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REVIEW ARTICLE

Micropropagation of fruit crops: A review

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Abstract

In vitro culture has the greatest commercial value in the extremely quick creation of clonal plants compared to conventional techniques of propagation. It has also proven to be of tremendous practical utility as assistance to the development and maintenance of disease-free planting material, germplasm conservation, and supplements to the regular methods of plant progress, such as plant genetic engineering. The discovery and implementation of current tissue culture techniques are thought to be paving the way for a second green revolution. Here, the standardization of techniques for the *in vitro* clonal propagation of fruit crops is highlighted. In addition, we summarize the effects of several surface sterilizing agents, plant growth regulators (PGRs), and anti-phenolic chemicals on culture establishment, shoot proliferation, *in vitro* rooting, hardening and economics of *in vitro* generation of true-to-type plants.

Keywords

Explant, surface sterilization, establishment of culture, shoot multiplication, rooting hardening, economics

Introduction

The majority of clonal planting material is commercially propagated through traditional methods like mound layering, which is totally seasonal, has a low multiplication rate, necessitates a lot of space and labour, and produces planting material that is not profitable for farmers. However, *in vitro* propagation techniques provide an efficient and effective method of creating quality planting material that is true to type in less time and space (1). For fruit breeders, micropropagation makes it possible to quickly create new varieties, breeding lines, or variants of existing ones. In order for transgenic lines to be successfully regenerated, this is a critical step in the process (2). Micropropagation in fruit plants can produce offspring year-round, disease-free plant material with less work and lower expenses, and it can proliferate genotypes that produce sterile seeds. A number of research groups have developed *in vitro* conditions suitable for the micropropagation of various apple cultivars and rootstock material over the last 2 decades, including (3-9). These *in vitro* propagation strategies produce four to 6 shoots on average from a single source shoot over a period of 4 weeks (the transfer generation). In the meanwhile, it can take several generations to produce enough clonal plants to make this technique financially viable.

In vitro techniques

Biotechnological challenges can be studied and solved using plant tissue

culture. For commercial use in the field of plant propagation, several techniques have gained traction. Organs, tissues, embryoids etc. can all be produced in plant tissue culture (Fig. 1). Explants from shoot tips, leaves, stems, cotyledons and microsporocytes have been used by (10) to describe plant regeneration. The initial explant utilization is the most critical factor in determining the quality and quantity of regenerated plants (11). Plant tissue culture is a significant alternative to the traditional method of vegetative propagation for the regeneration of elite and rare species (12). For large-scale propagule generation, plant tissue culture is the ideal option. This is especially true for endangered medicinal plant species, where explant material is scarce. Due to several reasons, including the presence of phenolic chemicals, exogenous and endogenous infection, maturity, juvenility, slow-growing habit and

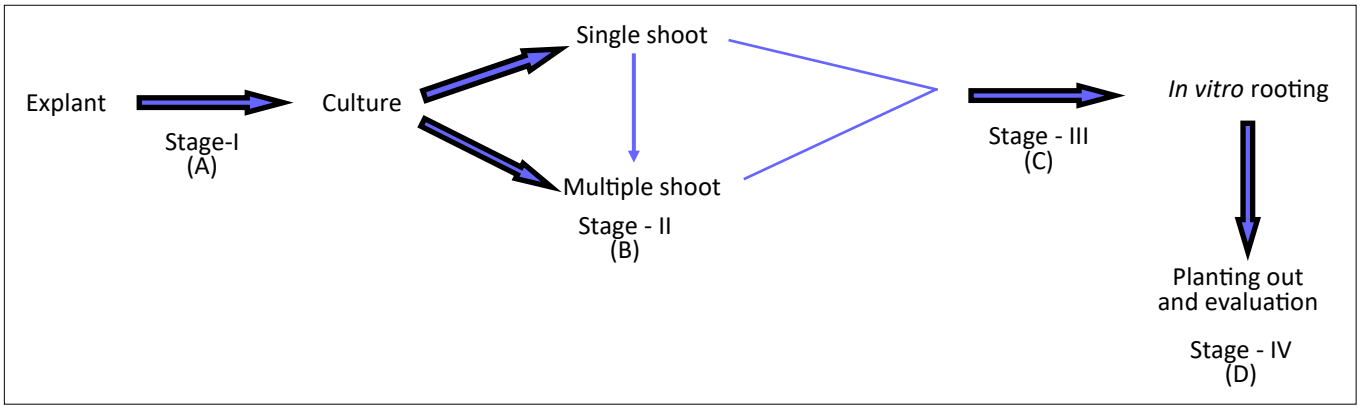

Fig. 1. *In vitro* cloning procedure in fruit crops (**A**) Culture setup (**B**) Shoot multiplication (**C**) *In vitro* rooting (**D**) Planting out and evaluation.

