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QTL mapping specific to *Thrips palmi* resistance in *Capsicum annuum*

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Abstract

Capsicum, more commonly known as red pepper or chili pepper, is an important vegetable and spice throughout the world. Thrips are insects that cause highly devastating losses to Capsicum production, resulting in both direct and indirect damage. Some resistance to *Thrips palmi* (*T. palmi*) has been identified in the *Capsicum* species, with tests revealing that resistance to thrips is species specific. In this study, a Quantitative Trait Loci (QTL) analysis was performed to determine resistance against *T. palmi* in an F_2 population derived from a cross between the highly resistant *C. annuum* AC 1979 and the highly susceptible *C. annuum* Berceo. In addition, a resistance test (Choice method) was used to study phenotypic data. One hundred sixty-one SNPs markers were used to construct a linkage map. An F_2 genetic linkage map, constructed with a JoinMap 4.0 program, consisted of 13 linkage groups with a total length of 783.84 cM. The interval mapping of the area under the disease progress curve (AUDPC) showed significance in the LG1 (Chromosome 3) and LG2 (Chromosome 12). The highly significant QTL in LG1 was located on the M238 SNP marker with about 12.2% explained phenotypic variance. The highly significant QTL in LG2 was located on the M171 single nucleotide polymorphism (SNP) marker with an explained phenotypic variance of 8.9%. These two QTLs may play a role in *T. palmi* resistance in *C. annuum.* Moreover, these M238 and M171 SNP markers will be used in pepper marker-assisted breeding for *T. palmi* resistance.

Keywords: *Thrips palmi*, Pepper, Resistance, Single nucleotide polymorphism (SNP), Quantitative trait loc (QTL)

1. Introduction

Capsicum species (2n=2x=24), including hot peppers as well as sweet and bell peppers, is an agriculturally and economically important vegetable crop worldwide. According to farmers, from 1998 to 2002 the average annual capsicum losses due to insects ranged from 7% in China to 56% in India [1]. Field and greenhouse cultivation of peppers were commonly subject to infestation by thrips. Thrips oviposit and feeds on the leaves and developing fruits [2]. This direct damage consequently decreases the plants' photosynthetic capacity [3]. The adverse effects on photosynthesis have led to reduced vitality and yield declines [4]. Indirect damage by thrips has also been observed through transmission of *Machlomovirus*, *Carmovirus*, *Tospovirus*, *Sobemovirus* and *Ilarvirus* [5].

Thrips species commonly found in *Capsicum* spp. include western flower thrips (*Frankliniella occidentalis*; *F. occidentalis*), melon thrips (*Thrips palmi*; *T. palmi*) and chilli thrips (*Scritothrips dorsalis*; *S. dorsalis*). The *F. occidentalis* has been found in Europe, whereas *T. palmi* and *S. dorsalis* present a more serious problem in subtropical and tropical regions, and have been designated as quarantine organisms in the EU [6]. Thrips are difficult to control with the use of insecticides because of their high reproductive rates, short life cycles, cryptic behaviors and their ability to rapidly develop resistance to insecticides [7]. Thus, development of cultivars resistant to thrips increase the efficiency of thrips control. The identification of resistant accessions is necessary for the successful and sustainable production of pepper. It has been reported that several pepper varieties have shown resistance to *F. occidentalis* [6-9], *Thrips parvispinus* [7], *T. palmi* and *S. dorsalisi* [6]. For example,

Capsicum annuum (*C. annuum*) AC 1979 and *C. annuum* Keystone Resistant Giant were *F. occidentalis*-resistant [7-9]. Although *C. annuum* Keystone Resistant Giant is susceptible to *T. palmi*, *Capsicum chinense* (*C. chinense*) No.4661 is resistant [6]. Only one study identified a *Capsicum spp*. with resistance to *T. palmi* [6], the major foliar feeding thrips species in Thailand.

Molecular marker linkage maps for thrips have been established for *Capsicum* spp. A quantitative trait loci (QTL) analysis for *F. occidentalis* resistance has been reported for an F_2 population of an interspecific cross between the highly resistant *C. annuum* AC 1979 and the highly susceptible *C. chinense* No. 4661. The damage scores and survival of larvae observed in a no-choice test or leaf dish assay were the parameters used for resistance. One QTL with an explained 50% genetic variation was identified on chromosome 6 [10]. The resistant capability of pepper accessions has a notable impact on thrips' larval mortality and oviposition rate [2]. Moreover, phytochemicals have been reported to play a role in the resistance of *C. annuum* AC 1979 to *F. occidentalis*. Metabolite QTL analysis revealed that diterpene glycosides and flavonoid compounds were correlated to thrips resistance [11].

