DETECÇÃO DE VÍRUS E VIROIDE EM SEMENTES E MUDAS

TERMINOLOGIA

TRANSMISSÃO: passagem do vírus de indivíduos infectados para sadios.

PERPETUAÇÃO: passagem de material infectado de uma geração clonal para outra através da multiplicação vegetativa ou sementes, implicando na continuidade da passagem do vírus

TRANSMISSÃO DE VÍRUS E VIROIDES POR SEMENTES

A. Características

- 1/4 dos vírus conhecidos são transmitidos por sementes.
- Muitos viroides são transmitidos por sementes
- Transmissão de 0 - 100%, maioria < 50%.
- Transmissão é função da hospedeira e do vírus.

EX: Vírus necrose branca do fumo

Não é transmitido por semente de fumo
É transmitido por semente de *Nicandra physaloides*

EX: Vírus do mosaico amarelo do feijoeiro

Não é transmitido por sem. de diversos *Phaseolus*
É transmitido por sem. de *Vigna sinensis*

- Época em que a planta foi infectada.
- Longevidade do vírus na semente: meses até anos.

TABLE 12.9 Examples of Relative Importance of Seed Transmission for Viruses of Various Virus Genera[a]

Virus Genus/ Group	No. Members		Type of Potential Injury[b]						% Seed Transmission
	In Group	Seed-Borne	A	B	C	D	E	F	
Alfamovirus	1	1	+	+	+				1–23
Bromovirus	6	1	+	+	+				+[c]
Capillovirus	4	1							1–60
Carlavirus	60	2							2–90
Carmovirus	18	2							10–40
Caulimovirus	34	1[d]							[d]
Closterovirus	28	1							+
Comovirus	15	6	+	+	+				1–90
Cryptovirus	31	31							100
Cucumovirus	3	3	+	+	+				<1–1
Dianthovirus	5	0							
Enamovirus	1	1							1–2
Fabavirus	4	0							
Geminivirus	102	1							+
Hordeivirus	4	1	+	+	+		+		+
Ilarvirus	17	8				+			1–90
Luteovirus	7	0							
Marafivirus	3	0							
Nepovirus	40	17	+	+	+				3–100
Plant reovirus	14	0							
Potexvirus	36	4							1–6
Potyvirus	179	16	+	+	+	+	+	+	<1–80
Rhabdovirus	15	1							+
Sobemovirus	14	4							1–80
Tenuivirus	11	0							
Tobamovirus	17	7	+	+	+				1–20[e]
Tobravirus	3	3	+	+	+				1–35
Tospovirus	13	1							Up to 95
Tombusvirus	13	1							+
Tymovirus	23	3							+
Viroids	15	5			+	+			+

Data from Stace-Smith and Hamilton (1988) and from AAB Descriptions of Plant Viruses.

[a] *Note that not all members of genus were tested for seed transmissibility.*

[b] *A, survival of inoculum; B, dispersal of inoculum; C, primary inoculum source; D, contamination of germplasm lines; E, contamination of virus-free planting material; F, direct crop losses due to plants arising from infected seed.*

[c] *+indicates that no % values were given.*

[d] *BSV is apparently seed transmitted in Musa but probably by activation of integrated viral sequences.*

[e] *Seed-transmission of TMV probably due to contamination.*

Hull, 2012

Legenda Tabela 21.1

A: sobrevivência do inóculo
B: disseminação do inóculo
C: fonte primária do inóculo
D: contaminação de germoplasmas
E: contaminação de material propagativo livre de vírus
F: dano direto na produção originário de sementes infectadas

B. Valor epidemiológico

- Perpetua o vírus sob condições adversas.

- Foco inicial de inóculo na cultura.

- Introdução e estabelecimento do vírus em novas áreas, países.

- Presença em bancos de germoplasma: efeito no melhoramento.

C. Tipos de transmissão de vírus e viroide por semente

1. Infecção da plântula por vírus ou aderido à parte externa da semente
   Eliminação: calor seco 70-76°C, 1 a 3 dias
   Solução 3% de ortofosfato trissódio, 30 minutos
   0,25% hipoclorito de sódio

2. Transmissão verdadeira ou embriogênica

D. Rotas para infecção do embrião:

Diretamente da planta mãe ou pólen

- Infecção do meristema floral (vírus crípticos)

- Infecção direta do embrião

Problema: isolamento do embrião dos tecidos maternos, ausência de ligações vasculares

Evidence of Non-Transmission of *Rice yellow mottle virus* (RYMV) through Rice Seed

Abo et al., 2004

TROPICULTURA, 2004, **22**, 3, 116-121

Sobemovirus
4 grupos/25 sementes

Table 2

Detection of the presence of RYMV by ELISA in the rice components of the rice seeds from infected plants in nature and from screen house

Rice cultivars	Mode of RYMV infection	Source of seeds	ELISA OD values of rice components tested for RYMV (A 405 nm)		
			Husk (seed coat-	Endosperm	Eembryo
BG 90-2	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Mechanically	Screenhouse	0.2(+)	0.1(-)	0.1(-)
Bouake 189	Mechanically	Screenhouse	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Mechanically	Screenhouse	0.4(++)	0.1(-)	0.1(-)
Bouake 189	Mechanically	Screenhouse	0.4(++)	0.1(-)	0.1(-)

(+)= Positive values at A 405 nm, virus particle present

(-)= Negative values at A 405 nm, virus particle absent

Table 1

Detection of RYMV in the organs of germinating rice seeds from infected plants using enzyme linked immunosorbent assay (ELISA) test

