

Silver Nanoparticles Promote Microtuberization in Potato (*Solanum tuberosum* L.) cv. Desirée

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Abstract: The utilization of nano-materials in plant physiology experiments as a factor represents a milestone in the field of nanobiotechnology. This study is the first report on the effect of the anti-ethylene silver in the crystalline form as engineered nanoparticles on microtuberization in potato compared to the ionic form Ag^+ as silver thiosulphate (STS). Chemical reduction method was used to prepare the engineered silver nanoparticles (AgNPs) which were monodispersed and spherical in shape and the most of the AgNPs about 80% were in a size range (2-4 nm) according to the Transmission Electron Microscope (TEM) analysis. Different concentrations (0, 0.5, 1, 2, 4, 8 and 16 mg/l) of AgNPs or Ag^+ as silver thiosulphate (STS) were used for investigating their influence on microtubers induction in microtubers induction medium (MIM) and microtubers formation in microtubers formation medium (MFM). The results exhibited that, the maximum average number of microtubers/jar (18.95) was recorded using 1 mg/l of AgNPs compared to (14.60) using 8 mg/l of AgNO_3 (STS). These increases in the average number of microtubers/jar represent 97.40% and 51.30% using AgNPs and AgNO_3 (STS) compared to the control, respectively. Also, these results revealed that the use of AgNPs at (1mg/l) promote the formation of microtubers in potato cv. Desirée at the highest level (97.40%) which strongly indicate to the superiority of AgNPs over AgNO_3 (STS). Therefore, these results proved that AgNPs at low concentration (1mg/l) were more effective for producing the highest promotion of microtuberization in potato plant, which may be attributed to the superior properties of the crystalline form of nano-silver as AgNPs compared to the ionic form Ag^+ as AgNO_3 (STS). In addition, these promising results are useful for the advancement in microtubers production technology at large scale in Egypt. Furthermore, these results represent an essential step for enhancing the research progress using the engineered nanomaterials in plant nanobiotechnology.

Key words: Silver nanoparticles • Anti-ethylene • Potato • *Solanum tuberosum* • Microtubers

INTRODUCTION

Potato plant is the most important food crop after cereals (rice and wheat) in the world, which cultivated in the total area exceeded 19 million hectares [1, 2]. Potato is

an economically important crop in Egypt used for food and industry.

In vitro culture of potato stems as explants for microtuberization has been used for rapid production of disease-free potato seeds. Potato microtubers represent

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an important plant material which can be easily stored, transport and used effectively either for producing minitubers or for direct planting into the field. The advancement in microtubers research has vast potential for improving microtubers production as an essential technology for the production of potato seed tuber, also for improving the programs of potato breeding and conservation. In addition to the high potentiality of microtubers as a source for exchange and commercialization [3-13].

Ethylene inhibits the growth and morphogenesis in several plant species [14]. It is well known that plant cells produce ethylene during *in vitro* culture [15]. Some plants are very sensitive to ethylene such as potato and the accumulated ethylene *in vitro* can strongly retard the growth of shoots and reduce the leaf size. Also, the concentrations about $0.1 \mu\text{l l}^{-1}$ or less of ethylene can cause growth distortion of potato plantlets [16].

Furthermore, AgNO_3 promoted regeneration and growth of shoots in sweet potato, canola, sesame and in jojoba [17-20]. In addition, AgNO_3 improved the induction of embryogenic callus and regeneration of buffalograss shoots, enhanced the development of roots on regenerated shoots of faba bean [21, 22]. Also, AgNO_3 elevated somatic embryogenesis in safflower plant and in rice plants [23-25].

The advent of nano-science era vastly increased the potentialities of research using the engineered nano-materials in various fields. Thus, these engineered nano-materials with a particular size and shape exhibit novel properties and provide a possible horizon to find effective solutions for complex problems [26]. The application of the engineered nano-materials in agriculture aims to produce high yield, reduce the amounts of fertilizers and chemicals used for plant protection [27]. The influence of the engineered nano-materials on plants including growth, development, yield and quality of plant product [28].

The utilization of nano-materials in plant physiology experiments as a factor represents a milestone in the field of nanobiotechnology. The application of AgNPs (50 ppm) in combination with nitroxin increased yield and weight of potato minitubers [29]. Furthermore, the use of nano-materials at the level of *in vitro* culture of plants has emerged and several reports indicated to the usefulness of nanoparticles if used in culture media at a suitable concentration [30]. For instance, the use of silver nanoparticles (AgNPs) in culture media at 10 mg/l increased callus formation, shoot induction and the number of regenerated shoots of *Tecomella undulata*

plant [31]. Also, the nodal segments of *T. undulata* cultured on MS medium supplemented with 60 mg/l of AgNPs significantly increased shoot regeneration (%) and the average number of shoots/explant [32]. The addition of 5 mg/l of BA, 3 mg/l of NAA and 8 mg/l of AgNPs (30-90 nm) to MS medium increased callus formation (89%) and callus fresh weight/explant of *Solanum nigrum* plant [33]. The effect of AgNPs on potato plants *in vitro* did not study until recently. Thus, Ehsanpour and Nejati [34] reported that AgNPs at 0.5, 1, 1.5 and 2 ppm increased leaf area of *in vitro* potato plants cv. White Desirée, but the shoot and root length were dramatically decreased.

So far there are no reports on the effect of silver nanoparticles on microtuberization in potato plant. Therefore, the aim of the present study was to investigate the effect of silver as anti-ethylene either in the crystalline form as silver nanoparticles (AgNPs) or in the ionic form Ag^+ as silver thiosulphate on microtuberization in potato cv. Desirée.

