Silver nanoparticles on dragon fruit *in vitro* germination and growth

Nanopartículas de prata na germinação e crescimento *in vitro* de fruta do dragão

Caroline de Oliveira Timoteo[1], Renato Paiva[1], Michele Valquíria dos Reis[1*], Diogo Pedrosa Corrêa da Silva[2], Bruna Raphaella da Silva[1], Juliano Elvis de Oliveira[3]

ABSTRACT

Silver nanoparticles (AgNPs) have different physicochemical properties compared to their counterparts that occur on the macro scale. Due to these properties, it has been used in a variety of medicinal products, pharmaceuticals, food packaging, dyes, cosmetics, biosensors, electronics and agriculture. However, although there are attempts to determine possible toxic effects of AgNPs, the results are still inconclusive, since the phytotoxicity of the nanoparticles in plant species is dependent on the species and the concentration of AgNPs to which they are exposed. Thus, the objective of this study was to evaluate the phytotoxicity of AgNPs on the *in vitro* cultivation of the cactus *Hylocereus undatus* (dragon fruit). For this purpose, dragon fruit seeds were inoculated in 1/4 MS culture medium supplemented by different concentrations of AgNPs. Subsequently, the seedlings were acclimatized. After 90 days of *in vitro* cultivation, it was observed that the presence of AgNPs did not affect dragon fruit germination, as well as the length of the cladodium. However, longer roots were observed in the presence of 8 mg L^{-1} of AgNPs. In acclimatization, the plants from *in vitro* cultivation with AgNPs showed a higher number of shoots. These results indicate that the tested concentrations of AgNPs showed no *in vitro* cultured dragon fruit toxicity.

Index terms: Nanotechnology; *Hylocereus undatus*; pitaya; tissue culture.

RESUMO

As nanopartículas de prata (AgNPs) apresentam propriedades físico-químicas distintas em comparação aos seus homólogos que ocorrem na escala macro. Devido a essas propriedades, vem sendo utilizada em uma variedade de produtos medicinais, farmacêuticos, em embalagens de alimentos, tinturas, cosméticos, biossensores, eletrônicos e na agricultura. Entretanto, embora existam tentativas para determinar possíveis efeitos tóxicos das AgNPs, os resultados ainda apresentam-se inconclusivos, uma vez que a fitotoxicidade das nanopartículas em espécies vegetais é dependente da espécie e da concentração de AgNPs a qual são expostas. Dessa forma o objetivo do presente estudo foi avaliar a fitotoxicidade das AgNPs no cultivo *in vitro* da cactácea *Hylocereus undatus* (fruta do dragão**)**. Para esse fim, sementes de fruta do dragão foram inoculadas em meio de cultura 1/4 MS suplementado por diferentes concentrações de AgNPs. Posteriormente as plântulas obtidas foram aclimatizadas. Após 90 dias de cultivo *in vitro,* observou-se que a presença de AgNPs não afetou a germinação da fruta do dragão, assim como o comprimento do cladódio. Entretanto, raízes de maior comprimento foram observadas na presença de 8 mg L^{-1} de AgNPs. Na aclimatização, as plantas provenientes do cultivo *in vitro* com AgNPs apresentaram maior número de brotos. Esses resultados indicam que as concentrações testadas de AgNPs não apresentaram toxidez em fruta do dragão cultivada *in vitro*.

Termos para indexação: Nanotecnologia; *Hylocereus undatus*; pitaia; cultura de tecidos.

INTRODUCTION

Silver is widely known for its antimicrobial properties and has been used since antiquity. However, with the advent of nanotechnology, this has become widely used on the nano scale (Prabhu; Poulose, 2012). Silver nanoparticles (AgNPs) are considered more efficient than silver in their macro or micro scale, due to their excellent catalytic and conductive properties and antibacterial activities (Alves Claro et al., 2018; Xu et al., 2018; Yin et al. al., 2018). As a result, these unique characteristics have led to their wide use in different products and formulations in different areas (Peters et al., 2016), and it is considered one of the most commonly used metallic nanomaterials in industries and agriculture, accounting for 24% of products which have nanoparticles in their compositions (Vance et al., 2015).

