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Tissue Culture in Plant Pathology: A Sustainable Approach to Disease Management

Jitendra Singh Rathore

Department of Botany, Government College Kherwara, Udaipur, Rajasthan (India) 313803

INTRODUCTION

Plants are constantly exposed to various pathogens that cause numerous diseases, ultimately reducing their productivity (Agrios, 2005; George *et al.*, 2008). These harmful agents include fungi, bacteria, viruses, phytoplasmas, viroids, nematodes, and others (Ingram & Helgeson, 1980; Hansen & Hildebrand, 1983). Understanding the interactions between host plants and pathogens is crucial, as it offers valuable insights into how pathogens affect plant physiology, their life cycles, and modes of survival (Gunckel & Sanford, 1979; Reinert & White, 1984). Such knowledge is essential for accurate disease diagnosis and the development of effective management strategies (Cassells & Curry, 2001).

The natural progression of plant diseases is influenced by multiple environmental and biological factors that vary across time and location, making consistent study challenging (Hartmann *et al.*, 2002). One effective approach to address these limitations is the application of plant tissue culture in plant pathology (Murashige, 1974; Pierik, 1990). Tissue culture techniques provide a controlled environment, offering unique advantages for examining host-pathogen interactions and understanding the mechanisms of disease development more precisely (George *et al.*, 2008; Thorpe, 1980).

Although plant tissue culture offers several significant advantages, it also presents certain limitations when applied to phytopathological studies. A primary challenge is that cultured plant tissues often differ both physiologically and genetically from whole, intact plants (Cassells & Curry, 2001). These differences in structural organization can limit the accuracy and reliability of comparisons made between *in vitro* cultures and natural plant systems (Reinert & White, 1984).

Another limitation lies in the limited availability of standardized *in vitro* protocols for a wide range of crop species, making it difficult to study their interactions with various pathogens (Preece & Sutter, 1991; Phillips & Garda, 2001). Most of the existing data pertains to model plants such as potato, tomato, tobacco, and carrot (Ingram & Helgeson, 1980). Despite these constraints, advancements in plant cell, tissue, and organ culture—including techniques like protoplast culture—over recent decades have significantly expanded their utility in plant pathology (Bhojwani & Razdan, 1996). These innovations have allowed researchers to explore more practical and targeted applications (Pierik, 1990; Thorpe, 1980).

Tissue culture techniques are currently being used for several important purposes in plant pathology. These include the cultivation of obligate parasites and symbionts under controlled conditions, the elimination of pathogens from infected plant tissues, and the detailed study of host-pathogen interactions (Walkey, 1991; Faccioli & Marani, 1998). Additionally, *in vitro* systems are valuable for selecting disease-resistant lines, screening germplasm for resistance traits, and improving crops through the transfer of disease resistance genes (Kaul & Bhatnagar, 1995; Verma & Dougall, 1981). Together, these applications highlight the potential of tissue culture as a powerful tool in sustainable disease management and crop improvement strategies (George *et al.*, 2008; Agrios, 2005).

2. *In vitro* Culture of Obligate Parasites: A Tool in Plant Pathology

Many vegetatively propagated plant species suffer from systemic infections by viruses, bacteria, fungi, and nematodes, with the inoculum often passed through successive generations, reducing yield and quality (Walkey, 1991; Agrios, 2005). In conventional field conditions, conducting pathogenicity tests or crop loss assessments is difficult without access to large quantities of pure pathogen cultures (Ingram & Helgeson, 1980; Hansen & Hildebrand, 1983). However, advancements in plant tissue culture have enabled researchers to grow these organisms under controlled laboratory conditions, providing a more efficient and reproducible system (Gunckel & Sanford, 1979; Pierik, 1990).

