Cellular and Molecular Biology

C M B Association Publisher

Journal homepage: www.cellmolbiol.org

The Effects of Nanosilver on Bacterial Contamination and Increase Durability Cultivars of *Rosa hybrida* L. Through of Stenting Method

Bahareh Shaafi[1], Danial Kahrizi [1*], Alireza Zebarjadi[1], Pejman Azadi[2]

[1]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, Razi University, Kermanshah, Iran*

[2]*Department of Plant Tissue culture and Genetic Engineering, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO). Karaj, Iran*

ARTICLE INFO

Review paper

Article history:
Received: December 08, 2021
Accepted: March 07, 2022
Published: March 31, 2022

Keywords:
Rosa hybrida, Natal Briar, Nanosilver, Nanotechnology, Agrobacterium; tissue culture

ABSTRACT

The rose flower (*Rosa hybrida* L.) is one of the world's most popular flowers among ornamental plants and the cut rose flower industry is the most important aspect of the rose culture industry in the world. The stenting method (cutting and grafting) uses a stem containing a leaf and a dormant bud as a scion, which is grafted onto an internode from the rootless rootstock to form the graft and the induction of the root on the rootstock are carried out simultaneously. In this method, Natal Briar is often used due to its desirable traits such as high rooting, the possibility of faster regeneration after cutting, the ability to adapt to different culture media and high resistance to root diseases. Most imported Natal Briar rootstocks, in addition to increasing the cost of producing hybrid roses, have a percentage of *Agrobacterium* contamination that can reduce the efficiency of stenting. Therefore, using the tissue culture technique to propagate the base is one of the important solutions. But one of the problems of tissue culture is the control of bacterial contamination so that these factors grow faster than explants and absorbing carbohydrate sources from the culture medium prevents regeneration of Natal Briar rootstocks. Nanotechnology today has expanded the field of application of nanomaterials due to the increase in the surface-to-volume ratio, electron transfer ability and surface reaction capability. Inhibit DNA replication and destroy the cellular structure of *Agrobacterium*. Therefore, the use of appropriate concentrations of Nanosilver in the culture medium of Natal Briar rootstocks increases the economic efficiency and durability of *Rosa hybrida* cultivars obtained by the stenting method.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.3.21

Introduction

Roses are one of the most popular and economically important horticultural crops. These plants are used for many different purposes, such as cut flowers, ornamental plants such as potted and garden plants, and the food, pharmaceutical and perfume industries (1). Rose sp. is one of the most widespread ornamental plants and has been selling well and in high demand for many years (2). Different classifications divide roses into two categories: cut flowers and garden flowers. To increase the cut flower yield of commercial roses, they are usually grafted on compatible introduction rootstocks. Rose plants are propagated by seeds and methods of vegetative propagation, such as stem cuttings, grafting, budding, cutting grafting (stenting), cutting-budding, root grafting and tissue culture. Asexual reproduction is the only way to maintain desirable traits in an elite variety, especially when it is heterozygous and polyploid (3).

The easiest and most common way to grow roses is to use stem cuttings. However, the stenting method is an effective technique for the rapid propagation of plants. This is an efficient technique for rose propagation in which cutting and grafting are carried out simultaneously, and the scion is grafted onto an unrooted rootstock. The formation of combined and adventitious roots on the rootstock co-occurs. The efficiency and flower productivity of grafted plants is higher than that of self-grown plants due to the use of rootstocks. Due to the different climate and soil conditions in other regions, it is suggested that different rootstocks should be the primary concern for the compatibility of rootstocks and scions (4).

Today, Natal Briar is the rootstock in the propagation of nearly all roses grown in countries like

*Corresponding author. E-mail: dkahrizi@razi.ac.ir

Ecuador, Colombia, Kenya, Ethiopia, and Uganda. Significant characteristics of Rose Natal Briar include improved production in grafted varieties, longer stem, better rooting rate, faster regeneration after the cut (harvest), ability to adapt well to different cultivation environments, and high resistance to root diseases.

Natal Briar is the rootstock for grafted roses, accounting for 60-70% of the world's planted area due to its easy rooting,good stem length , and head size. Production of cut roses is highly limited to commercial hybrid varieties grafted on rootstocks for sustainable relevance. It is now established as a suitable and consistent rootstock worldwide. The grafting rootstock is a widely cultivated Dutch rootstock (R. multiflora Natal Briar). ,However this rootstock has been successfully validated to increase rose cut flower yield, its susceptibility to Agrobacterium infection and high salinity limit roses (5,6).

Today, advances in nanotechnology in various fields of physiology and biochemistry have expanded the range of applications of nanomaterials, so the use of nanoparticles due to their unique properties, such as a large surface area for increased adhesion strength and better contact with microorganisms and resistance to contamination plant tissue medium. Bacterial infection is considered to be a significant problem during plant micropropagation. Due to the importance of contamination control to achieve tissue culture goals, many different approaches have been proposed, each with advantages and disadvantages. Nanosilver is antibacterial, antifungal, and antiviral with good bacteria removal potential (7,8).

