

Screening *Capsicum* Accessions for Tomato Necrotic Ringspot Virus Resistance

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ABSTRACT

A total of 52 Capsicum spp. accessions of three different species, C. annuum, C. baccatum and C. chinense, were screened against tomato necrotic ringspot virus, a new Tospovirus isolate in Thailand that causes severe yield losses in vegetable crops. A mechanical inoculation method was used to screen the accessions for resistance to the virus, and resistant plants were transplanted into a greenhouse for continued observation. The experiment was conducted in 2011 in Chiang Mai Province, Thailand. A total of eight accessions were identified with resistance to the virus: three lines of C. annuum (PY-4300, PY-4301 and PY-4302) and five lines of C. baccatum (CA1316, CA1998, CA2000, CA2008 and CA2009).

Keywords: Tospovirus, Pepper, *Capsicum*

INTRODUCTION

Thrips-transmitted *Tospoviruses* significantly reduce yield and quality of vegetables, legumes and ornamental crops in many parts of the world (Mumford et al., 1996). Currently, 19 formal and tentative *Tospovirus* species have been characterized and classified into three major serogroups: (i) *Tomato spotted wilt virus* serogroup (ii) *Watermelon silver mottle virus* serogroup and (iii) *Iris yellow spot virus* serogroup (Yeh et al., 2009).

Resistance to *Tospoviruses* has been reported earlier. *Tomato spotted wilt virus* resistance was found in several *C. chinense* accessions, including ‘PI152225’ and ‘PI159236’. The resistance was expressed as a hypersensitive response and controlled by the dominant gene *Tsw* (Black et al., 1991; Boiteux et al., 1994 and Moury et al., 1997). Unfortunately, the *Tsw* gene from ‘PI152225’ and ‘PI159236’ is not effective against other *Tospovirus* species including *Groundnut ringspot virus*, *Tomato chlorotic spot virus* (Boiteux et al., 1994), *Impatiens necrotic spot virus*

(Roggero et al., 1999) and *Capsicum chlorosis virus* (McMichael et al., 2002). The source of *Capsicum chlorosis virus* resistance, a single dominant gene, was found in 'PI290972', *C. chinense* from USDA/University of Georgia collections (Persley et al., 2005).

Tomato necrotic ringspot virus is a new *Tospovirus* isolate infecting field crops such as tomato and pepper in different regions of Thailand (Chiemsombat et al., 2010 and Seepiban et al., 2011). This newly discovered *Tospovirus* isolate has not been assigned to any serogroup and there are no reports on sources of resistance and the inheritance of resistance. This disease is transferred by thrips. The chemical or biological control of this vector is very difficult in both greenhouse and open field conditions. Many indigenous weeds form a natural host reservoir for this virus. The development of resistant cultivars would offer a better alternative in reducing crop losses from this virus. The main objective of this study was to screen *Capsicum* spp. accessions for resistance to tomato necrotic ringspot virus. Resistant accessions can then be used in pepper breeding programs.

MATERIALS AND METHODS

This research work was conducted at two research stations of Hortigenetics Research S.E. Asia Limited in Chiang Mai, Thailand during February-June 2011. The screening for tomato necrotic ringspot virus resistance source by mechanical inoculation was conducted at Farm Lert Phan Research Station, Sansai District, Chiang Mai, Thailand. After initial selection, the seedlings of the resistant accessions were transplanted to a greenhouse at Pongyeng Research Station, Maerim District, Chiang Mai, Thailand for further evaluation of symptom development. A randomized complete block design with four replications was used for the mechanical inoculation trial. One replication contained 18 plants. A total of 52 *Capsicum* accessions, of which 7 *C. annuum*, 24 *C. baccatum* and 21 *C. chinense*, were screened for resistance to the virus.