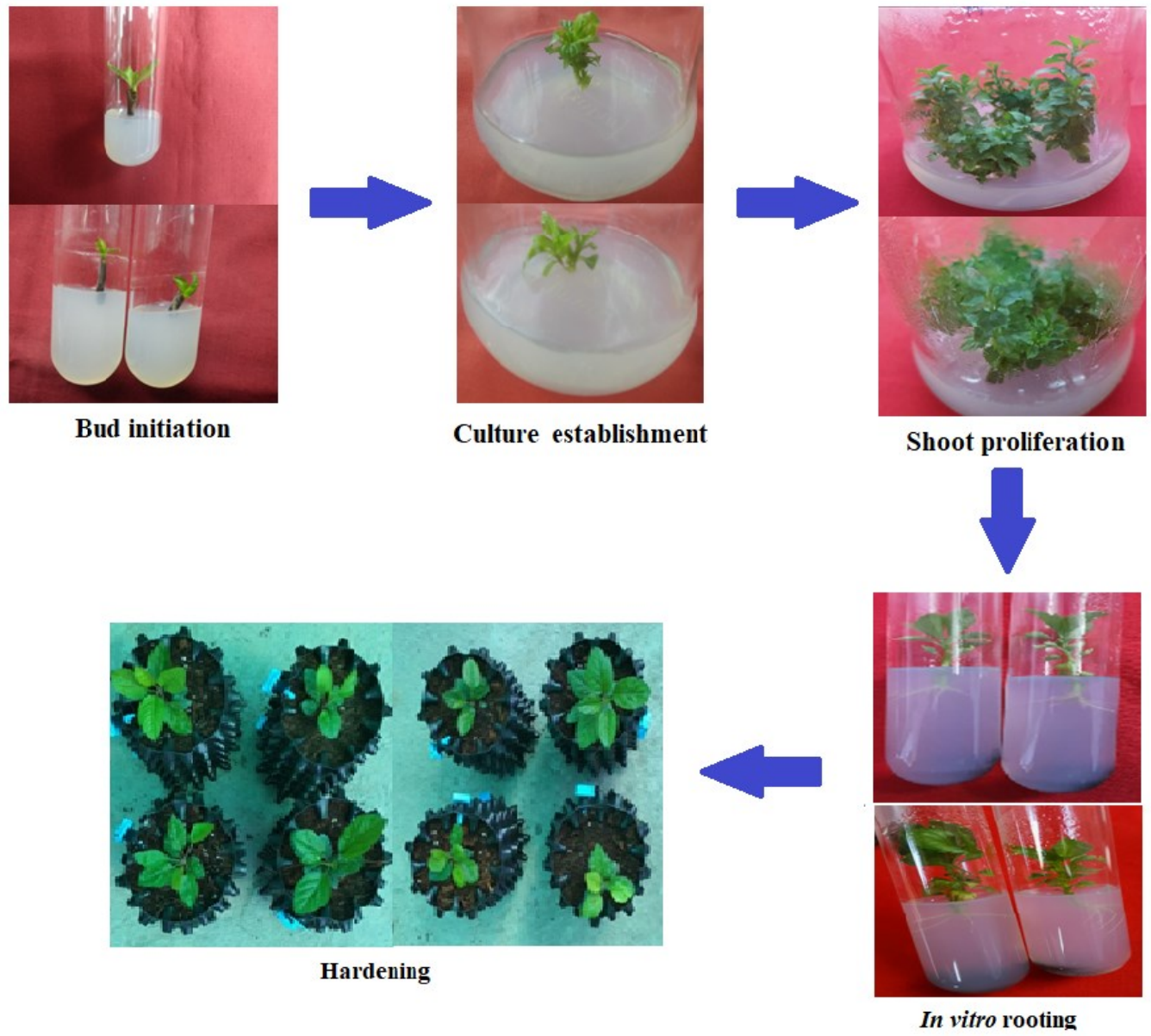

Fig. 2. Tissue culture stages in apple.

