REVIEW ARTICLE

Exploring the Applications and Implications of Silver Nitrate and Silver Nanoparticles in Plant Tissue Culture

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Abstract

Silver nitrate and silver nanoparticles have emerged as versatile tools in plant tissue culture, offering a range of applications in micropropagation, regeneration, and the production of metabolites. This review highlights the benefits and limitations of using silver nitrate and silver nanoparticles in plant tissue culture, including their role in enhancing shoot growth, regeneration, and secondary metabolite production. Additionally, their potential in large-scale propagation, preservation of ornamental plants, and salinity tolerance is discussed. While silver nitrate and silver nanoparticles have shown promise, their use also raises concerns about phytotoxicity and genetic instability. This review aims to provide a comprehensive understanding of the applications and implications of using silver nitrate and silver nanoparticles in plant tissue culture, shedding light on their potential for future research and applications.

Keywords: Keywords: Silver nitrate, Silver nanoparticles, Plant tissue culture, Metabolite production

Highlights:

- Role of silver nitrate and silver nanoparticles as an important additive in plant tissue culture.
- Review of silver nitrate along with its concentration on a specific explant of the plant and its response.
- Silver nanoparticles (AgNPs) are powerful regulators in micropropagation, regeneration, and metabolite synthesis.
- The incorporation of silver compounds stimulate elevated production of valuable phytochemicals, boosting the biochemical profile of in-vitro cultures.
- The review offers a comprehensive overview and outlines directions for future research and practical applications.

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Introduction

Plant tissue culture technique is the most constantly used biotechnological tool for fundamental and applied purpose categorized from exploration of plant developmental processes, commercial plant micropropagation, functional gene studies, plant breeding, and crop improvement, virus elimination from contaminated materials to delineate high-quality extensive flora, maintenance of germplasm vegetative propagated plant crops, and save endangered plant species (Vargas and Alejo, 2018). Furthermore, plant cell and organ cultures are of interest for the production of secondary metabolites of commercial and medicinal value. Advanced technologies, such as genome editing, merge with tissue culture and *Agrobacterium tumefaciens*-mediated transformation, are recently encouraging substitutes for the explicit genetic manipulation of desirable agronomical or industrial features in crop plants. Usages of omics (genomics, proteomics, and transcriptomics) to plant tissue culture will help untangle compound experimental procedures such as organogenesis (Skoog and Miller, 1957) and somatic embryogenesis, which will improve the regulation of the revitalization method of species (Us-Camas *et al.*, 2014). An important development in agricultural biotechnology is the incorporation of nanotechnology into plant tissue culture. By lowering contaminations, enhancing callus induction, and raising secondary metabolite yields through cell suspension cultures, nanoparticles increase the efficiency of micropropagation (Gunasena et al., 2024).

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Silver nitrate and silver nanoparticles (AgNPs) have unique properties that make them useful in various applications, including plant biotechnology and agriculture (Table 1) (Fig.1). Research has explored the effects of AgNPs on seed germination, plant propagation, and metabolite production in plant tissue culture. While AgNPs have shown potential in enhancing plant growth, outcomes have been inconsistent across different plant species and both positive and negative

Table 1: Effect of silver nitrate and silver nanoparticles in plant tissue culture

