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ORIGINAL ARTICLE

Silver nanoparticles improved explant disinfection, in vitro growth, runner formation and limited ethylene accumulation during micropropagation of strawberry (*Fragaria* × *ananassa*)

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Abstract

One of the common problems in strawberry (*Fragaria* × *ananassa*) micropropagation is the vitrification phenomenon (succulent plantlets, brittle stems, yellow leaves, etc.) leading to the reduction of plantlets quality and low survival rate in the greenhouse. In this study, the effects of silver nanoparticles (AgNPs) on explant disinfection, in vitro growth (shoot multiplication, and root formation), runner formation as well as ethylene accumulation during micropropagation of strawberry were investigated. The results showed that leaf explants treated with 200 mg/L AgNPs solution for 20 min was more effective in explant disinfection and shoot regeneration than using 1 g/L $HgCl_2$. In addition, AgNPs stimulated the growth of shoot and plantlet and as well shortened the duration of root formation (4 days) as compared to those in control without AgNPs during micropropagation. Besides, AgNPs reduced the ethylene gas accumulation in the culture's vessels of shoots (0.66 ppm) and plants (0.06 ppm) compared to controls (1.77 ppm; 0.15 ppm; respectively). Moreover, AgNPs combination with culture period (5; 10 or 15 days) effect root formation stage and acclimatization in the greenhouse. The plantlets that cultured on MS medium supplemethed with 0.5 mg/L AgNPs during 10 days showed higher survival rate (93.33%) after 15 days as well as runner formation per plant (8.00 runners) after 60 days in greenhouse than those in control.

Key message

AgNPs improved explant disinfection and in vitro growth. AgNPs improved runner formation in the greenhouse. AgNPs limited ethylene accumulation during micropropagation.

Keywords Ethylene accumulation · *Fragaria* × *ananassa* · Runner · Silver nanoparticles · Vitrification phenomenon

Abbreviations

AgNPs Silver nanoparticles
MS Murashige and Skoog medium (1962)

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Introduction

Micropropagation of strawberry (*Fragaria* × *ananassa*) is the appropriate method to produce a large number of homogeneous virus-free plants via meristem culture (Mir et al. 2010; Mozafari and Gerdakaneh 2012). In addition, stem nodes (Harugade et al. 2014), seeds (Mahmoud and Kosar 2014a, b) and leaves (Palei et al. 2017), etc. were also used as primary materials in the micropropagation of strawberry plants. Up to now, there are many studies on strawberry micropropagation focused on plant materials (Jan et al. 2013), disinfectants (Abbas et al. 2017; Oo et al. 2018), plant growth regulators on callogenesis (Palei et al. 2017; Abbas et al. 2017; Na et al. 2019), shoot regeneration (Palei et al. 2017) and root formation (Nam et al. 2016; Palei et al. 2017; Wafaa and Wahdan 2017).

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On the strawberry plants, mecury chlorite ($HgCl_2$) (Jan et al. 2013; Oo et al. 2018), hydroperoxide (H_2O_2) (Munir et al. 2015), hypochlorite calcium ($Ca(ClO)_2$) (Jan et al. 2013; Abbas et al. 2017) were used to disinfect explants from microbial contamination, but the efficiency was not optimal and safe (Mihaljević et al. 2013) Therefore, finding a new disinfectant that is effective and safe is essential. In addition, strawberry shoots or plants grown in closed culture flasks with high humidity, low light, no supplemental CO_2, high sucrose and nutrient medium induce some abnormal phenomena (vitreous, yellowing, deciduous, browning, etc.) (Hdider and Desjardins 1993; Veilleux and Johnson 1998; Mir et al. 2019). One of the common problems in micropropagated strawberry is the vitrification phenomenon (succulent plantlets, brittle stems, yellow leaves, etc.) leading to reduced plantlets quality and survival rate in the greenhouse, because of the production and accumulation of ethylene gas in closed vessel cultures during growth and development (Kevers and Gaspar 1985; Nehra et al. 1992; Hdider and Desjardins 1993; Veilleux and Johnson 1998; Palei et al. 2015). In order to overcome disadvantages, such as increase growth and development to improve plantlets quality, some studies used aeration vessels or added activated charcoal to the culture medium (Hdider and Desjardins 1993; Mir et al. 2019); however, the problems have not been completely solved.

In recent studies, AgNPs have been used to disinfect explants of seaweed *Kappaphycus striatus* (Mo et al. 2020), reduce microbial infection in microponic medium (Tung et al. 2018); overcome some abnormal phenomena and increase in vitro growth of *Stevia rebaudiana* Bertoni (Ramírez-Mosqueda et al. 2019), *Pennisetum alopecuroides* (Parzymies et al. 2019), lilies (Salachna et al. 2019), banana (El-Mahdy et al. 2019), *Caralluma tuberculata* (Ali et al. 2019), *Rosa hybrida* L. 'Baby Love' (Ngan et al. 2020).

In this study we used AgNPs as an explant disinfectant to replace $HgCl_2$, and show improvement in the the in vitro growth of shoots and plants, as well as assess the variation of ethylene gas content in culture flasks. In addition, this study also showed the optimal culture period combined with AgNPs supplementation in the culture medium on rooting stages to the acclimatization, growth and runner formation in greenhouse condition.

Materials and methods

Plant material

Three-month-old ex vitro leaves of *Fragaria* × *ananassa* plants that were grown in the greenhouse of Tay Nguyen Institute of Scientific Research (Dalat, Vietnam), were used in the study.

AgNPs solution preparation

Aqueous solution was prepared with AgNPs of average size less than 20 nm consists of silver ion source ($AgNO_3$), stabilizator (β-chitozan), and reducing agent ($NaBH_4$). The AgNPs solution is set according to the ratio: $AgNO_3$ less than 1000 ppm, 250–300 ppm β-chitozan, 200 ppm $NaBH_4$, the ratio mol $[NaBH_4]/[AgNO_3] = ¼$ and the dripping rate of $NaBH_4$ is 10–12 drops/min provided by the Institute of Environmental Technology (Hanoi, Vietnam) (Chau et al. 2008).

