DEVELOPMENT OF SNP MARKERS SPECIFIC TO THRIPS (*THRIPS PALMI*) RESISTANCE IN PEPPER

AREERAT SAEKO

MASTER OF SCIENCE IN GENETICS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN GENETICS
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APPROVED BY **Advisory Committee**

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ชื่อเรื่อง การพัฒนาเครื่องหมายโมเลกุลชนิด SNP ซึ่งจำเพาะต่อความต้านทานเพลี้ยไฟ (*Thrips palmi*) ในพริก
ชื่อผู้เขียน นางสาวอารีรัตน์ แซ่โก
ชื่อปริญญา วิทยาศาสตรมหาบัณฑิต สาขาวิชาพันธุศาสตร์
อาจารย์ที่ปรึกษาหลัก รองศาสตราจารย์ ดร.แสงทอง พงษ์เจริญกิต

บทคัดย่อ

เพลี้ยไฟ (*Thrips palmi; T. palmi*) เป็นศัตรูพืชที่สร้างความเสียหายจำนวนมากให้กับการผลิตพริกในประเทศไทย โดยการวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความต้านทานและค้นหาเครื่องหมายดีเอ็นเอที่มีความสัมพันธ์กับความต้านทานต่อ *T. palmi* ในพริก จากผลการศึกษาความต้านทานของพริกต่อ *T. palmi* จำนวน 17 สายพันธุ์ ด้วยการทดสอบความต้านทาน 2 แบบ คือ วิธีการคัดเลือกแบบอิสระและแบบไม่มีทางเลือก พบว่า *Capsicum annuum* (*C. annuum)* AC 1979 เป็นสายพันธุ์ที่มีความต้านทานสูง และ *C. annuum* Berceo เป็นสายพันธุ์ที่มีความอ่อนแอ ซึ่งทั้ง 2 สายพันธุ์ใช้เป็นพันธุ์พ่อแม่ในการสร้างประชากรลูกผสมรุ่นที่ 2 (F_2) เพื่อใช้ในการค้นหาตำแหน่งของยีนควบคุมลักษณะต้านทานต่อ *T. palmi* (Quantitative Trait Loci; QTLs) ด้วยวิธีการทดสอบความต้านทานแบบอิสระ จากการสร้างแผนที่พันธุกรรม (linkage map) ด้วยเครื่องหมาย Single Nucleotide Polymorphism (SNP) ที่แสดงความแตกต่างระหว่างพันธุ์พ่อแม่จำนวน 161 เครื่องหมายกับประชากรรุ่น F_2 จำนวน 195 ต้น ผลการวิเคราะห์ความสัมพันธ์ระหว่างแผนที่พันธุกรรมกับค่าดัชนีการเกิดโรค (DX) และพื้นที่กราฟใต้เส้นโค้งแสดงเปอร์เซ็นต์ต้นที่เป็นโรค (AUDPC) พบ QTL1 ตั้งอยู่บนโครโมโซมแท่งที่ 3 และ QTL2 ตั้งอยู่โครโมโซมแท่งที่ 12 ซึ่งสามารถอธิบายความแปรปรวนของความต้านทานได้ 12.9 และ 8 เปอร์เซ็นต์ ตามลำดับ จากข้อมูลดังกล่าว QTLs ทั้ง 2 ตำแหน่งนี้จึงสัมพันธ์กับความต้านทานต่อ *T. palmi* นอกจากนี้ยังพบเครื่องหมาย M238 และ M171 ใน QTL1 และ QTL2 ตามลำดับ ซึ่งสามารถนำไปใช้ในการปรับปรุงสายพันธุ์พริกให้มีความต้านทานต่อ *T. palmi* ต่อไป

คำสำคัญ : เพลี้ยไฟ, Thrips palmi, พริก, ความต้านทาน, เครื่องหมาย SNP

Title	DEVELOPMENT OF SNP MARKERS SPECIFIC TO THRIPS (*THRIPS PALMI*) RESISTANCE IN PEPPER
Author	Miss Areerat Saeko
Degree	Master of Science in Genetics
Advisory Committee Chairperson	Associate Professor Dr. Saengtong Pongjaroenkit

ABSTRACT

Thrips (*Thrips palmi; T. palmi*) are among the most pepper damaging pests in Thailand. The objectives of this work were to study a level of resistance and identify DNA markers that related to *T. palmi* resistance in pepper. Seventeen pepper accessions were classified for *T. palmi* resistance using two resistant test methods which are free choice and no choice. Resistance test revealed that *Capsicum annuum* (*C. annuum*) AC 1979 and *C. annuum* Berceo were most resistant and susceptible accessions, respectively. These 2 accessions were used as parental line to produce 195 plants F_2 population for a linkage mapping construction from 161 polymorphic SNP markers. The linkage map and Disease Index and the Area Under the Disease Progress Curve data from free choice method were analyzed for Quantitative Trait Loci (QTLs) . The highly significant QTL1and QTL2 were found, located on chromosome 3 and 12, with about 12.9 and 8% explained phenotypic variance, respectively. Therefore, these QTLs were associated with *T. palmi* resistance. Moreover, M238 and M171 SNP markers were identified in these QTLs which will be used in pepper marker-assisted breeding for *T. palmi* resistance.

Keywords : Thrips, Thrips palmi, Pepper, Resistance, Single Nucleotide Polymorphism markers

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Areerat Saeko

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CHAPTER 1

INTRODUCTION

Pepper or Chili is an important vegetable and spice crop in the world. It can be used in many forms such as fresh, cooked, herb or spices, and many kinds of processing products, as it contains a high nutritional value, for example, carotenoids (provitamin A) , ascorbic acid (vitamin C) , tocopherols, (vitamin E) , phenolic compounds, flavonoids and capsaicinoid (Kato, Hanada et al. 2000), (Topuz and Ozdemir 2004). Pepper is ranked as the first and third most important vegetable in Asia and the world, respectively as shown in Table 1 (Ali, 2006).

Table 1 Area planted to important vegetables in the world and Asia, 2003

Name of vegetables	Area (000 ha)		Relative rank	
	World	Asia	World	Asia
Total vegetables	49,948	35,786	-	-
Green peas	6,509	2,036	1	4
Tomato	4,201	2,385	2	2
Chili (fresh and pimento)	3,668	2,458	3	1
Cabbages	3,188	2,348	4	3
Onion dry	3,006	2,025	5	5
Cucumber	2,253	1,765	6	6
Eggplant	1,647	1,547	7	7

Source: FAOSTAT database.

Approximately 89% of the world's total pepper-growing areas are located on the Asian continent, with the main growing areas in India, China, Korea, Thailand, Vietnam, Sri Langka, and Indonesia (Pinto et al., 2016). The distribution of the sample by type of respondent per country is presented in Table 2. Mite and Thrips were the most devastating insects in the Chilean fields, with serious attacks occurring almost every three to five out of five years. From 1998 to 2002, average annual losses due to insects as perceived by farmers varied from 7% in China to 56% in India in Table 3 (Ali, 2006).

Up to date, thrips have become a significant pest worldwide, causing yield losses in farmer fields. They can cause direct damage by feeding and ovulating the leaves and growing fruits, resulting in their deformation (Maharijaya et al., 2012). Consequently, the photosynthetic capacity of the plant is reduced (Shipp et al., 1998). Photosynthesis is adversely affected, vitality is reduced, and yield reduction is appreciable (Reddy, 2016).