S. No.	*Additive*	*Concentration*	*Plant*	*Explants*	*Response*	*Reference*
1.	Silver nitrate	2.0 mg/L	*Anthurium andraeanum*	Shoot	To increase the quality of shoots obtained in multiple stages and to accelerate the rooting stage.	Cardoso, 2019
2.	Silver nitrate	1 mg/L	*Lantana camara*	Shoot	Healthy plantlets thrive in-vitro	Tien et al., 2019
3.	Silver nitrate	2.5 µM	*Moringa oleifera* Lam.	Shoot	Prevention of leaf fall, showcasing efficient micropropagation	Ravi et al., 2019
4.	Silver nitrate	40 µM	*Arabica coffee*	Leaf	Somatic embryos became rooted plants with 100% survival in the greenhouse	Lorz et al., 2019
5.	Silver nitrate	0.3 mg/L	St John's wort *(Hypericum perforatum L.)*	Shoot and root	Enhances visual culture in conservation	Syahid and Wahyuni, 2019
6.	Silver nitrate, Salicylic acid	30 µM	*Hyptis marrubioides*	Seedlings	Both abiotic elicitors boost rutin content	Pedroso et al., 2019
7.	Silver nanoparticles	15.4 mg/L	*Campomanesia rufa*	Shoot	AgNPs show minimal impact on in-vitro multiplication at low concentrations	Timoteo et al., 2019.
8.	Silver	10 µM	*Grapevine*	Leaf	Induces programmed cell death (PCD) in grapevine suspension cell cultures.	Fillippi et al., 2019
9.	Silver and Gold nanoparticles	10 and 30 ppm	*Chrysanthemum, gerbera*	Root and shoots	A high concentration of silver nanoparticles hindered the formation of adventitious shoots	Tymoszuk and Miller, 2019
10.	Silver nanoparticles	6-20 µM	*Bauhinia acuminate*	Flower	Induction of osteogenic differentiation and proliferation in mesenchymal stem cells (MSCs), contributing to the healing of meniscus injury.	Hu et al., 2019
11.	Silver nitrate	7.5 µM	*Caladium bicolor cv.*	Shoot	Reduced hyperhydricity occurred at high frequency in the shoot culture.	Isah, 2019
12.	Silver nitrate	0.5,1,3,10mM	*Berberis vulgaris*	Leaf and root	Clean, cost effectiveand safe biosynthesis of silver nitrate using its extract, without the use of toxic substances, leading to high antibacterial activity.	Behravan et al., 2019
13.	Silver nitrate	50 mg/L 0.5 mg/L	*Alternanthera sessilis L.*	Shoots	A specific growth hormone combination was determined for the large-scale production of genetically identical plantlets from internodal segment explants.	Sowmya et al., 2020
14.	Silver nitrate and Ammonium	1.9 mg/L, 550 mg/L	Perennially-Cultivated Ginger *(Zingiber officinale Roscoe)*	Shoot	Sucrose's positive impact on microrhizome formation in ginger	An et al., 2020
15.	Silver nitrate	10 µM	*Solanum tuberosum*	Shoot	To induce shoot organogenesis in internodal explants and increase the number of shoots.	Kaur and Kumar, 2020
16.	Silver nitrate	2 mg/L	*Capparis decidua*	Shoot	Optimal micropropagation protocol for C. decidua, paving the way for research in conservation and enabling genetic/ genomic studies in this significant species.	Ahlawat et al., 2020

17.	Silver nitrate	30 mg/L	Tomato *(Lycopersicon esculentum)*	Seedlings	Its exposure led to phytotoxicity and impaired antioxidative responses, highlighting the higher reactivity of the ionic silver form compared to NPs.	Noori et al., 2020
18.	Silver nitrate	10 mg/L	Eggplant *(Solanum melongena L.)*	Anther	Enhances antherviability, embryo quality, direct embryogenesis, regeneration speed and yields of embryos and in-vitro plantlets through a triple synergistic effect.	Vural and Ari, 2020
19.	Silver nitrate Silver nanoparticles	40 µM 20 µM 60 µM	Australian finger lime *(Citrus australasica)*	Leaf and shoots	Propagated finger lime explants and controlled leaf abscission.	Mahmoud et al., 2020
20.	Silver nitrate	50–75 mg/L	Wheat *(Triticum aestivum L.)*	Anther	Reduction of albinism and increase in embryo induction on 50-75 mg/L silver nitrate along with optimum doses of IAA and kinetin	Hassan and Islam, 2021
21.	Silver nitrate	5 mg/L	Oilseed rape *(Brassica napus L.)*	Callus Shoot	Activation of stress response was necessary to obtain shoots with higher frequency in the varieties Albina, Lancia, and Menthol.	Ramadan et al., 2021
22.	Silver nitrate	25, 50, 75, and 100 mg/L	*Rosa canina*	Axillary buds	The highest quality shoots with maximum growth vigor were observed in $AgNO_3$ containing media.	Samiei et al., 2021
23.	Silver nitrate	2 mg/L	Argan *(Argania spinosa* (L.) Skeels*)*	Shoots	High rooting and acclimatization percentages are crucial for the rapid and large-scale propagation of this endangered species.	Amghar et al., 2021
24.	Silver nitrate	2 mg/L	*Dalbergia sissoo* Roxb (Shisham)	Leaf	Optimal induction and shoot multiplication occurred on MS media.	Raturi and Thakur, 2021
25.	Silver nitrate	10 mg/L	Greenhouse Bell Pepper *(Capsicum annuum L.)*	Anther	The highest percentage of embryonic anthers occurred with the interaction of silver nitrate.	Zamani and Choukan, 2021
26.	Silver nitrate	34 µM	*Musa acuminata*	Bud	Rapid and mass multiplication demonstrates superior propagation efficiency and plantlet quality.	Shekhar et al., 2021
27.	Silver nitrate	2.5 mg/L	*Vanilla Planifolia*	Leaf	Enhanced somatic embryogenesis and facilitated plantlet regeneration.	Manokari et al., 2022
28.	Silver nitrate	20 mg/L	*Polygonum multiflorum*	Shoot	$AgNO_3$ treatment decreases the number of shoots and shoot length.	Park et al., 2022
29.	Silver nitrate	0, 5, 10 mg/L	Strawberry (*Fragaria ananassa)*	Shoot and root	Boosted shoot characteristics during multiplication, enhanced rooting parameters in the rooting stage and improved survival, plant height, leaves and dry weight in the acclimatization stage.	Nasir and Abdul Hussein, 2022
30.	Silver nitrate Silver nanoparticles	4.0 ml/L	Date palm (*Hayani* cv.)	Root	Enhancing both the number and length of shoots at 3.0 ml/L concentrations compared to the control treatment.	Elsayh et al., 2022
31.	Silver nitrate	50 µL/L 4 µM/L	*Capsicum annuum* (var. Chile Ancho and Misraty)	Callus	Reduces callus formation during shoot regeneration and also increases the number of plants.	Enfeshi et al., 2023