Rice cultivars	Mode of RYMV infection	Source of seeds	ELISA OD values of germinated seeds tested for RYMV (A 405 nm)		
			Endosperm/Husk	Plumule	Radicle
BG 90-2	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.2(+)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.4(++)	0.1(-)	0.1(-)
Serberang MR	Naturally	Field	0.4(++)	0.1(-)	0.1(-)

4 grupos/25 sementes

Table 3

Results of visual assessment and ELISA values of leaf samples of screenhouse reared plants raised from seeds of RYMV infected rice plants

Seeds from Rice cultivars	Mode of RYMV infection	Source of seeds		ELISA OD values (A 405 nm)		
			Location	21 DAS	42 DAS	63 DAS
BG 90-2	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Mechanically	Screenhouse	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Mechanically	Screenhouse	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Mechanically	Screenhouse	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Mechanically	Screenhouse	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
BG 90-2	Mechanically	Screenhouse	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
IR 1529-680-3	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
IR 1529-680-3	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
IR1529-680-3	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
IR1529-680-3	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
IR1529-680-3	Naturally	Field	Mali	0.1(-)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	Ivory Coast	0.1(-)	0.1(-)	0.1(-)
Bouake 189	Naturally	Field	Ivory Coast	0.1(-)	0.1(-)	0.1(-)

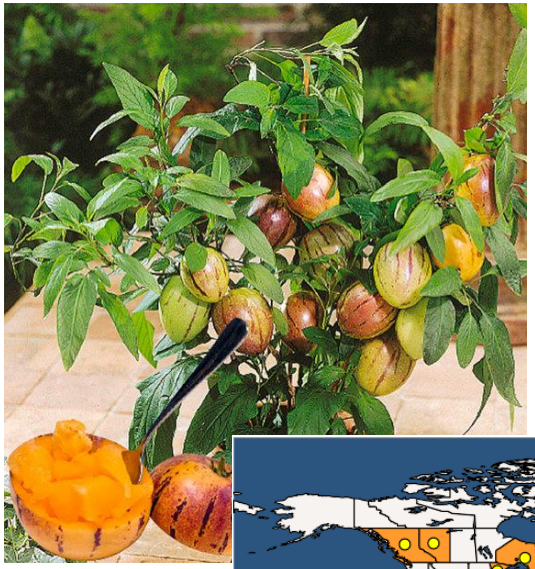
50 sementes/variedade

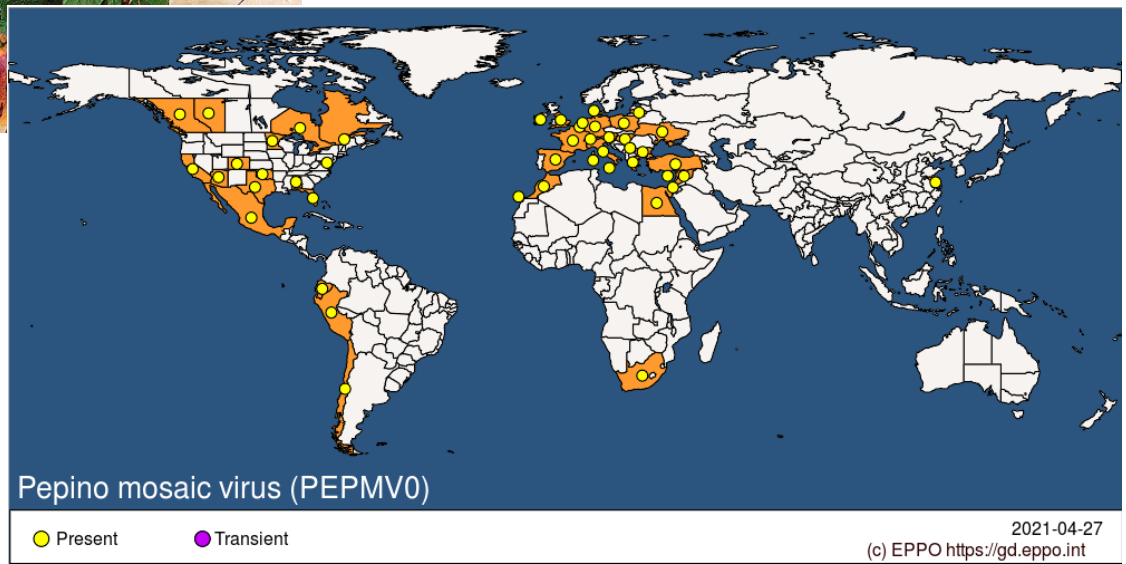
Pepino mosaic virus on Tomato Seed: Virus Location and Mechanical Transmission

Ling, 2008

Plant Disease / December 2008

MELÃO ANDINO - PEPINO DOCE - MELÃO DE ÁRVORE - MELÃO DE VASO - *Solanum muricatum* (Peru, 1980)





Potexvirus
Transmissão: contato, sementes, abelhas

Pepino mosaic virus on Tomato Seed: Virus Location and Mechanical Transmission

Ling, 2008

Potexvirus

Plant Disease / December 2008

Nenhum das 10.000 sementes germinadas deu origem a plantas sintomáticas, avaliadas por ELISA e transmissão mecânica para *Nicotiana benthamiana*.

O vírus foi transmitido mecanicamente para plantas de tomate e *N. benthamiana* com extratos de sementes.