Tissue culture offers unique advantages for the study of obligate parasites (George *et al.*, 2008; Thorpe, 1980). These organisms can now be co-cultured with host tissues *in vitro* under defined environmental parameters, allowing detailed investigations into host-parasite interactions (Simons & Hildebrand, 1981; Reinert & White, 1984). Additionally, this technique ensures a steady supply of contaminant-free cultures of parasites such as spores and nematodes (Kaul & Bhatnagar, 1995; Verma & Dougall, 1981). Such systems serve as ideal models to study infectivity, virulence, metabolism, and reproductive behavior of these pathogens (Gunckel & Sanford, 1979; Ingram & Helgeson, 1980).

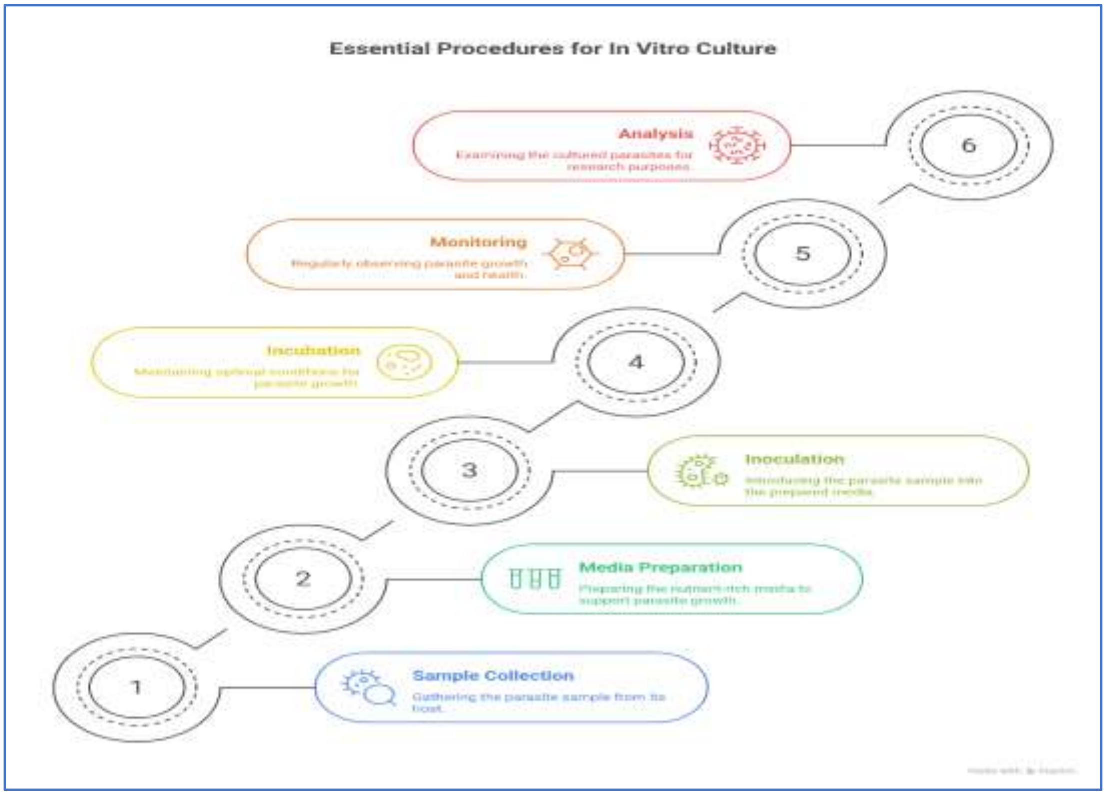

Figure 1. Steps in In Vitro Culture of Obligate Parasites

2.1 Fungal Pathogens and Dual Cultures

Fungal plant pathogens can be broadly classified into biotrophs and necrotrophs, based on how they derive nutrients from host tissues (Simons & Hildebrand, 1981; Agrios, 2005). Biotrophs feed on living cells, while necrotrophs consume dead or dying tissues (Ingram & Helgeson, 1980). Some fungi, such as *Phytophthora infestans*, are hemi-biotrophic and exhibit both modes during their lifecycle (Hartmann *et al.*, 2002). Biotrophic pathogens, which include powdery mildews and rusts, cannot be cultured independently and require host tissues (Pierik, 1990; Thorpe, 1980).

