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Plant Screening for Detection of Virus Resistance Traits

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SUMMARY

Plant viruses threaten global food security and are best managed through genetic resistance rather than conventional measures. While dominant R genes have historically been key, recent advances highlight recessive resistance genes as alternative pathways. Screening protocols using sap inoculation and insect vectors like whiteflies and aphids are essential for identifying resistant germplasm. These resistant sources serve as valuable material for breeding programs aimed at durable virus resistance.

INTRODUCTION

Plant viruses pose a significant threat to global food production and security (Singhal et al., 2021). Owing to their intracellular nature, they cannot be effectively managed through conventional prophylactic measures (Nabi et al., 2020). The most cost-effective and environmentally sustainable strategy lies in exploiting crop genetic resistance, which is governed by the complex mechanisms underlying plant–virus interactions (Yadav et al., 2023a). Over the past eight decades, remarkable progress has been achieved in understanding plant resistance to viral pathogens (Baranwal et al., 2020; Nabi et al., 2021). While most natural resistance genes identified historically have been dominant R genes encoding NBS-LRR proteins, the past decade has witnessed the cloning of numerous recessive resistance genes in crops, highlighting an alternative pathway to combat viral infections. Nevertheless, the development of robust and durable resistance capable of countering the extraordinary genetic variability of viruses remains a pressing challenge for the future (Yadav et al., 2023b). This article outlines the screening protocols used to identify resistant sources against plant viruses. Such sources may serve as valuable material for the discovery of novel *R* genes or as donor parents in resistance breeding programs.

Transmission of plant viruses

Plant viruses may be transmitted through sap, seeds, or insect vectors (Yadav et al., 2013). For disease screening, however, sap inoculation and insect-mediated transmission are the most commonly employed methods to assess plant resistance to viral infections.

Mechanical sap inoculation:

Mechanical inoculation involves bringing healthy plant tissues, typically leaves, into contact with a virus-containing inoculum to initiate infection (Dubey et al. 2020). While, many plant viruses can be transmitted mechanically, not all are amenable to this method. Successful infection requires the creation of minor wounds, usually achieved by applying a mild abrasive such as carborundum powder, since intact leaf surfaces with their protective cuticle and cell walls serve as strong barriers against viral entry. Following abrasion, the leaves are inoculated by gently rubbing them with freshly prepared inoculum. Although a single virus particle could theoretically infect a cell, in practice several hundred particles (often more than 500) are necessary to establish infection and produce visible lesions. This is likely because only a limited number of epidermal cells are wounded sufficiently to allow virus particles to penetrate into the cytoplasm.

Material required

The materials required for mechanical inoculation include virus-infected plants as the source of inoculum, test plants or germplasm for screening, an inoculation buffer (commonly phosphate buffer), sterilized mortars and pestles, a mild abrasive such as celite (diatomaceous earth) or carborundum, cheesecloth pieces or cotton swabs for application, and plant labels or markers for identification.

Procedure for screening of soybean germplasm against Cowpea mild mottle virus (CPMMV)

Grow the test plant genotypes in a soil mixture composed of soil, sand, and peat in a 1:1:1 ratio, using 8-10 inch earthen pots placed in the glasshouse. To ensure reliable screening, maintain the plants under insect-proof cages from germination until the appearance of viral symptoms.

Inoculum preparation

- Collect fresh soybean leaves infected with CPMMV and homogenize them in a small volume of cold homogenization buffer (0.1 M phosphate buffer, pH 7.0–7.6) using a sterile pestle and mortar to obtain a finely ground virus suspension (1:1 w/v).
- Apply the prepared inoculum onto carborundum dusted leaf surfaces of test plants at the first trifoliate stage using sterile glass rods or cotton swabs.
- Following mechanical inoculation, spray the leaves with water to remove excess inoculum and minimize evaporation.
- Monitor the trifoliate leaves regularly for symptom development, which typically appears 10–14 days post-inoculation depending on genotype.
- Record disease reactions (as described in Table 1) and classify the genotypes as resistant or susceptible accordingly.

Table 1. Categorization of genotypes by infection percentage (Cheruku et al. 2017)

Score	Resistant category
0%	Absolutely resistant
0.01-11.11%	Highly resistant
12.22-33.33%	Moderately resistant
34.44-55.55%	Moderately susceptible
56.6-77.77%	Susceptible
78.88-100%	Highly susceptible

Precaution to be followed

- Select young plants, preferably at the 3–4 leaf stage, for inoculation or screening.
- Use young leaves from infected plants as the inoculum source, as they contain the highest virus concentration.
- Ensure the mortar and pestle are thoroughly cleaned, autoclaved, and kept on ice before inoculum preparation.
- Apply carborundum carefully in the appropriate amount to avoid excessive injury to the plants.
- Label each plant clearly with details (such as host name, virus isolate, and date of inoculation).
- Perform inoculation in the late afternoon or evening, when plant resistance is at its lowest.

Important factors in sap transmission:

Abrasives

- Carborundum powder and celite are commonly used abrasives that create sub-lethal injuries, facilitating the entry of plant viruses. However, as the dust can irritate the eyes and skin, they should be applied with caution.

Buffer

- Mechanical inoculation typically uses a virus suspension in a liquid buffer, and the choice of buffer directly influences inoculum efficiency. Phosphate buffer is most commonly employed, as it stabilizes the virus and enhances infectivity in many cases. However, in certain host–virus combinations, alternative buffers may prove more effective.

pH of the buffer

- The pH of the inoculation buffer strongly influences viral infectivity. Optimal infectivity is achieved with phosphate buffer at pH 7.0–7.6. However, since virus–host combinations vary in their response, the optimum pH must be standardized for each case.

