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Research

The Effect of Silver Nitrate and Silver Thiosulphate on *In Vitro* Shoot Regeneration of Australian Pink Finger Lime (*Citrus australasica* cv. Mia Rose)

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ABSTRACT

Citrus australasica, commonly known as the finger lime, is a distinctive fruit indigenous to the coastal rainforest of Australia. It is renowned for its unique appearance, tangy flavor, and multiple medicinal properties. Additionally, its unique caviar-like pulp has garnered significant attention in the culinary industry. Conventional propagation of *C. australasica* is challenged by incompatibility issues during grafting and budding, its monoembryonic nature, and the lengthy time required to flower and bear fruits. Micropropagation on the other hand is an efficient alternative to conventional propagation for various commercial crops including citruses. However, the issue of leaf abscission was previously observed in *C. australasica* cultures, which has resulted in reduced shoot viability. The current study aimed to determine the effects of silver nitrate ($AgNO_3$) and silver thiosulphate (STS) on *in vitro* shoot regeneration of *C. australasica* cv. Mia Rose. Nodal explants were cultured on half-strength Murashige and Skoog (MS) media supplemented with $AgNO_3$ at 2.00, 4.00, 6.00, 8.00, and 10.00 mg/L and STS at 10.00, 20.00, 40.00, 60.00 and 80.00 µM in combination with 2.00 mg/L 6-benzylaminopurine (BAP). Results demonstrated that 4.00 mg/L $AgNO_3$ resulted in the highest shoot number and length (2.65 ± 0.18 and 0.27 ± 0.03 cm respectively), while treatment with 10.00 µM STS resulted in the highest number of shoots and shoot length (2.36 ±0.19 and 0.32 ± 0.04 cm respectively). No leaf abscission was observed for all treatments of $AgNO_3$ and STS after eight weeks of culture. This study underscored the efficacy of $AgNO_3$ and STS as potent ethylene antagonists in shoot cultures of *C. australasica* cv. Mia Rose, suggesting their potential utilization in the micropropagation of the finger lime species.

Key words: 6-benzylaminopurine (BAP), *Citrus australasica*, nodal explants, silver nitrate ($AgNO_3$), silver thiosulphate (STS)

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INTRODUCTION

Finger lime (*Citrus australasica*) is a Citrus species native to the sub-tropical rainforest bordering the Southeast region of Queensland and New South Wales of Australia. Renowned for its distinctive finger-like shape and separated pulp vesicles, resembling caviar, earning the name “citrus caviar”. The fruit grows up to 12 cm long and displays a wide spectrum of colors for its skin and pulps, ranging from green, dark red to black. The tree size ranges from a compact bush to a medium-sized tree depending on the variety. In addition to its appearance, finger lime serves as a traditional food source for the indigenous populations. Nowadays, fresh finger lime serves as a versatile garnish in culinary applications, substituting lemon or lime in various seafood dishes, salads, or cocktails (Hardy *et al.*, 2010). Apart from the food and beverages industries, finger lime has broadened its utilization in diverse industries. Like most Citrus, finger lime has been reported to have good antioxidant potential which is a powerful antioxidant essential for immune system function and collagen synthesis, due to the presence of vitamin C, E, lutein, and phenolic compounds in the fruit (Delort & Yuan, 2018). Moreover, it is also a rich source of dietary fiber, and healthy fats, along with essential minerals

like calcium, magnesium, phosphorus, and potassium, making it a potentially valuable ingredient in the pharmaceutical and nutraceutical industries (Richmond *et al*., 2019). The finger lime fruit is a niche fruit in the Australian market locally and for exportation, producing a volume of less than 100 tonnes annually and with a premium price range of AUD 59/kg at wholesale and AUD 84/kg at retail (Glover *et al*., 2021). Anticipating a progressive rise in demand throughout the year, it is projected that production will align with the increasing demand, prompting the initiation of commercial-scale cultivation initiatives both locally and globally.

