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Reduced incidence of tomato yellow leaf curl virus and leafminer in a tomato cultivar in northern Thailand

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV), a whitefly-vectored begomovirus, is a major limiting factor for tomato production worldwide, including Thailand. Field evaluation of commercial tomato cvs. for resistance to the virus endemic in northern Thailand has not been reported. Experiments conducted in northern Thailand in 2011–2013 compared the field performance of three tomato cvs. from the US with a commonly grown Thai cultivar. There were few significant differences observed among the cvs. in marketable yield, and the severity of root-knot and root rot. However there were significant differences noted in TYLCV incidence; virus incidence ranged from 38 to 100% and 8–41% in experiments one and two, respectively. In both experiments 'Husky Cherry Red' from the US had the lowest TYLCV incidence, leafminer damage (*Liriomyza* sp.), and progression of foliar necrosis primarily caused by thrips feeding and possibly exacerbated by air pollution. TYLCV infection was confirmed by a simplified method of nuclear inclusion visualization with light microscopy in experiment one, and by virus inclusion detection and PCR in experiment two.

1. Introduction

Begomoviruses vectored by the sweet potato whitefly [*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)] represent a significant challenge for production of many important food crops especially in the subtropics and tropics (Polston and Anderson, 1997; Varma and Malathi, 2003). Tomato yellow leaf curl virus (TYLCV) and tomato leaf curl virus (ToLCV) comprise a begomovirus group within the *Geminiviridae* that is a limiting factor for tomato production worldwide (Hanssen et al., 2010), and TYLCV has caused extensive crop losses in Thailand (Attathom et al., 1990). Tomato yellow leaf curl symptoms were first observed in tomato in Thailand in 1973 (Chandrasrikul, 1973); the causal agent was identified as a begomovirus 10 years later (Thanapas et al., 1983), and was molecularly characterized as a bipartite begomovirus in the early 1990s (Attathom et al., 1994; Rochester et al., 1994). Tomato yellow leaf curl virus-Thailand (TYLCTHV) is an unusual virus that may represent an evolutionary intermediate between monopartite and bipartite begomoviruses (Briddon et al., 2010). In Green et al., 2003, reported a second virus species, TYLKaV, causing yellow leaf curl symptoms in tomato and eggplant in Kanchanaburi province, western Thailand. TYLCTHV has spread from northern Thailand–Myanmar into southern China and also appears to be displacing ToLCV in Taiwan; TYLCKaV has spread from western Thailand–Vietnam to Java and Indonesia (Kenyon et al., 2014).

Tomato production in Thailand is primarily located in the northern, northeastern and western parts of the country (Nath et al., 2009), and the country's total tomato production was valued at 95.6 million USD in 2010 (FAO Statistics Division, 2015). One of the essential components in the integrated management of tomato begomoviruses is host resistance (Polston and Lapidot, 2007). A field study and greenhouse study in central Thailand and greenhouse studies in China and Taiwan have identified sources of resistance to TYLCTHV in tomato breeding lines (Choi et al., 2012; Chomdej et al., 2012; Kadirvel et al., 2013; Li, 1999). Although many TYLCV-resistant tomato cvs. have been developed

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worldwide, field evaluation of commercial tomato cvs. for resistance to the virus endemic in northern Thailand has not been reported. Current management of the virus in the country is based on vector control through insecticide applications; but reliance on this single strategy may lead to pesticide misuse and the development of insecticide resistance in *B. tabaci* and other arthropod pests. Agricultural pesticide misuse and overuse has been documented in Asia in general (Ketelaar and Kumar 2012) and in tomato production in northern Thailand in particular (Lamers et al., 2013). Our research objective was to compare the field performance of US tomato cvs. with a common Thai cv. in northern Thailand, including susceptibility to TYLCV and other plant pathogens, and arthropod pests.

2. Materials and methods

2.1. Plants

Two field experiments were conducted at a private research facility in Lampang, northern Thailand in 2011 through 2013. Dried cow manure was incorporated into the soil prior to planting at rates of 8 and 24 t/ha in experiments one and two, respectively. Seven-week-old transplants of US tomato cultivars Christmas Grape, Husky Cherry Red, and Roma, and the commonly grown Thai cv. Seeda were planted on 5 Dec., 2011 spaced 46 cm apart; 10-week-old transplants of Husky Cherry Red and Seeda were planted on 2 Dec., 2012 at a spacing of 30 cm. Plants were not pruned, but were staked and tied, and mulched with rice straw. Equal amounts of water were applied per plant as necessary, and plants were equally fertilized biweekly using a liquid fish fertilizer. No pesticides of any kind were applied in either experiment. A randomized complete block design with four replications consisting of four plants, and four replications of three plants was used in experiments one and two, respectively.