With the widespread use of AgNPs on a commercial scale, the possibilities of environmental release and the potential risks to the environment and human health have greatly increased. Thus, the interaction of

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[1]Universidade Federal de Lavras/UFLA, Departamento de Biologia/DBI, Lavras, MG, Brasil
[2]Universidade Federal de Lavras/UFLA, Departamento de Agricultura/DAG, Lavras, MG, Brasil
[3]Universidade Federal de Lavras/UFLA, Departamento de Engenharia/DEG, Lavras, MG, Brasil
[*]Corresponding author: mvreis@yahoo.com.br

silver nanoparticles-plants occurs, either indirectly, by discarding products in the nature that present these nanoparticles in their composition, or directly, applying AgNPs (Fabrega et al. al., 2011). Regardless of the form of exposure, there is not an extensive knowledge about the effects that nanoparticles have on the plant system yet.

In recent years, research has sought information on the effects of silver nanoparticles on plant germination and growth, bioaccumulation and contamination of natural resources in order to clarify the phytotoxicity potential of AgNPs in plant species (Liu et al., 2018; Zhang et al., 2018; Li et al., 2017; Baptista et al., 2015; Thuesombat et al., 2014). However, there are still gaps as to their toxicity due to controversial and inconclusive results. In this context, it is important to study the phytotoxicity of AgNPs in plants, as they form the basis of the food chain, so that rules and regulations for the use and disposal are established, in order to avoid environmental damage.

Thus, the aim of this study was to analyze the phytotoxicity potential of AgNPs in dragon fruit [*Hylocereus undatus* (Haw.) Britton & Rose], a cactus whose commercial cultivation has been increasing due to antioxidant, anticancer, antimicrobial, antihyperlipidemic, antidiabetic and cicatrizant properties (Ibrahim et al., 2018). Dragon fruit cultivation becomes even more promising due to the desirable agronomic characteristics of the crop, which shows tolerance to drought, allowing its cultivation in regions with low rainfall rates.

MATERIAL AND METHODS

Chemical and plant material

Silver nanoparticles (AgNPs) were synthesized according to the methodology described by (Turkevich; Stevenson; Hillier, 1951), with adaptations. A solution was prepared with silver nitrate (0.18 g L^{-1}) and sodium carboxymethylcellulose (0.6 g L^{-1}), which remained under constant heating and stirring. Upon reaching 95 °C, an aqueous sodium citrate solution (1%) was added. The concentration of the AgNPs solution was quantified by UV-VIS absorption spectroscopy (model UV-1800, Shimadzu).

Dragon fruit seeds (*Hylocereus undatus*) were extracted from ripe fruits and underwent a manual withdrawal process of mucilage under running water. The seeds were then involved in paper towel for 24 hours for drying at room temperature and stored in plastic tubes at 8 °C.

AgNPs *in vitro* culture

In a laminar flow chamber the seeds were disinfested in 70% alcohol (1 minute) and commercial sodium hypochlorite (2.5%) for 20 minutes (Lopes et al., 2017). The seeds were then inoculated in 1/4 MS culture medium (Murashige; Skoog, 1962), plus 30 g L^{-1} sucrose and 2.5 g L^{-1} phytagel (Lopes et al., 2017). Different concentrations of silver nanoparticles (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg L^{-1}) were added to the culture medium. The pH was adjusted to 5.7 ± 0.1 before being autoclaved. The culture medium was sterilized by autoclaving at 121 °C and 1 atm for 20 minutes.

The material was maintained in a growth room at 25 °C, photoperiod of 16/8 h (light / dark) under white fluorescent light with irradiance of 36 µmol m^{-2} s^{-1}. After 90 days of cultivation, germination (%), shoot length (cm), root length (cm) and fresh weight (g) of the seedlings were evaluated.

