Development of test methods and screening for resistance to thrips in *Capsicum* species

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Abstract
Thrips are one of the most damaging pest organisms in field and greenhouse pepper (*Capsicum*) cultivation. They can cause damage on pepper directly by feeding on leaves, fruits and flowers, and indirectly by transferring viruses, especially Tomato Spotted Wilt Virus (TSWV). No commercial pepper varieties are available with an effective level of resistance to thrips. Our research is aimed at the development of tools for breeding varieties with a broad resistance to thrips. This encompasses setting up of effective test methods, the identification of sources of resistance and mapping of QTLs for resistance. Thirty-two pepper accessions of four species of pepper (*Capsicum annuum, C. baccatum, C. chinense* and *C. frutescens*) originating from different geographic and climatic regions were tested for resistance using several screening methods. The tests were performed in Indonesia and the Netherlands with *Thrips parvispinus* and *Frankliniella occidentalis*, respectively. Accessions were tested under choice (screenhouse, greenhouse) and non-choice (leaf disc, detached leaf and cuttings) conditions. Screening methods were compared and correlations among these methods were assessed. We observed a large variation for resistance to thrips in pepper. Our results also indicate that the leaf disc test can be used as an efficient and predictive screening method for thrips resistance in pepper. An F2 population from a cross between a highly resistant and a susceptible accession was produced and currently we are studying the inheritance of resistance in this population.

Keywords: *Thrips parvispinus, Frankliniella occidentalis.*

Introduction

Pepper (*Capsicum*), one of the most widely grown vegetables in the world faces problems from thrips both in the tropics and temperate regions. At least 16 thrips species have been reported to occur on *Capsicum* (Capinera, 2001; Talekar, 1991). *Frankliniella occidentalis* is the most common thrips species in greenhouse cultivation in Europe (Tommasini and Maini, 1995), while *Thrips parvispinus* is the main species on *Capsicum* in Indonesia, Malaysia, the Philippines, Thailand and Taiwan (Reyes, 1994) Thrips do not only cause direct damage by feeding and laying eggs on pepper leaves and fruit, but also cause indirect damage by transmitting plant viruses, especially Tomato Spotted Wilt Virus (TSWV) (Ulman et al., 1992).

The main approach to control thrips in pepper is the use of pesticides. However, thrips rapidly develop resistance to pesticides. Also, pesticides are costly and have harmful effects on growers, consumers and the environment. As an alternative, integrated pest management (IPM) has started to be implemented. To help IPM become a success, (partially) resistant varieties are needed. Resistance to thrips not only decreases direct damage but may also be useful for controlling insect-transmitted plant viruses (Maris et al., 2004; Jones, 2005). Breeding programs for obtaining pepper varieties resistant to thrips involve the screening of potential sources of resistance and selection of promising plants or lines in more advanced stages. Screening tests with thrips can pose problems, however: thrips are difficult to contain and so may spread to other experiments. Therefore evaluation methods are needed that are easy to conduct; accurate; reproducible; require little space, time, and energy; and pose no risk of contamination. These characteristics are present in *in vitro* tests. Several tests have been described in the past, e.g. a leaf disc assay for thrips resistance in cucumber (Kogel et al., 1997), a detached leaf test for *Helicoverpa armigera* resistance in pea (Sharma et al., 2005) and a screen cage test for aphids resistance in sweet pepper (Pineda et al., 2007). Our objective in this study is to develop a good method for testing thrips resistance in pepper with the previously mentioned characteristics.

Materials and methods

Pepper accessions

Pepper accessions used in this study were collected from gene banks based on several previous studies and from East West Seed Indonesia (EWINDO). Thirty-two pepper accessions from four species of pepper (*Capsicum annuum, C. baccatum, C. chinense* and *C. frutescens*) were used in this study.

Thrips population

Two thrips species were used in this study: *Thrips parvispinus* and *Frankliniella occidentalis*. *F. occidentalis* was selected as it is the most prevalent thrips species in European pepper cultivation, while *T. parvispinus* was selected as representative of Asian thrips. *T. parvispinus* was collected from the field in Purwakarta (Java, Indonesia), while *F. occidentalis* was collected from glasshouses in the Netherlands.