Culture conditions

In vitro condition: The explants were cultured at 25 ± 2 °C with a 16 h photoperiod under fluorescent light (40–45 µmol m^{-2} s^{-1}) and humidity of 55–60%.

Ex vitro condition: The plantlets were grown in greenhouse at 25 ± 2 °C during the day and 15 ± 2 °C at night, the average humidity of about 75–80% and natural light with shade 40%.

Effects of AgNPs on explant disinfection and shoot regeneration

Three-month-old ex vitro leaves were washed under running tap water, then soaked in 70% alcohol for 30 s, washed three times with sterile distilled water, and disinfected with AgNPs solution (50; 100; 200; 500 mg/L). The exposure times were 5; 10; 15; 20; 30 min. The control treatments were sterilized with 1 g/L $HgCl_2$ for 4 min (Oo et al. 2018). These disinfected leaf explants were cut into circles (1 cm in diameter) and transplanted on Murashige and Skoog (1962) (MS) medium supplemented with 1 mg/L TDZ and 0.1 mg/L IBA, 30 g/L sucrose, and 8 g/L agar (Sutter et al. 1997) and assessment of shoot regeneration after 45 days of culture.

Effects of AgNPs on shoot multiplication

In vitro shoots derived from regenerated shoots (2.0 cm in height and three leaves) were cultured on MS medium supplemented with 0.5 mg/L benzyladenine (BA) (Danial et al. 2016) and AgNPs (0.2; 0.4; 0.6; 0.8; 1.0 mg/L). Control was the medium without AgNPs. The effects of AgNPs on shoot multiplication were obtained after 30 days of culture.

Effects of AgNPs on root formation

In vitro shoots with 3.0 cm in height and four leaves were cultured on root formation medium including MS medium supplemented with 0.02 mg/L α-naphthalene acetic acid, 30 g/L sucrose, 1 g/L activated charcoal, 8 g/L agar (Haddadi

et al. 2010), and AgNPs (0.25; 0.5; 1.0; 1.0; 1.5; 2.0 mg/L). Control was the medium without AgNPs. The root formation was recorded after 15 days of culture.

Ethylene accumulation content in culture vessels during shoot multiplication and root formation stages

Culture vessels of 15-day-old shoots and 30-day-old plantls derived from with/without AgNPs were used to measure the ethylene gas concentration by gas chromatography with flame ionization probes (Cristescu et al. 2012). Gas chromatography was performed using the Varian CP-3380 chromatograph (Walnut Creek, CA, USA).

AgNPs combination with culture period on root formation and acclimatization

Plantlets derived from optimal AgNPs and control treatment after 5; 10 or 15 days of culture were collected, washed, planted in blisters, and placed under greenhouse conditions. Plantlets are watered twice per day in the 1st week after planting, then watered once per day in the early morning, to avoid excessive stagnant water. Survival rate and growth indicators were obtained to assess the quality of plantlet derived from AgNPs treatments.

Statistical analysis

Each treatment was repeated three times with ten vessels/treatment (three explants/vessel). Data were processed and analyzed by Microsoft Excel 2010 and SPSS 16.0 software according to Duncan's and LSD's tests with $\alpha = 0.05$ (Duncan 1955).

Results and discussion

Effects of AgNPs and $HgCl_2$ on explant disinfection

The results showed that the ability of AgNPs to explant disinfection and shoot regeneration at different concentrations and treatment times differed from that of $HgCl_2$ treatment after 45 days of culture (Table 1).

The leaves disinfected with 50 mg/L AgNPs showed 100% contamination rate only after 5 days of culture. However, all explants treated for a short time (5 min) were contaminated; and the leaves became necrotic at a longer

Table 1 Effect of AgNPs and $HgCl_2$ on explant disinfection after 45 days of culture

Disinfection agent	Concentration (mg/L)	Exposure time (min)	Contamination rate (%)	Shoot regeneration rate (%)	No. of shoots		Characteristic of explant
					Total	> 1.5 cm in height	
AgNPs	50	5	100.00a*	–	–	–	Contamination
		10	100.00a	–	–	–	
		15	100.00a	–	–	–	
		20	100.00a	–	–	–	
		30	100.00a	–	–	–	
	100	5	100.00a	–	–	–	Contamination
		10	73.33e	22.22d	10.00e	0.00c	Small shoot (< 0.5 cm)
		15	63.33de	30.00cd	12.33de	0.00c	
		20	60.00de	33.33cd	11.33de	0.00c	
		30	–	–	–	–	Necrosis
	200	5	100.00f	–	–	–	Contamination
		10	60.00de	34.44cd	10.67de	0.00c	Large shoot
		15	37.78bc	56.67ab	15.67bc	4.00b	
		20	28.89ab	64.44a	21.00a	6.67a	
		30	–	–	–	–	Necrosis
	500	5	100.00f	–	–	–	Contamination
		10	48.88cd	45.55ab	13.00cd	0.00c	Large shoot
		15	36.67abc	61.11ab	13.33cd	4.00b	
		20	22.22a	57.78ab	19.00a	4.67b	
		30	–	–	–	–	Necrosis
$HgCl_2$	1000	4	49.99cd	46.67abc	16.33b	0.00c	Small shoot

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

time (30 min) (Table 1). The results suggested low concentration of AgNPs (50 mg/L) or a short-time (5 min) and long-time (30 min) are not affective in the sterilization process. Increasing exposure time and concentration of AgNPs significantly reduced the contamination rate, the lowest rate was obtained at 500 mg/L AgNPs in 20 min (22.22%), followed by 200 mg/L AgNPs in 20 min (28.29%). The contaminations were lower than those treated with $HgCl_2$ (49.99%).

In addition, leaf samples disinfected with 200 mg/L AgNPs for 20 min yielded higher biomass of callus induction than $HgCl_2$ treated after 21 days of culture (data not shown). In addition, the shoot regeneration rate (64.44%), the number of shoots/explant (21 shoots), the number of shoots with a size larger than 1.5 cm (6.67 shoots) observed in treatment with 200 mg/L AgNPs for 20 min after 45 days of culture were higher compared to other treatments and controls (Table 1).

