The effects of different concentrations of Nano-Silver on elimination of Bacterial contaminations and phenolic exudation of Rose (Rosa hybrida L.) in vitro culture

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ABSTRACT: Rose is known as the first and the most important cutflowers in all over the world. One of the common methods of mass production of this plant is propagation through tissue culture. The main limiting factor of rose tissue culture is Bacterial contaminations and phenolic exudation in the establishment phase that significantly makes most of explants spoiled. Nano-Silver is able to control and stop the bacterial contamination. In this experiment Nano-Silver was added to medium with concentrations of (0, 50, 100 and 150 ppm) and in the other experiments explants were immersed in solutions of Nano-Silver with concentrations (0, 100, 200 and 400 ppm) in complete random design. The experiments were carried out with four replications. The results showed that the concentration of 100 ppm which is directly added to the medium can reduce Bacterial contamination and phenolic exudation rate. Concentration of 200 ppm for 20 minutes after surface sterilization was the best treatment of immersion to control bacterial contaminations. High concentrations of Nano-Silver make regeneration of explants more and more slower and in some cases lead to destroy explants. In general, Nano-Silver had no effects on fungal contamination.

Keywords: Bacterial contamination, In vitro condition, Phenolic exudation, Nano_Silver

INTRODUCTION

Rose is one of the most important commercial crops. It is generally propagated by vegetative methods like cutting, layering, budding and grafting (Pati et al., 2006). Roses have gained the title of the world’s favorite flower in part due to their vast diversity in plant habit and floral characteristics. They have been bred and selected to serve a number of niches including flowering landscape shrubs, formal garden specimens, cut flowers, blooming potted plants, and sources of perfume and vitamin C. (Anderson., 2006). In vitro cultures are now being used as tools for the study of various basic problems in plant sciences. It is now possible to propagate all plants of economic importance in large numbers through tissue culture (Shabbir et al., 2009). Successful tissue culture of all plants depends on the removal of exogenous and endogenous contaminating microorganisms. A major problem of in vitro plant culture techniques is chronic contamination by micro organisms (Sarmast et al., 2011). Fungi and bacteria are the most common microorganisms to be found on or in plant tissues (Abdi et al., 2008). The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology.
New applications of nanoparticles and nanomaterials are emerging rapidly. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics, Catalysis and micro electronics (Jain et al., 2009). Nano_Silver has antimicrobial effects at low concentrations. However, so far there is no report on using NS to eliminate microorganisms in tissue culture procedures (Abdi et al., 2008). Nano_Silver has shown to have antibacterial, antifungal and antiviral effects (Nomiya et al., 2004). Using 100 mg/l of NS solution after surface sterilization resulted in the highest percentage of disinfected explants (Abdi et al., 2008). Nano_Silver could be applied without adverse effects on plant growth and development (Sarmast et al., 2011). (Safavi et al., 2011) found that the NS had a good potential for removing of the bacterial contaminant in tobacco plant tissue culture procedures. The present study was conducted to evaluate the potential of NS to eliminate fungal and bacterial contaminants in Rose (Rosa hybrida L.).

MATERIALS AND METHODS

This study was performed in tissue culture laboratory, Faculty of Agriculture, Tarbiat Modares University. Nodular stem segments (1-1.5 cm) were used in these experiments as explants. Explants collected were first washed in running tap water for 30 min and then kept in household detergent + disinfectant (Teepol + Savlon) for fifteen minutes followed by second washing with distilled water to remove all the traces of detergent. Thereafter, they were soaked in 0.1 % (w/v) bendomyl (fungicide) for half hour then washed thoroughly with sterile distilled water. The explants were then treated with 5% (v/v) sodium hypochlorite for 5 minutes. After discarding sodium hypochlorite, the explants were washed three times with sterilized distilled water to remove all the traces of sodium hypochlorite. After surface sterilization, Nano_Silver treatment includes immersion in concentrations (0, 100, 200 and 400 ppm) At the same time and In other experiments Nano_Silver as a direct addition to the culture medium at a concentration of (0, 50, 100 and 150 PPM) In a completely randomized block design with four replications and Each replicate includes four units trial (4 Glass and Each glass contains 2 explants) In two separate experiments were performed. After sterilization, about 1 cm single node explants were cultured on a modified MS (Murray and Skoog 1962) medium containing salts, organic constituents, 30 g/l sucrose, 8 g/l agar. The pH of media was adjusted to 5.8 by 0.1 N HCl before autoclaving for 15 min at 121_C and 1.5 kg cm^-2 pressure. Cultures were kept under a 16 h photoperiod of 30 mm m^-2 s^-1 light intensity emitted by two cool white fluorescent lamps at 25 ± 3_C.

