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ORIGINAL ARTICLE

Silver nanoparticles as an effective stimulant in micropropagation of *Panax vietnamensis*—a valuable medicinal plant

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Abstract

Effects of silver nanoparticles (AgNPs) on somatic embryogenesis and plantlets with rhizome of *Panax vietnamensis* were presented in this study. The highest number of somatic embryos (140) was obtained on MS medium containing 1 mg/L 2.4-D, 0.5 mg/L NAA, 0.2 mg/L Kin and 1.6 mg/L AgNPs. Plantlets with rhizome growth enhanced on SH medium supplemented with 1 mg/L NAA and 1.2 mg/L AgNPs and limited the ethylene gas content in the culture flasks. A low concentration of ethylene gas (0.92 ppm) stimulated plantlets with rhizome growth and development, and provided positive effect on the formation and quality of plantlets. The rhizome size of the plantlets in 1.2 mg/L AgNPs treatment was larger than those in the control without AgNPs. Plantlets acclimatized on the soilless substrate were higher survival rate (93.65%) as compared to the control (44.44%) after one year in the greenhouse. In particular, the rhizome saponin content was doubled compared with that in the planlets without AgNPs after 4-year planting in the greenhouse. These results may be scaled up for micropropagation of this important medicinal plant.

Key Message

AgNPs improved somatic embryogenesis. AgNPs enhanced plantlets with rhizome. AgNPs limited ethylene accumulation during micropropagation. AgNPs increased the survival rate of plantlets with rhizome in the greenhouse. AgNPs increased saponin content of plantlets with rhizome.

Keywords Ethylene · Rhizome · Saponin · Somatic embryos · Vietnamese ginseng

Abbreviations

MS	Murashige and Skoog (1962)
SH	Schenk and Hildebrandt (1972)
Kin	Kinetin
NAA	1-Naphthaleneacetic acid
AgNPs	Silver nanoparticles
2.4-D	2,4 Dichlorophenoxyacetic acid
TDZ	Thidiazuron
G-Rg1	Gingsenosid Rg1
M-R2	Majonoside-R2
G-Rb1	Gingsenosid Rb1

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Introduction

Ginseng, a perennial plant belonging to genus *Panax*, family Araliaceae, has been commonly known as a popular herbal medicine for thousands of years. Although there are many plants affiliated with the genus Panax, only five of them are used for promoting vitality and prolonging life, including Panax ginseng, American ginseng, Vietnamese ginseng, Japanese ginseng, and Pseudo ginseng. Ginseng's pharmacological effects are derived from multiple active ingredients, including ginsenosides, polysaccharides, peptides, phytosterols, polyacetylenes, polyacetylenic alcohols, and fatty acids (Gillis 1997). Vietnamese ginseng has been known to the world with the scientific name *Panax vietnamensis*

Ha *et* Grushv (Grushvitskii et al. 1987. *P. vietnamensis* contains high level of saponin—an important medicinal compound in the ginseng genus. In particular, the group of substances having the most decisive effect on the pharmacological effects of this ginseng species are triterpeneic saponins, which are represented by MR2, G-Rb1 and G-Rg1. One of the most interesting characteristics that distinguish *P. vietnamensis* from the other Ginsengs is its remarkably high MR2 content, accounting for more than 50 percent of the total saponin content and 42 times higher than the content of this compound in Japanese ginseng (Le et al. 2015). The presence of MR2 with high levels has contributed to the formation of some specific pharmacological effects of Ngoc Linh ginseng, including depression, diabetes, fatigue, aging, inflammation, internal degeneration, nausea, tumors, pulmonary problems, dyspepsia, vomiting, nervousness, stress, and ulcers (Hong et al. 2013). In contrast to other species of ginseng, Vietnamese ginseng was discovered quite recently (in 1973) (Grushvitskii et al. 1987) with a narrow geographical distribution (only in Ngoc Linh mountainous area). Since then, it has been over-exploited leading to the threat of extinction. With the aim of preserving the rare medicinal herbs of the country and developing large scale cultivation to supply raw materials for pharmaceutical applications, micropropagation of *P. vietnamensis* has been a potential research direction. Somatic embryogenesis has been used as a tool for micropropagation of herbal plants, especially in ginseng (Monteiro et al. 2002). There has been earlier research on Panax ginseng propagation by plant tissue culture and particularly by somatic embryogenesis in the early 1980s (Chang and Hsing 1980; Tirajoh et al. 1998; Kevers et al. 2000). Several studies on the embryogenesis, regeneration of callus and germination of shoots on *P. vietnamensis* had been reported (Nhut et al. 2017). However the quality of plantlets with rhizomes was not high due in part to the uncontrolled ethylene gas content in the culture flasks. Ag and its compounds have long been used as anti-bacteria agents in food preservation and injuries sterilization. $AgNO_3$ has been used to inhibit the activity of ethylene gas in somatic embryogenesis (Fuentes et al. 2000; Giridhar et al. 2004), shoot induction (Cardoso 2019), and in vitro rooting (Steinitz et al. 2010). In plant micropropagation, AgNPs were not routinely added to the culture medium as nutritious elements but were added as anti-microbial agents (Arab et al. 2014; Spinoso-Castillo et al. 2017) and inhibitors of ethylene activity, thereby successfully promoting morphogenesis, improving the growth, development and quality of plantlets (Thao et al. 2015). However, research on the application of AgNPs is still quite limited in medicinal plant micropropagation. Up to now, effects of AgNPs on *P. vietnamensis* micropropagation have not yet been investigated. In this study, AgNPs was supplemented to the culture medium to increase the somatic embryo formation, and enhance rhizome quality of *P. vietnamensis* cultured in vitro. This study was aimed to investigate the effects of AgNPs as a plant growth regulator on somatic embryo formation and proliferation. Further investigation on the effects of AgNPs on the plantlet growth with rhizome of *P. vietnamensis* via inhibiting the activity of ethylene gas was also carried out. In addition, the adaptability, growth and development of plantlets derived from AgNPs supplemented medium under greenhouse conditions were also investigated to evaluate the effects of AgNPs in the micropropagation process.