$HgCl_2$ and $Ca(ClO)_2$ are commonly used to disinfect explants and their effectiveness depends on the explant source (stem, petiole, stem, shoot-tip), exposure time treatment, and plant species (Oo et al. 2018; Bharti et al. 2018; Shukla et al. 2019); however, the disinfection efficiency and survival rate are low (less than 75%) because they have strong detergent and antimicrobial agent on fungal and bacterial cell walls resulting in low shoot regeneration (Mihaljević et al. 2013).

These results show that changes in AgNPs concentration (50; 100; 200; 500 mg/L) as well as exposure time (5–30 min) significantly affect the sterilization and regeneration of shoots. AgNPs used as disinfectant can easily move and penetrate through the cell layers and accumulate inside the cells of the explants via phloem, xylem (Kim et al. 2007; Navarro et al. 2008) and is metabolized and used to support metabolic processes within the cell, thereby having a positive impact on plant growth (Navarro et al. 2008). The results recorded in this study demonstrate that AgNPs are potential disinfectants and can replace common disinfectants.

Effect of AgNPs on shoot multiplication

Shoot multiplication efficiency increased proportional to the increase of AgNPs (0–0.2 mg/L) and reached the highest value in 0.2 mg/L AgNPs treatment such as number of shoots (12.67), shoot height (3.93 cm), shoots higher than 2.0 cm in height (8.67 shoots), dry weight (89.42 mg) and total chlorophyll (37.3 nmol/cm^2) (Table 2). However, shoot regeneration efficiency was decreased at AgNPs concentrations higher than 0.2 mg/L, meanwhile, the number of roots, root length, leaf length and leaf width were increased (Fig. 1a). In addition, the dry matter rate (4.95–7.81%) increased proportional to the increase in AgNPs (0–0.6 mg/L), this result proved that AgNPs increased dry matter rate and reduced water accumulation in shoots. Sharma et al. (2012) showed in their studies on *Brassica juncea* cultured on agar medium supplemented with 50 mg/L AgNPs increased the fresh weight, root length, shoot height, chlorophyll content of seedlings compared to control, but at high AgNPs concentration the growth was reduced. The effect of AgNPs at low concentrations (1–5 mg/L) in increasing shoot multiplication on Bananas was also recorded (El-Mahdy et al. 2019). Moreover, Ngan et al. (2020) also showed that AgNPs inhibited the activity of ethylene, increased the dry matter accumulation, thereby reducing the vitrification phenomenon in rose micropropagation.

Effect of AgNPs on root formation

The rooting rate reached 100% in treatments with/without AgNPs; however, rooting ability was earlier nearly 4 days at 0.5 mg/L AgNPs as compared to control without AgNPs (Fig. 2). Harugade et al. (2014) indicated strawberry root formation on medium supplemented with plant growth regulators after 7 days of culture. However, the results of

Table 2 Effect of AgNPs on the shoot multiplication after 30 days of culture

AgNPs (mg/L)	No. of shoots		Shoot height (cm)	Fresh weight (g)	Dry weight (mg)	Dry matter rate (%)	Leaf length (cm)	Leaf width (cm)	Total chlorophyll (nmol/cm^2)**
	Total	> 2 cm in height							
0	5.67d*	3.67de	3.43c	1315.40a	65.16b	4.95b	0.53d	0.47c	34.03c
0.05	8.67c	4.33cd	3.53bc	1193.17bc	83.00a	6.96a	0.53d	0.53c	35.43abc
0.10	10.33b	6.00bc	3.70b	1235.97ab	86.13a	6.97a	0.67cd	0.70b	36.80ab
0.20	12.67a	8.67a	3.93a	1260.87ab	89.42a	7.09a	0.77c	0.73b	37.30a
0.40	9.67bc	6.33b	3.63b	1130.43c	84.76a	7.05a	0.97b	0.77b	34.87bc
0.60	2.33e	2.00e	3.36c	408.10d	31.86c	7.81a	1.17a	0.93a	31.47d

