



# Silver nanoparticles in the micropropagation of *Campomanesia rufa* (O. Berg) Nied

Caroline de Oliveira Timoteo<sup>1</sup> · Renato Paiva<sup>1</sup> · Michele Valquíria dos Reis<sup>1</sup> · Pedro Ivo Cunha Claro<sup>2</sup> · Diogo Pedrosa Corrêa da Silva<sup>3</sup> · Jose Manoel Marconcini<sup>4</sup> · Juliano Elvis de Oliveira<sup>5</sup>

Received: 4 September 2018 / Accepted: 7 February 2019 / Published online: 13 February 2019  
© Springer Nature B.V. 2019

## Abstract

With the advancement of nanotechnology, nanomaterials are beginning to be employed in different areas of science, including plant tissue culture. Among nanomaterials, silver nanoparticles (AgNPs) are widely used because of their antibacterial effects. However, knowledge about the effects of AgNPs on in vitro cultivation of plant species is poor. The present study aims to analyse the effects of AgNPs on the in vitro propagation of *Campomanesia rufa*. Nodal segments were sectioned and cultured in MS medium to induce buds. The MS medium was supplemented with benzylaminopurine, different concentrations of AgNPs or AgNO<sub>3</sub>. AgNPs were synthesized and characterized as a function of time. The shoots were analysed for number, height and fresh weight. Biochemical and light microscopy and scanning electron microscopy were also performed. Data from the characterization of AgNPs demonstrate that the heating process for sterilization of the culture medium promotes an agglomeration of AgNPs. The lowest concentrations AgNPs (0.385, 0.77 and 1.54 mg L<sup>-1</sup>) did not affect the in vitro multiplication, since no significant differences were observed in relation to the control, as for the number, height and fresh weight of the shoots formed, however, in the treatments with 15.4 mg L<sup>-1</sup> AgNPs and AgNO<sub>3</sub>, a reduction in the number of shoots was observed. No biochemical, morphological or anatomical changes were observed in the shoots formed. Concludes, AgNPs did not affect the in vitro multiplication at low concentrations but may cause more damage to plant development than the use of AgNO<sub>3</sub>, depending on the concentration used.

## Key message

This manuscript reports that silver nanoparticles not affect the in vitromultiplication of *Campomanesia rufa*, and the results are important because explain thebehavior of AgNPs under high temperatures.

**Keywords** AgNPs · Nanotechnology · Gabiroba · Tissue culture

---

Communicated by M. Angeles Revilla.

✉ Caroline de Oliveira Timoteo  
carolineoliveira011@gmail.com

<sup>1</sup> Department of Biology, Sector of Plant Physiology, Federal University of Lavras, Lavras, MG, Brazil

<sup>2</sup> Graduate Program in Materials Science and Engineering (PPG-CEM), Federal University of São Carlos, São Carlos, SP, Brazil

<sup>3</sup> Department of Agriculture, Federal University of Lavras, Lavras, MG, Brazil

<sup>4</sup> National Nanotechnology Laboratory for Agribusiness (LNNA), Embrapa Instrumentation, São Carlos, SP, Brazil

<sup>5</sup> Department of Engineering, Federal University of Lavras, Lavras, MG, Brazil

## Introduction

Nanomaterials have unique physicochemical properties that differ distinctly from their macroscopic or even ionic counterparts. These properties usually result from their small size, shape, chemical composition, high surface area and reactivity (Ma et al. 2010). Because they exhibit such unique properties, nanomaterials have been widely used to improve seed germination (Lahiani et al. 2013; Siddiqui and Al-Whaibi 2014), increase plant growth and yield (Kole et al. 2013), to allow genetic modifications in plant cells (Torney et al. 2007) and to improve photosynthetic efficiency (Sharma et al. 2012).

In plant tissue culture, there are promising indications of the utility of nanotechnology. Iron nanoparticles, for example,

improved the in vitro development of strawberry seedlings submitted to water stress (Mozafari et al. 2018). CuO nanoparticles stimulated the in vitro production of bioactive compounds in *Stevia rebaudiana* (Javed et al. 2017) and *Brassica rapa* spp. *pekinensis* (Chung et al. 2018).

The effective micropropagation of crop species depends on a range of factors, including the type of culture medium and plant growth regulators (Satish et al. 2015; Venkatachalam et al. 2015; Scalzo et al. 2016; Yu et al. 2017). The application of nanomaterials may be an alternative to optimize protocols of somatic embryogenesis, organogenesis, induction of shoots and roots, superficial disinfection of explants for in vitro culture, and production of secondary metabolites and even to improve or reduce the somaclonal variation of in vitro explants (Kim et al. 2017).

Silver nanoparticles (AgNPs), due to their attractive physiological properties and their recognized antimicrobial action, may have potential use in superficial disinfection processes of explants for in vitro culture (Sarmast and Salehi 2016). AgNPs have been shown to be effective in reducing bacterial contamination in *Vanilla planifolia* (Spinoso-Castillo et al. 2017) and in the control of contaminants for the in vitro culture of *Valeriana officinalis* (Abdi et al. 2008), and *Olea europaea* (Rostami and Shahsavari 2009).

The AgNPs were also efficient in in vitro multiplication of *Alternanthera sessilis* (Venkatachalam et al. 2017), induction of somatic embryogenesis and plant regeneration of *Gloriosa superba* (Mahendran et al. 2017), and the promotion of *Brassica juncea* growth in vitro through the improvement of its antioxidant status (Sharma et al. 2012).

In addition to acting directly on in vitro propagation, silver nanoparticles may also act as inhibitors of the hormone ethylene (Syu et al. 2014; Sarmast et al. 2015). This phytohormone accumulates in culture vessels and may affect the growth and in vitro development of some species (Biddington 1992).

Silver nanoparticle exposure responses in plants have been studied mainly in relation to toxicity (Cvjetko et al. 2017), and less attention has been given to the possibility that silver nanoparticles may support in vitro growth of plant species.

In this context, the present study has the objective of analysing the effect of silver nanoparticles on the micropropagation of *Campomanesia rufa* (O. Berg) Nied. *C. rufa* is a fruit-bearing species of the Cerrado with potential for food commercialization, but it presents difficulties for propagation in the natural environment.