Increasing the shelf life of cut flowers is possible using modern genetic engineering methods. Aging-related genes (SAGs) that encode enzymes and transcription factors of this pathway are expressed by hormones such as ethylene, Abscisic acid, and jasmonic acid. They increase while cytokines reduce their expression and delay the aging stage in the plant. The role of ethylene in the aging process of flowers can also be investigated through genetic changes in the ethylene signaling pathway. The use of ethylene production inhibitors or action increases cut flowers' lifespan. It reduces aging, and silver is an ethylene inhibitor that binds to the ethylene enzyme acceptor site to block the ethylene acceptor and use this compound in the preservative solution. Reduces the negative effects of this hormone such as aging and wilting of cut flowers (9).

Silver ions react with sulfhydryl groups of cell membranes and replace them with sulfur and damage cell membranes, react with phosphorous compounds such as nucleic acids and prevent cell division, increase the leakage of protons and potassium ions, and prevent Respiratory chain processes causing cell death and exert their antibacterial properties. One of the problems of tissue culture of fine samples is the production of ethylene in the culture medium, which prevents the division and differentiation of cells. Nano silver reduces the activity of ethylene through two possible mechanisms, 1) enters the cell with the same structure and inactivates them as a coating on ethylene receptors. 2) Due to oxidation, it is converted to silver ion and reduces its activity by replacing silver with copper ion in the ethylene receptor (10). Therefore, an appropriate increase in nanosilver concentration will increase morphological indices. Because imported rootstocks are often infected with Agrobacterium, it is necessary to use the tissue culture technique to increase the production of Natal rootstock and also immunity from bacterial contamination. There are various methods for controlling environmental contaminants such as meristem culture, replication of culture and antibiotics, but antibiotic compounds are expensive, heat sensitive and toxic and have a limiting effect on the growth, regeneration, callus induction and survival of explants. So that by removing them from the culture medium and transferring the rootstocks to the re-bed, contamination appears. Prolonged exposure of plant cells also causes mutations, so finding an effective, low-cost, available, and safe substance to remove contaminants from the culture medium is essential because bacteria and fungi absorb more carbohydrate sources from the environment. Cultures can grow faster than plant cells and prevent them from regenerating (4).

The application of silver nanoparticles along with calcium due to reduced ethylene production and microbial activity creates more strength in the tissue of cut flowers, which increases cellular resistance and thus reduces ion leakage in cells. Combined treatments of silver nanoparticles and calcium nanoparticles often have a significant effect on reducing the decomposition of leaf chlorophyll. In fact, the increasing effect of silver nanoparticles on maintaining leaf chlorophyll content is more than calcium nanoparticles. Due to the fact that ethylene degrades chlorophyll, silver and calcium nanoparticles due to their anti-ethylene role lead to an increase in chlorophyll in the petals treated with these nanoparticles significantly increasing the amount of total chlorophyll in most cut flowers of roses (11).

The selection of rootstock and scion cultivars as well as their cultivation methods can be significant treatments on yield, so today most studies have focused on mass production and propagation of seedlings so that after transplanting and adaptation, flowering And the quality of the plants created are important traits for tissue culture (12). The adaptation of roses is often difficult due to the rapid drying of the seedlings and their susceptibility to diseases caused by high humidity. Therefore, considering the physiology of the mother plant, the source of explant tissue, the components of the culture medium and the level of growth regulators are the factors affecting flowering. Therefore, experimental design for mass propagation of contaminant-free rootstocks by using nanoparticles in tissue culture conditions and transferring them for stenting using *Rosa hybrid* cultivars and creating optimal conditions in the flowering phase, increasing shelf life and Reduction of post-harvest lesions is essential.

Preparation of culture medium

The rootstocks used in the greenhouse are transferred to the laboratory and after cutting the leaves from the petiole, the branches are divided into single nodes of 2 to 3 cm, then the isolated explants are subjected to disinfection treatments and cultured in MS medium. To prepare the culture medium containing different concentrations of silver nanoparticles, since these particles are heat resistant and insoluble in all solvents, so to prepare MS suspension containing silver nanoparticles, after preparing the culture medium and adding nanosilver, the culture medium is placed on a shaker. The silver nanoparticles are evenly distributed as they cool slowly. Survival percentage of explants and percentage of bacterial contamination are recorded after 4 weeks. In fact, the survival index is obtained by calculating the percentage of regenerated Natal Briar bases without total infection. Also, once a month, cultivation and transfer to an MS culture medium containing different concentrations of nanosilver are performed, and finally, after 2 months, morphological factors such as rooting percentage, length and number of roots are measured

Materials and propagation (stenting and stem cutting)

The plant materials used in the research should be obtained from commercial rose greenhouses. The greenhouse was equipped with a mist system to help adjust moisture levels. During the study cycle, daily average temperature and relative humidity were maintained at 20 ± 5 °C and 85 ± 5%, respectively (13). Flowering stems were collected early in the morning and kept under cool and moist conditions until transported to the work area. The flowering stems include mature leaves with open flowers. After harvesting, each stem is divided and cut into sections with five-leaf leaves and dormant buds. A single internode without buds was used per rootstock. The scions were grafted into 4.0 cm long internodes from semi-hardwood cuttings of the *Rosa hybrida* L.'Natal Briar' rootstock. In this method, scions and rootstocks with suitable flat cuts can be grafted together to form a maximum overlap of layers. The scion is selected according to the thickness of the stem rootstock. The scions are then grafted using the splice grafting method. Scions and rootstocks with a suitably smooth cut can be grafted together with a maximum overlap of cambium. Plastic tape is used to wrap the graft joint. For better rooting and to avoid suckers, remove the terminal shoots from the rootstock. Remove all leaves and shoots from rootstock cuttings. Shoot removal was done to promote better rooting and prevent sucker growth on rootstocks. The tops of the rootstock internodes and the bases of the scions are held together while cutting to facilitate the stand. The scion and rootstock are combined by the splicing grafting method. For proper graft growth, the scion and rootstock are kept in close contact by wrapping them with a piece of grafting tape. The bottom of the rootstock was immersed in different concentrations of IBA for 10 seconds. The stentings are planted in a mixture of coconut peat and perlite medium (1:2 ratio) and placed in a greenhouse under a mist system. The beds are disinfected every two weeks with the fungicide "Captan" at a concentration of 0.2% to prevent fungal infections. The scaffolds were grown under the above greenhouse conditions for 60 days.

