

Treatment for cleaning small seed lots of tomato and pepper seeds of surface contamination with viroids

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Background

- Viroids are small circular single-stranded RNAs of approximately 245-400 nt that are able to infect plants (Diener, 1999, Flores et al., 2005).
- There are two viroid families; Pospiviroidae (five genera) and Avsunviroidae (three genera). The genus *Pospiviroid* in the Pospiviroidae contains 10 species, nine of which infect solanaceous plants (Matsuura et al., 2010).
- In plants propagated via seeds, viroids may be transmitted mechanically or through seed or pollen. Only TPMVd is known to be efficiently transmitted by aphids (Ninth report of ICTV, 2011).
- Solanaceous plants infected with viroids can express severe or mild symptoms; some are even symptomless. Viroids have been reported to cause severe diseases and to adversely affect tomato production in some locations (Mackie et al., 2016; Matsuura et al., 2010).

- As Pospiviroids can be dispersed as contamination on the seed coat, or for some species within the embryo of seeds produced from an infected plant, their international spread through trade or exchange of seed is an increasing concern for many countries.
- Particularly for tomato and pepper seed, it is important to be able to reduce the level of viroid contamination of seed to reduce the risk of international spread. However, reliable protocols for cleaning seeds of viroids until now have not been publicly available.

Six most important viroids infecting solanaceous plants

Viroid species	Chinese name	Country requesting viroid negative declaration
<i>Potato spindle tuber viroid (PSTVd)</i>	馬鈴薯紡錘形塊莖類病毒	e.g. tomato and Capsicum spp. (Australia); tomato (India); tomato, pepper, eggplant, etc. (Korea)
<i>Pepper chat fruit viroid (PCFVd)</i>	番椒小果類病毒	e.g. tomato and Capsicum spp. (Australia)
<i>Tomato apical stunt viroid (TASVd)</i>	番茄莖頂矮化類病毒	e.g. tomato (Australia)
<i>Tomato chlorotic dwarf viroid (TCDVd)</i>	番茄褪綠矮化類病毒	e.g. tomato (Australia)
<i>Tomato planta macho viroid (TPMVd)</i>	番茄植株雄化類病毒	e.g. tomato (Australia)
<i>Columnea latent viroid (CLVd)</i>	金魚藤潛伏類病毒	e.g. tomato and Capsicum spp. (Australia)



Viroid infection of tomato can cause symptoms of small leaves, leaf chlorosis, and spindly shoots or stunting.

Research objective

To assess the efficacy of various treatments for cleaning small lots of tomato and pepper of seed surface contamination with viroids.

Viroid detection in tomato seed lot (T001) samples after different cleaning treatments

Treatment	Viroid detection (No. of positive sub-sample/No. of total sub-sample)					
	Replication I		Replication II		Replication III	
	Pospi1F/Pospi1R ¹	CLVd	Pospi1F/Pospi1R	CLVd	Pospi1F/Pospi1R	CLVd
untreated	5/5 ²	2/5	5/5	2/5	5/5	2/5
0.5N HCl ³ x 15min + 10%TSP ⁴ x1hr	0/5	0/5	4/5	0/5	1/5	0/5
20% Clorox ⁵ x 15 min & 80°Cx24 hrs	2/5	0/5	3/5	0/5	3/5	0/5
0.5N HCl x 15min + 10%TSPx1hr + 80°Cx24 hrs	0/5	0/5	1/5	0/5	0/5	0/5