## Materials and methods

### Synthesis of silver nanoparticles

Silver nanoparticles were synthesized according to the methodology described by Turkevich et al. (1951) with

adaptations. A solution was prepared with silver nitrate ( $0.18 \text{ g L}^{-1}$ ) and sodium carboxymethylcellulose ( $0.6 \text{ g L}^{-1}$ ), which was maintained under constant heating and stirring. At  $95 \text{ }^\circ\text{C}$ , an aqueous solution of sodium citrate (1%) was added thereto. The concentration of the silver nanoparticle solution was quantified by UV–Vis absorption spectroscopy (model UV-1800, Shimadzu).

### Characterization of silver nanoparticles

The culture media containing the silver nanoparticle solution at the corresponding concentrations, after autoclaving, were characterized by means of dynamic light scattering (DLS) and zeta potential, performed with the Malvern 3000 Zetasizer equipment.

Three replicates were used for each treatment. For analysis of the solutions, 0.5 mL of each sample suspension was combined with 100 mL of deionized water and sonicated in an ultrasonic tip (Brason) for 1 min at a power of 450 W.

### Bud induction

Buds (4 cm) induced from established in vitro plantlets of *Campomanesia rufa* were transferred to MS medium (Murashige and Skoog 1962) plus  $5.62 \text{ } \mu\text{M}$  BAP (6-benzylaminopurine) and  $30 \text{ g L}^{-1}$  sucrose (Sant'Ana et al. 2018). The medium was supplemented with different concentrations of silver nanoparticles (0.0, 0.385, 0.77, 1.54 and  $15.4 \text{ mg L}^{-1}$ ) or silver nitrate at a concentration of  $0.18 \text{ g L}^{-1}$ , which corresponds to the same concentration used for the synthesis of stock solution of AgNPs. This concentration is equivalent to  $0.114 \text{ g L}^{-1}$  of  $\text{Ag}^+$  ions.

The medium was solidified with  $7.0 \text{ g L}^{-1}$  agar, and the pH was adjusted to  $5.7 \pm 0.1$  before autoclaving at  $121 \text{ }^\circ\text{C}$  and 1 atm pressure for 20 min. The culture was maintained in a growth room for 90 days at a temperature of  $25 \text{ }^\circ\text{C}$  on a 16-h photoperiod (fluorescent lamps radiating  $36 \text{ } \mu\text{mol}$  of photons  $\text{m}^{-2} \text{ s}^{-1}$ ) for shoot multiplication and subsequent growth evaluations, biochemistry, light microscopy and scanning electron microscopy.

After 90 days of culture, the number and size of the shoots were analysed, and the total fresh mass of the shoots. The size of the shoots was measured using a ruler.

### Biochemical analysis of antioxidant metabolism

At 90 days of culture, samples of the shoots formed were removed and stored in a freezer at  $-80 \text{ }^\circ\text{C}$  until biochemical analysis.

The enzymatic extract for biochemical analysis was obtained by the maceration in liquid nitrogen of 200 mg of fresh material from the aerial part of the shoots, to which was added 1.5 mL of an extraction buffer composed of

potassium phosphate buffer (400 mM) in pH 7.8, 15  $\mu\text{L}$  EDTA (10 mM), 75  $\mu\text{L}$  ascorbic acid (200 mM) and 1035  $\mu\text{L}$  water (Biemelt et al. 1998). The extract was centrifuged at  $13,000\times g$  for 10 min at 4  $^{\circ}\text{C}$ , and the supernatant collected for analysis of superoxide dismutase (SOD) was evaluated according to Giannopolitis and Ries (1977).

SOD activity was assessed by the ability of the enzyme to inhibit nitrotriazolium blue photoreduction, NBT (Giannopolitis and Ries 1977). We added 5 mL aliquots of the supernatants from treatments with different concentrations of AgNPs and silver nitrate to incubation medium containing 100 mM potassium phosphate buffer (pH 7.8), 70 mM methionine, 10  $\mu\text{M}$  EDTA, 1 mM NBT, 0.2 mM riboflavin and water. Samples and a control composed of the same reaction medium without the sample were illuminated with a 20 W fluorescent lamp for 7 min. Readings were performed at 560 nm where a unit of SOD corresponds to the amount of enzyme required to inhibit the NBT photoreduction at 50%.

### Light microscopy

For the anatomical studies, leaf and stem samples from 90 days of culture were collected and stored in alcohol 70% (v/v) until the anatomical sections were taken. To prepare the slides, leaf and stem samples were sectioned at approximately 0.5  $\text{cm}^2$ , and these sections were dehydrated in increasing ethylic series and included in methacrylate (Historesin, Leica Instruments, Heidelberg, Germany).

Transverse sections of the leaves and stems were made with an 8  $\mu\text{M}$  thick microtome, stained with toluidine blue (O'Brien et al. 1964) and sealed using stained glass (Acrylic).

The prepared slides were observed and photographed under a Zeiss Scope AX10<sup>®</sup> microscope coupled to the digital camera and photomicrographed with Axio Vision R.L. 4.8<sup>®</sup> software. The presence or absence of cellular damage was determined.

### Scanning electron microscopy

Leaf and stem segments of the shoots were fixed in Karnovsky aqueous solution for 24 h, after which the material remained in glycerol for 30 min. After this period, the stem and leaf segments were segmented into liquid nitrogen (LN). Thereafter, dehydration was accomplished in increasing series of acetone (25, 50, 75, and 100%) for 10 min.

In the next step, the material was dried in the critical point equipment, and the cuts were adhered to the stubs and covered by a layer of metallic gold. The segments were observed with a JEOL T200 scanning electron microscope.

## Results

### Characterization of silver nanoparticles

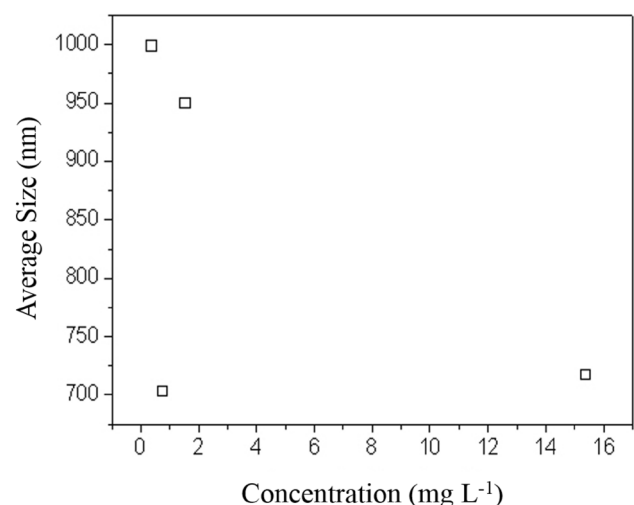
In DLS analysis, the presence of silver nanoparticles in the culture medium was observed in both the lowest (0.385  $\text{mg L}^{-1}$ ) and the highest concentration (15.4  $\text{mg L}^{-1}$ ), which indicates the presence of silver ions in the medium used to induce new buds in *C. rufa*.