The virus isolate used in this experiment was collected from infected tomato plants in the research farm of Hortigenetics Research in Sansai District. The isolate was screened by enzyme linked immune sorbent assays (ELISA) against tomato mosaic virus, capsicum chlorosis virus and tomato necrotic ringspot virus. The pure isolate of tomato necrotic ringspot virus was increased on the variety 'Early California Wonder'.

Seeds of 52 *Capsicum* accessions were sown in sowing trays with 72 cells. Klasman Deilmann substrate was used as growing medium. When the seedlings had fully expanded cotyledons and two true leaves began to appear, the plants were ready for mechanical inoculation. *C. annuum* cultivar 'Early California Wonder' and *C. chinense*, PI 159236 and PI 152225, were used as susceptible controls in this study. Prior to inoculation, plants were kept in a dark room for 6-12 hr. The inoculum was prepared by grinding 'Early California Wonder' infected leaves in 0.01 M phosphate buffer (pH 7.0) containing 0.01 M Na₂SO₃ 0.1% (Boiteux et al., 1993). The protocol was adjusted by adding Celite®545, particle size 125.3

µm., to the inoculum before inoculation, instead of dusting seedlings with 600-mesh Carborundum powder. The inoculum was gently rubbed on the leaves using cotton buds. Plants were rinsed with distilled water immediately after inoculation. Plants were re-inoculated two days later to prevent any chance of escape.

The inoculated plants were maintained at 25°C and a 12-hour photoperiod for a week and then transferred to the pathology greenhouse. Symptom development was scored on individual plants. Thirty-five days after inoculation, all plants were assayed for the presence of tomato necrotic ringspot virus using double-antibody sandwich–enzyme linked immune sorbent assays (DAS–ELISA) protocol. The polyclonal antiserum, prepared against the nucleoprotein of Thai tomato necrotic ring virus isolate, was kindly supplied by D. Peters (University of Wageningen, The Netherlands). The samples were considered as positive when the optical density (OD) values were at least twice the mean value of the healthy control at 405 nm. The plants were visually scored for tomato necrotic ringspot virus symptoms at 14, 21, 28 and 35 days after the second inoculation. Disease development was assessed by scoring of the disease severity of each plant using a rating scale from 0 to 4 (Figure 1), adjusted from the scale of Boiteux et al., (1993). Disease incidence and disease index were calculated from the individual disease severity ratings as follows:

$$\text{Disease incidence} = \frac{\text{Number of infected plant units}}{\text{Total number of plants unit assessed}} \times 100$$

$$\text{Disease index} = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e)}{(a + b + c + d + e)} \times \frac{100}{4}$$

Where a, b, c, d and e were the number of examined plants falling within categories 0, 1, 2, 3 and 4 (Cooke et al., 2006).

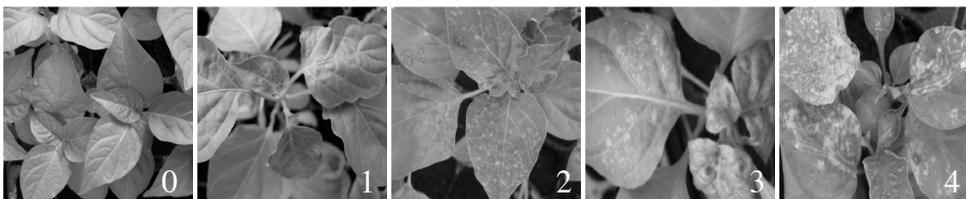


Figure 1. Disease severity scoring of tomato necrotic ringspot virus in *Capsicum*. Rating scale 0-4: 0 = no disease, 1 = weak apical leaves distortion, 2 = top distortion and weak mosaic in older leaves, 3 = strong leaves distortion and very clear mosaic symptoms in older leaves, 4 = severe stunting, top distortion and general necrosis.