However, at present, the propagation of finger limes predominantly relies on cutting and grafting, which is susceptible to compatibility issues, disease transmission between the plant materials, and a notable cost and labor intensity. In addition, finger lime produces zygotic monoembryonic seeds, which do not produce true-to-type plants (Delort & Yuan, 2018). In addition, a tree grown from seed is slower growing and might take up to 15 years to flower and 3 – 5 years to yield fruits. Furthermore, growing in a natural environment also exposes the plant to pest problems and fungal diseases. (Hardy *et al*., 2010; Delort & Yuan, 2018). Consequently, the limitations of these conventional propagation methods are hurdles in meeting the demands of large-scale production. This challenge is particularly significant for Citrus, which is recognized as the third most significant fruit crop globally. As a result, it has garnered global attention in Citrus improvement research through genetic transformation to enhance disease resistance and tolerance in both biotic and abiotic stress in Citrus plants (Sun *et al*., 2019). Therefore, the establishment of an efficient *in vitro* regeneration protocol for plant tissue is imperative to facilitate successful studies in gene transformation. Additionally, plant tissue culture serves as a potent tool for cultivating new cultivars, and seedless varieties, generating secondary metabolites, and conserving germplasm (Chamandoosti, 2017). With the progression of plant tissue culture technology, micropropagation has emerged as a pivotal technique for the clonal propagation of Citrus plants. This method enables the large-scale production of uniform, disease-free plantlets within controlled conditions, offering potential economic significance by enhancing vigor and yield beyond the utilization of conventional propagation methods.

Previous studies on the micropropagation of *C. australasica* were reported by Mahmoud *et al*. (2020), in which an efficacious micropropagation protocol of this species was established using nodal explants from their experimental accession of finger lime plants. The impediment of leaf abscission emerged as a significant challenge in the establishment of *C. australacisa* shoot culture, attributed to the accumulation of ethylene in the culture vessels. This ethylene buildup resulted in excessive leaf abscission, posing a potential threat to the viability of the tissue culture. Consequently, the application of silver-containing compounds, such as silver nitrate ($AgNO_3$) and silver thiosulphate (STS), serve as potent ethylene inhibitors to inhibit physiological action and regulate the biosynthesis of ethylene, therefore controlling leaf abscission in the culture. It was reported that the establishment of an efficient *in vitro* organogenesis protocol for finger lime was hindered by the complete leaf drop issue of the shoot culture, primarily due to the excessive ethylene production in the culture vessel (Mahmoud *et al*., 2020).

The Australian pink finger lime (*C. australasica* cv. Mia Rose) features a dark green skin that turns maroon when completely ripe, having pinkish red pulp, that has a slightly bitter taste with hints of grapefruit. Up to date, only a single complete micropropagation protocol has been documented for *C. australasica*, and studies on this protocol remain limited, especially in different cultivars. Despite the attractive features, and economical and medicinal properties of this plant, an effective micropropagation protocol specific to the Mia Rose cultivar has yet to be documented. Therefore, this study aims to assess the effect of silver-containing compounds, $AgNO_3$ and STS on the *in vitro* shoot regeneration and multiplication of the Australian pink finger lime (*C. australasica* cv. Mia Rose) using nodal explants.

MATERIALS AND METHODS

Explants selection and surface sterilization

Young shoot explants (5 – 6 cm) consisting of several nodal regions were excised from *C. australasica* cv. Mia Rose's mother plants are maintained at the Herbarium Unit of the School of Biological Sciences, Universiti Sains Malaysia. The shoot explants were subjected to pre-treatment in a solution with 4% (v/v) Sunlight® liquid detergent and Dettol® antiseptic disinfectant with 3 drops of Tween-20, followed by rinsing under running tap water for 30 min. Explants were then surface sterilized in 45% (v/v) Clorox® commercial bleach for 6 min, followed by 70% (v/v) ethanol for 5 min, and further rinsed with sterile distilled water. The surface sterilized explants were blotted dry on sterile filter paper and were excised into 1 cm in length before inoculation in half-strength Murashige and Skoog (MS) media including vitamins (Duchefa Biochemie, The Netherlands) (Murashige and Skoog, 1962), 1.5% (w/v) sucrose (Duchefa Biochemie, The Netherlands) and 0.8% (w/v) plant agar (Duchefa Biochemie,