Table . Preparation of stock (MS media)

Component	Chemical formula	Required concentration (mg/l) 1X	Volume 1000 ml (40X)
STOCK-1 (Macro nutrients) 40X			
Calcium chloride	$CaCl_2.2H_2O$	440.0	17.6 g
Potassium dihydrogen phosphate	KH_2PO_4	170.0	6.8 g
Potassium nitrate	KNO_3	1900.0	76.0 g
Magnesium sulphate	$MgSO_4.7H_2O$	370.0	14.8 g
Ammonium Nitrate	NH_4NO_3	1650.0	66 .0g
Note: Dissolve $CaCl_2$ separately and mix			
STOCK-2 (Micro nutrients) 400X			
Cobaltous chloride	$CoCl_2.2H_2O$	0.025	10 mg
Copper sulphate	$CuSO_4.5H_2O$	0.025	10 mg
Boric acid	H_3BO_3	6.200	2480 mg
Potassium iodide	KI	0.830	332 mg
Manganese sulphate	$MnSO_4.H_2O$	16.90	6760 mg
Sodium molybdate	$NaMoO_4.2H_2O$	0.250	100 mg
Zinc sulphate	$ZnSO_4.7H_2O$	8.600	3440 mg
STOCK-3 (Iron source) 200X			
Ferrous Sodium EDTA	FeNaEDTA	36.7	7.34 g
STOCK-4 (Organics) 400X			
Thiamine HCL	$C_{12}H_{17}CIN_4OS.HCL$	1	400 mg
Nicotinic acid	$C_6H_5NO_2$	0.5	200 mg
Pyridoxine HCL	$C_8H_{11}CINO_3.HCL$	0.5	200 mg
Myo-Inositol	$C_6H_{12}O_6$	100	40000 mg
Glycine	$C_2H_5NO_2$	2	800 mg

lengthy genetic variability, micropropagation of mature trees with vegetative explants has been a challenging process (13). Recent years have seen the widespread application of micropropagation techniques for propagating a variety of fruit and forest trees quickly and in great numbers (14). There are numerous applications for tissue culture techniques, including somatic hybridization and genetic modification. Using the shoot tips of M7 and M26 apple root stocks (Fig. 2), it was found that a single shoot apex could produce 60000 green shoots in under 8 months (15).

Murashige and Skoog revolutionized plant tissue culture in the early 1960s (Table) with the development of a standardized culture medium (16). MS medium is currently the most extensively used culture medium for a wide range of plant species. It's possible to grow just about any portion of a plant under aseptic conditions using plant tissue culture. Pathogen-free plants can be produced, maintained, multiplied and transported using *in vitro* procedures. Elite planting material with the necessary properties can be mass-produced using Plant Tissue Culture Technology (PTC). Subsidies and incentives were granted to encourage micropropagation of plants in India under the Act of 1951, which came into effect in 1991. During the VIII plan, the centrally supported Scheme on Integrated Development of Horticulture was used to promote this technology on a large scale. Tissue culture labs in the public sector are being supported at Rs. 2.1 million, while in the private sector, 20 % of the cost is covered, up to a maximum of Rs. 1.1 million per unit. Because of its outstanding effectiveness in micropropagation and speciation improvement in plants, the plant tissue culture technology is now widely used commercially. Tree clones can be grown using tissue culture since it has the ability to create vast numbers of plants in short order.

The study undertaken in India and abroad on the topic of micropropagation of fruit crops has been discussed under the following headings:

Surface sterilization and type of explants

Tissue culture relies on sterilization to ensure the *in vitro* propagation of desired genotypes free of external and internal contamination. It was found that 5 % NaOCl for 25 min followed by 0.05 % mercuric chloride ($HgCl_2$) for 5 min was effective for the maximum surface sterilization of walnut embryos, according to (16), whereas, 5 % sodium hypochlorite (NaOCl) for 25 min was effective for the maximum surface sterilization of cotyledons. It was noted that apical bud from axillary shoots of size (1.0-1.5 mm) showed highest survival rate (50 %) at establishment for both apple and pear cultivars (17). Explants cleaned with

