

## REVIEW OF FACTORS INFLUENCING THE MICROPROPAGATION OF FRUIT TREES

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### Abstract

*Explant type and size, surface sterilization, phenol exudation and control, culture medium, media (strength, kind, and condition), carbon supply and additions, light and temperature, plant growth regulators, pH, and agricultural media all influenced fruit tree micropropagation. Most plant species have virus-free in vitro culture with 0.5-mm explants like shoot tip culture. Surface sterilization was greatest for explants treated with (10-30%) clorox solution and two drops of tween 20 for 15-20 min. Explant type (shoot tip, axillary bud, one node cutting, leaf disc, flower buds and seeds). Antioxidants (150 mg LG1 citric acid+100 mg LG<sup>1</sup> ascorbic acid) for 2-24 h before culture are the best strategy to reduce phenol exudation. Several species developed explants and induced roots by lowering the medium pH to 5.7. Several explant species were cultured at 25-28EC with 16 h of artificial light and 8 h darkness. Sugar, the most common carbon and energy source for micropropagation, promotes growth. Full medium grew most explants. Date palm, mango, apple, and other culture explants release phenolic chemicals that thrive in liquid media during in vitro establishment and roots. Little auxins, cytokinins, and gibberellins are required. Most fruit micropropagation media uses BAP or <sup>2</sup>ip. NAA and IAB are the least stable in vitro rooting mediums. Auxin and cytokinins outperform gibberellins. Vermiculite: sand: peatmoss acclimatizes most plantlets (1:1:1).*

**Key words:** Micropropagation, fruit tree, explant type, medium, plant growth regulator.

### INTRODUCTION

Micropropagation from tissue culture produces many plants from little portions of the mother plant in a short time and small area. Micropropagation is a new method for mass multiplication of

commercial plant species like date palm<sup>1</sup>. Micropropagation provides bulk propagation and clean planting material. Micropropagation produces a sickness that banana propagation may cure<sup>2</sup>. Tissue culture micropropagation aids plant cloning. Tissue culture may generate a large number of genetically homogenous palms similar to other plants and typical fruit after 4 years after planting, date palm plants free of disease, and virtually 100% survival rate compared with vegetative shoots owing to their robust root systems<sup>1</sup>. Since most fruit plants from field sources are difficult to regulate for bacteria and fungus, surface sterilization is the most crucial stage in in vitro explant preparation<sup>3</sup>. In vitro date palm micropropagation depends on the number of leaves before transplantation in the greenhouse<sup>4</sup>. This review investigated fruit tree micropropagation variables.

**Mother plant (explants type, genotype and explants age):** MS medium supplemented with 6.0 mg LG1 BA and 0.1 mg LG1 NAA on leaf sections of pyrus communism produced the most shoots and best shoot regeneration.<sup>5</sup>Nodal shoot segment micropropagation of mature citrus limon plants<sup>6</sup>. Somatic embryo-derived young and aseptic plantlets did not cause phenolic exudation and contamination of guava c.v. "Banaras"<sup>7</sup>.

Shoot tips of three fig cultivars (Aboudi, Gizey, and Sultany) were cultivated separately on Murashige and Skoog (MS) media supplemented with 0.5 mg LG1 6-Benzylaminopurine to increase explant growth and reduce necrosis and browning<sup>8</sup>. Emam<sup>9</sup> discovered that MS medium generated pear rootstock shoot tips better than one-nodal cutting. Strosse et al.<sup>10</sup> found that banana shoot tips cultivated on MS media during establishment developed. Sutherland et al.<sup>11</sup> showed that banana shoot tips explants produced more homogeneous and disease-free plantlets. Baiea<sup>12</sup> generated two peach rootstock leaf disc explants on MS media (Fig. 1).

Axillary buds produced the most *Jatropha curcas* shoots (5.35/responding explant)<sup>13</sup>. Identical shoot tip, stem, and axillary bud explants micropropagated *Jatropha Curcas*<sup>14</sup>. Sumalatha<sup>15</sup> found that banana micropropagation worked with shoot tip explants. Jojoba clone from shoot tip and nodal segments had greatest shoot proliferation<sup>16</sup>.