MATERIALS AND METHODS

Synthesis of the Engineered Silver Nanoparticles: The engineered silver nanoparticles (AgNPs) in a size range of 1-5 nm were chemically synthesized according to the method described by Van Dong *et al.* [35] with some modifications. In brief, silver nitrate (AgNO_3) was reduced using sodium borohydride (NaBH_4) in the presence of trisodium citrate dihydrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3 \cdot 2\text{H}_2\text{O}$) and polyvinylpyrrolidone (PVP; Mw= 40,000) as stabilizing and capping agent, all materials were purchased from Sigma. Thus, one ml of 5 mM of an aqueous solution of AgNO_3 was added to 47.5 ml of deionized H_2O in a 100 ml flask with vigorous stirring at room temperature. After 5 min, 0.5 ml of 30 mM (trisodium citrate dihydrate) and 0.5 ml of 5 mg/ml (PVP) were added. Finally, a cold aqueous solution 0.5 ml of 50 mM (NaBH_4) was added and the color of the solution changed immediately to a light yellow due to the formation of the AgNPs.

Characterization of the Engineered Silver Nanoparticles: The engineered silver nanoparticles (AgNPs) were characterized by the surface plasmon peak using the UV/VIS spectrophotometer (JASCO Model-V530). Thereafter, the samples of the synthesized AgNPs were suspended in ethanol (70 %) and sonicated for full dispersion (15 min) before determining the size of the particles using the Transmission Electron Microscope (TEM) JEM 1400-JEOL-Japan.

Table 1: Composition of different media used for induction and formation of potato microtubers, (a) the original MS medium, (b) the modified MS medium containing low level of ammonium and (c) the improved media formula used for the microtubers induction medium (MIM) and the microtubers formation medium (MFM).

Substances	(a) MS mg/l	(b) Modified MS mg/l	(c) the improved media	
			MIM mg/l	MFM mg/l
Salts				
MS salts without (NH ₄ NO ₃ , KNO ₃ and KH ₂ PO ₄)	913.36	913.36	913.36	913.36
KNO ₃	1900	1900	2628.6	4044
NH ₄ NO ₃	1650	165	165	825
KH ₂ PO ₄	170	170	510	510
Amino acids				
Casein hydrolysate	-	-	300	1000
Glycine	2	2	-	-
Carbohydrates				
Sucrose	30,000	30,000	90,000	80,000
Myo-inositol	100	100	150	250
Vitamins				
Thiamine.HCl	0.1	0.1	10	10
Pyridoxine.HCl	0.5	0.5	1	1
Nicotinic acid	0.5	0.5	1	1
Pantothenate	-	-	0.5	0.5
Biotin	-	-	0.01	0.01
Riboflavin	-	-	0.01	0.01
Folic acid	-	-	0.01	0.01
Plant growth regulators				
6-Benzylaminopurine (BAP)	-	-	10	10
Gelrite	-	-	2,000	2,000
pH	-	-	5.8	5.8

Plant material: Potato (*Solanum tuberosum* cv. Désirée) virus-free seed tubers were provided from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Culture Media of Induction and Formation of Potato Microtubers: The culture media used in the present work were sustainably developed through a set of preliminary experiments conducted in our laboratories according to intensive studies on media composition used for microtuberization in potato. Thus, the developed protocol used in this study including two media; the first medium for microtubers induction and the second medium for microtubers formation.

Preconditioning of the explants for high capacity of microtubers induction was performed through the culture of the *in vitro* shoots of potato cv. Désirée as explants containing 3-5 nodes of potato for two weeks on the microtubers induction medium (MIM) containing 9 % sucrose, (10 mg/l) 6-Benzylaminopurine (BAP), B5 vitamins according to Gamborg *et al.* [36], in addition to the additional vitamins, myo-inositol and casein

hydrolysate as shown in (Table 1) in jars (200 ml) containing 25 ml/jar. The (MIM) medium containing a low level of total nitrogen (30 mM) compared to the original MS medium which containing (60.01 mM) [37]. Moreover, the ratio between Nitrate: Ammonium was adjusted to be; 14:1 (28 mM nitrate: 2 mM ammonium) which represents a very low level of ammonium 2 mM (165 mg/l of NH₄NO₃) which about 1/10 in MS medium, as the modified low ammonium MS medium (b), compared to (39.4 mM nitrate: 20.61 mM ammonium) in MS medium (a) as shown in Table 1. In addition to the elevated concentration of potassium (29.75 mM) and phosphorus (3.75 mM) compared to (20.04 mM of K) and (1.25 mM of P) in MS medium. Thereafter, the explants of potato were subcultured on microtubers formation medium (MFM) containing 8 % sucrose and (10 mg/l) 6-Benzylaminopurine (BAP) in jars (400 ml) containing 50 ml/jar for 6 weeks. The (MFM) medium containing total nitrogen 60 mM with nitrate: ammonium ratio; 5:1 (50 mM nitrate: 10 mM ammonium), a high concentration of potassium (43.75 mM) and a high concentration of phosphorus (3.75 mM) as shown in (Table 1).

Different concentrations (0, 0.5, 1, 2, 4, 8 and 16 mg/l) of AgNPs or AgNO₃ as silver thiosulfate (STS; which was freshly prepared and containing 2 mg/ml AgNO₃) according to Perl *et al.* [38] were added to the culture media, then the double concentrated solution (2x) of the culture medium (500 ml) was filter sterilized using a 0.22 µm filter (Millipore-GVWP04700), the medium was prepared by mixing the filter-sterilized solution with the double concentrated Gelrite (500 ml which were sterilized by the autoclave) and divided into a proper volume in sterile jars. After 6 weeks of culture on the (MFM), the produced microtubers of each treatment were collected and the data were recorded.