The AgNPs present in medium were larger than the AgNPs of the stock solution (155.2 nm). This increase in size is related to autoclaving of the culture medium. The high heating (121  $^{\circ}\text{C}$ ) for sterilization promoted the agglomeration of nanoparticles, which resulted in the increase in size compared to the solution of non-autoclaved AgNPs (Fig. 1).

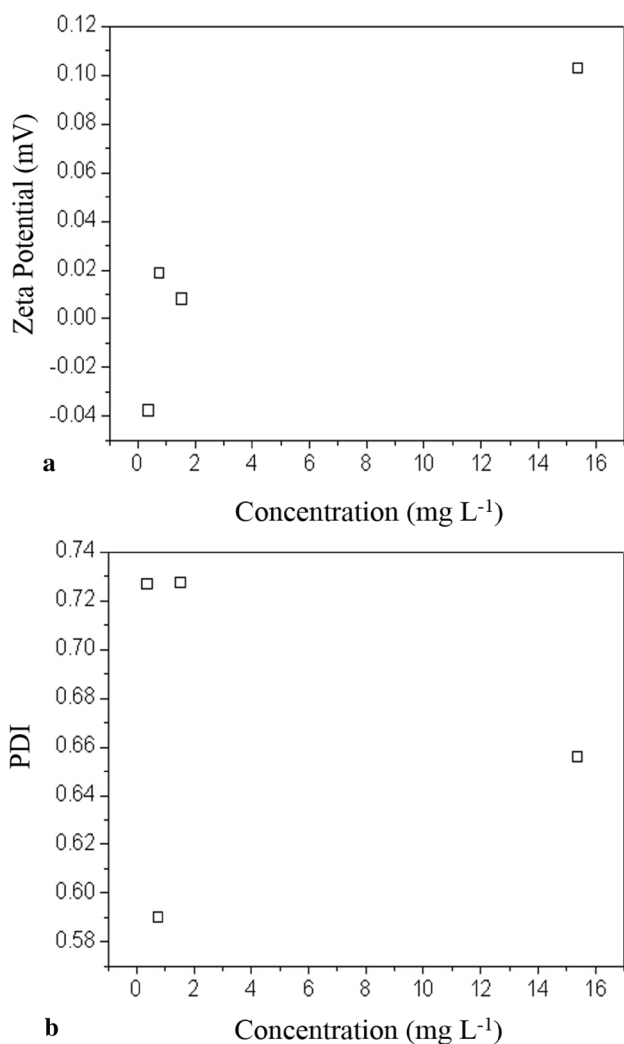
The Zeta Potential, close to zero, and the Polydispersity Index (PDI) show that the synthesized solution is unstable and tends to aggregate. The instability of the solution, associated with the high temperature to which the AgNPs were subjected during sterilization of the medium, contributed to the increase in size of AgNPs (Fig. 2a, b).

### Growth analysis

Exposure of *C. rufa* nodal segments to different concentrations of AgNPs did not affect the induction of buds at the concentrations of 0.385, 0.77, and 1.54  $\text{mg L}^{-1}$  compared to the conditions with no AgNPs. However, at the concentration of 15.4  $\text{mg L}^{-1}$  and in the silver nitrate treatment,



**Fig. 1** Mean diameter of AgNPs at concentrations of 0.385  $\text{mg L}^{-1}$ , 0.77  $\text{mg L}^{-1}$ , 1.54  $\text{mg L}^{-1}$  and 15.4  $\text{mg L}^{-1}$  in the culture medium after autoclaving, 10 days after the synthesis



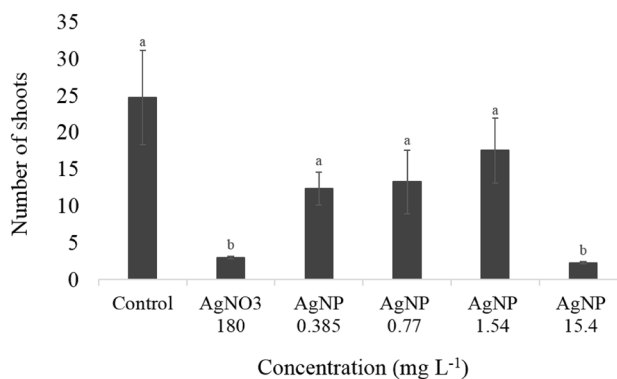
**Fig. 2** Zeta Potential (a) and Polydispersion Index (b) at concentrations of 0.385 mg L<sup>-1</sup>, 0.77 mg L<sup>-1</sup>, 1.54 mg L<sup>-1</sup> and 15.4 mg L<sup>-1</sup> of AgNPs in the culture medium, after autoclaving, 10 days after synthesis

there was a reduction of approximately 90% in the number of new shoots (Figs. 3, 4).

Consequently, with the reduction in the number of shoots, the total fresh mass was also reduced by approximately 80% in the silver nitrate and 15.4 mg L<sup>-1</sup> AgNPs conditions, relative to the control (Fig. 5). However, the length of shoots obtained (averaging 1.1 cm) was not influenced by exposure to silver nanoparticles.

### Biochemical analysis of antioxidant metabolism

No significant differences were observed among treatments in the superoxide dismutase activity (SOD) by the Scott-Knott test at the 5% probability level, which showed a mean activity of 0.51 U mg<sup>-1</sup> FM (Table 1).



**Fig. 3** Number of shoots obtained from *Campomanesia rufa* at 90 days of culture, induced by BAP (5.6 μM) and submitted to silver nitrate (180 mg L<sup>-1</sup>) or different concentrations of silver nanoparticles (0.385, 0.77, 1.54 and 15.4 mg L<sup>-1</sup>). The averages followed by the same letter do not differ significantly from one another by the Scott-Knott test at the 5% probability level

### Light microscopy

The photomicrographs of the leaf blade of *C. rufa* showed uniseriate epidermis with stomata present only on the abaxial face and dorsiventral heterogeneous mesophyll composed of a layer of palisade parenchyma and two layers of lacunae (Fig. 6).