Table 1. Virus distribution in tomato seed parts, floral organs, and fruits in a *Pepino mosaic virus* (PepMV) infected tomato plant

Tissue types	ELISA[x]	Real-time RT-PCR[y]	Bioassay[z]
Embryo	0.08 (–)	35.40 (–)	–
Seed coat	0.25 (+)	29.84 (+)	+
Seed membrane	1.04 (++)	18.49 (+)	+
Fruit pulp	1.63 (++)	17.84 (+)	+
Stigma	2.03 (+++)	24.94 (+)	+
Sepal	1.81 (++)	16.71 (+)	+
Petal	1.91 (++)	16.54 (+)	+
Anther	2.04 (+++)	16.79 (+)	+
Anther filament	1.59 (++)	24.19 (+)	+
Healthy check	0.08 (–)	35.00-No C_T	–
Threshold for positive	0.16	35.00	
PepMV-leaf	3.88 (++++)	16.04 (+)	+

3000 sementes em 10 grupos de 300, pré-germinadas em placa de Petri

Begomovirus Tomato Leaf Curl New Delhi Virus Is Seedborne but Not Seed Transmitted in Melon

Fortes et al., 2023

Plant Disease • 2023 • 107:473-479 • https://doi.org/10.1094/PDIS-09-21-1930-RE

ToLCNDV presence in melon floral tissues

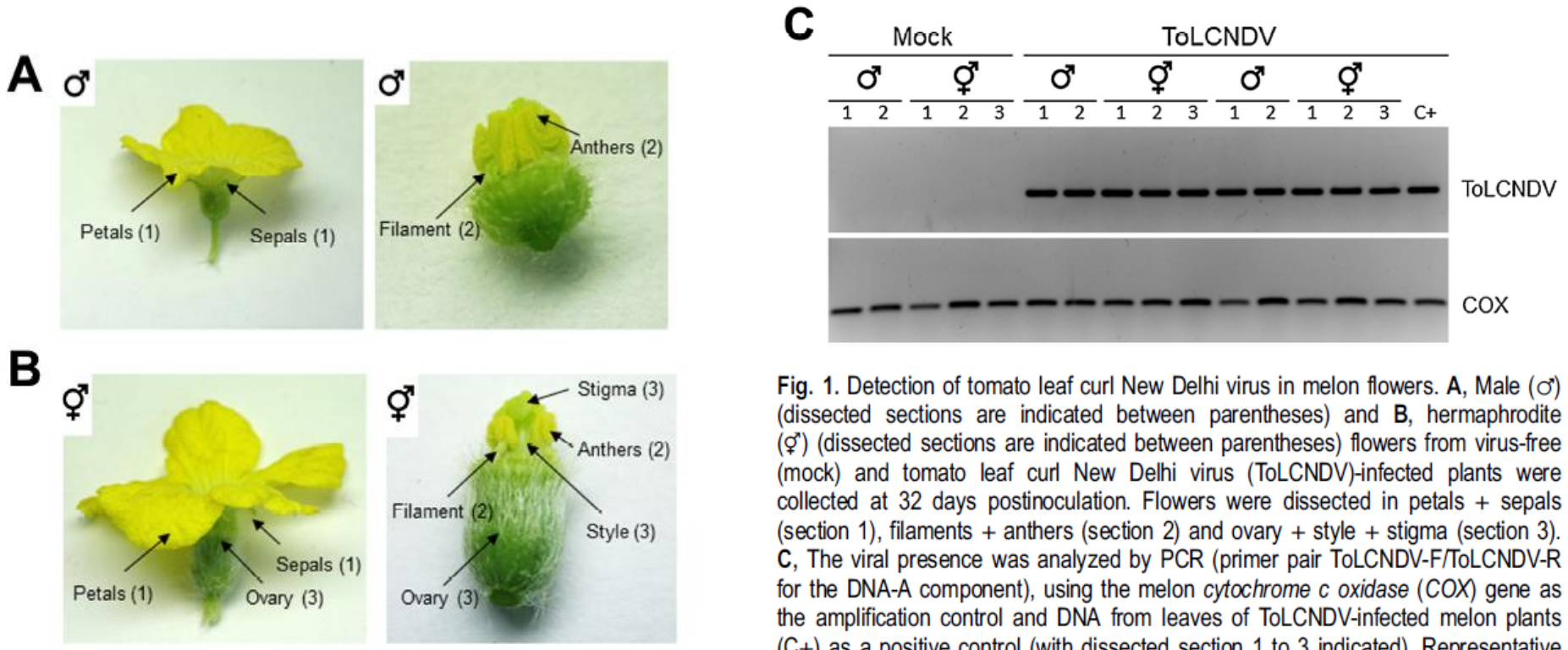


Fig. 1. Detection of tomato leaf curl New Delhi virus in melon flowers. **A**, Male (♂) (dissected sections are indicated between parentheses) and **B**, hermaphrodite (♀) (dissected sections are indicated between parentheses) flowers from virus-free (mock) and tomato leaf curl New Delhi virus (ToLCNDV)-infected plants were collected at 32 days postinoculation. Flowers were dissected in petals + sepals (section 1), filaments + anthers (section 2) and ovary + style + stigma (section 3). **C**, The viral presence was analyzed by PCR (primer pair ToLCNDV-F/ToLCNDV-R for the DNA-A component), using the melon *cytochrome c oxidase* (*COX*) gene as the amplification control and DNA from leaves of ToLCNDV-infected melon plants (C+) as a positive control (with dissected section 1 to 3 indicated). Representative results are shown.