2.0% sodium hypochlorite for 15 min and 0.1 % mercuric chloride for 7 min had the best significant survival value (97 %) for cultivar Stanley, according to (18). Axillary buds treatment with 2.0 % NaOCl for 3 min was noted to be the preeminent for sterilization that produced very less contamination (13.33 %) followed by (25.00 %) with 0.1 % $HgCl_2$ for 3 min in Gisela-5 cherry rootstock (19). Axillary buds (1.5 cm) were found better for higher minimum browning and higher culture establishment % (20). Explants of Flame seedless grape plants when shaked for 60 min in a solution containing 20 % Clorox with 50 drops/L Triton X-100 provided the best disinfection with 90 % tissue survival (21). Explants from peaches were sterilized with ethanol (70 %) for 30 seconds, $HgCl_2$ (0.1 %) for 3 min and NaOCl (1.0 %) for 3 min, in that order. This resulted in an aseptic culture with an explant survival rate of 63.34 % and an internodal segment survival rate of 80.00 %. In addition, increasing exposure duration to different sterilants significantly reduced explant survival (22). Treatment with $HgCl_2$ (0.1 %) for 3 min resulted in just 12.33 % contamination of shoot tips from Ganesh pomegranate trees. In contrast, $HgCl_2$ (0.1 %) for 5 min reduced contamination in nodal segment explants by 23.50% (20). Sodium hypochlorite (1.0 %) for 15 min followed by $HgCl_2$ (0.1 %) for 7 min was shown to be the most effective in vitro sterilizing strategy for banana micropropagation, according to a study by (23). Runner tips and nodal segments respectively were treated with sodium hypochlorite (15 %) for 20 min, followed by ethyl alcohol (70 %) for 30 seconds, which resulted in necrosis and tissue damage to the explants, resulting in a lower % of aseptic cultures (80 % and 76.66 % respectively). Nodal segments (0.3-0.8 cm) showed minimum contamination rate (48.1 % and 88.3 %) in Fereley Jaspi and Giesla pear rootstocks respectively after surface sterilization with 70 % ethanol for 1 min and 20 sec followed by soaking in bleach solution at 0.4 % for 12 min (24). Axillary buds (0.5-2.0 cm) of Merton 793 apple rootstock when surface sterilized with mercuric chloride ($HgCl_2$) for 4 min providing highest quantity of untainted buds (25), but the survival rate was more in exposure time of 3 min. Axillary buds of Douce de Djerba apple plants when surface sterilized with calcium hypochlorite ($CaOCl_2$) at 5 g/L for 5 min provided maximum uncontaminated % (96 %) (26).

After washing strawberry nodal segments in running tap water and again with Tween-20, followed by surface sterilization with mercuric chloride in a laminar air flow chamber for 5 min, the highest proportion of uncontaminated buds were generated (27). Minimum browning intensity and 60 % survival rate after 6 weeks of cultured was achieved in MM 111 apple rootstock using shoot tip of size 0.2 to 0.4 cm and 0.4 to 0.6 cm (28). Disinfection the shoot tips of *Citrus aurantifolia* with 1.0 % sodium hypochlorite followed by 1.0 % mercuric chloride for different time intervals and reported that maximum % of aseptic cultures were observed with sodium hypochlorite for 10 min followed by mercuric chloride for 5 min (29). Vegetative buds of strawberry when were treated with 0.5 % carbendazin and surface sterilized with solution of 0.1 % mercuric chloride for 2-3 min provided very less contaminated vegetative buds % (30). After 10 min of surface sterilization with sodium hypochlorite (HCL), Pajaro and Sweet Charlie strawberry explants showed 88 % uncontamination rate (31). The single node explants of Chinese gooseberry when surface sterilized with mercuric chloride for 3-8 min showed good % of un-contaminated cultures (32). Sterilization of internodal stem sections of citrus rootstocks with 1N HCL for 5 min significantly reduces contamination % (33).

In vitro culture establishment

Various studies have demonstrated that plant growth regulators are indispensable for establishment of explants, having role from incubation to establishment. When it comes to cultivating fruit (17) conducted an experiment on 8 apple and 2 pear rootstocks. As a consequence of this investigation, Murashige and Skoog (MS medium) enriched with BA, GA_3 and IBA had the highest survival rate (55 %) at establishment in the Principe Grande apple cultivar, which was tested. Maximum culture establishment % (68.5 %) with explant of the third node position was achieved using MS medium boosted with 1.0 mg/L 6-Benzylaminopurine (BAP) + 0.5 mg/L Naphthalene acetic acid (NAA) (20). Gisela-5 cherry rootstock explants showed 70 % *in vitro* establishment between the months of July and February on MS medium fortified with 0.5 mg/L BAP and 0.5 mg/L GA_3 (19). Maximum culture establishment was seen on MS medium boosted with BAP (1.5 mg/L) and malt extract (500 mg/L), according to (34). NAA (0.2 mg/L) and BAP (4 mg/L) were found to increase the % of grape cv. culture establishment in a study by (35). Explants of 0.2-0.4 cm and 0.4-0.6 cm presented little browning intensity and over 60% of explants survived after 6 weeks, according to (28). Thidiazuron (TDZ) at a concentration of 2-4.5 M was found to be beneficial for the development of *in vitro* cultures in muscadine grapes (36). Explants of grape cv. Noble and Tarheel were best cultured with Thidiazuron (0.45 to 2027 M) alone or in conjunction with Kinetin (0.5 or 1.0 M) or BAP (0.45 to 2027 M) (37). *Vitis* cultivated *in vitro* and sub -cultured shoot tips were studied for the effects of growth agents and photoperiod on shoot apices and sub-cultured shoot tips. According to the study's findings, adding NAA (5 10-7) and BAP (5 10-6) to the medium was helpful for Rougeon grape shoot culture establishment (38).

