Silver nanoparticles potently inhibit ethylene action more than silver thiosulfate and promote microtuberization in potato (Solanum tuberosum L.) cv. Spunta

Ahmed Shawky Ibrahim (shawky.ibrahim@gmail.com)
Cairo University Faculty of Agriculture https://orcid.org/0000-0002-0984-6470
Amr Mohamed Soliman
Benha University Faculty of Agriculture
Hosny Mohamed Abd El-Dayem
Benha University Faculty of Agriculture
Mohamed Ahmed Mady
Benha University Faculty of Agriculture
Rania Said Eid
Benha University Faculty of Agriculture

Research Article

Keywords: Potato, Solanum tuberosum, Microtubers, in vitro, silver nanoparticles, Ag-NPs, silver thiosulfate, STS

Posted Date: November 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3364238/v1

Abstract

The present study aimed to investigate the effect of silver nanoparticles (Ag-NPs) as an anti-ethylene agent on *in vitro* microtuberization in potato cv. Spunta. The concentrations; 0, 0.5, 1, 2, 4, 8, and 16 mg/l of Ag-NPs or silver thiosulfate (STS) were used to determine their influence on microtuberization. The results of Ag-NPs treatments showed that, the average number of microtubers/jar was elevated and reached the highest level (14.9 microtubers/jar) which represents about 83.95% over the control treatment (8.1 microtubers/jar) using the medium containing (1 mg/l) Ag-NPs. While the highest level (10.9 microtubers/jar) was recorded using (8 mg/l) STS, which is about 34.56% over the control treatment. Thus, these results confirmed that the low level of Ag-NPs (1 mg/l) was the most effective for inhibiting ethylene action on microtuberization in potato cv. Spunta. In addition, these findings complement and agree with those obtained in our previous study using the cv. Desirèe (Ibrahim et al. 2018a). Therefore, we report here that an efficient genotype-independent protocol for microtuberization in potato has been established. In addition, this developed protocol using Ag-NPs is important for enhancing potato microtuberization technology at the mass-production level in Egypt. Furthermore, these results are of great importance in plant physiology and nanobiotechnology research.

Key Message

The results showed that the use of Ag-NPs led to a remarkable increase in the average number of microtubers/jar (83.95%) in comparison with STS, which recorded only about 34.56%.

Highlights

1. Ethylene immensely inhibits *in vitro* microtuberization in potato.
2. Silver ions as silver thiosulfate (STS) or silver nanoparticles (Ag-NPs) potently inhibit the action of ethylene and promote microtuberization in potato.
3. Ag-NPs treatment (1 mg/l) led to a remarkable increase in the average number of microtubers/jar (14.9) which about 83.95% over the control treatment (8.1). Meanwhile, silver thiosulfate (STS) at high level (8 mg/l) recorded a moderate increase in the average number of microtubers/jar (10.9) which about 34.56% over the control treatment.
4. These results revealed that the crystalline form of silver as silver nanoparticles (Ag-NPs) at the low level (1 mg/l) was superior than the ionic form as silver thiosulfate (STS) at high level (8 mg/l) for inhibiting the action of ethylene and promoting microtuberization in potato cv. Spunta.
5. In addition, these findings complement and agree with those obtained in our previous study using the cv. Desirèe (Ibrahim et al. 2018a). Therefore, we report here that an efficient genotype-independent protocol for microtuberization in potato has been established. In addition, this developed protocol using Ag-NPs is important for enhancing potato microtuberization technology at the mass-production level in Egypt.

Introduction

Potato (*Solanum tuberosum* L.), which belongs to the family Solanaceae, is an important vegetable crop for local food consumption or for exportation to the world market because of its nutritional value for humans as a source of energy, starch, minerals, vitamins, and organic acids (Koch et al. 2020). Globally, potato is ranked as the fourth most essential food crop after wheat, rice, and maize (FAO, 2022a). Further, the total cultivated area of potato in 2022 reached 20.7 million hectares with an estimated productivity of about 437 million tons (FAO, 2022b). In Egypt, potato is an indispensable crop for food and industry and is ranked as the fourth most essential food crop, and its production is followed by the three major crops, wheat, rice, and maize (Ali et al. 2020). Thus, the production of potato in 2020 was approximately 5 million tons produced from about 175,161 hectares (FAO, 2022b). Potato is a favorable cash crop for producers in Egypt, although there are several problems related to viral diseases, pests, and the availability of virus-free seed tubers for the cultivation of potato plants. The majority of potato production in Egypt is locally consumed, although potato is an important vegetable crop for exportation; hence, approximately 350 thousand tons of potato were exported in 2017 (El-Anany et al. 2019).

In vitro microtuberization in potato using stem segments as explants has been utilized for the rapid mass-production of disease-free potato seed tubers. Potato microtubers are important plant materials used for the production of minitubers and for direct cultivation in the field, in addition to their high storage capacity and easy transport. The development of efficient protocols for microtuberization in potato is of great importance for achieving the mass-production technology of potato seed tubers as well as for sustaining the progress in potato breeding. In addition to increasing the potential for microtuber exchange and commercialization (Levy et al. 1993; Akita and Takayama 1994; Donnelly et al. 2003; Zhang et al. 2005; Badoni and Chauhan 2009; Wang et al. 2011; Halterman et al. 2012; Radouani and Lauer 2015; De Morais et al. 2018).

Ethylene is an important plant hormone that has a wide range of influences on plant growth and development (Abeles 1973; Lieberman 1979; Yang and Hoffman 1984; Yang 1985; Mattoo and Suttle 1991). The inhibition of growth and *in vitro* morphogenesis in response to the presence of ethylene has been documented in various plant species (Kumar et al. 1998). Ethylene is produced by plant cells during *in vitro* culture (Biddington 1992). Some plants such as potato are very sensitive to ethylene, and the accumulation of ethylene inside the culture vessel can extremely retard shoot growth and leaf expansion, thus, the plantlets have slim stems and small leaves. Also, for *in vitro* potato plantlets, minimal concentrations of ethylene about 0.1 µl l-1 or less can cause abnormal growth (Jackson et al. 1987). Several studies have confirmed the negative effects of ethylene on *in vitro* culture of different plants and have indicated that ethylene is a potent inhibitor of callus growth and morphogenesis, either through organogenesis (shoots and roots) or somatic embryogenesis (Purnhauser et al. 1987; Songstad et al. 1988; Roustan et al. 1989; Roustan et al. 1990; Pua and Chi 1993). Thus, the growth and development of *in vitro* plants can be improved by regulating the biosynthesis or action of ethylene in plant tissues (Beyer 1976b; Davies 1987; Purnhauser et al. 1987; Songstad et al. 1988; Bais et al. 2000; Giridhar et al. 2003). The biosynthesis of ethylene in plant tissues is inhibited by cobaltous ions (Lau and Yang 1976). Silver

ions have been shown to inhibit the action of ethylene (Beyer 1976a). Silver ions are thought to disturb the binding sites (receptors) of ethylene in the plasma membrane (Rodriguez et al. 1999). The ethylene receptor (ETR1) includes one binding site per homodimer and contains a single copper ion (Cu) as a co-factor, which mediates the binding of one ethylene molecule to the binding site. The presence of one silver ion in the ethylene-binding site instead of copper can lock the ethylene receptor into a dysfunctional conformation, thus suppressing ethylene responses (Zhao et al. 2002). Therefore, several studies have demonstrated the impact of silver ions as an inhibitor of ethylene action on plant regeneration (Beyer 1976b; Duncan et al. 1985; Davies 1987; Purnhauser et al. 1987; Songstad et al. 1988; Bais et al. 2000; Giridhar et al. 2003). Furthermore, silver nitrate ($AgNO_3$) enhanced the growth and shoot regeneration of different plants; potato (Adly et al. 2022), sweet potato plant (Gong et al. 2005), canola (Khan et al. 2003), sesame (Al-Shafeay et al. 2011 & 2018) and jojoba (Ibrahim et al. 2018b). In addition, the use of $AgNO_3$ led to improved regeneration capacity of buffalograss shoots (Fei et al. 2000), faba bean (Khalafalla and Hattori 2000). Also, $AgNO_3$ enhanced somatic embryogenesis in different plants; safflower (Mandal et al. 2001), rice (Ibrahim and El Shihy 2012a&b), wheat (Hassan and Shahinul Islam 2021), and olive (Bashir et al. 2022).

The impact of ethylene on microtuberization in potato was detected, Mingo-Castel et al. (1976) pointed out that ethylene markedly inhibits the formation of microtubers in potato. Perl et al. (1988) reported that during *in vitro* culture of potato plants, the ethylene was produced from potato shoots, accumulated in the closed vessels, and led to slim stems and small leaves. Accordingly, the addition of silver thiosulfate to the culture medium resulted in a remarkable increase in the development of shoots with large leaves compared with the control treatment. Vreugdenhil and Van Dijk (1989) also reported retardation effects of ethylene on shoot elongation and radial growth, as well as on microtuberization in potato using ethephon and ACC (ACC; 1 -aminocyclopropane- 1-carboxylic acid, the precursor of ethylene), whereas silver ions, such as silver thiosulfate (STS), reversed these effects. In addition, Zobayed et al. (2001) reported that the elimination of ethylene by a forced ventilation system enhanced the growth of *in vitro* potato plants, and this treatment improved the production of microtubers *in vitro* and the size of the produced microtubers in comparison with the closed system. In addition, the inclusion of $AgNO_3$ in the medium of the diffusive ventilation system led to an increase in the number of microtubers compared to the control treatment and the closed system. The differences in the number of microtubers produced between the forced ventilation system and the diffusive ventilation system with $AgNO_3$ were non-significant, while a remarkable increase in the fresh weight of microtubers was recorded in the forced ventilation system. Furthermore, the results of our previous study on microtuberization in potato plant cv. Desirée (Ibrahim et al. 2018a) revealed that the use of silver ions as silver thiosulfate (STS) 0, 0.5, 1, 2, 4, 8, and 16 mg/l in the culture medium led to a remarkable increase in the average number of microtubers/jar in comparison to the control treatment (9.60 microtubers/jar), and this increase reached a significant level at 2, 4, 8, and 16 mg/l. While the highest average number of microtubers/jar (14.60) was recorded using 8 mg/l (STS), this increase was about 51.3% over the control treatment. In addition, the average weight of microtuber and the average diameter of microtuber were increased and reached the highest levels (238.22 mg) and (8.12 mm) using 8 mg/l (STS) compared to the control treatment (123.11 mg) and (5.37 mm). El-Sayed

et al. (2021) reported that, the *in vitro* culture of potato shoots of three cultivars; Herms, Santana, and Spunta on MS medium supplemented with silver thiosulfate at different concentrations (0, 0.4, 0.8, 1.2, 1.6, or 2 mM of STS) resulted in the highest percentage of microtuberization under dark conditions using STS at 0.4 mM compared to 2 mM of STS under light conditions in comparison with the control treatments. While, the highest number of microtubers/plantlet was recorded with a 2.9-fold increase for Spunta under light with the treatment (0.4 mM STS) compared with that in the dark. Also, the same was recorded for Herms and Santana with the treatment of STS under light conditions. In addition, the treatments; 0, 1.2, 1.6, and 2 mM STS, in Spunta resulted in higher values of microtubers fresh weight under light conditions than in the dark.

