Abstract

In the research and development of disease-free *Citrus* spp. mother plant production for growing highland areas, the tissue culture process of three species of citrus including grapefruit, kumquat and lemon were investigated. The young branches of *Citrus* spp. were collected from Pongnoi Research Units, Royal Agricultural Station Pang Da, Chiang Mai province. The sample of these citrus leaves are tested the citrus greening disease by using PCR method and tristeza disease by using RT-PCR method in order to identify that the citrus plant is disease-free. For surface sterilization procedure of field-grown citrus, shoot or nodal section were sterilized by immersed in 0.5 % carbendazim solution for 20 min, dipped in 70% (v/v) alcohol for 30 sec, immersed in 10% (v/v) clorox solution and 0.1% (v/v) Tween-20 solution for 10 min, and then immersed in 5% (v/v) Plant Preservative Mixture (PPM^{TM}) solution for 20 min. These procedure showed the percentage of survival in grapefruit, kumquat and lemon at 50%, 60% and 60% respectively.

In the *in vitro* cultivation, the explants were cultured on nine semi-solid medium such as MS, WPM and LS medium which different strength of medium (½ and full) and concentration of sucrose (15 and 30 g/L). In the results, ½ LS media supplemented with 30 g/L of sucrose showed positive effect on shoot induction. From this medium, the shoot induction rate of grapefruit, kumquat and lemon was 70%, 100% and 70% respectively after 4 weeks of cultivation. Moreover, the cultivation on all those medium supplemented with 30 g/L of sucrose induced greater shoot induction rate than medium supplemented with 15 g/L of sucrose. For 12 weeks of cultivation, those medium did not certainly effect on shoot multiplication. However, shoots on ½ LS medium supplement with 30 g/L sucrose were produced healthy shoots and greener leaves.

The experiments were carried out to determine the effect of exogenous cytokinin on *in vitro* shoot induction and multiplication of *Citrus* spp. In the *in vitro* culture of kumquat, the suitable medium were ½ LS medium containing with 30 g/L of sucrose and supplemented with 3 mg/L of BAP. From this treatment, the shoot induction rate was 100% after 4 weeks and it was achieved the highest number of

healthy leaves after 12 weeks of cultivation. In case of lemon, the suitable medium were ½ LS medium containing with 30 g/L of sucrose and supplemented with 2 or 3 mg/L of BAP. Under these formulas, the shoot induction rate were 90% after 4 weeks and they were achieved high number of healthy greener leaves after 12 weeks of cultivation. When compared to the medium with absent of BAP (control treatment), the *in vitro* growth of citrus shoots on medium supplemented with BAP produced higher shoot induction rate, enhanced shoot elongation and healthy leaves multiplication. However all treatment could not induce shoot multiplication.