Statistical Analysis: Statistical analyses of the recorded data were performed according to Snedecor and Cochran [39]. Each treatment was replicated (20 replicates/treatment) and each replicate contains one jar (6 explants/jar). The least significant difference test (L.S.D.) at the level ($p \leq 0.05$) was used to compare the mean values ($\pm$ SE of twenty replicates; $n = 20$) of treatments.

RESULTS AND DISCUSSION

Characterization of the Engineered Silver Nanoparticles (AgNPs): The synthesis of the engineered silver nanoparticles (AgNPs) in this study was achieved using

the method of Van Dong *et al.* [35] with the described modifications in materials and methods which resulted in the production of ultrafine spherical AgNPs (the yellow solution of silver).

The aqueous solution of the engineered AgNPs was scanned using the spectrophotometer (UV/VIS - JASCO - V530) and the surface plasmon resonance peak of the AgNPs was appeared as a single peak at 400 nm and confirmed the presence of AgNPs (Fig. 1). This result of the surface plasmon resonance peak of AgNPs is in accordance with the results of Van Dong *et al.* [35]. Scanning of the solutions of metal-nanoparticles using the spectrophotometer UV/VIS absorbance is a useful technique and produce specific peaks related to the size and the shape of nanoparticles [40]. The use of the UV-Vis spectrum analysis is frequently used for characterization of AgNPs [35].

The size of the engineered AgNPs was determined using the TEM. Thus, the TEM micrograph in (Fig. 2) showed that the produced AgNPs were monodispersed and spherical in shape with a size range (1- 8 nm). Moreover, most of the AgNPs about 80% were in a size range (2-4 nm) as shown in (Fig 3). These results are in accordance with those obtained by Van Dong *et al.* [35] using the same method and they reported that the ultrasmall size (1-12 nm) of silver nanoparticles with an average diameter of 4 nm was obtained.

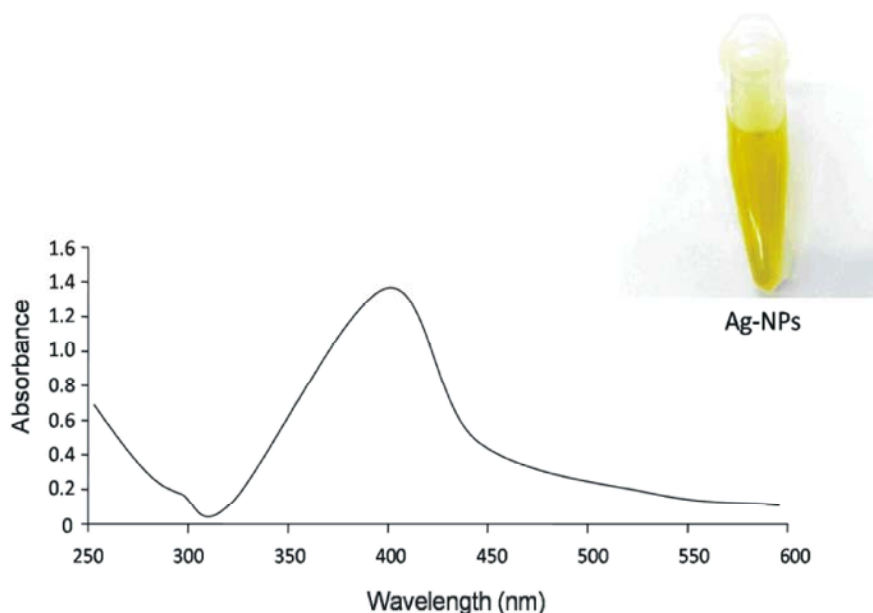


Fig. 1: The photograph of the engineered AgNPs using the absorption UV-visible spectrum. The sample was scanned and the peak of the engineered AgNPs appeared near 400 nm.