The emergence of the nano-science era using engineered nano-materials has immensely increased the potentialities of scientific research in different fields. The properties of these engineered nanoparticles vary depending on their size and shape, and they exhibit a wide range of novel properties and effectiveness. Therefore, they provide a new horizon for finding innovative solutions for complex problems. However, to achieve actual progress in the field of plant nanobiotechnology, intensive research is needed to integrate nanomaterials into experiments on plant physiology and plant tissue culture protocols (Ibrahim et al. 2018a, 2019). The use of engineered nanoparticles in agriculture has been successful in producing high yields, while reducing the amount of fertilizers and pesticides used for plant protection (Chen et al. 2013). Nevertheless, the use of engineered nano-materials affects plant growth and development, and consequently, the yield and quality of plant products (Gardea-Torresdey et al. 2014).

In the last decade, several studies reported the various effects of silver nanoparticles (Ag-NPs) including the stimulatory or toxic effects in different plants; *Zea mays* and *Phaseolus vulgaris* (Salama 2012), *Eruca sativa* (Vannini et al. 2013), *Tecomella undulate* (Aghdaei et al. 2012; Sarmast et al. 2015), *Solanum nigrum* (Ewais et al. 2015), *Arabidopsis thaliana* (Kaveh et al. 2013; Syu et al. 2014; Sun et al. 2017), vanilla (Spinoso-Castillo et al. 2017), peanut (Rui et al. 2017), *Linum usitatissimum* (Anjum et al. 2017; Zahir et al. 2019), rice (Gupta et al. 2018), *Swertia chirata* (Saha and Gupta 2018), *Campomanesia rufa* (Timoteo et al. 2019), *Lavandula angustifolia* (Jadczak et al 2019), *Echinacea purpurea* (Ramezannezhad et al. 2019), stevia (Castro-González et al. 2019; Ramirez-Mosqueda et al. 2020), *Rosa hybrida* L. 'Baby Love' (Ha et al. 2020), *Chrysanthemum* × *grandiflorum* (Tymoszuk and Kulus 2020), strawberry (Tung, et al. 2021), *Panax vietnamensis* (Cuong et al. 2021), grape (Elatafi and Fang 2022), potato (Tahmasbi et al. 2011; Ehsanpour and Nejati 2013; Baltazar Bernal et al. 2023), *Gaillardia pulchella* (Manokari et al. 2023), *in vitro* cultured seedlings of sugar beet, red clover alfalfa, rapeseed and white mustard (Tomaszewska-Sowa et al. 2023).

For instance, Tahmasbi et al. (2011) reported that, the application of silver nanoparticles (Ag-NPs) in a field experiment at a concentration (50 ppm) in combination with half amount of mineral nitrogen plus the Nitroxin biofertilizer led to a significant increase in the weight and yield of potato seed tubers produced from minitubers derived from virus-free *in vitro* cultured plantlets of cv. Sante. In addition, they suggested that this increase in tuber yield might be due to the antimicrobial effect of Ag-NPs which might

sustained growth of vigorous plants and healthy tubers for longer time in the soil. In addition, the *in vitro* use of nanoparticles has emerged, and some reports have studied the effect of nanoparticles used in culture media and indicated that nanoparticles could be useful if used at a suitable concentration (Kim et al. 2017). The addition of 8 mg/l of Ag-NPs (30–90 nm), 5 mg/l of BA, and 3 mg/l of NAA in the culture medium (MS) of *Solanum nigrum* plant increased the percentage of callus formation and the fresh weight of callus/explant (Ewais et al. 2015). Also, the inclusion of Ag-NPs (10 mg/l) in the culture medium of *Tecomella undulata* plant resulted in increased callus formation and the number of regenerated shoots (Aghdaei et al. 2012).

Moreover, several studies indicated that Ag-NPs caused some physiological effects, such as increasing the activity of the antioxidant enzymes in rice (Gupta et al. 2018) and peanut (Rui et al. 2017). In addition, it has been detected a remarkable downregulation in some genes of the ethylene signaling pathway in *Arabidopsis thaliana* plants (Kaveh et al. 2013) and *Tecomella undulata* plants (Sarmast et al. 2015; Sarmast and Salehi 2022). Furthermore, downregulation of auxin receptor-related genes in roots of *A. thaliana* exposed to Ag-NPs led to disturbance of the normal gravitropism of the roots (Sun et al. 2017).

Furthermore, Tung, et al (2021) reported that, the inclusion of AgNPs in culture media of strawberry (Fragaria × ananassa) for shoot multiplication and rooting led to a remarkable decrease of accumulated ethylene gas (0.66 ppm and 0.66 ppm) in the culture's vessels of shoots and plants compared to controls (1.77 ppm and 0.15 ppm), respectively. Thus, shoot multiplication reached to the highest level (12.67) using (0.2 mg/l) after 30 days. Meanwhile, AgNPs (0.5 mg/l) for 10 days in root formation medium was the best treatment for increasing the number of roots, root length, leaf length and leaf width. Therefore, the plantlets after 15 days showed higher survival rate (93.33%), as well as runner formation/plant (8.00 runners) after 60 days than those in control during the acclimatization in the greenhouse.

For potato *in vitro* culture, the use of Ag-NPs at concentrations; 0.5, 1, 1.5, and 2 ppm in the culture medium of potato plants cv. White Desirée led to an increase in leaf area and a dramatic decrease in the shoot and root length of *in vitro* plantlets (Ehsanpour and Nejati 2013). Baltazar Bernal et al (2023) reported that, the use of the gibberellin inhibitor paclobutrazol (PAC) 2 mg/l in combination with 50 mg/l of silver nanoparticles (AgNPs) as an anti-ethylene agent was the best treatment for *in vitro* conservation of potato plantlets for six months. Whereas after conservation the best combinations for *in vitro* multiplication of potato shoots were the treatments of AgNPs 50 mg/l with 1 or 2 mg/l of PAC.

Until recently, there were no reports on the effect of silver nanoparticles on microtuberization in potato plants. Thus, our previous study (Ibrahim et al. 2018a) was the first report performed to investigate the effect of silver nanoparticles or silver thiosulfate as anti-ethylene treatments on microtuberization in potato plant cv. Desirée. The results exhibited that, the use of Ag-NPs (1 mg/l) led to an increase in the average number of microtubers/jar to the highest level (18.95 microtubers/jar) which represents an increase of about 97.40% compared to the control treatment (9.60 microtubers/jar), while silver thiosulfate (STS) produced the highest level of increase (51.30%) for the average number of microtubers/jar (14.60 microtubers/jar) using 8 mg/l (STS) compared to the control.

Therefore, these results encouraged us to investigate the effect of silver nanoparticles on the formation of microtubers in another genotype of potato to determine whether this developed protocol is genotype-independent or not. Thus, the present study was conducted to investigate the influence of different concentrations of silver nanoparticles or silver thiosulfate as an anti-ethylene on the formation of microtubers in potato cv. Spunta. Also, in this study, we used the developed medium (microtubers formation medium; MFM), which was developed in our previous study (Ibrahim et al. 2018a), in addition to the concentrations of plant growth regulators (6 mg/l BAP and 1 mg/l TDZ) used recently in our last report on the effect of different concentrations of the plant growth regulators BAP and TDZ on microtuberization of potato cv. Spunta (Soliman et al. 2022) for establishing an efficient protocol for *in vitro* microtubers production in potato cv. Spunta, which is one of the important cultivars growing in Egypt, to sustain its cultivation by providing a stock of virus-free seed tubers from the *in vitro* seed tuber protocol. Moreover, this developed protocol using Ag-NPs is important for enhancing potato microtuberization technology at the mass-production level in Egypt.

Materials and Methods

Synthesis of silver nanoparticles:

The synthesis of silver nanoparticles 2–5 nm in size was performed according to Van Dong et al. (2012) with modifications as previously described in the previous report (Ibrahim et al. 2018a). In brief, sodium borohydride ($NaBH_4$) was used to reduce silver nitrate ($AgNO_3$) in the presence of polyvinylpyrrolidone (PVP; Mw = 40,0000) and trisodium citrate dihydrate ($C_6H_5O_7Na_3.2H_2O$) as stabilizing and capping agents. Accordingly, $AgNO_3$ (one ml of a 5 mM solution) was added to (47.5 ml of deionized H_2O) in a 100-ml flask on a magnetic stirrer at room temperature with vigorous stirring for 5 minutes. Then, 0.5 ml of (30 mM trisodium citrate dihydrate solution) and 0.5 ml of (5 mg/ml PVP) were added. After 5 minutes, 0.5 ml of a cold sodium borohydride solution (50 mM $NaBH_4$) was added, and the color immediately changed to a light-yellow color of silver nanoparticles solution (Ag-NPs).

Characterization of the synthesized silver nanoparticles:

The chemically synthesized silver nanoparticles (Ag-NPs) were carefully characterized first by the surface plasmon peak using the spectrophotometer (UV/VIS - JASCO Model-V530). Afterward, to determine the size of the particles; the suspension of Ag-NPs (in 70% ethanol) was sonicated (15 minutes) for full dispersion before being uploaded to the Transmission Electron Microscope (JEM 1400-JEOL-Japan).

Plant material and preparation of the explants:

In this study, we used virus-free seed tubers of potato (*Solanum tuberosum* L.) cv. Spunta, which were obtained from (Agricultural Research Centre), Ministry of Agriculture and Land Reclamation, Giza, Egypt. Potato tubers were prepared for breaking the dormancy: (a) the tubers were washed for 30 minutes with tap water followed by 20% sodium hypochlorite with a drop of Tween-20 for 15 minutes, then rinsed three times with sterile distilled water and kept in plastic bags for two months at 4°C and 80% humidity in

darkness; (b) the tubers were kept in paper bags for one month at 24°C for shoot sprouts. Thereafter, the produced shoots were isolated and surface sterilized by ethanol (70%) for 1 minute, followed by 20% sodium hypochlorite with one drop of Tween-20, then rinsed three times with sterile distilled water. Finally, the sterile shoots were cultured on MS medium (Murashige and Skoog 1962) for shoot multiplication and sub-cultured every 4 weeks on fresh medium.

Media composition for potato microtuberization

The work in this study contained two stages, as follows:

1. The first stage was to establish a stock culture of potato shoots to provide the explants for microtuberization experiments.
2. The second stage was to evaluate the impact of the anti-ethylene treatments on microtuberization using different concentrations; 0, 0.5, 1, 2, 4, 8, and 16 mg/l of silver nanoparticles (Ag-NPs) or silver thiosulfate (STS).