The Netherlands). The prepared media were adjusted to pH 5.80 before autoclaving at 121°C and 105 kPa for 15 min. All the cultures were maintained at a temperature of 25 ± 2°C, following a 16-hr light and 8-hr dark photoperiod, and were illuminated using a cool white fluorescent lamp (Philips TL-D, 36 W, Malaysia) with an intensity of 150 µmoL m^{-2} s^{-1}.

Preparation of STS

A weight of 1.58 g sodium thiosulfate ($Na_2S_2O_3$) (Sigma Aldrich, Germany) and 1.7 g $AgNO_3$ (Sigma Aldrich, Macedonia) were measured and dissolved respectively in 100 mL distilled water to create a 0.1 M stock solution. A 0.02 M stock solution of STS was prepared by adding $AgNO_3$ into $Na_2S_2O_3$ in a ratio of 1:4. The stock solution was then filter sterilized using Minisart© single-use filter unit (Sartorius, Germany) then was stored in dark in 1 – 8°C in a refrigerator.

Effect of silver compounds on shoot regeneration

Sterile nodal explants of *C. australasica* cv. Mia Rose was subjected to half-strength MS media supplemented with 2.00 mg/L 6-benzylaminopurine (BAP) in combination with $AgNO_3$ (Sigma Aldrich, Macedonia) at 2.00, 4.00, 6.00, 8.00, and 10.00 mg/L and STS at 10.00, 20.00, 40.00, 60.00 and 80.00 µM to assess its effect on shoot regeneration. The percentage of shoot induction, average number of new shoots, average length of new shoots, and percentage of leaf abscission were recorded after eight weeks of culture. Cultures were transferred into fresh media every four weeks. All the cultures were maintained at a temperature of 25 ± 2°C, following a 16-hr light and 8-hr dark photoperiod, and were illuminated using a cool white fluorescent lamp (Philips TL-D, 36 W, Malaysia) with an intensity of 150 µmoL m^{-2} s^{-1}.

Experimental design and statistical analysis

Each experiment was conducted with 10 explants per treatment and the experiment was replicated three times. The experiments were conducted in a completely randomized design (CRD). After eight weeks of incubation, the percentage of shoot induction, the number and length of new shoots, and the percentage of leaf abscission were calculated and recorded based on the formula below. The data collected was analyzed using IBM SPSS Statistics Software Version 27. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) and independent samples t-tests at the significance of $p \leq 0.05$ was employed to determine the difference between treatment means. The values presented are means and their standard error.

Percentage of Shoot Induction = (Number of Explants with New Shoots Formed)/(Total Number of Explants) × 100%

Percentage of Leaf Abscission = (Number of Explants with Abscissed Leaves)/(Total Number of Explants with New Shoots Formed) × 100%