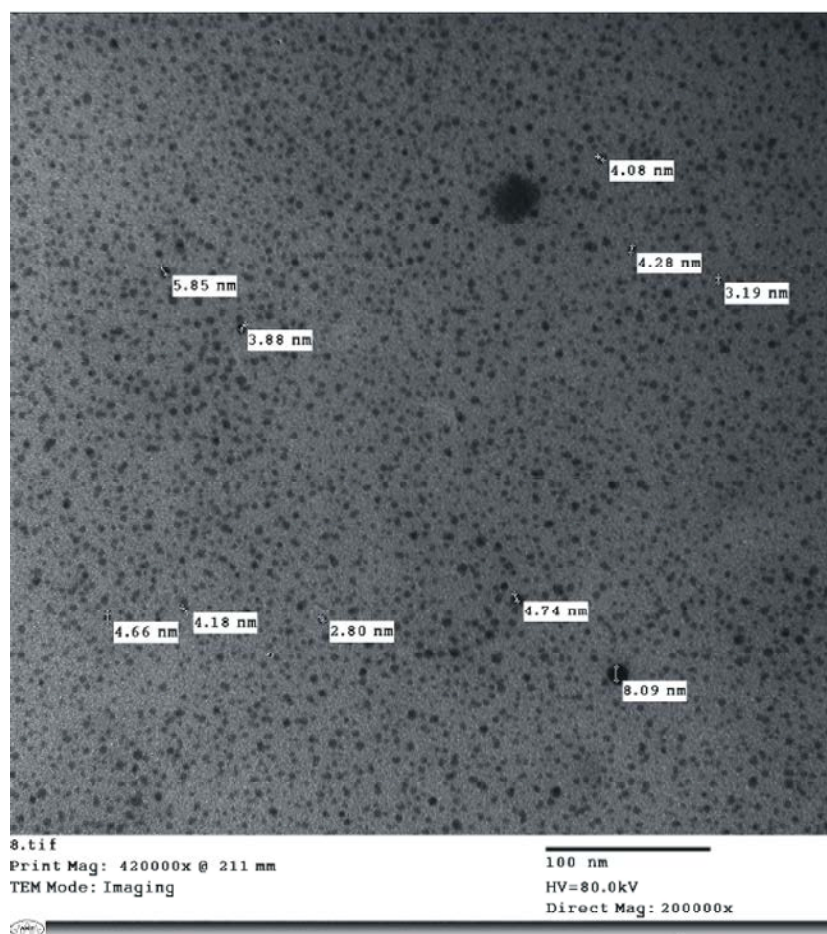


Fig. 2: The TEM micrograph of the engineered AgNPs with illustrated particle size. The monodispersed AgNPs are ultrafine and spherical in shape with a size range (1-8 nm). Scale bar; 100 nm.

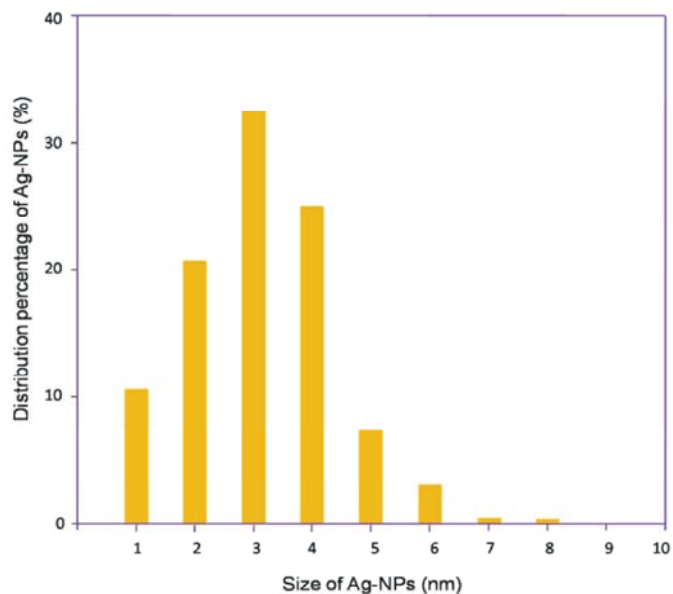


Fig. 3: The distribution percentage of the engineered AgNPs. The size of AgNPs (2-4 nm) represents about 80% of the produced silver nanoparticles.

Media Composition for the Production of Microtubers in Potato:

Preliminary experiments were conducted in our laboratories and resulted in the current improved protocol used in this study. Thus, the microtubers of potato were produced through two phases; the induction of microtubers (the explants were cultured on MIM for 2 weeks) and the formation of microtubers (the explants were cultured on MFM for 6 weeks).

In this study, the media used containing (8-9 % sucrose) were filter sterilized which mean that sucrose did not hydrolyze to glucose and fructose. Therefore, this protocol is standard to evaluate individual factors influencing the production of microtubers in potato. The high concentration of sucrose proved to be essential for microtuber formation in potato and also for producing microtubers in a large size [38, 41-47]. In addition, the growth of the induced microtubers is dependent on the availability of sucrose in the culture medium; the growth rate of microtubers was limited when sucrose was hydrolyzed to glucose and fructose [46]. The microtubers induction medium (MIM) containing a low level of total nitrogen (30 mM) and nitrate: ammonium ratio 14:1 (28 mM nitrate: 2 mM ammonium) as shown in (Table 1). This low level of ammonium is also important for microtuber induction in potato. Thus, in the microtubers induction medium (MIM) containing a ratio between total nitrogen and sucrose (30 mM nitrogen: 9% sucrose). In addition, the microtubers formation medium (MFM) containing 60 mM of total nitrogen and nitrate: ammonium ratio 5:1 (50 mM nitrate: 10 mM ammonium) and the ratio between total nitrogen and sucrose (60 mM nitrogen: 8% sucrose). Moreover, the concentrations of potassium and phosphorus were elevated to 43.75 mM and 3.75 mM in MFM, respectively.

These modifications are in accordance with findings of several workers which increased the formation of microtubers in different potato genotypes [48-54]. Accordingly, low level of total nitrogen in the culture medium is essential to induce microtuberization in potato [55, 56]. The ratio between the total nitrogen and sucrose (N:C) and the ratio between nitrate: ammonium is important for microtuberization in potato [48, 50]. The low levels of ammonium in culture media during the preconditioning of the potato plants used as a source for microtubers production resulted in increasing the number of the induced microtubers [49, 54, 57]. In addition, the high ratio (5:1) between nitrate:ammonium (50 mM nitrate:10 mM ammonium) promoted the growth of the produced microtubers (82%) weighed >1.0 g compared with only 22% weighed >1.0 g in the medium containing

the low ratio 2:1 (40 mM nitrate: 20 mM ammonium) according to Chen and Liao [57]. The higher concentrations of potassium and phosphorus than those in MS medium enhanced the microtuberization in potato cultivars (Nicola and Russet Burbank) according to Radouani and Lauer [12].