Table 2 Distribution of sample by respondent type and country

Country	Province/ State/ Region	Sample type and size							
		Chili farmer	CFHHW*	Non-chili farmer	NCFHHW**	CHHW+	Market agent	Processor	Total
China	Hunan, Sichuan and Guangdong	293	300	29	29	60	45	6	762
Indonesia	West, Central and East Java	256	243	50	46	62	16	6	679
India	Karnataka and Andhra Pradesh	291	256	41	45	50	5	4	692
Thailand	North, North-east & Central Thailand	255	219	30	48	40	11	3	606
Total		1,095	1,018	150	168	212	77	19	2,739

* CFHHW = Chili farmer household wife; ** NCFHHW = Non-chili farmer household wife;
+CHHW = City household wife.

Table 3 Major chili insects as perceived by farmers in selected chili-producing countries of Asia, 2002

Country	Rank				Occurrence (year out of 5)		Average annual losses (%)	
	1	2	3	4	1993-97	1998-2002	1993-97	1998-2002
China	W	M	A	T	5	5	8	7
India	M	T	C	A	3	3	48	56
Indonesia	T	M	A	C	4	4	11	25
Thailand	C	T	M	A	5	4	13	24

Note: A=Aphids (*Aphis gossypii* and *Myzus persicae*); C= Caterpillar (*Helicoverpa armigera* and *Spodoptera litura*); M=Mites (*Polyphagotarsonemus latus*); T=Thrips (*Scirtothrips dorsalis*); W=Tobbaco budworm (*Heliothis sp.*).

Thrips also cause indirect damage by transmitting plant viruses of the Tospovirus, Ilarvirus, Carmovius, Sobemovirus, and Machlomovirus genera (Jones, 2005). In Thailand, four tospovirus species, including Capsicum chlorosis virus (CaCV), Melon yellow spot virus (MYSV) and Watermelon silver mottle virus (WSMoV), have been identified as causing serious damage to various economically important crop such as tomato, pepper, peanut, watermelon, cantaloupe and cucumber (Chiemsombat et al., 2008). *Thrips palmi* (*T. palmi*) was reported to be a vector of Asian tospoviruses such as WSMoV, CaCV, Watermelon bud necrosis orthotospovirus (WBNV), Calla lily chlorotic spot virus (CCSV), Groundnut Bud Necrosis Virus (GBNV), and MYSV (Palmer et al., 1990; Lakshmi et al., 1995; Chen and Chiu, 1996; Kato et al., 2000; Persley et al., 2006).

A new tospovirus isolated from naturally infected tomato plants grown in Nakhon Pathom province (Thailand) was characterized as infected plants that showed symptoms consisting of necrotic spots, necrotic ringspots, and stem necrosis that this tospovirus isolate should be considered a member of a new species. The name tomato necrotic ringspots virus (TNRV) is proposed for this tospovirus. This high transmission competence of *T. palmi* suggested that it may contribute to the widespread of TNRV in Thailand with an abundance of this virus in the central, western and northern region in Thailand, where most of tomatoes and peppers are grown TNRV could be a serious threat to vegetable production in this country (Seepiban et al., 2011).

Marker-assisted selection (MAS) involves the transformation of phenotypic to genotype selection, which can improve the efficiency and accuracy of the selection of target traits and has been widely used for crop genetic improvement (Jena and Mackill 2008; Xu and Crouch 2008). There is an urgent need to identify molecular markers tightly linked with the genes governing resistance to major diseases and insects, tolerance to abiotic stresses, quality, and other agronomic traits. PCR-based markers and SNP markers should be markers of choice in MAS (Jain et al., 2002).

The objective of this study was to classified on *T. palmi* resistance level in pepper and develop the SNP markers linked to *T. palmi* resistance.

Objectives of the study

1. To identify accessions with different levels of *T. palmi* resistance in pepper
2. To determine the inheritance of *T. palmi* resistance in pepper
3. To develop SNP markers that specific to *T. palmi* resistance in pepper

Scope and Limitation

The study gathered and analyzed the information from the journals previously studied by researchers regarding the genes controlling thrips resistance in pepper. The molecular markers were designed primarily to the population of accession 3555 (Resistance line) and accession 3567 (Susceptible line, elite line), capable of detecting polymorphisms. The efficiency and reliability of the markers were also tested in different generations to check the inheritance of the genes. The development of the molecular markers that control thrips resistance in pepper can improve elite line and variety in the future to become thrips resistance with high yield.

Expected results

1. *T. palmi* resistance lines for pepper breeding program
2. SNP markers that specific to *T. palmi* resistance in pepper

CHAPTER 2

LITERATURE REVIEW

General information and its importance

Hot pepper is a member of the Solanaceae family. It is a diploid, facultative, self-pollinating crop and is closely related to potatoes, tomatoes, eggplant, tobacco, and petunia. Many members of the Solanaceae family have the same number of chromosomes (n = 12), yet differ drastically in genome size (Kim et al., 2014). *Capsicum* is native to the New World and comprises 33-34 species, five of which are domesticated including *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*. All *Capsicum* species are diploid, but two groups with distinct chromosome numbers are formed: with 2n = 24 and with 2n = 26 (da Costa Batista, 2016). Simply the taxonomic position of *Capsicum* can be represented as follows:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Solanales
Genus: *Capsicum*
Species: *chinense/annuum/pubescens*/etc.

Three of the domesticated species form an interesting complex. Eshbaugh et al. (1983) suggested that *C. annuum*, *C. frutescens*, and *C. chinense* form a closely linked group that evolved in the lowland tropics of Latin America and the Caribbean, with *C. annuum* eventually dominating Maxico, *C. frutescens* the Caribbean, and *C. chinense* Amazonas. Columbus and subsequent explorers of Mesoamerica were responsible for introducing *C. annuum* chilli peppers to Europe while Portuguese explorers introduced *C. chinense* to Eastern Europe, Africa, and Asia (Eshbaugh, 1983; Andrews, 1993). Andrew, 1984 provides maps of the hypothetical distribution of the

domesticated pepper species at the time of European discovery as extrapolated from Heiser (1976), Eshbaugh (1975), and McLeod et al. (1982) (Figure1).