2.2. Plant disease data

The incidence of TYLCV in each tomato cv. was assessed based on typical symptom expression (upward curling of leaves with yellow margins, plant stunting, and flower abortion), and was monitored at 4–12 d intervals for 103 and 116 d after planting (DAP) in experiments one and two, respectively. The area under the disease progress curve (AUDPC) for TYLCV was calculated. Nuclear inclusion formation is a diagnostic characteristic of infection by the Geminiviridae including begomoviruses (Christie et al., 1986). Begomovirus infection was confirmed in experiment one using a simpler method for nuclear inclusion visualization than that of Christie et al. (1986): 20-30 μm-thick longitudinal sections of midribs and petioles of expanding leaves were cleared for 5–10 s in 25% (v/v) lactic acid (Thermo Fisher Scientific Inc.) using a microwave oven; the tissue was rinsed three times in deionized water (dH_2O); stained for 1 min in a mixture containing equal volumes of 0.05% (w/v) toluidine blue O (Sigma-Aldrich Co. LLC) and 0.05% (w/v) basic fuchsin (Thermo Fisher Scientific Inc.); rinsed three times in dH_2O, mounted in dH_2O; and viewed with light microscopy at 1000x. In experiment two, begomovirus infection was confirmed by light microscopy as indicated above and PCR using the method of Atzmon et al. (1998) except that total genomic DNA was extracted with the DNeasy Kit (QIAGEN).

At the termination of the experiments, the root system of each tomato plant was rated for root-knot and root rot severity. Root-knot severity was determined by using the 0-to-5 Taylor-Sasser rating scale, where 0 = 0, 1 = 1 to 2, 2 = 3 to10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100galls per root system (Taylor and Sasser, 1978). Root rot severity was determined by using a 1-to-5 rating scale, where 1 = 0, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, and 5 = 51–100% root discoloration/loss. Detection and identification of fungi infecting the root systems were accomplished by mounting fine feeder root pieces in a calcofluor white solution (ENG Scientific, Inc., Clifton, NJ, USA), and examining discolored-healthy root tissue interfaces with epifluorescence microscopy using an excitation and barrier filter combination of 365 and 420 nm, respectively. The presence of typical sclerotia and mycelial mats was used for identification of *Sclerotium rolfsii*.

2.3. Leafminer data

Leafminer damage was assessed by counting the number of leaflets per tomato plant mined by *Liriomyza* sp. at 51 and 52 DAP, in experiments one and two, respectively. Because 'Husky Cherry Red' is considered a dwarf-indeterminate variety (Seminis, 2016) and may have fewer leaves per plant than the other varieties, in experiment two, the number of mines/leaf/plant were also counted at 52 DAP. These time points were chosen because they allowed for collection of leafminer data before it was obscured by the development of foliar necrosis.

2.4. Foliar necrosis data

Foliar necrosis, primarily caused by thrips feeding, and possibly exacerbated by air pollution from annual forest and other fires that occur in the region during the dry period (December to May), was estimated periodically from 57 to 95 DAP and 48 to 116 DAP in experiments one and two, respectively. A 1-to-5 rating scale was used where 1 = 0–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% foliar necrosis, and the area under the necrosis progress curve (AUNPC) was calculated for each cv.

2.5. Yield data

In experiment one, plants were harvested eight times at 2–7 d intervals, between 68 and 98 DAP, and the weight of marketable fruit per plant was recorded. In experiment two, plants were harvested and yield data collected six times at 7–9 d intervals, between 75 and 114 DAP.

2.6. Statistical analysis

The AUDPC for TYLCV and AUNPC for foliar necrosis were calculated using GraphPad Prism version 5.03. Treatment means were separated following ANOVA using Fisher's Protected LSD in experiment one and by T-test in experiment two ($P \leq 0.05$) (IBM SPSS Statistics 21).

3. Results

3.1. Plant disease data

Only viral symptoms typical of TYLCV were observed in both experiments. TYLCV infection was confirmed by the presence of often complex or branched nuclear inclusions observed by light microscopy in experiment one (Fig. 1), and by both nuclear inclusion visualization and PCR (data not shown) in experiment two; there was good agreement (>80%) between the two techniques in detection of the virus. No other type of virus inclusion was detected. TYLCV pressure in experiment one was high; incidences of the virus observed in 'Christmas Grape', 'Seeda' and 'Roma' ranged from 75 to 100%, while the incidence of the virus was significantly lowest in 'Husky Cherry Red' (37.5%) (Table 1). Although TYLCV pressure decreased in experiment two, virus incidence was again