The Effect of AgNPs or STS on *in vitro* Microtuberization in Potato cv. Desiree:

To enhance the production of microtubers in potato cv. Desiree in this study we used the developed protocol containing the improved media in addition to silver as anti-ethylene in two forms; the ionic form Ag^+ as silver thiosulphate (STS) and the crystalline form as silver nanoparticles (AgNPs). The influence of the inclusion of AgNPs or silver thiosulphate (STS) in culture media at different concentrations (0, 0.5, 1, 2, 4, 8 and 16 mg/l) on microtuberization in potato (cv. Desirée) are shown in (Table 2 and Fig. 4). The initiation of microtubers formation (days to microtubers initiation) was affected positively by the inclusion of silver in the culture medium. However, different concentrations of AgNPs significantly decreased the period to initiate the microtubers formation compared to AgNO_3 (STS) which recorded significant decreases with only four concentrations (2, 4, 8 and 16 mg/l). The short period to initiate the microtubers formation (10.95 days) was recorded using 1 mg/l of AgNPs compared to the control treatment (20.95 days). Moreover, the average number of microtubers was increased due to the addition of AgNPs or AgNO_3 (STS) in culture media. The maximum average number of microtubers/jar (18.95) was recorded using 1 mg/l of AgNPs compared to (14.60) using 8 mg/l of AgNO_3 (STS). This increase in the average number of microtubers/jar was about (97.40%) using 1 mg/l of AgNPs and about (51.30%) using 8 mg/l of AgNO_3 (STS) compared to the control treatments. In addition, the highest average number of the produced microtubers was accompanied with the highest average weight of microtuber (312.18 mg or 238.22 mg) and the highest average diameter of microtuber (10.15 mm or 8.12 mm) using 1 mg/l of AgNPs or 8 mg/l of AgNO_3 (STS), respectively.

The use of AgNPs at different concentrations resulted in significant increases in the average number of the produced microtubers while the use of AgNO_3 (STS) resulted in significant increases with only four concentrations (2, 4, 8 and 16 mg/l). Moreover, the results confirmed that the different concentrations of AgNPs were superior to AgNO_3 (STS) and recorded significant increases in the average number of the produced microtubers/jar.

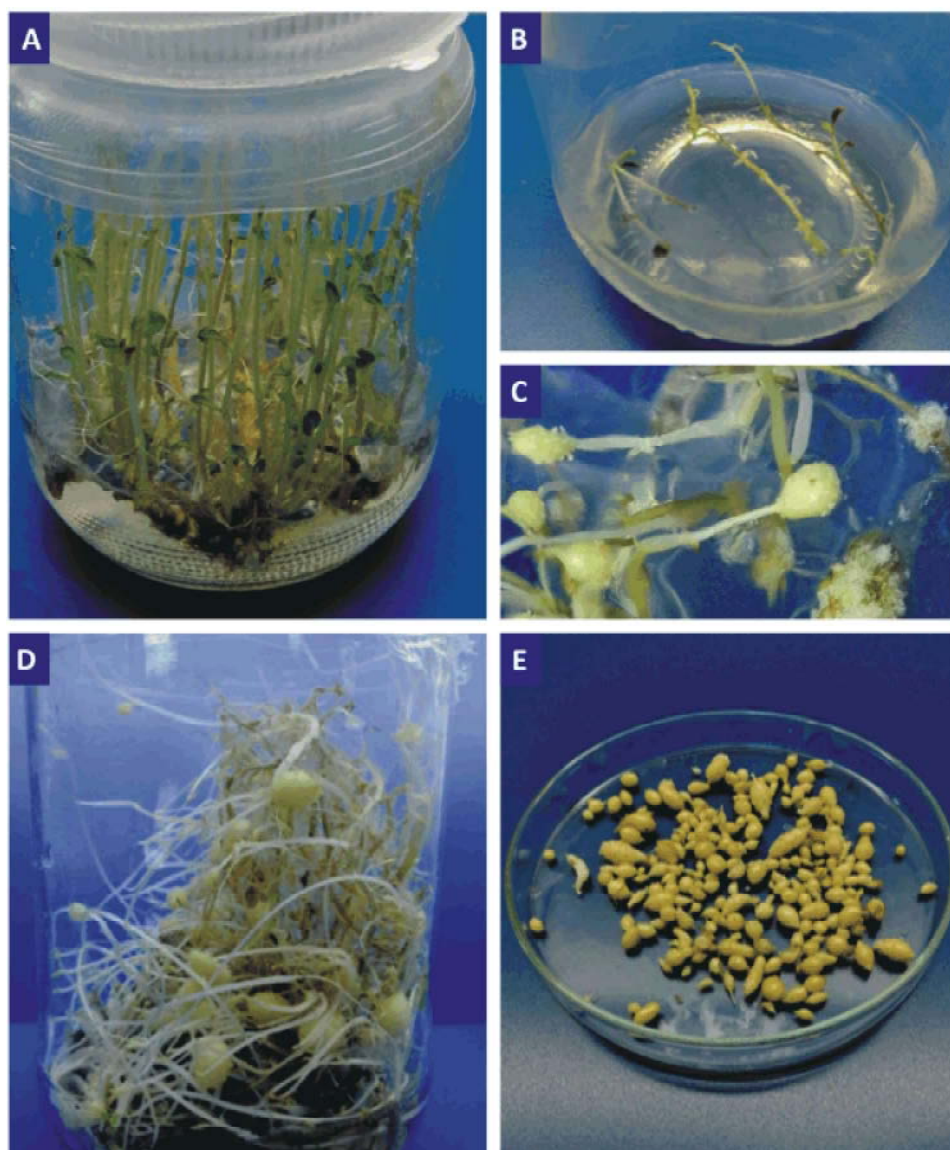


Fig. 4: Silver nanoparticles (AgNPs) promote microtubers formation in potato cv. Désirée. (A) Potato shoots used for microtuberization. (B) Shoot explants of potato cultured on microtubers induction medium (MIM) + AgNPs. (C) Initiation of microtubers after two weeks on MIM + AgNPs. (D) Production of microtubers after 6 weeks on MFM + AgNPs. (E) Harvest of microtubers after 6 weeks of culture on MFM + AgNPs.