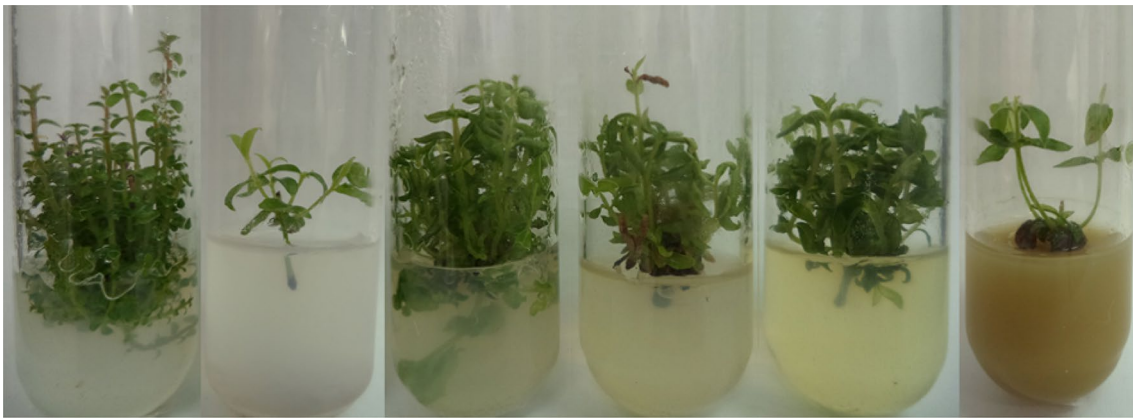
Light microscopy showed secretory glands abundant in the leaves and stem. In *Campomanesia xanthocarpa*, it was reported that these glands have lipid content (Gogosz et al. 2010). No cell damage was observed in the leaves and stems, regardless of the concentration of AgNPs or silver nitrate, in comparison to the control condition.

### Scanning electron microscopy

For all treatments, the electron photomicrographs reveal many simple and uniserial trichomes on both the adaxial and abaxial faces of the *C. rufa* leaf blades, which were grown in vitro. The trichomes are located both leaf veins and interveins (Fig. 7).

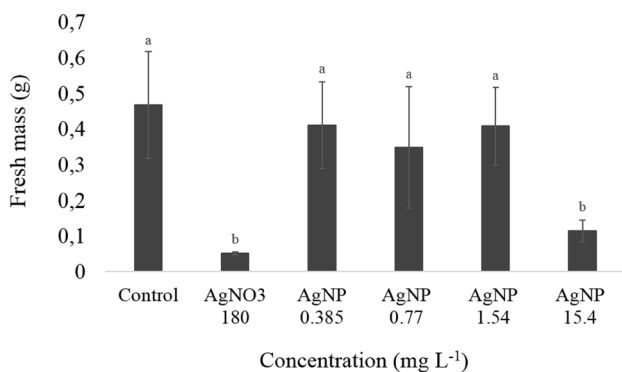
The stomata were observed only on the abaxial sides of the leaves and were classified as paracytic, with reniform guard cells, being more prominent than the fundamental epidermal cells (Fig. 7).

The stomata of the leaves treated with silver nitrate showed larger stomatal openings than seen in the control and in the treatments with AgNPs. No leaf surface damage was observed, regardless of the concentration of AgNPs or the presence of silver nitrate, in comparison to the control.



**Fig. 4** Appearance of *Campomanesia rufa* shoots at 90 days of culture induced by BAP (5.6  $\mu$ M) and submitted to silver nitrate ( $\text{AgNO}_3$ ) or different concentrations of silver nanoparticles (AgNPs).

From left to right, 0.0, 180  $\text{mg L}^{-1}$   $\text{AgNO}_3$ , 0.385, 0.77, 1.54 and 15.4  $\text{mg L}^{-1}$  AgNPs, respectively



**Fig. 5** Total fresh mass of the shoots obtained from *Campomanesia rufa* at 90 days of culture, induced by BAP (5.6  $\mu$ M) and submitted to silver nitrate (180  $\text{mg L}^{-1}$ ) or different concentrations of silver nanoparticles (0.385, 0.77, 1.54 and 15.4  $\text{mg L}^{-1}$ ). The averages followed by the same letter do not differ significantly from one another by the Scott-Knott test at the 5% probability level

**Table 1** Superoxide dismutase activity (SOD)

Concentration ( $\text{mg L}^{-1}$ )	U SOD $\text{mg}^{-1}$ fresh mass <sup>a</sup>
0	0.343a
AgNO <sub>3</sub> 180	0.623a
AgNP 0.385	0.561a
AgNP 0.77	0.534a
AgNP 1.54	0.473a
AgNP 15.4	0.554a
Average	0.514

<sup>a</sup>The averages followed by the same letter do not differ significantly from one another by the Scott-Knott test at the 5% probability level

## Discussion

No significant changes were observed among conditions without addition of AgNPs and treatments with low concentrations of AgNPs (0.385, 0.77 and 1.54  $\text{mg L}^{-1}$ ), regarding the number, height and fresh weight of the shoots.

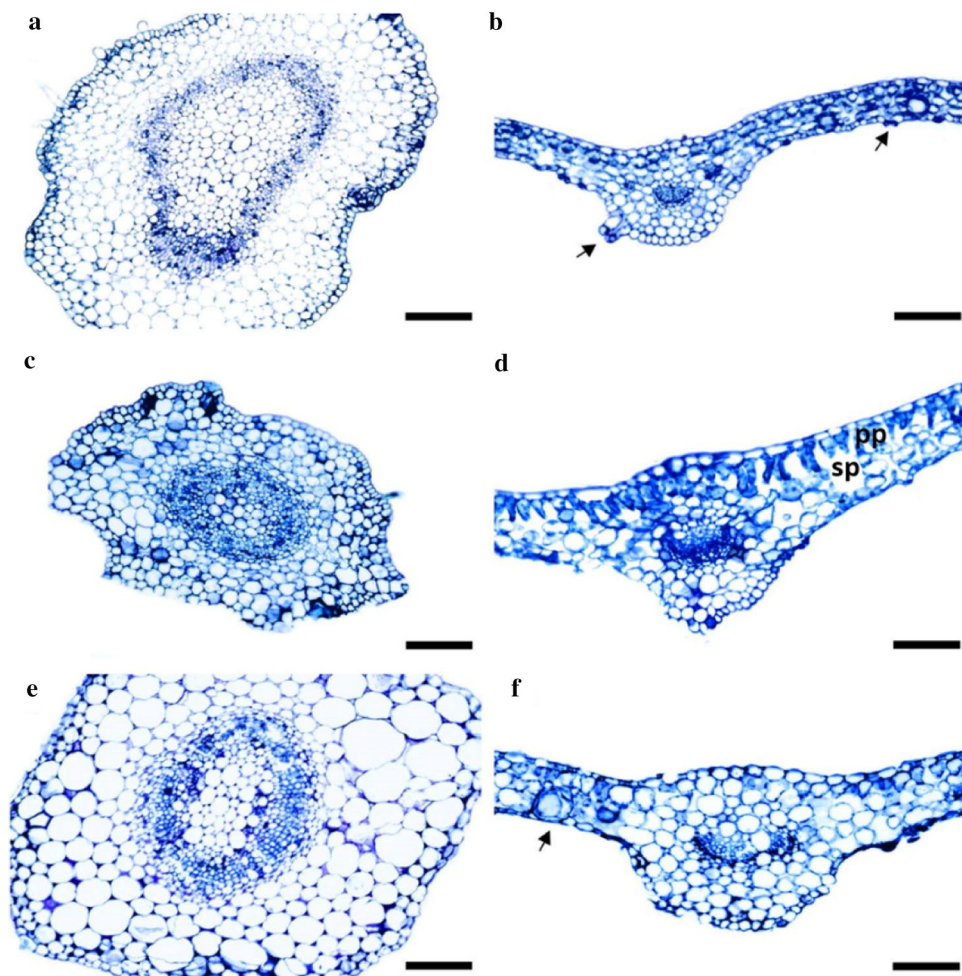
This low toxicity of the AgNPs may be related to the size of the AgNPs, as observed by the characterization of the culture medium the high temperature experienced by the medium during autoclaving may be promotes agglomeration of the nanoparticles, thus producing particles that exceed the nano scale (1 at 100 nm). As has been demonstrated, effects of AgNPs on plant cells vary with size of the nanoparticles (Geisler-Lee et al. 2012).

