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Virus-Free Strawberry Seedling Production: Advances in Elimination Technologies, Diagnostics and Standardized Propagation Systems

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Abstract

Strawberry is a globally important berry crop, but clonal propagation through runners facilitates the vertical transmission and accumulation of viruses and viroids, leading to seedling degeneration, reduced vigour, poor fruit quality, fruit deformity and yield losses. Strawberry viral diseases are difficult to control because of their high diversity, frequent latent and mixed infections, non-specific symptoms and long-distance dissemination through infected planting materials. In this review, "virus-free seedling" refers to planting material certified negative, within the detection limits of specified assays, for a defined panel of strawberry viruses and viroids at designated propagation stages; it does not imply freedom from all pathogens. Virus-free seedling production, primarily based on shoot-tip culture and supported by thermotherapy, chemotherapy, cryotherapy, anther culture, rapid propagation and molecular diagnostics, is an effective strategy for restoring seedling health and improving propagation quality. Recent advances in high-throughput sequencing, viromics, RT-PCR, RT-qPCR, multiplex PCR, isothermal amplification, lateral-flow assays and protected nursery systems have accelerated the shift from experience-based propagation to standardized, diagnostic-guided and certification-oriented production. This review summarizes the occurrence characteristics of strawberry viral diseases, the theoretical basis of virus elimination, key elimination technologies, molecular diagnostics, quality assessment, rapid propagation and industrial applications. It also proposes an integrated framework linking virus elimination, hierarchical diagnostics, genetic fidelity assessment, protected nursery management, vector control and traceable certification, providing a

reference for standardized production, quality control and industrial application of virus-free strawberry seedlings.

Keywords

Strawberry, Virus-Free Seedling, High-Throughput Sequencing, Viromics, Rapid Reproduction

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most widely cultivated and economically important berry crops worldwide. Its fruit is highly appreciated for its attractive appearance, distinctive aroma, balanced sweet-sour flavour and high nutritional value, including vitamin C, anthocyanins, phenolic acids, flavonoids and dietary fibre. With the rapid development of protected cultivation, substrate-based production, elevated nursery systems and agri-tourism-oriented farming, the demand for high-quality strawberry planting materials has increased substantially. In parallel, strawberry propagation systems have gradually shifted from conventional open-field runner propagation towards plug transplants, tissue-cultured plantlets, elevated nurseries and environmentally controlled production systems [1]. These changes have improved propagation efficiency and seedling uniformity, but they have also increased the need for standardized seedling health management, genetic fidelity control and certification-oriented production systems.

Commercial strawberry is predominantly propagated vegetatively through runners. Although clonal propagation is advantageous for maintaining cultivar identity and rapidly multiplying elite genotypes, it also facilitates the vertical transmission and progressive accumulation of viruses and viroids from mother plants to daughter plantlets. Once infected mother plants enter the nursery chain, viruses can be continuously disseminated through repeated runner multiplication, seedling transportation and regional exchange of planting materials [2]-[5]. Viral infection may lead to seedling degeneration, reduced plant vigour, impaired root development, poor floral bud differentiation, smaller fruit size, increased fruit deformity and yield losses. Therefore, the production of virus-free strawberry seedlings is not only a technical approach for virus and viroid elimination, but also a fundamental prerequisite for maintaining cultivar productivity, fruit quality and sustainable strawberry production.

For clarity, the term "virus-free seedling" in this review does not denote an absolute absence of all infectious agents. Rather, it refers to strawberry seedlings or propagation materials that have been regenerated, propagated or maintained under controlled conditions and have tested negative, within the detection limits of specified assays, for a defined panel of economically relevant strawberry viruses and viroids. This term is narrower than "certified virus-free", which would also include

fungi, bacteria, phytoplasmas, nematodes and other pests. This definition also distinguishes "virus-free seedling" from "healthy seedling", a broader term generally referring to visual vigour or agronomic performance without molecular confirmation, and from "detoxified seedling", which denotes material that has undergone elimination treatment but requires post-treatment diagnostic confirmation before being classified as virus-free.

Strawberry viral diseases are difficult to manage because of their high pathogen diversity, frequent latent infection, mixed infection and non-specific symptoms. More than 20 viruses have been reported to infect strawberry, including strawberry mottle virus (SMoV), strawberry mild yellow edge virus (SMYEV), strawberry crinkle virus (SCV), strawberry vein banding virus (SVBV), strawberry necrotic shock virus (SNSV), strawberry pallidosis-associated virus (SPaV), strawberry polerovirus 1 (SPV-1) and strawberry crinivirus 4 (SCrV-4) [5]-[10]. Among them, SMoV, SMYEV, SCV and SVBV are commonly detected in commercial strawberry production and are frequently associated with aphid-mediated transmission and vegetative propagation [10]-[12]. Some viruses may remain latent or induce only mild symptoms when present as single infections, whereas co-infection can aggravate plant decline and cause more severe losses in yield and fruit quality [13] [14]. In addition, the long-distance movement of infected planting materials can accelerate viral dissemination across production regions, making visual inspection alone insufficient for reliable seedling health assessment [15].

Virus-free seedling production provides an effective strategy for interrupting virus and viroid transmission in clonal propagation systems. The theoretical basis of this approach lies in the uneven distribution of viruses within infected plants. The shoot apical meristem generally contains relatively low viral titres because of rapid cell division, incomplete vascular differentiation and restricted viral movement into meristematic tissues [16] [17]. Accordingly, small shoot tips or meristematic tissues can be excised and regenerated *in vitro* to obtain plantlets with a reduced probability of viral infection. In strawberry, shoot-tip culture has become the most widely used and technically mature method for virus elimination and rapid propagation [18]-[20]. However, virus elimination efficiency is affected by target virus identity, cultivar genotype, explant physiological status, shoot-tip size, culture medium, plant growth regulator balance and post-regeneration testing [21]-[23]. Therefore, successful virus-free strawberry seedling production requires an integrated technical system rather than reliance on a single elimination method.

To improve virus elimination efficiency, shoot-tip culture is often combined with auxiliary treatments such as thermotherapy, chemotherapy, cryotherapy and alternative regeneration pathways. Thermotherapy can suppress viral replication and movement before shoot-tip excision, thereby reducing viral titres in meristematic tissues [21] [24] [25]. Chemotherapy can inhibit viral replication during *in vitro* culture, but its application requires careful optimization of antiviral compound concentration and exposure duration to avoid phytotoxicity and impaired

plantlet vigour [26] [27]. Cryotherapy has received increasing attention because it can contribute simultaneously to virus elimination and long-term germplasm conservation, particularly for valuable nuclear stock materials and genetic resources [21] [28]. In addition, anther culture and other regeneration pathways may provide complementary options for virus elimination and germplasm innovation, although their routine commercial application remains limited by genotype dependence, ploidy instability and somaclonal variation [22] [29].

Reliable virus detection is another core component of certified virus-free strawberry seedling production. Traditional methods, including indicator plant assays and enzyme-linked immunosorbent assay (ELISA), have contributed to early virus diagnosis, but they are often limited by long detection cycles, low sensitivity or dependence on symptom expression. Molecular diagnostic technologies, such as RT-PCR, RT-qPCR and multiplex PCR, have greatly improved the sensitivity, specificity and throughput of strawberry virus detection [10] [30] [31]. In particular, multiplex RT-PCR enables simultaneous detection of multiple major strawberry viruses and is suitable for large-scale screening of nursery materials [10]. High-throughput sequencing (HTS) has further expanded virus diagnosis from targeted detection to broad-spectrum virome analysis and novel virus discovery, making it especially valuable for nuclear stock screening, germplasm quarantine and certification of high-value materials [6] [32] [33]. Meanwhile, isothermal amplification methods, such as RT-LAMP and RT-RPA combined with lateral-flow strips, provide rapid and field-deployable tools for nursery monitoring and pre-dispatch seedling inspection [34]-[36].

Despite substantial progress, current research and application of virus-free strawberry seedling production remain limited by insufficient systematic comparisons among elimination and diagnostic methods, the separate evaluation of virus elimination efficiency from regeneration capacity, genetic fidelity, seedling vigour and commercial scalability, and the tendency to treat diagnostic technologies as independent tools rather than as components of a hierarchical quality-control strategy. Moreover, the links among virus elimination, molecular testing, protected nursery management, vector control, seedling certification and traceability have not yet been fully integrated into a unified framework, restricting the translation of laboratory-scale protocols into standardized, scalable and certification-oriented nursery production systems.

Therefore, this review summarizes recent advances in virus-free strawberry seedling production, with emphasis on the occurrence characteristics of strawberry viral diseases, the theoretical basis of virus elimination, key elimination technologies, molecular diagnostic methods, quality assessment strategies, rapid propagation systems and industrial applications. More importantly, this review proposes an integrated framework linking virus elimination, hierarchical diagnostics, genetic fidelity assessment, protected nursery production, vector management and traceable certification. By synthesizing technical progress and remaining challenges, this review aims to provide a theoretical and practical reference for the standard-

ized production, quality control and large-scale application of certified virus-free strawberry seedlings.

The overall conceptual framework of this review is summarized in Figure 1, which links virus elimination, molecular diagnostics, genetic fidelity assessment, protected propagation and traceable certification into an integrated production system.

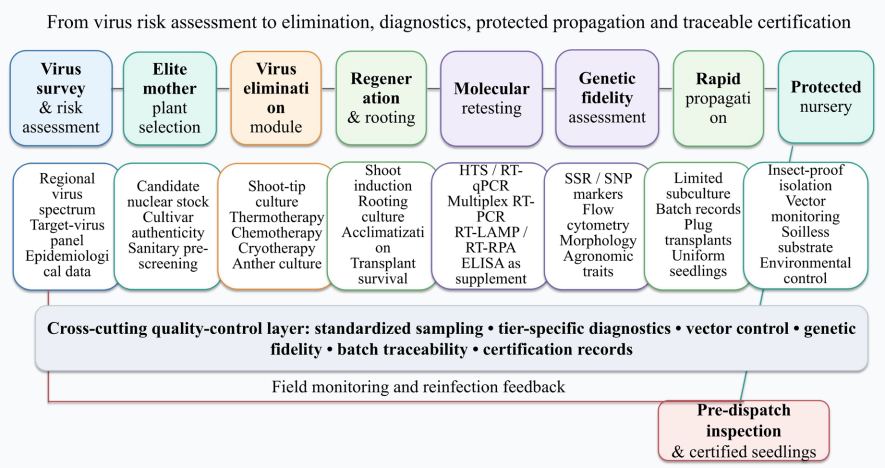

Figure 1. Integrated framework for certified virus-free strawberry seedling production.