In vitro shoot proliferation

Due to the phenomena of PGRs, *in vitro* shoot proliferation rates are quite high). In plant tissue culture, the growth regulators BAP and KN are commonly referred to as “shoot proliferation hormones.” The greatest percentage of shoot proliferation was seen in Red Fuji apple shoots cultured on MS medium boosted with 4.44 M BAP and 0.05 NAA after 15 and 45 days of culture (39). Explants of apple cv. Stanley were sterilized and *in vitro* propagated on MS medium boosted with 0.5 mg/l BAP combined with 0.1 mg/l IBA, resulting in an average number of 3.08 shoots per explant and 3.33 cm average shoot length per explant (18). In a study on micropropagation of new apple rootstock G814 (40), noted that BAP at 1.0 mg/l gives finest results at mul-

tiplication phase and produced a greater number of roots (2.0). On MS medium enriched with the cytokinin (0.5 and 1.0 mg/L) in conjunction with TDZ, while Principe Grande (55 %) and Barburina pear cultivars (50 %) had the highest rates of shoot initiation (17). In an experiment, development of tissue culture system for pear plant, (41) reported that the utmost shoot proliferation proportion was attained for Bitterbrine Lucas pear plants that produce maximum number of shoots (4.9) on MS medium augmented with 4.0 mg/l BAP. Gisela-5 cherry rootstock was propagated *in vitro* (19), who found that the rate of shoot rate and length increased with the sub-culturing passages, reaching a maximum of 1:19 and 6 cm after the third and fourth subculturing passages on MS medium boosted with 5 different concentrations and combinations of BAP, GA_3, IBA and KN. The maximum proliferated cultures (95.30 %) and shoot length (42.97 mm) were obtained using WPM boosted with 3.0 mg/L BAP (42). Study on *in vitro* culture in 3 elite clones of jackfruit was carried at Bagalkot, GKVK, Bengaluru and study revealed that chloramphenicol (60 mg/L) recorded least bacterial contamination (6.33 %) and highest survival (80.60 %) of the culture against basal medium (86.32 % bacterial contamination with culture survival of 7.16 %). Further, it was also observed that explants survival was highest when collected during January and chloramphenicol as antibiotic was effective in reducing bacterial contamination (43). Results from the study revealed that maximum shoot proliferation percentage in Azayesh-Esfahan, Morabbaee-Mashhad and M9 clonal rootstock was noted in MS medium boosted with 1.5 mg/L BAP. However, rootstocks Azayesh-Esfahan, Morabbaee-Mashhad produced maximum and minimum (4.6 and 3.66 shoots per explants) shoot proliferation values respectively (44). MS medium boosted with 1.5 mg/L of BAP and 500 mg/L malt extract resulted in the largest number of shoots (5.34) and the longest shoot per culture (3.557 cm) in the shortest period during shoot proliferation, according to (34). Medium enriched with 1.0 mg/L BAP + 1.5 mg/L kinetin produced similar types of apple cv. Topaz shoots per inoculated one i.e. 2.5 and 2.4 respectively (45). Further, there was a considerable increase in the number of shoots (3.6) when medium was added with 1 mg/L thidiazuron (TDZ). MS medium boosted with 3.0 mg/L BAP, 2.0 mg/L kinetin, 3.0 % sucrose and 0.8 % agar-agar (w/v) produced 85.7 % of clonal rootstock M9 shoot apices forming multiple shoots (46).