The 52 *Capsicum* accessions were classified into three groups, according to their reaction to the tomato necrotic ringspot virus. The accessions with a disease index of more than 50 were classified as susceptible. Accessions with a disease index between 25 and 50 were classified as medium resistant, while accessions with a disease index of less than 25 were classified as resistant. This classifica-

tion was adapted from the disease index system used for the evaluation of corn for resistance to Maize dwarf mosaic virus (Kuhn et al., 1977). All accessions that were classified as resistant or medium resistant, based on their disease index 35 days after inoculation, were transplanted into a greenhouse at the Pongyeng Research Station for continued observation. Final evaluation for tomato necrotic ringspot virus resistance was conducted 92 days after mechanical inoculation.

RESULTS

The susceptible control, 'Early California Wonder', showed development of tomato necrotic ringspot virus symptoms 14 days after inoculation. The controls 'PI 159236' and 'PI 152225' showed clear symptoms 35 days after inoculation. The leaves of infected plants were distorted, with general necrosis in the older leaves. Some plants showed strong stunting. Figure 2 shows the disease indexes of the three susceptible controls and the other *Capsicum* entries.

All accessions were tested for the presence of the tomato necrotic ringspot virus using DAS-ELISA to confirm the infection with the virus. After correction for the absorbance value of the healthy control plant, all the accessions tested positive for the presence of the tomato necrotic ringspot virus. The susceptible control 'Early California Wonder' showed highest ELISA values. The other susceptible control cultivars, 'PI 159236' and 'PI 152225', had much lower values. In many cases, the average disease index and virus concentration were not well correlated. Since a pure isolate was used, it could indicate that different accessions show different levels of symptom expression.

A total of 41 accessions that were classified as resistant or medium resistant, based on their disease index 35 days after inoculation, were selected and transplanted to the greenhouse for further evaluation (Table 1). During the final evaluation at fruiting stage (92 days after inoculation) a total of eight accessions remained resistant to tomato necrotic ringspot virus, based on the same classification. Two accessions, PY-4294 and CA-2006, could be classified as either resistant or intermediate resistant (Table 2).

The susceptible controls and eight other susceptible accessions expressed severe symptoms 35 days after inoculation and were correspondingly discarded. Due to the latent infection period of the virus, however, symptom expression continued after this. Ninety-two days after inoculation, another 17 accessions showed clear symptoms also.

Table 1. Classification of the *Capsicum* accessions into three groups based on their reaction to the tomato necrotic ringspot virus 35 days after inoculation (seedling observation, Figure 2).

Susceptible	Early California Wonder, PI 152225, PI 159236, CA1297, CA1299, CA1984, CA1993, CA1996, CA1999, CA2007, CA2010
Medium resistant	CA758, CA1316, CA1906, CA1979, CA1982, CA1986, CA1989, CA1998, CA2000, CA2005, CA2006, CA2009, CA752 CA1960, CA1961, CA1969, CA1975, PY-3566, PY-3567, PY-3969, PY-3997, PY-4289, PY-4292, PY-4295
Resistant	PY-4301, PY-4304, PY-4300, PY-4302, PY-4306 , CA1326, CA1997, CA2001, CA2008, CA1325, CA1891, CA1930, CA1966, CA1972, PY-4290, PY-4291, PY-4294

Table 2. Classification of the *Capsicum* accessions into three groups based on their reaction to the tomato necrotic ringspot virus at 92 days after inoculation (final observation, Figure 3).

Susceptible	PY-4306, CA1997, CA2001, CA1325, CA1891, CA1930, CA1960, CA1966, CA1972, CA1975, PY-3566, PY-3567, PY-3969, PY-3997, PY-4291, PY-4292, PY-4295
Medium resistance	PY-4304, CA1906, CA2000, CA2006 , CA1969, PY-4289, PY-4290, PY-4294
Resistance	PY-4300, PY-4301, PY-4302, CA1316, CA1998, CA2000, CA2008, CA2009

