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ROLE OF PLANT TISSUE CULTURE IN PRODUCTION OF VIRUS-FREE PLANTING MATERIAL IN HORTICULTURAL CROPS: A REVIEW

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ABSTRACT

Plant viruses and viroids are among the major constrains to sustainable cultivation of horticultural crops, especially those vegetatively propagated via tubers, bulbs, rhizomes, runners, suckers, cuttings, grafts and other clonal propagules. Continuous usage of infected planting materials leads to the build-up and spread of viruses from generation to generation, resulting in decreased vigor, yield, fruit quality, and life span of plants. Since systemic viruses are not easily controlled once they establish in perennial or vegetatively propagated crops, virus-free planting material becomes a key component in modern horticulture. Plant tissue culture, especially meristem and shoot-tip culture, offers an effective strategy for the elimination of viruses and the regeneration of healthy plants from infected plants. Efficiency of virus elimination can be increased by the combination of tissue culture with thermotherapy, chemotherapy, cryotherapy, electrotherapy, and micrografting. Efficiency of virus elimination reported in literature varies depending on crop, virus, genotype, explant size and treatment conditions. Recent reviews report about 50-100% success rate in various combinations. Molecular diagnostics like ELISA, PCR, RT-PCR, qRT-PCR, high throughput sequencing becomes an important part of virus elimination. Recent studies on apple, grapevine, pear, apricot and ornamentals also illustrate the importance of tissue culture combined with molecular indexing. This review discusses the principle, mechanism, methodologies, crop-wise application, advantages, limitations, and prospects of plant tissue culture for the production of virus-free planting material in horticultural crops.

KEYWORDS: Plant tissue culture, meristem culture, shoot-tip culture, thermotherapy, chemotherapy, micrografting, horticultural crops

1. INTRODUCTION

Crops of horticulture have significant role in ensuring food and nutritional security as well as income and diversity in agriculture. Production of fruits, vegetables, ornamentals, spices and plantation crops involves intensive commercial practices whereby the quality of planting materials plays crucial role in establishing and producing crop. An issue encountered in many horticultural crops is occurrence of systemic viruses and viroids in the vegetative propagation materials. Vegetative propagation generates plants which are genetically identical and thus capable of transmitting viral infections present in mother plants, (Singh *et al.,* 2011).

Clonally-propagated crops like potato, banana, strawberry, grapevine, apple, citrus, garlic, ginger and ornamentals are susceptible to accumulation of viruses. Infected tubers, bulbs, rhizomes, runners, suckers, cuttings and grafts are sources of viral infections for next generations. Application of diseased materials during propagation results in decline in performance of crop due to gradual increase in the levels of virus in clonal crops, (Megersa, 2017).

However, the notion of producing virus-free planting material in modern days goes beyond the concept of meristem culture. It constitutes a whole process that includes elite mother plant selection, pathogen indexing, thermotherapy or any other form of treatment suppressing viruses, meristem removal, in vitro regeneration, multiplication, rooting, acclimatization and post-regeneration molecular indexing. Such an integrated approach becomes more relevant for commercial horticulture, as the fact of getting a regenerated plant from an infected explant is no guarantee that the plant is virus-free, (Magyar-Tábori, *et al.,* 2021).

Thus, production of virus-free planting material is an important part of modern nursery and seed multiplication practices. (Wang *et al.* 2018) pointed out that virus-free plants should be obtained not only for disease control but also for safe movement of cultivar and germplasm exchange and international movement of breeding material.
Plant tissue culture serves as one of the main technological means of achieving this goal. The basic idea is application of small meristematic tissues containing less amount of virus compared to mature tissues. Thus, culture of meristems allows obtaining virus-free plants even from systemically infected source plants. However, virus exclusion is not always possible and depends on the interaction between host and pathogen, (Grout 1999).
Modern virus removal procedures combine culture of meristems and thermotherapy, chemotherapy, cryotherapy and other treatments. Recent comprehensive review by Thanuja *et al.* (2025) showed that conventional in vitro techniques allow removal of approximately 50–100% of virus in case of various virus-host combinations.

2. Principle of Virus Elimination by Tissue Culture

The basic principle of the meristem culture technique is due to the fact that a large number of viruses are present in a small concentration or absent in actively dividing cells of the apical meristem. The reasons for this phenomenon include rapid cell division, limitation of vascular differentiation, restriction of virus spread, specific antiviral defense system of meristem and RNA silencing, (Bhojwani and Dantu, 2013).
The relationship between the size of meristem and virus elimination plays a crucial role. Small meristems are more likely to be free of virus but harder to regenerate, while larger shoot tips are more likely to survive and regenerate but more likely to be infected with viruses. Thus, successful virus elimination is a combination of two factors: probability of being virus free and regenerability, (Wang, and Hu, 2005).
Conventional disease management methods are usually not capable of removing the virus infection that has been established in the tissues of the plant. For this reason, preventive measures involving the use of pathogen-tested seedlings have been found to be more effective than curing plants which have been already infected, (Paul Jr, F. (Ed.). (2012). The in vitro methods have become significant elements of the plant health program aimed at establishing pathogen-free mother plants and producing healthy propagules. According to Wang *et al.*, in vitro culture is one of the most effective methods in creating virus-free plants, and further investigations have proved that the combination of thermotherapy, shoot tip culture, chemotherapy, micrografting and cryotherapy can improve the effectiveness of virus elimination, (Kang *et al.,* 2025).