2. Occurrence Characteristics of Strawberry Viral Diseases and Theoretical Basis for Virus-Free Seedling Production

2.1. Diversity of Strawberry Viruses, Latent Infection and Mixed Infection

Strawberry is affected by a broad and continuously expanding spectrum of viruses, which represents one of the major biological constraints on the production and certification of certified virus-free planting materials. To date, more than 20 viruses have been reported to infect strawberry, including strawberry mottle virus (SMoV), strawberry mild yellow edge virus (SMYEV), strawberry crinkle virus (SCV), strawberry vein banding virus (SVBV), strawberry necrotic shock virus (SNSV), strawberry latent ringspot virus (SLRSV), strawberry pallidosis-associated virus (SPaV), strawberry polerovirus 1 (SPV-1) and strawberry crinivirus 4 (SCrV-4) [5]-[12]. These viruses differ markedly in genome organization, host range, vector specificity, tissue distribution, transmission route and pathogenicity. Among them, SMoV, SMYEV, SCV and SVBV are generally regarded as major viruses associated with commercial strawberry production because they can be transmitted by aphid vectors and further disseminated through vegetatively propagated nursery materials [10]-[14].

The rapid development of high-throughput sequencing, metagenomic analysis and multiplex molecular diagnostics has substantially expanded the known strawberry virome. Recent studies have identified novel or emerging strawberry-associated viruses and revealed that the viral spectrum in strawberry germplasm, pro-

duction nurseries and imported plant materials is more complex than previously recognized [5]-[9] [11]. For example, sequencing-based surveys have revealed new viral genetic diversity in strawberry germplasm and provided sequence resources for the development of improved RT-qPCR assays [5]. High-throughput sequencing has also been used in post-entry quarantine to detect strawberry viruses in imported materials, demonstrating its value for germplasm health assessment and biosecurity surveillance [6]. In addition, the identification of strawberry virus A, the regional spread of SPV-1 and the discovery of new strawberry-infecting rhabdoviruses further indicate that the composition of strawberry viral communities is still being updated [7]-[9] [11].

A key challenge in strawberry virus management is that viral symptoms are often non-specific, unstable or even absent under field conditions. Infected plants may show growth retardation, chlorosis, mottling, leaf crinkling, leaf curling, vein banding, reduced runner production, impaired floral bud differentiation, reduced fruit set, small fruit size and increased fruit deformity. However, these symptoms can be influenced by cultivar genotype, plant age, environmental conditions, nutritional status and co-infection status. Some viruses may remain latent or cause only mild symptoms when present as single infections, whereas mixed infections can intensify disease severity and accelerate seedling degeneration [12]-[14]. Therefore, visual diagnosis cannot provide reliable evidence for judging whether planting materials are truly virus-free.

Mixed infection is particularly important in strawberry because different viruses may coexist in the same mother plant or production seedling. In vegetatively propagated crops, infected mother plants can serve as long-term reservoirs of multiple viruses. During repeated runner propagation, these viruses may accumulate and spread to daughter plantlets, resulting in a continuous transmission chain from nuclear stock plants to commercial seedlings. Once infected seedlings are transported across production regions, they may introduce viruses into new nursery systems and production fields. Moreover, aphid vectors, whiteflies and nematodes can further promote secondary spread, depending on the virus species involved [10] [14] [15]. These characteristics explain why strawberry viral diseases are difficult to manage through field sanitation alone and why virus-free seedling production must be combined with molecular diagnostics, vector control, protected nursery management and certification systems.

To clarify the practical relevance of different viruses to virus-free seedling production, the major strawberry viruses associated with nursery propagation, their transmission routes, typical symptoms and recommended diagnostic methods are summarized in **Table 1**. This comparison provides a basis for selecting target viruses in regional certification programs and for designing hierarchical diagnostic strategies during mother plant screening, post-elimination testing and pre-dispatch inspection.

As shown in **Table 1**, aphid-transmitted viruses such as SMoV, SMYEV, SCV and SVBV should be prioritized in routine certification because they are closely

associated with vegetative propagation and nursery dissemination. Based on the virus characteristics summarized in Table 1, Figure 2 further illustrates the major transmission routes and certification risks associated with strawberry viral diseases. In contrast, SPaV, SCrV-4 and SLRSV require attention in regional monitoring programs because their symptoms may be inconspicuous and their occurrence can be underestimated when only visual inspection or single-virus testing is used. Therefore, the target-virus panel for certified virus-free strawberry seedlings should be determined according to regional virus prevalence, cultivar susceptibility, transmission vectors and the propagation tier being tested.

Table 1. Major strawberry viruses relevant to virus-free seedling production and certification.

Virus	Abbreviation	Main transmission route	Typical symptoms or infection features	Recommended diagnostic methods	Relevance to certification
Strawberry mottle virus	SMoV	Aphids; infected runners and nursery materials	Mottling, reduced plant vigour and yield loss; may occur in mixed infections	RT-PCR, RT-qPCR, multiplex RT-PCR, RT-RPA, HTS	High
Strawberry mild yellow edge virus	SMYEV	Aphids; infected runners and nursery materials	Mild yellowing of leaf margins; often latent or weakly symptomatic as a single infection	RT-PCR, RT-qPCR, multiplex RT-PCR, RT-RPA	High
Strawberry crinkle virus	SCV	Aphids; infected runners	Leaf crinkling, plant decline and reduced growth; disease severity may increase in mixed infections	RT-PCR, multiplex RT-PCR, HTS	High
Strawberry vein banding virus	SVBV	Aphids; infected runners and nursery materials	Vein banding, chlorosis, leaf distortion and growth reduction	RT-PCR, RT-qPCR, RT-LAMP, multiplex RT-PCR	High
Strawberry necrotic shock virus	SNSV	Thrips- or pollen-associated transmission; infected propagation materials	Necrotic symptoms and reduced vigour; symptom expression may vary with cultivar and environment	RT-PCR, RT-qPCR, HTS	Medium-High
Strawberry latent ringspot virus	SLRSV	Nematodes; infected propagation materials	Latent infection or ringspot-like symptoms; difficult to identify by visual inspection	RT-PCR, ELISA, HTS	Medium

Continued

Strawberry pallidosis-associated virus	SPaV	Whiteflies; infected propagation materials	Pallidosis, chlorosis and plant decline; often associated with mixed infection	RT-PCR, multiplex RT-PCR, HTS	Medium-High
Strawberry polerovirus 1	SPV-1	Aphids; infected propagation materials	Often latent or mildly symptomatic; regional occurrence has been increasingly reported	RT-PCR, multiplex RT-PCR, HTS	Medium-High
Strawberry crinivirus 4	SCrV-4	Whiteflies; infected propagation materials	Chlorosis and decline symptoms; may be underestimated in mixed infections	RT-PCR, multiplex RT-PCR, HTS	Medium

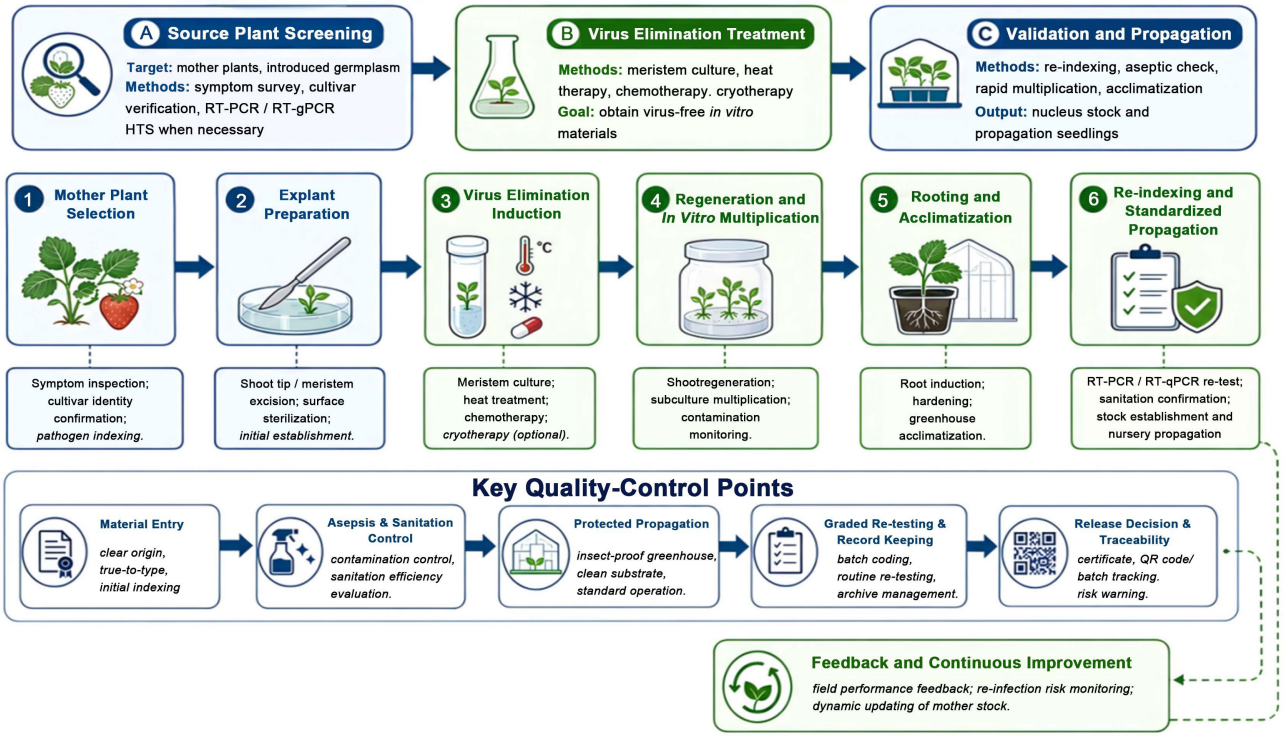

Figure 2. Major transmission routes and certification risks of strawberry viruses.