32.	Silver nitrate	2.5 mg/L	*Populus nigra L.*	Callus	Chronic phytotoxic effect of different concentrations of novel biofunctionalized silver nanoparticles and silver nitrate on Populus nigra L. It was reported that AgNPs were more toxic on poplar calli compared to $AgNO_3$.	Iori et al., 2023
33.	Silver nitrate and Silver nanoparticles	8 mg/L 2 mg/L	Wheat (*Triticum aestivum* L .)	Callus	Demonstrates the use of diverse machine learning models in cultivating mature wheat embryos under treatments and concentrations.	Turkoglu et al., 2023
34.	Silver nitrate	15.0 mg/L	Artichoke [*Cynara cardunculus* var. scolymus (L.) Fiori]	Callus	A promising strategy for promoting bioactive compounds from globe artichoke leaves, potentially benefiting large-scale industries, pharmacology and food supplements.	Kilic and Onus, 2023
35.	Silver nitrate	2 mg/L 500 mg/L	Sorghum (Sorghum bicolor L .)	Shoot	Optimize in-vitro regeneration and assess the effects of plant growth regulators.	Pawar et al., 2023
36.	Silver nitrate Thidiazuron	1.5 mg/L 1.0 mg/L	Carnation (Dianthus caryophyllus L .)	Shoot	Quick induction of roots and the maximum number of roots per culture were achieved.	Maurya et al., 2023
37.	Silver nitrate	2.0 mg/L	Hippophae salicifolia (Seabuckthorn)	Seedlings	Shoots generated in-vitro with silver nitrate-supplemented media failed to develop roots when subjected to rooting media.	Trivedi et al., 2023
38.	Silver nitrate Pyrazinamide	4 mg/L 4 mg/L	Tomato (Lycopersicon esculentum L)	Seedlings	Both enhanced tomato plantlets in-vitro salinity tolerance by affecting ethylene action or generation and boosting biochemical responses.	Zarei et al., 2023
39.	Silver nitrate	2.0 mg/L	Snapdragon (Antirrhinum majus L.) cv. Maryland Apple Blossom	Shoot	The significance of particular PGRs in shoot regeneration underscores $AgNO_3$'s role in mitigating hyperhydricity in in-vitro regenerated shoots.	Lee et al., 2023
40.	Silver nitrate 8-hydroxyquinoline	50/75/100ppm 100/150/200ppm	Lilium	Flower	Benefit researchers, farmers and end users in preserving the ornamental value of lilium-cut flowers for an extended period.	Brahma et al., 2023