In a study conducted with *Arabidopsis*, it was found that AgNPs of  $45 \pm 5$  nm promoted a higher growth rate with less induction of oxidative stress compared to AgNPs of  $8 \pm 2$  nm that caused the highest rate of inhibition of growth and higher levels of oxidative stress (Syu et al. 2014). In *Spirodela polyrhiza*, it was shown that silver microparticles did not cause oxidative stress to the detriment of silver nanoparticles; consequently, no changes in antioxidant metabolism were observed compared to the control (Jiang et al. 2014).

These results may be related to the size of pores in plant cell walls, which are generally in the range of a few nanometres (Carpita et al. 1979; Adani et al. 2011), much smaller than the size of the silver particles. The relatively large size of the silver particles may prevent them from easily penetrating plant cell walls and thus cause less damage than the smaller AgNPs that can pass through the pores.

In treatments with 15.4  $\text{mg L}^{-1}$  of AgNPs and with silver nitrate (180  $\text{mg L}^{-1}$ ), similar results were observed. In

**Fig. 6** Transverse cuts of *Campomanesia rufa* leaf and stem segments at 90 days of culture, resulting from the induction of BAP shoots ( $5.6 \mu\text{M}$ ), submitted to silver nitrate ( $0.18 \text{ g L}^{-1}$ ) or silver nanoparticles (AgNPs). **a** and **b** control, **c** and **d** silver nitrate, **e** and **f**  $0.77 \text{ mg L}^{-1}$  AgNPs. *PP* palisade parenchyma, *SP* spongy parenchyma. In **b** highlight for stomata and in **f** prominence for secretory gland. Bar:  $10 \mu\text{M}$



both treatments, a reduction in the number and the fresh mass of the shoots was observed. However, the concentration of  $\text{AgNO}_3$  is higher than the concentration of AgNPs, suggesting that AgNPs may be more toxic than  $\text{AgNO}_3$  for plant species.

In similar concentrations AgNPs have been observed to be more toxic to *Lolium multiflorum* plants than  $\text{AgNO}_3$ , and AgNPs toxicity caused significant reductions in root growth rate and changes in the morphology of root systems (Yin et al. 2011). Likewise,  $\text{AgNO}_3$  was shown to be more effective in improving growth rates (root and shoot length and leaf area) than the AgNPs for the in vitro culture of *Solanum tuberosum* (Bagherzadeh Homaei and Ehsanpour 2015).

In *Allium cepa*,  $\text{AgNO}_3$  was more toxic than AgNPs coated with citrate, polyvinylpyrrolidone (PVP) or cetyltrimethylammonium bromide (CTAB) (Cvjetko et al. 2017).  $\text{AgNO}_3$  was also shown to be more toxic than AgNPs in *Brassica* sp., due to the lower accumulation of AgNPs and better APX and CAT activities, which resulted in lower oxidative stress (Vishwakarma et al. 2017). In *Brassica juncea*, inhibition of root length and chlorophyll content in plants

exposed to  $\text{AgNO}_3$  at the same concentrations of AgNPs was observed (Pandey et al. 2014).