Tissue culture techniques enable the establishment of dual cultures where biotrophic fungi grow on host callus tissues (Simons & Hildebrand, 1981; George *et al.*, 2008). This system has been used to maintain aseptic fungal cultures, study pathogen-host interactions, and produce spores for further study (Reinert & White, 1984; Cassells & Curry, 2001). However, successful dual culture systems require careful optimization of conditions such as temperature, hormone concentrations, and inoculum density, which influence host susceptibility and pathogen growth (Thorpe, 1980; Pierik, 1990).

2.2 Powdery Mildews: Challenges and Attempts

Powdery mildew fungi are obligate parasites causing significant damage to crops like cereals, legumes, and fruit plants (Simons & Hildebrand, 1981; Agrios, 2005). Despite various attempts to infect callus cultures with these pathogens, consistent and sustained growth has remained elusive (Pierik, 1990; Thorpe, 1980). Factors like relative humidity, photoperiod, and host tissue vigor have been studied, with some success noted in grape tissue cultures (Hartmann *et al.*, 2002; George *et al.*, 2008). Still, the establishment of stable and reproducible powdery mildew cultures remains a significant challenge (Ingram & Helgeson, 1980; Cassells & Curry, 2001).

2.3 Axenic Culture of Parasitic Fungi

The cultivation of obligate parasitic fungi in the absence of host tissues has long been difficult (Simons & Hildebrand, 1981; Ingram & Helgeson, 1980). A breakthrough came with the axenic culture of *Sclerospora graminicola* on modified synthetic media (Gunckel & Sanford, 1979; Pierik, 1990). This method enabled researchers to study different fungal stages and physiological responses independently of host tissues (Thorpe, 1980; Reinert & White, 1984). Axenic cultures provide valuable material for research in pathogen physiology, infection mechanisms, and responses to environmental factors or treatments (George *et al.*, 2008; Cassells & Curry, 2001).

2.4 Culture of Phytoplasma and Spiroplasma

Phytoplasmas, once thought to be viruses, are now known to be wall-less bacteria that cannot currently be cultured on cell-free media. Their study relies heavily on molecular techniques. In contrast, spiroplasmas—another group of mollicutes—can be cultured *in vitro* and have been implicated in diseases like citrus stubborn and corn stunt. Cultured spiroplasmas are useful in pathogenicity studies, including transmission by insect vectors and toxin production (Faccioli & Marani, 1998; Gunckel & Sanford, 1979).

2.4 Mycorrhizae in Tissue Culture

Mycorrhizal fungi form beneficial symbiotic associations with plant roots, enhancing nutrient uptake and disease resistance. However, as obligate symbionts, they are difficult to culture axenically. Root organ cultures have emerged as promising alternatives for propagating vesicular-arbuscular mycorrhizal fungi. These systems could enable mass production of mycorrhizal inocula and open avenues for studying fungal roles in plant defense (George *et al.*, 2008).

2.5 Growth of Nematodes in Tissue Culture

Plant-parasitic nematodes are significant agricultural pests (Agrios, 2005; Kaul & Bhatnagar, 1995). Tissue culture allows the growth of nematodes on excised roots, callus, and seedlings from various plant species, offering a controlled system to study their behavior and physiology (Verma & Dougall, 1981; Thorpe, 1980). Certain nematodes, such as root-knot species, reproduce effectively in these systems, enabling investigations into host responses, hormone effects, and nematicide screening (Kaul & Bhatnagar, 1995; George *et al.*, 2008). However, challenges remain in sterilizing nematode inocula without affecting viability (Pierik, 1990).