From the perspective of healthy seedling production, the complexity of strawberry viral diseases has three practical implications. First, virus-free seedling production should not target only one or two major viruses, but should be based on

regional virus spectra and include the major viruses known to occur in local production systems. Second, diagnostic strategies should combine broad-spectrum detection for high-value materials with targeted and cost-effective assays for routine nursery testing. Third, virus elimination should be followed by repeated molecular retesting and protected propagation to prevent reinfection. Therefore, understanding the diversity, latency and mixed-infection characteristics of strawberry viruses provides the epidemiological foundation for designing standardized virus-free seedling production systems.

2.2. Theoretical Basis for Virus Elimination through Shoot-Tip Culture and Integrated Treatments

The production of virus-free strawberry seedlings is based on the uneven distribution of viruses within infected plants. Many plant viruses spread systemically through vascular tissues or cell-to-cell movement via plasmodesmata. However, the shoot apical meristem usually contains extremely low viral titres or may remain free of detectable viruses because of rapid cell division, incomplete vascular differentiation, high metabolic activity and limited viral movement into the youngest meristematic cells [16] [17]. This biological feature provides the theoretical basis for meristem culture and shoot-tip culture. By excising a sufficiently small shoot tip and regenerating it under aseptic *in vitro* conditions, it is possible to obtain plantlets with a greatly reduced probability of viral infection.

In strawberry, shoot-tip culture is the most established and widely used method for virus elimination and rapid propagation. The technique generally involves selection of healthy-looking mother plants, surface disinfection of explants, excision of shoot tips or meristematic tissues, primary culture, shoot induction, subculture multiplication, rooting, acclimatization and post-regeneration virus testing [18]-[20]. The size of the excised shoot tip is a critical factor. Smaller shoot tips usually have lower viral titres and therefore higher potential for virus elimination, but their survival and regeneration rates are often lower. In contrast, larger shoot tips generally regenerate more easily but carry a higher risk of residual viral infection. Thus, the practical success of shoot-tip culture depends on achieving a balance between virus elimination efficiency and plantlet regeneration capacity.

Virus elimination efficiency in strawberry is not determined by shoot-tip size alone. Different viruses vary in their tissue distribution, replication dynamics, movement ability and sensitivity to external stress, leading to virus-dependent differences in elimination efficiency [21]. Cultivar genotype, explant physiological status, culture medium, plant growth regulator composition, light conditions and subculture duration can also affect survival rate, multiplication coefficient, rooting capacity and genetic stability of regenerated plantlets [18]-[23]. Therefore, a standardized virus-free seedling system should not rely on a single universal protocol, but should optimize elimination and regeneration parameters according to target virus, cultivar and production purpose.

Auxiliary treatments, including thermotherapy, chemotherapy, cryotherapy and

alternative regeneration pathways, are often used to improve the efficiency and reliability of virus elimination [21] [24]-[29]. Thermotherapy can reduce viral replication and movement before shoot-tip excision, thereby increasing the probability of obtaining virus-free regenerants. Chemotherapy can inhibit viral replication during *in vitro* culture through antiviral compounds, although concentration and exposure duration must be carefully controlled to avoid phytotoxicity and reduced plantlet vigour. Cryotherapy can selectively damage virus-infected cells or tissues during liquid-nitrogen-based treatment while allowing a portion of meristematic cells to survive and regenerate; it also has value for long-term germplasm conservation. In addition, anther culture and other regeneration pathways may provide complementary strategies for virus elimination and germplasm innovation, although their commercial application is constrained by genotype dependence, ploidy variation and somaclonal variation.

The theoretical basis of virus-free seedling production should therefore be understood as a combination of three principles. The first is biological exclusion, which relies on the low viral titre or absence of viruses in meristematic tissues. The second is stress-assisted suppression, in which thermotherapy, chemotherapy or cryotherapy reduces viral activity or selectively eliminates infected cells. The third is diagnostic verification, which confirms the virus status of regenerated plantlets through sensitive molecular detection. Only when these three principles are integrated can laboratory virus elimination be translated into reliable nursery-scale production.

In addition to virus elimination, quality control of regenerated strawberry plantlets must include genetic fidelity and agronomic performance. Long-term subculture, excessive plant growth regulator exposure, somatic regeneration and anther culture may induce somaclonal variation or ploidy instability [20] [22] [29]. Therefore, regenerated plantlets should be evaluated not only for virus status, but also for cultivar authenticity, ploidy level, morphological uniformity, rooting ability, flowering performance and fruit quality. Molecular markers, flow cytometry and field evaluation can be incorporated into quality assessment, especially for nuclear stock plants and pre-basic stock materials. This broader quality-control concept is essential for developing certified virus-free strawberry seedlings that are negative for target viruses and viroids and genetically stable.

Overall, the occurrence characteristics of strawberry viral diseases and the biological basis of meristem-based virus elimination jointly indicate that virus-free seedling production should be designed as an integrated system. This system should include virus-spectrum investigation, mother plant selection, optimized elimination treatment, regeneration and rooting, molecular retesting, genetic fidelity assessment, protected propagation, vector control and traceable certification. Such an integrated framework provides the foundation for transforming strawberry seedling production from experience-based propagation to standardized, diagnostic-guided and certification-oriented healthy nursery production.

3. Advances in Key Technologies for Virus-Free Strawberry Seedling Production

3.1. Shoot-Tip Culture-Based Virus Elimination

Shoot-tip culture is the most established and widely applied technique for producing virus-free strawberry seedlings. Its effectiveness is based on the low viral titre or absence of detectable viruses in the shoot apical meristem and adjacent young tissues. In practice, the procedure generally includes mother plant selection, explant disinfection, shoot-tip excision, primary culture, shoot induction, subculture multiplication, rooting, acclimatization, transplantation and post-regeneration virus testing [18]-[20]. Because strawberry is mainly propagated through runners, runner-derived shoot tips are commonly used as explants for virus elimination and rapid propagation. This approach is technically feasible, relatively stable and compatible with large-scale tissue-culture-based propagation systems.

The size of the excised shoot tip is one of the most critical factors determining the balance between virus elimination efficiency and regeneration capacity. Smaller shoot tips generally contain lower viral titres and are therefore more favourable for virus elimination; however, they are more vulnerable to browning, dehydration, slow growth and regeneration failure. Larger shoot tips usually show higher survival and regeneration rates, but the probability of residual viral infection also increases. Therefore, the success of shoot-tip culture depends on identifying an appropriate explant size that maximizes virus elimination while maintaining sufficient plantlet regeneration. This trade-off is especially important in commercial production, where survival rate, multiplication coefficient, rooting capacity and operational stability must be considered together.

Recent studies have demonstrated that strawberry shoot-tip culture is highly genotype-dependent. Han *et al.* established a tissue culture and rapid propagation system using shoot tips of 'Benihoppe' and 'Suizhu' strawberry and showed that disinfection procedures, medium composition and plant growth regulator combinations affected shoot induction, multiplication and rooting performance [18]. Du *et al.* developed a virus-elimination and rapid propagation system for 'Benihoppe' strawberry and reported that low concentrations of 6-BA and NAA were suitable for shoot multiplication, while half-strength MS medium supplemented with IBA promoted rooting [19]. Naing *et al.* further demonstrated that *in vitro* propagation systems should be evaluated not only by multiplication efficiency but also by morphological and genetic stability of regenerated plantlets [20]. These findings indicate that genotype-specific optimization is essential for developing reliable virus-free strawberry propagation protocols.

From an industrial perspective, shoot-tip culture remains the core technology for strawberry virus-free seedling production because it can be integrated with rapid multiplication, rooting, acclimatization and protected nursery systems. However, its limitations should not be overlooked. The technique requires skilled micromanipulation, strict aseptic operation and repeated virus testing. In addition, elim-

ination efficiency varies among viruses and cultivars, and excessive subculture may increase the risk of somaclonal variation. Future improvement should focus on standardizing shoot-tip excision, optimizing genotype-specific culture media, shortening regeneration cycles, limiting subculture generations and integrating molecular diagnostics into each propagation tier.

3.2. Thermotherapy-Assisted Shoot-Tip Culture

Thermotherapy is commonly used as an auxiliary treatment to enhance virus elimination efficiency before shoot-tip culture. The principle of thermotherapy is to expose infected plant materials to elevated temperatures that suppress viral replication and movement while allowing host tissues to survive. By reducing viral titres in shoot tips or meristematic tissues, thermotherapy increases the probability of regenerating virus-free plantlets after shoot-tip excision [21] [24] [25]. In strawberry, thermotherapy is generally combined with shoot-tip or meristem culture rather than used as an independent elimination method.

The effectiveness of thermotherapy depends mainly on treatment temperature, exposure duration, cultivar heat tolerance, explant physiological status and target virus. Insufficient temperature or short exposure may fail to suppress viral replication, whereas excessive temperature or prolonged treatment may cause leaf chlorosis, tissue browning, necrosis, reduced shoot regeneration and poor rooting. Therefore, thermotherapy should be optimized according to cultivar and virus identity rather than applied as a uniform protocol. This is particularly relevant for strawberry, where cultivar-specific responses during tissue culture and regeneration are frequently observed [18] [19] [23].

Thermotherapy-assisted shoot-tip culture is especially useful when viruses are distributed beyond the smallest meristematic region or when direct shoot-tip culture alone results in unstable elimination efficiency. However, its industrial application is constrained by relatively long treatment periods, increased labour input and potential heat stress injury. To improve its practical value, future studies should explore temperature-gradient treatments, alternating-temperature regimes, short-term heat shock, antioxidant supplementation and light-environment regulation. Such refinements may help balance viral suppression with explant survival and regeneration capacity.

3.3. Chemotherapy and Antiviral Auxiliary Treatments

Chemotherapy refers to the use of antiviral compounds during *in vitro* culture to suppress viral replication and improve virus elimination efficiency. Nucleoside analogues such as ribavirin are commonly used in plant virus elimination systems, and other antiviral or growth-promoting compounds have also been explored in horticultural crops [26] [27]. In strawberry, chemotherapy should be considered an auxiliary strategy rather than a stand-alone method because the effectiveness of antiviral compounds may vary according to virus species, compound concentration, exposure duration, explant condition and cultivar tolerance.