Acclimatization

Seedlings from *in vitro* cultivation with AgNPs (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg L^{-1}) were acclimatized. Thus, 16 seedlings of each treatment were removed from the *in vitro* environment and washed in running water for removal of the culture medium. They were then transferred to plastic cups (250 mL) containing commercial substrate (Topstrato Hp®). The plastic cups with the plants were covered with a transparent plastic bag and kept in an acclimatization room at 25 °C and photoperiod of 16/8 h (light / dark) under white fluorescent light, with a mean irradiance of 56 µmol m^{-2} s^{-1}. Every 7 days, part of the plastic bag was cut and the plants watered and, at 21 days, they were completely removed and the plants were watered every two days up to 45 days. After 45 days of acclimatization, survival (%), shoot length (cm), root length (cm), fresh weight (g) and number of shoots were evaluated.

After the 45-day period in the acclimatization room, the plants were transferred to a nursery with 50% shade nets, where they remained for another 45 days, at the end of which plant survival was evaluated.

Statistical analysis

The experiments were performed in a completely randomized design (CRD). Data were analyzed by the SISVAR statistical software (Ferreira, 2014) by the Scott-Knott Test at 5% probability.

For the *in vitro* culture of dragon fruit, 100 seeds were used, distributed in 5 bottle, with 20 seeds per bottle of culture, and each seed was considered as a replication. For acclimatization, 16 seedlings *in vitro* were used, and each seedling was considered as a replication.

RESULTS AND DISCUSSION

AgNPs *in vitro* culture

The use of AgNPs did not affect the *in vitro* germination of dragon fruit, obtaining an average of 37% germination of the inoculated seeds (Table 1 and Figure 1).

However, the length of the cladode and fresh weight were not affected by the presence of AgNPs in the culture medium. Nevertheless, longer roots were observed in the treatment with 8 mg L^{-1} AgNPs (Table 1).

Similar results were observed in Arabidopsis seeds, whose germination was not affected by the presence of AgNPs in the culture medium at concentrations of 0.2, 0.5 and 3.0 mg L^{-1} (Qian et al., 2013). Similarly, AgNPs did not alter seed germination of *Raphanus sativus* at concentrations up to 500 mg L^{-1} (Zuverza-Mena et al., 2016). However, in *Brassica nigra*, concentrations above 50 mg L^{-1} led to a decline in germination (Amooaghaie; Tabatabaei; Ahadi, 2015).

Analyzing the growth parameters in *Brassica* sp. it was observed that the toxic effects of AgNPs were dose-dependent, leading to reductions in fresh weight and root and shoot length, with increasing AgNP concentration (Vishwakarma et al., 2017). This was also observed in *Saccharum* spp., in which the presence of AgNPs in the culture medium caused an increase in number of shoots at concentrations of 50 and 100 mg L^{-1} and reductions at the concentration of 200 mg L^{-1} (Bello-Bello et al., 2017). In *Vigna radiata* the length and weight of root and shoot were not altered in plants exposed to 5, 10 and 20 mg L^{-1} of AgNPs when compared to the control, but were reduced after exposure of 50 mg L^{-1} of AgNPs (Nair; Chung, 2015).

Table 1 – Growth analysis of dragon fruit seedlings after 90 days of cultivation, whose seeds were germinated *in vitro* in MS medium supplemented by different concentrations of silver nanoparticles.

AgNPs (mg L^{-1})	Germination (%)*	Cladode (cm)*	Root (cm)*	Fresh weight (mg)*
0	41.25 (0.086) a	4.03 (0.27) a	3.09 (0.41) b	0.34 (0.025) a
0.5	43.00 (0.076) a	3.94 (0.33) a	2.68 (0.25) b	0.30 (0.031) a
1	33.00 (0.082) a	3.58 (0.29) a	2.87 (0.31) b	0.26 (0.029) a
2	48.00 (0.072) a	3.75 (0.26) a	3.46 (0.26) b	0.27 (0.024) a
4	35.00 (0.081) a	3.64 (0.33) a	3.59 (0.40) b	0.34 (0.048) a
8	30.00 (0.084) a	3.54 (0.28) a	4.51(0.36) a	0.30 (0.035) a

*Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability. The numbers in parentheses correspond to the standard error.