When grown on agarized MS media containing 0.25 to 2.0 mg/L BAP, 0.50 mg/L NAA, or 0.50 mg/L kinetin, *Citrus megaloxycarpa* L. shoot tips developed many shoot buds, according to a study (47). For the *Pyro* dwarf pear rootstock, (48) found that the maximum multiplication index (2.99) was achieved on the medium boosted with 5 M BAP and 0.5 M IBA. Using 4.4 M BAP and 2.27 M thidiazuron (TZD) and 4.4 M BAP during the shoot multiplication phase, (49) reported the best shoot production in terms of shoot quantity and shoot quality. M27 had the largest multiplication rate (5.7) and the highest fresh weight (2.25 g/jar) compared to MM106, which had the lowest (0.7) per month multiplication rate. Douce de Djerba apple micropropagation was studied by (26), who found that MS medium enriched with 1.0 and 2.0 mg/L produced several shoots from axillary buds. Gulf-ruby shoot multiplication was obtained on WPM medium supplemented with IBA, BAP, KT and casein hydrolysate (50). On WPM medium with 0.05 mg/L IBA, 0.03 mg/L KT/BAP and 1.0 casein hydrolysate, they found that the growth of the shoot *in vitro* was facilitated. However, with 1.0 mg/L BAP and 1.0 mg/L kinetin, the most shoots (4.55), with a maximum shoot. Different mediums *viz*. MS, QL, WPM and DKW used for multiplication of *Malus seiboldii* and MS medium with an addition of iron showed highest proliferation rates ranged from 3.3 to 5.7 as well as shoot number (4.8 shoots/ explant) and shoot height i.e. 2.4 cm (51). In a study, (52) found that the MS medium supplemented with 2.22 M BAP produced the most shoots (6.8 + 0.80), the longest shoots (17.5 + 4.6 mm) and the highest sprouting % (85.0 %). An experiment for rapid clonal multiplication of apple rootstock MM111 utilizing axillary buds and reported that the combination of BA (1.0 mg/L) and GA3 (0.5 mg/L) produced longer shoots and the greatest multiplication rate (1.5) (28). To promote the best rate of shoot regeneration in the Kyobo grape, researchers used half MS medium supplemented with BAP and IBA (0.02 mg/L) (53). To generate the greatest number of shoots in the Calmeria grape variety, researchers used B5 medium with 1 mg/L BAP and 1 mg/L PP 333, as well as 0.05 mg IAA and 0.05 mg IAA (54). A study conducted by (55), it was found that the maximum % of shoot regeneration (806) was achieved with genotype S799 on a medium containing glucose and 8.9 11M 6-benzyladenine. When the cytokinin concentration (BAP or Kn) was increased to 9.0 mM, the growth of the shoots increased. On a medium supplemented with BAP at 9.0 mM, 94% of the explants showed shoot development within 78 days, with the longest shoot measuring 51 cm (56). Thidiazuron-assisted induction of shoots in apple cv. Gale gala and pear cv. Bartlett cultured stem slices was 37 % and 97 % higher than medium accompanied by BAP and kinetin in a study (57). Rootstocks produced 11.6+2.5 shoots (1.5+0.8 cm in length) per tube per month in micropropagation experiments including thirteen *Malus* cultivars and rootstocks, with antibiotics having the most significant effects on proliferation (8). To improve the quantity of shoots produced, TDZ was combined with BAP or Kinetin (1.0-5.0 M) (36). In all of the grape genotypes evaluated (58), BAP at 5-10 M led to greater shoot tip proliferation. A medium treated with cytokinins, BAP and zeatin both at 2.0 mg/L in Chenin Blanc grapes plant yielded the best shoot proliferation rates (59). In medium boosted with 80 mg/L adenine sulphate, 170 mg/L sodium phosphate and 3-4 mg/L BAP, it was found that grape French hybrid cv. Baco showed the highest rate of shoot production (60).

In vitro rooting

In plant tissue culture, PGRs such as auxins (IAA, IBA, 2,4-D, and NAA), which are commonly regarded as rooting hormones, are responsible for inducing roots of high quality *in vitro*. Among the several concentrations of NAA evaluated for Golden Delicious apple trees (39), only 0.53 M NAA supported the greatest roots (average of 7.66 number and length of 22 cm). A study was carried out (61) to standard-

ize the protocol for new apple rootstock series G814 using QL media boosted with different amount of BAP (0.5, 1, 1.5, 2 and 2.5 mg/l) and study revealed that BAP @ 2.5 mg/l resulted in highest rooting % (80) as well as length of the root (5 cm). It was found that Camoesa and Repinaldo rooted well in MS medium with 0.1 mg/L IBA without cytokinins (17). Maximum *in vitro* rooting (100%) in Gisela 5 - clonal cherry rootstock on full strength MS medium reinforced with 0.5 mg/L IBA (62). The best rooting (18.20%) was found in a one-step method on half-strength MS medium boosted with 0.5 mg/L IBA (19). It was found that citrus shoots rooted best in half-strength MS medium with IBA and NAA (63). IBA (1.0 mg/L) and NAA (1.0 mg/L) exhibited maximum rooting percent (83.33), number of roots per shoot (2.47) and longest root (3.57 cm). Rootstock Ferely Jaspi had 100% rooting, the most shoots (8.1) and the longest roots (3.08 cm) (24). It was observed that half strength MS media supplemented with 5 M IBA and 0.3 M gibberellic acid (GA_3) had the highest percentage (96%) and other rooting metrics, including root numbers (4.88) and length of the longest root (1.83) for shoots from BAP medium (64). Half-strength MS medium containing 5.4 M IBA and 1.2 M 2, 4-D produced considerable apple rootS (65). After 3 months, MM106 had % rooting while M9 had 11%. Inducing *Citrus magaloxpcarpa* L. roots with 0.50 mg/L NAA and 0.25 mg/L BAP was more successful (47). *In vitro*, Merton793 rooted better in MS media (1/2 strength) with 0.1 mg/L -naphthyl acetic acid (25). *Citrus* × *jambhiri* L. rooted well on MS medium supplemented with 1.0 mg/L NAA and 1.0 mg/L IBA (66). It was found that half strength MS medium boosted with 0.2-0.5 mg/l IBA, 15 g/L sucrose and 20-40 mg/L phoroglucinol (PG) had the highest rooting percentage (10.42%) in Chinese plum without phoroglucinol and 79.76% with it (67). Nearly 75% rooting was achieved on 12 MS medium with 0.1–0.5 mg/L IBA, 2% sucrose and 0.4% agar in one step. Inducing *Malus seiboldii* genotypes at night with 25 M IBA in liquid or agarized medium increased rooting % (51). Various concentrations of IBA initiate roots, but 2.0 and 2.5 mg/L in MM106 show the most (68).