Additionally, nematodes can often be propagated on callus tissues of non-host species, indicating a degree of flexibility in their host requirements under *in vitro* conditions (Verma & Dougall, 1981; Ingram & Helgeson, 1980). For instance, potato rot nematodes have been grown successfully on carrot and tobacco callus cultures (Kaul & Bhatnagar, 1995). Alfalfa callus has been widely used for nematode propagation due to its compatibility (Thorpe, 1980). Growth and reproduction of nematodes *in vitro* are influenced by several factors, including medium composition and plant hormones (George *et al.*, 2008; Bhojwani & Razdan, 1996). Auxins such as 2,4-D have been found to stimulate nematode reproduction (Preece & Sutter, 1991). Dual cultures of nematodes with excised roots or callus tissues allow continuous maintenance and enable researchers to investigate parasitic mechanisms, hormone responses, and altered host metabolism using biochemical or radiolabeling approaches (Verma & Dougall, 1981; Cassells & Curry, 2001).

These *in vitro* systems are invaluable not only for fundamental research but also for applied studies aimed at developing resistant plant varieties and sustainable disease control strategies (Gunckel & Sanford, 1979; Pierik, 1990). By mimicking natural host-parasite interactions under controlled conditions, tissue culture facilitates deeper insights into pathogenicity, supports the development of diagnostic tools, and aids in screening for resistance traits (George *et al.*, 2008; Reinert & White, 1984). As tissue culture technologies advance, their integration into plant pathology will continue to expand, supporting sustainable agricultural practices and resilient crop production systems (Agrios, 2005; Murashige, 1974).

3. Elimination of Phytopathogens from Infected Hosts

Many plant diseases caused by fungi and bacteria can often be managed effectively with chemical treatments such as fungicides and antibiotics (Agrios, 2005; Walkey, 1991). However, viral infections in plants present a unique challenge since viruses cannot be eradicated through conventional chemical means (Faccioli & Marani, 1998; Morel & Martin, 1952). These pathogens can cause both localized and systemic infections. In localized infections, viruses typically move from one cell to the next via plasmodesmata and do not spread extensively (Hu & Wang, 1983). On the other hand, systemic infections are more problematic, especially when viruses are transmitted throughout the plant by phloem-feeding insects like aphids (Walkey, 1991; Agrios, 2005). This type of infection becomes particularly serious in vegetatively propagated perennial crops such as potato, sugarcane, and ginger, as well as in many ornamental and fruit plants propagated via cuttings (Kartha, 1981; George *et al.*, 2008). Since there is no effective antiviral treatment for plants and destroying mature perennials is not a viable solution, alternative strategies are needed (Faccioli & Marani, 1998; Walkey, 1991).

One effective method for managing systemic viral infections is the use of meristem-tip culture, a technique that capitalizes on the ability of plants to regenerate from a small cluster of meristematic cells (Morel & Martin, 1952; Hu & Wang, 1983). These rapidly dividing cells often remain virus-free due to several inherent biological advantages (Kartha, 1981; Faccioli &

Marani, 1998). Firstly, the high metabolic activity of meristematic tissues makes it difficult for viruses to hijack the host's biosynthetic machinery (Murashige, 1974). Secondly, the absence of a fully developed vascular system in these tissues prevents the spread of phloem-restricted viruses (Walkey, 1991). Additionally, virus movement through plasmodesmata is a slow process, making it difficult for them to infect fast-dividing meristematic cells effectively (George *et al.*, 2008; Cassells & Curry, 2001). Lastly, higher auxin concentrations in meristematic tissues are believed to inhibit viral replication (Bhojwani & Razdan, 1996). As a result, healthy plants can often be regenerated by culturing meristematic tissues under sterile conditions (Hu & Wang, 1983; Kartha, 1981).

3.1 Meristem-Tip Isolation and Culture

The process involves aseptically isolating the apical meristem from the plant and culturing it on a sterile, nutrient-rich medium (Hu & Wang, 1983; George *et al.*, 2008). Once established, the meristematic cells continue to grow and differentiate, eventually forming complete plantlets (Murashige, 1974; Bhojwani & Razdan, 1996). This technique was first successfully used for virus elimination in *Dahlia* by Morel and Martin in the early 1950s (Morel & Martin, 1952; Faccioli & Marani, 1998).