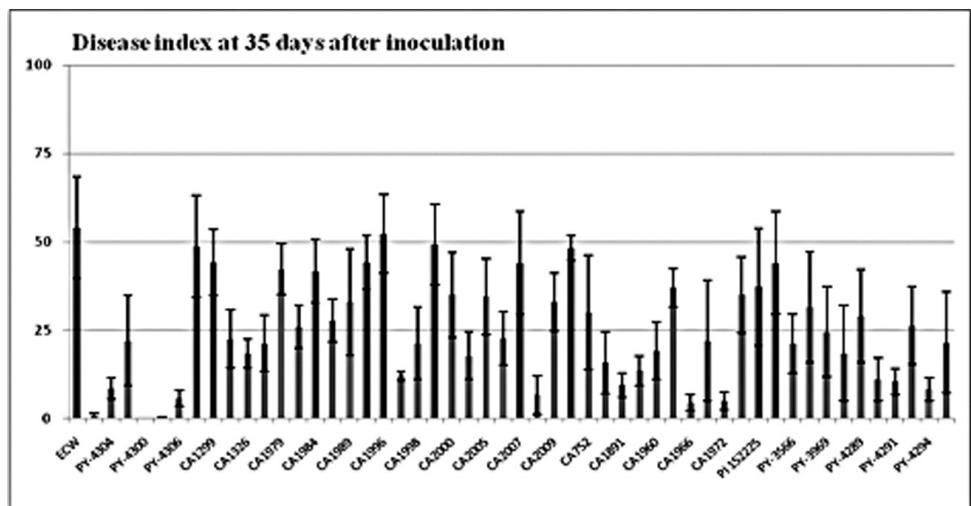


Figure 2. Mechanical inoculation reaction of 52 *Capsicum* accession to tomato necrotic ringspot virus after 35 days in greenhouse at Hortigenetic Research Station, Sansai District, Chiangmai, Thailand.

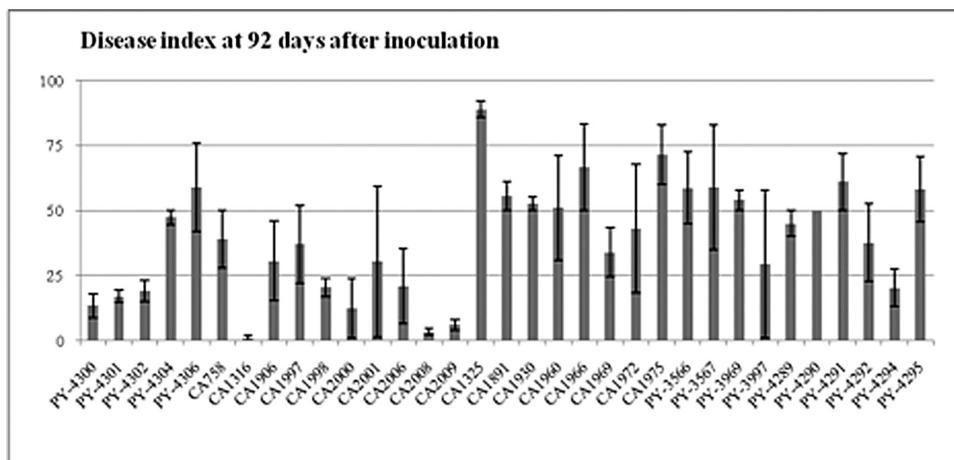


Figure 3. *Capsicum* spp. that shown very low disease index at 35 days after inoculation were selected for continued evaluation of symptom development at Pongyang Research Station, Maerim District, Chiang Mai, Thailand.

DISCUSSION

Although *Tospoviruses* can be mechanically transmitted under experimental conditions, *Tospovirus* dispersal and survival in nature depends on passage to plants by thrips vectors (Whitefield et al., 2005). The virus isolate loses its virulence if continued mechanical inoculation is used, resulting in a large number of plants that show little or no symptom development. Evaluation under greenhouse conditions using thrips as natural vector, will increase the efficiency of transmission and the expression of virus symptoms.