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

**Measured by chlorophyll meter (SPAD-502, Minolta Co., Ltd., Osaka, Japan)

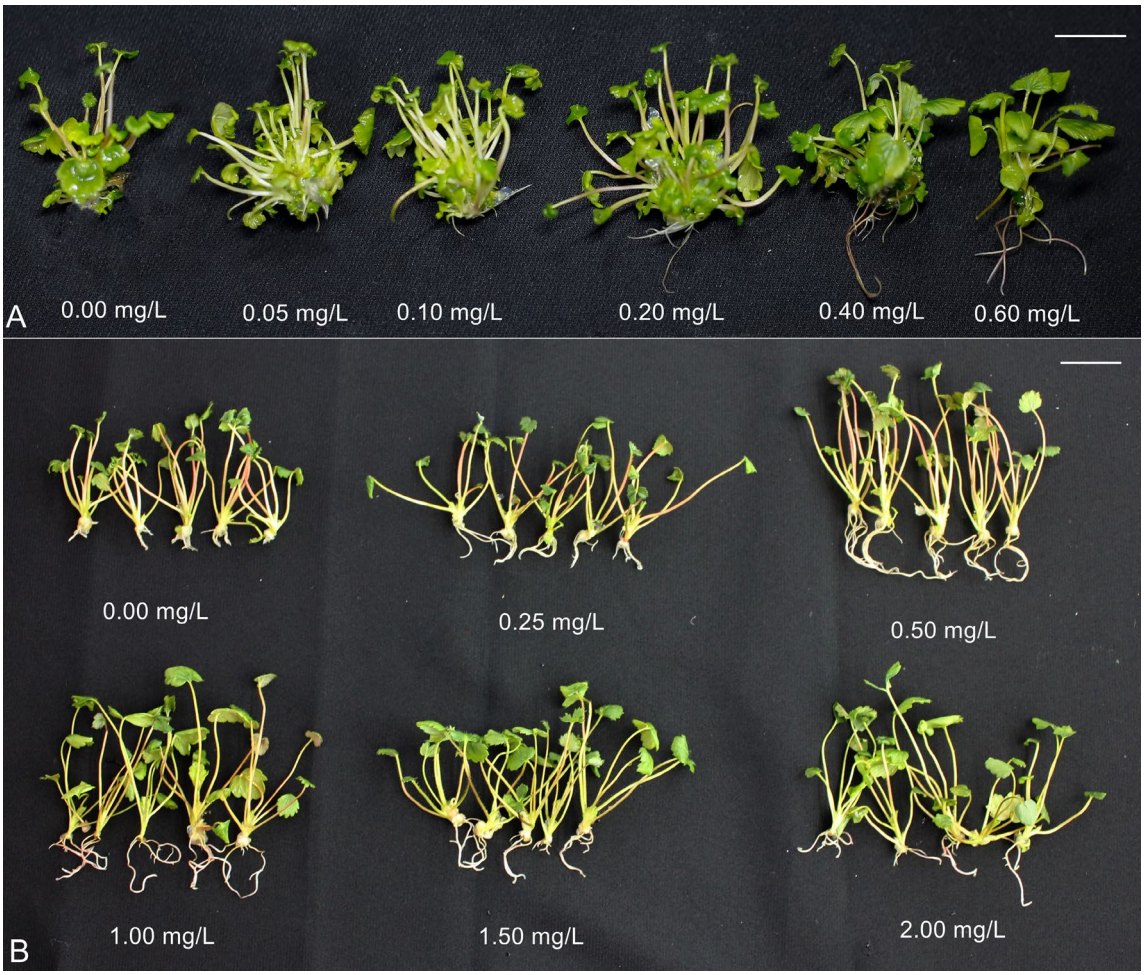

Fig. 1 Effect of AgNPs on the shoot multiplication stage (30-day-old) and rooting stage (15-day-old). *Bar*: 2 cm

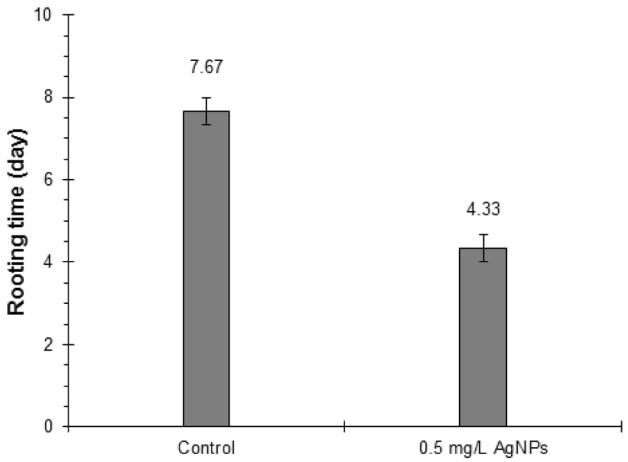

Fig. 2 AgNPs and control treatment on rooting time

our study showed that adding AgNPs to the culture medium shortened the root induction time (4.33 days) as compared to those in control without AgNPs (7.67 days), and the growth of 10-day-old plants derived from 0.5 mg/L AgNPs was similar to 15-day-old plant in the control. The results of this study show that it is possible to shorten the rooting time. This lead to saving eectricity, the costs of maintaining the plants, shortening the propagation time. Hence saving production costs and increasing production efficiency.

In the control treatment, the plant growth was lower than that of treatments supplemented with AgNPs (Fig. 1b, Table 3). Plant growth increased in proportion to the increase in AgNPs concentrations (0–0.5 mg/L); reached the highest 0.5 mg/L AgNPs treatment. However, plan growth slowed at higher than 0.5 mg/L AgNPs, (except for root length) (Table 3). Mahmoud and Kosar (2014a, b) reported that strawberry shoots cultured on MS medium supplemented with 4 mg/L $AgNO_3$ (2.56 mg/L Ag^+) had rooting rate (100%), plant height (5.3 cm) and number of roots (1.5 roots) reached the highest. This may be probably

Table 3 Effect of AgNPs on the rooting stage after 15 days of culture

AgNPs (mg/L)	Rooting rate (%)	Plant height (cm)	Fresh weight (mg)	Dry weight (mg)	No. of roots	Root length (cm)	No. of leaves	Total chlorophyll (nmol/cm^2)
0	100	4.60d	157.67d	14.00d	4.67b	0.50f	6.00b	31.53d
0.25	100	4.70cd	185.67c	27.00c	5.67ab	1.33e	6.67ab	33.47c
0.50	100	5.60a	242.67a	34.67a	6.67a	3.40b	7.67a	39.30a
1.00	100	5.67a	212.67b	31.33b	5.67ab	3.67a	6.67ab	36.63b
1.50	100	5.27b	197.00c	27.00c	4.67b	2.43c	6.67ab	32.60c
2.00	100	4.93c	161.33d	25.67c	2.33c	2.23d	7.00ab	31.50d

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

that Ag^+ may not be directly involved in plant growth but may have indirect effect through ethylene. However, AgNPs (1–100 nm) have shown to be more effective and active than ions (Ag^+) (Yin et al. 2011), so AgNPs are being applied in many fields of science and technology. In micropropagation, AgNPs may be reduced infection of fungi, bacteria, etc. (Arab et al. 2014; Spinoso-Castillo et al. 2017) and hence improved seedling growth, plant development and quality (Sarmast et al. 2015; Thao et al. 2015). In this study, the AgNPs concentration for rooting stage was only 0.5 mg/L AgNPs, which gave optimal results (lower than $AgNO_3$). Thus, the use of AgNPs has been effective in rooting and plant growth as well as earlier root induction times.

In the 2.0 mg/L AgNPs treatment, the results showed that the root morphology (almost browning) was different from the other treatments with lower AgNPs concentrations. This might be because the high AgNPs concentration may reduce the ability to absorb nutrients and water of the root system, resulting in slower plant growth than other treatments. Syu et al. (2014) showed that high AgNPs concentration causes oxidative stress that leads to an increase levels of ROS in the roots.