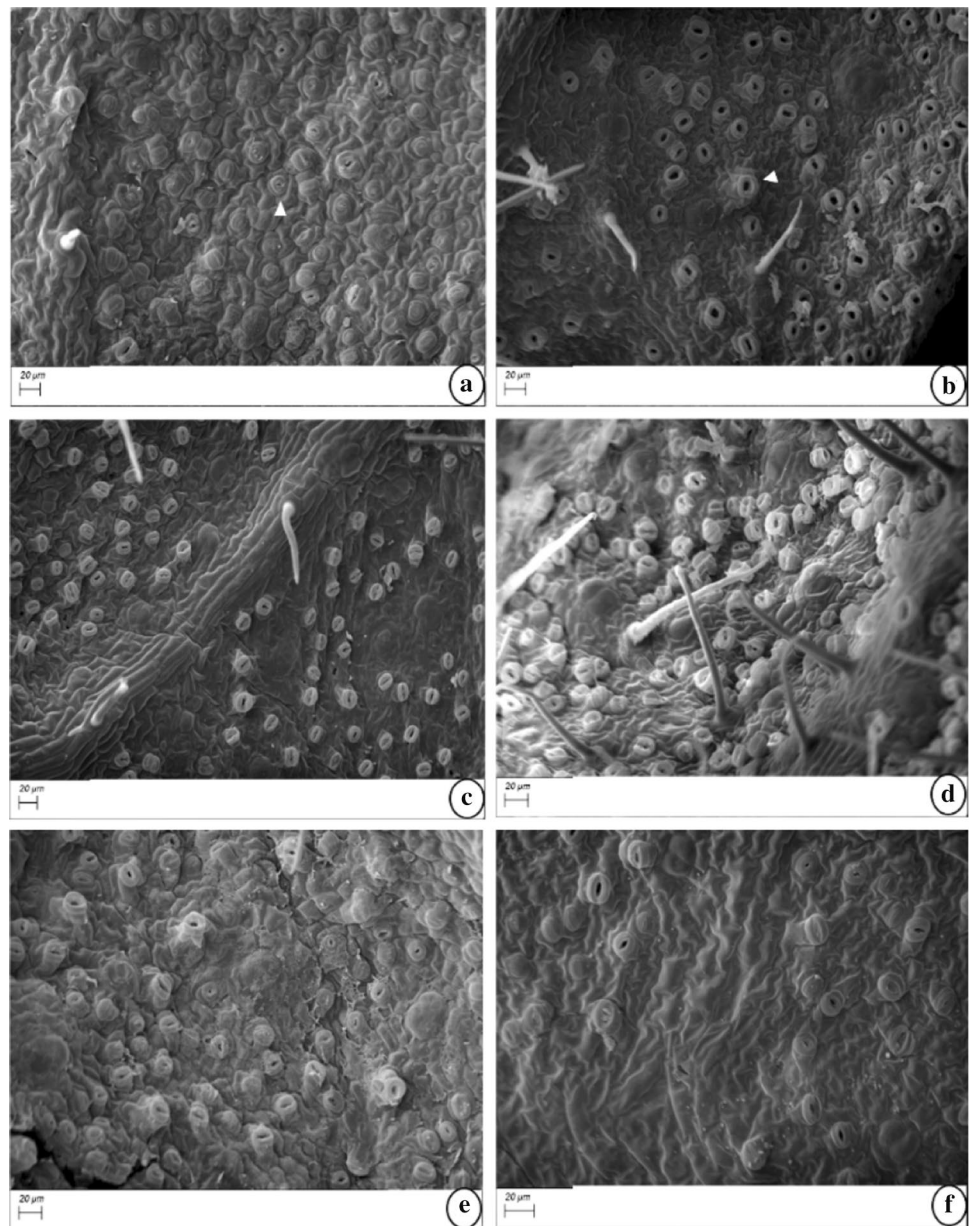
In *Cucumis sativus* cortical cell degeneration and disintegration of the endoderm in  $\text{AgNO}_3$  treatments were more prominent than in treatments with AgNPs, although both were toxic to the growth and development of this species (Tripathi et al. 2017).

In *Capsicum annuum*, both AgNPs and  $\text{Ag}^+$  ions similarly affected the development of the plant, decreasing its height and biomass as a result of the increase of total  $\text{Ag}^+$  content in the plant tissues (Vinković et al. 2017).

With these data, it is clear that the effect of AgNPs and silver nitrate is still controversial, with results that vary widely from species to species. These results make evident the need for further studies to understand the effects of AgNPs on plant cells and to better explore the intrinsic properties of nanoparticles.

No significant differences in antioxidant metabolism were observed among the treatments, regardless of the concentrations of AgNPs or silver nitrate. However, it has been reported that the exposure of plants to nanomaterials can increase the production of reactive oxygen species (ROS),

**Fig. 7** Scanning electron microscopy photomicrography of the abaxial surface of foliar segments of *Campomanesia rufa* at 90 days of culture, resulting from the induction of shoots from BAP (5.6  $\mu\text{M}$ ), submitted to silver nitrate (180  $\text{mg L}^{-1}$ ) or different concentrations of silver nanoparticles (AgNPs). **a** control, **b** silver nitrate, **c** 0.385  $\text{mg L}^{-1}$  AgNPs, **d** 0.77  $\text{mg L}^{-1}$  AgNPs, **e** 1.54  $\text{mg L}^{-1}$  AgNPs and **f** 15.4  $\text{mg L}^{-1}$  AgNP. Arrowhead: in **b** stomata and in **c** trichome



such as singlet oxygen ( $^1\text{O}_2$ ), superoxide ( $\text{O}_2^{\bullet-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\text{OH}^\bullet$ ) (Hossain et al. 2015).

ROS influence the growth and development of a plant, so they need to be rapidly metabolized by the antioxidant system to avoid oxidative stress conditions. Enzyme metabolism for ROS detoxification in plants is essential for the protection of plant cells and their organelles against the toxic effects of ROS (Mittler 2017). Thus, when a plant is subjected to some type of abiotic stress, the increase in the activity of antioxidant enzymes is remarkable (Gill et al. 2015). It is important to evaluate the physiological conditions of plant cells in this context, as these conditions effect their growth and development.

SOD is the first line of defence of the antioxidant system, catalysing the decomposition of  $\text{O}_2^{\bullet-}$  in  $\text{O}_2$  and  $\text{H}_2\text{O}_2$ , and CAT and APX catalyse the dismutation of  $\text{H}_2\text{O}_2$  in  $\text{H}_2\text{O}$  and  $\text{O}_2$  (Das and Roychoudhury 2014).

It is reported that  $\text{Ag}^+$  ions released from AgNPs induce oxidative stress through the generation of ROS. In vitro multiplication of sugarcane by temporary immersion, an increase of ROS and lipid peroxidation have been reported when different concentrations of AgNPs were added to MS medium (Bello–Bello et al. 2017). In *Solanum tuberosum*, an increase in  $\text{H}_2\text{O}_2$  and lipid peroxidation was also observed in explants cultured in vitro in the presence of AgNPs or silver nitrate (Bagherzadeh Homaei and Ehsanpour 2015).

However, in the present study, it is possible to verify that AgNPs were probably not provoking the production of ROS in *C. rufa* seedlings grown in vitro, since SOD activity was not significantly changed by the addition of AgNPs or silver nitrate. The analysis of the images obtained by light and scanning microscopy showed no cellular damage in the vegetal cells. However would have been expected if high concentrations of H<sub>2</sub>O<sub>2</sub>, (which can potentially cause plant cell death) had been present.

The greater opening of the stomata in the silver nitrate treatment is related to the positive effect of AgNO<sub>3</sub> in the reduction of hyperhydricity. It has been reported that the presence of AgNO<sub>3</sub> in the culture medium reduces the hyperhydricity of explants cultivated in vitro by promoting greater stomatal opening, resulting in greater water outflow from the plantlets and reducing the accumulation of water in the tissues (Gao et al. 2017).