The composition of the culture medium for establishing the stock culture of potato shoots was the original MS medium containing (3 mg/l) 6-Benzylaminopurine (BAP), 10 mg/l ($AgNO_3$) as silver thiosulfate, and 3% sucrose in 400 ml glass jars (50 ml/jar), thus, the nodal explants (three nodes/explant) were cultured (6 explants/jar) for 4 weeks under (16h/8h) dark/light at 22°C ± 2.

The medium for the second stage was the developed medium used in the previous work (Ibrahim et al. 2018a; Soliman et al. 2022), the microtubers formation medium (MFM), which contained 60 mM of total nitrogen with a ratio of 5:1 between nitrate and ammonium; 50 mM nitrate: 10 mM ammonium, in addition to a high concentration of phosphorus (3.75 mM), which about 3 times of the original MS medium, and a high concentration of potassium (43.79 mM), which represents 2.18 times of the original MS medium, as shown in (Table 1). The concentration of sucrose was 8%, and the plant growth regulators BAP (6 mg/l) and TDZ (1 mg/l) were used. The culture media were prepared as double concentrated solutions (2x), and then 500 ml (2X) of each culture medium containing Ag-NPs or $AgNO_3$ as silver thiosulfate was filter-sterilized using a sterile filter (0.22 µm; Millipore-GVWP04700). Silver thiosulfate (STS; containing 2 mg/ml $AgNO_3$) was freshly prepared according to Perl et al. (1988).

Then, the 500 ml (2X) filter-sterilized solution of each culture medium was mixed with the 500 ml (2X) gelrite (which was sterilized by the autoclave for 20 min) and divided into sterile jars of 400-ml in volume (50 ml/jar). Thus, the nodal explants (five nodes/explant) were cultured (6 explants/jar) and kept for 10 weeks in full darkness at 17°C ± 0.5. The experiment was repeated twice, with twenty replicates per treatment. Finally, the jars of each treatment were photographed, and the number of microtubers/jar, the diameter, and the weight of the microtubers were recorded.

Statistical analysis

Analyses of variance (ANOVA) of the completely randomized design (CRD) were performed on the collected data using SPSS software. The least significant difference test; L.S.D. was utilized at the level

($p \leq 0.05$) to compare the mean values of the treatments; mean ± SE of 20 replicates; n = 20, (Snedecor and Cochran 1980).

Table 1
Composition of the original MS medium (a), the modified MS medium containing a low level of ammonium (b), and the improved medium (c) the microtubers formation medium; MFM, for microtuberization in potato.

Substances	(a) MS	(b) Modified MS	(c) the improved media MFM
	mg/l	mg/l	mg/l
MS salts without (NH_4NO_3, KNO_3 and KH_2PO_4)	913.36	913.36	913.36
KNO_3	1900	1900	4044
NH_4NO_3	1650	165	825
KH_2PO_4	170	170	510
Casein hydrolysate	-		1000
Glycine	2	2	-
Sucrose	30,000	30,000	80,000
Myo-inositol	100	100	250
Thiamine.HCl	0.1	0.1	10
Pyridoxine.HCl	0.5	0.5	1
Nicotinic acid	0.5	0.5	1
Pantothenate	-	-	0.5
Biotin	-	-	0.01
Riboflavin	-	-	0.01
Folic acid	-	-	0.01
6-Benzylaminopurine (BAP)			6
Thidiazuron (TDZ)			1
Gelrite			2,000
pH			5.8

Results and Discussion

Characterization of the synthesized silver nanoparticles (Ag-NPs):

The aqueous solution of the synthesized silver nanoparticles (Ag-NPs) was prepared according to Van Dong et al. (2012) with modifications as previously described in the previous report (Ibrahim et al. 2018a). Thereafter, the aqueous solution of Ag-NPs was scanned spectrophotometrically using (JASCO - UV/VIS -Model: V530) and a single peak of the Ag-NPs surface plasmon resonance was detected at 400 nm as shown in (Fig. 1), which confirmed the presence of the synthesized Ag-NPs. This result of the Ag-NPs surface plasmon resonance peak agrees with the results of Ibrahim et al. (2018a) and Van Dong et al. (2012).

The use of the spectrophotometer UV/VIS absorbance analysis is frequently used as a useful technique for the characterization of Ag-NPs. Therefore, several reports confirmed that the scan of the solutions of nanoparticles by the spectrophotometer UV/VIS absorbance is practical and produces a distinct single peak correlated to the size and the shape of the prepared nanoparticles (Xiong et al. 2011, Van Dong et al. 2012, and Ibrahim et al. 2018a).

The results of the Transmission Electron Microscope (TEM) analysis confirmed that the synthesized silver nanoparticles (Ag-NPs) were spherical in shape and mono-dispersed, with a size ranging between 1 and 8 nm as shown in (Fig. 2). In addition, the distribution percentage of the Ag-NPs sizes revealed that the majority of Ag-NPs were in a size range (2–6 nm) and represented about 90% of the dispersed silver nanoparticles in the micrograph, as shown in (Fig. 3). These results are in accordance with our previous study (Ibrahim et al. 2018a), as well as with (Van Dong et al. 2012) who reported that the size of the synthesized Ag-NPs ranged between 1 and 8 nm with an average diameter of 4 nm.

The influence of (Ag-NPs) or (STS) on microtuberization in potato cv. Spunta:

To overcome the retardation effect of ethylene and improve microtuberization in potato cv. Spunta, we used the developed medium of our previous report (Ibrahim et al. 2018a) as shown in (Table 1), in addition to the plant growth regulators (6 mg/l BAP and 1 mg/l TDZ) used recently in our last report (Soliman et al. 2022). Also, in this study, experiments were conducted to evaluate the impact of the anti-ethylene treatments (different concentrations; 0, 0.5, 1, 2, 4, 8, and 16 mg/l of silver nanoparticles; Ag-NPs or silver thiosulfate; STS) on microtuberization in potato cv. Spunta. The results of the inclusion of Ag-NPs or STS in culture media at different concentrations resulted in an increase in the average number of produced microtubers/jar and this increase reached a significant level in all Ag-NPs treatments, while this increase was significant only at 8 mg/l of STS, as shown in (Table 2 and Fig. 4&5). The Ag-NPs treatment (1 mg/l) recorded the highest average number of microtubers/jar (14.9 microtubers/jar), while (10.9 microtubers/jar) were recorded using (8 mg/l) of STS. These increments were about (83.95%) using (1 mg/l) of Ag-NPs and about (34.56%) using (8 mg/l) of STS compared to the control treatments (8.1 microtubers/jar), as shown in (Table 2).

Table 2
Effect of silver nanoparticles (Ag-NPs) or $AgNO_3$ (STS) on the average number of microtubers/jar.

Treatments concentration mg/l	Average No. of microtubers/jar			
	STS	% of Difference	Ag-NPs	% of Difference
0	8.1 ± 0.10	0	8.1 ± 0.11	0
0.5	8.2 ± 0.11	+ 1.23	10.3 ± 0.15	+ 27.16
1	8.5 ± 0.12	+ 4.93	**14.9 ± 0.24**	**+ 83.95**
2	9.1 ± 0.14	+ 12.34	14.3 ± 0.21	+ 76.54
4	9.5 ± .015	+ 17.28	12.7 ± 0.19	+ 56.79
8	**10.9 ± 0.18**	**+ 34.56**	11.5 ± 0.18	+ 41.97
16	9.7 ± 0.16	+ 19.75	11.1 ± 0.16	+ 37.03
L.S.D at 0.05	1.68			
The mean (± SE) of twenty replicates				

Table 3
Effect of silver nanoparticles (Ag-NPs) or $AgNO_3$ (STS) on the average weight of microtuber (mg).

Treatments concentration mg/l	Average weight of microtuber (mg)			
	STS	% of Difference	Ag-NPs	% of Difference
0	118.15 ± 1.81	0	120.33 ± 1.97	0
0.5	122.12 ± 1.96	+ 3.36	154.35 ± 3.02	+ 28.27
1	135.25 ± 2.11	+ 14.47	**203.51 ± 4.18**	**+ 69.12**
2	149.61 ± 2.91	+ 26.62	188.69 ± 4.07	+ 56.81
4	153.83 ± 3.14	+ 30.19	181.17 ± 3.58	+ 50.56
8	**176.27 ± 3.27**	**+ 49.19**	178.28 ± 3.24	+ 48.15
16	164.94 ± 3.19	+ 39.60	172.73 ± 3.02	+ 43.54
L.S.D at 0.05	33.84			
The mean (± SE) of twenty replicates				

Table 4
Effect of silver nanoparticles (Ag-NPs) or $AgNO_3$ (STS) on the average diameter of microtuber (mm).

Treatments concentration mg/l	Average diameter of microtuber (mm)			
	STS	% of Difference	Ag-NPs	% of Difference
0	4.3 ± 0.04	0	4.3 ± 0.04	0
0.5	4.6 ± 0.05	+ 6.97	6.2 ± 0.06	+ 44.18
1	5.5 ± 0.05	+ 27.90	**8.3 ± 0.09**	**+ 93.02**
2	5.6 ± 0.6	+ 30.23	7.6 ± 0.08	+ 76.74
4	6.3 ± 0.06	+ 46.51	7.3 ± 0.07	+ 69.76
8	**6.5 ± 0.06**	**+ 51.16**	6.6 ± 0.06	+ 53.48
16	6.3 ± 0.06	+ 46.51	6.3 ± 0.06	+ 46.51
L.S.D at 0.05	0.54			
The mean (± SE) of twenty replicates				

In addition, the highest average weight of microtuber (203.51 mg) was recorded using (1 mg/l) of Ag-NPs compared to (176.27 mg) using (8 mg/l) of STS which represents an increase of about 69.12% and 49.19% in comparison to the control treatments, respectively, as shown in (Table 3). As well, the highest average diameter of microtuber (8.3 mm) was recorded using (1 mg/l) of Ag-NPs compared to (6.5 mm) using (8 mg/l) of STS, and these increments were about 93.02% and 51.16% in comparison to the control treatments, respectively, as shown in (Table 4). Thus, these results confirm the positive impact of the inclusion of silver in culture media as an anti-ethylene agent either in the ionic form Ag^+ as STS or in the crystalline form as Ag-NPs and proved the superiority of the use of Ag-NPs at the low level (1 mg/l) over the use of STS (8 mg/l), Fig. 6.

The results of this study are consistent with those obtained in the previous study (Ibrahim et al. 2018a) using the genotype cv. Desirèe. Therefore, these results of two different genotypes of potato (Spunta and Desirèe) strongly lead us to conclude that the use of silver nanoparticles (Ag-NPs) proved to be more effective than silver thiosulfate (STS) to alleviate the inhibition effect of ethylene on the formation of microtubers in potato.

