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Improvement of production efficiency of pathogen-free stock *via* shoot tip culture of chrysanthemum

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Objectives

The main objective of the present study was to develop an improved procedure for the production of virus-free stocks of chrysanthemum.

Materials and Methods

Establishment of virus-free plantlets was done by integrating shoot tip culture and chemotherapy. Shoot tips (ca. 0.2-0.3mm) were cultured in Murashige and Skoog (MS) medium with kinetin, NAA, and 3% sucrose solidified with 0.02% Phytagel. Antiviral agents Ribavirin and Amantadine were added to the medium at 25, 35 and 50mg/L concentrations. Further multiplication of the cultures was tested using different combinations and concentrations of NAA, Kinetin, BA and TDZ. Optimal photosynthetic photon flux density (50, 100, 200 $m.m^{2}.s^{1}$), sucrose concentration (1-5%), and inoculation density (6, 12, 18 plants/vessel) in vitro were determined. Leafy node cuttings of chrysanthemum were cultured photoautotrophically or photomixotrophically, enriched (1500ppm $C0_2$) or non-enriched in vitro.

Results and Discussion

An efficient production procedure for virus-free chrysanthemum stocks was established. Virus-free plantlets were obtained from shoot tip culture. The highest survival rate was achieved in shoot tip culture alone (80%) while relatively lower survival was obtained in treatments with Ribavirin or Amantadine. Multiple shoot formation was promoted in medium with 2mg/L kinetin and 0.02mg/L NAA. Further propagation was also possible using the same PGR combination. Optimal growth of chrysanthemum was achieved in cultures under 100 $m.m^{2}.s^{1}$ PPFD, 3.0% sucrose, and six explant/vessel density. Single leafy node cuttings of chrysanthemum showed better growth than non-leafy explants. Photoautotropy greatly improved the growth of chrysanthemum in vitro as compared to heterotrophic culture. Photoautotropically grown plantlets increased in shoot length and dry weight more than 70 and 50%, respectively. In contrast to heterotropic culture, plantlets from photoautotrophic culture showed normal stomatal structures. TKS (commercial peat moss) in combination with either perlite or vermiculite (1:1 v/v) could be used as substrate for further multiplication in the automatic culture chamber.

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