Nodal segments were better explants for callus induction than citrus leaf and root segments<sup>17</sup>. Guava c.v. "Banarasi" was micropropagated using somatic embryo-derived young and aseptic plantlets as explants<sup>7</sup>.

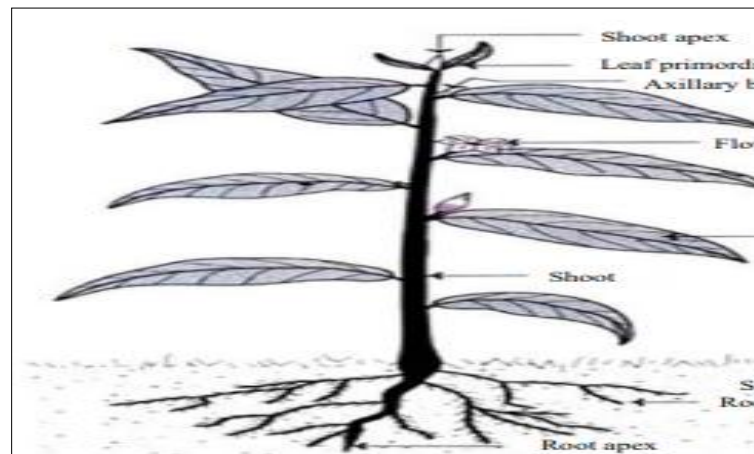
On WPM medium, the c.v. Golden delicious control and genotype 4566 had the longest roots.

**Surface sterilization of explants:** Banana plants were surface disinfected with 0.1% HgCl<sub>2</sub> solution containing tween 20 for 5 min and then rinsed with sterile, deionized water<sup>15</sup>. When sterilizing branch tip and nodal bud with sodium hypochlorite Clorox (NaOCl) for 20 min, pomegranate explants survived<sup>19</sup>. Axial pomegranate bud and segment sterilization with NaOCl and Na

methiolate for 20 min yielded 65% explant survival. <sup>20</sup>NaOCl (0.75%) had the highest Jack fruit survival rate (46.66%), followed by NaOCl (1.0%) with 26.66% and NaOCl (1.25%) with 6.66%.

In another investigation on mango in vitro growth, 10% sodium hypochlorite "Clorox" (5.25% NaOCl) and 0.05% mercuric chloride (HgCl<sub>2</sub>) for dipping periods of 7 and 10 min yielded the best survival rates and lowest contamination rates (Fig. 2). Surface sterilization with 10% sodium hypochlorite produced excellent explants with little rootstock visible contamination Mariana (*Prunus mariana*).

Surface sterilization using 10% sodium hypochlorite and surfactant droplets for 10 min effectively sterilized grape tissue (*Vitis vinifera*). Three dosages, 0.05, 0.1, and 0.2% of aqueous sodium hypochlorite or mercuric chloride solution, respectively.



**Fig. 1: Different types of explant**

10 min surface sterilized. 0.1-0.2% mercuric chloride enhanced aseptic culture establishment but impeded break owing to *Bambusa tulda* explant toxicity. Grape root stocks were sterilized by immersing explants in 15% sodium hypochlorite for 15 minutes.

**Phenol exudation and its control:** Activated charcoal in the culture medium

stimulated woody plant proliferation but absorbed growth regulators and lowered pH. Apple root stock shoots emit less phenolic chemicals after dark treatment for a few days. In another in vitro cultivation of pomegranate c.v. Mridula, nodal segments with sterile wax reduced phenol exudation and increased explant percentage<sup>28</sup>. Ascorbic acid reduced apple establishment oxidation best<sup>18</sup>.

Other techniques include maintaining explants in the dark after culture, adding antioxidants such citric acid (100 mg LG1) and ascorbic acid (150 mg LG1) for 30 min, and adding activated charcoal (3 g LG1). These treatments minimize olive explant browning and phenolic compound synthesis.

**Medium (type, strength and state):**

Coffee plantlets thrive in full-strength Murashige and Skoog medium.

MS media with Fe-EDDHA instead of Fe-EDTA maximized apple proliferation and shoot growth. Woody plant medium (WPM) prevents fungal, bacterium, and phenolic oxidation during establishment<sup>18</sup>.