Materials and methods

Materials

Explant source

The 6-week-old in vitro leaf-derived calli obtained on SH medium supplemented with 1 mg/L 2.4-D, 0.2 mg/L TDZ (Nhut et al. 2011) were used as original explants.

AgNPs solution

AgNPs solution (smaller than 20 nm in size) was obtained according to the ratio: [AgNO3] = 750–1000 ppm, [β-chitozan] = 250–300 ppm, [NaBH4] = 200 ppm, molar ratio [NaBH4]/[AgNO3] = ¼, the dripping rate of NaBH4 was 10–12 drops/min. AgNPs solution was provided by the Institute of Environmental Technology, VAST (Chau et al. 2008).

Culture media

MS medium and SH medium supplemented with 8 g/L agar, 30 g/L sucrose, 1 g/L activated charcoal and different growth regulators, depending on experimental purpose, were used for in vitro culture experiments. AgNPs at different concentrations (0.4, 0.8, 1.2, 1.6, 2.0 mg/L) were added to the culture medium. The culture medium was adjusted to pH 5.8 before autoclaving at 121 °C, 1 atm for 30 min.

Methods

Somatic embryo formation and proliferation

To investigate the effect of AgNPs on somatic embryo formation and proliferation; callus (1.5 × 1.5 cm, 0.5 g in weight) were cultured on MS medium containing 1 mg/L 2.4-D, 0.5 mg/L NAA, 0.2 mg/L Kin (Nhut et al. 2012) and AgNPs at different concentrations. The treatment without AgNPs was used for control. After 14 weeks, growth characteristics including number of somatic embryos (embryos/

explant), embryo-derived plantlets (plantlets/explant), embryo-derived plantlets greater than 3 cm in height (plantlets/explant), plantlet fresh weight (g), plantlet dry weight (mg) were recorded.

Plantlet growth with rhizomes

Plantlets (1.5 cm in height) of *P. vietnamensis* were cultured on SH medium added with 1 mg/L NAA (Nhut et al. 2017) and AgNPs at different concentrations to investigate the plantlet quality. The treatment without AgNPs was used for control. Data on the rhizome diameter (cm), rhizome length (cm), rhizome fresh weight (g), rhizome dry weight (g), SPAD (nmol/cm^2) plantlet morphology were recorded after 12 weeks of culture.

The content of ethylene gas in the culture flask

Ethylene accumulation content in culture flasks was determined by gas chromatography (GC) with fame ionization probes (Cristescu et al. 2013). 1 cm^3 (1 mL) of gas was collected directly from a tiny culture flask and pumped into GC with a syringe. GC system (GC-CP 3380), syringe (BD Tuberculin syringe 1 mL), needle (BD PrecisionGlide Needle) and specialized gas leak prevention patch were used for ethylene content analysis.

The ability to absorb AgNPs

The plantlets cultured on SH supplemented with 1.2 mg/L AgNPs after 12 weeks were used to measure Ag absorption. The culture medium was collected and decomposed with HNO_3/HCl acid, then the analyzed samples were converted to gases of free ions by using the suitable power source. Gas samples were analyzed by atomic absorption spectroscopy (AAS) and measured by absorption spectroscopy (Shimadzu, AAS-6650, Japan) (Kojuncu et al. 2004). Absorbed-Ag content was calculated by the following formula:

$$AgA = Ag0 - AgR$$

In which: AgA: absorbed-Ag rate after 12 weeks, Ag0: total Ag content in culture medium at the beginning (mg), AgR: residual Ag content in culture medium after 12 weeks (mg).

The acclimatization of plantlets in the greenhouse

Plantlets from the in vitro rooting experiment supplemented with 1.2 mg/L AgNPs and without AgNPs were collected, washed agar, transplanted into plastic basket containing a soilless mixture (Metro-Mix® 350, Scottsco, Manisville, Ohio) and placed in the greenhouse. After 1 year of cultivation, survival rate (%) and growth indicators including number of leaves, plantlet height (cm), leaf area (cm^2), fresh weight (g), total chlorophyll contents in leaves—SPAD (nmol/cm^2), leaves abscission were recorded to investigate the plantlet quality.

The saponin accumulation

After 4 years of cultivation, data on the fresh weight (g), saponin compounds [G-Rg1 (%), M-R2 (%), G-Rb1 (%) and G-Rg1 + M-R2 + G-Rb1 (%)] of plantlets derived from treatment supplemented with 1.2 mg/L AgNPs were compared with 4-year-old plantlets without using AgNPs and 4-year-old seedlings under homogeneous conditions.

The 4-year-old rhizomes were collected, washed, dried and ground into powder grade. The powder (0.5 g) was extracted in methanol by sonicator (10 mL methanol × 6 times). Then it was evaporated by an evaporator to dry residues. The concentrates were dissolved in 20 mL of water and fractionated with ether ethylic and n-butanol, respectively. The n-butanol was concentrated under vacuum pressure in order to yield the dried extract. The dried extract dissolved with a mixture of acetonitrile water solvent (2:1, v/v) and a volume of 5 mL was filtered through a 0.45 mm membrane. The filtrate was finally injected in the HPLC system for quantitative determination of saponins by using the calibration curve method. HPLC system: Supelco RP C18 column (250 × 4.6 mm; I.D. 5 mm) and a SPD-M20A-PDA detector (Shimadzu) were used. HPLC parameters: volume injection of 20 mL; flow rate of 0.5 mL/min. Column temperature was kept at 25 ℃ (Gui et al. 2007).

The scanning electron microscopy (SEM)

The embryos clusters were separated from the explant and soaked in solution of paraformaldehyde 4% at 4 ℃ to incubate overnight. The samples were rinsed 3 times in 25 mM sodium phosphate (pH = 7) before dehydration by soaking in ethanol at the concentrations 10% (30 min), 30% (30 min), and 50% (30 min), 65% (30 min), 75% (30 min), 90% (30 min), 95% (30 min), absolute ethanol (30 min, triplicates), absolute ethanol was incubated overnight at 4 ℃. The sample was processed to critical dryness via carbon dioxide, mounted on aluminum substrates and coated with a 60 nm palladium layer. Microscopic image was recorded with a scanning electron microscope (FE SEM S4800).

