ScienceDirect

Scientia Horticulturae
Volume 253, 27 July 2019, Pages 358-363

Silver nitrate enhances *in vitro* development and quality of shoots of *Anthurium andraeanum*

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https://doi.org/10.1016/j.scienta.2019.04.054
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Highlights

- $AgNO_3$ at 1 mg L^{-1} improved the diameter of leaves and maintain the shoot proliferation in two Anthurium genotypes.
- $AgNO_3$ acts as Plant Growth Regulator, by low concentration used and distinguished *in vitro Anthurium* response.
- $AgNO_3$ can revert the main observed *in vitro* symptoms of ethylene effects in *Anthurium* micropropagation.

- $AgNO_3$ at concentrations above 2,0 mg L^{-1} resulted in lately rooting induction and elongation of Anthurium and needs avoided.

Abstract

During the *in vitro* cultures of *Anthurium*, some disorders such as excessive stem elongation and reduced leaf area have been observed. In this study, the hypothesis that such disorders can be attributed to the accumulation of ethylene in an *in vitro* culture environment was tested using $AgNO_3$, an inhibitor of ethylene action. Different concentrations of $AgNO_3$ were added to the culture medium on the multiplication stage of two cultivars of *Anthurium andraeanum*. The Murashige and Skoog (MS) culture medium with half of the concentration of macronutrients (MS½) and containing 30 g L^{-1} sucrose, 0.1 g L^{-1} myo-inositol, 1.5 g L^{-1} of activated charcoal, and 0.5 mg L^{-1} of kinetin was used for the multiplication stage of *A. andraeanum*. The genotype was found to be an important factor affecting shoot regeneration from leaf segments. The addition of $AgNO_3$ in the culture medium suppressed excessive stem elongation and increased the leaf expansion in both *A. andraeanum* cv. Alabama and Dakota. The concentrations of 1.0 or 2.0 mg L^{-1} of $AgNO_3$ were the most optimum to increase the quality of shoots obtained in the multiplication stage and to accelerate the rooting stage.

Introduction

Araceae represents a family of economically important plants with 113 genera and 3324 species (The Plant List, 2018) naturally occurring in the tropical Americas (Haigh, 2009). *Anthurium* and *Spathiphyllum* are the two most popular ornamental genera of the family and have beautiful large and colored spathes (Henny et al., 2017). *Anthurium* has secured a great place in the global flower market, either for pot or as cut flowers (Wang, 1999; Assis et al., 2011). The main breeder companies developing new varieties of *Anthurium* are located in the Netherlands (Noman and Stiglitz, 2015).

Although seeds can be used for propagation in *Anthurium*, this method is basically used for breeding and/or conservation purposes because of the high heterozygosity, a small number of seeds per plant, and long juvenility period for seedling development (Dufour and Guerin, 2003; Bejoy et al., 2008). The clonal micropropagation of *Anthurium andraeanum*, using shoot organogenesis from leaf explants, is the main technique used for the large-scale propagation of commercial varieties producing large quantities of plantlets with high clonal fidelity in different cultivars (Cardoso and Habermann, 2014).

The plantlets of *Anthurium,* derived from micropropagation, produce a high number of shoots in less time and space and is the only method that produces disease- and pest-free large-scale plantlets (Chen and Henny, 2008) compared with other vegetative propagation techniques (Tsang et al., 2010). The main disadvantage of micropropagation is high cost due to the environmentally controlled conditions for *in vitro* maintenance and the labor involved in transferring shoots and plantlets to a new agar-based culture medium (Chen, 2016; Cardoso et al., 2018). The rooting of small (3–5 cm long) apical stem cuttings in temperature-controlled greenhouses, called 'mini-cuttings', was combined with micropropagated plantlets to reduce the costs associated with micropropagation techniques.