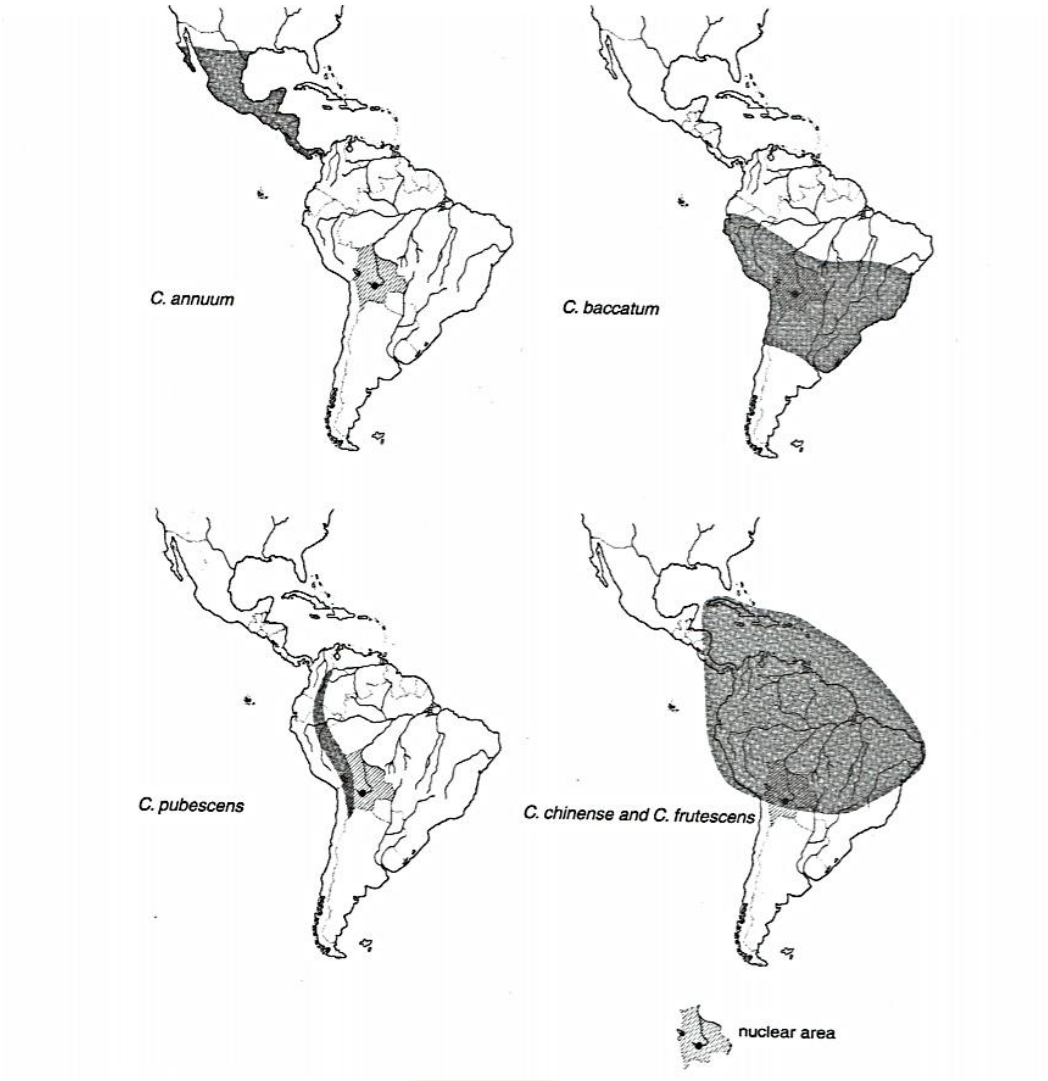

Figure 1 Hypothetical distribution of domesticated Capsicum peppers at the time of European discovery of the New World Hypothetical distribution of domesticated Capsicum peppers at the time of European discovery of the New World
(Adapted from Andrews, 1984; Eshbaugh, 1975; Heiser, 1976; and McLeod et al. 1982.)

Molecular studied by Walsh and Hoot (2001) showed that *C. annuum*, *C. frutescens*, and *C. chinense* were closely related (Figure 2). *C. chinense* is somewhat more distant from *C. annuum* with *C. galapagoense* inserted between *C. frutescens* and *C. chinense*. Placement of *C. galapagoense* raises the question as to which mainland species gave rise to this island endemic. Eshbaugh et al. (1983), using data from isozyme studies, proposed that *C. annuum*, *C. frutescens*, and *C. chinense* form a closely knit complex arising from an ancestral gene pool with the *C. frutescens* gene pool having given rise to *C. chinense* (Figure 3). Although taxonomists may be in a quandary on whether to recognize one, two, or three species within this complex, within the horticultural and commercial trade five distinct taxonomic species continue to be recognized.

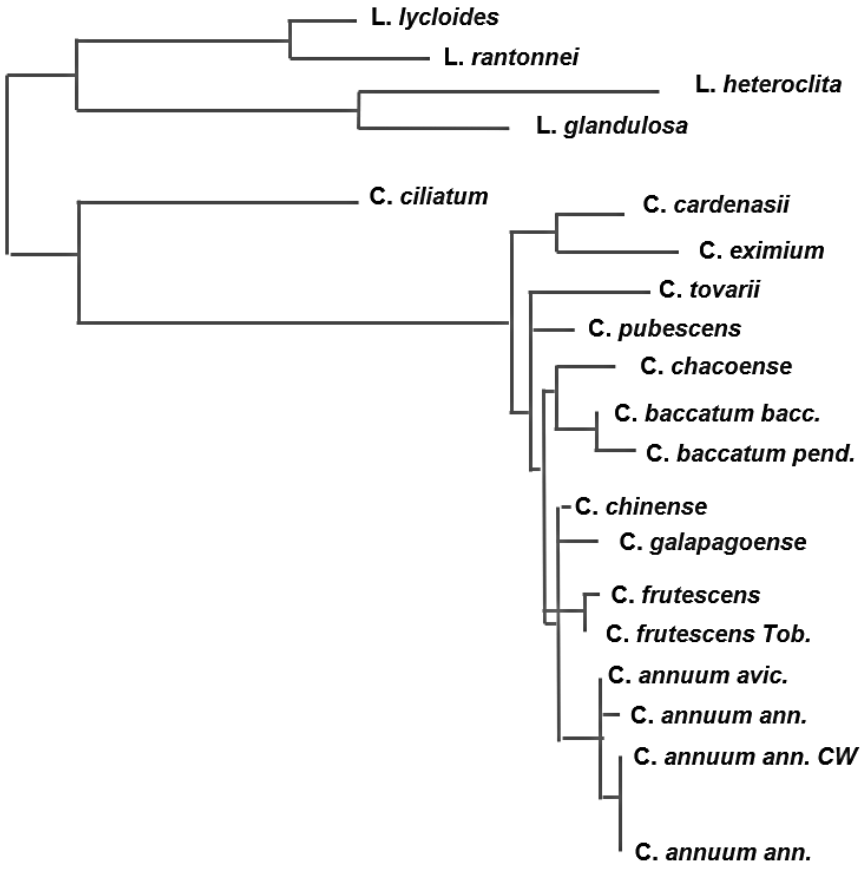

Figure 2 The shortest tree results from the combined atpB-rbcL spacer and waxy data.

bacc. = var. baccatum, pend. = var. pendulum, Tob = cv. Tobasco, avic. = var. aviculare = var. glabriusculum, ann. = var. annuum, and CW = cv. Early CalWonder. (From Walsh and Hoot, 2001)