The ethylene gas accumulation in culture vessels at shoot multiplication and root formation stages

The ethylene content significantly decreased when AgNPs were added to the culture medium and was always lower than that of the control treatment during shoot multiplication after 30 days of culture (Fig. 3a). In the 0.2 mg/L AgNPs treatment, the ethylene gas accumulation in the culture vessel was lowest (0.66 ppm) and 2.68 times lower than the control (1.77 ppm).

Sarropoulou and Maloupa (2016) suggested that the addition of ethylene inhibitors ($CoCl_2$) to culture media increased the shoot formation of *Sideritis raeseri*, meanwhile, adding 2 mg/L $AgNO_3$ increased cotton shoot multiplication (22.2 shoots) after 3 weeks of culture (Kumar et al. 2016).

During the rooting stage, the ethylene gas accumulation in culture vessels is inversely proportional to the concentration of AgNPs added to the culture medium from 0 to 0.5 mg/L. Ethylene gas accumulation (0.06 ppm) at 0.5 mg/L AgNPs treatment was lower than those in others and control treatments (0.15 ppm) (Fig. 3b). In addition, the plant growth and development in this treatment was higher (Table 3 and Fig. 1). A high AgNPs concentrations (1.0–2.0 mg/L)

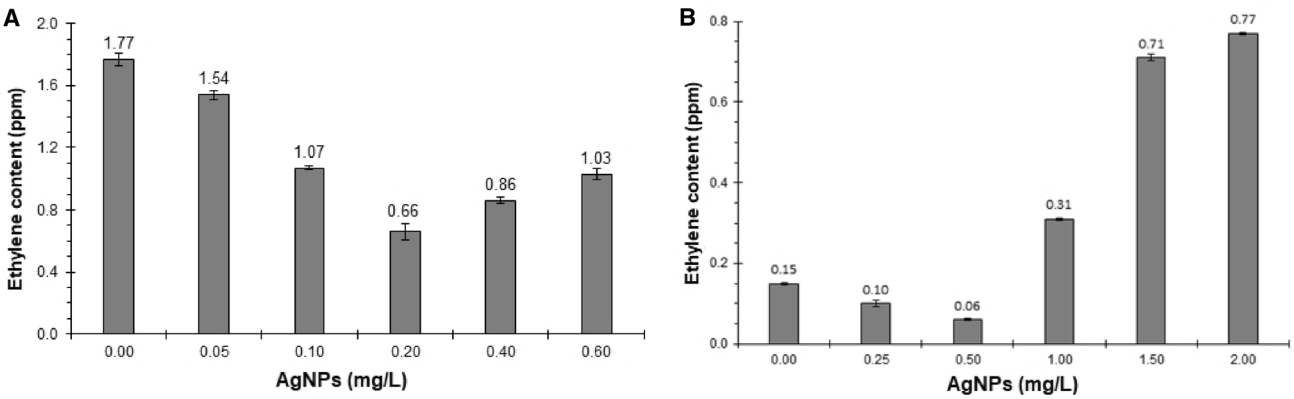

Fig. 3 Ethylene content in culture vessel's of 30-day-old shoots and 15-day-old plants. **a** 30-day-old shoots. **b** 15-day-old plants

increased the ethylene gas accumulation in culture vessels and inhibited plant growth. The plants showed yellowing and curly leaves. Plant height, root length and the number of roots decreased.

The result of this study showed the role of AgNPs in inhibiting the formation and activity of ethylene gas. AgNPs have the ability to inhibit the activity of ethylene gas by blocking the binding of ethylene to receptors in plant cells; which prevent the formation and activity of ethylene gas (Razavizadeh and Rostami 2015). Previous studies showed that the accumulation of ethylene gas had been significantly reduced by using $AgNO_3$, Ag_2SO_4 as inhibitors in micropropagation of cherry (Sarropoulou and Maloupa 2016), cotton (Kumar et al. 2016). In addition, aminoethoxy vinyl glycine, silver thiosulfate, and sodium nitroprusside have been used for stimulation of shoot formation and inhibiting ethylene effect on yellowing of leaves in roses (Park et al. 2016). The use of AgNPs to limit the adverse effects of ethylene on plants is still limited, only reported for *Swertia chirata* (Saha and Gupta 2018), rose (Ngan et al. 2020).

AgNPs combination with culture period on root formation and acclimatization

In the control treatment, plant height, fresh weight, dry weight, number of roots, root length, number of leaves, and total chlorophyll also increased significantly over time (5–15 days). However, this increase was low in the 0.5 mg/L AgNPs supplemented treatment at the same time (Table 4). In 0.5 mg/L AgNPs treatment, fresh weigth, dry weigth, number of roots, root length outperformed the control and about twofold to over fourfold after 5, 10 and 15 days of culture (Table 4).

After 15 days cultivating in the greenhouse, the results showed that the survival rate (86.67–93.33%) in 0.5 mg/L AgNPs treatment was higher than those in control (26.67–73.33%) (Table 5 and Fig. 4a, c, e).

Corresponding to in vitro growth, plantlets derived from 0.5 mg/L AgNPs had better growth and development than those in control treatments after 30 days in the greenhouse (Fig. 4b, d, f). Plant height, fresh weight, number of roots, root length, number of leaves, and total chlorophyll in 0.5 mg/L AgNPs treatment were significantly higher than compared to the controls.