Screening Methods

a. Screenhouse and glasshouse test

In the screenhouse test, pepper accessions were grown on raised beds in a screenhouse of EWINDO at Purwakarta, West Java, Indonesia. Seedlings were raised under insect-free conditions in a seedling bed and transplanted six weeks after germination. Six plants per accession were planted in a plot, with two replications in a randomized block design. Plants were spaced 75 cm between rows and 45 cm between plants in a row. Pepper plants were grown according to standard screenhouse pepper cultivation techniques (Rossel and Ferguson, 1979). Thrips infestation occurred naturally. Thrips were identified as *T. parvispinus*. After the thrips attack, peppers were rated for damage using a relative scale from 0 (no damage) to 3 (severe damage, i.e. strongly curled leaves, silvering and black spots).

In the glasshouse test, pepper accessions were grown at 25^{o}C under 16/8 hr day/night cycle under standard glasshouse conditions at Wageningen University and Research Centre, Wageningen, the Netherlands. Four plants per accession were planted in a plot, with two replications in a randomized block design. After a spontaneous thrips (*F. occidentalis*) infestation, plant were rated using relative scale from 0 (no damage) to 3 (severely curled leaves).

b. Leaf Disc Test

F. occidentalis were reared on susceptible Chrysanthemum cultivar Spoetnik (Fides, De Lier, the Netherlands) in a greenhouse at 25^{o}C and 70% relative humidity (Koschier et al., 2000), while *T. parvispinus* were collected from a pepper field at Purwakarta, Indonesia. Adult female thrips were starved for 24 hours in a chamber with only water. Leaf discs (4 cm in diameter) were cut from the youngest fully opened leaves using a leaf punch and placed in petri dishes on water agar (15g/l agar) with the lower (abaxial) side upward. Ten starved female adult thrips were placed on each leaf disc using a wet brush. Dishes were closed using air-permeable plastic (in the Netherlands) or silk-like textile (in Indonesia) and placed in a climate room at 24^{o}C, 16 h light, 70% RH. There were six replicates for each accession.

The extent of ‘silver damage’ and destruction by thrips feeding and secretion were rated using a relative scale from 0 (no damage) to 3 (severe damage) two days after inoculation.
c. Detached Leaf Test
The detached leaf tests were performed as the leaf disc test, except that intact leaves from each accessions were placed with their petioles in wet Oasis® (2cm x 5cm x 4cm) and were put in a jar. Jars were closed using air-permeable plastic (in the Netherlands) or silk-like textile (in Indonesia) and placed in a climate room at 24^{o}C, 16 h light, 70% RH. There were six replicates for each accession. The extent of ‘silver damage’ and destruction by thrips feeding and secretion were rated together using a relative scale from 0 (no damage) to 3 (severe damage) two days after inoculations.
d. Cutting Test
Three week old cuttings of pepper plants were grown at 25^{o}C, 16 h light under greenhouse conditions at Wageningen, the Netherlands. Two cuttings from the same accession were placed in one pot (20 cm diameter). Pollen grains were added to each pot before releasing 40 synchronized thrips larvae (L1 stages) per pot. To obtain L1, *F. occidentalis* were reared at 25^{o}C day and 20^{o}C night in glass jars containing small cucumber fruits and a few grains commercial pollen (Bijenhuis, Wageningen, the Netherlands). The use of pollen grain in this experiment is to stimulate thrips and larvae to feed. L1 stage were obtained by allowing female thrips to lay eggs in new fruits for one day, after which the adult thrips were brushed off and fruits were kept at 25^{o}C for three days, when the larvae emerged (Mollema et al., 1993). After releasing L1 thrips, each pot was enclosed in a thrips-proof cage to avoid thrips escape. There were four replicates for each accession. After three weeks the damage was rated using relative scale from 0 (no damage) to 3 (severely curled leaves).

We performed screenhouse and glasshouse tests under choice conditions, and leaf disc, detached leaf and cutting tests in non-choice situations. In a choice situation, thrips are allowed to move between accessions. In contrast, in a non-choice situation, thrips cannot move from the accession on which they are placed, and possible preference effects are excluded.