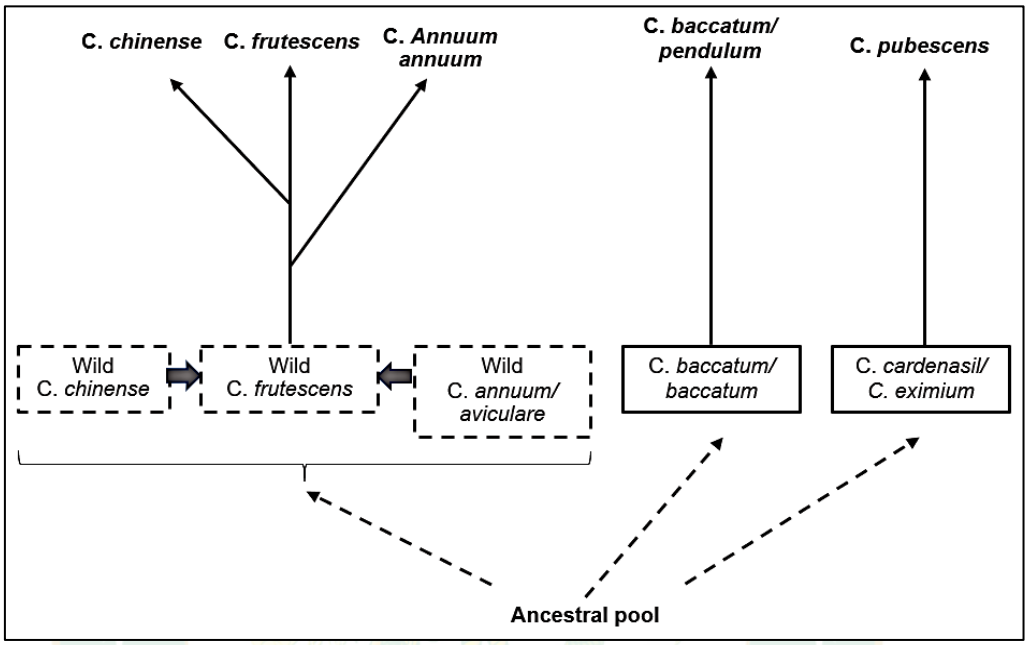

Figure 3 The hypothetical five-species model depicting evolution of domesticated Capsicum (Solanaceae).
(Originally published in the Journal of Ethnobiology by Eshbaugh et al., 1983) (NB *C. annuum* var. aviculare = *C. annuum* var. glabriusculum).

C. annuum var. annuum L

The species *C. annuum* L. var. annuum, including cultivars in the jalapeno, pablano, Anaheim, ancho, bell, big Jim, cayenne, and serrano types, was originally described by Linnaeus in Species Plantarum. *C. annuum* is a small shrub 2 m. with white to bluish-white flowers, most often one per node. Calyx teeth are lacking or short, rarely exceeding 0.5 mm. The chromosome number of *C. annuum* is 2n = 24. Tetraploids (2n = 48) are known from India.

C. chinense Jacq

C. chinense includes the cultivars in the habanero, Scotch bonnet, rocotillo, chili blanco types, and is the dominant domesticated pepper of Amazonas. This species is characterized as a small stout shrub up to 1.5 m. tall, glabrous to puberulent with two flowers, or more, at a node. The flowers are pendant (rarely erect) and have a prominent constriction between the base of the calyx and pedicel, especially when the fruit. The flower lacks calyx teeth. The corolla is dull white (rarely greenish white), spreading to recurved. Anthers are blue to violet, rarely yellow. The style and stigma are rarely exerted more than 1 mm. The fruit, of many different colors, contain seeds that are cream to yellow (D'Arey and Eshbaugh, 1974). The name *C. chinense* is an anomaly in that no Capsicum peppers were ever native to China. An Asian species, *C. anomalum*, was described from Japan in the 1800s but is now placed in the monotypic genus Tobocapsicum as *T. anomalum* was found in central China, Taiwan, Japan, Korea, the Philippines, and Bornero. It occurs in damp, evergreen forests from sea level to 700 m. and is distinct from Capsicum in its androecium and fruit structure (Hunziker, 2001).

C. frutescens L

C. frutescens contains cultivars of the tabasco, malagueta, Africa birdseye, piri-piri and Thai pepper types. This species is the source of Tabasco sauce, once the most famous hot sauce throughout the world. Today it has been supplanted by a multitude of hot sauces. Trying to distinguish this species from *C. annuum* and especially *C. chinense* is very difficult even for pepper experts.

C. frutescens is a species of the lowlands. It is a small shrub, or tree-like shrub, up to 2 meters tall. It can be herbaceous to woody. Plants range from glabrous to pubescent, being mostly puberulent. Typically, two or more flowers are present per node. Flowers lack a prominent constriction between the base of the calyx and pedicel. Calyx teeth are absent. The corolla is greenish white and spreading to recurved. Anthers are blue to violet, rarely yellow. The style and stigma are exerted 1.5 millimeters or more beyond the anthers. The immature fruit is green

without dark pigmentation, while the mature fruit is red or very rarely orange, erect, and deciduous. The seeds are cream to yellow.

C. baccatum var. pendulum (Willd) Eshbaugh

C. baccatum var. pendulum, known as aji, aji amarillo, cuerno de oro, cumbia, and others, is another distinct species (Eshbaugh, 1968, 1970) and the most common domesticated pepper in Peru. This lowland South American species has cream-colored flowers with paired gold or green markings. Typically, fruits are elongate with cream-colored seeds. The wild progenitor gene pool is *C. baccatum* var. baccatum known as arivivi. This taxon is common in Bolivia and northern Argentina with outlier populations in Peru and Paraquay. *Capsicum praetermissum* from Brazil was treated as a variety of *C. baccatum* (Hunziker, 1971) but is treated here as a distinct species.

C. pubescens Ruiz & Pavon

C. pubescens, the rocoto (Quechua = ruqutu), locoto (Aymara = lucutu), Chile manzana, and others, is distinct among domesticated peppers. This pepper was largely ignored by taxonomists until Eshbaugh's research (1979, 1982). It is morphologically and genetically different from all the other domesticated peppers. It has large rotate purple or white flowers, typically with five to eight lobes. The fruit contains dark brown or black seed unique among domesticated peppers. It is throughout the mid-elevation Andes between 1,500 and 3,000 m. *C. pubescens* has large rugose pubescent leaves. It can be enormous, growing horizontally across the ground or on supporting vegetation, attaining a length in excess of 18 m. Stems often have mixed green and purplish pigment giving them a striped appearance. Genetically, it is associated with *C. eximiun* (Bolivia and northern Argentina; Hunziker, 1950), *C. cardenasii* (Bolivia; Heiser and Smith, 1958) and *C. tovarii* (Peru; Eshbaugh et al., 1983).

The significant diagnostic characteristics of Capsicum are: annual or perennial glabrous herbs or undershrubs; Leaves that alternate, are entire or repand; Flowers

pedicelled, axillary, solitary or two-three together; Sepals connate in a subentire or minutely five-toothed calyx, much shorter than the fruit; Petals five, connate in a rotate corolla; tube short; lobes valvate in bud; Stamens five, adnate nearly to base of corolla-tube, filaments short; Anthers not exceeding filaments; dehiscence longitudinal; Carpels connate in a two-celled, rarely a three-celled ovary; style linear; stigma subcapitate; Fruit globose or elongated or irregularly shaped, many-seeded berry; Seeds discoid, smooth or subscabrous; Embryo peripheric (Basu et al., 2003).