MS medium with lower ammonium and potassium nitrates generated the most Citrus Limon shoots.<sup>6</sup> 3/4 medium-strength jojoba clones developed best<sup>16</sup>. Tissue-cultured plantlet acclimatization worked well. Subculturing rooted shoots in MS salts solution and increasing light intensity boosted plantlet photosynthesis and turned date palm heterotrophic to autotrophic.

Mazri discovered the highest date palm survival (70-86%) on half medium strength after 1 month pre-acclimation. Direct greenhouse transfer produced 12-28%. Half-strength MS medium generated the most almond plantlets, c.v. Nonpareil, with the longest roots.

Pomegranate<sup>19</sup> micro shoots rooted best on half-strength MS basal medium with 10.33 roots/shoot. Greening and explant development per micro flower bud were better on full and one-half medium strength compared to one-quarter medium strength of pear "Le Conte" flower buds.

In vitro root formation of date palm c.v. Boufeggous plantlets depends on medium before acclimatization. Liquid medium is best for transplanting plantlets to Barhee date palm greenhouses (solid or semisolid media). A liquid medium increased shoot quality before transporting plantlets to the date palm c.v. Najda greenhouse. Date palm c.v. Barhee plantlets on liquid Ms medium survived best in vitro. Apple plantlets grown in liquid organized media with 25 µM IBA rooted best on all genotypes, including M. domestic c.v. Golden delicious<sup>18</sup>.

**Supporting media:** Pineapple plantlets produced the most and longest shoots on MS medium with 2 g LG1 gelrite. During multiplication, 7 g LG1 agar outperformed gelrite<sup>40</sup>. Similar to banana (musa spp) c.v. Grand naine plantlets, decreasing agar from 0.8 to 0.4% improves in vitro root and shoot parameters<sup>41</sup>. Banana plants were micropropagated using gelling specialized agar at 5-8 g LG1 in the medium<sup>15</sup>.

**Carbon source and additive:** Papaya shoot tip explants thrived on 30 g LG1 sucrose media. Banana (Musa spp) c.v. Grand naine plantlets rooted best in 30 g LG1 sucrose.

MS medium with 20 g LG1 fructose increased shoot proliferation of two fig cultivars (Sultany and Aboudi) compared to explants and media without sucrose.

Jojoba plant shoot length and leaf number

rose with Murashige and Skoog medium fructose and glucose. Shot<sup>16</sup> had sucrose. Fructose produced the most dry weight compared to glucose, sucrose, and maltose, while 30-60 g LG1 sucrose improved date palm explant development and shoot growth.

Half-strength treatment boosted proliferation and shoot number.

Adenine sulphate (80 mg LG1) or yeast extract (300 mg LG1) boosted callus development and reduced Nemagaurd and Okinawa root stock necrosis and browning. Olive medium (OM) with 50 cc LG1 coconut water generated the most explants per month, 3.4. Banana pants micropropagation performed best with 30-40 g LG1 sucrose<sup>15</sup>.

**Light, temperature conditions and pH requirements:** In vitro date palm c.v. Barhi plantlets cultivated under 2000 lux increased most metrics under research, including root length, root number, shoot length, and greening, compared to other light intensities. When cultures were exposed to 3000 lux, shoot thickness increased<sup>4</sup>. Date palm in vitro plantlets photosynthetic better with high light intensity.

Tasted in vitro plantlets at high light intensity (4000-12000 lux) and temperature (26-36EC) may also scorch leaves and wilt<sup>45</sup>. In vitro root production of date palm c.v. Barhi showed greatest shoot thickness at 3000 lux. When cultures were exposed to 2000 lux, root number, root length, leaf number, and greening were reported.

The same finding indicated that various parameters impacting the in vitro root production of most fruit trees, including light intensity (4.000-12000 lux) and temperature (26-36EC), which may promote leaf charring and plantlet growth, produced the greatest results for root number and length. Light intensity

increased photosynthesis during date palm acclimatization.

In vitro banana plantlets were lit for 12-16 h<sup>15</sup> at 28EC. Cold explant preparation for 3 days at 5EC<sup>12</sup> worked best for peach root stocks.