Culture conditions

In vitro

The culture room was maintained at temperature of 25 ± 2 ℃, fuorescent lights with intensity of 40–45 μmol.m^{-2}.s^{-1}, photoperiod of 16 h/day, and 55–60% humidity.

Ex vitro

Plantlets were grown in greenhouse with daytime temperatures of 25 ± 2 ℃, night temperatures of 15 ± 2 ℃ at night, the average humidity of about 90–95%, using natural light with shade 90%, and soil pH about 6.5.

Statistical analysis

The experiments were conducted in triplicates, 30 explants per treatment. All data were processed by Microsoft Excel 2010 and SPSS 16.0 statistical analysis software basing on Duncan test method with $\alpha = 0.05$ (Duncan 1955).

Results and discussion

Somatic embryo formation and proliferation on medium containing AgNPs

The results in Table 1 showed that all callus explants formed somatic embryos and embryo-derived plantlets on the culture medium with or without AgNPs supplementation; however, the number of embryos and embryo-derived plantlets depended on the concentration of AgNPs. The number of somatic embryos (140) and the embryo-derived plantlets (14.66) at a concentration of 1.6 mg/L AgNPs were significantly 3–4 times higher than the ones of the control treatment (40.33; 4.33; respectively). Besides, embryo morphology recorded at a concentration of 1.6 mg/L AgNPs were globular, heart-shaped embryos, torpedo, cotyledonary (Figs. 1a, b, c and 2) with smooth surface, separated from each other; whereas only globular, adherent embryos were recorded in the control.

The number of embryo-derived plantlets greater than 3 cm in height and plantlet fresh weight reached optimum at AgNPs concentration from 0.8 to 1.6 mg/L (Fig. 1d). In which, the fresh weight of plantlets in the AgNPs supplement treatment at 0.8 mg/L (0.63 g) and 1.2 mg/L (0.56 g) were superior to that at 1.6 mg/L AgNPs (0.48 g), but the dry weight was significantly lower (56.33 and 62.00 mg) when compared to the treatment supplemented with 1.6 mg/L AgNPs (86.00 mg). Thus, the plantlets in the treatment with the addition of 0.8 and 1.2 mg/L AgNPs showed vitreous phenomena. On the other hand, parameters in the additional treatment of 2.0 mg/L AgNPs showed not only inhibition of new embryo formation but also the negative effect on the development of embryo-derived plantlets.

In theory, every living plant cell is capable of somatic embryogenesis. The use of $AgNO_3$ in improving the frequency of somatic embryogenesis has been studied in many different species: *Coffea canephora* (Giridhar et al. 2004), *Daucus carota* (Montague et al. 1979). In this study, AgNPs was used for the first time in *P. vietnamensis* somatic embryogenesis.

In previous studies, somatic embryogenesis was insignificant when culturing thin cell layers of rhizome of *P. vietnamensis* under the influence of plant growth regulators (Nhut et al. 2011). In 2012, Nhut et al. reported that the addition of spermidine to the culture medium conferred high efficiency in embryogenesis from in vitro rhizome thin cell layer-derived callus, but the embryos were mainly globular and adherent ones (Nhut et al. 2012). In the study we observed that AgNPs at optimal concentration had a positive effect on somatic embryo formation as well as proliferation from *ex vitro* leaf explant-derived callus of *P. vietnamensis*. In particular, these embryos had morphological diversity as well as they changed to differentiated state with smooth surface, gradually developing cotyledons suitable for the formation of high quality plantlets. This result also shows that there is a significant difference to the observations of Nhut et al (2011) where they used *ex vitro* leaf-derived callus to regenerate shoots (8.2

Table 1 Effects of medium containing AgNPs on somatic embryo formation and proliferation of *P. vietnamensis* after 14 weeks of culture

AgNPs (mg/L)	No. of somatic embryos	Embryo-derived plantlets		Plantlet fresh weight (g)	Plantlet dry weight (mg)
		Total	Embryo-derived plantlets > 3 cm		
0.0	40.33^{c}*	4.33^{d}	2.66^{c}	0.28^{c}	28.66^{d}
0.4	49.33^{c}	9.33^{b}	4.00^{bc}	0.35^{bc}	43.33^{bcd}
0.8	83.66^{b}	9.66^{b}	5.00^{ab}	0.63^{a}	56.33^{bc}
1.2	98.33^{b}	10.66^{b}	5.33^{ab}	0.56^{ab}	62.00^{b}
1.6	140.00^{a}	14.66^{a}	5.66^{a}	0.48^{abc}	86.00^{a}
2.0	40.66^{c}	6.66^{c}	0.00^{d}	0.31^{bc}	41.00^{cd}

*Different letters in the same column indicate significantly different means using Duncan's test ($p < 0.05$)