The major advantage of chemotherapy is that antiviral pressure can be maintained throughout the *in vitro* culture period. This may be beneficial for materials with systemic viral infection or for viruses that are difficult to eliminate by shoot-tip culture alone. However, chemical treatments can also cause phytotoxicity, including chlorosis, reduced shoot proliferation, abnormal morphology, hyperhydricity, delayed rooting and reduced plantlet vigour. Prolonged exposure or excessive concentration may further compromise genetic stability and subsequent field performance. Therefore, chemotherapy-based protocols must balance antiviral efficacy with plantlet quality.

Recent molecular studies on strawberry viruses have provided new insights into virus-host interactions and host antiviral defence. For example, studies on SVBV and SMoV have shown that viral movement proteins, viral silencing suppressors and host defence-related proteins can influence viral infection and host resistance [31] [37]-[39]. Although these studies do not directly represent commercial virus-elimination protocols, they provide a theoretical basis for developing targeted antiviral treatments, screening resistant germplasm and combining virus elimination with molecular breeding. In future strawberry virus-free seedling systems, chemotherapy may be more valuable when integrated with shoot-tip culture, thermotherapy, cryotherapy and sensitive post-treatment diagnostics.

3.4. Cryotherapy

Cryotherapy has emerged as an important advanced strategy for eliminating viruses from clonally propagated horticultural crops and conserving valuable germplasm. In strawberry, cryotherapy usually uses shoot tips, meristems or apical buds as explants and involves preculture, osmoprotection, vitrification-based dehydration, liquid nitrogen exposure, rewarming and plant regeneration. During ultra-low-temperature treatment, a portion of meristematic cells can survive and regenerate, whereas virus-infected cells or tissues may be selectively damaged. Thus, cryotherapy combines virus elimination with long-term germplasm preservation [21] [28].

Compared with conventional shoot-tip culture, cryotherapy may be particularly useful for valuable nuclear stock plants, germplasm collections and materials infected with viruses that are difficult to eliminate by meristem excision alone. Wöhner and Höfer evaluated cryotherapy and meristem isolation from stolons for eliminating viruses in Fragaria germplasm and reported that elimination efficiency differed among viruses [21]. This finding indicates that cryotherapy is not universally effective for all strawberry viruses; rather, its success depends on virus identity, explant size, dehydration level, vitrification solution toxicity, rewarming rate and regeneration capacity.

Despite its advantages, cryotherapy has several limitations for routine commercial seedling production. It requires specialized equipment, technically trained personnel and highly optimized regeneration protocols. In addition, excessive dehydration or vitrification may cause tissue injury and reduce plantlet recovery.

Therefore, cryotherapy is more suitable for high-value germplasm, nuclear stock plants and certification-source materials than for ordinary production seedlings. Future research should focus on genotype-wide protocol optimization, reduction of oxidative stress during rewarming, improvement of post-cryotherapy regeneration and integration of HTS or RT-qPCR testing after recovery.

3.5. Anther Culture and Alternative Regeneration Pathways

In addition to shoot-tip culture, alternative regeneration pathways such as anther culture, somatic embryogenesis and adventitious shoot regeneration have been explored for virus elimination and germplasm innovation in strawberry. Anther culture may reduce the transmission of certain viruses during gametophytic development or regeneration and can be combined with haploid or doubled-haploid breeding. Tian *et al.* established a virus-free rapid propagation system for strawberry 'Miaoxiang 7' through anther culture and evaluated regenerated materials using ploidy and genetic stability analyses [22]. This indicates that anther culture may have dual value in virus-free seedling production and germplasm improvement.

However, anther culture is not yet a routine commercial method for strawberry virus-free seedling production. Its efficiency is strongly affected by genotype, donor plant condition, microspore developmental stage, induction medium, plant growth regulators and culture environment. Moreover, regenerated plantlets may show ploidy variation, reduced uniformity or somaclonal variation. Therefore, plantlets derived from anther culture or somatic regeneration must be subjected to strict evaluation, including flow cytometry, molecular marker analysis, morphological assessment and agronomic performance testing [20] [22] [29].

From a practical perspective, alternative regeneration pathways should be positioned as complementary methods rather than substitutes for shoot-tip culture. They are more appropriate for research, germplasm innovation, breeding material development and specific cases where conventional shoot-tip culture is inefficient. For commercial propagation, their application should be restricted to carefully evaluated materials and should be accompanied by virus detection, genetic fidelity assessment and field performance verification.

3.6. Comparative Evaluation of Major Virus-Elimination Technologies

Different virus-elimination technologies have distinct advantages, limitations and application scenarios. For commercial strawberry seedling production, shoot-tip culture remains the foundational method because of its technical maturity and compatibility with rapid propagation. Thermotherapy and chemotherapy are mainly used as auxiliary treatments to increase elimination efficiency, especially for materials with high viral titres or unstable responses to direct shoot-tip culture. Cryotherapy is valuable for nuclear stock plants and germplasm conservation, while anther culture and other regeneration pathways are more suitable for research and

breeding-oriented applications. Therefore, the selection of virus-elimination technology should be based on target virus, cultivar genotype, material value, production scale, cost and certification requirements.

We compare virus-elimination technologies by four practical criteria: reliability (consistent removal of target viruses/viroids across species, cultivars, and batches), regeneration (explant survival, shoot/root formation, and vigor), scalability (standardization in commercial nurseries with acceptable labor/technique), and cost sensitivity (dependence on equipment, skilled staff, time, diagnostics, and regeneration losses). These criteria are interdependent; high elimination efficacy is impractical if regeneration is poor or costs are excessive.

Table 2. Comparative evaluation of major virus-elimination technologies for strawberry seedling production based on elimination reliability, regeneration capacity, scalability and cost sensitivity.

Technology	Basic principle	Main advantages	Main limitations	Suitable materials	Industrial applicability
Shoot-tip culture	Uses low viral titre or absence of virus in meristematic tissues to regenerate virus-free plantlets	Technically mature; compatible with rapid propagation; suitable for commercial production	Requires skilled excision; efficiency affected by shoot-tip size, virus species and genotype	Runner tips, shoot tips, meristematic tissues	High
Thermotherapy-assisted shoot-tip culture	Elevated temperature suppresses viral replication and movement before shoot-tip excision	Can improve elimination efficiency; useful for some systemic viruses	Long treatment period; heat stress, browning and reduced regeneration may occur	Infected mother plants, runners, *in vitro* plantlets	Medium-High
Chemotherapy-assisted culture	Antiviral compounds inhibit viral replication during *in vitro* culture	Continuous antiviral pressure; can be combined with shoot-tip culture	Phytotoxicity, abnormal growth and reduced plantlet vigour at high concentrations	*In vitro* shoots and explants	Medium
Cryotherapy	Liquid-nitrogen-based treatment selectively damages infected cells while preserving meristematic cells	Useful for difficult-to-eliminate viruses; also supports germplasm conservation	Requires specialized equipment; protocol optimization is difficult; recovery may be genotype-dependent	Shoot tips, meristems, germplasm resources, nuclear stock	Medium for production; high for germplasm conservation

As summarized in Table 2, no single technology can meet all requirements of virus-free strawberry seedling production. A practical and certification-oriented strategy should combine shoot-tip culture as the core elimination method with thermotherapy, chemotherapy or cryotherapy when necessary. After regeneration, all candidate plantlets should be subjected to molecular retesting, genetic fidelity assessment and protected propagation. This integrated approach can improve

the reliability, scalability and commercial value of virus-free strawberry seedling production.

4. Virus Detection and Quality Assessment of Virus-Free Strawberry Seedlings

4.1. Indicator Plants, Serological Assays and Preliminary Virus Screening

Reliable virus detection is a prerequisite for the production, certification and commercial application of virus-free strawberry seedlings. Because strawberry viruses often induce latent infection, mixed infection or non-specific symptoms, visual inspection alone cannot accurately determine the health status of mother plants, regenerated plantlets or commercial seedlings. Therefore, virus detection should be incorporated throughout the whole production chain, including mother plant selection, post-elimination verification, subculture multiplication, acclimatization, protected nursery production and pre-dispatch inspection [10] [12].

Early methods for strawberry virus detection mainly included indicator plant grafting, symptom observation and enzyme-linked immunosorbent assay (ELISA). Indicator plant assays can reflect viral biological activity and symptom expression, but they are time-consuming, labour-intensive, space-demanding and susceptible to environmental interference. ELISA is relatively simple, low-cost and suitable for preliminary screening of known viruses. However, its reliability depends strongly on antibody specificity, viral titre, sample quality and the physiological status of tested plants. For viruses with low titres or uneven tissue distribution, ELISA may produce false-negative results, particularly during early infection or latent infection [12] [15].

From a certification perspective, traditional detection methods should not be completely abandoned, but their role should be repositioned. Indicator plant assays and ELISA are more suitable for preliminary screening, biological validation or low-cost routine monitoring, whereas final certification of virus-free strawberry seedlings should rely on molecular diagnostic methods with higher sensitivity and specificity. This shift is especially important for nuclear stock plants and pre-basic materials, where false-negative detection may lead to long-term dissemination of infected propagation materials.

4.2. RT-PCR, RT-qPCR and Multiplex PCR Detection

Reverse transcription PCR (RT-PCR) remains one of the most widely used molecular methods for detecting strawberry viruses because of its high specificity, methodological maturity and relatively low cost. Compared with symptom observation and ELISA, RT-PCR can detect viral nucleic acids directly and is therefore more suitable for identifying latent or low-symptom infections. RT-qPCR further improves detection sensitivity and enables quantitative assessment of viral accumulation, which is useful for evaluating changes in viral titre before and after elimination treatments and for selecting candidate nuclear stock plants [30] [31].

The development of virus-specific RT-qPCR assays has improved the accuracy of strawberry virus detection. Zhao *et al.* established a TaqMan RT-qPCR assay for Brassica yellows virus isolated from strawberry, providing a sensitive molecular tool for viral detection and quantification [30]. Xu *et al.* developed singleplex and duplex TaqMan RT-qPCR systems for strawberry mottle virus (SMoV) and strawberry vein banding virus (SVBV), which improved the sensitivity and efficiency of detecting two major strawberry viruses [31]. These studies indicate that RT-qPCR is particularly suitable for post-elimination retesting, low-titre virus detection and quantitative evaluation of virus-removal efficiency.