Tissue blot hybridization, PCR e qPCR

4 flores masculinas
6 hermafroditas

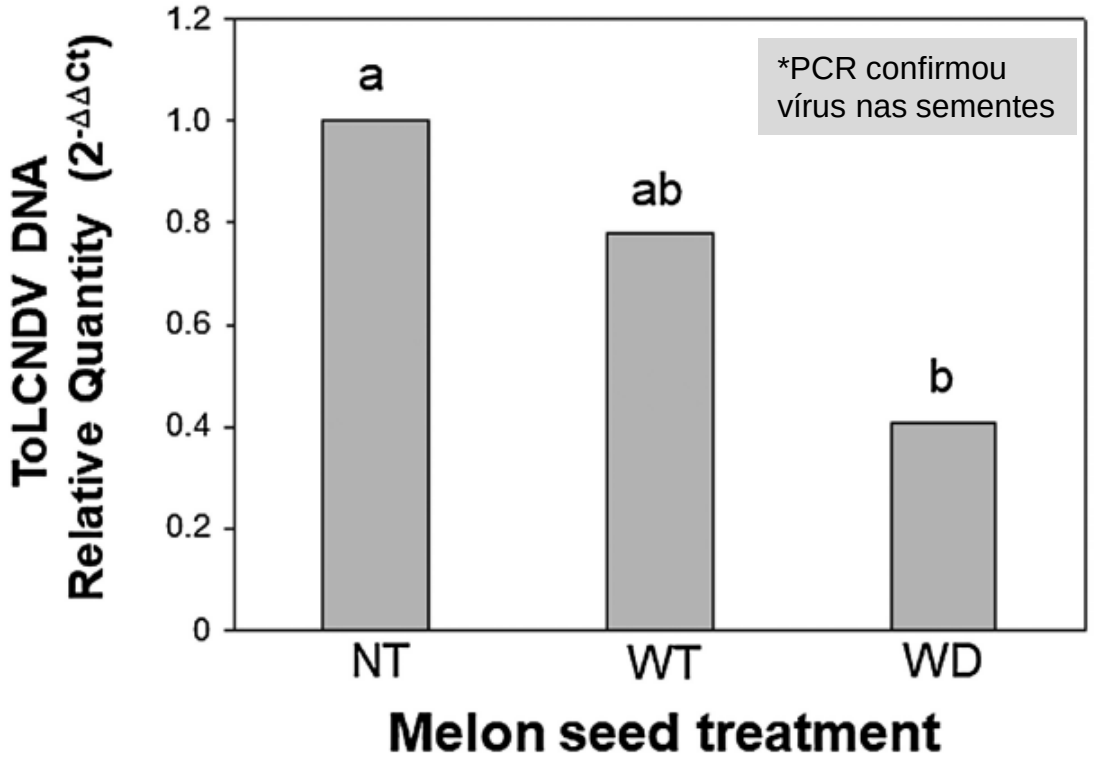


Fig. 2. Means of relative quantity (RQ) of tomato leaf curl New Delhi virus DNA in melon seeds. Melon seeds of Nesta (Nst) plants experimentally infected with tomato leaf curl New Delhi virus (ToLCNDV) at the two- to three-leaf stage were removed from ripe fruit without surface treatment (nontreated seeds, NT) or surface-treated by (i) washing with distilled water (washed seeds, WT) or (ii) washing with distilled water and surface disinfection by immersion in 50% bleach (about 1.5% sodium hypochlorite) followed by two washes with distilled water (washed + disinfected seeds, WD). Ten seeds of each NT, WT, or WD treatment and 10 control seeds from ToLCNDV-free Nst melon plants were individually tested. The viral DNA accumulation was estimated by the $2^{-\Delta\Delta Ct}$ method after real-time quantitative PCR detection of ToLCNDV using the melon *cytochrome c oxidase* (*COX*) gene as the amplification control to normalize the ToLCNDV cycle threshold values and the mean of the fold-change values of the NT sample as the reference value for RQ calculations. Mean RQ values in different treatments sharing a common letter are not significantly different (nonparametric median test comparisons, $P < 0.05$).