Thrips

Due to domestication, commercially grown hot and sweet pepper have lost their resistance to thrips, and as a result, Capsicum is infested by several thrips species (Talekar, 1991; Capinera, 2001; Ssemwogerere et al., 2013). Thrips species commonly found on Capsicum include *Frankliniella occidentalis* (*F. occidentalis*), *Thrips palmi* (*T. palmi*), *Scirtothrips dorsalis* (*S. dorsalis*) and, to a lesser extent, *Thrips tabaci* (*T. tabaci*) *T. palmi* and *S. dorsalis* are more problematic in tropical to subtropical regions (Kenneson and Cannon 2007). Controlling thrips on Capsicum with pesticides is difficult and the identification of resistant accessions is necessary for the successful and sustainable pepper production in the future. Thrips belong to the family Thripidae in the order Thysanoptera which contains nearly 7,700 described thrips specie. However, less than 1% of them are considered agricultural pests that directly cause crop damage by feeding and indirectly by transmitting tospoviruses (German et al., 1992). At present, 15 thrips species have been reported to transmit tospoviruses (Rotenberg et al., 2015; Schneweis et al., 2017). Among them, *F. occidentalis* is worldwide the most devastating invasive species, with a broad host range, transmitting multiple tospoviruses (genus Orthotospovirus, family Tospoviridae, Order Bunyavirales) including the economically important tomato spotted wilt virus (TSWV) (Riley et al., 2011). Melon thrips (*T. palmi*) originated in Southeast Asia (Mound, 2002). *T. palmi* is a widely distributed major agricultural pest in the tropics and subtropics, causing significant losses in cucurbit and solanaceous crops through feeding damage and transmission of tospoviruses (Gamage et al., 2018)

T. palmi

T. palmi is almost entirely yellow in coloration, and its identification is hampered by both its small size (1.0-1.3 mm.) and its remarkable similarity to certain other yellow or predominantly yellow species of Thrips (Clover et al., 2010). Adult thrips are about 1 mm. long, and females are usually a bit larger than males (Figure 4). At least 16 species of thrips attack Capsicum (Talekar, 1991; Capinera, 2001).

Figure 4 *T. palmi,* female (left) and male (right).
(photo: A. J. M. Loomans, PPS, Wageningen, the Netherlands; scale bar = 500 μm. = 0.5 mm.).

Taxonomy information

Name:	*T. palmi*, 1925
Taxonomic position:	Insecta, Thysanoptera, Terebrantia, Thripidae
Common name:	melon thrips

Since the late 1970s, *T. palmi* has become widely distributed in tropical and subtropical regions, including Southeast Asia, the Pacific Islands, the Caribbean Islands, and South America. *T. palmi* become the most serious pest of eggplant, cucumber, and sweet pepper both in greenhouses and open fields (Murai, 2002).

Thrips life cycle (Figure 5)

A whole generation may be completed in about 20 days at 30°C, but it is lengthened to 80 days when the insects are cultured at 15°C. Melon thrips are able to multiply during any season that crops are cultivated but are favored by warm weather. When crops mature, their suitability for thrips declines, so this thrips growth rate diminished even in the presence of warm weather (Capinera, 2008).

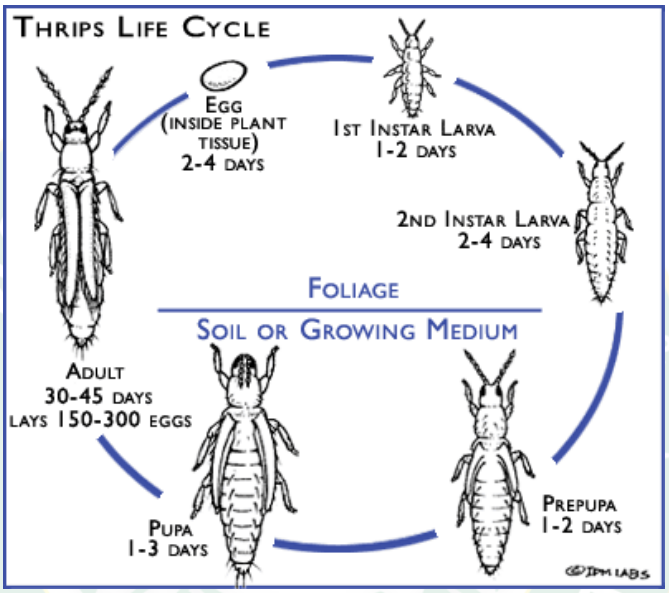

Figure 5 General life cycle of thrips.

Source: https://www.ipmlabs.com/thrips-damage/

Eggs: Eggs are deposited in leaf tissue, in a slit cut by the female. One end of the egg protrudes slightly. The egg is colorless to pale white in color, and bean-shaped in form. The duration of the egg stage is about 16 days at 15°C, 7.5 days at 26°C, and 4.3 days at 32°C.

Larvae: The larvae resemble the adults in general body form through they lack wings and are smaller (Figure 6). There are two instars during the larval period. Larvae feed in groups, particularly along the leaf midrib and veins, and usually on older leaves. Larval development time is determined principally by the suitability of temperature, but host plant quality also has an influence. Larvae require about 14, 5, and four days to complete their development at 15, 26, and 32°C, respectively. At

the completion of the larval instars, the insect usually descends to the soil or leaf litter, where it constructs a small earthen chamber for a pupation site.

Figure 6 Larvae of melon thrips, *T. palmi*
Photograph by FDACS-DPI

Pupa: There are two instars during the "pupal" period. The prepupal instar is nearly inactive, and the pupal instar is inactive. Both instars are nonfeeding stages. The prepupae and pupae resemble the adults and larvae in form, except that they possess wing pads. The wing pads of the pupae are longer than that of the prepupae. The combined prepupal and pupal development time is about 12, 4, and 3 days at 15, 26, and 32°C, respectively.

Adult: Adults are pale yellow or whitish in color, but with numerous dark setae on the body. A black line, resulting from the juncture of the wings, runs along the back of the body. The slender fringed wing is pale. The hairs or fringe on the anterior edge of the wing are considerably shorter than those on the posterior edge. They measure 0.8 to 1.0 mm. in body length, with females slightly larger than males (Figure 7). Unlike the larval stage, the adults tend to feed on young growth, and so are found on new leaves. Adult longevity is 10 to 30 days for females and seven to 20 days for males. Development time varies with temperature, with mean values of about 20, 17, and 12 days at 15, 26, and 32°C. Females produce up to about 200 eggs but an average of about 50 per female-both mated and females deposit eggs.

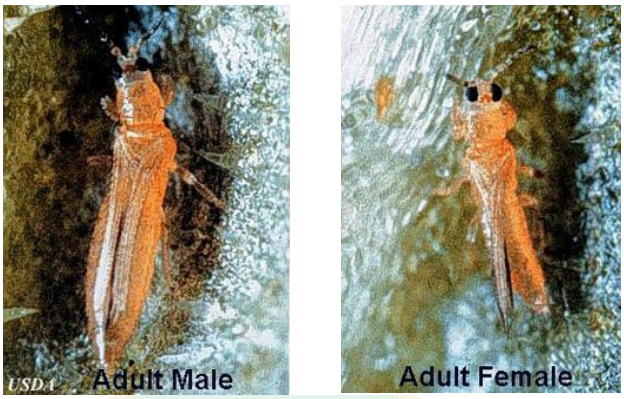

Figure 7 Adult melon *T. palmi*
Photograph by the University of Florida

Careful examination is required to distinguish melon thrips from other common species. The *Frankliniella* species are easily separated because their antennae consist of eight segments. In contrast, in Thrips species, there are seven antennal segments. To distinguish melon thrips from onion thrips, *T. tabaci* Lindeman, it is helpful to examine the ocelli. There are three ocelli on the top of the head, in a triangular formation. A pair of setae are located near this triangular formation, but unlike the arrangement found in onion thrips, the setae do not originate within the triangle. Also, the ocelli bear red pigment in melon thrips, whereas they are grayish in onion thrips (Figure 8). In general, the primary body color of adult melon thrips in yellow, but in onion thrips, it is yellowish gray to brown.