RESULTS AND DISCUSSION

Effect of $AgNO_3$ on shoot regeneration

To evaluate the effect of $AgNO_3$ on shoot regeneration of *C. australasica* cv. Mia Rose, the sterile nodal explants were culture in half-strength MS media with 2.00 mg/L BAP and supplemented with $AgNO_3$ at five concentrations (2.00, 4.00, 6.00, 8.00 & 10.00 mg/L) following an 8-week culture period. Regarding Table 1, results demonstrated that the addition of $AgNO_3$ in the treatments was effective at reducing leaf abscission in contrast to the control (38.38 ± 23.15%) (Figure 1a), resulting in no leaf abscission on the nodal explant at the treatment of $AgNO_3$ at 2.00 to 8.00 mg/L. While the highest concentration of $AgNO_3$ at 10.00 mg/L, resulted in 3.03 ± 4.25% of leaf abscission. However, no significant differences were observed in the number of new shoots and shoot length between the control and the treatments. Among the treatments, the treatment of 4.00 mg/L $AgNO_3$ (Figure 1b) resulted in a significantly higher number of shoots (2.65 ± 0.18) compared to the treatment of 2.00, 8.00 and 10.00 mg/L $AgNO_3$. The absence of leaf abscission in the treatment of 4.00 mg/L $AgNO_3$ indicated a better leaf retention effect compared to the control, despite not substantially promoting shoot regeneration over the control. Besides, the number of shoots and length of new shoots formed decreased progressively with the increase of concentrations of $AgNO_3$ from 4.00 to 10.00 mg/L, resulting in the least number of new shoots (1.72 ± 0.23) when 10.00 mg/L of $AgNO_3$ was supplemented. The treatment of 8.00 mg/L $AgNO_3$ (Figure 1c) has a significantly lower shoot length (0.15 ± 0.02 cm) compared to the control, indicating a negative effect on shoot elongation at this concentration.

Table 1. The effect of $AgNO_3$ in half-strength MS media in combination with 2.00 mg/L BAP on the shoot regeneration of *C. australasica* cv. Mia Rose nodal explant after eight weeks of culture

Concentrations (mg/L)	Percentage of Shoot Induction (%)	No. of New Shoots ($\bar{x}$ + S. E.)	Length of New Shoots (cm) ($\bar{x}$ + S. E.)	Percentage of Leaf Abscission (%)
Control (0.00)	71.63 ± 29.70^{a}	2.05 ± 0.22^{ab}	0.25 ± 0.03^{b}	38.38 ± 23.15^{b}
2.00	79.17 ± 11.02^{a}	1.95 ± 0.19^{a}	0.20 ± 0.02^{ab}	0.00 ± 0.00^{a}
4.00	70.45 ± 32.06^{a}	2.65 ± 0.18^{b}	0.27 ± 0.03^{b}	0.00 ± 0.00^{a}
6.00	74.60 ± 15.12^{a}	2.35 ± 0.20^{ab}	0.27 ± 0.04^{b}	0.00 ± 0.00^{a}
8.00	53.33 ± 38.44^{a}	1.73 ± 0.25^{a}	0.15 ± 0.02^{a}	0.00 ± 0.00^{a}
10.00	78.06 ± 14.68^{a}	1.72 ± 0.23^{a}	0.20 ± 0.02^{ab}	3.03 ± 4.25^{a*}

#Means with the same letter within columns are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Means with * are significantly different from the control treatments according to independent samples t-test

Fig. 1. Shoot regeneration of *C. australasica* cv. Mia Rose in half-strength MS media with 2.00 mg/L BAP in different concentrations of $AgNO_3$ after eight weeks of culture. (a) control, (b) 4.00 mg/L $AgNO_3$, (c) 8.00 mg/L $AgNO_3$ and (d) 10.0 0 mg/L $AgNO_3$. Scale bars represent 1 cm.

Multiple studies demonstrated that shoot development was enhanced by the presence of $AgNO_3$ in combination with cytokinin in *Citrus* during the multiplication stage. The findings in the current study is in accordance with an earlier study on lemon (*Citrus limon)* shoot tip and nodal culture, where results indicated that the addition of 3 mg/L $AgNO_3$ into 2 mg/L BAP and 0.5 mg/L GA_3 produced the highest length of shoots among all the treatments tested. However, they also reported that multiple shoot formation was not enhanced by the addition of $AgNO_3$ into the medium, whereby the medium added with BAP resulted in the highest response in terms of number of shoots (Kotsias & Roussos, 2001). According to a previous study by Eng *et al*. (2014), the attempt to regenerate shoots from kaffir lime (*Citrus hystrix)* shoot tip explant in MS medium in combination with varying concentrations of BAP alone resulted in leaf abscission, which intensifies with the increased BAP concentrations, suggesting a direct relationship between BAP concentration and the observed issue. Results from subsequent experiments were aligned with the current study which revealed that the incorporation of $AgNO_3$ into BAP-containing media substantially promoted the average number of new leaves and shoots formed, in contrast to the control treatment.