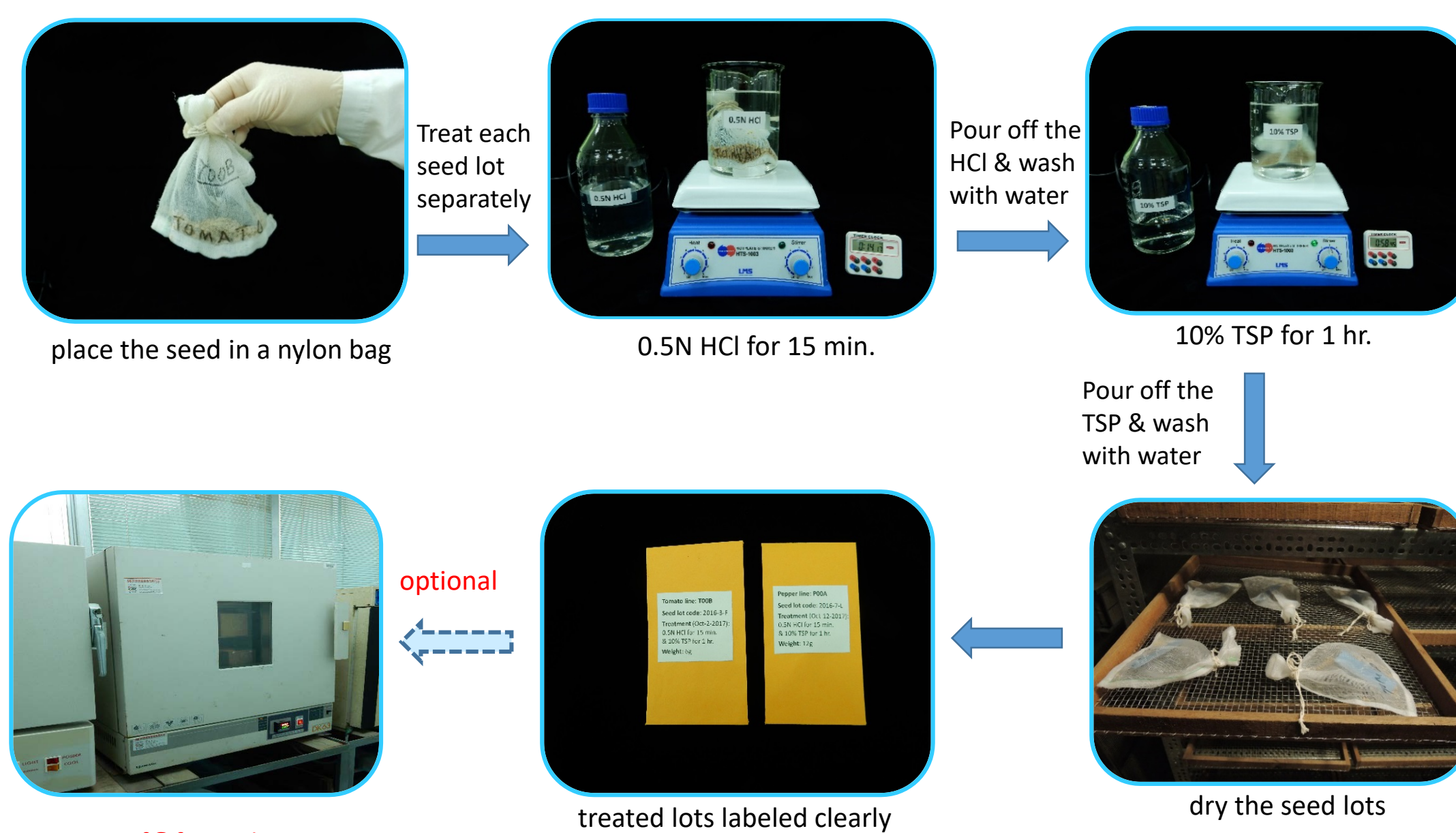
¹ primer pair (Verhoeven et al., 2004); ² each sub-sample comprises about 400 seeds; ³ HCl: Hydrochloric acid; ⁴ TSP: Trisodium Phosphate; ⁵Clorox: domestic bleach

Effect of seed cleaning treatments on short-term germination rate

Tomato line	Treatment	Germination Rate (%)
T00A	none	95
	0.5N HCl x 15min+ 10%TSPx1hr	95
T00B	none	92
	0.5N HCl x 15min+ 10%TSPx1hr	87
T00C	none	98
	0.5N HCl x 15min+ 10%TSPx1hr + 80°Cx24 hrs	91
T00D	none	100
	0.5N HCl x 15min+ 10%TSPx1hr + 80°Cx24 hrs	93

Protocols for cleaning tomato and pepper seeds of surface contamination with viroids

- Soak the seeds in 0.5N HCl for 15 minutes
- Pour off the used HCl and wash with seven changes of water
- Soak bags of seeds in 10% TSP for 1 hour
- Pour off the used TSP and wash with seven changes of water
- Dry at 28-30 °C in an oven for 20-24 hr followed by 7 days of additional drying in a cool room maintained at 15°C and 10% RH
- Additional for well dried Tomato seed only – treat in an oven at 80°C for 24 hours (optional)



Conclusions

- The preliminary results of tests using relatively small seed lots indicated that treating tomato and pepper seed with 0.5N HCl for 15 minutes followed by treating them with 10% TSP for 1 hour can usefully reduce the surface contamination of tomato and pepper seed with viroids.
- The heat treatment after HCl and TSP treatment has only been effective for tomato seeds and may cause much reduced viability in pepper seeds.
- The viability of tomato and pepper seed lots when tested soon after treatment with HCl and TSP remained good, but the viability of such treated seed after long-term storage has yet to be tested.