To initiate meristem-tip culture, shoot apices approximately 1–2 mm long are dissected from the plant, typically from segments containing a single node (Hu & Wang, 1983). These segments are surface sterilized, first by immersing them briefly in 70% ethanol and then treating with sodium hypochlorite solution, followed by multiple rinses with sterile water (George *et al.*, 2008; Preece & Sutter, 1991). Using a dissecting microscope, the surrounding leaflets are carefully removed, exposing the apical dome and one or two leaf primordia (around 0.2–0.5 mm in length) (Kartha, 1981). This small tissue fragment is then transferred to a semi-solid culture medium enriched with growth hormones such as auxins or cytokinins (Pierik, 1990; Phillips & Garda, 2001). Over time, these explants are subcultured and can be multiplied through micropropagation (Bhojwani & Razdan, 1996; George *et al.*, 2008).

One of the main limitations of this technique is the requirement for an extremely small meristem tip to avoid including virus-infected tissue (Walkey, 1991; Faccioli & Marani, 1998). However, such small explants sometimes fail to regenerate. In such cases, slightly larger meristem tips may be used, in combination with thermotherapy or chemotherapy, to deactivate the virus (Hu & Wang, 1983; Kartha, 1981). Thermotherapy involves exposing the donor plants to elevated temperatures (typically between 30–34°C) for a prolonged period (Faccioli & Marani, 1998). This stress inhibits viral activity and allows subsequent meristem culture to regenerate virus-free plants (Walkey, 1991; George *et al.*, 2008). This method has proven successful in crops like *Pelargonium* (Morel & Martin, 1952).

Alternatively, chemotherapy using antiviral compounds has been explored (Walkey, 1991). For instance, the antiviral compound Virazole (a nucleoside analogue) has shown some promise when applied to potato plants prior to meristem-tip culture (Faccioli & Marani, 1998). However, concerns remain about the potential mutagenic effects of such chemicals on plant tissues (Cassells & Curry, 2001). As a result, thermotherapy remains the more widely accepted and safer option for virus elimination in plant tissue culture (Kartha, 1981; George *et al.*, 2008).

