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Improvement of postharvest quality of cut rose cv. 'First Red' by biologically synthesized silver nanoparticles

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ABSTRACT

The efficacy of biologically synthesized silver nanoparticles (AgNPs) was evaluated for their potential to improve the postharvest quality of rose cut flowers cv. 'First Red'. AgNPs were applied as pulse treatment at 25, 50 and 100 mg L^{-1} for 24 h. Control flowers were pulsed in distilled water for the same period of time. The treated and untreated flowers were then transferred to distilled water for vase life evaluation. All levels of AgNPs significantly prolonged the vase life compared with the control. The microbial growth was suppressed in vase solution, while relative fresh weight (RFW), relative water content (RWC) and chlorophyll content as well as membrane stability index (MSI) were maintained as a result of using AgNPs. In addition, stomatal conductance, ethylene production and malondialdehyde (MDA) were decreased in response to AgNPs application. H_2O_2 production was decreased while antioxidant enzyme activities (CAT, SOD and POX) were increased in AgNPs treated flowers relative to the control. Among AgNPs treatments, the most effective level was 50 mg L^{-1}. The results suggest that the biologically synthesized AgNPs could be used for improving the postharvest quality of cut roses as a promising eco-friendly, non toxic and novel alternative source to chemical and physical AgNPs sources or common chemicals used in preservative solutions in rose flowers.

1. Introduction

Rose (*Rosa hybrida* L.), belongs to family Rosaceae, is the major cut flower crop for exporting all over the world. However, it has a limited commercial value due to early dehydration (van Doorn, 1997). 'First Red' cultivar is one of the most important cut roses which exhibited a climacteric like peak of ethylene production (Chamani et al., 2005). The most important aim of advancing in postharvest science is to provide information for the horticultural industry to enable them to supply consumers with attractive and long-lived flowers (Scariot et al., 2014). It is well known that blockage of xylem vessels is the main reason of rose vase life reduction (van Meetern et al., 2001). Occlusion of cut flower stems may occur as a result of vase solution microorganisms (Loubaud and van Doorn, 2004; He et al., 2006), air embolism (van Ieperen, 2007) and the physiological wound healing (Williamson et al., 2002). It has been reported that the main cause of the blockage in roses is bacterial growth (Put and Clerkx, 2001; van Meetern et al., 2001; Bleeksma and van Doorn, 2003; Balestra et al., 2005). Vascular occlusion limits vase water supply to the flowers and leaves (da Silva, 2003; Loubaud and van Doorn, 2004; Lu et al., 2010a) and hence leads to water stress and consequently reducing vase life (van Doorn, 1997; Lu et al., 2010a). It has been established that water relations are a very important factor affecting postharvest quality and longevity of cut flowers (Lu et al., 2010a) and the main reason of short vase life after harvest is water stress (van Doorn, 1997). The microorganism proliferation in the vase solution also causes water relation interruption as a result of occlusion in the basal end of cut flower stem (Bleeksma and van Doorn, 2003; He et al., 2006; Liu et al., 2009a).

Investigators have mainly focused on how to maximize the vase life of cut flowers (Fanourakisa et al., 2013). Therefore, controlling and reducing microbial proliferation and consequently its negative effect is a critical factor for improving quality and longevity of cut roses. Addition of biocides in the vase water is a common practice to control bacterial growth and increase vase life of cut flowers (Liao et al., 2000; Hassan et al., 2004; Solgi et al., 2009; Hassan and Ali, 2014). Some biocides have shown environmental risks and health hazards such as silver nitrate and silver

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thiosulphate (Damunupola and Joyce, 2006) and hence researchers are working to develop efficient alternative biocides. Recently, environmentally and health-friendly production methods have become crucial for reaching the goal of more sustainable plant production.

Silver nano-particles (AgNPs) are one of the most widely studied nanomaterials, because of their unique optical, catalytic, sensing and antimicrobial properties (Sharma et al., 2014). In the floristry, various antimicrobial agents have been recommended including AgNPs as a promising antimicrobial agent with broad spectrum activity (Marambio-Jones and Hoek, 2010; Li et al., 2011). AgNPs are considered more effective as germicides than the other Ag forms because of their higher surface area to volume ratio (Jiang et al., 2004; Rai et al., 2009). AgNPs were applied to cut flowers and have been found to be effective as an antimicrobial agent (Liu et al., 2009a; Solgi et al., 2009; Lu et al., 2010b; Basiri et al., 2011), an ethylene inhibitor (Kim et al., 2005), and a regulator of stomatal aperture (Lu et al., 2010a). Prolonging vase life as a result of AgNPs application was accompanied with better maintenance of relative fresh weight, suppression of bacterial growth in the vase water and stem-end or vascular blockage reduction which increased the water uptake and maintained the turgidity of flowers (Solgi et al., 2009; Liu et al., 2009a, 2012; Rafi and Ramezanian, 2013). Kazemi and Ameri (2012) reported that AgNPs treatment inhibited the growth of microorganisms in the vase solution and decreased malondialdehyde (MDA) content and hence prolonged the vase life of gerbera. The positive effects of AgNPs were observed whether applied as pulse or vase solution treatment in rose, carnation and gerbera cut flowers (Liu et al., 2009b).