In addition to the known effect of silver ions on inhibiting ethylene action, there is another effect on the activation of polyamine biosynthesis in plants that was confirmed in different plant species. Accordingly, Kumar et al. (2009) mentioned that silver nitrate ($AgNO_3$) was widely used *in vitro* due to its potent inhibition of ethylene action on plant growth and development, and consequently enhancing growth and morphogenesis in plant tissue culture through increasing the regeneration capacity of shoots or somatic embryogenesis. Furthermore, S-adenosyl-L-methionine is the precursor of both ethylene and polyamines (Evans and Malmberg 1989; Bais and Ravishankar 2002). The role of ethylene in inhibiting the activities

of both methionine decarboxylase and arginine decarboxylase S-adenosyl led to a decrease in the synthesis of polyamines in pea seedlings (Apelbaum et al. 1985; Smith1985).

Therefore, the promotive effect of $AgNO_3$ as a potent ethylene inhibitor on organogenesis could be more likely attributed to the activation of polyamine biosynthesis than the reduction of ethylene biosynthesis (Kumar et al. 2009). Furthermore, Kim et al. (2016), reported that the addition of $AgNO_3$ and putrescine to the regeneration medium of *Polygonum tinctorium* improved the regeneration of shoots. More recently, Adly et al. (2022), reported that the addition of different concentrations of $AgNO_3$ (0, 2, 4, 6, 8, and 10 mg/l) to MS medium resulted in a remarkable increase in shoot regeneration from callus derived from internodal explants of potato which recorded the highest increase in number of shoot/explant using 4 mg/l $AgNO_3$ (2.3 fold compared to the control treatment), followed by the treatment of 6 mg/l $AgNO_3$ (1.6 fold). These results were attributed to the positive role of Ag^+ ions on inhibition of ethylene action in addition to increasing the accumulation of polyamines in the regenerated shoots, which recorded the highest level of a 3.6-fold increase over the control using 8 mg/l $AgNO_3$ without increasing the content of H_2O_2, lipid peroxidation, or damage of DNA. This finding may explain the substantial success of the use of silver ions in experiments of *in vitro* culture for different plants; potato (Zobayed et al. 2001; Ibrahim et al. 2018a; El-Sayed et al. 2021; Adly et al. 2022), sweet potato (Gong et al. 2005), canola (Khan et al. 2003), sesame (Al-Shafeay et al. 2011 & 2018), jojoba (Ibrahim et al. 2018b), buffalograss (Fei et al. 2000), faba bean (Khalafalla and Hattori 2000), safflower (Mandal et al. 2001), rice (Ibrahim and El Shihy 2012a&b), wheat (Hassan and Shahinul Islam 2021) and olive plant (Bashir et al. 2022).

Although, many reports confirm the positive effect of silver ions ($AgNO_3$ or silver thiosulfate; STS) as anti-ethylene on the growth of *in vitro* shoots (Perl et al. 1988) and on the formation of microtubers in potato plants (Vreugdenhil and Van Dijk 1989; Zobayed et al. 2001; El-Sayed et al. 2021). Up to the present, there are only two reports on the effect of (Ag-NPs) on the growth of *in vitro* cultured potato plants (Ehsanpour and Nejati 2013; Baltazar Bernal et al. 2023). Thus, Ehsanpour and Nejati (2013) reported that Ag-NPs at (0.5, 1, 1.5, and 2 ppm) led to an increase in the leaf area of *in vitro* potato plants cv. White Desirée, but the length of shoots and roots dramatically decreased. In addition, Baltazar Bernal et al (2023) disclosed that, the use of the gibberellin inhibitor paclobutrazol (PAC) 2 mg/l in combination with 50 mg/l of AgNPs as an anti-ethylene agent was the best treatment for *in vitro* conservation of potato plantlets for six months. Whereas after conservation the best combinations for *in vitro* multiplication of potato shoots were the treatments 50 mg/l of AgNPs with 1 or 2 mg/l of PAC.

Moreover, Syu et al. (2014) reported that silver nanoparticles AgNPs inhibit ethylene action and retard ethylene biosynthesis in *A. thaliana*. Sarmast et al. (2015), studied the effect of silver nanoparticles at concentrations; 0, 30, 60, or 120 mg/l (AgNPs) on gene expression of (*ACS* gene) of the key enzyme ACC synthase; 1-aminocyclopropane-1-carboxylate synthase (*ACS*) for the production of ethylene in leaf tissues of regenerated (*Tecomella undulata*) shoots. The results revealed that the expression of the *TuACS* gene was reduced in the leaves of *in vitro*-treated shoots with (AgNPs), which exhibited a remarkable delay in the senescence of explants. More recently, Sarmast and Salehi (2022), reported that

the addition of AgNPs (50 or 150 mg/l) to MS medium of *in vitro* seedlings of tobacco, resulted in a remarkable increase in leaf fresh weight, the height of plants, and root length for tobacco seedlings cultured on MS medium containing 50 mg/l of AgNPs compared to the control without AgNPs. Further, this treatment (50 mg/l of AgNPs) led to a decrease in the production of ethylene in closed vessels of *in vitro* tobacco seedlings, according to gas chromatography analysis. In addition, qRT-PCR analysis confirmed that the tissues of tobacco treated with (50 mg/l AgNPs) exhibited a downregulation of the key ethylene signaling genes *ETR1*, *ERS1*, and *CTR1* as well as a downregulation of the ethylene-synthesizing gene *ACS2*. Thus, these results provide evidence that AgNPs cause inhibition of ethylene action in addition to downregulation of ethylene production.

Finally, so far, we did not find any other reports on the effect of silver nanoparticles (Ag-NPs) on potato microtuberization, and this study is the second report after our previous report (Ibrahim et al. 2018a) using Ag-NPs for enhancing microtuberization in potato plants. Hence, these results confirmed the superiority of the use of Ag-NPs at the low level (1 mg/l) over the use of STS (8 mg/l) to alleviate the inhibition effect of ethylene on the formation of microtubers in potato. Therefore, the obtained results in this study on the genotype cv. Spunta, together with the results of the previous report (Ibrahim et al. 2018a) on cv. Desirée, proved that an efficient genotype-independent protocol for microtuberization in potato has been established using Ag-NPs. Accordingly, further molecular and physiological studies for enhancing potato microtuberization are in progress. In addition, these results are important for enhancing potato microtuberization technology at the mass-production level in Egypt.

Conclusions

The results of the current study substantially proved the positive effect of the inclusion of silver in culture media for promoting microtuberization in potato cv. Spunta, as an anti-ethylene agent either in the ionic form Ag^+ as STS or in the crystalline form as Ag-NPs. These results confirmed the superiority of the use of Ag-NPs at the low level (1 mg/l) over the use of STS (8 mg/l) to alleviate the inhibition effect of ethylene on the formation of potato microtubers. Thus, these findings complement and agree with those obtained in our previous study using the cv. Desirèe (Ibrahim et al. 2018a). Therefore, we report here that an efficient genotype-independent protocol for microtuberization in potato has been established. In addition, this developed protocol using Ag-NPs is important for enhancing potato microtuberization technology at the mass-production level in Egypt. Furthermore, these results are of great importance in plant physiology and nanobiotechnology research. As well as, these results strongly confirm the usefulness of using engineered nanomaterials to solve existing *in vitro* culture problems that hinder the progress in plant biotechnology and genetic engineering research. Thus, it could increase the progress of plant genetic improvement for achieving sustainable agriculture by producing novel high-yield plants capable of withstanding and tolerating the dangerous climate and environmental changes that threaten life on Earth.

Declarations

Acknowledgments

We would like to express our sincere gratitude and thanks to the Egyptian Government, the Ministry of Higher Education and Scientific Research represented by Science, Technology & Innovation Funding Authority (STDF) in collaboration with Egyptian Knowledge Bank (EKB) for their participation in the Transformative Agreement (plus fully OA Agreement) with Springer Nature for open access publishing with fees covered. We are truly appreciative of their support and commitment to advancing knowledge and research in Egypt.

Author contribution

Authors share equally; designed the research, conducted the experiment, analyzed Data, wrote the manuscript and all authors have read and approved the final manuscript.

Funding

This study was funded by Cairo University-Plant Biotechnology Research Laboratories (CU-PBRL) *Center of Excellence & Innovation in Plant Biotechnology*, Plant Physiology Department, Faculty of Agriculture, **Cairo University** in collaboration with Department of Agricultural Botany, Faculty of Agriculture, **Benha University** – Egypt.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interests.

Human and animal rights

This research did not involve experiments with human or animal participants.

Informed consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Data available with the paper or supplementary information:

All data supporting the findings of this study are available within the paper and its Supplementary Information. Example from: DOI: 10.5829/idosi.jhsop.2018.129.139
https://idosi.org/jhsop/10(3)18/4.pdf &
https://assjm.journals.ekb.eg/article_263821_6eefed816cb5a15678e6f2818440f638.pdf

References

1. Abeles FB (1973) Ethylene in plant biology. Academic press New York, 302 p. ISBN 978-0120414505.
2. Adly WM, Mazrou YA, EL-Denary ME, Mohamed MA, Abd El-Salam ET, Fouad AS (2022) Boosting Polyamines to Enhance Shoot Regeneration in Potato (*Solanum tuberosum* L.) Using AgNO3. Horticulturae 8(113):1–13. https://doi.org/10.3390/horticulturae8020113
3. Aghdaei M, Salehi H, Sarmast MK (2012) Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. micropropagation. Adv Hortic Sci 26:21–24.
4. Akita M, Takayama S (1994) Stimulation of potato (*Solanum tuberosum* L.) tuberization by semicontinuous liquid media surface level control. Plant Cell Rep 13:184–187.
5. Ali AM, Awad MY, Hegab SA, Gawad AE, Eissa MA (2020) Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. J Plant Nutrition 44(3):411–420. doi:10.1080/01904167.2020.1822399
6. Al-Shafeay AF, Ibrahim AS, Nesiem MR, Tawfik MS (2011) Establishment of regeneration and transformation system in Egyptian sesame (*Sesamum indicum* L.) cv Sohag 1. GM Crops 2(3):1–11
7. Al-Shafeay AF, Ibrahim AS, Nesiem MR, Tawfik MS (2018) Dehydration Stress Tolerance in Sesame Plants (*Sesamum indicum* L.) Overexpressing the *Arabidopsis thaliana*-Molybdenum Cofactor Sulfurase *LOS5/ABA3* Gene Under Greenhouse Conditions. Biosci Res 15(4):3131–3143
8. Anjum S, Abbasi BH, Hano C (2017) Trends in accumulation of pharmacologically important antioxidant-secondary metabolites in callus cultures of *Linum usitatissimum* L. Plant Cell Tiss Organ Cult 129:73–87. DOI 10.1007/s11240-016-1158-3
9. Apelbaum A, Goldlust A, Icekson I (1985) Control by Ethylene of Arginine Decarboxylase Activity in Pea Seedlings and Its Implication for Hormonal Regulation of Plant Growth. Plant Physiol 79:635–640.
10. Badoni A, Chauhan JS (2009) Microtuber: A Source of Germplasm Conservation. Report and Opinion 1(3):69–71
11. Bais HP, Ravishankar G (2002) Role of Polyamines in the Ontogeny of Plants and Their Biotechnological Applications. Plant Cell Tiss Organ Cult 69:1–34
12. Bais HP, Sudha G, Suresh B, Ravishankar GA (2000) $AgNO_3$ influences *in vitro* root formation in *Decalepis hamiltonii* Wight, Arn. Current Sci 79:894–898
13. Baltazar Bernal O, Spinoso-Castillo JL, Mancilla-Álvarez E, Muñoz-Márquez Trujillo RA, Bello-Bello JJ (2023) *In Vitro* Conservation and Regeneration of Potato (*Solanum tuberosum* L.): Role of Paclobutrazol and Silver Nanoparticles. Horticulturae, 9:676, 1–11. https://doi.org/10.3390/horticulturae9060676
14. Bashir MA, Silvestri C, Salimonti A, Rugini E, Cristofori V, Zelasco S (2022) Can Ethylene Inhibitors Enhance the Success of Olive Somatic Embryogenesis?. Plants 11(168):1–10. https://doi.org/10.3390/plants11020168
15. Beyer EM (1976a) A potent inhibitor of ethylene action in plants. Plant Physiol 58(3):268–271

