



Q-bank Plant Viruses and Viroids

Protocol for mechanical inoculation of test plants

Buffers and chemicals

Phosphate buffer (10x):

2,72 g	KH_2PO_4
14.20 g	$\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$
800 ml	demineralised water

set pH to 7.4 with NaOH
add demineralised water to 1000 ml total volume

Inoculation buffer (0.02 M PB):

200 ml	Phosphate buffer (10x)
[2% (w/v) PVP]	20 g Polyvinylpyrrolidone (PVP; MW 10,000)
800 ml	demineralised water

Disinfection solution:

25 ml	sodium hypochlorite solution (commercial bleach)
[1% (v/v) hypochlorite]	75 ml demineralised water

*For some virus species additional components are recommended. If applicable specific information is provided on species records.

Equipment and materials

Carborundum powder (500 mesh)	
Extraction bags	e.g. Bioreba 410100
Hand model homogeniser	e.g. Bioreba 400010
Petri dishes	
Gloves	
Protecting mask	
Labels	
Water resistant marker	

Procedure

Mechanical inoculation

- Select plants to be inoculated and place them on the right spot in the greenhouse
[Table 1 indicates optimum stage of most common test plant species]
- Dust carborundum powder onto leaves of test plants (use mask to protect mouth and nose)
- Put about 0.5 g plant material into extraction bag
- Add about 5 ml inoculation buffer
- Fold edge extraction bag 2 times to close
- Grind plant material with homogeniser
- Pour inoculum into petri dish
- Moisten one or two fingers in inoculum and rub gently onto the leaves (use gloves), while supporting them with the other hand (preferably covered with tissue paper)
- Rinse inoculated plants with tap water (within 2-5 minutes)
- Grow test plants at 18-25 °C with supplementary illumination for a day length of at least 14 hours
- Inspect test plants for symptoms at least twice a week for at least 3 weeks

Prevention of cross contamination

- Change gloves between samples
- Separate test plants of different samples
- Clean contaminated equipment and surfaces

Table 1. Optimum stage for inoculation of test plants

Test plant species	Number of leaves	Remarks
<i>Ammi majus</i>	1-2	
<i>Brassica campestris</i>	2-3	
<i>Capsicum annuum</i>	2-3	
<i>Chenopodium amaranticolor</i>	3-4	> for only local symptoms
<i>Chenopodium quinoa</i>	3-4	> for only local symptoms
<i>Cucumis sativus</i> 'Chinese slangen'	2 cotyledons	remove leaves, except top leaf
<i>Datura metel</i>	2-3	
<i>Datura stramonium</i>	2-3	
<i>Gomphrena globosa</i>	about 6	
<i>Nicotiana benthamiana</i>	3-4	
<i>Nicotiana bigelovii</i>	3-4	
<i>Nicotiana debneyi</i>	2-3	
<i>Nicotiana glutinosa</i>	3-4	
<i>Nicotiana glauca</i>	3-4	
<i>Nicotiana glauca</i> 67A	4-6 expanded	
<i>Nicotiana miersii</i>	2-3	
<i>Nicotiana occidentalis</i> -37B	4-6 expanded	
<i>Nicotiana occidentalis</i> -P1	4-6 expanded	
<i>Nicotiana ruscifolia</i>	1-2	
<i>Nicotiana tabacum</i> 'Samsun'	1-2	
<i>Nicotiana tabacum</i> 'White Burley'	1-2	
<i>Nicotiana tabacum</i> 'Xanthii'	1-2	
<i>Petunia hybrida</i>	4	
<i>Phaseolus vulgaris</i> 'Dubbele witte zonder draad'	2	remove stem and leaves when >2 leaves
<i>Physalis floridana</i>	2-3	
<i>Pisum sativum</i> 'Kelvedon Wonder'	4-6	
<i>Solanum lycopersicum</i> 'Money-maker'	1-2	
<i>Vicia faba</i> 'Witkiem'	2-4	

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