Results of this study also showed that AgNPs affected rooting ability and the development of runners in the

Table 4 The combination of AgNPs and culture period on plant growth at rooting stage

Culture period	AgNPs	Plant height (cm)	Fresh weight (mg)	Dry weight (mg)	No. of roots	Root length (cm)	No. of leaves	Total chlorophyll (nmol/cm^2)
5	0	3.83d*	87.33d	8.40f	0.33d	0.04e	4.67 c	35.00c
	0.5	4.57b	146.67c	18.67c	4.33bc	0.80c	5.00c	41.40a
10	0	4.07c	94.33d	12.00e	3.67c	0.47d	5.33bc	35.73c
	0.5	4.70b	161.33b	21.00b	5.33b	2.37b	6.00b	42.60a
15	0	4.60b	158.33b	14.00d	4.67bc	0.53d	6.00b	31.87d
	0.5	5.63a	242.67a	34.67a	6.67a	3.53a	7.67a	39.13b

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

Table 5 The combination of AgNPs and culture period on rooting stage after 15 days in the greenhouse

Culture period	AgNPs	Plant height (cm)	Survival rate (%)	Fresh weight (mg)	No. of roots	Root length (cm)	No. of leaves	Total chlorophyll (nmol/cm^2)
5	0	4.40e*	26.67c	165.00e	4.67b	4.27d	5.00c	30.87d
	0.5	4.97c	86.67a	323.33c	8.33a	6.40b	7.33b	37.53bc
10	0	4.83d	60.00bc	201.67d	5.33b	5.50c	6.00c	36.03c
	0.5	6.67a	93.33a	433.33a	8.67a	9.07a	7.67ab	38.77b
15	0	4.87cd	73.33b	201.67d	5.33b	4.37d	5.33c	36.67c
	0.5	6.37b	90.00a	376.67b	9.33a	8.73a	8.67a	41.90a

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

Fig. 4 The growth of planlet derived from combination of AgNPs and culture period in the greenhouse. *Bar*: 5 cm. **a** Plantlets derived from 5 day-rooting after 15 days at the greenhouse. **b** Plantlets derived from 5 day-rooting after 30 days at the greenhouse. **c** Plantlets derived from 10 day-rooting after 15 days at the greenhouse. **d** Plantlets derived from 10 day-rooting after 30 days at the greenhouse. **e** Plantlets derived from 15 day-rooting after 15 days at the greenhouse. **f** Plantlets derived from 15 day-rooting after 30 days at the greenhouse. **0**: Control. **1** 0.5 mg/L AgNPs

Table 6 The growth of runner derived from AgNPs and control after 60 days in the greenhouse

Culture period	AgNPs (mg/L)	Runner time (day)	Runner rate (%)	No. of runner per plant	Runner			
					Runner height (cm)	Fresh weight (g)	No. of leaves	Total chlorophyll (nmol/cm^2)
5	0	45.67a	40.00d	1.33c	5.36c	2.75c	1.67b	35.13c
	0.5	41.67b	78.33c	5.33b	7.98b	3.04bc	2.67ab	38.73b
10	0	41.33b	74.00c	4.67b	7.91b	3.09b	3.00ab	38.07b
	0.5	36.67d	100.00a	8.00a	11.21a	3.55a	3.67a	40.37a
15	0	39.67c	88.33b	4.33b	8.15b	2.82bc	3.00ab	37.53b
	0.5	38.67c	100.00a	8.33a	8.59b	2.90bc	3.67a	40.63a

*Different letters (a, b,…) in the same column represent statistically significant differences at $\alpha = 0.05$ (Duncan's test)

Fig. 5 The growth of runner derived from AgNPs and control after 60 days in the greenhouse. *Bar*: 5 cm. **a** 5 day-rooting (control). **b** 10 day-rooting (control). **c** 15 day-rooting (control). **d** 5 day-rooting (0.5 mg/L AgNPs). **e** 10 day-rooting (0.5 mg/L AgNPs). **f** 15 day-rooting (0.5 mg/L AgNPs)

greenhouse. We observed earliest runner development time (36.67 days) and rooting time (10 days) in the 0.5 mg/L AgNPs treatment. In the control treatments runners formed late (45.67 days) and rooting was early (5-days) (Table 6). Besides, the rooting rate (100%), number of runner (8.00 and 8.33 runners), number of leaves per runner (3.67 leaves), total chlorophyll (40.37 and 40.63 nmol/cm^2) at 0.5 mg/L AgNPs (derived from 10-day and 15-day rooting) were higher than those in others (Table 6 and Fig. 5). Moreover, the runner height (11.21 cm) and runner fresh weight (3.55 g) derived from 0.5 mg/L AgNPs (10-day rooting) were the highest compared to others. From the above results, in vitro strawberry plantlets derived from 0.5 mg/L AgNPs (10-day rooting) showed good growth and development under in vitro and ex vitro conditions as well as the ability to runner formation. Furthermore, runners derived from 0.5 mg/L AgNPs treatment showed good growth and development as well as the ability to flower and fruit after 60 days of planting. Flowers and fruits did not have any deformations and they were similar to those obtained from control treatment.

Conclusion

This study showed that the addition of AgNPs in culture medium significantly improved, explant disinfection, shoot multiplication, plantlets quality as well as runner formation of strawberry in the greenhouse. In addition, AgNPs was effective in reducing ethylene gas accumulation during shoot multiplication and rooting stages.

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Author contributions HTT acquired data wrote the manuscript. TTT, DMC, VQL, VTH, TH, NBN, HTNP, BVTV participated in interpretation of data and revision for intellectual content. DTN and HTT conceptualized and designed the study. All authors discussed the results and contributed to the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Abbas KH, Al-Sallihy AAM, Inrahim KM (2017) Contamination free callus cultures in strawberry (*Fragaria* × *ananassa* Dutch cv.). Int J Chemtech Res 10(9):633–640

Ali A, Mohammad S, Khan MA, Raja NI, Arif M, Kamil A, Mashwani ZUR (2019) Silver nanoparticles elicited in vitro callus cultures for accumulation of biomass and secondary metabolites in *Caralluma tuberculata*. Artif Cells Nanomed Biotechnol 47(1):715–724. https://doi.org/10.1080/21691401.2019.1577884

Arab MM, Yadollahi A, Hosseini-Mazinani M, Bagheri S (2014) Effects of antimicrobial activity of silver nanoparticles on in vitro establishment of G×N15 (hybrid of almond×peach) rootstock. Genet Eng Biotechnol J 12(2):103–110. https://doi.org/10.1016/j.jgeb.2014.10.002

Bharti N, Kapoor B, Shaunak I, Sharma P, Sharma R (2018) Effect of sterilization treatments on in vitro culture establishment of tomato (Solanum lycopersicum L. Int J Chem Stud 6(5):1165–1168