Contrary to the current study, Mahmoud *et al*. (2020) corroborated the leaf abscission issue in *C.*

australasica shoot culture in their preliminary study and proceeded to evaluate the effect of $AgNO_3$ as an ethylene antagonist in MS medium supplemented with 2.2 µM BAP. Results indicated a negative effect on *in vitro* shoot regeneration and produced 24 ± 7.48% leaf abscission on the shoot culture. Besides *C. australasica*, leaf abscission was also encountered in the shooting culture of various *Citrus* species including grapefruit (*Citrus* × *paradisi*) and sweet orange (*Citrus sinesis*), therefore the utilization of $AgNO_3$ was included in their study (Marutani-Hert *et al*., 2010; Prusty *et al*., 2023). This was consistent with the study of Park *et al*. (2022) who reported that the addition of $AgNO_3$ and BAP harmed shoot development, in terms of shoot quantity and length on internode explants of *Polygonum multiflorum*. It was reported that the optimal shoot development occurred at control treatment of MS medium supplemented solely with 2 mg/L BAP, with results gradually deteriorating by a higher concentration of $AgNO_3$. At the highest concentration of 20 mg/L $AgNO_3$, the lowest numbers of shoots and shortest shoot length (1.4 ± 0.2 and 9.7 ± 1.6 mm, respectively) were recorded. Nevertheless, the combination of BAP and $AgNO_3$ was also found to stimulate *in vitro* flowering, inducing and reducing callus formation on various explants such as in tomato, Brazil spinach, and Indian rosewood (Shah *et al*., 2014; Sowmya *et al*., 2020; Raturi & Thakur, 2021). In addition, Nasir & Abdulhussein (2022) investigated the effect of $AgNO_3$ in stimulating *in vitro* rooting in strawberry (*Fragaria* × *ananassa* Duch.) runner tip explants, demonstrating a significantly higher rooting percentage, number of roots formed and root length in contrast to the treatments without the addition of $AgNO_3$. Besides organogenesis, several studies also demonstrated that the addition of $AgNO_3$ promotes the induction of somatic embryogenesis. In *Coffea* sp., the presence of a small amount of $AgNO_3$ solely in culture media, or in combination with BAP was found to improve the regenerative capacity of embryo yield during somatic embryogenesis using leaf explants (Fuentes *et al*., 2000; Rojas-Lorz *et al*., 2019). In addition, the promoting effect of the application of $AgNO_3$ in combination with BAP in somatic embryogenesis was also observed in *Vanilla planifolia* (Manokari *et al*., 2022).

Silver ion in the form of nitrate, is widely utilized in the regeneration of *in vitro* shoot and root organogenesis as well as somatic embryogenesis due to its solubility, easy availability, and stability (Kumar *et al*., 2009). Ethylene accumulation in plant tissue culture is often identified as a key player in triggering leaf abscission, which occurs after leaf senescence, which negatively impacts the overall health and survival of the plant. In efforts to counteract ethylene-induced leaf abscission, ethylene biosynthesis inhibitors such as silver-containing compounds like $AgNO_3$ and STS are commonly used. A previous study by Beyer (1975) demonstrated that ethylene was known to initiate cell separation in the abscission zone of leaves by enzymatic action, leaf blades were the initial target tissue when exogenously ethylene is applied. Therefore, throughout the developmental stages of leaves, the accumulation of ethylene can trigger the senescence process. This process encompasses the programmed-cell death and the activation of nutrient recycling from senescing leaves to other organs (Iqbal *et al*., 2017). Therefore, silver ion as an ethylene antagonist was corroborated to substitute the role of copper ion co-factor at the ethylene binding sites during ethylene biosynthesis, which is then transported in the cell, thus inhibiting the previous steps in the pathway, thereby inducing ethylene-insensitivity in the cell (Zhao *et al*., 2002). Consequently, the application of $AgNO_3$ has been extensively documented in plant tissue culture, not only to regulate morphogenesis in plants from *in vitro* shoot induction, rooting, flowering, and somatic embryogenesis, as well also to modulate ethylene activity in plant systems and advert premature abscission of leaves, fruits and flowers (Kumar *et al*., 2009).