Multiplex PCR is especially valuable for large-scale nursery testing because it can simultaneously detect multiple viruses in a single reaction. Wang *et al.* developed a multiplex RT-PCR assay for six strawberry viruses, including strawberry mild yellow edge virus (SMYEV), strawberry vein banding virus (SVBV), strawberry mottle virus (SMoV), strawberry polerovirus 1 (SPV-1), strawberry pallidosis-associated virus (SPaV) and strawberry crinivirus 4 (SCrV-4) [10]. This strategy reduces detection cost and time, improves testing efficiency and helps identify mixed infections that may be missed by single-virus assays.

In practical production, RT-PCR and multiplex RT-PCR are suitable for batch screening of basic stock and certified production seedlings, whereas RT-qPCR is more appropriate for high-value materials, post-elimination verification and samples suspected of low viral titre. However, the reliability of these methods depends on standardized sampling position, nucleic acid extraction quality, primer specificity, positive and negative controls, and interpretation criteria. Therefore, molecular detection protocols should be standardized according to target virus, propagation tier and regional virus prevalence.

4.3. High-Throughput Sequencing and Viral Genomics Detection

High-throughput sequencing (HTS) represents a major advance in plant virus diagnosis because it enables broad-spectrum detection without prior knowledge of viral sequences. Unlike targeted PCR-based methods, HTS can detect known viruses, divergent viral variants and novel viruses simultaneously. This feature is particularly important for strawberry, in which viral diversity is continuously expanding and mixed infection is common [5]-[9] [12].

HTS has been increasingly applied to strawberry virus discovery, germplasm health assessment, post-entry quarantine and plant health certification. Diaz-Lara *et al.* used sequencing of a strawberry germplasm collection to reveal new viral genetic diversity and to provide a basis for developing improved RT-qPCR assays [5]. Nunes-Leite *et al.* demonstrated the value of HTS for detecting strawberry viruses in post-entry quarantine materials, highlighting its potential in preventing the introduction of infected germplasm [6]. In broader plant health systems, HTS has also been recognized as a powerful tool for virus and viroid detection and for supporting certification programs [32] [33].

Nevertheless, HTS is not suitable as a routine detection method for every com-

mercial seedling batch because of its relatively high cost, bioinformatic requirements and incomplete standardization in threshold setting and result interpretation. A more practical strategy is to apply HTS to nuclear stock plants, germplasm resources, imported materials and diagnostically unclear samples, while using RT-qPCR or multiplex PCR for routine production batches. In this way, HTS can function as a front-end discovery and risk-assessment tool within a hierarchical diagnostic framework rather than as a universal replacement for PCR-based detection.

4.4. Isothermal Amplification, Lateral-Flow Assays and Field-Deployable Detection

Isothermal amplification technologies, including reverse transcription loop-mediated isothermal amplification (RT-LAMP), reverse transcription recombinase polymerase amplification (RT-RPA) and recombinase-aided amplification, have attracted increasing attention because they do not require complex thermal cycling instruments. These methods are characterized by rapid amplification, simple operation, visual readout and compatibility with lateral-flow strips, making them suitable for on-site detection in nurseries and production fields [34]-[36].

RT-LAMP has been developed for the detection of strawberry vein banding virus and provides a rapid molecular tool for specific virus identification [34]. Zou *et al.* developed RT-RPA assays combined with lateral-flow strips for rapid detection of strawberry mottle virus and strawberry mild yellow edge virus, respectively [35] [36]. These methods are particularly useful for nursery-stage screening, field monitoring, rapid investigation of suspected infection and pre-dispatch inspection of planting materials.

However, field-deployable assays should be used with a clear understanding of their limitations. Although RT-RPA, RT-LAMP and lateral-flow assays are rapid and convenient, they may be less suitable for comprehensive virus-spectrum screening or quantitative evaluation of viral load. Therefore, positive or doubtful field-test results should be confirmed by RT-PCR, RT-qPCR or HTS when used for certification decisions. In virus-free seedling production, rapid assays are best positioned as production-side supplements rather than the sole basis for certification.

4.5. Hierarchical Diagnostic Strategy for Certified Virus-Free Strawberry Seedlings

Virus-free strawberry seedling production should move from single-stage detection towards a hierarchical and whole-process diagnostic strategy. Different propagation tiers have different risk levels, material values and testing objectives; therefore, the diagnostic method should be selected according to the purpose of each production stage. For example, nuclear stock plants require broad-spectrum and highly sensitive detection, whereas certified production seedlings require cost-effective batch testing and rapid pre-dispatch inspection.

Table 3. Hierarchical diagnostic strategy for certified virus-free strawberry seedling production.

Production stage	Material type	Main detection objective	Recommended diagnostic methods	Suggested testing point
Stage I	Germplasm resources and candidate nuclear stock plants	Broad-spectrum virus discovery and exclusion of unknown or emerging viruses	HTS + RT-qPCR validation	Before establishment of nuclear stock
Stage II	Regenerated plantlets after virus elimination	Verification of virus elimination efficiency	RT-PCR, RT-qPCR, multiplex RT-PCR	After shoot-tip culture, thermotherapy, chemotherapy or cryotherapy
Stage III	Pre-basic stock and basic stock plants	Routine monitoring of major regional viruses and mixed infection	Multiplex RT-PCR, RT-qPCR	During multiplication and before transfer to nursery
Stage IV	Certified production seedlings	Large-scale batch screening and quality control	Multiplex RT-PCR, RT-PCR, ELISA as supplementary method	Before dispatch or commercial distribution
Stage V	Nursery and field monitoring	Rapid detection of suspected infection and reinfection risk	RT-RPA, RT-LAMP, lateral-flow assays, followed by PCR confirmation when necessary	During protected nursery production and field surveillance

As shown in Table 3, HTS should be prioritized for high-value materials such as germplasm resources and nuclear stock plants, where the risk of introducing unknown or emerging viruses must be minimized. RT-qPCR and multiplex RT-PCR should serve as the core tools for post-elimination retesting and routine nursery screening. RT-RPA, RT-LAMP and lateral-flow assays are more suitable for rapid field diagnosis, nursery monitoring and pre-dispatch inspection. This tiered strategy can reduce detection cost while maintaining diagnostic reliability across different stages of virus-free strawberry seedling production.

4.6. Quality Assessment beyond Virus Detection

Certification of virus-free strawberry seedlings should not be limited to confirming the absence of detectable viruses. Tissue culture, long-term subculture, plant growth regulator exposure, somatic regeneration, cryotherapy and anther culture may induce morphological variation, ploidy changes or somaclonal variation. Therefore, quality assessment should include virus status, cultivar authenticity, genetic fidelity, ploidy stability, physiological vigour, rooting capacity, transplant survival, flowering performance and fruit quality [20] [22] [29].

Genetic fidelity can be evaluated using molecular markers such as SSRs or SNPs, while ploidy stability can be assessed using flow cytometry or chromosome analysis. Morphological and agronomic evaluations should also be conducted after acclimatization and field establishment, especially for nuclear stock plants and

pre-basic stock. Tian *et al.* evaluated ploidy level and genetic stability in an anther culture-based virus-free rapid propagation system of strawberry, demonstrating the necessity of integrating genetic stability assessment into virus-free seedling quality control [22]. Naing *et al.* also emphasized the importance of producing morphologically and genetically stable plants during strawberry *in vitro* propagation [20].

A complete quality-control framework should therefore combine four dimensions: virus and viroid status, genetic stability, physiological quality and production traceability. Virus and viroid status determines whether the seedling meets the basic requirement of virus-free production. Genetic stability ensures that the regenerated material maintains cultivar identity. Physiological quality determines whether seedlings can survive transplantation and perform well in commercial production. Traceability ensures that each propagation batch can be linked to its mother plant source, detection record, propagation tier and nursery management history.

In summary, virus detection should be integrated with genetic fidelity assessment, seedling physiological evaluation and traceable certification. Such a system can prevent the misclassification of "apparently healthy" but infected materials as virus-free seedlings and can also avoid the commercial use of virus-free but genetically unstable regenerants. Therefore, the future development of virus-free strawberry seedling production should depend on a diagnostic-guided quality-control system that links molecular detection, propagation management and certification standards.

5. Rapid Propagation Systems and Industrial Application of Virus-Free Strawberry Seedlings

5.1. Subculture Multiplication and Maintenance of Virus-Free Status

After virus elimination and molecular confirmation, large-scale propagation of virus-free strawberry materials depends on the coordinated integration of *in vitro* multiplication and protected nursery production. Tissue-culture-based rapid propagation generally includes subculture multiplication, rooting culture, acclimatization, transplantation and maintenance of mother plants. At the multiplication stage, the main objective is not only to increase the number of plantlets, but also to maintain virus-free status, cultivar identity, genetic fidelity and physiological vigour throughout repeated propagation cycles [18]-[20].

Subculture multiplication is usually performed on MS or modified MS medium supplemented with appropriate concentrations of cytokinins and auxins, such as 6-BA, KT, NAA or IBA. Appropriate cytokinin levels can promote axillary bud break and multiple shoot formation, thereby improving the multiplication coefficient. However, excessive cytokinin concentration or prolonged subculture may cause shoot dwarfing, hyperhydricity, chlorosis, abnormal morphology and reduced rooting capacity. These problems are particularly important for virus-free

seedlings because the commercial value of regenerated plantlets depends not only on rapid multiplication but also on uniformity and long-term field performance [20] [29].

Strawberry cultivars often show genotype-dependent responses during *in vitro* multiplication. Han *et al.* reported that 'Benihoppe' and 'Suizhu' differed in their responses to disinfection procedures, medium composition and plant growth regulator combinations during shoot-tip culture and rapid propagation [18]. Du *et al.* showed that low concentrations of 6-BA and NAA promoted shoot multiplication in 'Benihoppe', while half-strength MS medium supplemented with IBA improved rooting performance [19]. Liang *et al.* also emphasized that cultivar-specific optimization is necessary during shoot-tip culture and rapid propagation of 'Suizhu' strawberry [23]. These findings indicate that a universal multiplication protocol is unlikely to be optimal for all strawberry cultivars.

In commercial virus-free seedling production, the number of subculture cycles should be strictly controlled. Excessive subculture may increase the risk of somaclonal variation, physiological decline and loss of propagation uniformity. Therefore, nuclear stock plants and pre-basic stock should be maintained with a limited number of subculture cycles, strict batch records and periodic molecular retesting. For certified production seedlings, multiplication efficiency may be moderately increased, but it should remain within a quality-control framework that includes morphology, rooting ability, genetic fidelity and virus status.