Table 2: Effect of different concentrations of silver nanoparticles (AgNPs) or AgNO_3 (STS) on the initiation of microtubers formation (days), the average number of the produced microtubers/ jar, the average weight of microtuber (mg) and the average diameter of microtuber (mm).

Conc. mg/l	Days to microtubers initiation		Avg. No. of microtubers/ jar		Avg. weight of microtuber (mg)		Avg. diameter of microtuber (mm)	
	(STS)	AgNPs	(STS)	AgNPs	(STS)	AgNPs	(STS)	AgNPs
Control (0)	20.90±0.25	20.95±0.26	9.65±0.13	9.60±0.12	123.11±1.91	123.12±1.89	5.37±0.05	5.45±0.06
0.5	20.10±0.22	12.60±0.14	10.80±0.14	13.65±0.17	178.54±2.82	217.33±4.07	6.79±0.07	7.91±0.11
1	18.80±0.21	10.95±0.12	11.50±0.16	18.95±0.23	211.47±3.11	312.18±5.65	7.18±0.08	10.15±0.18
2	16.90±0.20	11.85±0.13	12.70±0.18	18.10±0.21	217.58±4.13	289.14±5.46	7.51±0.09	9.65±0.17
4	14.85±0.16	11.95±0.14	13.75±0.19	17.85±0.18	226.87±4.82	274.28±5.14	7.64±0.11	9.39±0.15
8	13.30±0.14	12.30±0.15	14.60±0.21	16.95±0.17	238.22±5.43	266.95±4.71	8.12±0.13	9.06±0.14
16	14.20±0.15	12.65±0.15	14.35±0.20	16.45±0.16	235.48±5.12	257.46±4.56	7.84±0.12	8.81±0.12
L.S.D. at 0.05	2.24		1.98		40.53		0.92	

The mean (±SE) of twenty replicates

The advancement in microtubers research has vast potential for improving microtubers production as an essential technology for the production of potato seed tuber, also for improving the programs of potato breeding and conservation. In addition to the high potentiality of microtubers as a source for exchange and commercialization [3-13].

This is the first report on the effect of silver nanoparticles on microtuberization in potato. The obtained results confirmed the positive influence of AgNPs on the average number of the produced microtubers which recorded the highest increase (97.40%) using 1 mg/l of AgNPs compared to the control treatment. Therefore, these results may be attributed to the role of AgNPs in reducing the impact of ethylene on potato explants during microtuberization. Also, these results indicated that AgNPs were superior to AgNO₃ (STS) in alleviating the retardation effect of ethylene on microtuberization in potato plant. These results are in accordance with reports on the effect of silver thiosulphate as anti-ethylene on growth and microtuberization in potato plant. Ethylene is released from potato shoots cultured *in vitro* in closed boxes and resulted in small leaves and slim stems [38]. Therefore, the use of ethylene inhibitors such as AgNO₃ can enhance the response of plant tissues grown *in vitro*. Thus, the use of silver thiosulphate immensely increased the development of potato shoots with large leaves compared to the control [38]. Mingo-Castel *et al.* [58] pointed to that ethylene markedly inhibits *in vitro* tuberization in potato. Zobayed *et al.* [59] concluded that removal of ethylene by the forced-ventilation system effectively enhanced the growth of *in vitro* potato cultures as well as this ventilation treatment improved the tuberization *in vitro* with an increase in the size of the produced tubers compared to the closed system. In addition, the use of AgNO₃ in the culture medium of the diffusive-ventilation system resulted in an increase in the size of potato leaves and the number of the produced tubers compared to the control without AgNO₃ and the sealed system. In addition, no significant differences were recorded in the number of the produced tubers between the diffusive-ventilation system with AgNO₃ and the forced-ventilation system, while the fresh weight of the tubers was markedly increased in the forced-ventilation system.

CONCLUSIONS

We report here for the first time that, the anti-ethylene silver in the crystalline form as engineered nanoparticles

(AgNPs) in a size (2-4 nm) can strongly promote microtuberization in potato compared to the ionic form Ag⁺ as silver thiosulphate (STS). Thus, the use of AgNPs at (1 mg/l) promote the formation of microtubers in potato cv. Désirée at the highest level (97.40%) compared to (51.30%) which was recorded using (8 mg/l) of (STS). The results showed that the highest average number of microtubers/jar (18.95) was recorded using 1 mg/l of AgNPs compared to (14.60) using 8 mg/l of AgNO₃ (STS). These increases in the average number of microtubers/jar represent 97.40% and 51.30% using AgNPs and AgNO₃ (STS) compared to the control, respectively. Also, these results revealed that the use of AgNPs at (1 mg/l) promote the formation of microtubers in potato cv. Désirée at the highest level (97.40%) which strongly indicate to the superiority of AgNPs over AgNO₃ (STS). Therefore, these results proved that AgNPs at low concentration (1 mg/l) were more effective for producing high promotion of microtuberization in potato plant, which may be attributed to the superior properties of the crystalline form of nano-silver as AgNPs compared to the ionic form Ag⁺ as (STS). In addition, these promising results are useful for the advancement in microtubers production technology at large scale in Egypt. Furthermore, the utilization of nano-materials in plant physiology experiments as a factor represents a milestone in the field of nanobiotechnology.