Effect of STS on shoot regeneration

On the other hand, STS was applied in the shoot regeneration of *C. australasica* cv. Mia Rose where the sterile nodal explants were cultured in half-strength MS media with 2.00 mg/L BAP and supplemented with STS at five concentrations (10.00, 20.00, 40.00, 60.00 & 80.00 µM) following a 8-week culture period. Regarding Table 2, it was found that the addition of 10.00 up to 40.00 and 80.00 µM of STS in the culture successfully mitigated leaf abscission to 0.00% throughout the shoot regeneration stage even after eight weeks of culture. Even though a significant difference was not observed between the control (Figure 2a) and the treatments in terms of shoot quantity and length increment, it was evident that with the addition of minimal concentration of STS at 10.00 µM (Figure 2b), it could effectively reduce the leaf abscission percentage from 60.00% to 0.00%. The lack of significant difference in the number of new shoots and shoot length between the control and the treatment suggested that the application of STS at the tested concentrations did not substantially enhance or inhibit the shoot regeneration of *C. australasica*. However, the treatment of 10.00 µM STS in combination with 2.00 mg/L of BAP produced the highest number (2.36 ± 0.19) and length of new shoots (0.32 ± 0.04 cm) among all treatments, indicating the beneficial effect of a minimal amount of STS. In addition, the independent samples t-test

revealed a significantly lower number of new shoots between the treatment of 40.00 µM STS (Figure 2c) and the control, producing only 1.83 ± 0.12 new shoots per explant after eight weeks of culture, suggesting that 40.00 µM STS negatively affected shoot regeneration in *C. australasica*.

Table 2. The effect of STS in half-strength MS media in combination with 2.00 mg/L BAP on the shoot regeneration of *C. australasica* cv. Mia Rose nodal explant after eight weeks of culture

Concentrations (µM)	Percentage of Shoot Induction (%)	No. of New Shoots ($\bar{x}$ + S. E.)	Length of New Shoots (cm) ($\bar{x}$ + S. E.)	Percentage of Leaf Abscission (%)
Control (0.00)	66.67 ± 57.74^{a}	2.27 ± 0.14^{a}	0.25 ± 0.03^{a}	60.00 ± 14.14^{b}
10.00	80.00 ± 34.64^{a}	2.36 ± 0.19^{a}	0.32 ± 0.04^{a}	0.00 ± 0.00^{a}
20.00	76.67 ± 40.42^{a}	2.22 ± 0.17^{a}	0.30 ± 0.03^{a}	0.00 ± 0.00^{a}
40.00	80.00 ± 34.64^{a}	1.83 ± 0.12^{a*}	0.23 ± 0.02^{a}	0.00 ± 0.00^{a}
60.00	76.67 ± 40.42^{a}	2.18 ± 0.15^{a}	0.26 ± 0.03^{a}	4.76 ± 8.25^{a*}
80.00	100.00 ± 0.00^{a}	2.14 ± 0.07^{a}	0.27 ± 0.032^{a}	0.00 ± 0.00^{a}

#Means with the same letter within columns are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Means with * are significantly different from the control treatments according to independent samples t-test