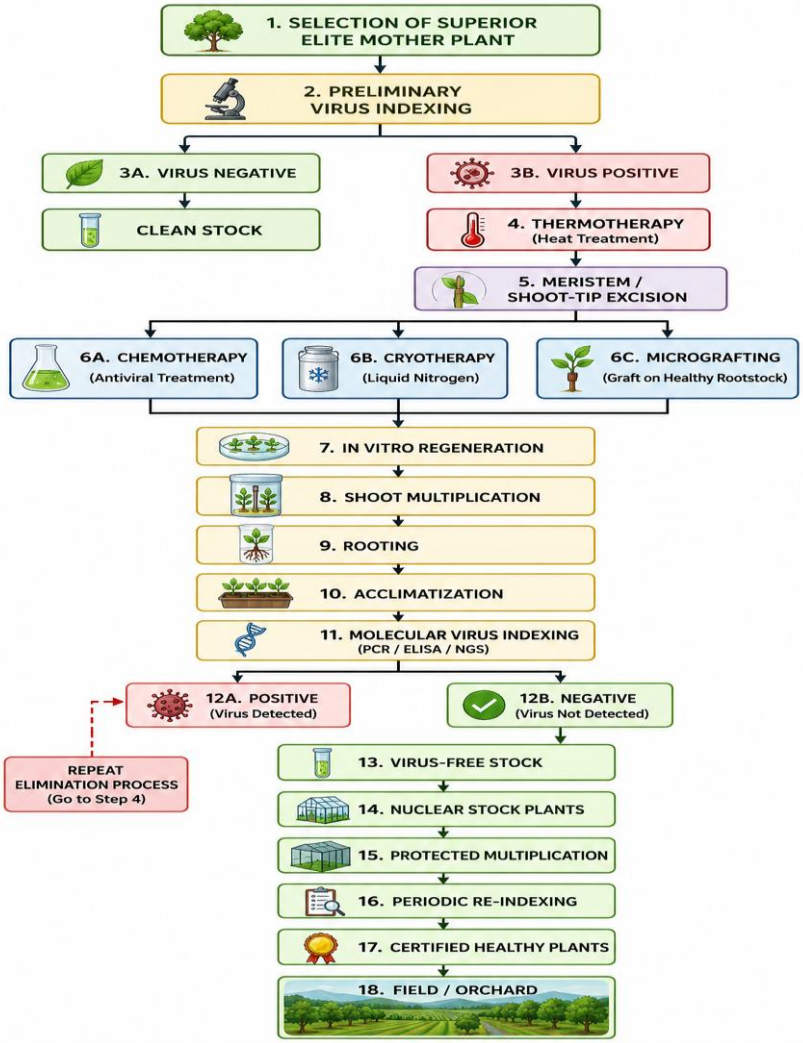


Figure-1: Flow chart of Virus-Free Horticultural Planting Material

3. Important Tissue Culture Techniques

The use of virus-free planting material is especially critical in crops which are vegetatively propagated for commercial purposes, (Martin, 1994). Seed-propagated crops are usually inefficient at transmitting viruses through their true seeds, but the ability to maintain infections from one generation to another in vegetatively propagated crops means that the health of the mother plant directly affects the health of the planting material, (Taşkın, *et al.*, 2013).

Infected banana suckers are able to transmit viruses between planting cycles in bananas. Infected potatoes are important for maintaining viral infections in seed production systems in potato. Strawberry plants infected with viruses can pass on the pathogens using the runners. Grapevines and apple plants can spread the infection via infected scions or budwood used for propagation. Garlic and ginger plants infected with viruses or viroids can maintain a population of pathogens from one generation to the other, (Smith, and Drew, 1990).

Using virus-free planting material reduces the amount of inoculum introduced into production systems, leading to better establishment, more vigorous plants, better yields, and higher quality products. In perennial horticulture crops, the benefits last many years as the use of healthy planting material sets up the foundation for productive orchards and vineyards, (Bhat, And Rao 2020).

It should be noted that the importance of using disease-free planting material does not apply only to individual farmers. It has implications for nursery certification, germplasm exchange, quarantine, international transport of planting materials, and the long-term health of horticultural production systems.

3. Plant Tissue Culture as a Tool for Virus Elimination

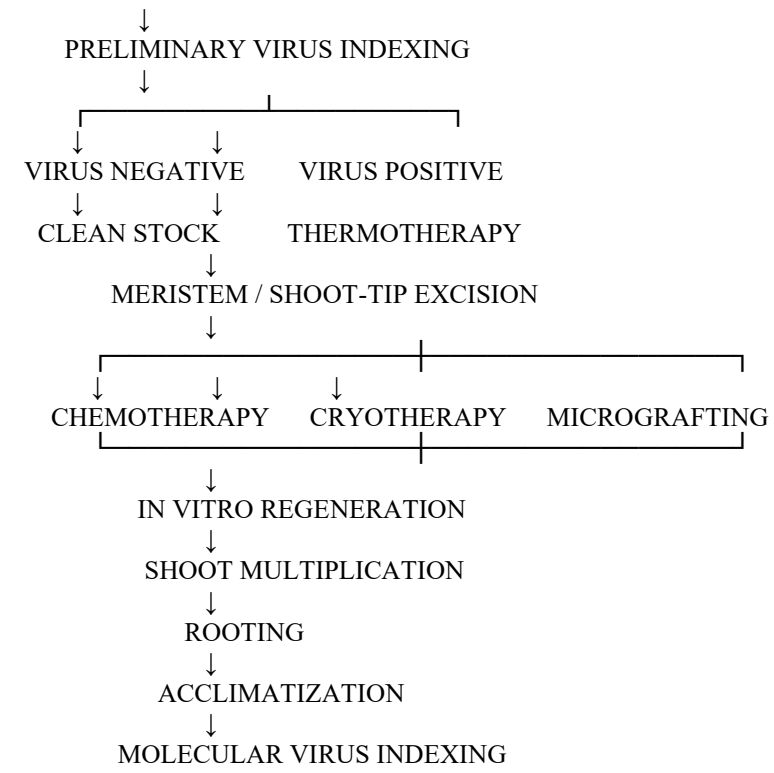
Plant tissue culture provides the means to cultivate small tissues isolated from infected plants under sterile conditions. Meristem culture is especially useful in eliminating viruses among the different methods of tissue culture.

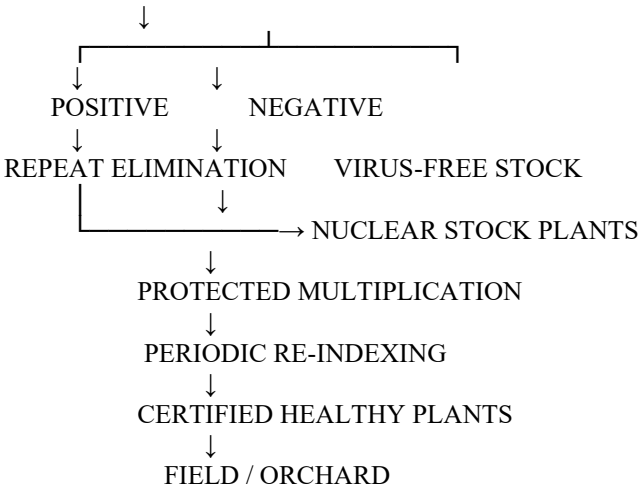
The apical meristem consists of cells that undergo division to promote primary growth in plants. Most viruses are transferred through plasmodesmata and vessels, and such rapid transfer into the meristem of plants may be difficult. Moreover, antiviral resistance and RNA-silencing may prevent virus propagation in the meristematic tissues. As a result, a very tiny apical meristem is capable of regenerating a virus-free plant from a plant that carries viruses.