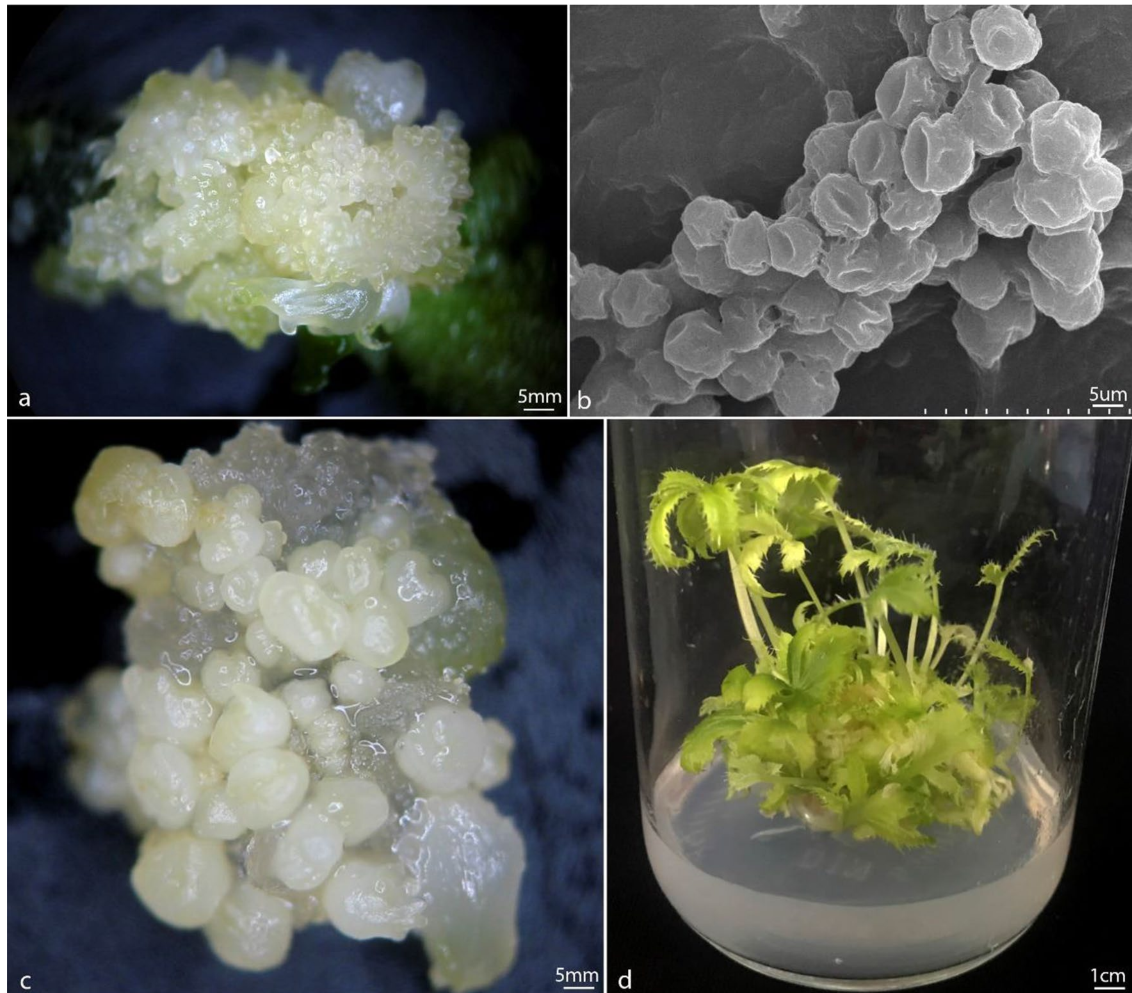

Fig. 1 The embryogenic calli induction and somatic embryo formation of *P. Vietnamensis* on the medium containing 1.6 mg/L AgNPs at different times of culture. Embryogenic calli after 6 weeks of culture that were taken by fluorescence microscope (**a**) and scanning electron microscope (SEM) (**b**). Somatic embryo images taken by fluorescence microscope on the medium containing after 8 weeks of culture (**c**). Somatic embryo-derived plantlets after 14 weeks of culture (**d**)

shoots/explant) and forming of plantlets without via somatic embryogenesis (Nhut et al. 2011).

So far there have been no reports on the influence of silver ions affecting the process of somatic embryogenesis. However, there are studies that demonstrate silver nitrate as an inhibitor of the activity of ethylene gas (Beyer 1976), and using SAM precursor for polyamine reactions which influenced somatic embryogenesis (Kong and Yeung 1994). In this study, the somatic embryogenesis observed under the influence of AgNPs may provide evidence that AgNPs promotes somatic embryogenesis and proliferation of *P. vietnamensis*.

Effects of medium containing AgNPs on in vitro rhizome growth, AgNPs absorption ability of plantlet and ethylene gas accumulation in culture flask

The results showed that the rhizome size and weight was different in various concentrations of AgNPs added to the culture medium (Table 2). The rhizome diameter (1.16 cm), rhizome length (2.43 cm), rhizome fresh weight (1.27 g) and rhizome dry weight (0.74 g) at concentration 1.2 mg/L AgNPs (Fig. 4b) were significantly higher than the control