Measurement of tested indicators and analyzed data

At the end of the study, the plants are taken out from the medium. Their morphological and biochemical traits included transplant success rate, rooting rate, number of roots, length of longest root, fresh and dry weight of roots, branch traits, branch traits (including the number of leaves, number of branches) and longest shoot length), number, chlorophyll (a, b) and total carotenoid content is evaluated. The collected data cultivars (*R. hybrida* L.) are statistically analyzed and the results are compared using the new Duncan multi-range test at the 5% probability level using the SAS program (version 9.2)

Results and discussion

Cut flowers are among the flowers that are sensitive to gravity. During the maintenance of cut flowers, the buds are bent due to gravity and block the flow of water to the buds and flowers are half open and accelerate their placentation. Resistance to geotropism depends on the flower stem's strength and the branches' torsion due to the amount of solution adsorption (14,15). Using microbial agents and preservative compounds prevents the activity of bacteria and other microbial agents and increases the adsorption of the solution. Accumulating calcium in plant tissues strengthens the polymer bond between the middle septa of the pectocellulose membrane, enhancing the cell wall network, resulting in increased tissue mechanical strength, reduced electrolyte leakage and reduced stem curvature. Also, calcium reduces the evapotranspiration, increases the turbidity and reduces the petals' wilting by affecting the pores' opening and closing mechanism (16).

In vitro multiplication or micropropagation of roses allows for rapid propagation, production of disease-free plants, the application of genetic engineering to test gene function, accelerated breeding and tissue culture protocols, and the combination of new rootstocks can improve the yield and economic benefits of cut flowers. The production of good-quality roses depends on several factors. Several factors are related to the progress of breeding programs to introduce new varieties with improved characteristics, including combinations with new floral aromas, new flower colors and longer harvest periods. Despite these factors, some undesired barriers may limit high-quality rose production, such as Agrobacterium infection, sensitive rootstocks, low-quality substrates, and saline irrigation supplies (17,18).

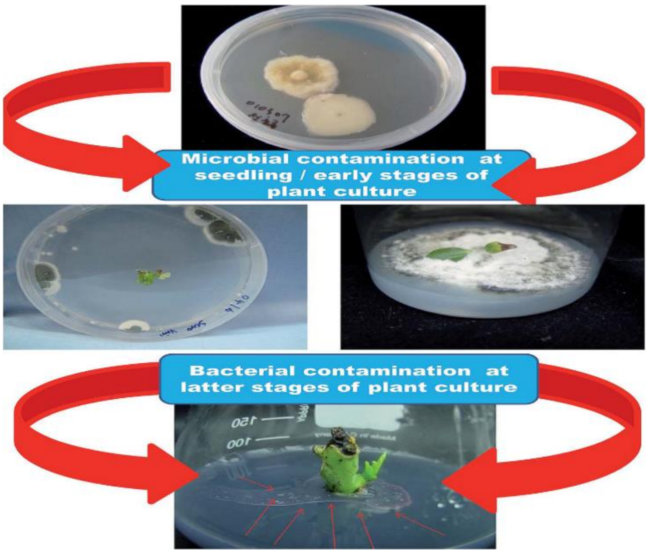

Figure 1. Slides showing microbial contamination during different phases of the plant culturing process.

Aging in plants is an evolutionary and planned process that includes structural, biochemical and molecular changes, all of which are signs of planned cell death. In general, aging and programmed cell death (PCD) both refer to the mechanisms that trigger the planned end of individual cells. There are many reasons for the onset of aging, including the involvement of oxygen free radicals (ROS) and oxidative stress. Accordingly, it can be stated that aging is an oxidative process in which ROS and the antioxidant system are involved (19). The aging process of flowers is associated with several morphological, physiological and biochemical changes. Petal wilting is one of the most common signs of aging. Also, the lack of the whole opening of flowers and curvature are among the symptoms seen in rose cultivars. One of the metabolic signs of petal aging is a decrease in its protein content. The breakdown of tissue proteins is a sign of membrane destruction that occurs at the same time as aging. During the aging process, degradation of other large biological molecules such as nucleic acids and lipids is also observed. Several factors control the aging process, and by manipulating each of these factors, the aging process can be controlled to some extent. Through this, the durability of cut flowers in rose cultivars can be increased (20,21).