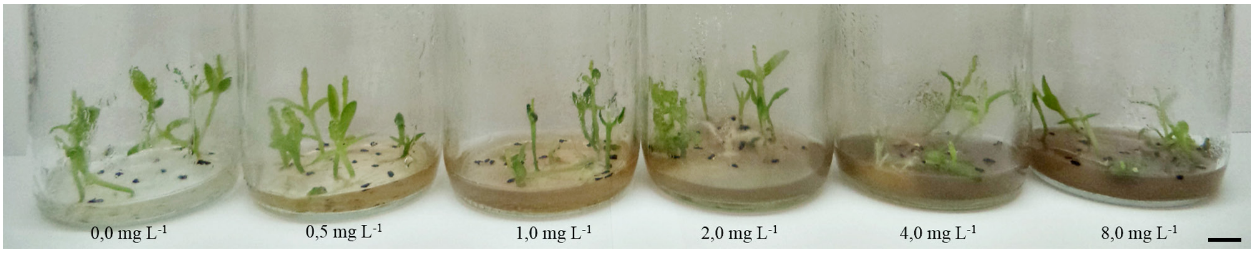

Figure 1 – Dragon fruit seedlings, after 90 days of *in vitro* culture, whose seeds were germinated in MS medium supplemented by different concentrations of silver nanoparticles (AgNPs). Bar = 1 cm.

Similar results were observed in *Solanum tuberosum*, where AgNPs reduced shoot length, but significantly increased root length, leaf area and dry weight of shoot and root at 2 mg L^{-1} concentration (Bagherzadeh Homaee; Ehsanpour, 2015). In *Cucurbita pepo*, exposure of AgNPs at concentrations of 500 and 1000 mg L^{-1} reduced their biomass by 57% and 71%, respectively, and transpiration by 78% and 79%, respectively (Stampoulis; Sinha; White, 2009). In *Arabidopsis* and *Eruca sativa*, exposure to AgNPs caused an increase in root elongation (Syu et al., 2014; Vannini et al., 2013).

As shown, the effects of AgNPs on plant growth and development are closely related to the concentration of nanoparticles in the growing environment, which can be toxic or stimulate root and shoot growth. In this study, low concentrations of AgNPs were used in the culture medium, in relation to other studies that address the phytotoxicity potential of AgNPs. As no phytotoxic effects of AgNPs were observed on dragon fruit, it is believed that these results may be related to the concentration used.

Another factor that is related to AgNP toxicity is the size of the AgNPs. Studies with *Arabidopsis* and poplar have shown that smaller particles tend to be more toxic than larger particles (Wang et al., 2013). Smaller nanoparticles have a larger surface area, leaving a greater number of exposed atoms on the surface, which leads to the occurrence of redox, photochemical, biochemical reactions and intercellular physicochemical interactions (Marambio-Jones; Hoek, 2010).

Due to the reduced size, smaller nanoparticles are easier to penetrate the walls of the plant cells and to be transported (Navarro et al., 2008), since their transport in the cells occurs via apoplast or symplast and are transported from one cell to another through plasmodesm (Rico et al., 2011). In studies conducted with *Arabidopsis*, it was observed that only 20 and 40 nm nanoparticles were found in the cell wall and in the plasmodesm of the cells, and caused more toxic effects in the roots, when compared to AgNPs of 80 nm (Geisler-Lee et al. 2012).