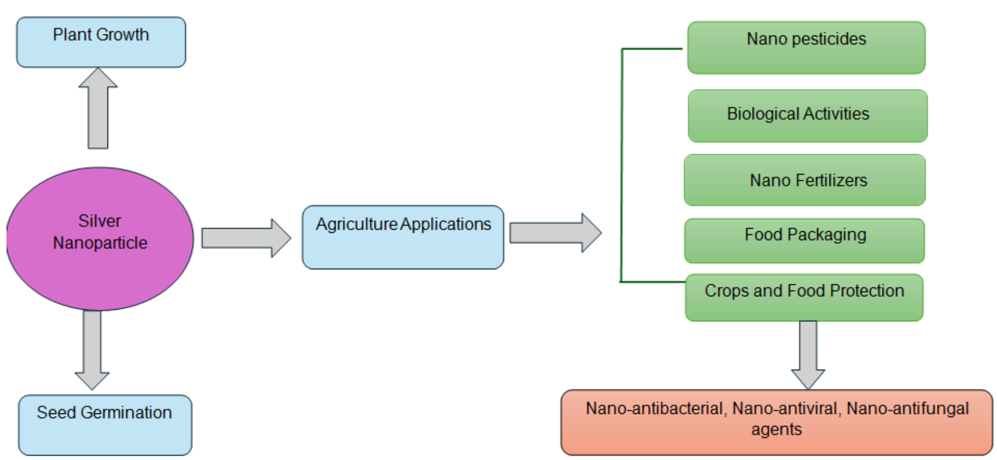

Fig. 1: The impact of silver nanoparticles on the growth of plants
(Source: https://doi.org/10.1016/j.heliyon.2023.e16928)

effects have been observed (Mahajan *et al*., 2022). Further research is needed to understand the complex interactions between AgNPs and plants, including their mechanism of action and potential phytotoxicity, to fully harness their benefits and address limitations in commercial applications. In order to illuminate the possibilities for further study and applications, this review attempts to present a thorough grasp of the uses and implications of employing silver nitrate and silver nanoparticles in plant tissue culture.

Nutrition Aspect in Plant Tissue Culture

Plant tissue culture media significantly impact plant growth and development. Over the years, various formulations have been devised for numerous plant species, with a selection of a few commonly employed in the majority of tissue culture work. While Murashige and Skoog formulation (1962) stands out as the prevailing base medium today, other effective media, such as those by White (1963), Gamborg's B-5 (1968), Schenk and Hildebrandt (1972), Nitsch and Nitsch (1969) and Woody Plant Medium (1980) are frequently utilized. These formulations can be prepared from liquid stock solutions of salts and vitamins or obtained pre-blended in powder form. The tissue culture technique must mainly contain the following components: nutrient salts, vitamins, amino acids or nitrogen supplements, carbon sources, growth regulators, solidifying agents and undetermined organic supplements.

Nutrient salts provide essential "macro" and "micro" elements for plant growth. Macronutrients (nitrogen, phosphorus) are needed in larger amounts, while micronutrients (iron, zinc) are required in smaller quantities. Sodium and chlorine, though not essential, are sometimes included. These nutrients are typically supplied in easily absorbable salt forms, such as calcium nitrate or potassium chloride. Chelated forms of iron and zinc, using agents like EDTA or EDDHA, are occasionally added to enhance availability and prevent precipitation (Bonnart *et al*., 2022).

Vitamins produced by plants cultivated *in-vitro* frequently exhibit an incapacity, unlike those grown in soil or alternative substrates (Chimdessa, 2020). To address this, plant tissue culture media are supplemented with various vitamins in the range of 0.1-10 mg/L. These supplements, such as thiamine, nicotinic acid, pyridoxine and myo-inositol, serve to stimulate specific metabolic functions.

Amino acids contribute to plant tissue culture media, aiding in plant cell growth by providing a readily accessible nitrogen source, often more easily absorbed than inorganic alternatives. Amino acid mixtures like casein hydrolysate or individual amino acids such as glycine, glutamine, or adenine are incorporated into the media at rates ranging from 2-1000 mg/L.

In-vitro, plants cannot produce their carbohydrates like they do in nature. Hence, a carbohydrate source, commonly sucrose, but also glucose, fructose, or maltose at rates of 20-30 g/L, must be provided for plant growth. The impact of the carbohydrate source and quantity varies among plant species.