Although cut flowers have high economic value, they have post-harvest lesions and a short lifespan, so any attempt to prolong their shelf life by controlling aging using genetic manipulation or treatments chemical can effectively reduces waste after harvest. During the aging process, the rate of photosynthesis decreases and the rate of respiration increase so that the activity of hydrolyzing enzymes such as proteases also increases. Large degradation of biomolecules such as proteins, nucleic acids and lipids also occurs during the aging process (22). Application of ethylene biosynthetic inhibitors such as amino oxyacetic acid or inhibitors of its activity such as silver thiosulfate and 1-methylcyclopropene increase the post-harvest life of ethylene-sensitive plants. Shutting down genes in the ethylene biosynthesis pathway through genetic engineering can also increase the shelf life of cut flowers (23).

During the aging process of the petals, the increase of free radicals causes the destruction of phospholipids and the release of fatty acids, followed by peroxidation. This increases the membrane's permeability, leading to a more significant water content loss in the petals. One of the biochemical signs of aging is the accumulation of proline, especially in the petals of hybrid rose cultivars. Proline acts as an osmotic substance in the cell and protects enzymes and some large molecules against stress. When the plant is stressed, proline levels rise faster than other amino acids (24). One of the physiological signs of aging, peroxidation of membrane lipids and their destruction, is affected by oxygen free radicals. As a result of this process, the amount of malondialdehyde (MDA) increases. Increasing the activity of enzymes such as lipoxygenase (LOX) increases the synthesis of malondialdehyde in old age. MDA is used as an indicator of age and physiological resistance. Studies show that the production of MDA in the petals of hybrid rose cultivars is higher in the aging stage than in the flower bud stage. Also, in the aging stage, the application of oxidative stress increases the synthesis of malondialdehyde in the petals (25).

In terms of other postharvest characteristics, such as ethylene susceptibility, there are differences between different cultivars of a species, which provides a reasonable basis for implementing breeding programs to increase the shelf life and reduce ethylene induced by ethylene. Cut roses with different postharvest life are also other in terms of various morphological and anatomical features. Among the types of plant hormones, ethylene is the most important stimulating hormone in the aging process in plants, so its presence in the environment reduces the appearance of plants and their durability (26, 27).

Ethylene is produced from methionine in a series of reactions. Methionine is converted to S-adenosyl methionine (SAM) by the enzyme adenosyl methionine synthase (SAM synthase). SAM is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthio-adenosine (MTA) by the enzyme ACC-synthase. MTA can be recycled to methionine, ACC can be converted to malonyl ACC (MACC) by the enzyme ACC-N-malonyltransferase, or it can be oxidized by the enzyme ACC oxidase to ethylene to form carbon dioxide and cyanide (HCN) to give. HCN is converted to beta-cyanolanin by the enzyme beta-alanine synthase to prevent toxicity from cyanide accumulation during the synthesis of high levels of ethylene (28). After ethylene synthesis, this hormone is perceived by ethylene receptors and its message through the message transmission system produces specific biological responses (Figure 2).

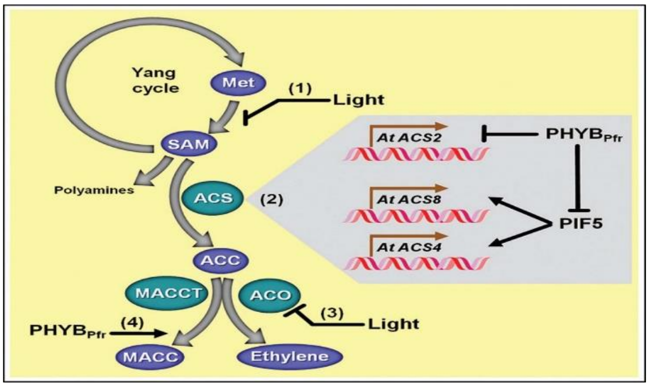

Figure 2. Ethylene biosynthesis pathway

Inhibitors are used to reduce the harmful effects of ethylene. Ethylene inhibitors include two groups inhibitors of ethylene biosynthesis and inhibitors of ethylene action. Amino oxyacetic acid (AOA1) and amino ethoxy vinyl glycine (AVG5) are inhibitors of ethylene biosynthesis (29). These two compounds inhibit the conversion of SAM to ACC and inhibit the production of ethylene by disrupting the action of the enzyme ACC synthase. These compounds are often expensive and do not alter the plant's sensitivity to external ethylene. Therefore, they are not effective in preventing the effects of foreign ethylene during storage and transportation. Ethylene inhibitors include silver-containing compounds such as silver nitrate, silver nanoparticles, and silver thiosulfate (STS), which can increase the shelf life of cut flowers (30).

Chlorophyll a and b content with appropriate concentration of Nanosilver improves photosynthetic pigment status, increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and thus reduces the synthesis of hydrogen peroxide and inhibits the peroxidation of cell lipid membranes, reduces the synthesis of positive ethylene binders through binders. Ethylene protein and increase the protein content of plant leaves, delaying aging and preventing yellowing and leaf fall. The results showed that the induction of oxidative stress due to high concentrations of silver nanoparticles in barley increases the content of proline, as an antioxidant that can be an inhibitory factor in the regeneration of explants because increasing this amino compound due to Nanosilver treatment causes Glutamine, as a common precursor of chlorophyll and proline synthesis, is less involved in chlorophyll biosynthesis, which ultimately reduces the synthesis of chlorophyll in plants treated with Nano silver (31).