Results and discussion

We observed large differences in thrips damage among pepper accessions. In all of the tests, accession means for damage varied from 0.0 (no symptoms) to 3.0 (severe damage) and Kruskal-Wallis tests for accession effects were always significant. All tests resulted in relatively high heritability values (0.68 to 0.92), except the cutting test (0.34).
The damage observed in this study was different in tests using whole plants (screenhouse, greenhouse and cuttings) or *in vitro* leaves (leaf discs and detached leaf). The symptoms in the tests using whole plants were silvering, stunting, curling and deformation, while those in the leaf (disc) tests consisted of silvering and the incidence of black spots caused by thrips feeding and secretion (Figure 1). These differences are probably due to the nature of the material used in the tests. Stunting, curling, and deformation of leaves only occur when the leaves are still growing, so they could not be observed in the leaf disc and the detached leaf tests. These damages can be classified into four classes as shown as Figure 1.

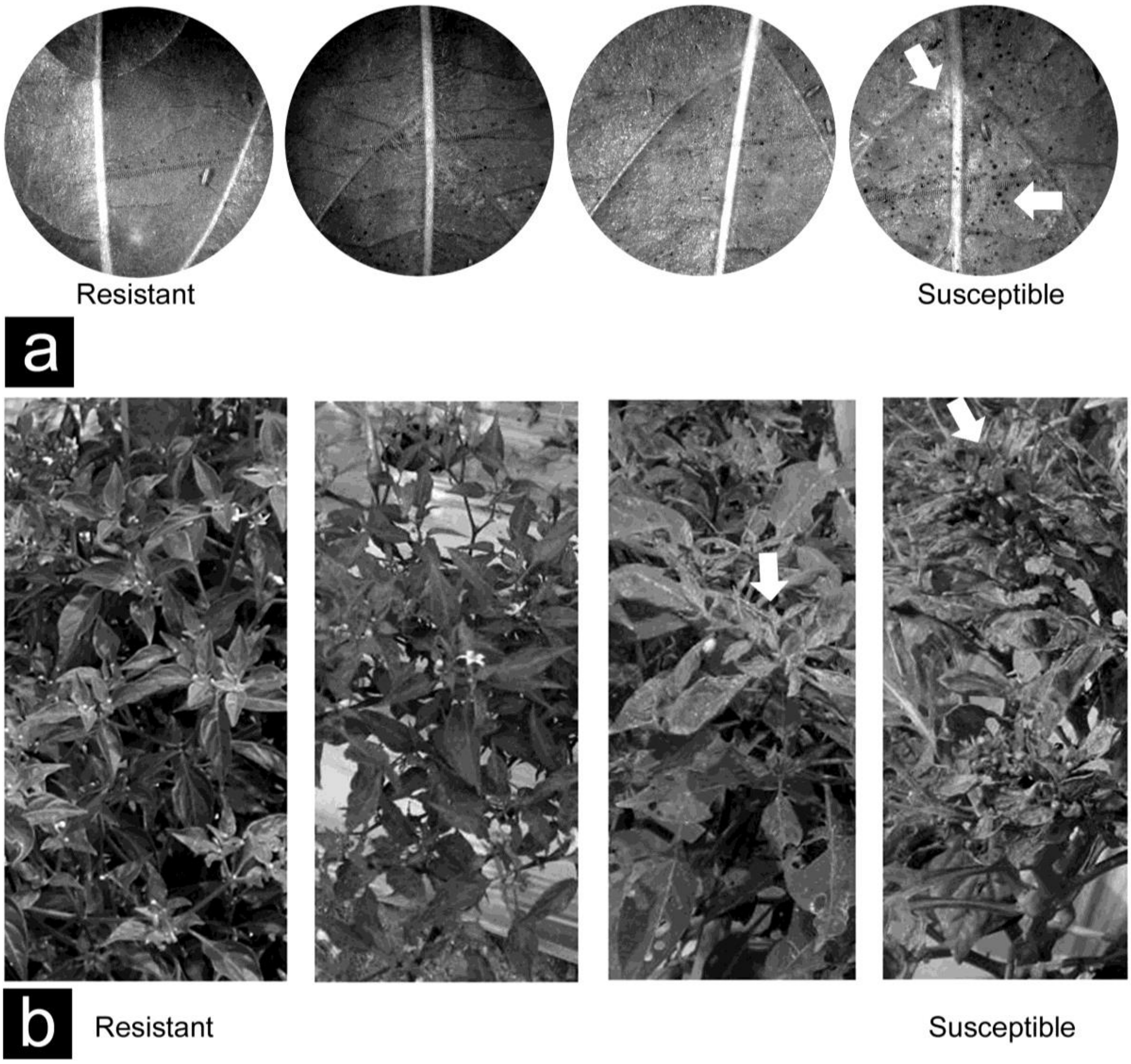

Figure 1. Damage caused by thrips in screening methods. (a) silver damage caused by thrips feeding and black spots caused by fecal material in the leaf disc test (indicated by arrows) and (b) leaf curling and deformation in the screen house test (indicated by arrow).

Damage caused by *F. occidentalis* and *T. parvispinus* was very similar in all the tests in our study. There were no differences between the symptoms caused by *F. occidentalis* and *T. parvispinus* in the leaf disc and detached leaf tests. The symptoms in the screenhouse (*T. parvispinus*) and glasshouse tests (*F. occidentalis*) were also identical. In the literature we found no reports of specific differences in damage caused by different thrips species on pepper. One report mentions that feeding injury caused by *F. occidentalis* is similar to that caused by *T. tabaci* Lindeman (Capinera, 2001).

Although the symptoms observed in the leaf disc and detached leaf tests were different from those found in the other tests, the damage scores of almost all tests were significantly correlated (Table 1).. This shows that preference effects, which were possible in the screenhouse and greenhouse tests but not in the other tests, must be small compared with antibiosis (non-preference) differences.