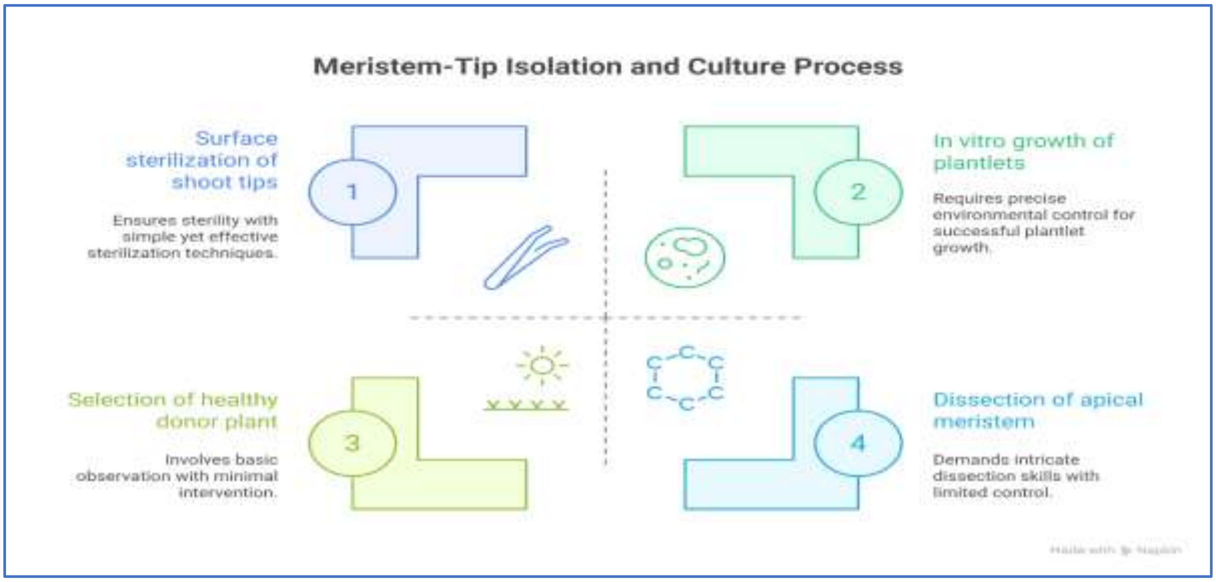

Figure 2. Meristem Tip isolation and culture process

4. Study of Host-Parasite Interaction

Plant tissue culture has emerged as a valuable tool for investigating host-parasite interactions in a variety of plant diseases caused by fungi, bacteria, and nematodes (Gunckel & Sanford, 1979; Hansen & Hildebrand, 1983). This technique provides a simplified and highly controlled experimental system where environmental conditions—both physical and chemical—can be precisely regulated (George *et al.*, 2008; Thorpe, 1980). Tissue culture enables researchers to expose large populations of host cells to specific pathogens without causing significant damage to the tissues (Reinert & White, 1984; Cassells & Curry, 2001). It also allows for accurate control over cell numbers and pathogen inoculum, and offers the flexibility to add or remove specific compounds, such as metabolic precursors or products, to study their influence on host-pathogen dynamics (Pierik, 1990; Phillips & Garda, 2001).

Although early studies raised concerns about the reliability of tissue culture systems for studying disease resistance—for example, in cases where resistance genes in potato against *Phytophthora infestans* appeared to function normally but later biochemical analysis revealed anomalies—subsequent research has proven the value of well-designed *in vitro* systems (Ingram & Helgeson, 1980; Agrios, 2005). Investigations into crown-gall disease physiology and the expression of resistance to *Phytophthora parasitica* in tobacco tissue cultures have shown that, when appropriately defined, tissue culture models can yield important insights (Schlegel & Kado, 1987; Gunckel & Sanford, 1979).

These systems offer a platform to explore various aspects of host-pathogen relationships, including mechanisms of resistance and host recognition, the physiological basis of tumor development, the effects and pathways of pathogen-produced toxins and enzymes, the nature of systemic infections, and the dynamics of nutrient exchange—especially relevant in symbiotic interactions like mycorrhizae (Cassells & Curry, 2001; Thorpe, 1980; George *et al.*, 2008). While tissue culture may not provide complete answers to the complexities of host-parasite relationships, it undoubtedly opens up numerous avenues for generating novel and meaningful insights that can advance our understanding of plant pathology (Reinert & White, 1984; Agrios, 2005). Many possibilities remain unexplored, but the existing research already underscores the significant potential of *in vitro* approaches in this field (Murashige, 1974; Pierik, 1990).

5. *In vitro* Cell-Line Selection

In vitroIn vitro cell-line selection is a promising approach for developing disease-resistant plants, especially when conventional methods like breeding, mutagenesis, or hybridization face limitations (Thorpe, 1980; Preece & Sutter, 1991). Traditional methods often struggle due to inefficient screening, limited genetic diversity, and environmental variability (Agrios, 2005; Cassells & Curry, 2001). Challenges such as uneven disease pressure, high escape rates, and the difficulty of selecting for both major and minor resistance genes simultaneously hinder progress (George *et al.*, 2008). Moreover, conventional induced mutagenesis requires screening large populations due to the rarity of useful mutations, while clonal crops with polyploidy and sterility further complicate breeding (Bhojwani & Razdan, 1996).

Tissue culture techniques help overcome these issues by enabling controlled regeneration from selected cells (Murashige, 1974; Thorpe, 1980). Early studies demonstrated that plant cells could be selected *in vitro* for resistance to pathogen-derived toxins, with whole plants later regenerated from those resistant cells (Reinert & White, 1984; Ingram & Helgeson, 1980). This strategy has since shown promise in cases where conventional breeding has failed (Gunckel & Sanford, 1979; George *et al.*, 2008). Recurrent selection *in vitro*—especially without using mutagens—has been successfully applied in crops like tobacco and maize, where cells were selected for resistance to specific toxins, and regenerated plants exhibited enhanced disease resistance (Thorpe, 1980; Cassells & Curry, 2001).