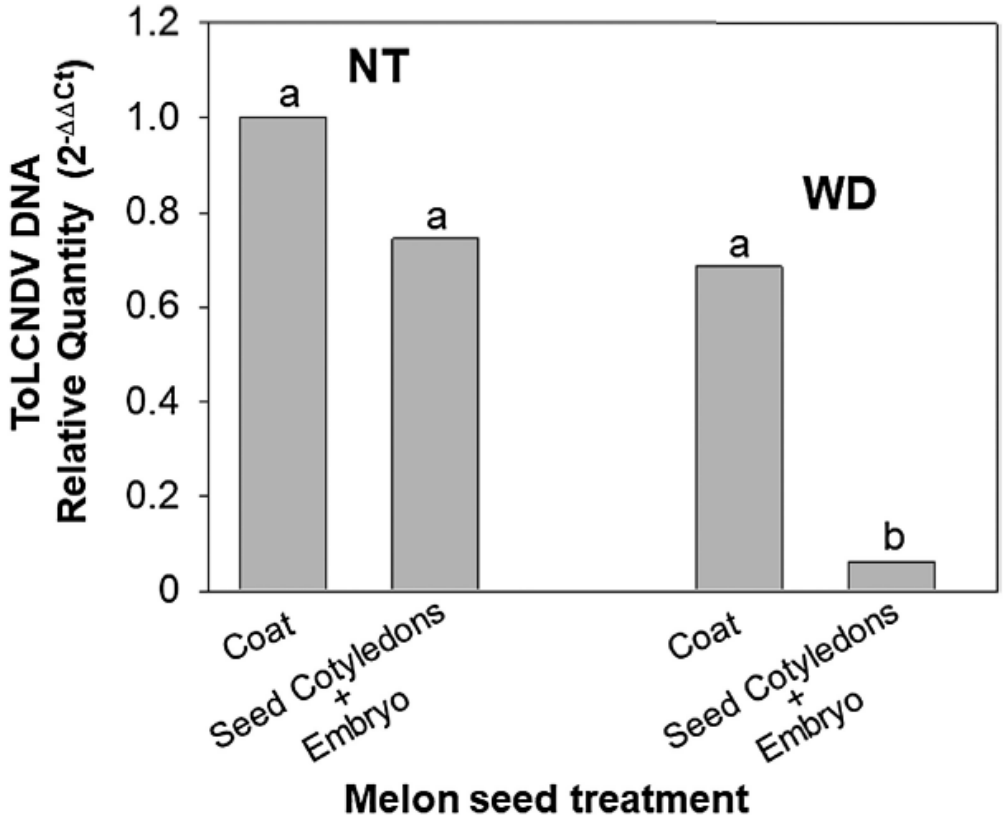


Fig. 3. Means of relative quantity (RQ) of tomato leaf curl New Delhi virus DNA in external and internal fractions of melon seeds. Melon seeds of Nesta plants experimentally infected with tomato leaf curl New Delhi virus (ToLCNDV) at the two- to three-leaf stage were removed from ripe fruit without treatment (nontreated seeds, NT) or surface disinfected after thorough washing with distilled water by immersion in 50% bleach (about 1.5% sodium hypochlorite) followed by two washes with distilled water (washed + disinfected seeds, WD). Ten seeds of each NT or WD treatment were dissected in external (seed coat) and internal (seed cotyldedons + embryo) fractions that were individually analyzed for the detection of ToLCNDV. The viral DNA accumulation was estimated by the $2^{-\Delta\Delta Ct}$ method after real-time quantitative PCR detection of ToLCNDV using the melon *cytochrome c oxidase* (*COX*) gene to normalize the ToLCNDV cycle threshold values and the mean of the fold-change values of the seed coat fraction of the NT sample as the reference value for RQ calculations. Mean RQ values in different treatments sharing a common letter are not significantly different (nonparametric median test comparisons, $P < 0.05$).

Table 2. Results of hybridization, PCR, or quantitative real-time PCR (qPCR) analysis to evaluate seed-to-progeny plant transmission of tomato leaf curl New Delhi virus (ToLCNDV) in melon[a]

	Assay 1, location 1			Assay 2, location 2				Assay 3, location 2			
		qPCR			Hybridization	PCR			Hybridization	PCR	
Cultivar	Plants[b]	Bulks[c]	Pos/bulk[d]	Plants[b]	Pos/plants[e]	Bulks[c]	Pos/bulk[d]	Plants[b]	Pos/plants[e]	Bulks[c]	Pos/bulk[d]
BR	100	20	0/20	96	0/96	10	0/10	70	0/70	7	0/7
Nst	100	20	0/20	83	0/83	9	0/9	67	0/67	7	0/7
MY	100	20	0/20	94	0/94	10	0/10	65	0/65	7	0/7
BR+[f]	5	1	1/1	10	9/10	1	1/1	5	4/5	1	1/1
Nst+[f]	5	1	1/1	10	10/10	1	1/1	5	5/5	1	1/1
MY+[f]	5	1	1/1	10	10/10	1	1/1	5	5/5	1	1/1
Nst–[f]	5	1	0/1	10	0/10	1	0/1	10	0/10	1	0/1

[a] Progeny plants derived from nontreated seeds collected from fruit of ToLCNDV-infected melon cultivars Brimos (BR), Mayor (MY), or Nesta (Nst) plants were individually analyzed at 60 days postplanting for ToLCNDV presence in the youngest, newly emerged leaf. The analysis was conducted by tissue-blot hybridization of squash blots of leaf petioles with a probe specific to ToLCNDV or by PCR (primer pair ToLCNDV-A-F/ToLCNDV-A-R) or qPCR (primer pair ToLCNDV-F/ToLCNDV-R; probe ToLCNDV-P) analysis of total DNA extracts from bulked leaf samples (5 to 10 leaves per bulk). Assays were conducted in location 1 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain) and location 2 (Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Málaga, Spain).

[b] Total number of analyzed plants.

[c] Total number of analyzed bulks, from 5 to 10 young leaves per bulk.

[d] Number of positive bulks/total number of analyzed bulks.

[e] Number of positive plants/total number of analyzed plants.

[f] Control plants (BR+, Nst+, and MY+) were experimentally inoculated by *Agrobacterium tumefaciens*–mediated inoculation using an infectious clone of the isolate ES-Alm-661-Sq-13 of the "Spain" (ES) strain of ToLCNDV (DNA-A and DNA-B, GenBank accession numbers KF749223 and KF749226, respectively) (BR+, Nst+, and MY+), or were mock inoculated (Nst–) (two- to three-leaf stage) and analyzed at equivalent time of plants derived from seeds.

