

Micropagation of citrus by using olive culture techniques

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This investigation was carried out at the Tissue Culture laboratory, Horticulture Department, Faculty of Agriculture, Moshtohor, Zagazig University during the period 1997 to 1999. The aim was to find out the best possibilities of culturing ovule and anther of both Washington navel orange and Red Khalili orange explants for regenerating haploid plantlets either directly or indirectly. Resultant callus and plantlets were subjected to different doses of gamma rays irradiation to enhance genetical variabilities, which valuable for the breeder to select the best cytological combinations as well as proliferating and rooting of non and irradiated plantlets. Also, RAPD fingerprint was done to assure the genetical category of the resulted plantlets. On this concern, mother bearing Washington navel orange trees and Red Khalili orange trees were selected and the flowers were taken just before opening. Then sterilized by using 10% Clorox with two DROPs of Tween-20 for 15 minutes and washed 3 times then cultured on different nutrient medium types. Testing of cold pre-treatment, flower size, medium strength, additives, different BAP concentrations, and irradiation was carried out during the establishment stage. In-addition, different cytokinin types, and different concentrations of BAP were studied during proliferation stage. Moreover, medium strength, GA₃, medium state, and auxin type with different concentrations were evaluated during rooting stage. Besides, bulked sergeant analysis was used to analyze DNA extracts with four random primers with RAPD-PCR technique for Red Khalili citrus orange tree Red Khalili citrus in tissue culture (callus, plantlet produced from callus). Four random primers were included i.e.: B5, C7, D20 and E7. The obtained results can be summarized as follow: I- Establishment stage: 1-Ovules surpassed anthers explants in all parameters under study (necrosis, explant development, callus production, and direct regeneration). 2-Washington Navel orange ovules showed better performance than Red Khalili orange as necrosis was low, while other parameters were superior. 3-Murashige and Tucker medium was more preferable than both Murashige and Skoog and modified MS medium in increasing explant development while modified MS was suitable for callus production. 4- Storing ovules in the refrigerator (5°C) for 7 days before culturing succeeded in reducing necrosis and increasing explant development, direct regeneration and greening. However, callus production increased by using room temperature. 5-Large flower size encouraged ovule development and maximized direct regeneration as well as reduced necrosis while the reverse was true for callus production. 6-One-fourth and one-eighth medium strengths succeeded in reducing necrosis and callus production while increased ovule development, direct regeneration, shootlength, and greening. 7-Supplementation the culture medium with 200mg/L of either malt extract or casein hydrolysate encouraged maximum explant development and plantlets regeneration. 8-The lower concentration of BAP (0.5mg/L) increased the number of regenerated plantlets, growth, and greening while reduced necrosis, browning and callus production. II-Effect of irradiation treatments: 1-Higher doses of gamma radiation had an adverse effect on either callus or regenerated plantlets. However, non-radiated (control) or 2.0Krad dose increased number of leaves, number of plantlets produced and greening while decreased necrosis. III-Proliferation stage: 1- Kinetin surpassed BAP in improving growth and greening as well as reducing necrosis while, BAP maximized proliferation.