Chau NH, Bang LA, Buu NQ, Dung TTN, Ha HT, Quang DV (2008) Some results in manufacturing of nanosilver and investigation of its application for disinfection. Adv Nat Sci: Nanosci Nanotechnol 9(2):241–248

Cristescu SM, Mandon J, Arslanov D, De Pessemier J, Hermans C, Harren FJ (2012) Current methods for detecting ethylene in plants. Ann Bot 111(3):347–360. https://doi.org/10.1093/aob/mcs259

Danial GH, Ibrahim DA, Omer MS (2016) Response of running shoot tips of strawberry (*Fragaria* × *ananasa*) for in vitro propagation in Kurdistan region of Iraq. Int J Environ Agric Biotechnol 1(2):164–169. https://doi.org/10.22161/ijeab/1.2.11

Duncan DB (1955) Multiple range and multiple F tests. Biometrics 11(1):1–42

El-Mahdy MT, Radi AA, Shaaban MM (2019) Impacts of exposure of banana to silver nanoparticles and sliver ions in vitro. Middle East J Appl Sci 9(3):727–740

Haddadi F, Aziz MA, Saleh G, Rashid AA, Kamaladini H (2010) Micropropagation of strawberry cv. Camarosa: prolific shoot regeneration from in vitro shoot tips using thidiazuron with N6-benzylamino-purine. HortScience 45(3):453–456. https://doi.org/10.21273/HORTSCI.45.3.453

Harugade S, Tabe RH, Chaphalkar S (2014) Micropropagation of strawberry (*Fragaria* × *ananassa* Duch.). Int J Curr Microbiol App Sci 3(3):344–347

Hdider C, Desjardins Y (1993) Prevention of shoot vitrification of strawberry micropropagated shoots proliferated on liquid media by new antivitrifying agents. Can J Plant Sci 73(1):231–235. https://doi.org/10.4141/cjps93-037

Jan A, Bhat KM, Bhat SJA, Mir MA, Bhat MA, Imtiyaz A, Rather JA (2013) Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture. Afr J Biotechnol 12(39):5749–5753. https://doi.org/10.5897/AJB2013.12918

Kevers C, Gaspar T (1985) Vitrification of carnation in vitro: changes in ethylene production. ACC level and capacity to convert ACC to ethylene. Plant Cell Tissue Org Cult 4(3):215–223. https://doi.org/10.1007/BF00040195

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwany CY, Kim YK, Lee SY, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol 3(1):95–101. https://doi.org/10.1016/j.nano.2006.12.001

Kumar GP, Sivakumar S, Siva G, Vigneswaran M, Kumar TS, Jayabalan N (2016) Silver nitrate promotes high-frequency multiple shoot regeneration in cotton (*Gossypium hirsutum* L.) by inhibiting ethylene production and phenolic secretion. In Vitro Cell Dev Biol Plant 52(4):408–418. https://doi.org/10.1007/s11627-016-9782-5

Mahmoud O, Kosar M (2014a) In vitro achievement of strawberry roots formation using $AgNO_3$. Am Eurasian J Agric Environ Sci 14(11):12453. https://doi.org/10.5829/idosi.aejaes.2014.14.11.12453

Mahmoud O, Kosar M (2014b) In vitro achievement of strawberry roots formation using $AgNO_3$. Am Eurasian J Agric Environ Sci 14(11):1281–1286. https://doi.org/10.5829/idosi.aejaes.2014.14.11.12453

Mihaljević I, Dugalić K, Tomaš V, Viljevac M, Pranjić A, Čmelik Z, Puskar B, Jurković Z (2013) In vitro sterilization procedures for micropropagation of 'Oblačinska'sour cherry. J Agric Sci 58(2):117–126

Mir JI, Ahmed N, Rashid R, Wani SH, Mir H, Sheikh MA (2010) Micropropagation of strawberry (*Fragaria* × *ananassa*). J Crop Improv 37(2):153–156

Mir H, Rani R, Ahmad F, Sah AK, Prakash S, Kumar V (2019) Phenolic exudation control and establishment of in vitro strawberry (*Fragaria* × *ananassa*) cv. Chandler. Curr J Appl Sci Technol 33(3):1–5. https://doi.org/10.9734/CJAST/2019/v33i330071

Mo VT, Cuong LK, Tung HT, Huynh TV, Nghia LT, Khanh CM, Lam NN, Nhut DT (2020) Somatic embryogenesis and plantlet regeneration from the seaweed *Kappaphycus striatus*. Acta Physiol Plant 42:104. https://doi.org/10.1007/s11738-020-03102-3

Mozafari AA, Gerdakaneh M (2012) Influence of media and growth regulators on regeneration and morphological characteristics of strawberry cvs Kurdistan and Merck (*Fragaria* × *ananassa* Duch.). Int J Plant Physiol Biochem 4(5):99–104. https://doi.org/10.5897/IJPPB11.008

Munir M, Iqbal S, Baloch JUD, Khakwani AA (2015) In vitro explant sterilization and bud initiation studies of four strawberry cultivars. J Appl Hortic 17(3):192–198. https://doi.org/10.37855/jah.2015.v17i03.36

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Plant Physiol 15(3):473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Na H, Kim BS, Kim J (2019) Anther-derived callus induction based on culture medium, myo-inositol, $AgNO_3$ and Fe-EDTA in 'Seolhyang' strawberries. J Plant Breed Crop Sci 11(1):26–32. https://doi.org/10.5897/JPBCS2018.0779

Nam NB, Huy NP, Luan VQ, Tung HT, Nhut DT (2016) Application of wireless power transmission led lighting system in propagation of chrysanthemum and strawberry. Planta Daninha 34(4):617–630. https://doi.org/10.1590/s0100-83582016340400001

Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quiagg A, Santschi PH, Sigg L (2008) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17(5):372–386. https://doi.org/10.1007/s10646-008-0214-0

Nehra NS, Kartha KK, Stushnott C, Giles KL (1992) The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry. Plant Cell Tissue Org Cult 29(3):257–268. https://doi.org/10.1007/BF00034361