Plant growth regulators like auxins and cytokinins are added for organogenesis and their balance depends on the chosen tissue culture technique. They play an active role in stem elongation, tropism and apical dominance. The common auxins used in plant tissue culture media include IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid) and NAA (Naphthalene acetic acid). IAA is the only essential auxin occurring in plant tissues. Auxins vary in their physiological activity and in the range to which they transfer through tissue and are metabolized. Cytokinin commonly used in culture media includes BAP (Benzylaminopurine), kinetin and TDZ (Thidiazuron). Cytokinin energizes cell division, produces the formation of seed and axillary shoot multiplication and slows down root formation. It boosts the growth of the callus and helps prolongation of dwarf plantlets. Other growth regulators are sometimes added to tissue culture media as abscisic acid, a compound that vitalizes callus growth, depending upon the species. Although growth regulators are the most high-end additives, they have less effect on the growth medium optimization because they are necessary in very small concentrations (Saad and Elsahad, 2012).

Tissue culture media usually contain solidifying agents, like agar or gellan gum, to provide physical support. Agarose, from specific red seaweeds, is used less frequently, especially in pollen culture. The choice of this agent can impact plant growth and testing is advisable as preferences may vary among plant

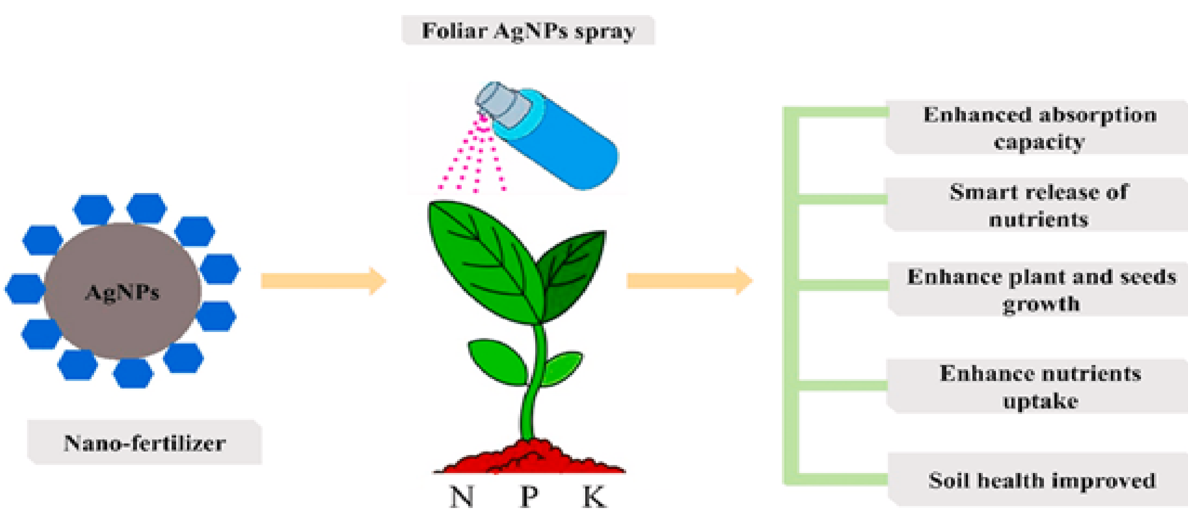

Fig. 2: The foliar spray of AgNPs as nano-fertilizers enhances nutrient uptake and plant growth.
(**Source:** https://doi.org/10.1016/j.heliyon.2023.e16928)

species. Factors like the manufacturer or purity of the gelling agent can also influence plant responses. For suspension cell cultures, continuous shaking, or for shoot cultures, a support system like a paper "raft" may be necessary if no gelling agent is used.

Role of Additives in Plant Tissue Culture

Additives are used for experimental studies like micropropagation, regeneration, androgenesis and enhancement in the value of secondary metabolites and bioconversion of cells and also certain plant species. There are many kinds of additives, namely, activated charcoal, casein hydrolysate and silver nitrate (Bansal and Gokhale, 2012).

Activated charcoal is a powdery wood charcoal supplemented with tissue culture media to initiate changes in the dilution rate. "By enabling the removal of toxic substances and phenolic compounds, it creates a conducive environment for cell growth and development, leading to improved embryogenesis and organogenesis in tissue culture". It plays a vital role in micropropagation, seed germination, somatic embryogenesis, anther culture, rooting, stem elongation, synthetic seed production and protoplast culture in various plants.