Table 1. Spearman correlation coefficients and significance between damage score in screening methods of thrips resistance in pepper.

		T. parvispinus		*F. occidentalis*			
		Leaf disc	Detached leaf	Glasshouse	Leaf disc	Detached leaf	Cutting
T. parvispinus	Screen house	0.77 ***	0.80 ***	0.76 ***	0.65 **	0.70 ***	0.53 *
	Leaf disc		0.87 ***	0.71 ***	0.71 ***	0.71 ***	0.45 *
	Detached leaf			0.73 ***	0.70 ***	0.69 ***	0.50 *
F. occidentalis	Glasshouse				0.77 ***	0.73 **	0.48 *
	Leaf disc					0.77 ***	0.41
	Cutting						0.64 **

Top figure: correlation coefficient
Bottom figure: significance. *, **, and *** indicate significance at $P<0.05$, $P<0.01$, and $P<0.001$ respectively

Compared to the screenhouse or glasshouse tests, leaf disc and detached leaf tests are relatively easy to conduct. A small climate room is sufficient to test many accessions. Also they require little time: damage can be scored two days after inoculation. An additional advantage is that the plants from which leaves are tested remain uninfested by thrips. Finally, environmental factors during these tests are better controlled than in screenhouses or glasshouses. In this study, the leaf disc and detached leaf tests were compared to see if the wounding involved in obtaining leaf discs would have any effect on the response to thrips. We did not observe any difference in the type of symptoms on leaf discs versus whole leaves, nor in the general amount of damage. The correlation between leaf disc and detached leaf test was high and significant. An advantage of the leaf disc test over the detached leaf test is that the sample size is more standardized.

Based on the test results we selected two accessions having contrasting damage scores, and crossed these to obtain a segregating F2 population. We are currently screening the F2 population using the leaf disc test as phenotyping method in order to perform QTL mapping of thrips resistance in pepper.

Our results also show that *in vitro* tests for evaluating thrips resistance in pepper are reliable and deliver results similar to whole plant tests. Similar tests might be developed for other insect pests as well, which will strongly facilitate resistance breeding. The leaf disc test is the most suitable for assessing the resistance of a large number of pepper accessions to thrips.

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