The stability of silver nanoparticles also influences the toxicity of AgNPs, where unstable particles tend to aggregate, which decreases their biocidal activity (Kvítek et al., 2008). Conditions of the culture medium such as pH, ionic strength, presence of complexing agents and organic matter affect the stability of AgNPs, given that the nanoparticles tend to aggregate when submitted to high salt concentrations and pH values close to the isoelectric point. (Elzey; Grassian, 2010; Marambio-Jones; Hoek, 2010).

Considering that concentration, size and stability of AgNPs affect their toxicity and since no toxic effects were observed in dragon fruit plants exposed to different concentrations of nanoparticles, these results are believed to be related to the aggregation potential of AgNPs in the medium, together with the low concentration used, which potentially decreased the toxic effects of AgNPs on dragon fruit seedlings.

Acclimatization

After the acclimatization period, 100% of the seedlings from *in vitro* cultivation at different concentrations of AgNPs survived. No significant differences were observed between the plants regarding shoot and root length (Table 2 and Figure 2). However, there were differences in the number of shoots. The treatments with 2, 4 and 8 mg L^{-1} AgNPs showed a larger number of shoots, but they did not present differences in length (Table 2). The fresh weight was not affected by seed germination in the culture medium with AgNPs (Table 2).

After 45 days in the nursery with 50% shade nets, 100% survival of the acclimatized plants was observed. These results indicate that the AgNPs were not phytotoxic to dragon fruit, since seedlings obtained by *in vitro* germination showed 100% survival in the *ex vitro* environment.

Beneficial aspects of AgNPs in plant cells have been observed in several species. As in dragon fruit, where a larger number of shoots were observed in plants that were germinated in the presence of AgNPs, the application of AgNPs in MS medium increased the efficiency of shoot regeneration in *Swertia chirata* due to the inhibitory action of AgNPs on ethylene (Saha, Dutta Gupta, 2018).

AgNPs also induced an increase in root length and an increase in vigor index of *Brassica juncea* seedlings due to improvements in photosynthetic quantum efficiency and antioxidant potential, reducing the levels of reactive oxygen species (Sharma et al., 2012). They induced a shoot increase in *Vanilla planifolia*, as a consequence of the increase in the accumulation of N and Mg, elements associated with the biosynthesis of chlorophyll, whose molecule is necessary for the photosynthetic activity (Spinoso-Castillo et al., 2017).

However, some studies report phytotoxic effects of AgNPs. In cytotoxic studies using *Allium cepa*, it was reported that AgNPs at 100 mg L^{-1} decreased mitotic index and caused chromosomal aberrations such as viscosity, breaks and cell wall disintegration (Kumari; Mukherjee; Chandrasekaran, 2009). In *Arabidopsis*, seedlings treated with AgNPs had shorter roots than seedlings of the control treatment and these showed visible brown tips, with particles smaller than 80 nm, causing greater impact on plant growth (Geisler-Lee et al., 2012).

Growth of *Phaseolus radiatus* and *Sorghum bicolor* seedlings exposed to AgNPs of 40 mg L^{-1} also showed a reduction of 20% and 47%, respectively, when compared to seedlings without exposure to AgNPs, whose growth was reduced due to damage in the AgNPs system in the cells (Lee; Kwak; An, 2012). In *Lemna minor*, it was observed that low concentrations of AgNPs (160 µg L^{-1}) reduced the number of fronds and, consequently, the relative growth rate (Gubbins; Batty; Lead, 2011).

These results indicate that the phytotoxicity of AgNPs is intrinsically related to the species, since their effects depend on the intrinsic characteristics of the species and can greatly vary from ecosystem to ecosystem.

Table 2 – Dragon fruit growth analyses after 45 days of acclimatization, whose seeds were germinated *in vitro* in MS medium supplemented by different concentrations of silver nanoparticles.