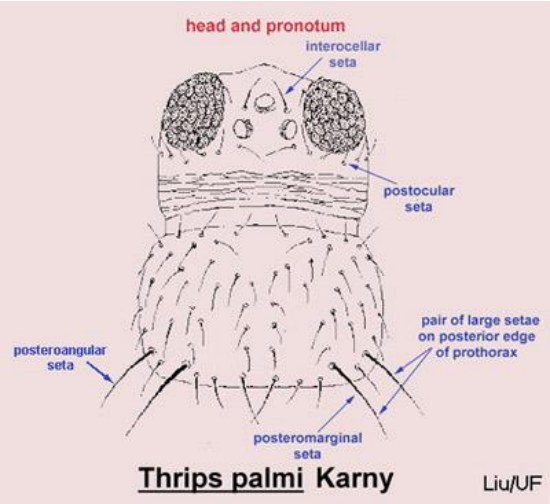

Figure 8 Head and pronotum of the melon thrips, *T.palmi*, with setae identified. Graphics by T.X. Liu, Texas A&M.

The most complete summary of melon thrips biology and management is presented in Capinera (2008). Developmental biology is given by Tsai et al. (1995) and Capinera (2008), and field biology by Kawai (1990) and Capinera (2008). Keys for identifying common thrips were presented by Palmer (1990), Oetting et al. (1993) and Capinera (2008).

Categories of resistance

Three categories of plant resistance to insects were described by Painter (1951) to classify plant-pest insect interactions. They include antibiosis, antixenosis, and tolerance. Antibiosis and antixenosis resistance categories describe the reaction of an insect to a plant, while tolerance resistance describes the reaction of a plant to insect infestation and damage.

Antixenosis

Kogan and Ortman (1978) proposed the term 'antixenosis' to describe more accurately term of non-preference (Painter, 1951) insects for a resistant plant. Antixenosis, a term derived from the Greek word xemos (guest), describes the inability of a plant to serve as a host to an insect herbivore. As a result, insect pests

are forced to select an alternate host plant. Both antixenosis and non-preference denote the presence of morphological or chemical plant factors that adversely alter insect behavior, resulting in the selection of an alternate host plant.

Antibiosis

Antibiosis is the category of resistance that includes those adverse effects on insect life history, which result after a resistant host plant is used for food (Painter, 1951). Both chemical and morphological plant defenses mediate antibiosis and antibiotic effects of these resistant plants ranging from mild to lethal. The impacts of antibiosis are measured as the death of early instars, reduced size or low weight, a prolonged period of development of the immature stages, reduced adult longevity and fecundity, death in the prepupal or pupal stage, and abnormal (wandering or restless) behavior.

Tolerance

Plants may also be resistant to insect attack by possessing the ability to withstand or recover from damage caused by insect populations equal to those on a susceptible cultivar. According to Painter (1951) tolerance is a "basis of resistance in which the plant possesses an ability to grow and reproduce or to repair an injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host. The expression of tolerance is determined by the inherent genetic ability of a plant. Unlike antixenosis and antibiosis, tolerance involves only plant characteristics and is not part of an insect-plant interaction. However, tolerance often occurs in combination with antibiosis and antixenosis. Because of its unique nature in plant resistance to insects, the quantitative measurement of tolerance is accomplished by using entirely different experimental procedures from those used to study antibiosis antixenosis (Smith et al., 1993).

Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are a DNA sequence variation occurring when a single nucleotide (A, T, C or C) differs among members of a species. SNPs are the most abundant marker system both in animal and plant genomes and has recently emerged as the new generation molecular marker for various applications. Being binary or co-dominant status, they are able to efficiently discriminate between homozygous and heterozygous alleles. Moreover, unlike microsatellites, their power comes not from the number of alleles but from the large number of loci that can be assessed. Once the rare SNPs are discovered in a low diversity species, the genetic population discrimination power can be equivalent to the same number of loci in a genetically diverse species. The more evolutionary conserved nature of SNPs makes them less subject to the problem of homoplasy. Most importantly, SNPs are amenable to high throughput automation, allowing rapid and efficient genotyping of large numbers of samples.

In a plant, SNP markers can be designed from ESTs and single stranded pyrosequencing. A high-throughput genome analysis method called diversity array technology (DArT), based on a microarray platform, has been developed for the analysis of plant DNA polymorphism. Eijk et al. (1994) described a novel SNPs genotyping technique, SNPs Wave. Chip-based SNPs arrays use thousands of oligonucleotide probes attached to a solid surface (e.g., glass, silicon wafer), allowing a large design to interrogate up to 10 SNPs at known locations on one to 10 DNA templates in a single tube. The basic principle of the SNPs and detection method is illustrated in Figure 9. In brief, the protocol includes the preparation of sample reactions using template and primer, performing SNaPshot reactions by thermal cycling, and conduction of post-extension treatment of the products. Then automated electrophoresis of the samples and finally, analyzing the data (Foster et al., 2010).

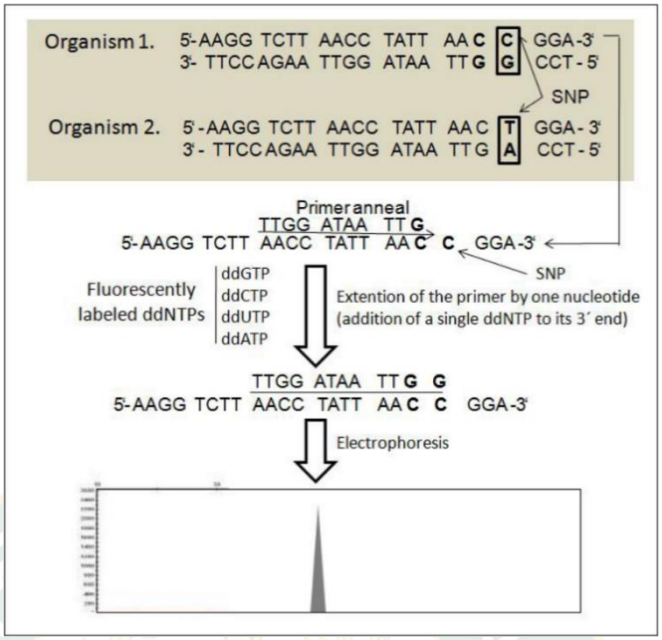

Figure 9 A flow-chart showing the basic principle of SNPs method.

Research related

Fery and Schalk (1991) used a replicated greenhouse study to confirm the availability of resistance to Western Rower thrips in pepper germplasm. Host-plant resistance ratings confirmed earlier observations that there was a considerable amount of variability within pepper germplasm for reaction to *F. occidentalis*. Plants of Keystone Resistant Giant, Yolo Wonder L, Mississippi Nemaheart, Sweet Banana, and California Wonder were resistant to the insect and exhibited only mild symptoms of damage.