Following this study, the identified resistant lines from *C. annuum*, PY-4300 and PY-4301, are being used to breed resistance against tomato necrotic ringspot virus. The inheritance of resistance from these accessions was studied. The study revealed tomato necrotic ringspot virus resistance was controlled by a single recessive gene (Puangmalai et al., 2013).

The most resistant *Capsicum* accessions to tomato necrotic ringspot virus isolate by mechanical inoculation were five accessions of *C. baccatum*, CA1316, CA1998, CA2000, CA2008 and CA2009 and three accessions of *C. annuum*, PY-4300, PY-4301 and PY-4302.

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REFERENCES

- Black, L. L., H.A. Hobbs, and J.M. Gatti. 1991. Tomato spotted wilt virus resistance in *Capsicum chinense* PI 152225 and 159236. *Plant Dis.* 75:863.
- Boiteux, L.S., T. Nagata, W.P. Dutra, and M.E.N. Fonseca. 1993. Sources of resistance to tomato spotted wilt virus (TSWV) in cultivated and wild species of *Capsicum*. *Euphytica* 67:89-94.
- Boiteux, L.S., and A.C. De Avila. 1994. Inheritance of a resistance specific to tomato spotted wilt tospovirus in *Capsicum chinense* 'PI 159236'. *Euphytica* 75:139-142.
- Chiemsombat, P., M. Sharman, K. Srivilai, P. Campbell, D. Persley, and S. Attathom. 2010. A new tospovirus species infecting *Solanum esculentum* and *Capsicum annuum* in Thailand. *Australasian Plant Disease Notes* 5:75–78.
- Cooke, B.M., D. Gareth Jones, and B. Kaye. 2006. *The epidemiology of plant disease* second edition. Springer. Dordrecht, The Netherlands. 576p.
- Kuhn, C.W., and T.H. Smith. 1977. Effectiveness of a disease index system in evaluating corn for resistance to maize dwarf mosaic virus. *Phytopathology* 67:288-291.
- McMichael, L.A., D.M. Persley, and J.E. Thomas. 2002. A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia. *Australian plant pathol.* 31:231-239.
- Moury, B., A. Palloix, K. Selassie-Gebre, and G. Marchoux. 1997. Hypersensitive resistance to tomato spotted wilt virus in three *Capsicum chinense* accessions is controlled by a single gene and is overcome by virulent strains. *Euphytica* 94:45-52.
- Mumford, R.A., I. Barker, and K.R. Wood. 1996. The biology of the tospoviruses. *The Annals of Applied Biology* 128:159-183.
- Persley, D.M., M. Sharman, D. McGrath and S. Garland. 2005. Developing capsicum and with Resistance to Tospoviruses in Australia. *In VIII International Symposium on Thysanoptera and Tospoviruses* September 11-15,2005, Asilomar, Pacific Grove, California. 49pp. *Journal of insect sciences* 7:28.
- Puangmalai, P., N. Potapohn, A. Akarapisarn, and H. J. Pascha. 2013. Inheritance of Tomato Necrotic Ring Virus Resistance in *Capsicum annuum*. *Journal of Agricultural Science* 5(2):129-133.
- Roggero, P., G. Dellavalle, M. Ciuffo, and S. Pennazio. 1999. Effects of temperature on infection in *Capsicum* sp. And *Nicotiana benthamiana* by impatiens necrotic spot tospovirus. *Eur. J. Plant Pathol.* 105:509-512.
- Seepiban, C., O. Gajanandana, T. Attathom, and S. Attathom. 2011. Tomato ring spot virus, a new of tospovirus isolated in Thailand. *Archives Virology* 156:263-274.

- Whitfield, A.E., D.E. Ullman, and T.L. German. 2005. Tospovirus-thrips interactions. *Annu. Rev. Phytopathol* 43:459-489.
- Yeh, S.D., and T.C. Chen. 2009. Detection and identification of thrips-borne tospoviruses by serological and nucleic acid tools. *In* Asian seed congress Tospovirus and thrips vectors workshop for breeder, Bangkok, Thailand.