Roses are propagated by stem cutting, budding, grafting, stenting (simultaneous cutting and grafting), root grafting, in vitro micropropagation, and in some cases by seed in the stenting method, a stem containing one leaf and a dormant bud is used as a scion that is grafted onto an internode of the non-rooted rootstock. Formation of graft union and root induction on the rootstock occurs concurrently and in the case of rose hybrids within three weeks. Rose propagation by the stenting method is an efficient technique with many advantages used worldwide by rose producers (32). Rootstock plays an important role in successful propagation via stenting. Both environmental factors and the physiology of plants can affect the healing of rootstock and scion in the stentings. The suitable environmental conditions helped in the rapid flow of plant sap in rootstock and scion, leading to the formation of the cambium layer, vascular tissue and grafting success. Physiologically, stenting is more complicated than cutting propagation because the formation of the graft union and rooting should occur simultaneously, and there are influenced by photosynthesis, bud development, and root formation. Using this method, the growers could propagate roses in a significantly shorter time compared with the cutting method. Moreover, higher quality flowers could obtain using the stenting method (4).

Figure 3. The course of stenting used in *Rosa hybrida*: A, preparation of harvested scion and rootstock

The use of nanosilver, on the one hand, can save the explants by killing harmful microorganisms and on the other hand in high concentrations the production of reactive oxygen species and the effect on genetic material can cause abnormalities in plant tissues. The appropriate concentration and time of these nanoparticles can be a useful tool to eliminate tissue culture contamination. The application of silver nanoparticles along with calcium in many cases has a better effect on the growth indices of cut flowers so that the use of silver nanoparticles in solutions that maintain cut flowers increases the shelf life of these flowers and calcium nanoparticles. They also delay the aging of plant tissues (33). Due to the fact that calcium plays a role in the structure and functions of cell membranes by binding proteins with enzymatic and non-enzymatic roles to cell membrane phospholipids, thus the activity of ethylene-producing enzymes, which have a protein structure and bind to cell membranes. Are reduced and eventually with less ethylene production, which stimulates the activity of cell wall hydrolyzing enzymes, the cell wall is less destroyed (9).

The purpose of genetic engineering in using wild-type cultivars is to contain valuable genes for resistance to living and non-living stresses, adaptation to arid and semi-arid climates, salinity, and bacterial infections. Substrate diseases, salinity and pH limit the growth and yield of rose rootstocks. Considering the role of rootstock in vegetative growth rate, early maturity, yield and disease resistance, choosing the right rootstock can play an important role. In reproduction, roses have quality cut branches. Due to the fact factors such as heat and high humidity can increase the growth rate of Agrobacterium tumefaciens and the systematic movement of bacteria into the seedlings, which reduces the yield and quality of plants (21,34).

The thriving tissue culture of all plants depends on removing exogenous and endogenous contaminating microorganisms. A significant problem with plant micropropagation is the chronic contamination of microorganisms. Fungi and bacteria are the most common agents of contamination. Nanotechnology is one of the most active research fields in modern materials science. Nanoparticles exhibit new or improved properties based on specific properties such as size, distribution, and morphology. New applications for nanoparticles and nanomaterials are emerging rapidly. Nanocrystalline silver particles have tremendous applications in high-sensitivity biomolecular detection and diagnostics, antibacterial and therapeutic agents, catalysis, and microelectronics (35). Studies have shown that silver ions interact with the sulfhydryl (-SH) groups of proteins and DNA bases, thereby inhibiting the respiration process or DNA unwinding. Inhibition of cell division and disruption of bacterial cell envelopes have also been documented, and interactions with hydrogen bonding processes have been shown to occur. It has been demonstrated that nano-sized silver can also control bacterial infection under tissue culture conditions (36,37).

Silver nanoparticles (SNPs) are relatively new as a method of pulsing cut flowers and have demonstrated their importance as antibacterial agents that can kill 650 species of bacteria in water. SNPs release silver ions, Ag+ (29), which replace hydrogen cations (H+) on sulfhydryl or Thiel groups (-SH) on bacterial cell membrane surface proteins, thereby reducing membrane permeability and ultimately leading to cell death (35). This study evaluated the antibacterial effects of silver nanoparticles (SNP, 33 nm) and silver nitrate (AgNO3) on the vase life of red ribbon cut flowers. They were pulsed with 50 mg of L-1 SNP and AgNO3, with or without 5% sucrose. Microscopic observation showed cutting of xylem vessels at the stem end five days after pulse treatment. There were few visible particles in the vessel pulsed with SNP and AgNO3. SNPs anchor or penetrate bacteria's cell walls and disrupt their cell membranes. Control flowers exhibited more vascular occlusion than treated flowers. Panels A and B are stem apexes pulsed in 50 mg L-1 SNP for 1 hr; panels C and D are stem apexes of control flowers. Vascular obstruction is thought to be primarily due to microbial proliferation. The efficacy of Ag+-carrying nanoparticles as antibacterial agents has been well established. The antimicrobial activity of SNPs is partly a function of particle size, with higher surface-to-volume ratios increasing the proportion of grain boundary atoms. In our study, SNP inhibited the hydraulic conductivity of rose-cut flower stems, which was associated with a reduction in the number of bacteria at the stem ends (35).