Further mechanisms have reported for AgNPs: AgNPs treatment extends the vase life of Movie Star roses by reducing transpiration and decreasing stomatal opening and hence improving water relations (Lu et al., 2010a). AgNPs treatment maintained membrane stability, retained chlorophyll content, retarded weight loss, improved solution uptake and prevented vase solution microbial proliferation without any evident side effect on cut flowers (Jowkar et al., 2013). Moreover, stem hydraulic conductance suppression (Lu et al., 2010a), transpiration and stomatal opening reduction (Rafi and Ramezanian, 2013) were demonstrated in response to AgNPs.

Although using AgNPs as a biocide has been reported, more information are still required on the possible physiological mechanisms of AgNPs in improving postharvest quality of cut flowers such as effects on ethylene, membrane stability, malondialdehyde content, antioxidant enzyme activity and reactive oxygen species. Moreover, the previous literature focused on the effects of chemical and physical sources of AgNPs on the postharvest quality of cut flowers. However, increasing concentration of nanosilver with varied physical and surface properties, could pose a threat to human and environmental health (Panyala et al., 2008). Hence, extracellular biological synthesis of AgNPs provides a promising eco-friendly, non toxic and simple alternative source to chemical and physical sources (Narayanan and Sakthivel, 2010; Fayaz et al., 2011). To the best of our knowledge, no information was available in the literature concerning the effect of biological source of AgNPs on postharvest quality of cut flowers. Therefore, this study was an attempt to investigate the possible effects of biologically synthesized AgNPs as a novel material on improving the postharvest quality of rose cut flowers cv. 'First Red'.

2. Materials and methods

2.1. Plant materials

Rosa hybrida cv. 'First Red' was used in this experiment. The flowers were obtained from a greenhouse of a commercial grower in Jeddah, Saudi Arabia during 2013 season and were immediately stood in buckets contained tap water and directly transported to the laboratory of Faculty of Science, Taif University. At the laboratory, flower stems were re-cut under distilled water to 50 cm length. All leaves were removed other than the upper two leaves which were retained on the stem.

2.2. AgNPs source

2.2.1. Biosynthesis of AgNPs

Biosynthesis of AgNPs was carried out using *Alcaligenes faecalis* bacteria as previously described (Bhainsa and D'Souza, 2006; El-Deeb et al., 2013). Briefly, bacteria were grown in a 500 mL Erlenmeyer flask that contained Mueller–Hinton Broth. The flasks were incubated for 21 h in a shaker set at 120 × *g* and 37 °C. After the incubation period, the culture was centrifuged at 10,000 × *g* and the supernatant used for the synthesis of AgNPs. Two test tubes, the first containing $AgNO_3$ (Sigma, USA, 99.9% pure) without the supernatant and the second containing the supernatant and $AgNO_3$ solution at a concentration of 1 mM were incubated at 30 °C. The biosynthesis of AgNPs was monitored by visual observation of the test-tubes for a change in the color of the culture medium from a clear to brown, and by measurement of the peak exhibited by AgNPs in the UV–vis spectra.

2.2.2. UV–vis spectroscopy analysis of AgNPs

Characterization of the synthesized particles was carried out according to the method previously described by Bhainsa and D'Souza (2006). The biosynthesized silver nanoparticles using the cell free supernatant were characterized by UV–vis spectroscopy (Perkin-Elmer, Lambda 25) instrument scanning in the range of 200–900 nm, at a resolution of 1 nm. All samples were prepared by centrifuging an aliquot of culture supernatant (1.5 mL) at 10,000 × *g* for 10 min and diluted 10-fold for all experiments involving measurement of UV–vis spectra. Cell free supernatant without addition of silver nitrate was used as a control throughout the experiment.

2.2.3. Transmission electron microscopy (TEM) analysis of AgNPs

Samples for transmission electron microscopy (TEM) analysis were prepared on carbon-coated copper TEM grids. Studies of size, and morphology of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 kV accelerating voltage (JTEM-1230, Japan, JEOL) with selected area electron diffraction (SAED). Finally, the obtained images were processed using the software ImageJ (Rasband, 1997).

2.3. AgNPs treatments

Rose stems were divided into four groups and individually pulsed with AgNPs at 0, 25, 50 and 100 mg L^{-1} for 24 h before transfer to distilled water in conical flasks. Control flowers were pulsed for the same period with distilled water. The mouths of conical flasks were covered with plastic film to minimize evaporation and prevent contamination (Li et al., 2012; Liu et al., 2012). AgNPs solutions were prepared at the beginning of the experiment and were not renewed during the experiment. Four treatments with five replicates were applied and each replicate consists of three flowers.

2.4. Vase life evaluation

Vase life of cut roses was evaluated at 20 ± 2 °C, 65 ± 5% RH and 12 h photoperiod with 10–12 μmol m^{-2} s^{-1} irradiance from cool-white fluorescence lamps. During the vase life period, the flowers were assessed daily. Vase life was defined as the period from the beginning of the experiment to the time when 50% of floret petal