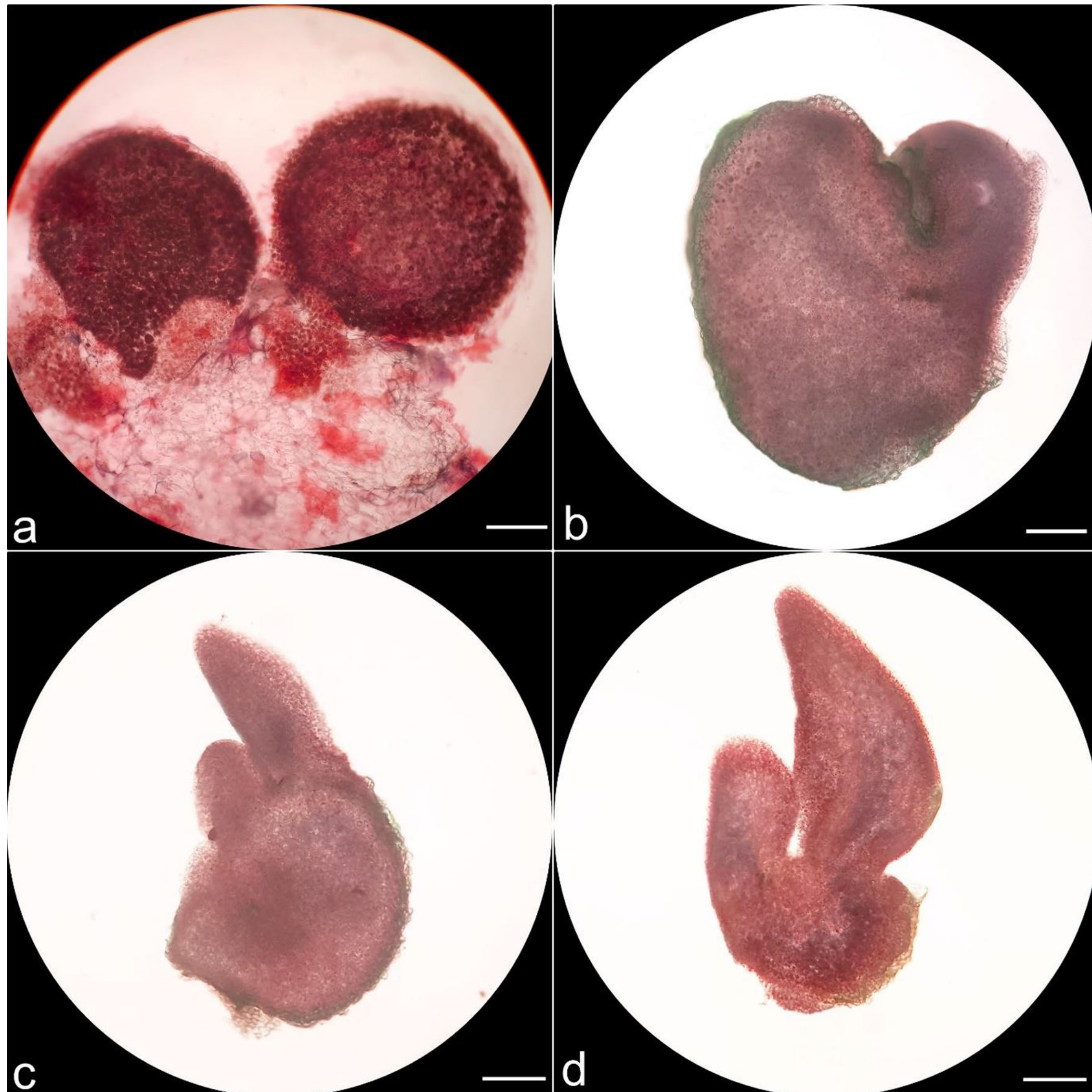

Fig. 2 The morphological and structural of somatic embryo on the medium containing 1.6 mg/L AgNPs were observed by microscope with magnification × 40 after 8 weeks of culture. Anatomical morphology of globular (**a**), heart-shaped (**b**), torpedo (**c**), cotyledonary embryo (**d**). Bar 1 mm

Table 2 The effect of medium containing AgNPs on the plantlet growth with rhizome after 12 weeks of culture

AgNPs (mg/L)	Rhizome diameter (cm)	Rhizome length (cm)	Rhizome fresh weight (g)	Rhizome dry weight (g)	SPAD (nmol/cm^2)	Note
0.0	0.43^{d}	1.50^{c}	0.43^{d}	0.10^{d}	18.56^{bc}	Yellowish leaf
0.4	0.70^{bc}	1.63^{c}	0.59^{c}	0.19^{c}	22.70^{abc}	Green leaf
0.8	0.86^{b}	2.03^{b}	0.91^{b}	0.43^{b}	24.10^{ab}	Green leaf
1.2	1.16^{a}	2.43^{a}	1.27^{a}	0.74^{a}	24.56^{a}	Green leaf
1.6	0.73^{bc}	1.90^{bl}	0.89^{b}	0.40^{b}	18.80^{abc}	Green leaf
2.0	0.66^{c}	1.53^{c}	0.43^{d}	0.15^{cd}	16.93^{c}	Light green leaf

Different letters in the same column indicate significantly different means using Duncan's test ($p < 0.05$)

(0.43 cm; 1.50 cm; 0.43 g; 0.10 g; respectively) (Fig. 4c) and other AgNPs supplementary treatments. Besides, the results were also recorded on plantlet morphology in all treatment containing AgNPs. In the control, plantlets were tall with yellowish leaves and low SPAD value while in the medium containing 0.4–1.6 mg/L AgNPs gave vigorous plantlets with green leaves and high SPAD value. In the medium containing 2 mg/L AgNPs showed the light green leaf and low SPAD value (Table 2 and Fig. 4a).

The results in Fig. 3 showes that the amount of AgNPs absorbed by the plants was closely related to the amount of ethylene gas produced in the culture flask. The greater the amount of AgNPs added to the culture medium, the greater the concentration of AgNPs absorbed in the plant. The results also showed that the appropriate amount of AgNPs (2.33 µg) absorbed in the plants gave the lowest amount of ethylene gas produced in the culture vessel. This initiated the plantlets in this treatment to develop rhizomes which grow better than in the treatments with other concentrations of AgNPs and control (without AgNPs). Ethylene gas content in the culture flask gradually decreased when adding from 0.4 mg to 1.2 mg/L AgNPs. However, the addition of silver at high concentrations (1.6, 2 mg/L) increased ethylene accumulation and was higher than that of the control (without AgNPs) (Fig. 3). Besides, AgNPs content absorbed by the plants was low; this content may be acceptable for medicinal plants.

In this study, the result showed the ethylene gas content at 1.2 mg/L AgNPs was lower compared to other AgNPs treatments and the control and this was inversely proportional to the plantlet growth and development in the culture flask. Thus, ethylene is an important factor in plantlet growth and development. So far, there are no reports on the effects of AgNPs on rhizome formation. However, there have been reports of the effect of AgNPs on root formation and plantlet quality enhancement. The study of Bais et al. (2000) on *Decalepis hamiltonii*, claiming that addition of 40 µM $AgNO_3$ caused inhibition of ethylene action which improved root initiation and elongation (Bais et al. 2000). Another study also showed that the accumulation of ethylene gas was signifcantly reduced to increase plantlet quality when using 60 µ/L AgNPs in micropropagation of *Tecomella undulata* (Sarmast et al. 2015). Therefore, the development of this effective micropropagation technology in combination with AgNPs will be a new direction in enhancing *P. vietnamensis* plantlet quality.