16. Beyer EM (1976b) Silver ion: a potent anti-ethylene agent in cucumber and tomato. HortScience 11(3):175–196.
17. Biddington NL (1992) The influence of ethylene in plant tissue culture. Plant Growth Regulation 11:173–187
18. Castro-González CG, Sánchez-Segura L, Gómez-Merino FC, Bello-Bello JJ (2019) Exposure of stevia (*Stevia rebaudiana* B.) to silver nanoparticles *in vitro*: Transport and accumulation. Sci. Rep 9:10372.
19. Chen HC, Roco MC, Son JB, Jiang S, Larson CA, Gao Q (2013) Global nanotechnology development from 1991 to 2012: patents, scientific publications, and effect of NSF funding. J Nanopart Res 15:1951–1971. https://doi.org/10.1007/s11051-013-1951-4
20. Cuong DM, Cong Du P, Tung HT, Ngan HTM, Luan VQ, Phong TH, Khai HD, Phuong TTB, Nhut DT (2021) Silver nanoparticles as an effective stimulant in micropropagation of *Panax vietnamensis* a valuable medicinal plant. Plant Cell Tiss Organ Cult 146:577–588. https://doi.org/10.1007/s11240-021-02095-2
21. Davies PJ (1987) The plant hormones: their nature, occurrence, and functions. In: DAVIES P.J. ed. Plant Hormones and Their role in Plant Growth and Development, Ch. Al. Boston: Martinus Nijhoff
22. De Morais TP, Asmar SA, Silva HF, Luz JM, de Melo B (2018) Application of tissue culture techniques in potato. Biosci JUberlândia 34(4):952–969
23. Donnelly D, Coleman WK, Coleman SE (2003) Potato Microtuber Production and Performance: A Review. Amer J Potato Res 80:103–115
24. Duncan DR, Williams ME, Zehr B, Widholm JM (1985) The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta 165(3):322–332
25. Ehsanpour AA, Nejati Z (2013) Effect of nanosilver on potato plant growth and protoplast viability. Biological Lett 50(1):35–43
26. El-Anany AM, Abdel-Aziz F, Khafagy, EY (2019) Potato cultivation and production. Technical issue No. 1376 (In Arabic), Central Administration of Agricultural Extension, Ministry of Agriculture, Egypt
27. Elatafı E, Fang J (2022) Effect of Silver Nitrate ($AgNO_3$) and Nano-Silver (Ag-NPs) on Physiological Characteristics of Grapes and Quality during Storage Period. Horticulturae 8:419, 1-17. https://doi.org/10.3390/horticulturae8050419
28. El-Sayed SF, Taha SS, Darwish OS, Mwessongo SZ (2021) Effect of silver thiosulphate and photoperiod on *in vitro* tuberization of three potato (*Solanum tubersum* L.) cultivars. Plant Archives 21(1):308–317
29. Evans PT, Malmberg RL (1989) Do Polyamines Have Roles in Plant Development?. Annu Rev Plant Biol 40:235–269
30. Ewais EA, Desouky SA, Elshazly EH (2015) Evaluation of Callus Responses of *Solanum nigrum* L. Exposed to Biologically Synthesized Silver Nanoparticles. Nanosci Nanotechnol 5:45–56
31. FAOSTAT. Food and Agriculture Organization of the United Nations (2022a) COMMITTEE ON AGRICULTURE, Proposal for an International Day of Potato. COAG/2022/19. Available online:

https://www.fao.org/3/nj015en/nj015en.pdf

32. FAOSTAT: Food and Agriculture Organization of the United Nations. World Food and Agriculture – Statistical Yearbook (2022b) Available online: https://www.fao.org/3/cc2211en/cc2211en.pdf
33. Fei SZ, Read PE, Riordan TP (2000) Improvement of embryogenic callus induction and shoot regeneration of buffalograss by silver nitrate. Plant Cell Tiss Organ Cult 60:197–203
34. Gardea-Torresdey JL, Rico CM, White JC (2014) Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. Environ Sci Technol 48:2526–2540
35. Giridhar P, Indu E, Vijaya Ramu D, Ravishankar G (2003) Effect of silver nitrate on *in vitro* shoot growth of coffee. Trop Sci 43:144–146
36. Gong Y, Gao F, Tang K (2005) *In vitro* high frequency direct root and shoot regeneration in sweet potato using the ethylene inhibitor silver nitrate. South African J Botany 71(1):110–113
37. Gupta SD, Agarwala A, Pradhan S (2018) Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: an insight from antioxidative enzyme activities and gene expression patterns. Ecotoxicol Environ Saf 161:624–633
38. Ha NTM, Manh Do C, Hoang TT, Ngo ND, Bui LV, 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 Tiss Organ Cult 141:393–405. https://doi.org/10.1007/s11240-020-01796-4
39. Halterman D, Charkowski A, Verchot J (2012) Potato, viruses, and certification in the USA to provide healthy propagated tubers. Pest Technology 6(1):1–14
40. Hassan MF, Shahinul Islam SM (2021) Effect of silver nitrate and growth regulators to enhance anther culture response in wheat (*Triticum aestivum* L.). Heliyon 7:1–6. e07075 https://www.cell.com/heliyon/pdf/S2405-8440(21)01178-6.pdf
41. Ibrahim AS, El Shihy OM (2012a) High-throughput regeneration from mature embryos of eleven commercial rice (*Oryza sativa* L.) cultivars through somatic embryogenesis using a novel genotype independent protocol. Res J Agric Biol Sci 8(2):336–354
42. Ibrahim AS, El Shihy OM (2012b) An efficient *Agrobacterium tumefaciens*-mediated transformation of some elite Egyptian rice cultivars. Res J Agric Biol Sci 8(3):355–368
43. Ibrahim AS, El Shihy OM, Hamdy BM (2018b) An Improved Regeneration Protocol of Female Jojoba(*Simmondsia chinensis*) Plants from Callus Derived from Leaf Explants. J Horti Sci Ornam Plants 10(2):81-90
44. Ibrahim AS, Fahmy AH, Ahmed SS (2018a) Silver Nanoparticles Promote Microtuberization in Potato (*Solanum tuberosum* L.) cv. Desirée. J Hort Sci Ornamen Plants 10(3):129–139. DOI: 10.5829/idosi.jhsop.2018.129.139 https://idosi.org/jhsop/10(3)18/4.pdf
45. Ibrahim AS, Fahmy AH, Ahmed SS (2019) Copper nanoparticles elevate regeneration capacity of (*Ocimum basilicum* L.) plant via somatic embryogenesis. Plant Cell Tiss Org Cult 136:41–50

46. Jackson MB, Abbott AJ, Belcher AR, Hall KC (1987) Gas exchange in plant tissue cultures. In: Jackson MB, Mantell S, Blake J, eds. Advances in the chemical manipulation of plant tissue cultures. BPGRG Monograph 16. Bristol: British Plant Growth Regulator Group 57–71
47. Jadczak P, Kulpa D, Bihun M, Przewodowski W (2019) Positive effect of AgNPs and AuNPs in *in vitro* cultures of *Lavandula angustifolia* Mill. Plant Cell Tiss Organ Cult 139:191–197. https://doi.org/10.1007/s11240-019-01656-w
48. Kaveh R, Li YS, Ranjbar S, Tehrani R, Brueck CL, Van Aken B (2013) Changes in *Arabidopsis thaliana* gene expression in response to silver nanoparticles and silver ions. Environ Sci Technol 47:10637–10644
49. Khalafalla MM, Hattori K (2000) Ethylene inhibitors enhance *in vitro* root formation on faba bean shoots regenerated on medium containing thidiazuron. Plant Growth Regulation 32:59–63
50. Khan MR, Rashid H, Ansar M, Chaudry Z (2003) High frequency shoot regeneration and *Agrobacterium*-mediated DNA transfer in Canola (*Brassica napus*). Plant Cell Tiss Org Cult 75:223–231
51. Kim DH, Gopal J, Sivanesan I (2017) Nanomaterials in plant tissue culture: the disclosed and undisclosed. RSC Adv 7:36492–36505
52. Kim JK, Baskar TB, Park SU (2016) Silver nitrate and putrescine enhance *in vitro* shoot organogenesis in *Polygonum tinctorium*. Biosci Biotechnol Res Asia 13:53–58
53. Koch M, Naumann M, Pawelzik E Gransee A, Thiel H (2020) The importance of nutrient management for potato production Part I: Plant nutrition and yield. Potato Res 63:97–119
54. Kumar PP, Dimps Rao C, Goh CJ (1998) Influence of petiole and lamina on adventitious shoot initiation from lamina explants of Paulownia fortunei. Plant Cell Rep 17:886–890
55. Kumar V, Parvatam G, Ravishankar GA (2009)$AgNO_3$: a potential regulator of ethylene activity and plant growth modulator. Electron J Biotechnol 12: 8–9
56. Lau O-L, Yang SF (1976) Inhibition of ethylene production by cobaltous ion. Plant Physiol 58(1):114–117
57. Levy D, Seabrook JA, Coleman S (1993) Enhancement of tuberization of axillary shoot buds of potato (*Solanum tuberosum* L.) cultivars cultured *in vitro*. J Exper Botany 44:381–386
58. Lieberman N (1979) Biosynthesis and action of ethylene. Ann Rev of Plant Physiol 30:533–591
59. Mandal AK, Gupta SD, Chatterji AK (2001) Factors affecting somatic embryogenesis from cotyledonary explants of safflower. Biol Plant 44:503–507
60. Manokari M, Raj MC, Dey A, Faisal M, Alatar AA, Joshee N, Shekhawat MS (2023) Silver nanoparticles improved morphogenesis, biochemical profile and micro-morphology of *Gaillardia pulchella* Foug cv. 'Torch Yellow'. Plant Cell Tissue Organ Cult 153:1–13.
61. Mattoo AK, Suttle JC (1991) The plant hormone ethylene. CRC press. Boca Raton, Florida, 337 p. ISBN 0-8493-4566-9.