5.2. Rooting Culture, Acclimatization and Transplant Quality

Rooting culture is a critical stage connecting *in vitro* multiplication with *ex vitro* nursery production. Well-developed root systems are essential for water uptake, nutrient absorption, transplant survival and early seedling establishment. In strawberry tissue culture, rooting is commonly induced on half-strength MS or other low-salt media supplemented with suitable concentrations of auxins such as IBA or NAA [18] [19]. The optimal rooting medium may vary among cultivars, and excessive auxin may result in abnormal root morphology or reduced shoot quality.

Acclimatization is required because *in vitro* plantlets are produced under high humidity, low irradiance, limited gas exchange and aseptic conditions. Such plantlets often have a thin cuticle, weak stomatal regulation and immature root absorption capacity, making them vulnerable to dehydration, wilting and pathogen infection after transfer to *ex vitro* conditions. Therefore, acclimatization should include gradual vessel opening, moderate light transition, high but decreasing relative humidity, improved ventilation, disease prevention and careful substrate moisture management [40]-[43].

The acclimatization substrate should be loose, well aerated, moderately water-retentive and certified virus-free. Peat, perlite, vermiculite and coconut coir are commonly used alone or in mixtures. Du *et al.* reported that regenerated 'Be-

nihoppe' plantlets rooted effectively and showed good transplant survival in a mixed substrate of peat, vermiculite and perlite [19]. Similar studies on strawberry micropropagation and acclimatization also indicate that substrate type, humidity control and gradual adaptation strongly influence survival rate and subsequent seedling quality [40] [41].

For virus-free seedlings, transplant quality should be evaluated using both physiological and sanitary indicators. Physiological indicators include root number, root length, shoot height, leaf number, leaf colour, plantlet uniformity and transplant survival. Sanitary indicators include the absence of visible disease symptoms, negative virus detection results and freedom from pest contamination during acclimatization. Seedlings that survive transplantation but show weak growth, poor rooting or abnormal morphology should not be used as nuclear stock or prebasic stock, even if they test negative for target viruses.

5.3. Protected, Factory-Based Nursery Production and Reinfection Prevention

The industrial application of virus-free strawberry seedlings requires protected nursery systems that can maintain seedling health after virus elimination. Traditional open-field runner propagation is vulnerable to soil-borne pathogens, nematodes, aphids, whiteflies and environmental fluctuations. In contrast, modern propagation systems, including elevated nurseries, substrate-based cultivation, plug transplants, isolated greenhouses, insect-proof screen houses and factory-based seedling production, can reduce reinfection and vector pressure and improve seedling uniformity [1] [44] [45].

Protected nursery systems are particularly important because virus-free status is not permanent. Once seedlings are exposed to virus-transmitting vectors or infected surrounding plants, reinfection can occur during nursery multiplication or field production. Therefore, virus-free strawberry seedling production should not be understood as a one-time laboratory event, but as a continuous health-management process. This process requires insect-proof isolation, vector monitoring, removal of suspected plants, tool disinfection, substrate sanitation, environmental regulation and periodic virus testing [10] [12] [35] [36].

Factory-based nursery production provides a practical route for improving propagation efficiency and reducing reinfection risk. Temporary immersion bioreactors may reduce labour input and increase multiplication efficiency, while elevated and substrate-based nursery systems can decrease soil-borne disease and nematode pressure. Plug transplants are convenient for transportation, mechanized planting and uniform field establishment. In addition, precise regulation of temperature, light, humidity and fertigation can improve root development and plantlet uniformity, which are essential for commercial seedling quality [1] [44] [45].

Vector management should be integrated into every stage of protected propagation. Aphids are particularly important because they transmit several major

strawberry viruses, including SMoV, SMYEV, SCV and SVBV. Whiteflies and nematodes may also contribute to the spread of specific viruses or virus-like disease complexes. Therefore, nursery facilities should combine insect-proof nets, sticky traps, regular scouting, weed control, sanitation barriers and targeted pest management. Vector control should be linked with molecular monitoring because the absence of visible symptoms does not guarantee the absence of viral infection.

5.4. Hierarchical Propagation and Certification-Oriented Quality Control

A standardized virus-free strawberry seedling system should be organized as a hierarchical propagation structure. Nuclear stock plants should originate from elite mother plants that have passed strict virus testing, cultivar identification and genetic fidelity assessment. Pre-basic stock should be propagated from nuclear stock under protected conditions and subjected to repeated molecular testing. Basic stock should be produced from pre-basic stock and used for controlled multiplication. Certified production seedlings should be generated from basic stock and tested before commercial distribution. This hierarchical system can reduce the risk of virus accumulation and maintain traceability across propagation generations.

The quality-control objectives differ among propagation tiers. For nuclear stock plants, the priority is maximum health security, genetic fidelity and long-term conservation. For pre-basic and basic stock, the emphasis is on maintaining virus-free status and propagation uniformity. For certified production seedlings, the key requirements are batch consistency, transplant performance, freedom from major regional viruses and compliance with nursery standards. Therefore, the testing intensity and diagnostic methods should be adjusted according to propagation tier, material value and risk level.

Table 4. Hierarchical propagation and quality-control strategy for virus-free strawberry seedlings.

Propagation tier	Material source	Main objective	Key quality-control measures	Recommended detection strategy
Nuclear stock	Elite mother plants after virus elimination	Maintain highest sanitary and genetic quality	HTS or broad-spectrum screening, RT-qPCR validation, genetic fidelity assessment, strict isolation	HTS + RT-qPCR; periodic retesting
Pre-basic stock	Nuclear stock-derived plantlets	Maintain virus-free status and cultivar identity	Limited subculture cycles, protected propagation, morphology and ploidy assessment	RT-qPCR + multiplex RT-PCR
Basic stock	Pre-basic stock-derived plants	Controlled multiplication for nursery use	Batch records, vector exclusion, substrate sanitation, periodic testing	Multiplex RT-PCR or RT-qPCR

Continued

Certified production seedlings	Basic stock-derived plants	Commercial distribution and field planting	Pre-dispatch inspection, batch sampling, pest-free nursery management, traceability	Multiplex RT-PCR, RT-PCR, RT-RPA/RT-LAMP as rapid supplements
Nursery and field monitoring materials	Plants under nursery or early field production	Monitor reinfection risk	Vector surveillance, removal of suspected plants, rapid screening and confirmatory testing	RT-RPA/RT-LAMP followed by RT-PCR or RT-qPCR confirmation

As summarized in Table 4, the production of certified virus-free strawberry seedlings should follow a top-down propagation system. The higher the propagation tier, the stricter the detection and genetic fidelity requirements should be. Conversely, production seedlings require cost-effective batch testing and pre-dispatch inspection. This tiered model can balance diagnostic accuracy, production cost and commercial scalability.

The exact diagnostic intensity, sampling frequency and certification threshold should be adjusted according to national or regional nursery regulations, local virus prevalence and the intended propagation tier.

5.5. Traceability, Batch Management and Industrial Application

Traceability is essential for the industrial application of virus-free strawberry seedlings. Each seedling batch should be linked to its mother plant source, virus-elimination method, diagnostic records, subculture generation, propagation tier, nursery location, vector-control measures and pre-dispatch testing results. Such documentation can help identify the source of infection if reinfection occurs and can also improve confidence among growers, nursery enterprises and certification agencies.

Standardized production depends on whole-process quality control. First, mother plant sources must be traceable and cultivar authenticity must be verified. Second, regenerated plantlets should be tested after virus elimination and again before transfer to protected nursery production. Third, the number of subculture cycles should be limited to reduce the risk of genetic variation. Fourth, nuclear stock, pre-basic stock, basic stock and certified production seedlings should be maintained separately according to propagation tier. Fifth, nursery facilities should establish systems for insect-proof isolation, tool disinfection, environmental monitoring, vector control and batch documentation.

Taken together, the industrialization of virus-free strawberry seedlings depends on the integration of tissue culture, molecular diagnostics, protected propagation, vector management and traceable certification. A successful system should not only produce virus-free plantlets *in vitro*, but also maintain their sanitary status, genetic stability and commercial performance throughout multiplication, nursery production and seedling distribution.

6. Challenges, Future Perspectives and Conclusions

6.1. Current Challenges in Virus-Free Strawberry Seedling Production

Despite substantial progress in virus-elimination technologies, molecular diagnostics and protected nursery production, the large-scale application of virus-free strawberry seedlings still faces several scientific, technical and industrial challenges. These challenges are not isolated; rather, they are interconnected across virus biology, *in vitro* regeneration, diagnostic reliability, nursery management and certification systems.

First, virus elimination efficiency remains strongly virus-dependent. Different strawberry viruses vary in tissue distribution, replication dynamics, movement ability and sensitivity to elimination treatments. Wöhner and Höfer reported that cryotherapy and meristem isolation showed different efficiencies in eliminating major strawberry viruses from Fragaria germplasm, indicating that a single elimination protocol cannot be universally applied to all virus-cultivar combinations [21]. Therefore, future virus-elimination systems should be optimized according to target virus, cultivar genotype and explant physiological status.

Second, virus elimination efficiency often conflicts with regeneration capacity and seedling quality. Smaller shoot tips or stronger stress treatments may improve the probability of virus elimination, but they can also reduce survival rate, delay regeneration, increase browning or necrosis and impair rooting capacity. Conversely, larger explants or milder treatments may improve regeneration but increase the risk of residual infection. This trade-off remains one of the key technical bottlenecks in commercial virus-free strawberry seedling production [18]-[21].

Third, diagnostic systems remain insufficiently standardized. Differences in sampling position, sampling season, target-virus range, nucleic acid extraction quality, primer design, assay sensitivity and interpretation criteria can reduce comparability among laboratories and production systems. Although RT-PCR, RT-qPCR, multiplex PCR, HTS and isothermal amplification have all improved strawberry virus detection, their application should be standardized according to propagation tier, regional virus prevalence and certification purpose [10] [12] [30]-[36].