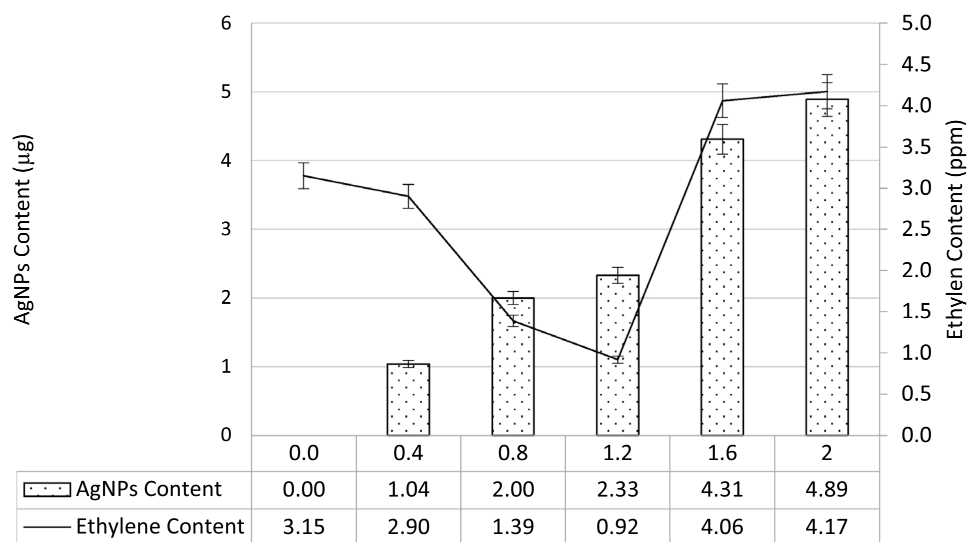

Fig. 3 The ability to absorb AgNPs and accumulate ethylene gas of *P. vietnamensis* plantlets after 12 weeks of culture

Table 3 The acclimatization and growth of plantlets derived from AgNPs in the greenhouse after 1 year in the greenhouse

Plantlets derived from AgNPs	Survival rate (%)	No. of leaves	Plantlet height (cm)	Leaf area (cm^2)	Fresh weight (g)	SPAD ($nmol/cm^2$)	Leaves abscission
Control	44.44 ± 6.34	1.33 ± 0.33	1.33 ± 0.16	0.40 ± 0.09	1.16 ± 0.16	19.07 ± 0.96	After 1 week
1.2 mg/L	93.65 ± 1.58	4.00 ± 0.57	5.66 ± 0.72	5.77 ± 0.55	4.53 ± 0.08	24.61 ± 0.24	After 4 weeks

Different letters in the same column indicate significantly different means using Duncan's test ($p < 0.05$)