Casein hydrolysate is a non-toxic nitrogen additive consisting of a combination of amino acids. As a valuable provider of available nitrogen, it has gained widespread application as an additive in the creation of embryo culture media. Its effectiveness surpasses that of a blend of amino acids. The belief is that the cost-effective fulfilment of nitrogen deficiency through its addition is likely due to the presence of certain stimulatory factors that have not been identified yet.

Silver Nitrate and Silver nanoparticles and their application

Silver nitrate emerges as a significant regulator of plant growth, particularly influential in the realm of plant tissue culture, where its impact spans a broad spectrum of micro-propagation techniques, encompassing callus culture, somatic embryogenesis, multiple shoot induction, shoot regeneration, as well as shoots and root formation, as elucidated in various studies (Fig.2). Notably, the profound influence of silver nitrate becomes apparent in the growth and modifications of explants, attributed to the inhibitory effect of $AgNO_3$ on ethylene action, a phenomenon extensively explored in the scientific literature. The proficiency of silver ions in explicitly obstructing the action of externally applied ethylene is a key facet, influencing classical responses such as senescence, abscission, and growth interruption, contributing to a comprehensive understanding of its regulatory role in plant development. Moreover, the effect of silver nitrate has been thoroughly examined in the micro-propagation of diverse plant species, with a particular focus on shoot growth, somatic embryogenesis, and direct somatic embryogenesis, underscoring its versatile and pivotal role in shaping plant physiological processes.

$AgNO_3$ is a widely used inhibitor of ethylene action, while AgNPs have unique physicochemical properties that make them useful in biotechnology and agriculture. Silver nanoparticles have been successful in controlling microbial contaminants, aiding in callus induction, organogenesis and somatic embryogenesis, improving the genetic transformation and secondary metabolites production. Nanoscience helps us understand how nanoparticles interact with plants, leading to improved growth and development (Mahendran *et al*., 2019).

Quality Enhancement

Quality enhancement of plants refers to the healthy root and shoot development with more numbers of branches and leaves. It also includes the development of resistance against disease and various types of stresses in plant tissue. In the process of various activities of plant tissue culture, silver nitrate is used as an additive to increase the quality of shoots and roots. Healthy shoots were obtained in multiple stages to accelerate the rooting stage in *Anthurium andraenum* (Cardoso, 2019). In *Capsicum annuum* var. Chile Ancho and Misraty, the addition of $AgNO_3$ reduces callus formation during shoot regeneration and increases the number of plants bred *in-vitro* at the concentration of 30 µM/L (Enfeshi *et al*., 2023). Optimal induction and shoot multiplication occurred in *Dalbergia sissoo* Roxb by adding silver nitrate in the MS media. For *ex vitro* rooting, the best outcome was achieved with a pulse treatment of 200 mg/L IBA (Raturi and Thakur, 2021). Rapid and mass multiplication by the addition of silver nitrate, as it demonstrates superior propagation efficiency and plantlet quality in *Musa acuminata* (Shekhar *et al*., 2021). Silver nitrate at 100 mg/L induced the longest shoot and maximum number of leaves in *Rosa canina* (Samiei *et al.,* 2021).

Regeneration of genetically identical plants-

Genetically identical plants can be regenerated through a process where new genes are introduced into plant cells, which then replicate to form a mass of identical cells. This process ensures genetic uniformity since all cells originate from the same parent plant cell (Sowmya *et al*., 2020). Researchers have successfully regenerated genetically identical plants in various species, including *Arabica coffee* plants, using silver nitrate as an additive, achieving 100% survival in the greenhouse (Lorz *et al*., 2019). Silver nitrate has also been used for large-scale production of identical plantlets from internodal segment explants in *Alternanthera sessilis L*. and to promote bioactive compounds in *Cynara cardunculas var. scolymus* (L.) *Fiori* for industrial, pharmacological, and food supplement applications (Kilic and Onus, 2023). Silver nitrate has been used to analyse genetic fidelity in *Dianthus caryophyllus L*. microplants using ISSR markers (Maurya *et al*., 2023) and to optimize *in-vitro* regeneration and study plant growth regulator effects in Sorghum bicolor *L*. (Pawar *et al*., 2023) and *Vanilla Planifolia* (Manokari *et al*., 2022). Additionally, silver nitrate has been used to induce shoot organogenesis in *Solanum tuberosum*, enhancing shoot regeneration capacity (Kaur and Kumar, 2020). These studies demonstrate the effectiveness of silver nitrate in regenerating genetically identical plants and its potential applications in various fields.