Fourth, genetic fidelity and cultivar authenticity are still underemphasized in some virus-free propagation systems. A plantlet that tests negative for target viruses is not necessarily suitable for certification if it has undergone somaclonal variation, ploidy instability or cultivar misidentification. Long-term subculture, excessive plant growth regulator exposure, cryotherapy and alternative regeneration pathways may affect genetic stability. Therefore, virus detection should be integrated with ploidy analysis, molecular marker verification and agronomic evaluation, especially for nuclear stock and pre-basic stock materials [20] [22] [29].

Fifth, production cost and operational complexity remain major constraints for industrial application. Virus-free strawberry seedling production requires skilled

tissue-culture operation, repeated molecular testing, protected nursery facilities, vector control, batch documentation and quality certification. These requirements increase production costs compared with conventional runner propagation. Cost reduction should therefore rely on optimized tissue-culture protocols, automated or semi-automated propagation, temporary immersion bioreactors, multiplex detection and standardized batch management [1] [18] [19] [44] [45].

Finally, reinfection remains a persistent risk after virus elimination. Virus-free status is not permanent once seedlings are exposed to infected plants or virus-transmitting vectors. Aphids, whiteflies and nematodes may contribute to virus spread depending on the virus species involved. Therefore, virus-free seedling production must be combined with protected nursery management, vector monitoring, insect-proof isolation, sanitation measures and periodic virus testing [10] [12] [14] [35] [36].

Several evidence gaps remain, including limited cultivar coverage, inconsistent experimental and diagnostic standards across studies, and scarce direct comparisons among elimination technologies under identical virus-cultivar backgrounds. Therefore, current recommendations should be viewed as practical guidance based on available evidence rather than universally optimized protocols for all strawberry cultivars, viruses and production systems.

6.2. Future Perspectives

Future development of virus-free strawberry seedling production should move from technique-centered optimization towards system-level integration. The central objective should not be limited to obtaining virus-free regenerants *in vitro*, but should extend to maintaining sanitary status, genetic stability, propagation efficiency and traceability throughout the whole production chain.

First, virus-elimination strategies should become more precise and virus-specific. Shoot-tip culture should remain the core technology, but thermotherapy, chemotherapy and cryotherapy should be selectively combined according to target virus and cultivar response. For viruses that are difficult to eliminate by direct shoot-tip culture, integrated protocols combining stress pretreatment, micro-shoot-tip excision and repeated molecular retesting may improve reliability [21] [24]-[28].

Second, diagnostic systems should be developed into hierarchical and certification-oriented frameworks. HTS is most suitable for nuclear stock plants, germplasm resources, imported materials and diagnostically unclear samples, whereas RT-qPCR and multiplex RT-PCR are more appropriate for post-elimination verification and batch testing of nursery materials. RT-RPA, RT-LAMP and lateral-flow assays can serve as rapid tools for field monitoring and pre-dispatch inspection, but positive or doubtful results should be confirmed by PCR-based methods [5] [6] [10] [30]-[36].

Third, quality assessment should extend beyond virus detection. Future certification systems should include cultivar authenticity, genetic fidelity, ploidy stabil-

ity, morphological uniformity, rooting ability, transplant survival, flowering performance and fruit quality. Molecular markers, flow cytometry and field evaluation should be incorporated into the quality-control process, particularly for regenerated materials derived from long-term subculture, cryotherapy or anther culture [20] [22] [29].

Fourth, protected and factory-based nursery systems should become the main route for industrial-scale production. Elevated nurseries, plug transplants, soilless substrates, temporary immersion bioreactors, isolated greenhouses, insect-proof screen houses and precision environmental control can improve propagation efficiency while reducing pathogen and vector pressure [1] [44] [45]. In the future, digital monitoring of temperature, humidity, light, fertigation, pest occurrence and batch movement may further improve nursery management and seedling traceability.

Fifth, regional virus-spectrum databases and standardized certification protocols should be established. Because the prevalence of strawberry viruses varies among cultivars, regions and production systems, the target-virus panel for certification should be based on local epidemiological data. Standardized protocols should define mother plant selection, sampling position, testing frequency, detection methods, acceptance criteria, subculture limits, nursery isolation requirements and pre-dispatch inspection standards [10] [12] [14].

Overall, future progress will depend on the coordinated development of precise virus elimination, hierarchical diagnostics, genetic fidelity assessment, protected propagation, vector management and traceable certification. Such integration will be essential for transforming virus-free strawberry seedling production from laboratory-scale technical practice into a stable, scalable and industry-oriented production system.

6.3. Conclusions

Virus-free seedling production is a key strategy for reducing strawberry seedling degeneration, interrupting virus transmission and improving the sustainability of strawberry production. Shoot-tip culture remains the technical foundation of virus elimination, while thermotherapy, chemotherapy, cryotherapy and anther culture provide complementary options for improving elimination efficiency or supporting germplasm innovation. However, the value of virus-free seedling production cannot be evaluated solely by the success of virus elimination. A reliable system must also ensure regeneration capacity, genetic fidelity, physiological vigour, protected propagation and certification-based traceability.

Advances in RT-PCR, RT-qPCR, multiplex PCR, HTS, RT-LAMP and RT-RPA have promoted the transition of strawberry virus detection from symptom-based diagnosis to molecular, multi-target and field-deployable testing. Nevertheless, different diagnostic technologies should be used according to propagation tier and production purpose. HTS is most appropriate for high-value materials and virus discovery, RT-qPCR and multiplex PCR are suitable for routine nursery testing and

post-elimination verification, while isothermal amplification methods can support rapid field monitoring and pre-dispatch inspection.

In the future, the large-scale application of certified virus-free strawberry seedlings should be based on an integrated framework linking virus elimination, hierarchical diagnostics, genetic stability assessment, protected nursery production, vector control and traceable certification. This certification-oriented framework can improve the reliability, scalability and commercial value of virus-free strawberry seedling production and provide technical support for high-quality and sustainable development of the strawberry industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Lee, Y.H., Yeoung, H.S., Mezzetti, B. and Yeoung, Y. (2026) Strawberry Propagation: Progress on Propagation Methods, Environmental Regulation, and Disease Management Strategies over the Past 20 Years. *Horticulturae*, **12**, Article 351. https://doi.org/10.3390/horticulturae12030351

[2] Li, Y. (2025) Analysis of the Cultivation and Application of Drugfree Strawberry Seedlings. *Seed Science & Technology*, **43**, 119-121.

[3] Banerjee, D., Singh, V. and Thakur, R. (2023) Micro Propagation on Strawberry: A Review. *E3S Web of Conferences*, **453**, Article 01019. https://doi.org/10.1051/e3sconf/202345301019

[4] Li, D., Sujata, S., Kang, K., Pang, H., Li, Y., Hou, C., *et al.* (2024) Polysaccharide Peptide Treatment Eliminates Strawberry Viruses and Promotes Strawberry Plant Growth and Rooting in Tissue Culture Media. *Plant Disease*, **108**, 2027-2033. https://doi.org/10.1094/pdis-10-23-2226-re

[5] Diaz-Lara, A., Stevens, K.A., Klaassen, V., Hwang, M.S. and Al Rwahnih, M. (2021) Sequencing a Strawberry Germplasm Collection Reveals New Viral Genetic Diversity and the Basis for New RT-qPCR Assays. *Viruses*, **13**, Article 1442. https://doi.org/10.3390/v13081442

[6] Nunes-Leite, L., Liefting, L.W., Waite, D.W., Khan, S. and Thompson, J.R. (2024) High-Throughput Sequencing Methods for the Detection of Two Strawberry Viruses in Post-Entry Quarantine. *Viruses*, **16**, Article 1550. https://doi.org/10.3390/v16101550

[7] Koloniuk, I., Přibylová, J., Čmejla, R., Valentová, L. and Fránová, J. (2022) Identification and Characterization of a Novel Umbra-Like Virus, Strawberry Virus A, Infecting Strawberry Plants. *Plants*, **11**, Article 643. https://doi.org/10.3390/plants11050643

[8] Fránová, J., Lenz, O., Přibylová, J., Čmejla, R., Valentová, L. and Koloniuk, I. (2021) High Incidence of Strawberry Polerovirus 1 in the Czech Republic and Its Vectors, Ge-

netic Variability and Recombination. *Viruses*, **13**, Article 2487. https://doi.org/10.3390/v13122487

[9] Medberry, A.N., Srivastava, A., Diaz-Lara, A., Rwahnih, M.A., Villamor, D.E.V. and Tzanetakis, I.E. (2023) A Novel, Divergent Member of the *Rhabdoviridae* Family Infects Strawberry. *Plant Disease*, **107**, 620-623. https://doi.org/10.1094/pdis-05-22-1078-sc

[10] Wang, Y., Zeng, X., Xiao, G., Zhang, D., Wen, X., Zhou, X., *et al.* (2024) Development of a Multiplex RT-PCR Detection for Six Viruses Infecting Strawberry. *Viruses*, **16**, Article 1858. https://doi.org/10.3390/v16121858

[11] Cultrona, M., Bonini, N., Margaria, P., Menzel, W., Pacifico, D. and Tessitori, M. (2024) First Report of Strawberry Polerovirus 1 in Strawberry in Italy. *Plant Disease*, **108**, Article 1122. https://doi.org/10.1094/pdis-01-24-0239-pdn

[12] Yan, B., Lu, M., Han, J., Cao, Y., Yan, F. and Song, X. (2025) Molecular Insights and Diagnostic Advances in Strawberry-Infecting Viruses. *Frontiers in Microbiology*, **16**, Article 1655696. https://doi.org/10.3389/fmicb.2025.1655696

[13] Mozafari, A.A., vafaee, Y., Hajizadeh, M., Saed-Moucheshi, A., Shahidi, P., Zandan, N.G., *et al.* (2022) Strawberry Crinkle (SCV) and Strawberry Mottle (SMoV) Viruses Affect Yield and Physio-Biochemical Responses of Three Strawberry Cultivars under Different Seasonal Conditions. *Molecular Biology Reports*, **49**, 8651-8661. https://doi.org/10.1007/s11033-022-07701-w

[14] Koloniuk, I., Matyášová, A., Brázdová, S., Veselá, J., Přibylová, J., Fránová, J., *et al.* (2022) Transmission of Diverse Variants of Strawberry Viruses Is Governed by a Vector Species. *Viruses*, **14**, Article 1362. https://doi.org/10.3390/v14071362

[15] Dholi, P., Khatiwada, P., Basnet, B. and Bhandari, S. (2023) An Extensive Review of Strawberry (Fragaria × Ananassa) Diseases and Integrated Management Approaches: Current Understanding and Future Directions. *Fundamental and Applied Agriculture*, **8**, 655-667. https://doi.org/10.5455/faa.136385

[16] Bradamante, G., Mittelsten Scheid, O. and Incarbone, M. (2021) Under Siege: Virus Control in Plant Meristems and Progeny. *The Plant Cell*, **33**, 2523-2537. https://doi.org/10.1093/plcell/koab140

[17] Mori, K. and Hosokawa, D. (1977) Localization of Viruses in Apical Meristem and Production of Virus-Free Plants by Means of Meristem and Tissue Culture. *Acta Horticulturae*, **78**, 389-396. https://doi.org/10.17660/actahortic.1977.78.49

[18] Han, R., Chang, J., Zhao, J., *et al.* (2022) Establishment of Tissue Culture and Rapid Propagation System of Strawberry Stem Tips. *Journal of Shanxi Agricultural Sciences*, **50**, 15-21.

