

Effect of irradiation and other treatments on genetic behaviour of some fruit trees in vitro

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This investigation was carried out at the Tissue Culture Unit, Horticulture Department, Faculty of Agriculture, Moshtohor, Zagazig University during the period 2002 to 2005. Explants (shoot tip and one node cuttings) for plum rootstock (Marianna 2624) was taken in April and subjected to running water for 15 minutes then sterilized by using 10% Clorox (commercial bleach) with two DROPs of Tween-20 for 10 minutes and washed 3 times with sterilized distilled water for 5 minutes. The explants were cultured on different medium types and explant types, testing of different antioxidant treatments and cold pretreatment as well as different additives. Beside different mutagens (colchicine, sodium azide and gamma rays) were evaluated for both plum rootstock (Marianna 2624) and pear rootstock (*P. betulaefolia*). Cytokinin types and 2-iP concentration for Mariana, and BAP in *P. betulaefolia*. However, medium strength, GA3 concentration, auxin type and IBA concentration were evaluated during rooting stage. Different levels of salinity by adding NaCl, CaCl₂ and combination between NaCl and CaCl₂ to the culture medium. Furthermore, drought tolerance was studied by using different concentrations of agar as solidifying agent as well as mannitol and polyethylene glycol as osmosis inducers. Tissue culture parameters, chemical analysis, and leaf mineral content under salinity treatments were also studied. In addition, bulked seedling analysis was used to analyze DNA with RAPD-PCR technique for plum rootstock. The obtained results can be summarized as follows:

5.1- Establishment stage:

- 1- Murashige and Skoog showed the most superior medium for decreasing necrosis and browning and increasing explant development and greening of Mariana 2624 plum rootstock.
- 2- Shoot tip surpassed one-nodal cutting in reducing necrosis and browning while increasing explant development.
- 3- Accumulation of phenolic compounds which caused oxidation were decreased to the lower most level when combined treatment of anti-oxidant solution and PVP was used and in turn all parameters under study are improved.
- 4- Cold pretreatment of the explant for 3 days at 5°C encouraged the best responses of the explant.
- 5- Addition of either adenine sulphate or yeast extract was effective in improving of both greening and growth development and reduced necrosis and browning.
- 6- Gamma rays at low dose improved all tissue culture parameters (necrosis, callus production, proliferation, growth and greening).
- 7- Irradiation with gamma rays enhanced the highest percentages of variabilities among treated plantlets specially when 2.0 Kr dose was used for plum rootstock (Marianna 2624) while 4.0 Kr was effective in *P. betulaefolia* pear rootstock.
- 8- Increasing irradiation dose up to 2.0 Kr maximized percentage of plantlets with large leaf area and verified plantlets while the percentage of plantlets with dwarfed stem were increased when 3 Kr dose was used for plum rootstock. However, increasing irradiation dose up to 4.0 Kr maximized percentage of plantlets with large area in *P. betulaefolia* pear rootstock.
- 9- Irradiation was the best mutagen that induced the highest variabilities followed with sodium azide then colchicine in both Marianna 2624 plum rootstock and *P. betulaefolia* pear rootstock.

5.2. Proliferation stage:

- 1- 2-iP was superior in enhancing proliferation while kinetin was effective in improving growth and greening parameters of plum rootstock while BAP was the best cytokinin in the case of pear rootstock.
- 2- Using 2.0 mg/L 2-iP maximized proliferation and greening parameters of plum rootstock. However, 2.0 mg/L of BAP is recommended for pear rootstock.

5.3. Rooting stage:

- 5.3.1. Shoot elongation: 1- Shoot elongation and greening of both plum rootstock and pear rootstock were increased by using one half medium strength.
- 2- Adding 0.3 mg/L of

GA3 to the culture medium enhanced a noticeable increase in shoot elongation and greening of plum rootstock while using of 2.0 mg/L GA3 was preferred for pear rootstock.