At 8+5 weeks, X0.5 and X1.0 MS medium with 14.70 m IBA showed the highest rooting response (20.8% and 75.9%). X0.5 and X1.0 MS mediums combined with IBA at 29.40 m and 14.70 m at 4+1 week showed highest rooting initiation (20.8% and 95.3%) (52). Transferring micro cuttings to auxins-free solid medium following root initiation on half strength MS media containing 0.5 mg/L IBA was found to be better in apple rootstocks MM111 (28). *In vitro* rooting was best in MS medium with 60 g/L sucrose (69). It was found that Pummelo rooted best in half-strength MS medium with 1.0 mg/L NAA. IBA alone induced up to 50% rooting in Nagpur mandarin, however 4.92 M and 1.11 M BAP induced 78% rooting and the most roots per shoot (70). 1.0 mg/L NAA alone or 0.5 mg/L NAA combined with 2.0 mg/L NAA and IBA generated the most roots in Kagzi lime (71), whereas 2.0 mg/L NAA and IBA produced the most roots overall. Jaffa mosambi plantlets grew the longest root (5.33 cm) on half-strength MS media with 0.5 mg/L NAA and 0.5 mg/L IBA (72). M9 apple rootstock rooted best in half-strength MS medium containing 0.5 mg/L IBA, 20 g/L sucrose and 8 g/L agar (73). Citrus rootstocks *Troyer citrange* and *Carrizo* rooted best in MS medium boosted with NAA and GA_3 (74). Regenerated sweet orange shoots rooted best in MS media boosted with NAA (0.75 mg/L) or NAA (0.5 mg/L) plus IBA (2.0 mg/L). *In vitro* regenerated pomegranate shoots rooted best in 0.54 mM NAA medium, with 92% rooting and 10.3 roots per shoot (56). Half MS or half Quoirin and Lepoivre (QL) medium with 1% sucrose and low IBA was optimum for *in-vitro* rooting of apple clonal rootstocks (75). Lower IBA concentrations induce roots in liquid medium rather than solid medium for most *Malus cultivars* (8). Apple *in vitro* raised shoots rooted 70-80% when treated with 1.5% sucrose and 0.3 mg/L IBA in the dark for 7 days (76).

Acclimatization / Hardening

In vitro-rooted plantlets must adjust to their new environment following transplantation, which is called acclimatization. Because *in vitro* plant material does not quickly adapt to *in vivo* circumstances, it is critical for *in vitro* grown plantlets (77). When acclimatizing to new conditions, elements including humidity, the composition of the potting mixture, the pH of the potting mixture, and water application all play a role. *Pyrus elaeagrofolia* established *in vitro* exhibited a strong response to acclimatization in pots with an autoclaved mixture of peat (70 %), perlite (12 %), sand (12 %) and orchard soil (6.0 %) coated with plastic film (78). All of the *ex-vitro* hardening conditions were tested for banana cv. Meitei Hei and recorded 98–100 % survival rate (2:1) (79). Only river sand potting media had a 100% survival rate. A plastic film-covered mixture of perlite (12%), sand (12%) and orchard soil (6.0%). It was found that out of different compositions of potting mixture (46), the highest survival rate (60.5%) was observed in sand: soil: perlite (1:1:1) for apple plantlets. In addition, maximum shoot biomass (2.45 $\pm$ 0.23 g) and number of leaves (24.4 $\pm$ 0.23) were also recorded in sand: soil: perlite (1:1:1). Acclimatization under mist systems in greenhouses of rooted shoots was effective (above 90%) for most of the plum rootstocks (24). During an experiment on *in vitro* multiplication of rough lemon (*Citrus jambhiri* Lush.), it was found that out of different potting mixtures, the maximum survival rate (93.33) was achieved in soil + sand + vermiculite (1:1:1) (63). 90.90 % of *Pyro dwarf* pear rootstock plants were acclimatized and survived in a greenhouse mist system with satisfactory morphological response (64). An 80 % survival rate was observed in a study by (25), who showed that 30 Merton 793 plantlets were successfully toughened after only 7 weeks of growth. There were no phenotypic anomalies in the regenerated shoots, which developed vigorously in the greenhouse (50). Douce de Djerba micropropagation earlier studied (26), revealed that regenerated shoots of apple had a 60% survival rate when transported to the field. For the *Malus Seiboldii* genotypes studied by (51), an acclimatization regimen implemented in the greenhouse yields survival rates of between 90 and 100 % for *ex vitro* plants grown in the greenhouse. The survival rate of transplanted *in vitro* produced M9 plants to polyvinyl cups or polybags was re-