In *Bacopa monnieri*, no morphological changes or toxic effects were observed in plants treated with AgNPs or silver nitrate by scanning electron microscopy. However, in light microscopy, small changes were observed in xylem elements within stems and roots of plants treated with AgNPs and AgNO<sub>3</sub> (Krishnaraj et al. 2012). In *Oryza sativa*, the presence of AgNPs of 150 nm at 10 and 100 mg L<sup>-1</sup> caused alterations in the anatomy of plantlets' leaves (Thuesombat et al. 2014). These results demonstrate that both the size and concentration of AgNPs may affect the development of plant species. There is need for further study to better understand the effects of AgNPs, since in the literature, the results remain controversial and inconclusive.

## Conclusions

AgNPs did not affect the in vitro multiplication of *C. rufa* at low concentrations, and the results for silver nitrate (180 mg L<sup>-1</sup>) were similar to the results for the 15.4 mg L<sup>-1</sup> concentration of AgNPs. These results indicate that AgNPs may cause more damage to plant development than AgNO<sub>3</sub>, depending on the concentration employed.

The characterization of the culture media containing AgNPs shows that the heat treatment drastically increases the size of the AgNPs and that this can significantly alter the unique properties present only in nanoparticles. These results are important because they help explain the behaviour of AgNPs under high temperatures and suggest opportunities for future research on the effects of heat-treated AgNPs on plant cells.

**Acknowledgements** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. Sincere thanks to the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) and the National Council for Scientific and Technological Development

(CNPq), for financial support. We are also grateful to the National Laboratory of Nanotechnology for Agribusiness - Embrapa Instrumentation - São Carlos-SP, where the silver nanoparticles were characterized.

**Author contributions** CT, MR planned the study, performed the experiments, analyzed the results and wrote the manuscript. RP research supervisor, contributed with scientific advice, corrected and revised the final version of the manuscript. JO, JM, PC and DS assisted in the planning and supervision of the study, performed the experiments, analyzed the results and corrected the manuscript. PC assisted performed experiments and analyzed the results. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors state that the present study was conducted in the absence of commercial or financial relationships that could result in a potential conflict of interest.

## References

- Abdi G, Salehi H, Khosh-Khui M (2008) Nano silver: A novel nanomaterial for removal of bacterial contaminants in valerian (*Valeriana officinalis* L.) tissue culture. *Acta Physiol Plant* 30:709–714. <https://doi.org/10.1007/s11738-008-0169-z>
- Adani F, Papa G, Schievano A et al (2011) Nanoscale structure of the cell wall protecting cellulose from enzyme attack. *Environ Sci Technol* 45:1107–1113. <https://doi.org/10.1021/es1020263>
- Bagherzadeh Homae M, Ehsanpour AA (2015) Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Indian J Plant Physiol* 20:353–359. <https://doi.org/10.1007/s40502-015-0188-x>
- Bello-Bello JJ, Chavez-Santoscoy RA, Lecona-Guzmán CA et al (2017) Hormetic response by silver nanoparticles on in vitro multiplication of sugarcane (*Saccharum* spp. Cv. Mex 69–290) using a temporary immersion system. *Dose Response* 15:1559325817744945. <https://doi.org/10.1177/1559325817744945>
- Biddington NL (1992) The influence of ethylene in plant tissue culture. *Plant Growth Regul* 11:173–187. <https://doi.org/10.1007/BF00024072>
- Biemelt S, Keetman U, Albrecht G (1998) Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. *Plant Physiol* 116:651–658. <https://doi.org/10.1104/pp.116.2.651>
- Carpita N, Sabulase D, Montezinos D, Delmer DP (1979) Determination of the pore size of cell walls of living plant cells. *Science* 205:1144–1147. <https://doi.org/10.1126/science.205.4411.1144>
- Chung I-M, Rekha K, Rajakumar G, Thiruvengadam M (2018) Production of bioactive compounds and gene expression alterations in hairy root cultures of chinese cabbage elicited by copper oxide nanoparticles. *Plant Cell Tissue Organ Cult*. <https://doi.org/10.1007/s11240-018-1402-0>
- Cvjetko P, Milošić A, Domijan A-M et al (2017) Toxicity of silver ions and differently coated silver nanoparticles in *Allium cepa* roots. *Ecotoxicol Environ Saf* 137:18–28. <https://doi.org/10.1016/j.ecoenv.2016.11.009>
- Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci* 2:53. <https://doi.org/10.3389/fenvs.2014.00053>
- Gao H, Xu P, Li J et al (2017) AgNO<sub>3</sub> prevents the occurrence of hyperhydricity in *Dianthus chinensis* L. by enhancing water loss