The goals of this study include elucidating the thrips (*T. palmi*) resistance levels in pepper accessions and developing Single nucleotide polymorphism (SNP) markers linked to the QTLs of *T. palmi* resistant gene in *C. annuum*. This information will be used in future breeding programs to develop thrips-resistant pepper varieties.

2. Materials and methods

2.1 Preparation of plants

Pepper accessions with possible resistance to thrips were selected based on findings from available literature [6-9] and supplemented with other accessions of various species and geographic origins. Greenhouse-based testing methods were used to evaluate the resistance levels of ten pepper accessions from three *Capsicum* spp. (e.g., *C. annuum*, *C. baccatum* and *C. chinense*) [8] (Table 1). Seeds were obtained from the Center of Genetic Resources (CGN), Wageningen University and the Research Center (Netherlands). As Berceo is economically important to the breeding of sweet peppers, seeds used for developing F_2 populations were obtained from East-West Seed Thailand Ltd. (Chiang Mai, Thailand). A cross between a *C. annuum* AC 1979 (female parent) and a *C. annuum* Berceo (male parent) generated one hundred and ninety-five F_2 plants for a linkage mapping construction. The parents were selected based on the evaluation of resistance against *T. palmi* (Table 1). An F_1 and an F_2 population as well as two parental lines were grown together without application of pesticides.

2.2 Thrips populations

The *T. palmi* parental stocks were obtained from a natural population found on pepper flowers in Suphan Buri Province, Thailand. The external morphology [12,13] and a PCR amplification of an internal transcribed spacer 2 region [14] were used to identify *T. palmi*. Adult thrips on pepper flowers were introduced into rearing boxes using okra pods (*Abelmoschus esculentus*) as the rearing medium and left overnight. The pepper flowers were then removed. The rearing boxes were incubated at 25 ˚C with 70% relative humidity for 16/8 day/night. Newly emerged adult thrips were inoculated on eggplant seedlings. Then, the transplanted eggplants were maintained in a greenhouse until being used as spreader plants.

2.3 Resistance test

The resistance tests were conducted in a greenhouse and were based on damage scores or whole plant damage scores. The tests were slightly modified from a method described by Fery and Schalk [8]. Eggplant transplants infested with adult thrips were placed in a screening greenhouse as a border row. Pepper seedlings were transplanted 30 d after sowing (DAS) and those with 4-6 true leaves in a pot were used in the damage assay. A Randomized Complete Block Design (RCBD) was used and replications at 14, 28 and 42 d after transplant (DAT) were evaluated. The RCBD was designed to identify which of the parental lines to be used were the most resistant and most susceptible. The amount of damage caused by thrips was rated using the following scale: symptomless (0), 1-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4) (Figure 1). The damage scores were transformed to a Disease Index (DI) and the Area Under the Disease Progress Curve (AUDPC) [15]. The Statistical Tool for Agricultural Research (STAR) was used for data analysis with ANOVA used to calculate means. Replicate comparisons were made after conducting a phenotypic analysis.

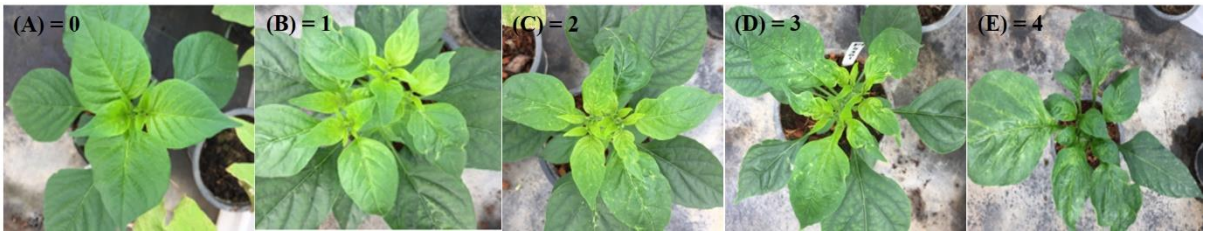

Figure 1 Examples of damage scores (0-4) of *T. palmi* in *C. annuum.* (A) = 0 (Symtomless), (B) = 1 (1 to 25% of leaf area is infected), (C) = 2 (26 to 50% of leaf area is infected), (D) = 3 (51 to 75% of leaf area is infected), (E) = 4 (76 to 100% of leaf area is infected or death of the plant).