62. Mingo-Castel AM, Smith OF, Kumamoto J (1976) Studies on the carbon dioxide promotion and ethylene inhibition of tuberization in potato explants cultured *in vitro*. Plant Physiol 57:480–485
63. Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant 15:473–497
64. Perl A, Aviv D, Galun E (1988) Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. Plant Cell Rep 7:403–406
65. Pua EC, Chi GL (1993) De novo shoot morphogenesis and plant growth of mustard (*Brassica juncea*) *in vitro* in relation to ethylene. Physiol Plant 88:467–474
66. Purnhauser L, Medgyesy P, Czakó M, Dix PJ, Márton L (1987) Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia* Viv. tissue cultures using the ethylene inhibitor $AgNO_3$. Plant Cell Rep 6:1–4
67. Radouani A, Lauer FI (2015) Effect of NPK media concentrations on i*n vitro* potato tuberization of cultivars Nicola and Russet Burbank. Am J Potato Res 92:294–297
68. Ramezannezhad R, Aghdasi M, Fatemi M (2019) Enhanced production of cichoric acid in cell suspension culture of *Echinacea purpurea* by silver nanoparticle elicitation. Plant Cell Tiss Organ Cult 139:261–273. https://doi.org/10.1007/s11240-019-01678-4
69. Ramírez-Mosqueda MA, Sánchez-Segura L, Hernández-Valladolid SL, Bello-Bello E, Bello-Bello JJ (2020) Influence of silver nanoparticles on a common contaminant isolated during the establishment of *Stevia rebaudiana* Bertoni culture. Plant Cell Tiss Organ Cult 143:609–618. https://doi.org/10.1007/s11240-020-01945-9
70. Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB (1999) A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science 283:996–998
71. Roustan JP, Latche A, Fallot J (1989) Stimulation of *Daucus carota* somatic embryogenesis by inhibitors of ethylene biosynthesis: cobalt and nickel. Plant Cell Rep 8(3):182–185
72. Roustan JP, Latche A, Fallot J (1990) Control of carrot somatic embryogenesis by $AgNO_3$ an inhibitor of ethylene action effect on arginine decarboxilase activity. Plant Sci 67(1):89–95
73. Rui M, Ma C, Tang X, Yang J, Jiang F, Pan Y, Xiang Z, Hao Y, Rui Y, Cao W, Xing B (2017) Phytotoxicity of silver nanoparticles to peanut (*Arachis hypogaea* L.): physiological responses and food safety. ACS Sustain Chem Eng 5:6557–6567
74. 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 Tiss Organ Cult 134:289–300. https://doi.org/10.1007/s11240-018-1423-8
75. Salama HM (2012). Effects of Ag nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Int Res J Biotechnol 3:190–197
76. Sarmast M, Niazi A, Salehi H, Abolimoghadam A (2015) Silver nanoparticles affect ACS expression in *Tecomella undulata in vitro* culture. Plant Cell Tiss Org Cult 121:227–236

77. Sarmast M, Salehi H (2022) Sub-lethal concentrations of silver nanoparticles mediate a phytostimulatory response in tobacco via the suppression of ethylene biosynthetic genes and the ethylene signaling pathway. *In Vitro* Cellular Devel Biology – Plant 58:1–14
78. Smith T (1985) Plant Polyamines Metabolism and Function Polyamine Synthesis. In Polyamines and Ethylene: Biochemistry, Physiology and Interaction; American Society of Plant Physiologists: Rockville, MD, USA, pp. 1–23
79. Snedecor GW, Cochran WG (1980) Statistical methods, 7th edn. Iowa State University Press, Ames
80. Soliman AM, Abd El-Dayem HM, Mady MA, Ibrahim AS, Eid RS (2022 Effect of Thidiazuron and 6-Benzylaminopurine on *in vitro* microtuberization in potato cv. Spunta.Annals Agric Sci Moshtohor 60(3):799–808.https://assjm.journals.ekb.eg/article_263821_6eefed816cb5a15678e6f2818440f638.pdf
81. Songstad DD, Duncan DR, Widholm JM (1988) Effect of 1-aminocycopropane-1-carboxilic acid silver nitrate and norbornadiene on plant regeneration from maize callus cultures. Plant Cell Rep 7(4):262–265
82. Spinoso-Castillo JL, Chavez-Santoscoy RA, Bogdanchikova N, Pérez-Sato JA, Morales-Ramos V, Bello-Bello JJ (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 Organ Cult 129:195–207
83. Sun J, Wang L, Li S, Yin L, Huang J, Chen C (2017) Toxicity of silver nanoparticles to *Arabidopsis*: inhibition of root gravitropism by interfering with auxin pathway. Environ Toxicol Chem 36:2773–2780
84. Syu Y, Hung J-H, Chen J-C, Chuang H (2014) Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. Plant Physiol Biochem 83:57–64
85. Tahmasbi D, Zarghami R, Azghandi AV, Chaichi M (2011) Effects of nanosilver and nitroxin biofertilizer on yield and yield components of potato minitubers. Int J Agric Biol 13:986–990
86. Timoteo Cd, Paiva R, dos Reis MV, Claro PIC, Corrêa da Silva DP, Marconcini JM, Elvis de Oliveira J (2019) Silver nanoparticles in the micropropagation of *Campomanesia rufa* (O. Berg) Nied. Plant Cell Tiss Organ Cult 137:359–368. https://doi.org/10.1007/s11240-019-01576-9
87. Tomaszewska-Sowa M, Rewers M, Bocian K, Sliwinska E (2023) Endoreduplication changes in five *in-vitro*-grown crops upon treatment with silver nanoparticles. Plant Cell Tiss Organ Cult. https://doi.org/10.1007/s11240-023-02563-x
88. Tung HT, Thuong TT, Cuong DM, Luan VQ, Hien VT, Hieu T, Nam NB, Phuong HTN, Vinh BVT, Khai HD, Nhut DT (2021) Silver nanoparticles improved explant disinfection, *in vitro* growth, runner formation and limited ethylene accumulation during micropropagation of strawberry (*Fragaria* x *ananassa*). Plant Cell Tiss Organ Cult 145:393–403. https://doi.org/10.1007/s11240-021-02015-4
89. Tymoszuk A, Kulus D (2020) Silver nanoparticles induce genetic, biochemical, and phenotype variation in chrysanthemum. Plant Cell Tiss Organ Cult 143:331–344. https://doi.org/10.1007/s11240-020-01920-4

90. Van Dong P, Ha CH, Binh LT, Kasbohm J (2012) Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. Intern Nano Letters 2:1–9
91. Vannini C, Domingo G, Onelli E, Prinsi B, Marsoni M, et al. (2013) Morphological and Proteomic Responses of *Eruca sativa* Exposed to Silver Nanoparticles or Silver Nitrate. PLoS ONE 8(7):1–8. e68752. doi:10.1371/journal.pone.0068752
92. Vreugdenhil D, Van Dijk W (1989) Effects of ethylene on the tuberization of potato (*Solanum tuberosum*) cuttings. Plant Growth Regulation 8:31–39
93. Wang B, Ma Y, Zhang Z, Wu Z, Wu Y, Wang Q, Li M (2011) Potato viruses in China. Crop Protection 30:1117–1123
94. Xiong J, Wang Y, Xue Q, Wu X (2011) Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid. Green Chem 13:900–904
95. Yang SF (1985) Biosynthesis and action of ethylene. HortScience 20(1):41–45
96. Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol 35:155–189
97. Zahir A, Nadeem M, Ahmad W, Giglioli-Guivarc'h N, Hano C, Abbasi BH (2019) Chemogenic silver nanoparticles enhance lignans and neolignans in cell suspension cultures of *Linum usitatissimum* L. Plant Cell Tiss Organ Cult 136:589–596. https://doi.org/10.1007/s11240-018-01539-6
98. Zhang ZJ, Zhou WJ, Li HZ (2005). The role of GA, IAA and BAP in the regulation of *in vitro* shoot growth and microtuberization in potato. Acta Physiol Plant 27:363–369
99. Zhao XC, Qu X, Mathews DE, Schaller GE (2002) Effect of ethylene pathway mutations upon expression of the ethylene receptor ETR1 from *Arabidopsis*. Plant Physiol 130:1983–1991
100. Zobayed SM, Armstrong J, Armstrong W (2001) Micropropagation of potato evaluation of closed, diffusive and forced ventilation on growth and tuberization. Annals of Botany 87:53–59

Figures

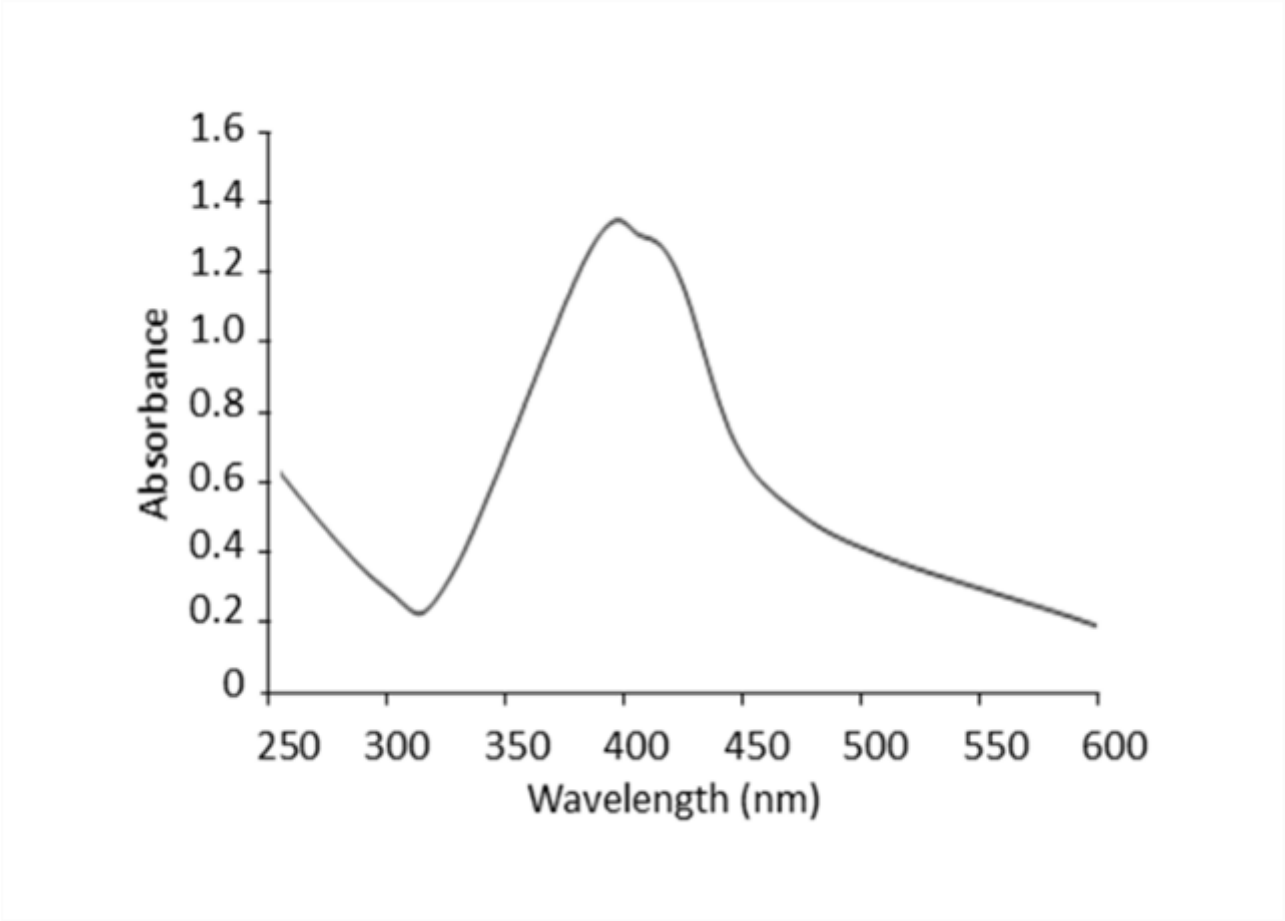

Figure 1

Photograph of the scanned solution of the engineered Ag-NPs using the spectrophotometer UV/VIS absorbance; the peak appeared near 400 nm

