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Development of Surface Sterilization Protocol for Nodal Explants Collected from Field Plants of Pomegranate

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Abstract

Pomegranate is an economically important fruit crop of the tropical and subtropical regions of the world. Hard wood cuttings are the traditional method for pomegranate propagation. However, there are a number of drawbacks to the conventional propagation approach, including poor success rates, delayed propagation and the inability to guarantee healthy, disease-free plants. The use of micropropagation technology as an alternative to traditional vegetative propagation method is growing in popularity nowadays. Hence, this study was aimed to develop a protocol for surface sterilization method to produce planting materials of the Kalipitiya hybrid variety. Initially nodal explants were washed with running tap water for 45 min then they were washed with liquid vim (teepol) solution for 15 min and followed by dipping in 0.06% fungicide for 45 min. Finally, streptomycin solution (100 mg/L) treatment was also used to explants for 20 min. After these steps, nodal segments were subjected to the following twelve treatments which included three different concentrations (5%, 10%, and 15%) of sodium hypochlorite (NaOCl) without or with 05 % (w/v) concentration of silver nitrate ($AgNO_3$) in the different exposure times (10 and 15 mins). In this experiment, the high fungal and less bacterial contaminations were observed after 10-14 days of culture. Among the treatments, contamination percentage was less in treatment containing 15% NaOCl with 0.05% $AgNO_3$ for 15 mins which has resulted 26.7% fungal contamination but bacterial contamination was not noted at two weeks of culture. It was noted that NaOCl with $AgNo_3$ showed less fungal and bacterial contaminations than without silver nitrate ($AgNo_3$). The lowest percentage (6.7%) of browning was recorded in the treatment containing 5% NaOCl with 0.05% $AgNO_3$ for 15 mins after two weeks. The survival rate of 73.3% was recorded after two weeks with 15% NaOCl and 0.05% $AgNO_3$ treatment for 15 mins which was the best surface sterilant concentrations for reducing the contamination and increasing the survival of the cultured explants of pomegranate. Sprouting of the survived nodal explants was started after five weeks of culture and high bud formation from nodal explants (53.3%) was achieved in the treatment 15% NaOCl and 0.05% $AgNO_3$ for 15 mins. Hence, it can be concluded that 15% NaOCl with 0.05% $AgNO_3$ for 5 mins procedure was the best treatment for surface sterilization of nodal explants Kalpitiya hybrid variety of pomegranate (*Punica granatum* L.). The results indicate the possibility of mass production of pomegranate using locally available planting material with tissue culture technology.

***Keywords**:* Micropropagation, Pomegranate, Silver nitrate, Sodium hypochlorite