Micropropagation is affected by different factors, such as plant growth regulators (PGRs) commonly used in the culture medium to control the *in vitro* development of plantlets. Generally, the cytokinins such as 6-benziladenine and kinetin, combined with 2,4-dichlorofenoxiacetic acid (2,4-D), stimulate the leaf organogenesis, whereas the isolated cytokinins promote high shoot proliferation. Further, some auxins such as indole-butyric acid (IBA) and naphthaleneacetic acid were used in the rooting phase for adventitious root induction (Gu et al., 2012; Cardoso and Habermann, 2014; Thokchom and Maitra, 2017). In addition, another PGR, ethylene gas, is naturally produced by the *in vitro* shoots and plantlets and can be accumulated in closed flasks (containers) and in growth rooms due to the limited gaseous exchange between the *in vitro* and *ex vitro* environment, causing some physiological disorders in the *in vitro* growing plantlets (Yasmin et al., 2013).

Among the most common disorders caused by ethylene accumulation under *in vitro* conditions are the excessive callus production, leaf abscission, and growth inhibition by production of etiolated shoots and early yellowing of leaves (Biddington, 1992; Santana-Buzzy et al., 2006). This reduces multiplication, growth, and efficiency of micropropagation, thus limiting and increasing the costs of micropropagation.

Similar symptoms were also observed during *Anthurium andraeanum* micropropagation in a commercial laboratory (JCC, Laboratory of Plant Physiology and Tissue Culture), especially in the multiplication stage, leading to the excessive callus production at the basal region of the shoots and the presence of etiolated shoots with a long stem and reduced leaf area. These symptoms in shoots delayed plantlet development in the next stages of rooting and acclimatization. Limited information is available on the effects of ethylene on *in vitro Anthurium* development. Teixeira da Silva et al. (2005) observed a considerable increase in the fresh weight of leaves of *A. andraeanum* (2-fold) when control bottles were replaced with Neoflon® PFA film Culture Packs, which offered better gas permeability and exchange (Tanaka, 1991). A peak of the ethylene accumulation was reported inside the flasks, with some symptoms in plantlets attributable to ethylene effects, whereas a ventilated system maintained a highly reduced level of ethylene with no symptoms in plantlets.

Other alternatives that can overcome the effects of ethylene on *in vitro* shoots and plantlets include the use of inhibitors of ethylene biosynthesis and action. Some Ag^+ ion containing chemicals can block ethylene receptors and are used for different purposes in agriculture, such as to extend the shelf-life of cut flowers (Ahmad et al., 2016), to reduce the microorganism proliferation in solutions (Pandian et al., 2010), and also for *in vitro* plant development (Teixeira da Silva, 2013; Sarropoulou et al., 2016). Ag^+ ions can act on *in vitro* growing plants and can improve plant regeneration, root formation (Steinitz et al., 2010; Tamimi, 2015), chlorophyll content (Tamimi, 2015), axillary shoot proliferation (Lai et al., 2000), and prevent excessive callus formations and mitigate other symptoms caused by ethylene (Santana-Buzzy et al., 2006).

This study aimed to test the micropropagation of two *Anthurium* cultivars, with the addition of silver nitrate ($AgNO_3$) to test its effects in preventing the morphological symptoms in shoots, which reduce the quality and delay the micropropagation of this important ornamental species.

Section snippets

In vitro establishment of leaf segments of *A. andraeanum* cultivars

The donors of explants for *in vitro* culture were adult plants in the phase of full blooming of *A. andraeanum* cv. Dakota and Alabama, both with red spathes, cultivated under greenhouse conditions with light intensity of 17–25,000 lx, temperature of 15–28 °C, air relative humidity > 50%, 4-mm/day drip irrigated, and fertirrigated weekly with 19–19–19 fertilizers (1.5 g L^{-1}) in pots (17 cm diameter) containing organic substrate coconut fiber.

For *in vitro* establishment, young leaves with…

In vitro establishment of leaf segments of *A. andraeanum* cultivars

At the establishment stage, the contamination rate was extremely low for cv. Alabama (10%) and nil (0%) for cv. Dakota. Although cv. Alabama showed higher regeneration, with 10.9 adventitious buds and 7.6 shoots per leaf segments, cv. Dakota showed recalcitrance to adventitious bud formation and resulted in only 2.5 adventitious buds and 0.6 shoots per leaf segments.