Notwithstanding the above facts, the meristem does not exclude all kinds of viruses. There are cases where viruses infect meristematic tissues, while other infections can be scattered in different parts of the plant body. As a consequence, the chances to get a virus-free plant depend on the virus, host species, meristem size, physiological status of meristem, growing conditions and detection method used, (Wang, and Hu, 2005).

Using small explants is helpful in getting rid of systemic viruses but at the same time reduces the viability and regenerative capacity of explants. It is, thus, important to find the right size of the explant which will provide an acceptable trade-off between virus elimination and regenerative ability.

Figure 2. Integrated Process for Production of Virus-Free Horticultural Planting Material





3.1. Selection of Elite Mother Plants

The development of virus-free planting materials starts with the selection of elite mother plants. The donor plant should possess horticulturally desirable attributes like high yield, good fruit or flower quality, right maturity period, disease tolerance, desired plant architecture, and adaptability to the intended production environment, (Smith, and Drew, 1990).
However, the mother plant should be checked for viral and viroid infection before starting the tissue culture process. A seemingly healthy plant may be carrying the latent viruses. Thus, visual inspection can't be relied upon as the only technique for selection of pathogen free donor material.

Mother plants are indexed through proper diagnostic techniques in advance propagation schemes. Infected but horticulturally superior plants are then eliminated of their viruses, while virus-free elite plants can be used directly for clean stock maintenance, (Allan 1981).

3.2 Apical Meristem Culture
In apical meristem culture, the excised apical meristem consisting of the apical dome and one or two leaf primordia is cultured on an appropriate culture medium which contains the minerals, vitamins, carbon source, and growth regulators (Ramgareeb, *et al,* 2010). Morphogenesis of the regenerated shoot followed by multiplication, rooting, and hardening of the plantlets is done prior to molecular indexing. Apical meristem culture is particularly important for bananas, potatoes, strawberries, garlic, grape vines, apples, and ornamentals.
Meristem culture is by far the most well-known tissue-culture technique of virus elimination. It entails the removal of a small piece of the apical meristem, usually comprising of the meristematic dome with one or few leaf primordia, and culture on suitable nutrient medium. Meristem culture highly relies on the size of the explants used. Small-sized meristems may possess high chances of being virus free but are quite difficult to isolate and have low chances of survival and regeneration. Large sized shoot tips may possess higher chances of survival and regeneration, but have more likelihood of being infected, (Hong, and Wang, 2026).

Regenerated meristem is cultured under controlled conditions until a full shoot is produced. The plantlets are then multiplied, rooted and acclimatized. The produced plant should then be tested for the presence of target viruses and viroids, (Sastry, and Zitter, 2014). Meristem culture plays a vital role in vegetative crops like banana, potato, garlic, strawberry, grapevine and several ornamental plants. Several literatures have continually stressed the importance of meristem culture in virus-free plant production.

3.3. Shoot Tip Culture
Shoot tip culture is similar to meristem culture but includes a somewhat larger piece of the apex of the shoot compared to the latter. This method offers a higher amount of tissue and can therefore be more successful than meristem cultures that use very small explants (Navarro, *et al.*, 1982). Shr-tip culture is often used in combination with thermotherapy. In this method, virus-infected plants or shoot cultures are subjected to an increase of temperature during a certain time period, and the shoot tips are then removed for culturing. Thermotherapy followed by shoot tip culture has been one of the most widely used methods for virus elimination in both herbaceous and woody horticultural plants, (Pérez-Caselles, *et al.*, 2025).
(Wang *et al.* 2018) provided many examples from plants such as potato, garlic, raspberry, apple, pear, peach, cherry, and others.

3.4 Thermotherapy
Thermotherapy entails the exposure of infected plants or in vitro shoots to high temperatures for a specified duration. This process inhibits viral replication and spread but ensures that the plant tissues survive enough to

allow regeneration (Hong, and Wang, 2026). (Wang *et al.* 2018) noted that 35–42°C temperatures for 4-6 weeks have been commonly applied, although the treatment parameters should be adjusted based on the virus-host interaction. Thermotherapy has proved especially successful when followed by meristem or shoot-tip culture.
Thermotherapy may work through inhibition of viral replication, degradation of viral RNA or protein and hindering the movement of virus towards new meristem formation. Some recent studies have demonstrated that thermotherapy could also affect the host defense systems as well as RNA-silencing pathway. However, the major disadvantage of thermotherapy is that the temperature at which the virus is inhibited might damage the plant, (Magyar-Tábori, *et al.*, 2021)

3.5 Thermotherapy along with Meristem Culture

Thermotherapy coupled with meristem culture technique is perhaps the most frequently used method of virus eradication. The heat treatment initially suppresses the viral replication and spread. Afterwards, the tiny meristems are removed from the shoots and then cultured using aseptic procedures, (Kang, *et al.*, 2025).
In one of the experiments, thermotherapy treatment at 37-40°C for a period of four weeks and followed by the meristem culture procedure was effectively used for the virus elimination in apple variety Oregon Spur-II. The meristems measuring 0.3-0.6 mm successfully eliminated apple chlorotic leaf spot virus, apple mosaic virus, apple stem grooving virus, and apple stem pitting virus (Vivek, and Modgil, 2018).
From the results of the current study, it could be observed that DAS-ELISA is quite an efficient technique for virus indexing and it can be used to develop virus-free planting material. In 'Oregon Spur-II' apple cultivar, effective virus elimination was achieved through subjecting the plant to thermotherapy at 37-40°C for 4 weeks along with the in vitro culturing of meristematic explants measuring 0.6mm in size. Future research can be done on prolonging the period of heat treatment and testing its efficiency in virus elimination with large sized explants, (Vivek, M. 2013).

3.6 Chemotherapy

Chemotherapy consists of using antiviral substances in the tissue-culture medium. Ribavirin is the most common substance used among the antiviral substances studied. The use of chemotherapy is able to prevent virus multiplication and could enhance the elimination of viruses along with thermotherapy and meristem culture. But it should be noted that the use of antiviral chemicals can interfere with the development and regeneration of plants. (Thanuja *et al.* 2025) identified chemotherapy as one of the important conventional in vitro approaches for virus elimination in horticultural crops. For this reason, the use of chemotherapy alone is not as effective as that when combined with meristem culture or thermotherapy. According to the recent literature, chemotherapy is one of the proven complementary methods of virus elimination, (Van den Houwe *et al.*, 2020).