Ngan HTM, Cuong DM, Tung HT, Nghiep ND, Le BV, Nhut DT (2020) The effect of cobalt and silver nanoparticles on overcoming leaf abscission and enhanced growth of rose (*Rosa hybrida* L. 'Baby Love') plantlets cultured in vitro. Plant Cell Tissue Org Cult 141(2):393–405. https://doi.org/10.1007/s11240-020-01796-4

Oo KT, Oo KS, Mon YY (2018) Establishment of efficient surface sterilization protocol on different types of field grown strawberry explants (*Fragaria* × *ananassa* Duch.). J Sci Innov Res 7(3):70–74

Palei S, Das AK, Rout GR (2015) In vitro studies of strawberry—an important fruit crop: a review. J Plant Sci Res 31(2):115–131

Palei S, Rout GR, Das AK, Dash DK (2017) Callus induction and indirect regeneration of strawberry (*Fragaria* × *ananassa*) Duch. cv. Chandler. Int J Curr Microbiol Appl Sci 6(11):1311–1318. https://doi.org/10.20546/ijcmas.2017.611.157

Park JS, Naing AH, Kim CK (2016) Effects of ethylene on shoot initiation, leaf yellowing, and shoot tip necrosis in roses. Plant Cell Tissue Org Cult 127(2):425–431. https://doi.org/10.1007/s11240-016-1066-6

Parzymies M, Pudelska K, Poniewozik M (2019) The use of nano-silver for disinfection of *Pennisetum alopecuroides* plant material for tissue culture. Acta Sci Pol Hortorum Cultus 18(3):127–135. https://doi.org/10.24326/asphc.2019.3.12

Ramírez-Mosqueda MA, Sánchez-Segura L, Hernández-Valladolid SL, Bello-Bello E, Bello J (2019) Antifungal activity of silver nanoparticles during in vitro culture of Stevia rebaudiana Bertoni. bioRxiv. https://doi.org/10.1101/846733

Razavizadeh R, Rostami F (2015) Risks and benefits assessments of silver nanoparticles in tomato plants under in vitro culture. Eng Res J 3(7):51–55

Saha N, Gupta SD (2018) Promotion of shoot regeneration of *Swertia chirata* by biosynthesized silver nanoparticles and their involvement in ethylene interceptions and activation of antioxidant activity. Plant Cell Tissue Org Cult 134(2):289–300. https://doi.org/10.1007/s11240-018-1423-8

Salachna P, Byczyńska A, Zawadzińska A, Piechocki R, Mizielińska M (2019) Stimulatory effect of silver nanoparticles on the growth and flowering of potted oriental lilies. Agronomy 9(10):610. https://doi.org/10.3390/agronomy9100610

Sarmast M, Niazi A, Salehi H, Abolimoghadam A (2015) Silver nanoparticles affect ACS expression in *Tecomella undulata* in vitro culture. Plant Cell Tissue Org Cult 121(1):227–236. https://doi.org/10.1007/s11240-014-0697-8

Sarropoulou V, Maloupa E (2016) Effect of the NO donor "sodium nitroprusside"(SNP), the ethylene inhibitor "cobalt chloride"(CoCl2) and the antioxidant vitamin E "α-tocopherol" on in vitro shoot proliferation of Sideritis raeseri Boiss. & Heldr. subsp. Raeseri. Plant Cell Tissue Org 128(3):619–629. https://doi.org/10.1007/s11240-016-1139-6

Sharma P, Bhatt D, Zaidi MGH, Saradhi PP, Khanna PK, Arora S (2012) Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotechnol 167(8):2225–2233. https://doi.org/10.1007/s12010-012-9759-8

Shukla S, Singh M, Kumar A, Rajbhar Y, Kumar M, Singh SP (2019) Effects of surface sterilization agents under in vitro culture of banana (Musa paradisiaca L.) variety "Udhayam". J Pharmacogn Phytochem 8(1):843–846

Spinoso-Castillo J, Chavez-Santoscoy R, Bogdanchikova N, Pérez-Sato J, Morales-Ramos V, Bello-Bello J (2017) Antimicrobial and hormetic effects of silver nanoparticles on in vitro regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. Plant Cell Tissue Org Cult 129(2):195–207. https://doi.org/10.1007/s11240-017-1169-8

Sutter EG, Ahmadi H, Labavitch JM (1997) Direct regeneration of strawberry *Fragaria* × *ananassa* Duch. from leaf disks. Acta Hortic 447:243–245. https://doi.org/10.17660/ActaHortic.1997.447.52

Syu Y, Hung JH, Chen JC, Chuang H (2014) Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. Plant Physiol Biochem 83C:57–64. https://doi.org/10.1016/j.plaphy.2014.07.010

Thao NP, Khan MIR, Thu NBA, Hoang XLT, Asgher M, Khan NA, Tran L-SP (2015) Role of ethylene and its cross talk with other signaling molecules in plant responses to heavy metal stress. Plant Physiol 169(1):73–84. https://doi.org/10.1104/pp.15.00663

Tung HT, Nam NB, Huy NP, Luan VQ, Hien VT, Phuong TTB, Le TD, Nhut DT (2018) A system for large scale production of Chrysanthemum using microponics with the supplement of silver nanoparticles under light-emitting diodes. Sci Hortic 232:153–161. https://doi.org/10.1016/j.scienta.2017.12.063

Veilleux RE, Johnson AA (1998) Somaclonal variation: molecular analysis, transformation interaction, and utilization. In: Janick J (ed) Plant breeding reviews, vol 16. Wiley, Hoboken, pp 229–268. https://doi.org/10.1002/9780470650110.ch6

Wafaa AF, Wahdan HM (2017) Influence of substrates on in vitro rooting and acclimatization of micropropagated strawberry (*Fragaria* × *ananassa* Duch.). Middle East J Agric Res 6(3):682–691

Yin L, Cheng Y, Espinasse B, Colman BP, Auffan M, Wiesner M, Rose J, Liu J, Bernhardt ES (2011) More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. Environ Sci Technol 45(6):2360–2367. https://doi.org/10.1021/es103995x