2.4 Single nucleotide polymorphism (*SNP) genotyping*

The SNP collections were chosen from a *C. annuum* genome database (http://peppergenome.snu.ac.kr/). KASPar™ genotyping assays were conducted using SNP markers. The system was a competitive allele-specific PCR dual FRET based assay. Two FRET cassettes, on which a primer was conjugated with a fluorescent dye (VIC or FAM) were used. DNA samples were extracted from young leaves. A total genomic DNA was extracted from the leaves of each plant using a cetyltrimethyl ammonium bromide (CTAB) method as described by Nishiguchi [16]. The Genomic DNA was amplified with allele-specific primers. When the FRET cassette primer hybridized to DNA, a separation of fluorescent dye and quencher was conducted [17].

2.5 Genetic linkage mapping

Initially, 572 SNP markers were analyzed to identify the marker that shows polymorphic between two parents. The resulting find of 161 SNP markers that showed polymorphic of two parental lines were subsequently surveyed and these results were used to analyze the segregation in 195 F_2 populations. The genetic linkage map was constructed in JoinMap 4.1 using Kosambi's regression mapping function with a recombination fraction smaller than 0.50 and a log of odds (LOD) score greater than 5 [18]. Linkage groups were verified using a regression mapping algorithm with a maximum level of 5. Ungrouped markers were deleted to obtain a final linkage map.

2.6 QTL analysis

The QTLs for *T. palmi* resistance were identified using a MapQTL 6.0 package [19]. Potential QTL regions were identified after performing an interval mapping analysis. Next, multiple-QTL mapping (MQM) was done using additional markers treating these regions as co-factors. The linkage map was prepared with MapChart 2.3 [20]. Finally, the candidate gene in the QTL region was identified using significant SNP markers that were BLAST-searched against the *C. annuum* genome database (http://peppergenome.snu.ac.kr/).

3. Results

3.1 Resistance study

Leaf deformation, curling and silvering damage were observed in susceptible lines (Figure 2). Visible symptoms started to appear two weeks after transplanting. Damage scores were noted and calculated under DI and AUDPC. A low value represents a resistance reaction, whereas a high value represents susceptibility to thrips infestation. The DI and ADUPC values were used to classify resistance into three levels (resistant, intermediate and susceptible) as shown in Table 1. The DI/ADUPC of *C. annuum* CGN16975/AC 1979 and Berceo were 4.17±2.6/145.80±45.4 (most resistant) and 74.50±20.8/2,281.23±321.8 (most susceptible) respectively. These 2 accessions were selected as parental lines to produce an F_2 population. The *C. annuum* AC 1979 was used as a donor for its resistance gene, whereas *C. annuum* Berceo had a high market value with important economic traits for sweet peppers such as good fruit quality, color and shape. Most of the resistance screening for *Capsicum* spp. involved studies of *F. occidentalis* [6-9]. To date, only Visschers [6] has studied *T. palmi* resistance. Thrips resistance has been investigated in various accessions, as shown in Table 1.

Table 1 DI and AUDPC in resistance level test from 10 accessions. The data presented represent the means ±S.D.

Accession code/ Accession name	Species	Resistance test		Resistance level
		DI	AUDPC	
CGN16975/AC 1979 [7]^{R} [9]^{R}	*C. annuum*	4.17±2.6^{e}	145.80±45.4^{d}	Resistant
CGN21557/ No.4661 [7]^{S} [9]^{S} [6]^{R}	*C. chinense*	9.03±5.3^{cde}	325.70±92.8^{cd}	Resistant
CGN17042/No.1553 [7]^{R} [9]^{R}	*C. baccatum*	12.50±8.3^{bcde}	398.60±131.5^{cd}	Resistant
CGN17220/Miscucho Colorado [7]^{S}	*C. chinense*	22.22±8.7^{bcde}	719.43±161.3^{bcd}	Intermediate
CGN17222 [7]^{S} [9]^{S}	*C. chinense*	25.27±10.5^{bcd}	933.00±168.2^{bc}	Intermediate
CGN21513	*C. baccatum*	29.17±2.9^{bc}	962.50±30.9^{bc}	Intermediate
CGN20503/Bisbas [7]^{R} [9]^{R}	*C. annuum*	32.08±13.4^{bc}	1,093.77±224.2^{b}	Intermediate
CGN23222 [8]^{R} [7]^{R} [6]^{S}	*C. annuum*	65.56±15.0^{a}	2,072.8±243.6^{a}	Susceptible
CGN23765 [7]^{R} [6]^{S}	*C. annuum*	68.06±6.4^{a}	2,012.5±113.9^{a}	Susceptible
Berceo (Blocky pepper)	*C. annuum*	74.50±20.8^{a}	2,281.23±321.8^{a}	Susceptible