3.7 Cryotherapy

Cryotherapy entails the exposure of shoot tip or meristematic tissue to very low temperature levels through cryopreservation processes that use liquid nitrogen. This process capitalizes on the difference in survival capacity between infected tissue and meristematic tissue under cryogenic conditions. Cryotherapy may also be used in germplasm storage, (Wang, and Valkonen, 2008)
According to (Wang *et al.* 2018), cryotherapy has been effectively integrated with thermotherapy for elimination of hard-to-eliminate viruses. Raspberry bushy dwarf virus and apple stem grooving virus have been treated through this combination.
The sustainable production of pome fruit crops depends largely upon the availability of healthy planting material that is virus-free. The multiplication and distribution of plants through virus- and viroid-free sources is not only important for controlling viruses in pome fruits but also for long-term breeding programmes and international transfer of plant genetic material. Different biotechnological techniques including shoot tip culture, micrografting, thermotherapy, chemotherapy, and cryotherapy of shoot tips have been utilized with different levels of success for the elimination of viruses in pome fruit species, (Bettoni, *et al.*, 2016). The efficiency of virus elimination can also be improved by combining two or more techniques in the process. Accurate detection technique is also necessary for verifying the successful elimination of virus and developing proper disease management strategy. Advances in biotechnology have helped in the development of several sensitive and reliable virus-detection techniques. This review discusses the recent advances in biotechnological techniques for producing healthy and virus-free pome fruit planting material, (Bettoni *et al.*, 2024).

3.8. Electrotherapy

Electrotherapy is a new approach where electrical treatment is done on infected plant parts in a controlled way. It may affect the virus particles and cells infected by the virus but allows selective regeneration of tissues. Even though there is some potential in electrotherapy, it is less standardized than meristem culture and thermotherapy. Electrical strength, treatment time, tissues and genotype of plant affect the result. Since too much electrical treatment might damage tissues, the process should be optimized prior to commercialization. Current reviews discuss electrotherapy as one of complementary in vitro approaches to virus elimination, (Singh and Kaur 2016).

3.9 Micrografting

Micrografting involves the grafting of small shoot tip or meristem onto a suitable rootstock using in vitro technique. It is very useful in woody fruit species, where isolated meristems regenerate poorly, (Wang, *et al.,* 2022). It has been used for elimination of viruses in citrus since a long time back. Wang *et al.* provided a review

of micrografting for citrus and other woody species and showed that micrografting may be combined with thermotherapy for better pathogen elimination. The technique is also useful since it offers a physiological link with rootstock, (Hussain, *et al.*, 2014).

Table-1: Examples of in vitro thermotherapy treatment followed by shoot tip culture for virus elimination

Plant species and cultivars or genotypes	Viruses	Temperature and duration	Shoot survival after thermotherapy (%)	Size of shoot tips (mm)	Shoot tip survival (%)	Virus-free frequency (%)
Grapevine 'Bidaneh Sefid' and 'Shahroodi'	GFLV	40 °C/30 °C, (day/night), 7 weeks	NT	NT	NT	100
Grapevine 'Agiorgitiko'	GLRaV-1 and GRSPaV	40 °C/37 °C, (day/night), 1 weeks	53	0.1–0.2 (Meristem) 5.0 (Shoot tip)	56 (Meristem) 88 (Shoot tip)	62 (Meristem) 38 (Shoot tip)
Grapevine 'Kober 5BB'	GVA, GFLV, grapevine fleck virus (GFkV), GLRaV-1 and GLRaV-3	37 °C, 48 days	100	NS	NT	100 (GFLV), 70 (GVA), 25 (GFLaV-1), 25 (GLRaV-3) and 0 (GFKV)
Fig 'Biadi' and 'Aswad'	Fig leaf mottle-associated virus-1 and 2 (FLMaV-1 and FLMaV-2), and fig mosaic virus (FMV)	35 °C, 30 days	NT	6.0	39 (Biadi) 42 (Aswad)	0–81
Raspberry 'Gatineau'	Raspberry bushy dwarf virus (RBDV)	37 °C, 21 days	NT	0.2–0.3	NT	39
Black raspberry (cultivars not specified)	Black raspberry necrosis virus (BRNV)	29 °C and 38 °C (in 4-h interval), 5 weeks	NT	NS	90	100
Caper (cultivars not specified)	Caper latent virus (CapLV)	38 °C, 45–60 days	97	0.3–0.4	60–93	89–93

NS not specified, *NT* not tested
Source, Wang *et al. (2018*

Table-2: Some examples of in vitro chemotherapy followed by thermotherapy for virus eradication

Plant species and cultivars or genotypes	Viruses	Antiviral chemicals (mg/L)	Survival of the treated shoots (%)	Survival of shoot tips (%)	Virus-free frequency (%)
Herbaceous crops					
Garlic (10 accessions)	OYDV, LYSV, GCLV, shallot latent virus (SLV) and MbFV	Ribavirin (50)	NT	NT	100 (OYDV, SLV and LY 90 (GLCV) and 88 (Mb
Oca (8 accessions), ulluco (5 acces-sions), arracacha 'Racacha Blanca'	Papaya mosaic virus (PapMV), arra-cacha virus B (AVB) and ullucus mild mottle virus (UMMV) in Oca; PapMV, ullucus virus *C* (UVC), UMMV, ullucus mosaic virus (UMV) in ulluco; arracacha A virus (AVA) in arracacha	Ribavirin (50)	≥80%	NT	(% not specified) free of vir in 7 accessions, and infected with UMMV in accession in oca, free viruses in 5 accessions in ulluco, and free of viru arracacha
Potato 'Baraka'	PVY	Ribavirin (20)	NT	NT	83
Potato 'F9-99'	PLRV and PVY	Ribavirin (20)	45–85	NT	26–60 (PLRV) 98–100 (PVY)
Potato 'Diamond'	PVY	Ribavirin (20)	26	NT	43
Begonia × semperflorens	PNRSV	Ribavirin (20)	NT	NT	58
Chrysanthemum 'Regol Time'	Chrysanthemum B virus (CVB)	2-Thiouracil (40)	NT	NT	27
Chrysanthemum 'Regol Time'	Tomato aspermy virus (TAV)	Ribavirin (10)	NT	NT	52
Herbaceous crops					
Cassava 'Cidade Rica', 'Riqueza II', 'Mandioca Lagoa', 'CM-425/7', 'Sabará', 'Sauma'	Cassava frogskin disease (CFSD) and cassava vein mosaic virus (CsVMV)	Tetracycline (5–15)	100	NT	100 (CFSD) 98 (CsVMV)
Woody plants					
Apple 'JonagoId', pear 'Pierre Cor-neille', raspberry 'Norna'	ACLSV and ASGV in apple, ACLSV in pear and raspberry vein chlorosis virus (RVCV) in raspberry	Ribavirin (50)	42 (apple), 80 (pear) and 34 (rasp-berry)	NT	100 (ACLSV and ASGV apple), 83 (ACLSV in pear (RVCV in raspberry)
Apple 'Xinhongjiangjun'	ASPV, ASGV and ACLSV	Ribavirin (25)	90	59 (apical shoots) and 100 (axillary shoots)	94 (apical shoots) 100 (axillary shoots)
Pear 'Pierre Corneille'	ACLSV	Ribavirin (50)	71	NT	90
Pear 'Jinshui no. 2'	ASGV, ACLSV	Ribavirin (25)	100	71	100