[19] Du, L., Ma, Z., Zhang, Y., *et al.* (2024) Establishment of a Detoxification and Rapid Propagation System for Red Strawberry Tissue Culture. *Hans Journal of Agricultural Sciences*, **14**, 1083-1087. https://doi.org/10.12677/hjas.2024.1410136

[20] Naing, A.H., Kim, S.H., Chung, M.Y., Park, S.K. and Kim, C.K. (2019) *In Vitro* Propagation Method for Production of Morphologically and Genetically Stable Plants of Different Strawberry Cultivars. *Plant Methods*, **15**, Article No. 36. https://doi.org/10.1186/s13007-019-0421-0

[21] Wöhner, T. and Höfer, M. (2023) Evaluation of Cryotherapy and Meristem Isolation from Stolons to Eliminate Viruses in Fragaria Germplasm. *Journal of Plant Pathology*, **106**, 729-735. https://doi.org/10.1007/s42161-023-01570-3

[22] Tian, R., Chen, S., Guo, J., Liu, K., Li, Z., Meng, L., *et al.* (2026) Establishing a Virus-Free Rapid Propagation System for Strawberry 'Miaoxiang 7' through Anther Culture. *Horticulturae*, **12**, Article 227. https://doi.org/10.3390/horticulturae12020227

[23] Liang, Z., Huo, H., Jia, M., *et al.* (2022) Study on Tissue Culture and Rapid Propagation of Stem Tips for Suizhu Strawberry. *Journal of Shanxi Agricultural Sciences*, **50**, 613-619.

[24] Alavijeh, M.K., Bayat, H., Kianpour, D., Kalantari, S. and zarei, A. (2025) Optimization of *in Vitro* Propagation and Virus Eradication Using Meristem Culture and Thermotherapy in Two Geranium Species *Pelargonium* × *hortorum* ('Zonal') and *Pelargonium* × *domesticum* ('Regal'). *BMC Plant Biology*, **25**, Article No. 9. https://doi.org/10.1186/s12870-024-06027-y

[25] Szabó, L.K., Desiderio, F., Kirilla, Z., Hegedűs, A., Várallyay, É. and Preininger, É. (2023) A Mini-Review on *in Vitro* Methods for Virus Elimination from *Prunus* sp. Fruit Trees. *Plant Cell, Tissue and Organ Culture* (*PCTOC*), **156**, Article No. 42. https://doi.org/10.1007/s11240-023-02670-9

[26] Zhang, A., Bettoni, J.C., Shi, X., Liu, Y., Yang, B. and Liu, Z. (2024) *In Vitro* Chemotherapy-Based Methods for Virus Elimination from Actinidia Macrosperma. *Scientia Horticulturae*, **337**, Article 113543. https://doi.org/10.1016/j.scienta.2024.113543

[27] Turcsan, M., Jaksa-Czotter, N., Nagyne Galbacs, Z., Olah, K., Olah, R., Varallyay, E., *et al.* (2025) Effects of Chemotherapy on the Elimination of Various Viruses and Viroids from Grapevine. *Horticulturae*, **12**, Article 46. https://doi.org/10.3390/horticulturae12010046

[28] Bettoni, J.C. and Volk, G.M. (2024) Cryotherapy Using Shoot Tip Cryopreservation. In: Volk, G.M., Ed., *Training in Plant Genetic Resources*: *Cryopreservation of Clonal Propagules*, Colorado State University, 30-37.

[29] Ramlal, A., Quan, P.W., Rajendran, A. and Subramaniam, S. (2025) Impact of Biophysicochemical Factors on Micropropagation, Haploidy and Doubled Haploidy in Strawberry (*Fragaria* sp.): A Critical Revisit. *Current Plant Biology*, **44**, Article 100555. https://doi.org/10.1016/j.cpb.2025.100555

[30] Zhao, X., He, C., Gao, D., Xu, T., Li, X., Liu, J., *et al.* (2022) Construction of Infectious cDNA Clone of Brassica Yellows Virus Isolated from Strawberry and Establishment of Taqman RT-qPCR. *Plants*, **11**, Article 3380. https://doi.org/10.3390/plants11233380

[31] Xu, T., Gao, D., Wu, M., Wang, H. and He, C. (2025) Establishment of Singleplex and Duplex TaqMan RT-qPCR Detection Systems for Strawberry Mottle Virus (SMoV) and Strawberry Vein Banding Virus (SVBV). *Plants*, **14**, Article 2330. https://doi.org/10.3390/plants14152330

[32] Maina, S., Donovan, N.J., Plett, K., Bogema, D. and Rodoni, B.C. (2024) High-Throughput Sequencing for Plant Virology Diagnostics and Its Potential in Plant Health Certification. *Frontiers in Horticulture*, **3**, Article 1388028. https://doi.org/10.3389/fhort.2024.1388028

[33] Villamor, D.E.V., Keller, K.E., Martin, R.R. and Tzanetakis, I.E. (2022) Comparison of High Throughput Sequencing to Standard Protocols for Virus Detection in Berry Crops. *Plant Disease*, **106**, 518-525. https://doi.org/10.1094/pdis-05-21-0949-re

[34] Ren, J., Zhang, J., Wang, Q., Zhou, Y., Wang, J., Ran, C., *et al.* (2022) Molecular Characterization of Strawberry Vein Banding Virus from China and the Development of Loop-Mediated Isothermal Amplification Assays for Their Detection. *Scientific Reports*, **12**, Article No. 4912. https://doi.org/10.1038/s41598-022-08981-9

[35] Zou, X., Dong, C., Ni, Y., Yuan, S. and Gao, Q. (2022) Rapid Detection of Strawberry Mottle Virus Using Reverse Transcription Recombinase Polymerase Amplification with Lateral Flow Strip. *Journal of Virological Methods*, **307**, Article 114566. https://doi.org/10.1016/j.jviromet.2022.114566

[36] Zou, X., Dong, C., Ni, Y. and Gao, Q. (2022) Rapid Detection of Strawberry Mild Yellow Edge Virus with a Lateral Flow Strip Reverse Transcription Recombinase Polymerase Amplification Assay. *Current Microbiology*, **79**, Article No. 365. https://doi.org/10.1007/s00284-022-03045-7

[37] Yang, X., Feng, J., Xu, K., Han, J., Zhang, M., Cheng, A., *et al.* (2025) RD21 Enhances Resistance to the Strawberry Vein Banding Virus by Promoting Autophagy-Mediated Degradation of the Viral Silencing Suppressor p6 Protein. *Plant Biotechnology Journal*, **23**, 3597-3611. https://doi.org/10.1111/pbi.70168

[38] Fan, L., He, C., Gao, D., Xu, T., Xing, F., Yan, J., *et al.* (2022) Identification of Silencing Suppressor Protein Encoded by Strawberry Mottle Virus. *Frontiers in Plant Science*, **13**, Article 786489. https://doi.org/10.3389/fpls.2022.786489

[39] Xu, T., Zhan, B., Zhang, Z., Fan, L., Gao, D., Dong, Z., *et al.* (2025) *FaNDUFB9* Attenuates Strawberry Mottle Virus Infection by Inhibiting the Activity of the Viral Gene Silencing Suppressor, Pro2Glu. *Molecular Plant Pathology*, **26**, e70061. https://doi.org/10.1111/mpp.70061

[40] Neri, J.C., Meléndez-Mori, J.B., Tejada-Alvarado, J.J., Vilca-Valqui, N.C., Huaman-Huaman, E., Oliva, M., *et al.* (2022) An Optimized Protocol for Micropropagation and Acclimatization of Strawberry (*Fragaria* × *ananassa* Duch.) Variety 'Aroma'. *Agronomy*, **12**, Article 968. https://doi.org/10.3390/agronomy12040968

[41] Dwiyani, R., Fitriani, Y., Ana, F.G.S. and Bimantara, P.O. (2024) A Study of Acclimatization Media on Strawberry (*Fragaria* × *ananassa* Duch.) Plantlets Produced from Meristem Culture. *AgriHealth*: *Journal of Agri-Food*, *Nutrition and Public Health*, **5**, 85-91. https://doi.org/10.20961/agrihealth.v5i2.85776

[42] Selivanova, M., Aisanov, T., Romanenko, E. and Esaulko, N. (2025) Survival and Development of Strawberry Plants on Various Substrates at the Stage of Adaptation. *BIO Web of Conferences*, **194**, Article 01025. https://doi.org/10.1051/bioconf/202519401025

[43] Grzelak, M., Pacholczak, A. and Nowakowska, K. (2024) Challenges and Insights in the Acclimatization Step of Micropropagated Woody Plants. *Plant Cell*, *Tissue and Organ Culture* (*PCTOC*), **159**, Article No. 72. https://doi.org/10.1007/s11240-024-02923-1

[44] Nasir, S.M. and Abdulhussein, M.A.A. (2022) Strawberry Shoots Multiplication under Temporary Immersion System (TIS) Using Plant Form Bioreactor. *NeuroQuantology*, **20**, 3186-3193.

[45] Lopez, L., Addison, T., Archambault, M., *et al.* (2023) Pest Management Strategic Plan for Strawberry in North Carolina, Virginia, South Carolina, Georgia, and Florida. National IPM Database. https://ipmdata.ipmcenters.org/source_report.cfm?view=yes&sourceid=2489