Roses are labeled as potted or cut flowers. Vase life for cut roses is usually short. These cut flowers wilt and the rachis bend under the flower head. Water balance is a significant factor in determining the quality and lifespan of cut flowers. It is influenced by water absorption and transpiration, which balance these two processes. Clogging of the stem ends is considered the main cause of water imbalance in cut flowers. These symptoms are thought to be caused by vascular blockage, which inhibits the flower's water supply (13). The rose is the first and most important cut flower in the world. One of the standard methods for mass production of this plant is propagation through tissue culture. The major limiting factors for rose tissue culture are bacterial contamination and phenolic exudates at the establishment stage, which significantly deteriorates most explants. Nanosilver can control and stop bacterial contamination. High concentrations of nanosilver slowed the regeneration of the explants and, in some cases, led to the destruction of the explants. In general, nanosilver does not affect fungal contamination (38).

Auxins and cytokines are compounds involved in plant growth regulation; indole-3-butyric acid (IBA), the most well-known auxin, has been widely used in stimulating the rooting of cuttings in many plant species. It is non-toxic to plants over a wide range of concentrations. The different levels of endogenous auxin in cutting and genotype characteristics might be a reason for variation in the rooting efficiency of cuttings among genotypes. The role of auxin in callus induction, cell division stimulation, cambium formation and vascular tissue differentiation is well established. Auxin also stimulates adventitious root formation in many species by promoting the transfer of carbohydrate and nitrogen compounds to the base of cleavage induced by stimulating root primordia (19).

All stages of graft conjugation formation (vascular cambium alignment of rootstock and scion, wound healing response, callus bridge formation, cambium formation, and vascular tissue formation) are influenced by auxin. Exogenous auxins were shown to have an essential role in the rooting formation of cuttings and affect the rooting speed and percentage of cuttings. Plants produce natural auxin in their fresh shoots and leaves; however, synthetic auxin must be used for successful rooting to prevent cuttings from dying and has been reported in many species (39).

Advances in nanoparticle (NP) production and the need for nanoscale system control have significantly impacted tissue engineering. NPs with low toxicity, contrast agent properties, customizable properties, the potential for targeting/stimulus-response delivery, and precise control of behavior (via external stimuli such as magnetic fields) make them potentially useful for improving engineered tissues and overcoming terminology. Functional tissue and organ replacements require high spatial and temporal control over biological events and their real-time monitoring. The controlled presentation and local delivery of biologically active substances (growth factors, chemokines, inhibitors, cytokines, genes, etc.) and contrast agents are essential for controlling and monitoring engineered tissues (40). Physiologically, stenting is more complex than cutting propagation because the formation of graft bonds must occur concurrently with rooting, and there is an interplay between photosynthesis, root formation, and shoot development. Therefore, after the leaves are formed on the scion, carbohydrates and natural hormones are produced and passed from the leaves to the rootstock for growth.

The purpose of studying the post-harvest physiology of cut flowers is to understand the complex biological relationships in the plant to reduce crop waste in the production chain because during the post-harvest period, due to unfavorable conditions such as low light intensity, inappropriate temperature, Low relative humidity, the plant is exposed to stressful situations (40). The applied stress often leads to adverse changes in the plant, such as wilting, discoloration, and shedding parts of the plant such as flowers, petals and buds. Therefore, understanding plants' complex biological relationships, such as respiration, ethylene production, transpiration, hormonal balance and the activity of enzymes associated with flower paleness and leaf yellowing, is essential to developing technical strategies to prevent quality degradation. The biological importance of ethylene in ornamental products, its signaling pathway in plants, and methods to reduce its destructive effects on the quality of ornamental products have been extensively studied (41).

Nanoparticles are at the forefront of nanotechnology development in biomedical research (35). Nanoparticles have a high surface area to volume ratio, which endows them with unique properties and enhances their catalytic, magnetic, mechanical, and optical properties, thereby expanding their potential biomedical applications (13,26). Among various metals, silver has long been recognized for applications, including food preservation, antibacterial therapy, and water purification. Recent advances in nanotechnology have led to the widespread use of silver nanoparticles (AgNPs) as antibacterial, anti-inflammatory, and anticancer agents. Applications because AgNPs possess unique optical, magnetic, catalytic and electronic properties (42,43).

Calcium is one of the essential elements in increasing and maintaining the quality of cut flowers. Accumulating calcium in plant tissues strengthens the polymer bond between the middle septa of the pectocellulose membrane, which strenhancese the cell wall network and ultimately increases the Mechanical strength of tissues and reduces stem curvature. Studies have also shown that calcium plays a role in the aging process and increases the shelf life of cut flowers of roses by several mechanisms because it has an inhibitory effect on ACC oxidase and consequently reduces ethylene production in the petals and increases the activity of proton pumps in the membrane. Plant cytoplasm, decrease in electrolyte leakage, increase in initial fresh weight of flower by increasing water uptake and decrease in transpiration during the postharvest period are the applications of calcium nanoparticles in the cultivation of cut flowers of roses (44).

Several factors reduce the life of cut flowers, including the reduction of carbohydrates and the accumulation of microorganisms in the preservative solution. Over time, the microbial population in the solution holding cut flowers increases, which can be an important reason for reducing their post-harvest life.

Still, the use of combined treatments of silver and calcium nanoparticles significantly reduces the microbial population (45).

Nanosilver can be used as a substitute for toxic substances and chemical compounds for environmental disinfection and pest control. Silver has been known for thousands of years as a metal that poses no health hazard to humans and the environment. However, this metal in the form of large particles is a low reactivity metal, its tiny particles with nanometer-size show interesting bactericidal effects. This astonishing effect is due to the large surface area of small nanoparticles when encountering microorganisms. They exhibit powerful antimicrobial effects through their impact on cellular metabolism and hindering microorganisms' respiration, growth and reproduction (46).