Resistance test scores appearing in the same column followed by the same letters imply no significant difference (P>0.05).
The symbol 'R' indicates resistance while 'S' stands for susceptibility to thrips.

Figure 2 Evidence of damage caused by *T. palmi* in Berceo (susceptible line) (A) leaf curling and distortion and (B) silvering symptoms. The complete plants are shown in (C) susceptible (left) and resistant (right).

3.2 Genetic linkage mapping

The 572 SNP markers were screened using *C. annuum* AC 1979 and *C. annuum* Berceo as 2 parental lines for polymorphic markers. The screening revealed 161 polymorphic SNP markers. These polymorphic markers were used for F_2 population genotyping and linkage map construction. Linkage groups were designated as 'CM334' *C. annuum* chromosomes. Thirteen linkage groups (LGs) were identified on 12 chromosomes. Most of the LGs were located on one single chromosome except for LG8 and LG9, which were both located on chromosome 10. The distance for the linkage groups varied from 4.35 to 146.35 cM, with a total length of 783.8 cM (Table 2).

Table 2 Relationship between linkage groups and chromosomes *T. palmi* resistance.

LG	Chromosome	Distance (cM)
1	Chr 3	146.349
2	Chr 12	90.613
3	Chr 11	39.112
4	Chr 1	52.195
5	Chr 8	88.637
6	Chr 4	105.627
7	Chr 6	102.515
8	Chr 10a	19.795
9	Chr 10b	4.350
10	Chr 2	19.601
11	Chr 7	43.766
12	Chr 5	5.438
13	Chr 9	65.842
	Total	783.840

3.3 QTL analysis

An interval mapping of DI and AUDPC revealed 2 significant QTL regions, with QTL1 detected on LG1 and QTL2 found on LG2. The mapping of QTL1 revealed an M238 SNP marker with LOD 5.67 and phenotypic variance explained at 12.2%. The M238 SNP marker was located between regions 122.859-132.455 cM or on the physical map at 240,151,347 bp of chromosome 3. Moreover, QTL2 showed an M171 SNP marker with LOD scores of 4.21 and an explained phenotypic variance of 8.9% (Figure 3). The M171 SNP marker was located between regions 2.563-12.089 cM or on the physical map at 227,940,867 bp of chromosome 12 (Table 2). For MQM mapping, the same patterns and markers were found, including the M238 SNP marker in LG1 and the M171 SNP marker in LG2. Therefore, there was a major QTL where LG1 had the highest significance, while LG2 was only a minor QTL of *T. palmi* resistance in this study. The primer sequences of M238 and M171 are presented in Table 4. The M238 SNP marker showed the differences between the resistant and susceptible groups (Figure 4). A search of an CM334 *C. annuum* v.1.55 reference genome sequence revealed that M238 nearby genes included *cytochrome c oxidase* (CA03g27940), *apocytochrome f* (CA03g27970), *nitrate transporter* (CA03g28020) and *homeobox protein* (CA12g19340) and *class III peroxidase* (CA12g19300).