Source, Wang *et al. (2018)*

Table 3. Some examples of thermotherapy followed by in vitro micrografting or shoot-tip cryotherapy for virus eradication

Method	Plant species and cultivars or genotypes	Viruses	Shoot-tip size (mm)	Survival of treated shoots (%)	Success of micrografting or shoot regrowth in cryotherapy (%)	Vi fr (%
Thermotherapy + micrografting	Citrus 'Early satsuma mandarin', 'Yuzu', 'Shiranuhi'	Citrus tristeza virus (CTV), satsuma dwarf virus (SDV),	0.3	NT	13–75	76 (S (C

		citrus tatter leaf virus (CTLV)				
Thermotherapy + micrografting	Citrus hybrid 'Kinnow'	Indian citrus ringspot virus (ICRSV)	0.7	42 (50°C for 120 min, water bath); 28 (50°C for 120 min, hot air); NT (38°C for 9 days, growth chamber)	38 (water bath); 26 (hot air); 40 (growth chamber)	37 ba air (g ch
Thermotherapy + micrografting	Citrus 'Ehimekashi dai28go', 'Setoka', 'Shiranuhi', 'Haraejosaeng', 'Pungkwang', 'Samdajosaeng'	Citrus tristeza virus (CTV)	NS	NT	NT	10
Thermotherapy + cryotherapy	Raspberry genotype 'Z13'	Raspberry bushy dwarf virus (RBDV)	1.0	NT	30 (shoot regrowth)	35
Thermotherapy + cryotherapy	Garlic 'Jonas'	Onion yellow dwarf virus (OYDV), shallot virus Y (SYSV), garlic common latent virus (GCLV)	NS	NT	40 (shoot regrowth)	90 10 80

Source, Wang *et al. (2018)*

Note: NT = not tested; NS = not specified. Virus-free frequency indicates the proportion of regenerated or grafted plants confirmed free from the respective virus. Thermotherapy conditions, explant size and genotype strongly influence both regeneration and virus-eradication efficiency.

Table-4: Virus and viroid diseases infecting pome fruit trees

Family	Genus	Species (abbreviation)	Genome	Particle shape	Disease
Viruses					
Betaflexiviridae	*Capillovirus*	Apple stem grooving virus (ASGV)	(+) ssRNA	Filamentous	Mostly latent on apple cultiva healthy; stem grooving on se as *Malus pumila* 'Virginia Crab
	Foveavirus	Apple stem pitting virus (ASPV)	(+) ssRNA	Filamentous	Mostly latent on apple cultivar stem appears on susceptible ap and 'Reinette Clochard' and plants *M. pumila* 'Virginia Crab
		Apple green crinkle associated virus (AGCaV)	(+) ssRNA	Filamentous	Potential causal relationship to
		Apricot latent virus (ApLV)	(+) ssRNA	Filamentous	–
	Tepovirus	Prunus virus T	(+) ssRNA	Filamentous	Latent and asymptomatic transmissible to *M. domestica* a
	Robigovirus	Pome virus Greece	(+) ssRNA	Filamentous	Latent and asymptomatic transmissible to *M. domestica* a
Bromoviridae	*Ilarvirus*	Apple mosaic virus (ApMV)	(+) ssRNA	Icosahedral	Pale yellow to bright cream ir along major veins on spring become necrotic on severely exposure to summer sun and he
		Apple necrotic mosaic virus (ApNMV)	(+) ssRNA	Spherical	Mosaic symptoms on apple symptoms caused by ApMV
		Apple ilarvirus 1	–	–	–
		Apple ilarvirus 2	–	–	–
		Prunus necrotic ringspot virus (PNRSV)	(+) ssRNA	–	–

		Solanum nigrum ilarvirus (SNIV)	–	–	–
Fimoviridae	*Emaravirus*	Pear chlorotic leaf spot-associated virus (PCLSaV)	(–) ssRNA	Spherical	Associated with pear chlorotic l
Geminiviridae	Unassigned	Apple geminivirus (AGV)	ssDNA	Twinned	–
Luteoviridae	*Luteovirus*	Apple luteovirus 1 (ALV-1)	(+) ssRNA	Icosahedral	–
	Unassigned	Apple-associated luteovirus (AaLV)	(+) ssRNA	Icosahedral	–
Partitiviridae	*Alphapartitivirus*	*Pyrus pyrifolia* partitivirus 2 (PpPV2)	dsRNA	Icosahedral	–
	Deltapartitivirus	*Pyrus pyrifolia* cryptic virus (PpCV)	dsRNA	–	–
Phenuiviridae	*Coguvirus*	Citrus virus A (CiVA)	(+) ssRNA	–	–
	Phlebovirus	Citrus concave gum-associated virus (CCGaV)	(–) ssRNA	Filamentous	–
	Rubodvirus	Apple rubbery wood virus 1 (ARWaV-1)	(–) ssRNA	–	Potential association with apple
		Apple rubbery wood virus 2 (ARWaV-2)	(–) ssRNA	–	Potential association with apple
Secoviridae	*Cheravirus*	Apple latent spherical virus (ALSV)	(+) ssRNA	Spherical	Latent on apple cultivars
	Nepovirus	Cherry leaf roll virus (CLRV)	(+) ssRNA	Isometric	–
		Tomato ringspot virus (ToRSV)	(+) ssRNA	Icosahedral	Associated with apple union ne
Tombusviridae	Unassigned	Apple tombus-like virus 1	–	–	–
		Apple tombus-like virus 2	–	–	–
Unassigned	Unassigned	Temperate fruit decay-associated virus (TFDaV)	ssDNA	–	–
		Apple picorna-like virus 1	–	–	–
Viroid					
Avsunviroidae	*Pelamoviroid*	Apple hammerhead viroid (AHVd)	ssRNA	Rod-like or quasi-rod-like	–
Pospiviroidae	*Apscaviroid*	Apple dimple fruit viroid (ADFVd)	ssRNA	Rod-like or quasi-rod-like	Discolored spots more or less d
		Apple scar skin viroid (ASSVd)	ssRNA	Rod-like or quasi-rod-like	Scar skin or dapple symptoms
		Pear blister canker viroid (PBCVd)	ssRNA	Rod-like or quasi-rod-like	Pear blister canker