REFERENCES

1. Tanios, S., A. Eyles, R. Tegg and C. Wilson, 2018. Potato Tuber Greening: a Review of Predisposing Factors, Management and Future Challenges. American Journal of Potato Research, 95:248-257.
2. FAO 2017. FAOSTAT. <http://www.fao.org/faostat/en/#data/QC>
3. Levy, D., J.E.A. Seabrook and S. Coleman, 1993. Enhancement of tuberization of axillary shoot buds of potato (*Solanum tuberosum* L.) cultivars cultured *in vitro*. Journal of Experimental Botany, 44: 381-386.
4. Akita, M. and S. Takayama, 1994. Stimulation of potato (*Solanum tuberosum* L.) tuberization by semicontinuous liquid media surface level control. Plant Cell Rep., 13: 184-187.
5. Zaki, M., 1997. Biological basis for *in vitro* microtuberization of potato (*Solanum tuberosum*). Egypt J. Bot., 37: 43-58.
6. Gopal, J., J.L. Minocha and H.S. Dhaliwal, 1998. Microtuberization in potato (*Solanum tuberosum* L.). Plant Cell Rep., 17: 794-798.

7. Donnelly, D., W.K. Coleman and S.E. Coleman, 2003. Potato Microtuber Production and Performance: A Review. *Amer. J. of Potato Res.*, 80:103-115.
8. Zhang Z.J., W.J. Zhou and H.Z. Li, 2005. The role of GA, IAA and BAP in the regulation of *invitro* shoot growth and microtuberization in potato. *Acta Physiol. Plant* 27:363-369.
9. Badoni, A. and J.S. Chauhan, 2009. Microtuber: A Source of Germplasm Conservation. *Report and Opinion*, 1(3): 69-71.
10. Wang, B., Y. Ma, Z. Zhang, Z. Wu, Y. Wu, Q. Wang and M. Li. 2011. Potato viruses in China. *Crop Protection*, 30: 1117-1123.
11. Halterman, D., A. Charkowski and J. Verchot. 2012. Potato, viruses and certification in the USA to provide healthy propagated tubers. *Pest Technology*, 6(1): 1-14.
12. Radouani, A. and F.I. Lauer, 2015. Effect of NPK Media Concentrations on In Vitro Potato Tuberization of Cultivars Nicola and Russet Burbank. *Am. J. Potato Res.*, 92: 294-297.
13. De Moraes, T.P., S.A. Asmar, H.F. Silva, J.M.Q. Luz and B. de Melo, 2018. Application of Tissue Culture Techniques in Potato. *Biosci. J.*, Uberlândia, 34(4): 952-969.
14. Kumar P.P., C. Dimps Rao and C.J. Goh, 1998. Influence of petiole and lamina on adventitious shoot initiation from lamina explants of *Paulownia fortunei*. *Plant Cell Reports*, 17: 886-890.
15. Biddington, N.L., 1992. The influence of ethylene in plant tissue culture. *Plant Growth Regulation*, 11: 173-187.
16. Jackson M.B., A.J. Abbott, A.R. Belcher and K.C. Hall, 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, pp: 57-71.
17. Gong, Y., F. Gao and K. Tang 2005. *In vitro* high frequency direct root and shoot regeneration in sweet potato using the ethylene inhibitor silver nitrate. *South African Journal of Botany*, 71(1): 110-113.
18. Khan M.R., H. Rashid, M. Ansar and Z. Chaudry, 2003. High frequency shoot regeneration and *Agrobacterium*-mediated DNA transfer in Canola (*Brassica napus*). *Plant Cell, Tissue and Organ Culture*, 75: 223-231.
19. Al-Shafeay, A.F., A.S. Ibrahim, M.R. Nesiem and M.S. Tawfik, 2011. Establishment of regeneration and transformation system in Egyptian sesame (*Sesamum indicum* L.) cv Sohag 1. *GM Crops*, 2(3): 1-11.
20. Ibrahim, A.S., O. M. El Shihy and B.M. Hamdy, 2018. 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.
21. Fei S.Z., P.E. Read and T.P. Riordan, 2000. Improvement of embryogenic callus induction and shoot regeneration of buffalograss by silver nitrate. *Plant Cell, Tissue and Organ Culture*, 60: 197-203.
22. Khalafalla, M.M. and K. Hattori, 2000. Ethylene inhibitors enhance *in vitro* root formation on faba bean shoots regenerated on medium containing thidiazuron. *Plant Growth Reg.*, 32: 59-63.
23. Mandal, A.K.A., S.D. Gupta and A.K. Chatterji, 2001. Factors affecting somatic embryogenesis from cotyledonary explants of safflower. *Biologia Plantarum*, 44: 503-507.
24. Ibrahim, A.S. and O.M. El Shihy, 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.
25. Ibrahim, A.S. and O.M. El Shihy, 2012b. An efficient *Agrobacterium tumefaciens*-mediated transformation of some elite Egyptian rice cultivars. *Res. J. Agric. Biol. Sci.*, 8(3): 355-368.
26. Ibrahim, A.S., A.H. Fahmy and S.S. Ahmed, 2018. Copper nanoparticles elevate regeneration capacity of (*Ocimum basilicum* L.) plant via somatic embryogenesis. *Plant Cell Tiss. Org. Cult.*, pp: 1-10 <https://doi.org/10.1007/s11240-018-1489-3>.
27. Chen, H.C., M.C. Roco, J.B. Son, S. Jiang, C.A. Larson and Q. Gao, 2013. Global nanotechnology development from 1991 to 2012: patents, scientific publications and effect of NSF funding. *J. Nanopart. Res.*, 15: 1951.
28. Gardea-Torresdey, J.L., C.M. Rico and J.C. White, 2014. Trophic transfer, transformation and impact of engineered nanomaterials in terrestrial environments. *Environ Sci Technol.*, 48: 2526-2540.
29. Tahmasbi, D., R. Zarghami, A.V. Azghandi and M. Chaichi, 2011. Effects of nanosilver and nitroxin biofertilizer on yield and yield components of potato minitubers. *Int. J. Agric. Biol.*, 13: 986-990.
30. Kim, D.H., J. Gopal and I. Sivanesan, 2017. Nanomaterials in plant tissue culture: the disclosed and undisclosed. *RSC Adv* 7:36492-36505.