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Leus L, Van K, De Riek J, Van H. Rose. In: Van Huylenbroeck J (ed) Ornamental crops. Springer, Switzerlan 2018; 85(1):23-29.
2. Nybom H, Werlemark G. Realizing the potential of health-promoting rosehips from dogroses (*Rosa* sect. *Caninae*). Curr Bioact Compd 2017; 13:3–17.
3. Zhou N N. Genetics and genomics of prickles on rose stem. PhD thesis (University of Angers, Angers, France. Doctor dissertation 2021.
4. Pourghorban M, Khaghani S, Azadi P, Mirzakhani A and Changizi M. Propagation of *Rosa hybrida* L. cv. 'Dolce Vita' by Stenting and Stem Cutting Methods in Response to Different Concentrations of IBA. Advances in Horticultural science 2019 ;33(1): 105-111.
5. Miguelez-Sierra Y, Hernández-Rodríguez A, Acebo-Guerrero Y, Baucher M, El Jaziri M. In vitro micrografting of apical and axillary buds of cacao. J Hortic Sci Biotech 2017; 92(1):25–30.
6. Vujović T, Ruzić D, Cerović R. In vitro shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. Hort Sci (Prague) 2012; 39(3):101–107.
7. Fouad AF, Hafez RM. Effect of cobalt nanoparticles and cobalt ions on alkaloids production and expression of CrMPK3 gene in *Catharanthus roseus* suspension cultures. Cell Mol Biol 2018; 64(12):62–69.
8. Tymoszuk A, Kulus D. Silver nanoparticles induce genetic, biochemical, and phenotype variation in chrysanthemum. Plant Cell Tissue Organ Cult; 2020 143:331–344.
9. Otiende MA, Fricke K, Nyabundi JO. et al. Involvement of the auxin–cytokinin homeostasis in adventitious root formation of rose cuttings as affected by their nodal position in the stock plant. Planta 2021; 254**:** 65.
10. Hatami M, & Ghorbanpour M. Defense enzyme activities and biochemical variations of *Pelargonium zonal* in response to Nano silver application and dark storage. Turkish Journal of Biology 2014; 38(1) ; 130-139.
11. Khalil MA, El-Shanshoury AE-RR, Alghamdi MA, Alsalmi FA, Mohamed SF, Sun J and Ali SS. Biosynthesis of Silver Nanoparticles by Marine Actinobacterium *Nocardiopsis dassonvillei* and Exploring Their Therapeutic Potentials. Front. Microbiol 2022;12:705673.
12. Bu Van L, Nguyen HA. simple, economical, and high efficient protocol to produce in vitro miniature rose. In Vitro Cellular and Development Biology-Plant 2020 ; 56(3): 362-368.
13. Pourghorban M, Azadi P, Khaghani S, Mirzakhani A, Changizi M and EdrisI B. Propagation of Three Cultivars of Rosa hybrida L. through Stenting Method. International Journal of Horticultural Science and Technology 2019; 7(1):27-36.
14. Ahn E Y, Jin H and Park Y. Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green synthesized by plant extracts. Materials Science & Engineering C 2019; 101, 204–216.
15. Zhou, N N. et al. Genetic determinism of prickles in rose. Theor. Appl. Genet 2020; 133, 3017–3035.
16. Thi Hong ,Traud W, Thomas D. Genetic analysis of callus formation in a diversity panel of 96 rose genotypes. Plant Cell, Tissue and Organ Culture (PCTOC) 2020; 142:505–517.
17. Bin-Jumah M, Al-Abdan M, Albasher G, and Alarifi S. Effects of green silver nanoparticles on apoptosis and oxidative stress in normal and cancerous human hepatic cells in vitro. Int. J. Nanomed 2020; 15, 1537–1548.
18. YangSH, Lian G. ROS and diseases: role in metabolism and energy supply. Mol Cell Biochem 2020; 467(1): 1–12.
19. Gurunathan S, Qasim M, Park C, Yoo H, Kim J.-H and Hong K. Cytotoxic potential and molecular pathway analysis of silver nanoparticles in human colon cancer cells HCT116. Int. J. Mol. Sci 2018;. 19:2269.
20. Khalil M, Ismail M M and El Shafay SM. Evaluation of antibacterial activity of macroalgae extracts as adjunctive therapy in neonates sepsis induced by Klebsiella pneumoniae. Arab. J. Sci. Eng 2020; 45, 4599–4607.
21. Liu G, Yuan Y, JiangH, BaoY, Ning G, ZhaoL, ZhouX, Zhou H, GaoJ, Ma N. Agrobacterium tumefaciens-mediated transformation of modern rose (Rosa hybrida) using leaf-derived embryogenic callus.Hortic Plant J 2021; 7(4): 359-366.