(Source, Bettoni, *et al.*, 2024)

4. Factors that Influence Efficiency of Virus Elimination

The procedure for virus elimination is not standardized as the efficiency of the chosen therapy method varies depending on certain biological and technical factors. One of the most important factors is the virus species as different virus strains vary in terms of their replication, mobility, thermosensitivity and localization in the plant tissue, (Cooper, 2013). While some viruses can easily be eliminated from shoot tips, others are capable of persisting in meristem tissues. The size of an explant plays an important role in the process as smaller meristems usually have higher chances of being free from viruses although they survive less often. Larger meristems usually regenerate easier but can contain viruses (Wang, and Hu, 2005) Regeneration ability and sensitivity to thermotherapy, chemotherapy and cryotherapy of the host genotype plays an important role so the protocol created for one cultivar cannot necessarily be used for another cultivar. Temperature and duration of treatment should also be strictly regulated since the increase of temperature and/or treatment period will lead to increased elimination efficiency and increased tissue damage. Physiological state of the donor plant is also significant, (Hong, and Wang, 2026).

6. Phytotoxicity and Tissue Damage

The problem of plant tissues damage is one of the main disadvantages of in vitro virus-elimination protocols. The methods are created to make such conditions which will suppress or kill the virus in plant tissue but it can continue growing. Thermotherapy can cause chlorosis, necrosis, retarded growth and decreased regeneration ability. Chemotherapy can impair cell division and shoots formation, (Awad, *et al.*, 2019). Cryotherapy can result in cryoinjury due to improper dehydration and cooling. Electrotherapy can cause tissue damage due to high electrical intensity. Phytotoxicity associated with in vitro virus-elimination protocols has been reviewed by researchers and authors have pointed out that regeneration capacity, morphology, plant development can be negatively influenced by such treatments. Genotype, the length of treatment, temperature, concentration of chemicals and electrical intensity are key factors of tissue damage. Therefore, the success of virus elimination should not be evaluated by virus-negative plants only. For the protocol to be successful, it must produce vigorous and genetically stable plants, (Magyar-Tábori, *et al.*, 2021).

7. Molecular Diagnostics of Virus-Free Plants

Confirmation of virus-free state is one of the key stages of the propagation procedure. It is possible that the plant which looks healthy in tissue culture still has latent viruses. ELISA is a common serological technique for detection of a variety of plant viruses. Polymerase chain reaction and reverse-transcription PCR increase sensitivity in case of DNA and RNA viruses respectively. Quantitative RT-PCR can give more information about the virus titer, (Devi, *et al.,* 2024). High throughput sequencing and metagenomics open opportunities for detection of different types of pathogens including unexpected, (Roy, *et al.,* 2024). Advances in detection technology for apple and grapevine have proved the effectiveness of indexing due to increased sensitivity, speed and precision especially in low-titer and latent infection. Thus, the virus-free propagation procedure must be combined with molecular diagnostics techniques, (Verma, *et al.,* 2004).

8. Crop-Wise Role of Tissue Culture in Virus-Free Planting Material

8.1 Banana

Another key example of applications of tissue culture in obtaining virus-free planting material is banana. This plant is mainly propagated through vegetative propagation via suckers and thus the utilization of infected planting material may result in increased accumulation and spread of viruses. The main methods in obtaining plants from this crop are based on meristem culture of small apical parts of selected mother plants. Thermotherapy and chemotherapy may be applied prior to or during meristem culture to enhance virus elimination process, (Helliot, *et al.*, 2004). The significance of tissue culture in banana has not been limited to only the process of virus elimination but also includes rapid multiplication, conservation of germplasm and genetic improvement, (Suman, 2017). Some recent reviews consider tissue culture as a platform for biotechnological improvement of banana.

8.2 Potato

Potato is an example of one of the plants where virus-free planting materials are crucial since the plant propagates through tubers and therefore viruses can be accumulated over several generations.

According to Wang *et al.* (2018), thermotherapy followed by shoot tip culture eliminated viruses in 33% of potato variety Diamond which was infected with PVY. In varieties Burren and Binella, frequencies of 56-81% virus free plants were achieved. In case of PVX, heat therapy at 32/42°C day/night for 35 days achieved 80% virus-free plants in the genotype tested.

8.3 Strawberry

Strawberry is usually propagated using runners; therefore, the health of mother plants is crucial for nursery production since virus complexes may negatively affect plant vitality, runner formation, and fruit production, (Lee, *et al.*, 2026) Meristem culture, shoot tip culture, and thermotherapy are some methods for cleaning strawberries. Cryotherapy is another option that offers chances for virus elimination and germplasm conservation. Recent review by (Thanuja *et al,* 2025). features strawberry as one of the most important horticultural crops for development of in vitro virus-elimination approaches.

8.4 Grapevine

Grapevine is a perennial crop where infections caused by viruses and viroids may persist for a long time, (Martelli, 2017). As vineyards are established using cuttings, grafting material, and certified nursery plants, the state of propagation material is especially important.

Grapevine may be infected by a number of viruses, including grapevine leafroll-associated viruses, grapevine fanleaf virus, and other viruses, (Burger, *et al.*, 2017). Tissue culture sanitation includes thermotherapy, shoot tip culture, chemotherapy, and sometimes micrografting. As point out, the use of virus-free planting material is crucial for sustainable production of apple and grapevine crops and highlights the importance of integration of eradication treatments with virus-indexing techniques, (Kang, *et al.*, 2025).

8.5 Apple and Pear

Apple and pear are perennial fruit trees where systemic virus and viroid infections can last for a lot of years. Since orchards require considerable investment and plants remain productive for a number of years, the use of infected nursery material can lead to long-term results, (Hong, and Wang, 2026).