31. Aghdaei, M., H. Salehi and M.K. Sarmast, 2012. Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. micropropagation. Adv. Hort. Sci., 26: 21-24.
32. Sarmast, M.K., A. Niazi, H. Salehi and A. Abolmoghadam, 2015. Silver nanoparticles affect ACS expression in *Tecomella undulata* in vitro culture. Plant Cell Tiss. Org. Cult., 121: 227-236.
33. Ewais, E.A., S.A. Desouky and E.H. Elshazly, 2015. Evaluation of Callus Responses of *Solanum nigrum* L. Exposed to Biologically Synthesized Silver Nanoparticles Nanosci. Nanotechnol., 5: 45-56.
34. Ehsanpour, A.A. and Z. Nejati, 2013. Effect of nanosilver on potato plant growth and protoplast viability. Biological Lett., 50(1): 35-43.
35. Van Dong, P., C.H. Ha, L.T. Binh and J. Kasbohm, 2012. Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. International Nano Letters, 2: 1-9.
36. Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50(1): 151-158.
37. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant, 15: 473-497.
38. Perl, A., D. Aviv and E. Galun, 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.
39. Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods, 7th edn. Iowa State University Press, Ames.
40. Xiong, J., Y. Wang, Q. Xue and X. Wu 2011. Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid. Green Chem., 13: 900-904.
41. Abbott, A.J. and A.R. Belcher, 1986. Potato tuber formation *in vitro*. In: LA Withers, PG Alderson (eds), Plant Tissue Culture and its Agricultural Applications. Butterworths, London. pp: 113-122.
42. Garner, N. and J. Blake, 1989. The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Ann. Bot., 63: 663-674.
43. Forti, E., G. Mandolino and P. Ranalli, 1991. *In vitro* tuber induction: influence of the variety and of the media. Acta Hort, 300: 127-132.
44. Simko, I., 1994. Sucrose application causes hormonal changes associated with potato tuber induction. J. Plant Growth Reg., 13: 73-77.
45. Struik, P.C. and S.G. Wiersema, 1999. Production of pre-basic seed. In: Seed Potato Technology. Wageningen Pers. pp: 173-216.
46. Yu, W.C., P.J. Joyce, D.C. Cameron and B.H. McCown, 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep., 19: 407-413.
47. Gopal, J., A. Chamaïl and D. Sarkar, 2004. *In vitro* production of microtubers for conservation of potato germplasm: Effect of genotype, abscisic acid and sucrose. In vitro Cel. Dev. Biol. Pl., 40: 486-90.
48. Avila, A., L. de, S.M. Pereyra and J.A. Argüello, 1998. Nitrogen concentration and proportion of NH₄-N affected potato cultivar response in solid and liquid media. Hort. Sci., 33: 336-338.
49. Charles, G., L. Rossignol and M. Rossignol, 1995. Mise au point d'un modèle de développement et de tubérisation contrôlés et synchrones chez la pomme de terre cultivée *in vitro*. Acta Bot. Gal., 142: 289-300.
50. Chen, J.J. and Y.J. Liao, 1993. Nitrogen-induced changes in the growth and metabolism of cultured potato tubers. J. Am. Soc. Hort. Sci., 118: 831-834.
51. Garner, N. and J. Blake, 1989. The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Ann. Bot., 63: 663-674.
52. Saker, M.M., T.A.A. Moussa, N.Z. Heikal, A.H.A. Abo ELLil and R. M.H. Abdel-Rahman, 2012. Selection of an efficient *in vitro* micropropagation and regeneration system for potato (*Solanum tuberosum* L.) cultivar Désirée. African Journal of Biotechnology, 11(98): 16388-16404.
53. Sarkar, D. and P.S. Naik, 1998. Effect of Inorganic nitrogen nutrition on cytokinin-induced potato microtuber production *in vitro*. Potato Res., 41: 211-217.
54. Zarrabeitia, A., X. Lejarcegui, J. Veramendi and A.M. Mingo-Castel, 1997. Influence of nitrogen supply on micropropagation and subsequent microtuberization of four potato cultivars. Am. Potato J., 370: 369-378.
55. Stallknecht, G.F. and S. Farusworth, 1979. The effect of nitrogen on the coumarin-induced tuberization of potato axillary shoots cultured *in vitro*. Am Potato J., 56: 523-530.
56. Wattimena, G.A., 1983. Micropropagation as an alternative technology for potato production in Indonesia. Ph.D. Thesis. University of Wisconsin-Madison.

57. Vreugdenhil D., Y. Boogaard, R.G.F. Visser and S.M. de Bruijn, 1998. Comparison of tuber and shoot formation from *in vitro* cultured potato explants. *Plant Cell Tiss. Org. Cult.*, 53:197-204.
58. Mingo-Castel, A.M., O.F. Smith and J. Kumamoto, 1976. Studies on the carbon dioxide promotion and ethylene inhibition of tuberization in potato explants cultured *in vitro*. *Plant Physiology*, 57: 480-485.
59. Zobayed, S.M.A., J. Armstrong and W. Armstrong, 2001. Micropropagation of potato evaluation of closed, diffusive and forced ventilation on growth and tuberization. *Ann. Botany*, 87: 53-59.