5.3.2. Root formation:

- 1-Indole-3-butyric acid was the most effective auxin as it enhanced rooting of both plum rootstock (Marianna 2624) and pear rootstock (*P. betulaefolia*).
- 2-Addition of low concentration (1.0 mg/L) IBA maximized rooting of plum rootstock (Marianna 2624) while 2.0 mg/L of IBA was the best for pear rootstock.

5.4. Stress tolerance:

5.4.a. Drought tolerance:

- 1-Increasing mannitol concentrations had an adverse effect on growth, number of shoots while gave the best results with shoot thickness, No. of roots of plum rootstock (Marianna 2624) while shoot thickness and root length in pear rootstock (*P. betulaefolia*) were improved.
- 2-Chlorophyll-B, total indoles and total phenols were maximized by increasing mannitol concentration up to 100 gm/L for plum rootstock and pear rootstock while reducing sugar was increased in pear rootstock.
- 3-Growth parameters i.e. growth, number of shoots, root length, shoot length, number of roots and shoot length responded positively with the lowest PEG concentration while shoot thickness tolerated higher concentration of PEG in both plum rootstock and pear rootstock.
- 4-Chlorophyll-B, reducing sugar in both plum rootstock and pear rootstock were increasing of by using the higher concentration of PEG.
- 5-Drought resulted from different concentrations of agar was greatly tolerated as appeared from the survival level of all tissue cultural parameters.
- 6-Culturing on medium supplemented with 12 g/L agar enhanced reducing sugars of plum rootstock and pear rootstock.

5.4.b. Salinity tolerance:

- 1-The adverse effect of salinity on tissue culture parameters of plum rootstock and pear rootstock appeared greatly by higher concentrations of NaCl followed with CaCl₂ and their combination (NaCl and CaCl₂).
- 2-Increasing NaCl concentration more than 1000 ppm induced a bad effect on all parameters under study up to the lethal concentration caused by 2000 ppm which caused almost death of the *In vitro* plantlets. However, the effect of CaCl₂ with different levels was less toxic to the plantlets compared with the similar levels NaCl. Meanwhile, combination of NaCl + CaCl₂ reduced the toxicity of NaCl.
- 3-Vegetative growth parameters were greatly affected by different salinity agents (NaCl & CaCl₂) than rooting parameters.
- 4-Increasing salinity resulted in increasing total indoles, total phenols, and proline.

5.5. Acclimatization stage: Using of combination from the peat moss and sand (3:1 v/v) maximized acclimatized plum and pear rootstock plantlets.

5.6. DNA finger print: RAPD analysis detected genetical differences in plum rootstock occurred from irradiation treatments with different doses of gamma rays.

CONCLUSION Culturing of Mariana 2624 pretreated with cold treatment and antioxidant solution. Shoot tip on Murashige and Skoog medium supplemented with P.V.P and adenine sulphate is preferred for establishment stage. Also using of irradiation is effective in inducing mutations and variations in both Marianna 2624 and *P. betulaefolia*. In addition, using the previous medium with adding 2.0 mg/L 2-iP maximized proliferation. Moreover, half MS medium strength supplemented with either 0.3 or 2.0 mg/L GA3 was valuable for shoot elongation of either Mariana 2624 or *betulaefolia* pear respectively. However, addition of either 1.0 or 2.0 mg/L IBA was recommended for Marianna 2624 and *betulaefolia* pear respectively. Meanwhile, Marianna 2624 is less tolerant for drought and salinity than *betulaefolia* pear. Also, most tissue culture and chemical parameters were adversely affected by increasing drought and salinity levels in the medium. On the other hand, total indoles and phenols as well as proline contents had increased with increasing salinity. In the meantime, finger print showed a genetical variabilities occurred in Marianna 2624 as a result of mutagen treatments specially those irradiated with 2 Kr. RAPD analysis detected genetical differences occurred from irradiation treatments with different doses of gamma rays.