C. Tipos de transmissão de vírus por semente

1. Infecção da plântula por vírus aderido à parte externa da semente
   Eliminação: calor seco 70-76°C, 1 a 3 dias
   Solução 3% de ortofosfato trissódio, 30 minutos
   0,25% hipoclorito de sódio

2. Transmissão verdadeira ou embriogênica

D. Rotas para infecção do embrião:

Diretamente da planta mãe ou pólen

- Infecção do meristema floral (vírus crípticos)

- Infecção direta do embrião

Problema: isolamento do embrião dos tecidos maternos, ausência de ligações vasculares

Bean common mosaic virus infecting manteiguinha cowpea (*Vigna unguiculata*): seed transmission, evaluation of yield loss, and genetic resistance

Boari et al., 2022

Tropical Plant Pathology (2022) 47:450–455

Fig. 1 Plants exhibiting leaves with mosaic, epinasty, leaf blade reduction, rolling, and stunting caused by bean common mosaic virus (BCMV)

Potyvirus
Transmissão por afídeos: não persistente

Cultivar TUC 170:
Taxa de transmissão por sementes 34,8%

Table 2 Means and standard deviations (S) of treatments infected and not infected with bean common mosaic virus (BCMV-Br:Para:1) for the variables grain production per plant (GrProd; g), number of pods per plant (NumPods), and number of grains per pod (NumGrPod), in manteiguinha cowpea

Treatment	GrProd		NumPods		NumGrPod		W100Gr	
	Mean	S	Mean	S	Mean	S	Mean	S
Not infected	11.31	3.91	11.23	3.19	9.81	1.85	9.57	1.49
Infected	5.31	3.61	5.95	3.58	8.95	1.97	9.70	1.39
Difference between treatments (%)	53%**		47%**		8.8%*		$1.4\%^{ns}$	

S standard deviation. **Significant difference at 1% of probability according to the Fisher-Snedecor F-test. *Significant difference at 2.5% of

Seed transmission of potato spindle tuber viroid and its distribution in reproductive organs in *Solanaceae* weed species

Matsushita & Kubota, 2023

Eur J Plant Pathol (2023) 167:315–322

Table 1 Seed transmission of PSTVd in *Solanum nigrum* and *Physalis angulata*

Plant species	PSTVd variant	GenBank accession no	Experiment			
			exp. 1	exp. 2	exp. 3	exp. 4
Solanum nigrum	VP35	LC523658	1/148[a] (0.6%)	5/62 (8.1%)	3/111 (2.7%)	0/152 (0%)
	VP72-1	LC523663	23/46 (50.0%)	10/95 (10.5%)	nt	nt
Physalis angulata	VP72-1	LC523663	118/118 (100%)	237/264 (89.7%)	nt	nt

[a]Number of test plants infected with viroid/total number of plants. nt, not tested

Plantas infectadas de *S. nigrum* e *P. angulata* foram assintomáticas.

Viroide transmitido mecanicamente para tomateiros.