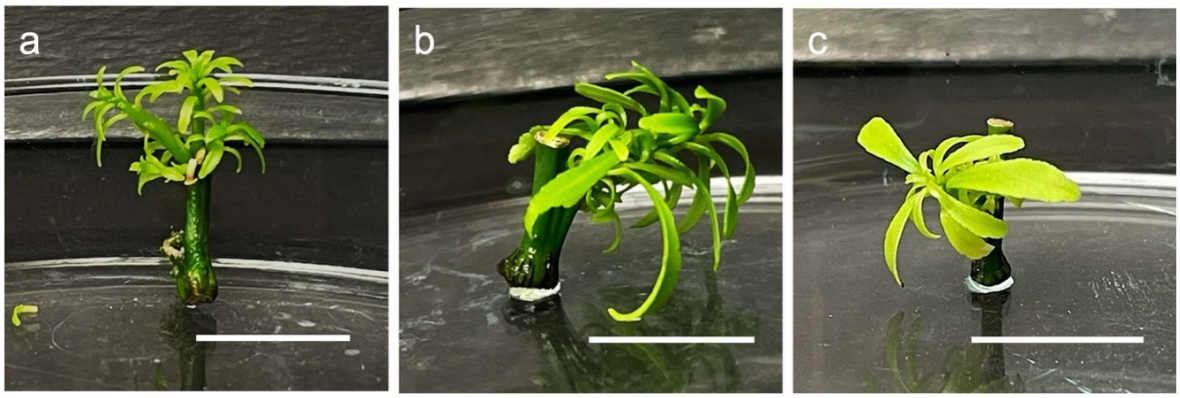

Fig. 2. Shoot regeneration of *C. australasica* cv. Mia Rose in half-strength MS media with 2.00 mg/ BAP in different concentrations of STS after eight weeks of culture. (a) control, (b) 10.00 µM STS, (c) 40.00 µM STS. Scale bars represent 1 cm.

The existing literature on the application of BAP in combination with STS in *Citrus* culture was still limited, indicating a significant gap in elucidating their combined effects on plant physiology and development in *Citrus*. In a previous study, Mahmoud *et al*. (2020) observed the occurrence of complete leaf abscission in an *in vitro* shoot culture of *C. australasica* due to excessive ethylene production, which eventually led to the mortality of the shoots. The application of 2.2 µM BAP and STS at all concentrations in MS medium has successfully addressed the leaf abscission problem (0%), in contrast to the control treatment (100%). In addition to mitigating issues in leaf abscission in the culture, the supplementation of 5 mg/L STS, alongside 2.5 mg/L BAP and 10 mg/L GA_3 has successfully eliminated premature microshoot abscission in Kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*) shoot culture. This resulted in 0% abscission as well as the greatest length increment of new shoots (7.81 cm) and the highest leaf count (11.82) (Murugan *et al*., 2022). In addition, the supplementation of 20 µM STS in combination with 2 mg/L BAP and 1 mg/L GA_3 in *C. limon* shoot culture significantly increased both the number of buds and the shoot length (Navarro-García *et al*., 2016). In the shoot culture of sweet orange (*C. sinesis* cv. Mosambi), it was observed that the impact of $AgNO_3$ on promoting shoot regeneration, in terms of both shoot number and length, surpassed that of STS, producing a result of 2.14 new shoots and 3.2 cm length increment in new shoots at a concentration of 17.66 µM $AgNO_3$. Whereas the effect of STS exhibited notable efficacy in mitigating leaf abscission, exhibiting an abscission control rate 2.34 times higher than the control treatment. Furthermore, the addition of STS in the culture led to an improvement in leaf chlorophyll content in contrast to the control treatment and all concentrations tested in the $AgNO_3$ treatment (Prusty *et al*., 2023).