Thermotherapy along with apical meristem culture has been used for elimination of viruses, including apple stem grooving virus and apple chlorotic leaf spot virus. The recent literature indicates that thermotherapy along with meristem culture can be used to obtain virus-free apple material (Kang, *et al.*, 2025). Similarly, thermotherapy and meristem culture have been used for elimination of viruses from pears. In particular, in 2026, a special protocol has been published on thermotherapy, meristem culture, and transplantation of virus-free pear.

8.6 Citrus

Citrus species are mainly propagated using grafting or budding techniques that make the health status of scions and rootstocks highly important. Citrus tristeza virus and other systemic pathogens can be spread by infected propagation material. Micrografting and shoot-tip culture are especially useful for sanitation of citrus as small shoot tips can be propagated on clean rootstocks, (Singh, *et al.*, 2019) Thermotherapy can be used to suppress the reproduction of viruses before shoot tip excision. The historical use of micrografting for removal of virus infection in citrus is well known, and further research extended the use of this technique to other virus and viroid diseases of citrus, (Abbas, *et al.*, 2008).

8.7 Garlic and Ginger

Garlic and ginger are examples of vegetatively propagated spices that suffer from accumulation of virus complexes through propagation cycles. Therefore, infected cloves or rhizomes can continuously spread virus infection. Meristem and shoot-tip culture will give virus free starting material while thermotherapy will increase the chances of virus elimination (Krishna, *et al.*, 2022). Garlic is a very good example of such plants as different viruses can appear in one plant, (Benke, *et al.*, 2023) The results of Wang *et al.* for several garlic varieties showed that frequencies of virus free plants were 63-100% for onion yellow dwarf virus and 80-100% for leek yellow stripe virus using thermotherapy and shoot-tip culture.

3.8 Ornamentals

Ornamental plants are also often propagated vegetatively that makes them prone to accumulation and spreading of systemic viruses. Chrysanthemum, carnation, gerbera, orchid, begonia, lily and bulbous ornamentals can be sanitized using tissue culture. Commercial value of these plants makes fast growing of healthy plants very important. Meristem culture provides rapid multiplication of selected plants after virus elimination, (Mehbub, *et al.,* 2022).

The Pelargonium species displayed varying sensitivity to PGR treatments. The highest percentage of bud shoots, plantlet formation, plantlet height, and root formation were recorded in 'Zonal' and 'Regal' pelargonium on media A and C, respectively. RT-PCR analysis indicated the presence of viruses in plantlets grown from buds. However, the combined use of thermotherapy and meristem culture resulted in 70% and 60% virus-free plantlets of 'Regal' and 'Zonal' Pelargonium, respectively, (Alavijeh, *et al.*, 2025).

9. Role of Genetic Fidelity in Virus-Free Plant Production

Elimination of the virus is not sufficient if the regenerated plant does not maintain the genetic composition of the superior mother plant. Thus, genetic fidelity becomes an integral part of a good virus-free plant propagation system, (Wu *et al.,* 2026).

Direct regeneration through meristem or axillary tissues is preferable since continued callus culture increases the possibility of somaclonal variation. The regenerated plants are evaluated morphologically while molecular markers are used to confirm their genetic fidelity. Therefore, a commercial virus-free plant should meet two criteria; it should be free of pathogens and genetically pure.

10. Acclimatization of Virus-Free Plantlets

Plantlets produced under laboratory conditions have physiological difference from those grown under field conditions, (Lu, *et al.*, 2024). They are produced in a humid, controlled light environment and a medium rich in nutrients. Direct transfer to field conditions may lead to transplant shock. Acclimatization involves reducing humidity and light controlled exposure while adapting to ex vitro media. The plantlets are transplanted in sterile or pathogen-free media and kept under protected conditions prior to being introduced into nursery conditions. Acclimatization becomes a very important phase in virus-free plant programs since failure of healthy plantlets at this stage makes the whole process uneconomical ornamental crops, namely begonia and chrysanthemum.

11. Establishment of Virus-Free Nuclear Stock

The final goal of tissue culture based sanitation is the establishment of virus free nuclear stock for apple and pear, (Zuļģe, *et al.*, 2017). If the regeneration plant is tested negative for the target pathogens, then it is kept under protected conditions.

This stock is used as a source of material for further multiplication stages. Indexing is required periodically due to reinfection by the vector, contaminated instruments, neighboring infected plants etc. In the production system, this requires maintaining sanitation and vector exclusion at all levels during nursery multiplication.

12. Advantages of Tissue Culture for Virus-Free Planting Material

Tissue culture gives some advantages as compared to conventional propagation method in case when healthy planting material is the main goal. The first major advantage is the availability of elite plants that are infected but carry desirable horticultural traits. These plants do not have to be discarded, but rather small meristematic parts can be separated and regenerated. The second major advantage is rapid multiplication since once virus free plant is produced, it allows multiplying shoots and producing many propagules in a relatively short time period. The third advantage is the ability to produce year-round because the production process does not depend on seasonal propagation but takes place in the controlled environment, (Roy, 2025).

The fourth advantage is the potential to combine pathogen elimination with molecular indexing and genetic fidelity testing. The fifth advantage is its compatibility with long-term germplasm storage through cryopreservation.

Table 5. Major Micropropagation Techniques and Their Applications in Horticultural Crops

Technique	Description	Key Advantages	Limitations	Major Horticultural Crops
Meristem Culture	Isolation and in vitro culture of apical or axillary meristems to regenerate healthy, virus-free plants.	Produces pathogen-free planting material; maintains genetic fidelity.	Skilled handling required; relatively low initial multiplication rate.	Banana, potato, sugarcane, strawberry
Axillary Bud Proliferation	Multiplication of shoots through the activation and development of axillary buds.	High clonal fidelity; suitable for large-scale commercial propagation.	Requires repeated subculturing; contamination may occur.	Ornamentals, banana, grape
Organogenesis	Induction of shoots and/or roots directly from explants or indirectly through a callus phase.	High regeneration potential; useful for genetic transformation and plant improvement.	Prolonged callus phase may cause somaclonal variation.	Tomato, pepper, apple, citrus
Somatic Embryogenesis	Formation and development of embryo-like structures from somatic cells under suitable culture conditions.	Suitable for mass propagation and automation; enables synthetic seed production.	Strong genotype dependence; possibility of somaclonal variation.	Mango, citrus, coffee, ornamentals
Temporary Immersion Systems (TIS)	Periodic immersion of explants in liquid culture medium using automated immersion cycles.	High multiplication rate; improved nutrient uptake; reduced hyperhydricity and labor requirement.	Requires specialized equipment and monitoring; contamination can spread rapidly.	Banana, stevia, orchids, medicinal plants
Bioreactor-Based Propagation	Large-scale multiplication of plant tissues, shoots or embryos in automated liquid-culture bioreactors.	High production capacity; efficient use of labor and culture medium; cost-effective at large scale.	High initial investment; requires technical expertise and process control.	Banana, ornamentals, spices
Synthetic Seed Technology	Encapsulation of somatic embryos, shoot buds or other propagules in a protective artificial seed coat for storage, transport and planting.	Easy handling and transportation; potential for short- to medium-term storage and distribution.	Low conversion or regeneration rate in some species; storage protocols are species-specific.	Orchids, medicinal plants, citrus