AgNPs (mg L^{-1})	Survived (%)*	Cladode (cm)*	Root (cm)*	Number of shoots*	Average shoot size (cm)*	Fresh weight (mg)*
0	100 a	6.71 (0.67) a	3.89 (0.49) a	2.81 (0.41) b	1.91 (0.30) a	1.35 (0.28) a
0.5	100 a	7.98 (0.78) a	4.08 (0.64) a	2.40 (0.64) b	1.30 (0.43) a	1.83 (0.33) a
1	100 a	6.07 (0.64) a	3.16 (0.30) a	2.79 (0.90) b	1.69 (0.46) a	1.26 (0.33) a
2	100 a	8.01 (0.83) a	3.64 (0.40) a	4.25 (0.75) a	1.67 (0.29) b	2.36 (0.51) a
4	100 a	6.53 (0.64) a	3.99 (0.57) a	5.25 (0.94) b	2.27 (0.35) b	2.17 (0.24) a
8	100 a	5.56 (0.62) a	4.09 (0.53) a	4.94 (0.88) b	1.71 (0.26) b	1.62 (0.32) a

*Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability. The numbers in parentheses correspond to the standard error.

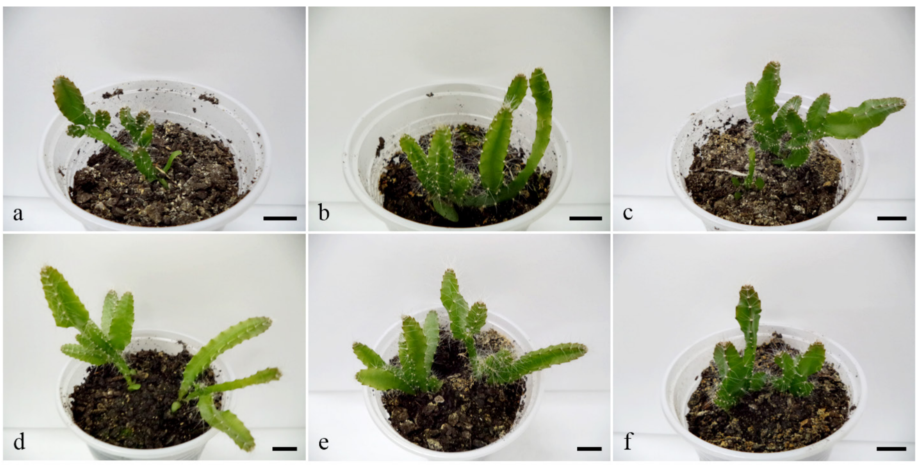

Figure 2 – Dragon fruit plants germinated *in vitro* in the presence of different concentrations of silver nanoparticles (AgNPs) after 45 days of acclimatization. Em a) 0.0, b) 0.5, c) 1.0, d) 2.0, e) 4.0 and f) 8.0 mg L^{-1} of AgNPs. Bar = 1 cm.

Once plants are the basis of the food chain and are able to accumulate AgNPs in their tissues, it is believed that bioaccumulation could occur at higher trophic levels, as evidenced in wheat and peanut studies, where an AgNPs accumulation was reported in the grains (Yang et al., 2018; Rui et al., 2017). However, in spite of the phytotoxic effects, it is reported that AgNPs can also stimulate plant growth depending on the concentration used, leading to improvements in the antioxidant system, eliminating reactive oxygen species and stimulating plant growth (Gupta; Agarwal; Pradhan, 2018). In this context, it is undisputed that the use and disposal of AgNPs in the environment should be carried out with caution and restrictions in order to avoid disastrous and irreparable damage to the environment and at the same time to ensure the conscious use of AgNPs that can lead to improvements in agricultural production.

CONCLUSIONS

AgNPs do not show toxicity in the *in vitro* germination of dragon fruit at the concentrations used in this study. AgNPs induced positive effects on root length and number of shoots. However, as the toxicity potential of AgNPs is related to their size, concentration, stability and mainly to the intrinsic characteristics of the species; the need for more complex studies is evident to determine the phytotoxicity of AgNPs in plant species.

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