ported to be 80 % (52) under a carefully controlled decreasing RH regime of 95 % to 70+5 % over a period of 5+1 weeks. Research shows that peat-filled paper cups can be used for the transfer of *in vitro* rooted apple rootstock plantlets within 3-4 weeks, allowing them to adapt to their new environment with no morphological difference (28). Regenerated Chandler strawberry plants in polythene bags with FYM (1:1) soil had the highest survival rate during the hardening period (80).

Transplanting banana plantlets 2 or 3 weeks after root initiation in a culture chamber at 26+2 °C resulted in a 90% survival rate (81) and that using polythene sheets resulted in an 80% survival rate. A mixture of sand, dirt and vermiculite (1:1:1) yielded the highest survival rate (95 %) in a lath housing, according to the researchers. Using sterile perlite soaked in quarter-strength MS salt and 0.5 % sucrose, (82) acclimated *in vitro* plantlets in greenhouse conditions by moving the plantlets 4 weeks previous to greenhouse transfer into sterile perlite. Northern Spy plantlets had the best chance of survival in coarse sand because of the increased aeration provided by the sand. apple cv. Golden Delicious and rootstock M26 and MAC9 were acclimatized using a mixture of perlite, peat, sand and commercial compost (83). For Golden Delicious and MAC9, the survival and growth rates were highest in optima + perlite and the least in peat + perlite for these two varieties. Peat + perlite was found to be superior for M26. The experimental investigation found that survival and growth rely on the kind of substrate and the cultivar.

Economics of in vitro production of plantlets

Economics consideration assumes prodigious importance in any commercial attempts of mass multiplication of plants. However, very few workers have worked out the economics analysis of *in vitro* produced plantlets. Explants from a 2-3-year-old mother block of clonal apple rootstock MM 111 were projected to cost Rs. 36.95 per plantlet, which includes Rs. 34.29 for tissue culture production and a further 2.66 rupees for greenhouse production (1). Using conventional methods, the projected production cost of an *ex-vitro* seedling is Rs. 80.00, but. (83) found that the cost *per in vitro* generated plantlet from an explant was Rs. 44.56, according to their study. Tissue-cultured banana, according to one report (70), is more profitable for farmers than sucker-propagated banana. He also stated that resources in Tissue Cultured Banana may be used effectively (TCB). One shoot explant of *C. reticulata* produced 180000 shoots per year, while the cost of one *in vitro* grown plantlet was estimated to be 165 Yen. The unit cost up to polygreen house stage of one guava plantlets obtained from seedling explant source was estimated to be Rs. 1.95 (71); whereas, the unit cost of one guava plantlet obtained from mature tree explant source was estimated to be Rs. 2.05. Each banana plantlet costs Rs. 2.22 at the greenhouse stage, which includes the first 4 weeks of growth (81). On an average 19200 papaya plants could be produced in every 3 weeks cycle from 250 initial shoot tips (72). They also reported that *in vitro* rooting band greenhouse stages are the most expensive items and estimated that the cost of papaya plantlet ready for field planting would be Rs. 0.85.

Conclusion

Micropropagation can be used to propagate superior cultivars, introduce novel cultivars with desirable traits, and multiply disease-free, healthy propagation material. Fruit cultivars, rootstocks, and scions can be micro propagated. Browning or blackening of culture media due to phenolic leaching, microbial contamination and *in vitro* tissue recalcitrance require attention. Understanding the biological processes that allow *in vitro* morphogenesis manipulation and studying plant hormones will improve our understanding and provide details needed to solve *in vitro* recalcitrance or plant growth and development.

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Authors contributions

Conceptualization, ML; investigation and resources, ML, SK, SS and NS; original draft preparation, ML; final draft preparation, ML; supervision, ML, MJ, PB and YS. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

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