RESULTS AND DISCUSSION

The analysis of variance resulted that there are significant differences in contamination rate, both among treatments and between control and treatments. Results Showed that the use of Nano_Silver as an immersion and as added directly to the culture medium Significantly reduce bacterial contamination internal Compared with the control (Table I,II). So that the use of the concentration 100 ppm the method of direct addition to the culture medium reduced Bacterial contamination rate from 70 % to 17.5% and explant survival percentage was 87.50% of in this treatment. The highest percentage of explants survival (100%) Related to the control. However, because of high concentration explants regenerated, destroyed (Fig 1). the use of the Nano_Silver concentration of 200 ppm for 20 minutes method immersion after Surface Cause sterilized Bacterial contamination was reduced to the level of to 20%. and in this treatment had the highest regeneration explants. (Fig 2). In general, higher concentrations are although more effective in reducing contamination But are caused Destroyed and regeneration lack of explants. In these experiments it was found that treatment of Nano_Silver as a direct addition To the culture medium is better than immersion treatment. Nano_Silver was not effects on fungal contaminations phenolic secretions. the use of the 200 ppm Nano_Silver Concentration as immersion method Reduced bacterial contamination in Araucaria excels (Samast et al., 2011). (Abdi et al., 2008) Reported Which Nano_Silver surface disinfection reduced the bacterial contamination in Araucaria excels. The 200 mg L-1 nano-silver solution had successfully controlled bacterial and fungal contamination and had no undesired effects on regeneration of plantlets (Fakhhrfeshani et al., 2012)

Bacterial infections are considered to be a main problem in the process of plant micropropagation (Panyala et al., 2008). Because of the importance of contamination controlling for achieving the goals of tissue culture, many different methods with its own advantages and disadvantages have been suggested till now. Applying Sodium hypochlorite, Mercury Chloride, alcoholic and antibiotic solutions are the most common examples. However the environmental side effects of Mercury chloride (Mutter et al., 2005, Counter and Buchanan ,2004). Nano_Silver has shown to have antibacterial, antifungal and antiviral effects (Nomiya et al., 2004; Sondi and Salopek- Sondi, 2004). (Safavi et al., 2011) reported NS had a good potential for removing of the bacterial.
contaminants in plant tissue culture procedures. (Rostami and Shahsavar, 2009) showed using very low concentrations of Nano_Silver as a disinfecting agent in plants tissue culture media is recommended. Nano_Silver can be used as a replacement for toxic substances and chemical compounds for the disinfestation of the environment, prevention and cure of diseases and pests. Silver has been known by man for several thousand years as a no dangerous metal for human and environmental health. Although this metal in the form of large particles is a metal with low reaction, however, its minute particles having nano dimensions exhibit an interesting sterilizing effect. This astonishing effect is due to the vast surface area which minute nano particles have when they encounter the microorganisms and exhibit a strong antimicrobial effect by their action on the cellular metabolism and hindrance of respiration, growth and propagation of microorganisms The use of Nano_Silver solution does not hinder plant growth (Panyala et al., 2008). Studies have demonstrated that silver ions interact with sulfydryl (–SH) groups of proteins as well as with the bases of DNA leading either to the inhibition of respiratory processes or DNA unwinding. Inhibition of cell division and damage to bacterial cell envelopes are also recorded and interaction with hydrogen bonding processes has been demonstrated to occur (Davod et al., 2011). Our results showed that silver in nano size can similarly control the bacterial infection in tissue culture conditions. In general, using NS solution after surface sterilization had acceptable influence on the bacterial contaminants control without any adverse effects on growth characters in micropropagation of valerian. However, it was not effective in controlling the fungi in this experiment. the substitution of NS as a new generation of antimicrobial agent for tissue culture can be proposed, however in the other hand, according to the limited studies on the application methods, advantages and disadvantages of NS through the process of plant and animal tissue culture, it seems that much experimental trials are needed to understand the NS toxicity, its activity as a medium component or their effects on the other explant and pathogen species.

Table I: effects of Nano-silver on Contaminant and Regeneration percentage of rosa hybrida (Added to medium)

<table>
<thead>
<tr>
<th>Nano-silver(ppm)</th>
<th>Contaminant % Mean ± S.E.</th>
<th>F-value</th>
<th>Regeneration % Mean ± S.E.</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42.5 ± 2.5 a</td>
<td></td>
<td>100 ± 0 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.462**</td>
<td></td>
<td>88.400**</td>
</tr>
<tr>
<td>50</td>
<td>25 ± 2.88 b</td>
<td></td>
<td>95 ± 2.89 a</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>17.5 ± 2.5 bc</td>
<td></td>
<td>87.5 ± 2.5 b</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>12.5 ± 2.5 c</td>
<td></td>
<td>52.5 ± 2.5 c</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE, means with different letter within columns indicate significant differences by ANOVA followed by Duncan test at p<0.05

Table II: effects of Nano-silver on Contaminant and Regeneration percentage of rosa hybrida (Immersion)

<table>
<thead>
<tr>
<th>Contaminant % Mean ± S.E.</th>
<th>F-value</th>
<th>Regeneration % Mean ± S.E.</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55 ± 2.88 a</td>
<td>100 ± 0 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.667**</td>
<td></td>
<td>9.333**</td>
</tr>
<tr>
<td>100</td>
<td>35 ± 2.88 b</td>
<td>95 ± 2.88 a</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>20 ± 4.08 c</td>
<td>90 ± 5.77 a</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>10 ± 4.08 d</td>
<td>75 ± 2.88 b</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE, means with different letter within columns indicate significant differences by ANOVA followed by Duncan test at p<0.05
Figure 1. Effects of Nano-silver on Contaminant and Regeneration percentage of rosa hybrida (Added to Medium)

Figure 2. Effects of Nano-silver on Contaminant and Regeneration percentage of rosa hybrida (Immersion)

Figure 3.

REFERENCES