To begin *in vitro* selection, a suitable culture system must be established. Callus cultures are most commonly used because they are easy to initiate and maintain, and allow for direct inoculation and microscopic monitoring of pathogen interactions (Pierik, 1990; Phillips & Garda, 2001). Callus tissue also permits analysis of biochemical responses such as phytoalexin production and often yields a higher frequency of resistant variants (George *et al.*, 2008; Hansen & Hildebrand, 1983). Other selection units like protoplasts or embryoids may be used, offering unique advantages such as single-cell origin and non-chimeric regenerants, though each system has technical challenges related to regeneration and stability (Bhojwani & Razdan, 1996; Preece & Sutter, 1991).

5.1 *In vitro* Selection for Toxin Resistance

Many fungal and bacterial plant pathogens produce low molecular weight toxins that mimic the disease symptoms when applied to plant tissues or cells *in vitro* (Agrios, 2005; Hansen & Hildebrand, 1983). These toxins are typically classified as pathogenicity factors, virulence factors, or disease non-determinants, and play critical roles in infection initiation, colonization, and symptom development (Reinert & White, 1984; Cassells & Curry, 2001). For example, the AK-toxin from *Alternaria alternata* facilitates host penetration, while T-toxin from *Helminthosporium maydis* promotes disease spread in maize (Gunckel & Sanford, 1979; Ingram & Helgeson, 1980).

In vitro selection for disease resistance is often conducted using toxin-containing media (Thorpe, 1980; Pierik, 1990). Toxins may be used in crude or purified form, with purified toxins preferred when their role in disease is well-characterized (George *et al.*, 2008). Despite limitations, crude culture filtrates are widely used, and success has been documented in crops like potato, tobacco, alfalfa, and oilseed rape (Phillips & Garda, 2001; Bhojwani & Razdan, 1996). The main advantage of using toxins over live pathogens is that toxins act at the cellular level, enabling the selection of resistant variants across a broader range of plant species and tissues (Thorpe, 1980; Cassells & Curry, 2001).

Selection typically involves exposing callus or cell cultures to sub-lethal toxin concentrations in successive cycles (Preece & Sutter, 1991; George *et al.*, 2008). Resistant cells continue to grow and divide despite toxin presence. Over time, these cells are regenerated into whole plants and screened for stable resistance (Murashige, 1974; Pierik, 1990). While careful calibration of toxin dosage is required to avoid false positives or excessive cell death, *in vitro* toxin-based selection has proven effective in isolating resistant lines, particularly when combined with somaclonal variation from prolonged culture (Bhojwani & Razdan, 1996; Thorpe, 1980).

CONCLUSION

In vitro techniques have revolutionized the study and management of plant-pathogen interactions by providing controlled and reproducible systems for experimentation. The use of meristem-tip culture has proven particularly effective in eliminating systemic viral infections from vegetatively propagated crops, offering a viable alternative to traditional plant protection strategies where no chemical treatments are available. Furthermore, tissue culture-based systems serve as powerful tools for dissecting host-parasite relationships, enabling detailed investigations into resistance mechanisms, pathogen behavior, and plant defense responses. *In vitro* cell-line selection, especially for toxin resistance, presents a promising avenue for developing disease-resistant cultivars. This approach allows for the precise application of selective pressure and facilitates the recovery of stable, non-chimeric resistant variants. Through strategies like callus culture, protoplast selection, and recurrent exposure to pathogen-derived toxins, researchers have successfully regenerated resistant plants in species where conventional breeding has shown limited success.

Overall, the integration of plant tissue culture with plant pathology has not only enhanced our understanding of disease processes but also provided practical solutions for the development of healthier and more resilient crop varieties. Continued refinement and application of these techniques hold great potential for sustainable agriculture and food security in the face of evolving plant disease challenges.

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