Inhibitors of infection

- Compounds such as polyphenols, tannins, and mucilage can hinder sap transmission. To counteract their effects, chemicals like sodium sulphite, ascorbic acid, or 2-mercaptoethanol (0.2–1%) are added to the inoculum. These agents enhance infectivity by reducing the impact of inactivating substances; for example, 2-mercaptoethanol acts effectively as a reducing agent.

Whitefly transmission

Whiteflies (*Bemisia tabaci*, Hemiptera*: Aleyrodidae),* a complex of morphologically indistinguishable species, are major vectors of plant viruses such as Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus. These viruses, though not replicating in the insect, are efficiently transmitted from plant to plant through feeding

or probing. Since, many cannot be transmitted by other means, whiteflies are essential for maintaining virus cultures, characterization, ecology studies, and resistance screening. Their use in breeding programs is particularly challenging, as effective selection requires complete inoculation of large plant populations to identify the few resistant genotypes.

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Rearing and Maintenance of Whiteflies

Whiteflies multiply rapidly under warm conditions, especially when natural enemies are absent and host plants favor outbreaks. Eggplant is the most commonly used host for *B. tabaci*, though tobacco, blackgram, and soybean can also serve as alternatives. For rearing, eggplants are first sown in cell trays and transplanted at the two-leaf stage into pots containing a vermiculite–soil mixture (1:1 v/v). Plants are maintained in growth chambers at 20–22 °C, 80% relative humidity, and a 16:8 h light–dark cycle to ensure optimal growth. Pupal stage whiteflies collected from the field are identified to species level and transferred onto potted eggplants. The insects are allowed to develop into adults on disease-free plants, after which healthy adults are collected and maintained on caged plants for about eight weeks to establish a non-viruliferous colony for experimental use.

Procedure of Transmission of viruses through whitefly (persistent virus)

- Use three-four day-old adult whiteflies, as they are most active and efficient in virus transmission.
- Transfer non-viruliferous whiteflies onto virus-infected plants for feeding.
- Allow an acquisition access period of 18–24 hours on diseased leaves for virus uptake.
- Collect viruliferous whiteflies using an aspirator and transfer them to test plants for resistance screening.
- Release 8–10 whiteflies per plant and maintain them within a cage system.
- Provide an inoculation access period of 18–24 hours on test plants, repeating the process three times.
- After feeding, remove the test plants and spray imidacloprid to eliminate the insects.
- Monitor plants regularly for symptom development and record disease reactions.
- Classify resistant lines based on symptom expression, virus presence after inoculation, pod setting, and yield performance.

Precaution

- Gently shake plantlets to prevent whiteflies from clustering and ensure proper feeding.
- During the inoculation period, check at least once to confirm probing/feeding by turning leaves inside the cage.
- Verify infection status by PCR three weeks post-inoculation.
- Use only plants showing clear diagnostic symptoms for experiments.
- Grow virus-infected and mock-inoculated plants under identical conditions but in separate greenhouse sections, and use them four weeks after inoculation.

Aphid transmission

A majority of plant viruses rely on vectors for transmission and survival, with insects being the most common. Among these, aphids are responsible for transmitting nearly half of all insect-vectored viruses. Their effectiveness as vectors is attributed to several unique traits: (1) the polyphagous nature of certain species such as *Myzus persicae*, enabling them to feed on diverse plant hosts and spread viruses across many crops; (2) their capacity for parthenogenetic reproduction, which allows rapid population growth; and (3) their needle-like stylet, capable of piercing plant cell walls and directly introducing viruses into host cells.

Rearing and maintenance of aphids

Aphids are maintained on suitable host plants, such as cotton or turnip for *M. persicae*. Females are collected, identified to species level, and released onto host plants kept under cage conditions. Populations should be monitored regularly for predators or parasites. Optimal rearing conditions include a temperature of 23–25 °C and a 16-hour light period.

Transmission by aphids

- Collect aviruliferous aphids and subject them to a 1-hour pre-acquisition starvation period.
- Use polymerase chain reaction confirmed virus-infected leaves as inoculum source.
- Allow aphids to feed on infected leaves for an acquisition access period (AAP): 1 hour for non-circulative transmission and 24 hours for circulative transmission.
- After AAP, transfer viruliferous aphids to test plants.

- Release 10–15 aphids per plant and provide an inoculation access period (IAP) of 5–20 minutes for non-circulative transmission and 12–24 hours for circulative transmission.
- Following IAP, remove aphids by spraying systemic insecticides.
- Maintain plants under optimal growth conditions and monitor regularly for symptom development.
- Identify resistant lines based on symptom expression, virus detection after inoculation, pod set, and yield performance.

Precaution

- Avoid overcrowding in aphid cultures.
- Introduce fresh, healthy host plants regularly.
- Minimize honeydew accumulation and associated sooty mold.
- Inspect cultures frequently for predators or parasites.

CONCLUSION

Effective management of plant viruses relies on precise screening methods using sap inoculation and insect vectors such as whiteflies and aphids. These protocols enable identification of resistant germplasm, which is crucial for breeding durable resistance against diverse viral pathogens. By standardizing inoculation techniques and maintaining vector colonies under controlled conditions, researchers can ensure reliable resistance evaluation. Ultimately, such efforts strengthen crop improvement programs and safeguard food security against viral threats.

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