Microrhizome germination

Microrhizome germination refers to the process by which tiny rhizomes, which are underground stems of plants, begin to grow and develop into new plants. Silver nitrate is used to produce sucrose's positive impact on microrhizome formation in *Zingiber officinale roscoe* (An *et al*., 2020). The addition of silver nitrate at 7.5µM reduces hyperhydricity occurring at high frequency in the shooting culture in *Caladium bicolor* (Isah, 2019).

Anther culture

Anther culture means plant regeneration from the haploid microspore cells with the aim of haploid and diploid plant production. Supplementation of silver nitrate enhances anther viability, embryo quality, direct embryogenesis, regeneration speed, and yields of embryos and *in-vitro* plantlets in *Solanum melongena L*. through a triple synergistic effect (Vural and Ari, 2020). Silver nitrate enhances anther culture responses and key outcomes related to regeneration when appropriate concentrations of chemicals are utilized. The most remarkable improvements in the formation of embryo-like structures and the production of regenerated green plants were observed through the synergistic action of $AgNO_3$ (50 mg/L), IAA (1.0 mg/L), and kinetin (0.5 mg/L) in *wheat (Triticum aestivum)*, (Hassan and Islam, 2021). In Greenhouse Bell Pepper *(Capsicum annum L.)*, anther culture formed the highest percentage of embryonic anthers occurring with the interaction of silver nitrate. Additionally, among the silver nitrate concentrations studied, the highest percentage of regenerated plantlets (11.71%) was achieved with 10 mg/L silver nitrate (Zamani and Choukan, 2021).

High acclimatization

High acclimatization is a process where plantlets or shoots in culture vessels adjust to a new micro-environment, adapting to the conditions of their surroundings. Silver nitrate boosted shoot characteristics in *Fragaria ananassa* during multiplication, also enhanced rooting parameters in the rooting stage, and improved survival, plant height, leaves and dry weight in the acclimatization stage (Nasir and Abdul Hussein, 2022).

Secondary Metabolite enhancement

Metabolite enhancement involves the augmentation or increase in the production of metabolites within a biological system, often achieved through various methods such as genetic modifications, environmental adjustments, or optimized growth conditions. The addition of silver nitrate as an abiotic elicitor in the *in-vitro* cultivation of *Lantana camara* plantlets can enhance their growth and overall health (Tien *et al*., 2019). The development of suspension culture from callus applies to the industrial production of secondary metabolites. The addition of silver nitrate (1mg/L) in suspension culture enhanced cell growth and the level of flavonoid baicalein in a rare medicinal tree, *Oroxylum indicum* (L.) Vent (Gokhale *et al*., 2022).

AgNPs

AgNPs have antimicrobial properties**,** making them useful in various applications. It shows minimal impact on *in-vitro* multiplication at low concentrations but may harm plant development more than $AgNO_3$, depending on the concentration in *Campomanesia rufa* (Timoteo *et al*., 2019). Silver nanoparticles (AgNPs) have been shown to promote the healing of meniscus injuries in *Bauhinia acuminate* by enhancing the osteogenic differentiation and proliferation of mesenchymal stem cells (MSCs) (Hu *et al*., 2019). In plant tissue culture, a high concentration of silver nanoparticles hindered the formation of adventitious shoots in *Chrysanthemum gerbera* (Tymoszuk and Miller, 2019). The cell suspension cultures could offer a sustainable option for the green synthesis of AgNPs in *Randia aculeata L*. (Antonio *et al*., 2022). The application of AgNPs to date palm (Hayani cv.) results in a significant increase in concentrations during the regeneration stage (Elsayh *et al*., 2022), enhancing both the number and length of shoots at 3.0 mg/L concentrations compared to the control treatment.