Ahmed et al. discovered that Banana plantlets c.v. Grand naine root best and quickest at PH 5.5 during medium preparation.

**Plant growth regulator:** After 30 days, Jatropha Curcas<sup>14</sup> produced the most shoots with BA (0.5 mg LG1) and IBA (0.25 mg LG1). As indicated in Fig. 7a-c, shoot bud differentiation required cultural media supplementation with 0.5 mg LG1 BA and IBA. Just 1.0 mg LG1 IBA elongated Jatropha Curcas shoots. Similar to 6.00 mg LG1 IBA, 3.0 g LG1 IBA increased coffee plantlet multiplication.

In another investigation, 3 mg LG1 BA and 0.5 mg LG1 NAA in the culture media stimulated citrus plant model segment regeneration (71.89%). Modal segment-derived callus cultivated on Ms medium with 0.5 mg LG1 NAA and 3 mg LG1 BA17 had the highest rooting rate (71%). The growth medium with 2.0 mg LG1 BA and 0.0, 0.5, or 1.0 IBA mg LG1 produced the most Nemagaurd or Okinawa rootstock plantlets<sup>12</sup>.

Almond plantlets c.v. Nonpareil multiplied best in Ms medium with 1.0 mg LG1 BA. 8.0 mg LG1 indole acetic acid increased root quantity and length/plantlets.

Apple plantlets were best cultured in vitro with 1.0 mg LG1 BAP and 1.0 mg IBA. The culture media supplemented with 1.0 and 1.5 mg LG1 BAP and 1.0 mg LG1 IBA increased the survival rate of MM 106 (96.7%) and Anna apple plantlets (93.3%). A dwarfing cherry root stock produced 5.37 shoots/explant in Ms medium with 0.5 mg LG1 BAP. The Olive medium

supplemented with 2.22 mg LG1 BAP produced the most explants after 30 days, averaging 3.4. OM with 3 g LG1 IBA has 85% rooting. The highly cytokinin-rich best joboba shot multiplication. Auxins or cytokinin<sup>16</sup> produced callus best.

Pomegranate shoot development needs cytokinin<sup>6</sup> benzyl aminopyrine (BAP). MS medium with 2.0 mg LG1 IBA19 is needed for in vitro rooting. Quorin and lepoivre medium with BA 0.4 mg LG1 and IBA 0.05 mg LG1 20 produced the most shoots.

0.5 mg LG1 IBA produced the most roots (93.33%) and longest roots (3.29 cm). Cherry laurel prunus laurocerasus L-rich MS medium also caused high-frequency shoot elongation.

Shoot proliferation was significant in sweet cherry cultivar "Lapins" basal medium with low BAP. BAP enhanced shoot elongation. 2.1 mg LG1 BAP in the medium boosted olive shoot proliferation. GA<sup>3</sup> in culture media may reduce BAP levels during proliferation. MS growth medium with 1.5 mg LG1 BAP generated the most Jack fruit shoots (4.66) per proliferating explant.

**Agricultural media:** According to Fig. 8, coffee plantlets acclimatized in a combination of sand and peat moss (1:1) had the best survival rates, plant height, thickness, and leaves/plant. Date palm c.v. Barhee plantlets acclimatized well in containers with 25% vermiculite, 50% peat moss, and 25% vermiculite. Rooted shoots (plantlets) were transplanted in tiny peat moss and perlite (2:1) pots and put in plastic tunnels or greenhouses. After three months in larger Maktom c.v. Date palm pots, 85% survived.

When transplanted to pots with vermiculite: Perlite 3: 1 (V/V) substrate, olive plantlets had the best survival rate

(95%) following acclimatization. After three months, date palm c.v. Maktom plantlets transplanted in tiny pots with a 2:1 peat moss-perlite combination and put in greenhouse plastic tunnels had the best survival rate (85%).

## CONCLUSION

This research found that 0.5-mm explants like shoot tip culture are virus-free for most plant species. The best way to control phenol exudation is to add antioxidants before culture. Adjusting the medium pH to 5.7 improved explant development and root induction in numerous species. Sugar provides energy and carbon for growth and development during micropropagation. Most explant species thrived in full medium. Auxins, cytokinins, and gibberellins are needed in little amounts.

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