- and antioxidant capacity. *Vitr Cell Dev Biol* 53:561–570. <https://doi.org/10.1007/s11627-017-9871-0>
- Geisler-Lee J, Wang Q, Yao Y et al (2012) Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology* 7(3):323–337. <https://doi.org/10.3109/17435390.2012.658094>
- Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol* 59:309–314. <https://doi.org/10.1104/PP.59.2.309>
- Gill SS, Anjum NA, Gill R et al (2015) Superoxide dismutase—mentor of abiotic stress tolerance in crop plants. *Environ Sci Pollut Res* 22:10375–10394. <https://doi.org/10.1007/s11356-015-4532-5>
- Gogosz AM, Cosmo NL, Bona C, Souza LA de (2010) Morfoanatomia da plântula de *Campomanesia xanthocarpa* O. Berg. (Myrtaceae). *Acta Bot Brasilica* 24:613–623. <https://doi.org/10.1590/S0102-33062010000300003>
- Hossain Z, Mustafa G, Komatsu S (2015) Plant responses to nanoparticle stress. *Int J Mol Sci* 16:26644–26653. <https://doi.org/10.3390/ijms161125980>
- Javed R, Mohamed A, Yücesan B et al (2017) CuO nanoparticles significantly influence in vitro culture, steviol glycosides, and antioxidant activities of *Stevia rebaudiana* Bertoni. *Plant Cell Tissue Organ Cult* 131:611–620. <https://doi.org/10.1007/s11240-017-1312-6>
- Jiang H-S, Qiu X-N, Li G-B et al (2014) Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrrhiza*. *Environ Toxicol Chem* 33:1398–1405. <https://doi.org/10.1002/etc.2577>
- Kim DH, Gopal J, Sivanesan I (2017) Nanomaterials in plant tissue culture: the disclosed and undisclosed. *RSC Adv* 7:36492–36505. <https://doi.org/10.1039/C7RA07025J>
- Kole C, Kole P, Randunu KM et al (2013) Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC Biotechnol* 13:37. <https://doi.org/10.1186/1472-6750-13-37>
- Krishnaraj C, Jagan EG, Ramachandran R et al (2012) Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. *Process Biochem* 47:651–658. <https://doi.org/10.1016/j.procbio.2012.01.006>
- Lahiani MH, Dervishi E, Chen J et al (2013) Impact of carbon nanotube exposure to seeds of valuable crops. *ACS Appl Mater Interfaces* 5:7965–7973. <https://doi.org/10.1021/am402052x>
- Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Sci Total Environ* 408:3053–3061. <https://doi.org/10.1016/J.SCITOTENV.2010.03.031>
- Mahendran D, Kishor PBK, Geetha N, Venkatachalam P (2017) Phycomolecule-coated silver nanoparticles and seaweed extracts induced high-frequency somatic embryogenesis and plant regeneration from *Gloriosa superba* L. *J Appl Phycol*. <https://doi.org/10.1007/s10811-017-1293-1>
- Mittler R (2017) ROS are good. *Trends Plant Sci* 22:11–19. <https://doi.org/10.1016/J.TPLANTS.2016.08.002>
- Mozafari A, Havas F, Ghaderi N (2018) Application of iron nanoparticles and salicylic acid in in vitro culture of strawberries (*Fragaria × ananassa* Duch.) to cope with drought stress. *Plant Cell Tissue Organ Cult* 132:511–523. <https://doi.org/10.1007/s11240-017-1347-8>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* 15:473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59:368–373. <https://doi.org/10.1007/BF01248568>
- Pandey C, Khan E, Mishra A et al (2014) Silver nanoparticles and its effect on seed germination and physiology in *Brassica juncea* L. (Indian Mustard) Plant. *Adv Sci Lett* 20:1673–1676. <https://doi.org/10.1166/asl.2014.5518>
- Rostami AA, Shahsavari A (2009) Nano-Silver particles eliminate the in vitro contaminations of olive “Mission” explants. *Asian J Plant Sci*. <https://doi.org/10.3923/ajps.2009.505.509>
- Sant'Ana CRO, Paiva R, Reis MV et al (2018) In vitro propagation of *Campomanesia rufa*: an endangered fruit species. *Ciência e Agrotecnologia* 42(4):372–380. <https://doi.org/10.1590/1413-70542018424011018>
- Sarmast MK, Salehi H (2016) Silver nanoparticles: an influential element in plant nanobiotechnology. *Mol Biotechnol* 58:441–449. <https://doi.org/10.1007/s12033-016-9943-0>
- Sarmast MK, Niazi A, Salehi H, Abolmoghdam A (2015) Silver nanoparticles affect ACS expression in *Tecomella undulata* in vitro culture. *Plant Cell Tissue Organ Cult* 121:227–236. <https://doi.org/10.1007/s11240-014-0697-8>
- Satish L, Rameshkumar R, Rathinapriya P et al (2015) Effect of seaweed liquid extracts and plant growth regulators on in vitro mass propagation of brinjal (*Solanum melongena* L.) through hypocotyl and leaf disc explants. *J Appl Phycol* 27:993–1002. <https://doi.org/10.1007/s10811-014-0375-6>
- Scalzo J, Donno D, Miller S et al (2016) Effect of genotype, medium and light on in vitro plant proliferation of *Vaccinium* spp. *New Zeal J Crop Hort Sci* 44:231–246. <https://doi.org/10.1080/01140671.2016.1206946>
- Sharma P, Bhatt D, Zaidi MGH et al (2012) Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl Biochem Biotechnol* 167:2225–2233. <https://doi.org/10.1007/s12010-012-9759-8>
- Siddiqui MH, Al-Wahaibi MH (2014) Role of nano-SiO<sub>2</sub> in germination of tomato (*Lycopersicon esculentum* seeds Mill.). *Saudi J Biol Sci* 21:13–17. <https://doi.org/10.1016/J.SJBS.2013.04.005>
- Spinoso-Castillo JL, Chavez-Santoscoy RA, Bogdanchikova N et al (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. <https://doi.org/10.1007/s11240-017-1169-8>
- 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. <https://doi.org/10.1016/J.PLAPHY.2014.07.010>
- Thuesombat P, Hannongbua S, Akasit S, Chadchawan S (2014) Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol Environ Saf* 104:302–309. <https://doi.org/10.1016/j.ecoenv.2014.03.022>
- Torney F, Trewyn BG, Lin VS-Y, Wang K (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat Nanotechnol* 2:295–300. <https://doi.org/10.1038/nnano.2007.108>
- Tripathi A, Liu S, Singh PK et al (2017) Differential phytotoxic responses of silver nitrate (AgNO<sub>3</sub>) and silver nanoparticle (AgNPs) in *Cucumis sativus* L. *Plant Gene* 11:255–264. <https://doi.org/10.1016/J.PLGENE.2017.07.005>
- Turkevich J, Stevenson PC, Hillier J (1951) A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss Faraday Soc* 11:55. <https://doi.org/10.1039/d19511100055>
- Venkatachalam P, Kalaiarasi K, Sreeramanan S (2015) Influence of plant growth regulators (PGRs) and various additives on in vitro plant propagation of *Bambusa arundinacea* (Retz.) Wild. A recalcitrant bamboo species. *J Genet Eng Biotechnol* 13:193–200. <https://doi.org/10.1016/J.JGEB.2015.09.006>
- Venkatachalam P, Malar S, Thiyagarajan M et al (2017) Effect of phytochemical coated silver nanocomplexes as novel growth-stimulating compounds for plant regeneration of *Alternanthera*

- sessilis* L. J Appl Phycol 29:1095–1106. <https://doi.org/10.1007/s10811-016-0977-2>
- Vinković T, Novák O, Strnad M et al (2017) Cytokinin response in pepper plants (*Capsicum annuum* L.) exposed to silver nanoparticles. Environ Res 156:10–18. <https://doi.org/10.1016/j.envres.2017.03.015>
- Vishwakarma K, Shweta G, Upadhyay N et al (2017) Differential phytotoxic impact of plant mediated silver nanoparticles (AgNPs) and silver nitrate (AgNO<sub>3</sub>) on *Brassica* sp. Front Plant Sci 8:1501. <https://doi.org/10.3389/fpls.2017.01501>
- Yin L, Cheng Y, Espinasse B et al (2011) More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. Environ Sci Technol 45:2360–2367. <https://doi.org/10.1021/es103995x>
- Yu R, Baniaga AE, Jorgensen SA, Barker MS (2017) A successful in vitro propagation technique for resurrection plants of the Selaginellaceae. Am Fern J 107:96–104. <https://doi.org/10.1640/0002-8444-107.2.96>

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.