22. Gupta J, Dubey RK. Factors affecting post-harvest life of flower crops. Int J Curr Microbiol App Sci 2018; 7(1):548–557.
23. Nguyen THN, Tänzer S, Rudeck J, Winkelmann T, Debener T. Genetic analysis of adventitious root formation in vivo and in vitro in a diversity panel of roses. Sci Hortic 2020; 266:109277.
24. Soufflet-FreslonV. Diversity and selection of the continuous-flowering gene, RoKSN, in rose. Horticulture Res 2021; 8, 1–11.
25. Swarnkar MK, KumarP, DograV & Kumar. Prickle morphogenesis in rose is coupled with secondary metabolite accumulation and governed by canonical MBW transcriptional complex. Plant Direct 2021; 5, e00325.
26. Goel N, Ahmad R, Singh R, Sood S and Khare SK. Biologically synthesized silver nanoparticles by Streptomyces sp. EMB24 extracts used against the drug-resistant bacteria. Bioresour. Tchnol. Rep 2021; 15:100753.
27. Nikzamir M, Akbarzadeh A and Panahi Y. An overview on nanoparticles used in biomedicine and their cytotoxicity. J Drug Deliv Sci Technol 2021; 61:102316.
28. Liao C, Li Y and Tjong SC. Bactericidal and cytotoxic properties of silver nanoparticles. Inter. J. Mol. Sci 2019; 20:449.
29. Kubiec K, Nowakowska K, Ilczuk A, Łukaszewska J. Optimizing micropropagation conditions for a recalcitrant ninebark (Physocarpus opulifolius L. maxim.) cultivar. In Vitro Cellular & Developmental Biology Plant 2021;57:281–295.
30. Nayak BK, Chitra N and Nanda A. Comparative antibiogram analysis of AgNPs synthesized from two Alternaria Spp. with amoxicillin antibiotics. J. Chem. Pharm. Res 2015; 7, 727–731.
31. Mohanta YK, Panda SK, Bastia AK and MohantaTK. Biosynthesis of silver nanoparticles from Protium serratum and investigation of their potential impacts on food safety and control. Front. Microbiol 2017; 8:626.
32. Faisal M, Ahmad N, Anis M, Alatar AA, QahtanAA. Auxin and cytokinin synergismin in vitro producing genetically stable plants of Ruta graveolens using shoot tip meristems. Saudi J Biol Sci 2018 ; 25:273–277.
33. Bahlol H S, Foda MF, Ma J and Han H. Robust synthesis of sizedispersal triangular silver nanoprisms via chemical reduction route and their cytotoxicity. Nanomaterials (Basel) 2019 ; 9:674.
34. Singh A, Gautam PK, Verma A, SinghV, Shivapriya PM, Shivalkar S., et al. Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: a review. Biotechnol. Rep 2020;25:e00427. doi: 10.1016/j.btre.2020.e00427.
35. Makvandi P, Baghbantaraghdari Z, Zhou W, ZhangY, Manchanda R, Agarwal T.Gum polysaccharide/nanometal hybrid biocomposites in cancer diagnosis and therapy. Biotechnol 2021; Adv. 48:107711. doi: 10.1016/j.biotechadv.2021.107711.
36. Siddiqi KS, Husen A and Rao RA. A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnol 2018; 16, 1–28.
37. Safavi K. Effect of titanium dioxide Nano particles in plant tissue culture media for enhance Resistance to bacterial activity. Bulletin of Environment, Pharmacology and Life science 2014;3(5), 163-166.
38. Augural M, Ibrahim M, Yang J, Abdul Hussein M, Xin Du, Hardy G, and Ran Y. Plant Growth Regulators Improve the Production of Volatile Organic Compounds in Two Rose Varieties. J Plant Res 2019 ; 8(2): 35-43.
39. Naing AH, Park JS, Kim CK. Effects of ethylene on shoot initiation, leaf yellowing, and shoot tip necrosis in roses. Plant Cell Tissue Organ Cult 2016; 127(2):425–431.
40. Shokri S, Babaie AR & Ahmadian Arab MM. The effects of different concentrations of nano-silver on elimination of bacterial contaminations and phenolic exudation of Rose (Rose hybrid L.) in vitro culture. International Journal of Farming and Applied Sciences2014; 1(3), 50-54.
41. Saratale RG, Saratale GD, Shin HS, Jacob JJ, PugazhendhiA, BhaisareM, Kumar G. New insights on the green synthesis of metallic nanoparticles using plant and waste biomaterials: Current knowledge, their agricultural and environmental applications. Environ. Sci. Pollut. Res 2018; 25, 10164–10183.
42. Nazary R, Aghayeh M, Abedy B,Balandari A, Samiei L, Tehrani A. The First Successful Report: Control of Browning Problem in in vitro Culture of Iranian Seedless Barberry, a Medicinally Important Species. Erwerbs-Obstbau 2021 ; 63:619-623.
43. Otari SV, PatilRM, GhoshSJ, ThoratND and Pawar SH. Intracellular synthesis of silver nanoparticle by actinobacteria and its antimicrobial activity. Spectrochim. Acta A Mol. Biomol. Spectrosc 2015; 136, 1175–1180.
44. Waheed A, Al-Mayahi M. In vitro plant regeneration system for date palm (Phoenix dactylifera L.): effect of chelated iron sources. J Genet Eng Biotechnol 2021; 19(83): 9-18.
45. Manimaran M and Kannabiran K. Actinomycetes-mediated biogenic synthesis of metal and metal oxide nanoparticles: progress and challenges. Lett. Appl. Microbio 2017;. 64, 401–408.
46. Khalil M A, El Maghraby GM, Sonbol FI, Allam NG, Ateya PS and Ali S. S. Enhanced efficacy of some antibiotics in presence of silver nanoparticles against multidrug resistant Pseudomonas aeruginosa recovered from burn wound infections. Front. Microbiol 2021;12:648560.