Maris et al. (2003) studied pepper accessions, thrips population, and virus isolate. Seven Capsicum accessions (Pikante Reuzen, Perla RZ, Mazurka RZ, CPRO-1, CPRO-2, PI 152225, and PI 159236) were used in this study. Accessions PI 159236, PI 152225 and Perla RZ (J. Haanstra, personal communication) were known to be resistant to TSWV. To select a thrips resistant pepper accession, seven accessions were assessed and tested for preference by thrips, for development of feeding damage, and for supporting the reproduction of a *F. occidentalis* population. The levels of thrips resistance of the pepper accessions were evaluated in "choice" and

“non-choice” tests. The seven pepper accessions tested showed considerable differences in resistance to thrips. When plants of all accessions were batchwise exposed to thrips in a choice test, feeding damage was observed on all accessions irrespective of whether they were preferred by thrips. Severe feeding damage was recorded on the accessions Pikante Reuzen and PI 159236, with moderate damage on Mazurka RZ and Perla RZ, and only mild damage on CPRO-1, CPRO-2, and PI 152225. Damage on the accessions which had only mild damage recorded did not increase 2 weeks after the release of thrips. Thrips resistance levels of two of the most promising accessions, CPRO-1 and PI 152225, were further analyzed in a nonchoice test, along with the most thrips susceptible accessions Pikante Reuzen and PI 159236 in a manner preventing any dispersal of thrips between the accessions. *F. occidentalis* reproduced most efficiently on plants of Pikante Reuzen, while only a few thrips accumulated on plants of the accessions CPRO-1, PI 152225, and PI 159236 during the entire test period of 8 weeks. The high numbers of thrips found on PI 159236 in the choice test might be explained by dispersal of thrips from the more preferred accessions or by the reproduction of these dispersing adults on this accession.

Maharijaya et al. (2011) developed practical and reliable screening methods for thrips resistance in pepper and identifying pepper accessions showing strong resistance to thrips. Thirty-two pepper accessions from four species of pepper (*C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens*) and two species of thrips (*F. occidentalis* and *T. parvispinus*) were used in this study. The results indicate that the laboratory based on leaf disc test and the detached leaf test can be used as reliable screening methods for thrips resistance in pepper. Six pepper accessions (*C. annuum* AC 1979, *C. annuum* Bisbas, *C. annuum* Keystone Resistant Giant, *C. annuum* CM 331, *C. baccatum* no. 1553, and *C. baccatum* Aji Blanco Christal) are identified as good sources for resistance against *T. parvispinus* and *F. occidentalis*. Six accessions are identified as susceptible accessions to both *T. parvispinus* and *F. occidentalis* (*C. annuum* Long Sweet, *C. chinense* Miscucho Colorado, *C. chinense* PI 281428, *C. chinense* no. 4661, *C. chinense* no. 4661 selection and *C. chinense* PI 315023).

Visschers et al. (2019) studied the screening for robust and general resistance to thrips encompasses testing different *Capsicum* accessions under various conditions and with different thrips species. Eleven Capsicum accessions (*C. annuum* and *C. chinense*) were screened for resistance to *F. occidentalis* at three different locations in the Netherlands. Next, the same 11 accessions were screened for resistance to *T. palmi* and *S. dorsalis* at two locations in Asia. This resulted in a unique analysis of thrips resistance in Capsicum at five different locations around the world. Finally, all accessions were also screened for resistance to *F. occidentalis* in the Netherlands using a leaf disc choice assay, allowing direct comparison of whole plant and leaf disc assays. Resistance to *F. occidentalis* was only partially consistent among the three sites in the Netherlands. The most susceptible accessions were consistently susceptible, but which accession was the most resistant differed among sites. In Asia, one *C. chinense* accession was particularly resistant to *S. dorsalis* and *T. palmi*, but this was not the most resistant accession to *F. occidentalis*. Overall, resistance to *F. occidentalis* correlated with *S. dorsalis*, but not with *T. palmi* resistance in the *C. annuum* accessions. Damage inflicted on leaf discs reflected damage on the whole plant level. Their study showed that identifying broad-spectrum resistance to thrips in *Capsicum* may prove to be challenging. The breeding program should focus on developing cultivars suitable for growing in defined geographic regions with specific thrips species and abiotic conditions.

Maharijaya et al. (2015) found the QTL analysis was carried out for *F. occidentalis* resistance in an F_2 population consisting of 196 plants derived from an interspecific cross between the highly resistant *C. annuum* AC 1979 as the female parent and the highly susceptible *C. chinense* 4661 as the male parent. Fifty-seven SSR, 109 AFLP and 5 SNPs markers were used to construct a genetic map with a total length of 1,636 cM. Damage caused by larvae and the survival of first and second larval stages observed in a no-choice test were used as parameters, all co-localizing near the same marker on chromosome 6. The use of this marker as cofactor in a multiple QTL mapping analysis failed to uncover any additional QTLs. This QTL explained about 50% of the genetic variation, and the resistance allele of this QTL was inherited from the resistant parent. The resistance of pepper accessions has a

significant effect on oviposition rate and larval mortality. Seven compounds were identified that correlate with susceptibility to thrips. Some of these compounds, such as tocopherols, were previously shown to have an effect on insects in general. Also, some specific secondary metabolites (alkanes) seemed to be more abundant in susceptible accessions and were induced by thrips infestation.

Frel et al. (2005) found quantitative trait loci (QTLs) for resistance to thrips in common bean, using $F_{5:7}$ recombinant inbred lines (RILs) as a mapping population. The RILs, derived by single seed descent (SSD) of the cross of two Mesoamerican bean lines, BAT 881 and G 21212, were found to show transgressive segregation for thrips resistance in the field. Correlations between damage and reproductive adaptation (RA) score were significant within and between seasons. The QTLs for both traits were located based on single interval mapping (IM) and joint interval mapping (JIM) analysis using a genetic map constructed with microsatellite and random amplified polymorphic DNA (RAPD) markers. Eight of eleven resulting linkage groups (LGs) were shown to be homologous to the chromosome of the integrated linkage map of common bean. A major QTL for thrips resistance located on LG b06 explained up to 26.8 % of the variance for resistance in a single season and was named Tpr61. The JIM across several seasons revealed various QTLs on LGs b02, b03, b06, and b08, some of which were located at regions of genes encoding for disease resistance.

CHAPTER 3
MATERIALS AND METHODS

The methods were divided into three parts: I) Identify accessions with different levels of *T. palmi* resistance in pepper II) Determine the inheritance of *T. palmi* resistance in pepper and III) Develop SNP markers that specific to *T. palmi* resistance in pepper.

1. Plant materials

Seeds of 17 accessions were obtained from the Center of Genetic Resources, the Netherlands, Plant Research International, Wageningen, and East West Seed (R&D), Chiang Mai, Thailand. A total of 17 pepper accessions from three species: *C. annuum, C. chinense* and *C. baccatum* were used to select the parental lines and developed a mapping population (Table 4). Pepper accessions with possible resistance to thrips were selected on the basis of available literature (Fery and Schalk 1991; Eigenbrode and Trumble 1994; Maris et al., 2003; Maharijaya et al., 2011) and supplemented with other accessions of various species and geographic origins. The mapping population was developed from a cross between extremely resistant parents and extremely susceptible parents. The F_2 population was grown together with P1, P2, and F_1 without any pesticide to study thrips resistance gene inheritance.