At the multiplication stage, *Anthurium andraeanum* cv. Dakota showed a higher number of leaves (4.4/shoot) and leaf diameter…

Genotype-dependent response of *in vitro* shoot organogenesis and shoot proliferation

The genotype is one of the main endogenous factors influencing the *in vitro* shoot development. Cardoso and Habermann (2014) tested four cultivars of *Anthurium* for shoot regeneration from leaf segments and showed that the percentage of shoot regeneration ranged from 0 to 8.13 shoots/leaf segment.

In our study, most of the shoot organogenesis from *Anthurium* leaf segments occurred during the intermediary phase of cream callus type, observed in leaf segment margins. *A. andraeanum* cv. Dakota showed…

Conclusions

The micropropagation of the two cultivars of *A. andraeanum*, Alabama and Dakota, was improved by the use of an ethylene inhibitor, $AgNO_3$, at low concentrations in the culture medium. The comparison between the treated or non-treated plantlets indirectly confirmed that *in vitro Anthurium* plantlets are susceptible to ethylene accumulation in flasks. The main symptoms of ethylene observed in non-treated plantlets were reversed by low concentrations of $AgNO_3$.…

Acknowledgements

JCC acknowledges CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the Processes 304174/2015-7 and 311083/2018-8 and to Uniplant Co. for infrastructural support.…

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Silver nitrate reduces hyperhydricity in shoots regenerated from the hypocotyl of snapdragon cv. Maryland Apple Blossom
2023, Scientia Horticulturae

Citation Excerpt :

…However, we observed an indication of slight improvement in shoot regeneration with 2.0 mg/L AgNO3, and the shoots at this concentration appeared healthier than others. This is consistent with the findings of Cardoso (2019) and Lian et al. (2020), who observed a positive effect of 2.0 mg/L AgNO3 on the shoot regeneration of Anthurium andraeanum and snapdragon. Xiaohan et al. (1995) and Lee et al.

(1997) also found that the addition of AgNO3 markedly increased the number of shoots per explant in chrysanthemum, especially in a medium containing a greater concentration of cytokinins than auxins.…

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2022, South African Journal of Botany

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…In vitro culturing of cells and tissues in closed containers may result in the accumulation of ethylene, which have adverse effects on differentiation and organogenesis (Faria et al., 2017). Ethylene also reduces the expansion of photosynthetic tissues, promotes necrosis, leaf senescence (Iqbal et al., 2017), and morpho-structural and physiological abnormality in the regenerants (Rodrigues et al., 2014; Cardoso, 2019). As per literature, the incorporation of ethylene inhibitors gradually improved organogenesis, somatic embryogenesis, and in vitro flowering (Zhang et al., 2001; Morshed et al., 2016; de Matos et al., 2021).…

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Informative title: Development of lighting intensity approach for shoot proliferation in Phalaenopsis amabilis through combination with silver nanoparticles

2022, Scientia Horticulturae

Citation Excerpt :

…Compounds containing silver, as inhibitors of ethylene signaling and therefore its action, can improve in vitro regeneration and shoot organogenesis (Vinod et al., 2009; Mahmoud et al., 2020). The beneficial effect of compounds containing silver (silver nanoparticles, AgNO3, etc) in micropropagation protocols has been systematically shown in several plants including Epidendrum denticulatum (Orchidaceae) (Juras et al., 2020), Alternanthera sessilis L. (Sowmya et al., 2020), Anthurium andraeanum (Cardoso et al., 2019) Swertia chirata (Saha and Dutta Gupta, 2018), and Cosmos bipinnatus (Jaberi et al., 2018). Furthermore, it has also been reported that AgNPs increase the explant longevity and shoot proliferation of Tecomella undulata in the in vitro condition (Sarmast et al. 2015).…

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> …These signs are the basic indications suggesting ethylene accretion in the culture vessels (Biddington, 1992; Santana-Buzzy et al., 2006). Similar response has been reported in many other species such as Capsicum chinense Jacq., Anthurium andraeanum and Rosa hybrid L. (Santana-Buzzy et al., 2006; Cardoso, 2019; Ha et al., 2020). To overcome such abnormalities, the effect of silver nitrate along with Kn fortified in MS medium was analyzed.…

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