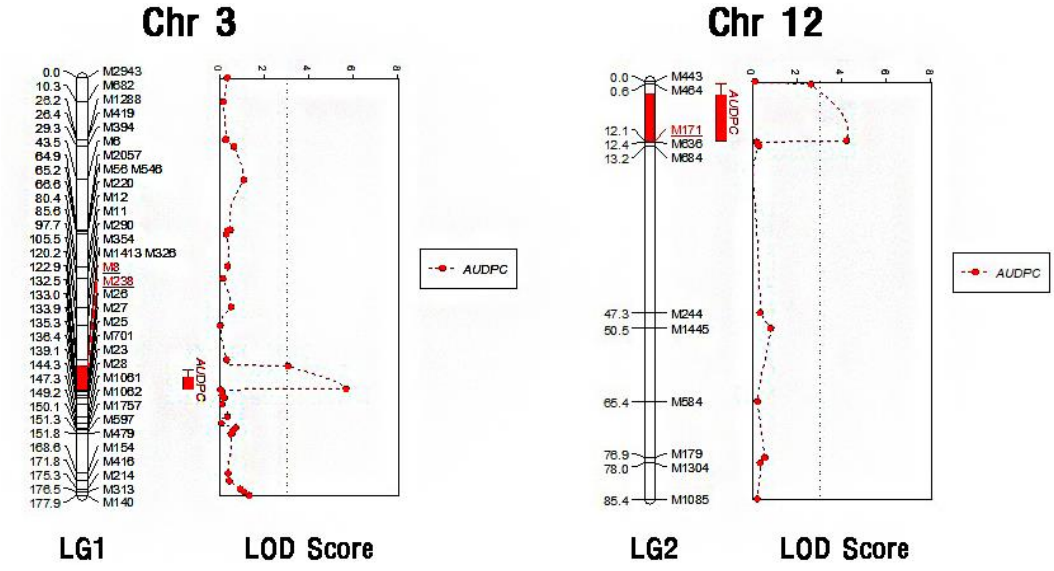

Figure 3 LOD profiles and Linkage group support intervals for AUDPC on Chromosome 3 (Left) and Chromosome 12 (Right). The dotted line at LOD 3.0 indicates a common LOD threshold.

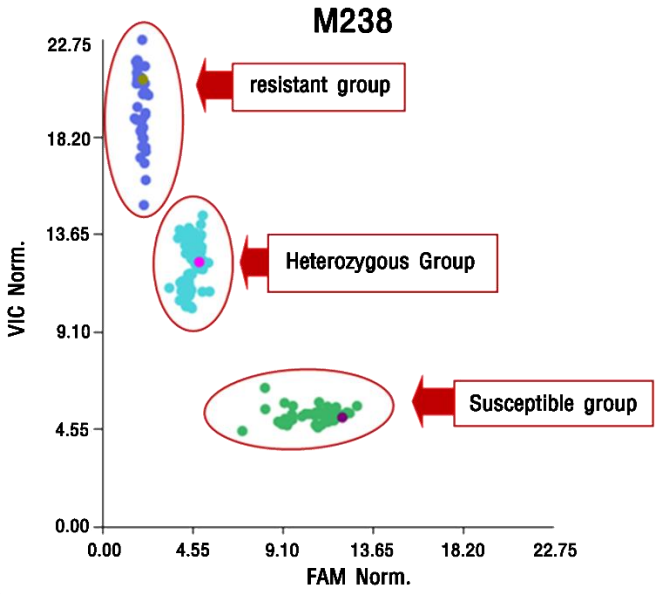

Figure 4 The M238 SNP Polymorphism image of F_2 population screening.

Table 3 Effect of QTLs on *T. palmi* resistance as detected in the F_2 population (*C. annuum* AC 1979 x *C. annuum* Berceo).

QTL	Chr.	Position (cM)	Significant marker	LOD	R^2 (%)	Physical map (Pepper 1.55) (bp)
QTL1	3	122.859 - 132.455	M238	5.67	12.2	240,151,347
QTL2	12	2.563 - 12.089	M171	4.21	8.9	227,940,867

Table 4 Primer sequences of M238 and M171 SNP markers from a *C. annuum* genome database.

Marker name	Primer sets	
	Primers	Sequences (5′- 3′)
M238 A/ G SNP	F1	GTA GCC CAA ACA GCA TTC AGA CA
	F2	GTA GCC CAA ACA GCA TTC AGA CG
	R	ACA TAA TTT GGT CGT CGA TGG AG
M171 A/ G SNP	F1	AAT CAT TCA CAA AAA TGG CAT TAC A
	F2	AAT CAT TCA CAA AAA TGG CAT TAC G
	R	TGG AGT AAT TCA AAG AGA AAT GGT TG

4. Discussion

4.1 Species specificity of thrips resistance

Most *T. palmi* resistance levels found in this experiment were consistent with previous reports, which noted the resistance of CGN16975/ *C. annuum* AC 1979 and CGN17042/ *C. baccatum* No.1553 to *F. occidentalis* [7, 9]. The most resilient line in this study, *C. annuum* AC 1979, showed good resistance against *T. palmi* and was selected as a donor line to create a population for marker development.