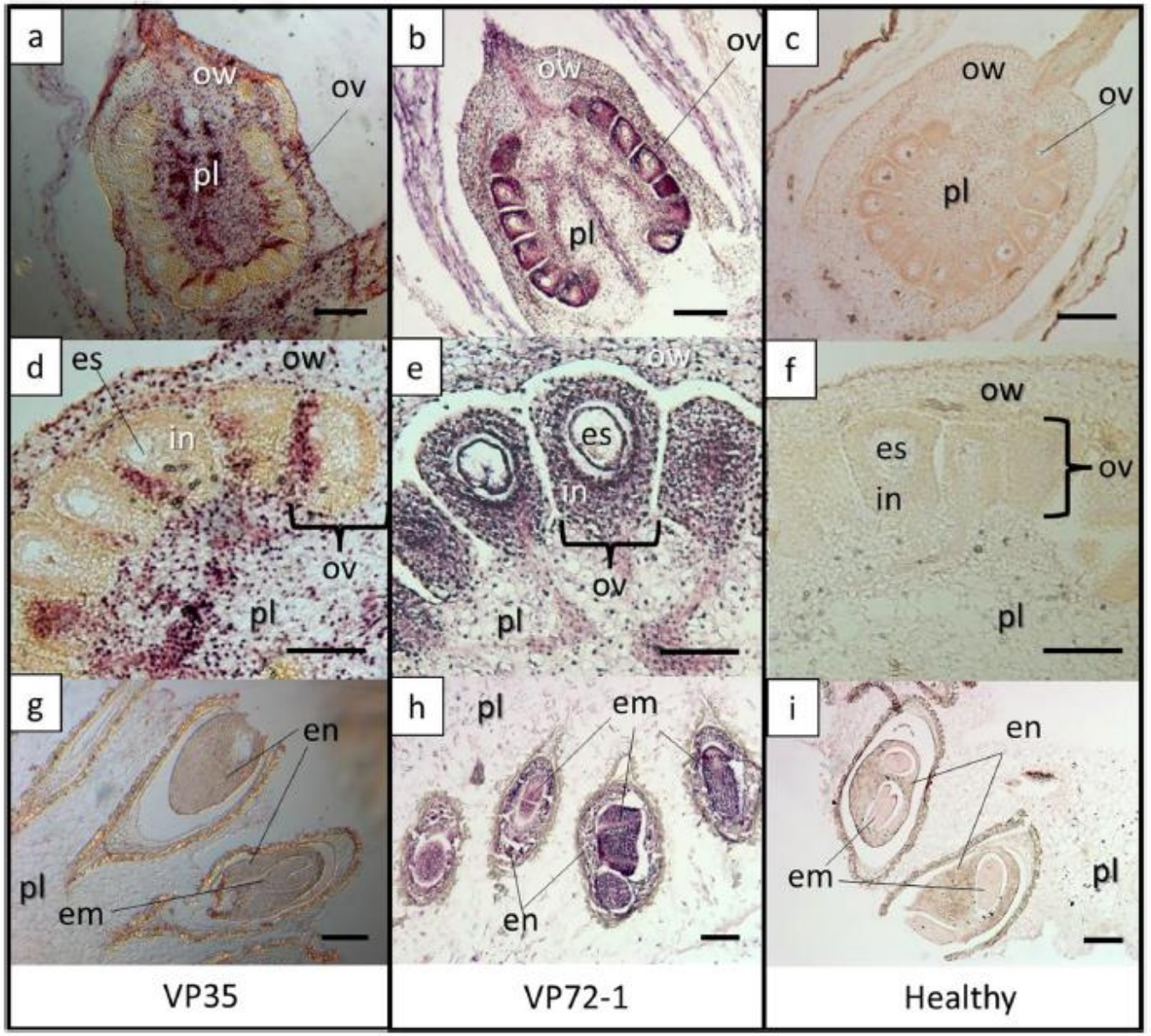


Fig. 1 Distribution of PSTVd in the flower buds and in the developing seeds of an infected *Solanum nigrum*. PSTVd isolate VP35-infected plant (a, d, g), PSTVd isolate VP72-1-infected plant (b, e, h), uninoculated healthy plant (c, f, i). Longitudinal section of a flower bud at the flower opening stage (a–c), the magnification of ovule at the flower opening stage (d–f) and developing seed (g–i). em, embryo; en, endosperm; es, embryo sac; in, integuments; ov, ovule; ow, ovary wall; pl, placenta. Bars indicate 200 μm (a–b, g–i) and 100 μm (d–f)

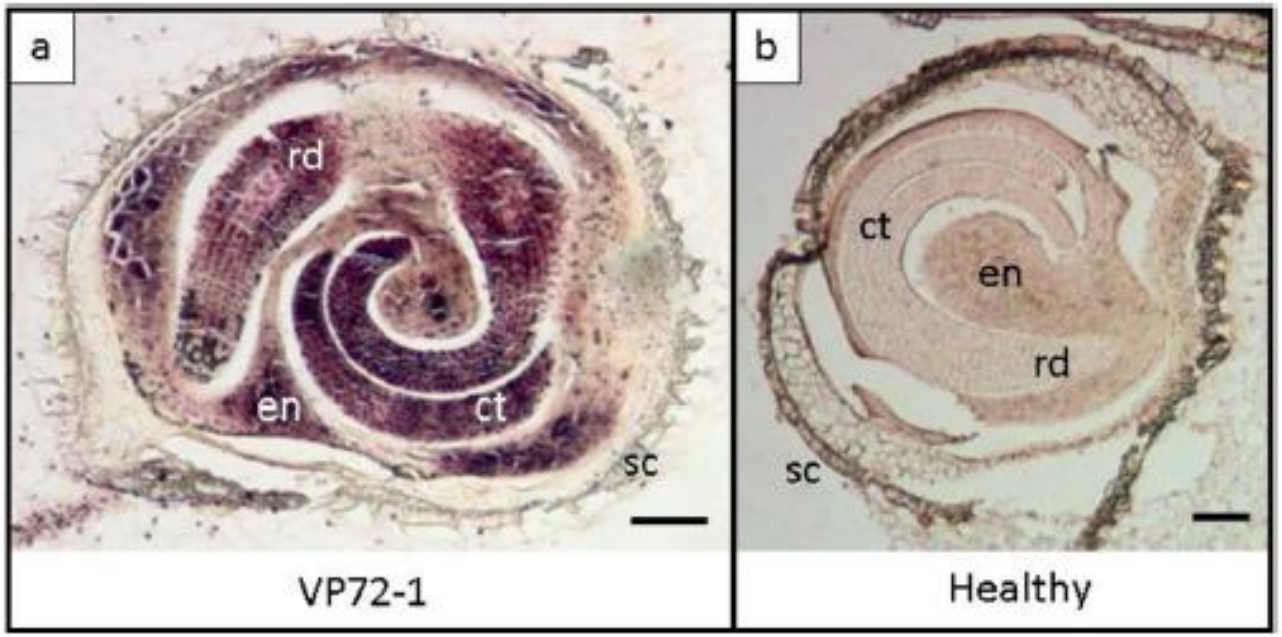


Fig. 2 Distribution of PSTVd in the mature seed of an infected *Solanum nigrum*. (a) Transverse section of a seed infected with PSTVd isolate VP72-1 (b) Transverse section of a seed from a healthy plant. ct, cotyledon; en, endosperm; rd, radicle; sc, seed coat. Bars indicate 200 μm

MÉTODOS DE DETECÇÃO DE VÍRUS/VIROIDE

- Biológico
- Sorológico (ELISA, Dot blot, Tissue printing)
- Molecular (PCR, RT-PCR, RTqPCR)
- Hibridização de ácido nucleico

Method for the Detection of *Lettuce mosaic virus* on Lettuce Seed and Seedlings

Crop: *Lactuca sativa*
Pathogen: *Lettuce mosaic virus* (LMV)

Revision history: Version 4.2, April 2015

Sample and sub-sample size

The minimum sample size of the seedling assay is 2,000 seedlings and the maximum sub-sample size is 100 seedlings. For the seed assay the minimum sample size is 10,000 seeds and the maximum sub-sample is 500 seeds.