Negative effect

Negative effects in tissue culture include genetic instability, somaclonal variations and abnormal growth patterns. Silver nitrate shows a negative effect by inducing programmed cell death (PCD) in *Vitis vinifera* suspension cell cultures through caspase-3-like activity, oxidative stress and disruption of the ubiquitin-proteasome complex, suggesting a shared pathway with animal cells in response to toxic metal in tissue culture (Fillippi *et al*., 2019). Shoots generated *in-vitro* with silver nitrate-supplemented media failed to develop roots when subjected to rooting media in *Hippophae salicifolia* (Trivedi *et al*., 2023). The significance of silver nitrate in *Antirrhinum majus L*. shows shoot regeneration and underscores $AgNO_3$'s role in mitigating hyperhydricity in *in-vitro* regenerated shoots. These insights enhance our understanding of hyperhydricity mechanisms and the proposed protocol is anticipated to aid in the *in-vitro* shoot regeneration and genetic transformation of the snapdragon cultivar studied (Lee *et al*., 2023). In *Polygonum multiflorum* highest concentration (20 mg/L) of $AgNO_3$ treatment showed a decrease in the number of shoots (1.4 ± 0.2 mm) and shoot length (9.7 ± 1.6 mm) (Park *et al*., 2022).

Large-scale propagation and preservation of ornamental plants

Growing ornamental plants on a large scale requires keeping their unique and desirable features intact. Adding silver nitrate to the growth process helps preserve the beauty of ornamental plants, like *Lilium* cut flowers, for a longer time (Brahma *et al*., 2023). Silver nitrate also helps preserve the visual appeal of St John's Wort (*H. perforatum* L.) in plant cultures (Syahid and Wahyuni, 2019). Additionally, silver nitrate can reduce excessive water retention and promote healthy growth in *Caladium bicolor* (Isah, 2019). It also enhances root growth and adaptation in *Argania spinosa* (L.) Skeels, allowing for rapid and large-scale propagation (Amghar *et al*., 2021). Furthermore, using silver nitrate in *Capparis decidua* plant cultures optimizes the growth process, enabling research and genetic studies in this important species (Ahlawat *et al*., 2020).

Large-scale micropropagation

A reliable technique for mass-producing healthy plants in a lab is called large-scale micropropagation. This method uses a special solution, including silver nitrate, to grow many identical plants that are free from diseases. This approach has been successfully used in Moringa plants (Ravi *et al*., 2019). Adding silver nitrate, silver nanoparticles or silver thiosulphate to the solution helps grow many shoots and prevents leaves from falling off in certain plants like *Citrus australasica* (Mahmoud *et al*., 2020). This method allows for efficient mass production of high-quality plants.

Salinity tolerance

Salinity tolerance is the plant's potential to grow and flourish its life cycle in high saline environments, via adding silver nitrate-enhanced *Lycopersicon esculentum* plantlets' *in-vitro* salinity tolerance by affecting ethylene action or generation and boosting biochemical responses (Zarei *et al*., 2023). Analysis of six-week-old calli revealed that silver nitrate enhanced the concentration of photosynthetic pigments, which is beneficial for cell regeneration. These findings suggest that activation of stress response in Oilseed rape (*Brassica napus L*.) was significant for achieving increased shoot frequency in the Albina, Lancia and Menthol varieties (Ramadan *et al*., 2021).

Economically effective

The cultivation and characteristics of economically viable plants vary depending on their intended uses and applications. Research has demonstrated the eco-friendly, cost-effective and safe production of silver nitrate using *Berberis vulgaris* aqueous extract, without the need for harmful substances, resulting in high antibacterial activity (Behravan *et al*., 2019). However, studies have also shown that the use of silver nitrate and silver nanoparticles can have detrimental effects on plant growth and development. For instance, *Populus nigra* L. exposed to silver nitrate and silver nanoparticles exhibited chronic phytotoxicity, with silver nanoparticles showing greater toxicity than silver nitrate (Iori *et al*., 2023). Additionally, exposure to 30 mg/L silver nitrate led to phytotoxicity and impaired antioxidant responses in *Lycopersicon esculentum* (Noori *et al*., 2020).

Conclusion

Silver nitrate and silver nanoparticles present the groundbreaking role in plant tissue culture, offering various benefits such as enhanced shoot growth, regeneration and secondary metabolite production. They have been successfully used in micropropagation, anther culture, microrhizome germination and large-scale propagation of various plant species. Additionally, silver nitrate has been shown to improve salinity tolerance and promote economically effective plant growth. However, it is crucial to note that excessive use or high concentrations of silver nitrate and silver nanoparticles can have negative effects on plant development, leading to phytotoxicity and genetic instability. Therefore, optimal concentrations and careful application are essential to harness the benefits of these additives in plant tissue culture. Further research is needed to fully understand their mechanisms and potential applications in biotechnology and agriculture.

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Conflict Of Interest

On behalf of all the authors, the corresponding author states that there is no conflict of interest.

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