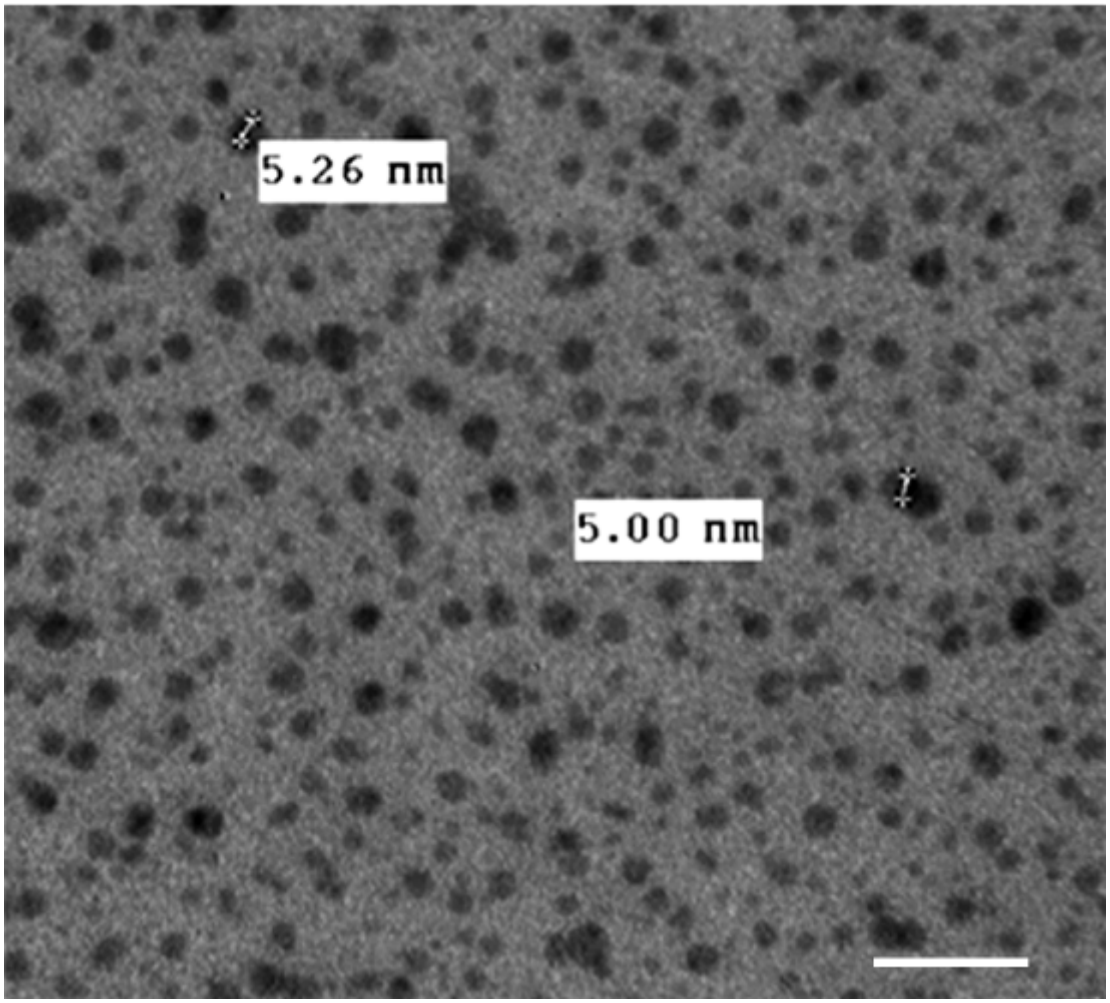

Figure 2

The engineered Ag-NPs with illustrated particle sizes. The monodispersed Ag-NPs are ultrafine and spherical in shape, with a size range (1–7 nm). The TEM micrograph scale bar is 20 nm

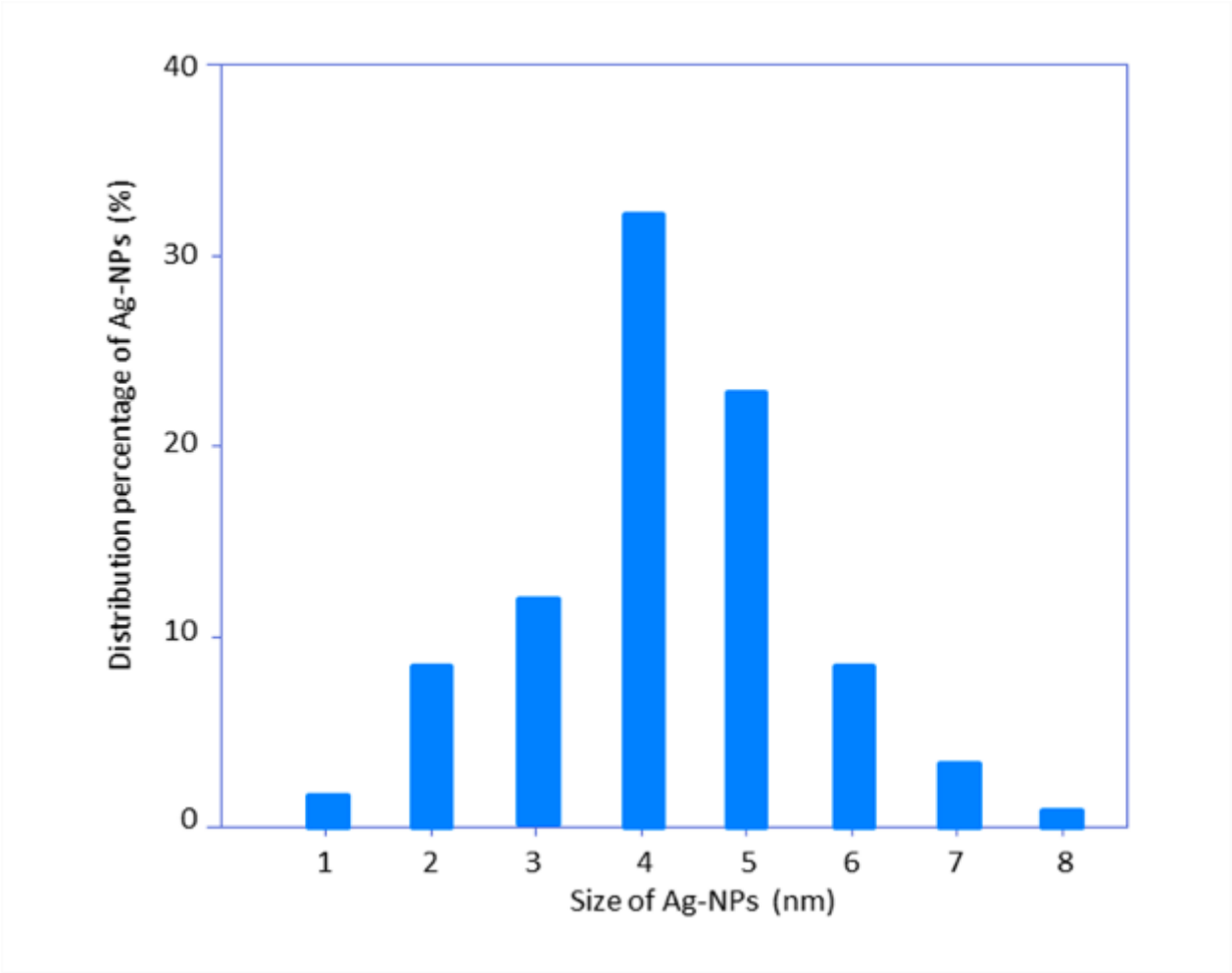

Figure 3

The percentage of the engineered silver nanoparticles Ag-NPs distributed in the TEM micrograph. The majority of the dispersed Ag-NPs were in the size range of (2–6 nm) and represented about 90% of silver nanoparticles in the micrograph

