0.77^{bc}
7	56.67^{a}	1.67^{a}	1.13^{a}
9	33.33^{b}	1.00^{b}	0.83^{bc}

*Different letters (a, b,…) in the same column represent statistically significant differences at $p < 0.05$ (Tukey's test)

Effects of AgNPs on in vitro fruiting

The results revealed that most of the in vitro flowers contained complete reproductive organs such as ovary, stigmas, and anthers; these in vitro flowers were capable of pollinating and forming young fruit after 90 days of culture (Fig. 6C, E, F, G). The flowers that did not bear fruit showed signs of wilting after 10 days of bloom (Fig. 6D). The fruiting rate in the treatments with AgNPs at the suitable concentrations was significantly higher than that in the control. In which, the treatment with 7 mg/L AgNPs gave the highest of fruiting rate (56.67%), number of fruits (1.67 fruits/plant) and fruit diameter (1.13 cm) (Table 3). However, when the

concentration of AgNPs increases to 9 mg/L, the fruiting rate tended to decrease (33.33%) (Table 3).

The present results showed the first young fruiting under in vitro conditions of *P. edulis* Sim f. *edulis*. Similarly, in vitro fruiting also has been observed in several plant species such as *Phyllanthus niruri* (Liang and Keng 2006), *Cleome viscosa* (Rathore et al. 2013), *Scrophularia takesimensis* (Jeong and Sivanesan 2015), *Withania somnifera* (Sivanesan and Won 2015), *Capsicum* spp. (Haque et al. 2016), *Solanum* (Haque et al. 2018). Several reports have shown that the accumulation of AgNPs in plants had long-term effects on some subsequent plant growth stages. For example, Cuong et al. (2021) reported that the addition of AgNPs not only increased in vitro rhizome formation but also improved the plantlet survival rate and saponin content in Ngoc Linh ginseng. AgNPs accumulation in the shoot multiplication stage also positively affected the rooting stage of strawberry plants (Tung et al. 2021). Our results indicated that AgNPs added to the culture medium not only promoted in vitro flowering stage but also positively influenced the fruiting of purple passion fruit. Pollination and fruiting in many plant species are highly dependent on the longevity of the flower (Amasino et al. 2017). However, flower longevity depends on many factors, especially ethylene level (Dar et al. 2021). Silver ion has been believed to inhibit ethylene production, thereby slowing down wilting of exogenous ethylene-sensitive in vitro flowers (Haque et al. 2016). Sreelekshmi and Siril (2021) reported an increase in vitro flower longevity in *Dianthus chinensis* cultured in medium supplemented with silver nitrate. The synergy between silver thiosulphate and BA was also reported to improve the in vitro fruit set in *Solanum americanum* and *Solanum villosum* (Haque et al. 2018). On the other hand, AgNPs have been reported to act as an effective inhibitor of ethylene formation in the micropropagation of many plant species (Saha and Gupta 2018; Ngan et al. 2020; Cuong et al. 2021; Tung et al. 2021), therefore, the addition of AgNPs in the culture medium probably influenced this process, thereby improving the in vitro fruiting.

In several plant species, such as *Ipomoea quamoclit* (Haque and Ghosh 2013), in vitro self-pollination was inhibited by lack of pollinators. In contrast, in vitro fruiting can occur without pollinators in *Withania* (Sivanandan et al. 2015). *P. edulis* Sim f. *edulis* is a self-fertile species, but the common genetic mechanism in passion fruit (self-incompatibility) can prevent this process under in vitro conditions (Madureira et al. 2014). In this study, the initiation of fruit development could be an expression of the fertilization that was performed. However, the setting seed, as well as the growth ability of seed, need to be studied further in the future. Despite this, the present works initially demonstrated in vitro fruiting in this plant species and evoked a new potential for future research to improve in vitro fruiting through artificial interventions, such as hand pollinations or in vitro flowering cultures together between varieties without barriers to fertilization.

Conclusion

In this study, AgNPs positively affected the regeneration and multiplication of shoots derived from ex-vitro internode-TCLs. However, adding of AgNPs to culture medium at high concentration can reduce shoot proliferation. In vitro flowering and fruiting of *P. edulis* Sim f. *edulis* were first recorded by using shoot tips cultured in the medium supplemented with AgNPs. In addition, the in vitro flowers were capable of pollinating and fruiting in vitro. The present results contribute to the study of flowering and fruiting mechanisms as well as to improving the breeding of passion fruit in the future.

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Author contributions DTN and THP designed the study. THP conducted experiments. TH, HTT, NTNM, HDK, DMC, VQL, and NBN participated in interpretation of data and revision for intellectual content acquired data wrote the manuscript.

Declarations

Conflict of interest The authors have no conflict of interest.

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