Principle

Lettuce seeds or seedlings (according to the choice of the laboratory) are ground in a buffer solution to extract the virus. The extract is tested using DAS ELISA for the detection of LMV.

Sensitivity and Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical processes for disinfestation or seed that has been treated using chemicals for disinfestation provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- This test method has not been validated for seed treated with protective chemicals or biological substances. If a user chooses to test treated seed using this method, it is the responsibility of the user to determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on the method results.
- In the seed assay, one infected seed can be detected in a sub-sample of 500 seeds.
- A comparison of antibodies is recommended (1).
- In the seedling assay, one infected seedling can be detected in sub-sample of 100 seeds.

Method for the Detection of *Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV) on Cucurbit seed

Crop:	Cucurbits (Watermelon (*Cirtullus lanatus* var. *lanatus*), Cantaloupe (*Cucumis melo* var. *catalupensis*) and melon (*Cucumis melo*))
Pathogens:	*Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV)
Date:	August 2011

Sample and sub sample size

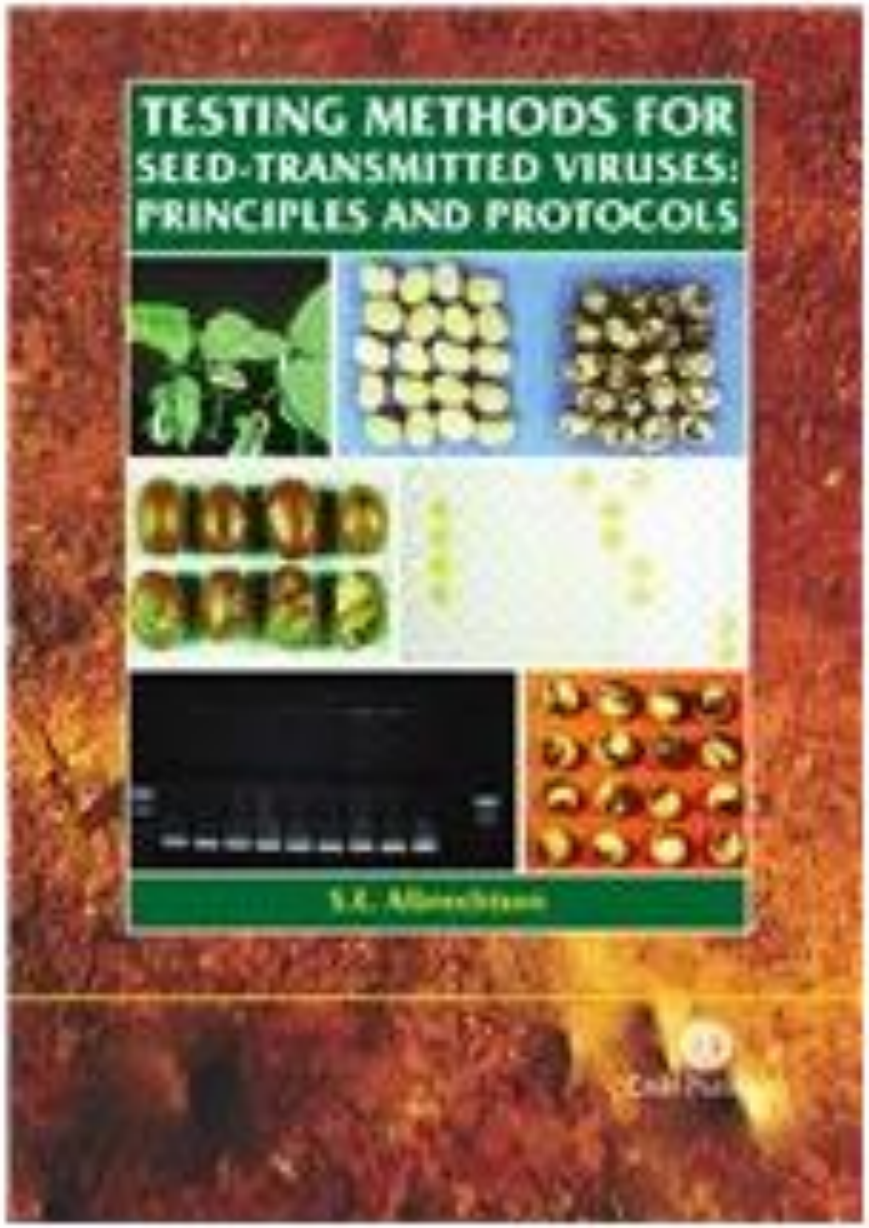
The recommended minimum sample size is 2,000 seeds with a maximum sub-sample size of 100 seeds.

Principle

The method, using ground seed in a DAS-ELISA, provides the option to simultaneously detect SqMV, CGMMV and MNSV in a single extract. The extract is tested in separate microtiter plates, one for each pathogen of interest; SqMV, CGMMV and/or MNSV. It can detect externally and internally located virions as well as infectious and non-infectious virions.

Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical processes for disinfestation or seed that has been treated using chemicals (such as hydrochloric acid, peroxyacetic acid, etc.) for disinfestation provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- Although ELISA is compatible with some seed treatment chemicals (Pataky et al., 2004), seed treatments may affect the performance of this test. It is the responsibility of the user to check for such antagonism and or inhibition by analysis, sample spiking, or experimental comparisons.



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