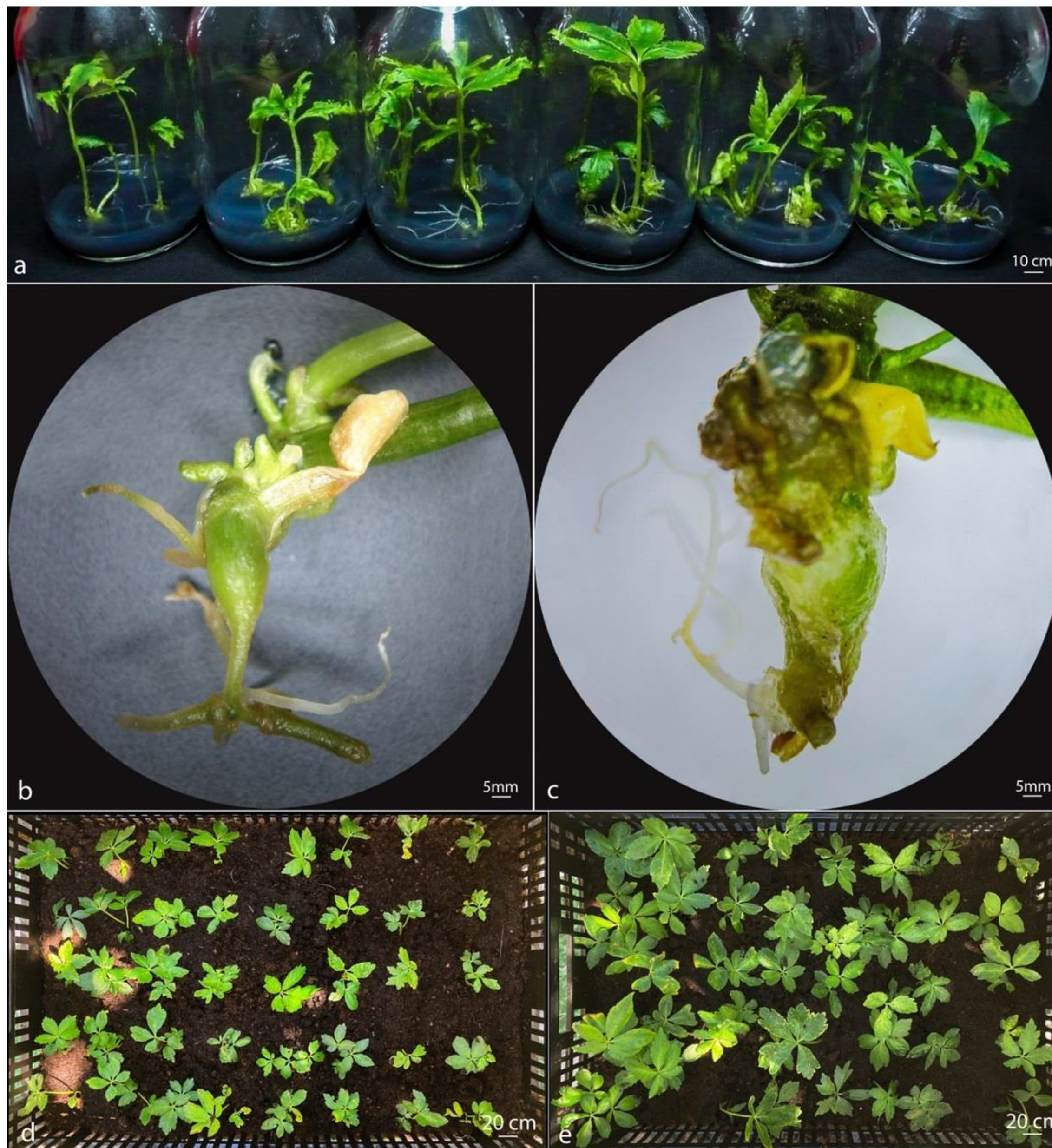

Fig. 4 The effects of AgNPs on plantlet growth of *P. Vietnamensis*. **a** The plantlets cultured on medium containing AgNPs at different concentrations (0, 0.4, 0.8, 1.2, 1.6, 2 mg/L, from left to right) after 12 weeks, **b** and **c** The rhizome formation of plantlets on the medium with or without 1.2 mg/L AgNPs after 12 weeks of culture, **d** and **e** Plantlets derived from in vitro culture in greenhouse condition after 1 year (**d** without AgNPs, **e** with 1.2 mg/L AgNPs)

Effects of AgNPs on acclimatization and growth of *P. vietnamensis* plantlets in the greenhouse

After 12 weeks of culture of plantlets in 1.2 mg/L AgNPs treatment and control (without AgNPs) they were transferred to the greenhouse to observe adaptation and survival (Table 3).

Results showed that after one week of planting and growing under homogeneous conditions, the aerial shoots of the plantlets originating from the control treatment (without AgNPs) showed abscission of leaves; whereas in the 1.2 mg/L AgNPs-derived treatment this phenomenon occurred after the 4th week. It can be explained that plantlets derived in vitro on medium supplemented with 1.2 mg/L AgNPs were better than those without AgNPs (Table 2). Ngan et al (2020a, b) also showed that acclimatization and growth of carnation and rose plantlets at the greenhouse depended on the plantlet quality on medium containing metal nanoparticles. AgNPs also showed to be effective in strawberry micropropagation, the addition of

AgNPs to the culture medium not only improved plantlet quality, but also enhanced acclimatization, growth and runner formation (Tung et al. 2021). This result once again demonstrated the role of metal nanoparticles in micropropagation. The results in Table 3 showed that the survival rate (93.56%) of plantlets derived from 1.2 mg/L AgNPs was double comparing to the control (44.44%). This result was higher than that was observed in the study of Nhut et al (2011) where they studied shoot regeneration and micropropagation of *Panax vietnamensis* Ha *et* Grushv. from *ex vitro* leaf-derived callus where they observed survival rate of 85% under natural habitat (Nhut et al. 2011). Besides, our results recorded that number of leaves (4.00), plant height (5.66 cm), leaf area (5.77 cm^2), fresh weight (4.53 g) and total chlorophyll contents (24.61 $nmol/cm^2$) in the treatment derived from 1.2 mg/L AgNPs (Fig. 4e) were also significantly higher than the control (1.33; 1.33 cm; 0.40 cm^2; 1.16 g; 19.07 $nmol/cm^2$, respectively) (Fig. 4d). This result is consistent with the observations made by Giridhar et al. (2004) where they showed the effectiveness of $AgNO_3$ which not only induced shoot multiplication but also influenced rooting of vanilla explants. The plantlets obtained on medium containing 40 µM $AgNO_3$ exhibited 100% survival (Giridhar et al. 2004). Thus AgNPs may be one of the best media supplement to develop efficient plantlets with superior rhizomes and rooting. This could also be considered as desirable propagation protocol for the conservation of this endangered plant.

Effects of AgNPs on saponin accumulation in 4-year rhizome of *P. vietnamensis* plants in the field

After 4 years of cultivation under greenhouse conditions, the results showed that the rhizome fresh weight (Fig. 5) and saponin accumulation capacity (Fig. 6) of *P. vietnamensis* derived from in vitro culture using AgNPs was significantly different from the ones in the control (seeds-derived seedlings and without AgNPs-derived plantlets). The fresh weight (77.5 g) of the rhizome derived from AgNPs treatment was higher than that from the seeds-derived seedlings (38.12 g) and the without AgNPs-derived plantlets (43.5 g). The weight of rhizomes derived from in vitro culture using AgNPs (about 12 to 13 fresh rhizomes reached 1 kg) was higher than that of the control (about 22 to 27 fresh rhizomes reached 1 kg) (Fig. 7).

The results showed that the rhizome derived from in vitro culture using AgNPs had G-Rg1, M-R2, G-Rb1, total saponin (1.550, 3.120, 1.370, 6.040%, respectively) significantly higher than that of the seeds-derived seedlings (1.149, 1.389, 0.988, 3.526%, respectively) and the without AgNPs-derived plantlets (1.284, 1.417, 1.012, 3.677%, respectively). In particular, the typical and characteristic compound of *P. vietnamensis* M-R2 was more than twice as high as the control. AgNPs has been studied as elicitors (also called nanoelicitors) in the production of paclitaxel in *Taxus chinensis* (Choi et al. 2001), tanshinone in *Salvia miltirorrhiza* (Zhao et al. 2010), phenol in *Bacop amonnieri* (Krishnaraj et al. 2012), Artemisinin in the culture of hairy root *Artemisia annua* (Zhang et al. 2013).