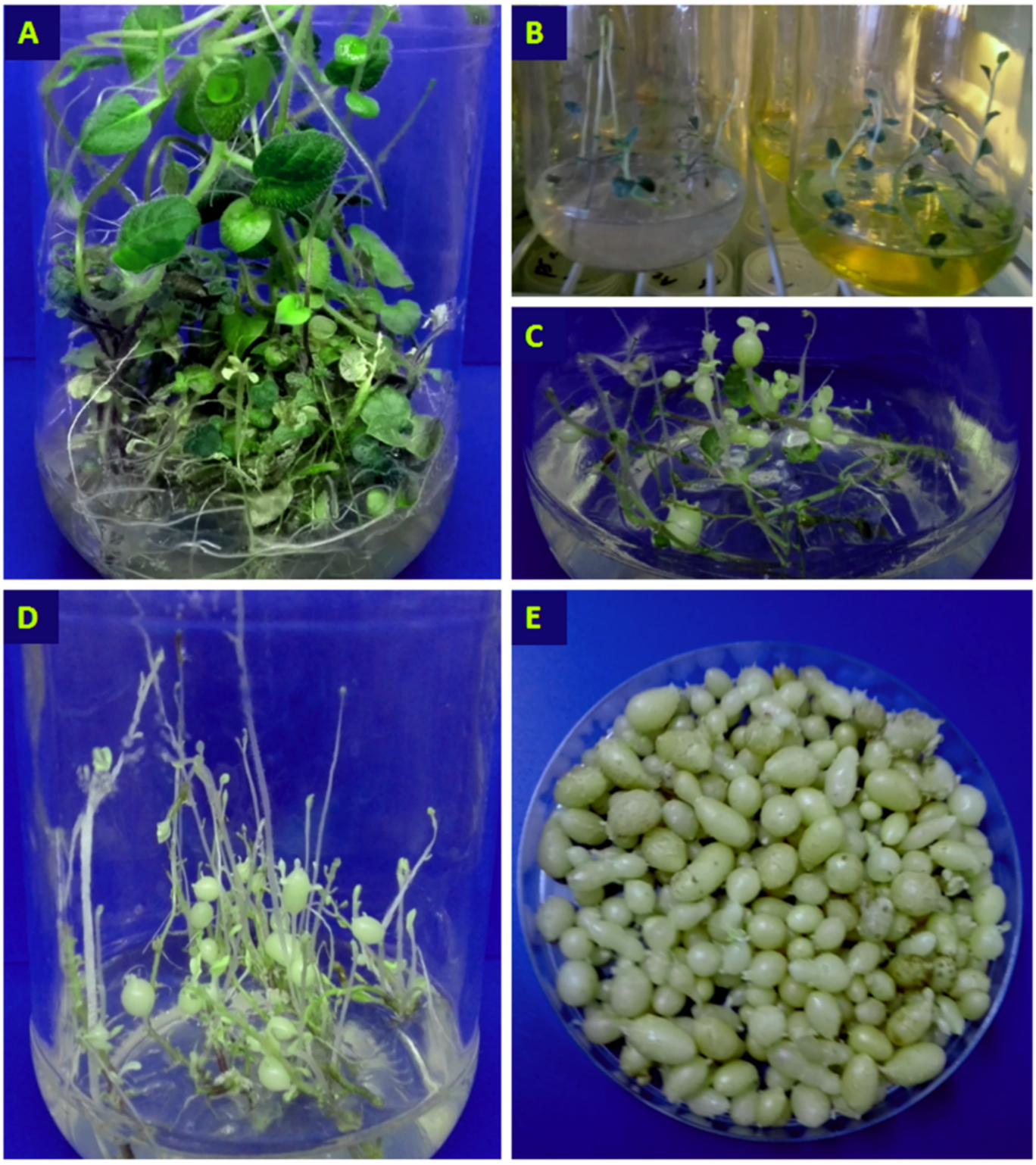

Figure 4

Promotion of microtubers formation in potato cv. Spunta using silver nanoparticles (Ag-NPs). (A) Potato shoots were used for microtubers production. (B) Shoot explants of potato cultured on microtubers formation medium (MFM) + Ag-NPs. (C) Initiation of microtubers after two weeks on MFM + Ag-NPs. (D) Production of microtubers after 10 weeks on MFM + Ag-NPs. (E) Harvest of microtubers after 10 weeks of culture on MFM + Ag-NPs

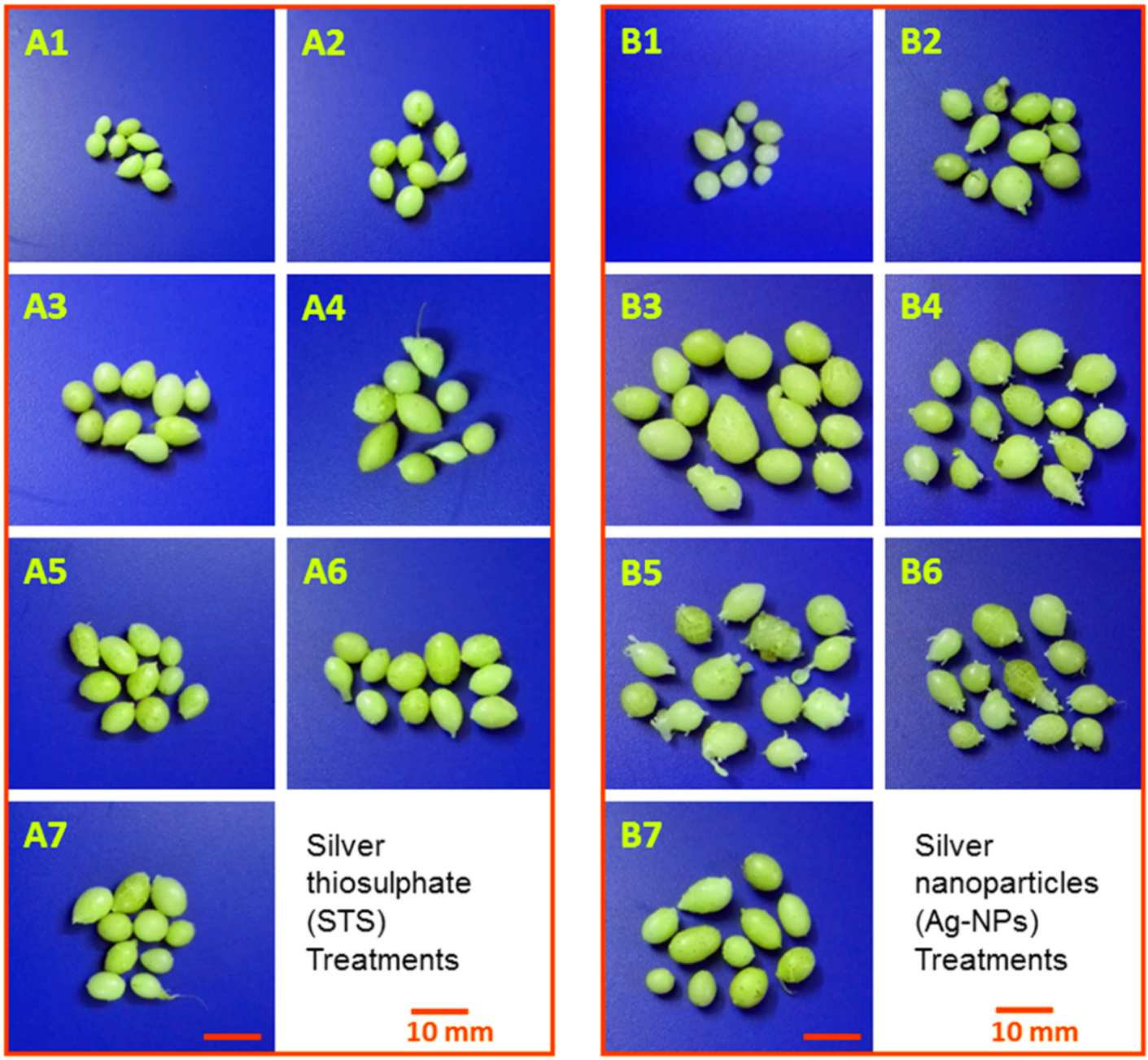

Figure 5

The effect of silver nanoparticles (Ag-NPs) on microtubers formation in potato cv. Spunta in comparison with silver thiosulfate (STS), the treatments of STS (concentrations A1 – A7; 0, 0.5, 1, 2, 4, 8, and 16 mg/l), the treatments of Ag-NPs (concentrations B1 – B7; 0, 0.5, 1, 2, 4, 8, and 16 mg/l)

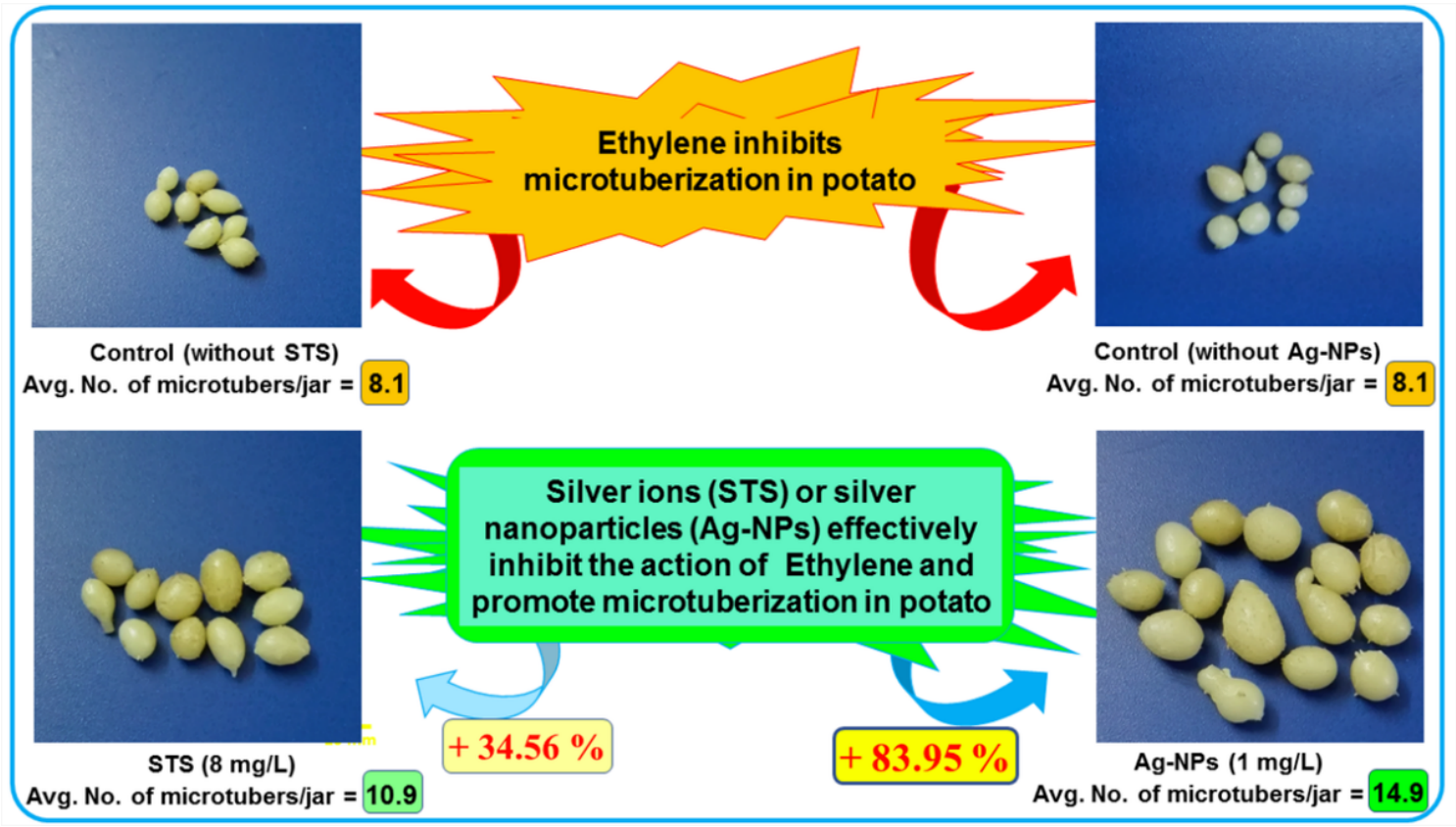

Figure 6

The inclusion of silver thiosulfate or silver nanoparticles in culture media alleviates the inhibitory impact of ethylene on microtuberization in potato. The use of the crystalline form of silver as silver nanoparticles (Ag-NPs) at the low level (1 mg/l) was superior to the ionic form as silver thiosulfate (STS) at a high level (8 mg/l) for inhibiting the action of ethylene and led to increasing the average number of microtubers/jar (14.9 and 10.9) which about 83.95% and 34.56% respectively, over the control treatment (8.1)