Some accessions showed resistance levels that were dissimilar to those identified in previous research. This might have resulted from different testing methods and/or species of thrips. The resistance test in this study used whole plants to test for *T. palmi* damage, whereas the previous study only conducted a leaf dish test for *F. occidentalis* damage [7]. This variation could also have been related to environmental factors, as reported by Visschers [6].

The thrips species specificity was also observed in accession CGN21557/ *C. chinense* No .4661 and CGN23222/*C. annuum* Keystone Resistant Giant. *C. chinense* No .4661 was resistant to *T. palmi* but susceptible to *F. occidentalis*. In contrast, *C. annuum* Keystone Resistant Giant was susceptible to *T. palmi* but resistant to *F. occidentalis* [7]. The CGN21557/ *C. chinense* No.4661 was also reported as being resistant to *T. palmi* and *F. occidentalis* susceptible accession [6]. This suggests that the thrips resistance found in *Capsicum* spp. might be driven by a variety of defense mechanisms. Therefore, resistance to *F. occidentalis* is not correlated with resistance to *T. palmi*.

4.2 Different QTLs associated with thrips resistance in various genetic backgrounds of Capsicum species

The QTL mapping of *T. palmi* resistance in F_2 populations between *C. annuum* AC 1979 x *C. annuum* Berceo was investigated. The QTLs found in those two parameters (DI and AUDPC) were co-localized near the same markers: M238 on chromosome 3 and M171 on chromosome 12. These two QTLs explained about 20% of the genetic variation. However, the major QTL on chromosome 6 with a 50% explained genetic variation was reported from QTL mapping of *F. occidentalis* resistance in F_2 populations between *C. annuum* AC 1979 x *C. chinense* No.4661 [10]. Still, this major QTL was not detected in this study. Although the *C. annuum* AC 1979 was used as the resistant parent in both studies, the susceptible parents used (*C. annuum* Berceo and *C. chinense* No.4661) were different. This study and the findings of Visschers [6] showed that *C. chinense* No.4661 was resistant to *T. palmi* (Table 1). Different defense mechanisms could be used by different *Capsicum* species and thrips species, as suggested by Visschers [6]. Moreover, the major QTL on chromosome 6 was effective exclusively against larvae in a leaf dish assay resistant test. However, this experiment tested resistance by looking at the damage to the whole plant in a greenhouse. Therefore, these 2 QTLs on chromosome 3 and chromosome 12 might play a role in *T. palmi* resistance in *C. annuum* rather than QTL on chromosome 6 as reported by the previous study [10].

Most of the genes examined from these 2 QTLs on *C. annuum* 334 were genes for photosynthesis, respiration and plant growth, such as *apocytochrome f*, *chlorophyll an oxygenase*, *cytochrome c oxidase* and *homeobox*. Genes of interest included nitrate transporters that respond to plant abiotic stress resistance [21], an apyrase-like protein that mediates biotic and abiotic stress responses [22] and class III peroxidases that are involved in plant defense reactions [23]. These genes might play a role in *T. palmi* resistance in *C. annuum*.

5. Conclusion

The resistance screening revealed that *C. annuum* AC 1979 was the accession most resistant to *T. palmi* and *F. occidentalis* infestation. *C. annuum* AC 1979 is a dominant pepper crop species that will support the breeder introgressing in a breeding program. In this study, the thrips species-specific was observed in accessions CGN21557/ *C. chinense* No. 4661 and CGN23222/ *C. annuum* Keystone Resistant Giant. *C. chinense* No .4661 was resistant to *T. palmi* but susceptible to *F. occidentalis*. In contrast, *C. annuum* Keystone Resistant Giant was susceptible to *T. palmi* but resistant to *F. occidentalis* [7]. The resistance to thrips within *Capsicum* species might be driven by different defense mechanisms.

The QTL mapping of *T. palmi* resistance in F_2 populations between *C. annuum* AC 1979 x *C. annuum* Berceo revealed two QTLs detected on chromosome 3 (QTL1) and chromosome 12 (QTL2). These two QTLs explained about 20% of the genetic variation. These 2 QTLs conferred resistance against *T. palmi* in *C. annuum*, a finding inconsistent with the previous study finding a QTL conferring *F. occidentalis* resistance on chromosome 6. The detected M238 and M171 SNP markers in QTL1 and QTL2, respectively, will be employed in pepper marker-assisted breeding for *T. palmi* resistance.

6. Acknowledgements

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