Protoplast Culture and Fusion	Isolation of plant protoplasts followed by regeneration or fusion of genetically different protoplasts to produce somatic hybrids.	Enables novel hybrid development; useful for genetic manipulation and genome-editing research.	Regeneration is difficult; highly species- and genotype-dependent.	Citrus, brassicas, ornamentals

(Source, Bhardwaj, *et al.*, 2025)

13. Integration of Tissue Culture and Molecular Diagnostics

The combination of tissue culture and molecular diagnostics is one of the most significant innovations in modern virus-free planting material production. The traditional way was very much based on symptom observation and use of indicator plants. The modern systems are increasingly using ELISA, PCR, RT-PCR, qRT-PCR and sequencing technologies.

This combination can be depicted as:

Infected elite plant → virus elimination through tissue culture → regenerated plant → molecular indexing → virus free nuclear stock.

The advantage of this approach is that tissue culture either eliminates or suppresses the pathogen and molecular diagnostics checks whether it worked. According to Kang *et al.* 2025), the advances in virus detection technologies improved sensitivity, speed and accuracy of virus indexing of tissue cultured apple and grapevine plants.

Table 6. Major diagnostic techniques used to confirm virus-free planting material

Diagnostic technique	Basic principle	Major advantage	Limitation	Application
ELISA	Antibody-based detection of viral proteins	Relatively simple and suitable for large numbers of samples	Requires suitable antibodies; sensitivity may be lower for low-titer infections	Routine virus indexing
PCR	Amplification of viral DNA	High sensitivity and specificity	Mainly suitable for DNA viruses unless preceded by reverse transcription	Detection of DNA viruses
RT-PCR	Reverse transcription followed by PCR amplification	Highly useful for RNA viruses	Requires RNA extraction and careful handling	Detection of RNA viruses
qRT-PCR	Real-time amplification and fluorescence detection	High sensitivity and quantitative capability	More expensive and technically demanding	Low-titer and latent infections
High-throughput sequencing	Sequencing of nucleic acids present in the sample	Can detect multiple known and unknown viruses	Relatively expensive and requires bioinformatic analysis	Comprehensive virus/viroid indexing
Indicator plants	Biological response following inoculation	Can detect infectivity and biological effects	Slow and sometimes insensitive	Historical/confirmatory indexing

14. Limitations

The first major limitation of virus elimination through tissue cultures is that full virus elimination is not assured for each crop-virus combination since some viruses may infect meristematic tissues whereas others might escape diagnosis due to low sensitivity of the technique.

The second major limitation is the problem of tissue damage that occurs during thermotherapy, chemotherapy, cryotherapy and electrotherapy due to over-application of treatments.

The third limitation is genotype specificity which means that different cultivars of the same species may behave differently in relation to the same culture medium and the virus-elimination technique.

The fourth limitation is that the technology requires considerable investment in laboratories and personnel.

The fifth limitation is contamination during multiplication and acclimatization.

The sixth limitation is the necessity of continuous indexing because virus-free plants can become infected after leaving tissue-cultures.

15. Emerging Technologies

The future of production of virus-free planting material will be more and more connected with combining conventional tissue-culture technology with biotechnological techniques.

Cryotherapy may be considered as an interesting combination of pathogen elimination and conservation of germplasm. Temporary immersion system may be effective for increasing the multiplication phase after virus elimination by eliminating manual work and increasing propagation.
High throughput sequencing is capable of detecting complex virus communities and unknown pathogens. It is especially relevant for perennial fruit crops that may carry multiple latent viruses.
RNA interference may also be considered as one of the possible approaches for virus suppression. Genome-editing technology (CRISPR/Cas systems) opens the ways for creation of virus resistant cultivars though its efficient application usually requires tissue culture system for regeneration.

16. An Integrated System for Commercial Production

A good commercial production system for producing virus-free planting material must involve a series of stages of strict control.
Selection of the elite mother plant with desired horticultural features should be done first. Then indexing for viruses and viroids should take place. In case of detection of infection, the elimination procedure should follow (thermotherapy + meristem culture).
Then the regenerated plants should be multiplied under aseptic conditions, rooted and acclimatized. Further, molecular indexing is carried out with the use of diagnostic methods. Virus-negative plants only should be introduced to the nuclear stock.
Protected conditions should be maintained in relation to the nuclear stock in order to produce the foundation planting material. Nursery multiplication should be done under vector exclusion and sanitation conditions with periodic indexing. Thus, virus-free planting-material production can be seen as an ongoing health management system, not just a tissue culture treatment.

17. CONCLUSION

Plant tissue culture has become one of the major technologies for the production of virus-free planting material in horticultural crops. This is especially true for vegetatively propagated crops because viruses and viroids can accumulate in them during successive generations. Meristem and shoot-tip culture are based on the ability of actively growing apical tissues to have lower numbers of systemic pathogens. Thermotherapy, chemotherapy, cryotherapy, electrotherapy and micrografting can significantly increase the efficiency of the meristem-based technique if used either separately or in combination. Experiments with several horticultural crops show that combined methods are much more efficient compared to individual techniques for certain virus-host pairs. Tissue culture alone does not guarantee the presence of virus-free status of the plant. Molecular indexing through ELISA, PCR, RT-PCR, qRT-PCR and sequencing is needed for detecting target viruses and viroids. Modern research on apples and grapevines shows increasing significance of combining virus-elimination treatments and sensitive diagnostic methods.
The main future direction of development lies in combining the technology of tissue culture with cryotherapy, temporary immersion systems, high-throughput sequencing, RNA interference, genome editing, automation and environment control. Such integration will change conventional micropropagation laboratories into modern plant health production facilities.

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