Using of biotechnology in in vitro propagation and development of some deciduous frutt rootstocks

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This investigation was conducted in the Tissue Culture Unit, Horticulture Dept. Fac. of Agric. Moshtohor, Benha university during the period from 2004 to 2007 to study the best way for maximizing protoplast yield and increasing number of viability protoplast as well as enhancing protoplast development of pear rootstocks (Pyrus betulaefolia and Pyrus communis) .The following procedures were studied : I-Pre-protoplast isolation:In vivo leaves of both pear rootstocks (betulaefolia and communis pear) were subjected to levels evaluation phenolic compound during the year round. Then sterilized the leaves and treated with different anti-oxidant treatments.New leaves from in vitro and sterilized in vivo pear rootstocks were divided into small sections and soaked in different plasmolysis treatments in combination treats with different protoplast sources (in vitro and in viva) . II-Protoplasts isolation:Small sections from in vitro were treated by different enzyme combination (mixtures) in combination with protoplast sources. Then different digestive enzyme media and osmotic pressure factor .as well as different incubation periods and different shaking speeds and periods. last purification:Different pore sizes, as well as centrifugation speeds and periods were used during purification stage , IV- Protoplast development:Different medium types and different protoplasts densities and hormonal balances were employed to find out the best procedures for protoplast development.The obtained results can be summarized as follow : 5.1. Pyrus betulaefolia : 5.1.a. Pre-protoplast isolation :1-It proved that March sample showed the lowest phenolic compounds either totals, free or conjugated contents which assured that the best time for taking explants.2-It is clear that using of anti-oxidant solution (0.1% ascorbic acid + 0.15 citric acid) was effective in reducing phenolic compounds and reducing their harmful effect.3-It is recommended to use plasmolysis treatment 9 g/100 ml mannitol for half hour then 13 g/100 ml mannitol for furthermore half hour to enhance successful protoplast isolation.4-In vitro protoplast source was surpassed in vivo protoplast source as it maximize protoplast yield. 5.1. b.Protoplast isolation :5-Immersing in vitro explants in enzyme solution supplemented (1.5% cellulase + 1.5% Macerozyme + 0.5% pectinase) was more effective in improving the protoplast yield. 6-The highest protoplast yield was obtained when CPW digestive medium was used.7-Addition of mannitol to the digestive enzyme medium was preferred in increasing the protoplast yield.8-Incubation of the explants for 20 hours enhance the highest protoplast yield.9-Shaking the incubated explants in enzyme solution at 75rpm for 30 min. encourage the best protoplast yield. 5.1.c. Purification:10-Using sieve at pore size 251.tm encourage the highest number of viable protoplasts.11-Centrifugation protoplast with speed rate 1000 rpm for 7.5min. inducted the greatest number of viable protoplasts. 5.1.d. Protoplast development:12-Murashig & Skoog medium was superior for the best protoplast development than both Gamborg and KM medium types.13-Using protoplast at density rate 2 X 105 in the culture medium enhanced an improvement in protoplast development.14- Supplementation of the culture medium with the combination of 1.00 mg/L NAA and 0.3mg/L BAP induced the highest protoplast development