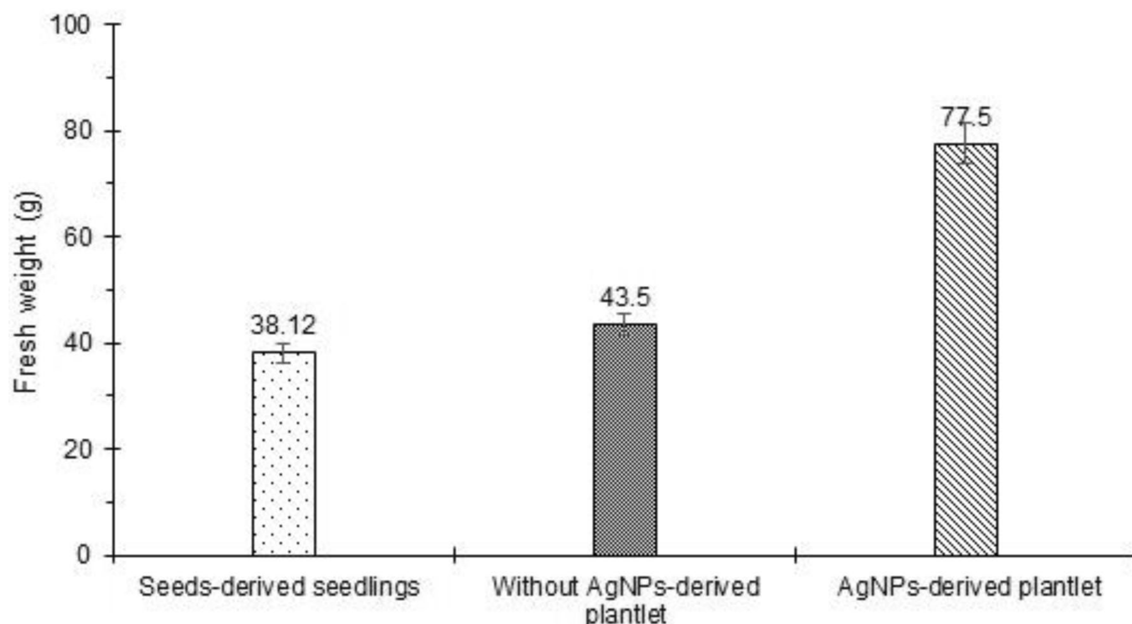

Fig. 5 The rhizome Fresh weight of *P. vietnamensis* plants derived from in vitro culture with or without AgNPs after 4 years of cultivation

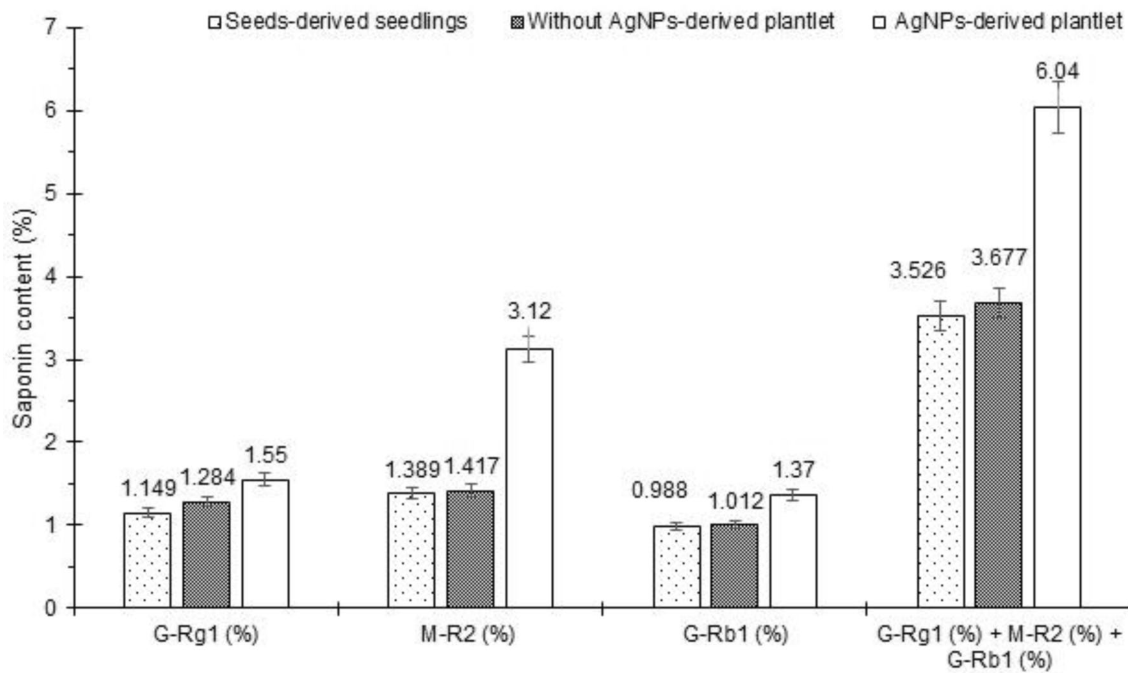

Fig. 6 Saponin content of *P. vietnamensis* plants derived from in vitro culture with or without AgNPs after 4 years of cultivation

However, more investigations are needed to find out the exact concentration, proper supplementation method and timing of application of AgNPs in order to realize growth potential and yield for other crops.

Fig. 7 Four-year-old plants of *P. Vietnamensis* in the greenhouse. The seed-derived seedling, the plantlet derived from treatment without AgNPs, the plantlet derived from treatment with AgNPs (from left to right). Bar 5 cm

Conclusion

The results of this study showed the positive effect of AgNPs on Vietnamese ginseng micropropagation. For somatic embryogenesis stage, 1.6 mg/L AgNPs adding to culture medium increased the number of somatic embryos formation and proliferation. For in vitro rhizome stage**,** the medium supplemented 1.2 mg/L AgNPs reduced amount of ethylene gas as well as improved the quality of plantlets with rhizome leading to increasing the percentage survival rate and total saponin content of these plantlets in greenhouse.

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Author contributions Do Manh Cuong acquired data wrote the manuscript. Phan Cong Du, Hoang Thanh Tung, Ha Thi My Ngan, Vu Quoc Luan, Truong Hoai Phong, Hoang Dac Khai, Truong Thi Bich Phuong participated in interpretation of data and revision for intellectual content. Duong Tan Nhut conceptualized and designed the study. All authors discussed the results and contributed to the final manuscript.

Declarations

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.

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