Several studies have indicated that the supplementation of STS in different species has improved regeneration capacity. It was also reported that the proliferation of adventitious buds was enhanced by the addition of STS in date palm (*Phoenix dactylifera*) culture, producing the best result at 90 µM STS, whereas the highest concentration of 120 µM reduced the number of shoots (Bader & Khierallah, 2009). The findings of the present study were aligned with a study on *Mentha piperita* shoot explants where the addition of STS in lower concentration in MS media could enhance the shoot regeneration effect,

generating the highest number of new shoots (4.75 ± 0.39) at 50 µM STS as well as a highest shoot induction percentage (90%) (Sujana & Naidu, 2011). In addition, Ricci *et al*. (2020) reported a similar finding in peach (*Prunus persica*) leaf culture, where the addition of 10 µM STS to Woody Plant Medium (WPM) supplemented with 15.5 µM BAP doubled the number of regenerating shoots per explant in contrast to control. However, STS concentration higher than 10 µM inhibited adventitious shoot regeneration in the culture. According to Salla *et al*. (2012), it was found that the addition of solely STS into MS media stimulated the proliferation of shoots, producing the highest number of shoots (3.2) and highest length increment (4.7 cm) at 50 µM in *Asclepias curassavica* nodal explants. Furthermore, STS was incorporated in potato (*Solanum tuberosum*) shoot culture as an ethylene antagonist to facilitate normal development of *in vitro* plants, thereby fostering the production of healthy leaves before their removal for use in protoplast culture. The supplementation of STS has been demonstrated to produce larger leaves and consequently improve both the yield and viability of protoplast. In their study, it was revealed that the inclusion of STS in the shoot culture resulted in leaf sizes approximately twice as large as the control group (Ehsanpour & Jones, 2001). Several studies also highlighted the role of STS in mitigating ethylene-induced flower abscission and stimulating the proliferation of somatic embryos (Martinez *et al*., 2014; Hyde *et al*., 2020).

Cardoso (2019) revealed that ethylene build-up in the culture altered plant growth and development, leading to plant disorders such as excessive callus formation, etiolated shoots, and yellowing of leaves, resulting in a loss in micropropagation efficiency. Therefore, silver in the form of thiosulphate could be a tool to inhibit the physiological action of ethylene and control ethylene biosynthesis in plant tissue culture. It was reported that STS is more effective in tissues than $AgNO_3$ because of its faster mobility in plant transport systems and less phytotoxicity nature compared to $AgNO_3$. Unlike $AgNO_3$, STS in its anionic complex form was found to exhibit faster transportation through the xylem compared to silver ions in the nitrate form. This rapid transport allows for the retention of silver ion functionality within the complex, subsequently delaying the senescence of flowers and petals (Veen & Van de Geijn, 1978; Veen, 1983). Based on various research findings, employing STS as a foliar spray in *ex vitro* conditions has been shown to enhance the longevity of flowers. This effect is attributed to the transportation of silver ions from mature leaves to the shoot apex, where flower buds are located. This mechanism protects flowers from ethylene-induced senescence and abscission, as evidenced in several flowering species including cassava (*Manihot esculenta*) and fresh-cut *Chrysanthemum morifolium* (Hyde *et al*., 2020; Sedaghathoor *et al*., 2020).

Based on our study, we found that the addition of $AgNO_3$ was more effective than STS in promoting the *in vitro* shoot regeneration of *C. australasica* cv. Mia Rose nodal explants based on the results for both the number and length of new shoots formed after eight weeks of culture. Notably, the addition of both silver compounds effectively mitigated the leaf abscission in the shoot culture, suggesting their potential as beneficial additives for enhancing leaf retention in *C. australasica* cv. Mia Rose.

CONCLUSION

The current study assessed the effect of silver-containing compounds, including $AgNO_3$ and STS, on the *in vitro* shoot regeneration of the Australian pink finger lime (*C. australasica* cv. Mia Rose) using nodal explants. Notably, the combination of 4.00 mg/L $AgNO_3$ or 10.00 µM STS with half-strength MS media supplemented with 2.00 mg/L BAP generated the highest number and length of induced shoots in both experiments respectively, achieving 0.00% leaf abscission in the shoot culture after eight weeks of culture. This study underscored the efficacy of $AgNO_3$ and STS as potent ethylene antagonists in *C. australasica* cv. Mia Rose shoot culture. Future work will include the induction of roots from regenerated shoots before acclimatization, aiming to establish a complete micropropagation protocol for *C. australasica* cv. Mia Rose.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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