Table 4 The accession name, species, description, and reaction of 17 numbers used in this study.

Accessions	Species	Description	Reaction	References
#3553	*C. baccatum*	904750135 - Radboud University	N/A	-
#3555	*C. annuum*	CGN16975: AC 1979	Resistant	Maharijaya et al., 2011
#1534	*C. chinense*	CGN21557: No.4661; PI 159236	Susceptible	Maharijaya et al., 2011
#9267	*C. baccatum*	CGN17042: No. 1553; PI 238061	Resistant	Maharijaya et al., 2011
#3236	*C. annuum*	MP LOCAL-15	N/A	-
#3559	*C. annuum*	PBC145, Tiwari, dought-R	Resistant	Eigenbrode and Trumble, 1994
#6444	*C. annuum*	7303F F5	N/A	-
#3560	*C. chinense*	Miscucho colorado; PI 152225	Susceptible	Maharijaya et al., 2011
#3564	*C. chinense*	PI 315023 (Royal Gold)	Susceptible	Maharijaya et al., 2011
#3067	*C. annuum*	HPR99F	N/A	-
#3547	*C. baccatum*	CGN21513 - PI260580	N/A	-
#3558	*C. annuum*	CGN20503: Bisbas	Resistant	Maharijaya et al., 2011
#3064	*C. annuum*	Syn#1900 F4	N/A	-
#3556	*C. annuum*	CGN23222: Keystone Resistant Giant	Susceptible	Fery and Schalk, 1991, Maharijaya et al., 2011
#2090	*C. annuum*	ECW	N/A	-
#3557	*C. annuum*	CGN23765: CM 331; Criollos de Morelos	Resistant	Maharijaya et al., 2011
#3567	*C. annuum*	SWP-PY-10723 (Berceo) OP	N/A	-

2. Thrips population

This research used *T. palmi* in the experiment. Thrips parental stocks were obtained from the natural population on pepper in Thailand greenhouse conditions for the free choice method and obtained from nature on watermelon in the Philippines greenhouse conditions for no choice method. The thrips species were identified based on external morphology (Palmer et al., 1989; Mehle and Trdan, 2012) and a protocol of Seepiban et al. (2015). In the free choice method, adult thrips were introduced in thrips rearing boxes using okra pod as rearing medium overnight after 24 hrs. removed the pepper flowers and adult thrips. The adult which emerged from the eggs was almost the same age suitable for screening studies. Optimum laboratory conditions for thrips rearing was 25°C and relative humidity of 70 % at 16hrs : 8hrs light and dark regimes. In the no choice method, thrips were collected from a watermelon in a poly-house. Five young thrips nymphs (1^{st}instar) were directly inoculated into leaf discs. Five replicates were prepared for each accession and placed in a climate room at 25°C, 16hrs : 8hrs, 70 % RH.

3. Resistance test

3.1 Free choice method

Pepper and eggplant seeds were sown in plastic, 72 cell trays with media, and coconut peat ratio 1:1. Eggplants fulfilled with adult thrips were transplanted in the screening greenhouse as a border row. Eighteen plants, 30 Day after sowing (DAS) of pepper seedling or 4-6 true leaves were transplanted in a pot were used. Randomized Complete Block Design (RCBD) were used in this experiment; 17 treatments, 6 plants per replication and 3 replications were evaluated 3 times, 14, 28 and 42 DAT (Day after transplant). The thrips damage scoring was modified from a disease severity scale by Riera-Ruiz et al. (2018). The symptom was classified from symptomless, 1-25 %, 26-50 %, 51-75 % and 76-100 % to scored 0 to 4 respectively. The resistance test was the greenhouse test based on damage scores, called the free choice method. Please see as figure 10.

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Figure 10 The thrips damage scoring was modified from a disease severity scale.

0 = Symptomless

1 = 1 to 25 % of leaf area is infected

2 = 26 to 50 % of leaf area is infected

3 = 51 to 75 % of leaf area is infected

4 = 76 to 100 % of leaf area is infected or death of the plant

The Disease Index (DX) was calculated using the above formula: Where a, b, c, d, e the number of plants examined which fell into the categories, 0, 1, 2, 3, 4 respectively (Puangmalai et al., 2013).

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

The area under the disease progress curve (AUDPC) was calculated by trapezoidal integration of the disease severity over time, considering the whole period evaluated as follows: Where X is the disease severity (percentage of plants diseased), n the number of evaluations, and t_{i+1}-t_i the time interval (days) between two consecutive evaluation (Campbell and Madden, 1990). Statistic analysis was performed statistically using Statistical Tool for Agricultural Research (STAR).

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

3.2 No choice method

The leaf discs (1.5 cm. in diameter) were taken from fully opened leaves using a leaf punch and placed inside a tightly fitted petri dish with agar at the center (15 grams/litre) and the lower side (abaxial) of the leaf facing upward. Five young thrips nymphs (1stinstar) were directly inoculated into leaf discs. Five replicates were prepared for each accession and placed in a climate room at 25^{o}C, 16hrs : 8hrs, 70 % RH. Nymphs survival on a leaf disc counted under a microscope at 8 days for recorded living or dead thrips (Figure 11). The data was analyzed statistically using Statistical Tool for Agricultural Research (STAR).

Figure 11 No choice method.

No choice method. (a) the lower (abaxial) side of the leaf facing upward, (b) nymph survival after 8 days was observed, (c) nymph dead after 8 days was observed.

4. SNP markers development

4.1 Plant material and DNA extraction

A mapping population was developed from a cross between the resistance line and the susceptible line as a parent. The two parents were chosen based on screening results from the free choice and the No choice resistance assay against *T. palmi* in the greenhouse. Total genomic DNA was extracted from leaves of each plant with the Cetyltrimethyl ammonium bromide (CTAB) method as described by Nishiguchi et al., (2002). Genomic DNA (10-15 ng/µL) of F_2 population was prepared to conduct the KASPar array technique.

4.2 SNP markers survey

SNP markers were chosen from multiple sources. First set was SNP markers from Maharijaya et al. (2015) that was highly significant with larval development on chromosome 6. SNP markers was designed to cover the region that was highly significant. Second set was 549 SNPs markers that cover all 12 chromosomes of *C. annuum* after that screened parental lines with SNP markers in house were screened, and SNP markers were designed. The markers that showed polymorphism in parental lines were used to screen F_2 population in the free choice and no choice method.

4.3 SNP genotyping assay and linkage map

Genotyping the SNP markers was performed using a KASPar array in 1.62 µL. reaction mixture containing 0.8 ug. template, 0.8 µL. assay, 0.02 µL. bulked primers (two specific forward primers of each allele and common reverse primer). KASPar array was set up and PCR was carried out using an Array Tape platform Nexar® In line Liquid Handling and Assay Processing System and Soellex® High Throughput PCR Thermal Cycler. The SNPs were detected by Araya® In line Fluorescence Detection System and Array Tape®. A linkage map was constructed using Join Map 4.1 software (Van Ooijen, 2006). Mapping within linkage groups was carried out with the regression algorithm and a maximum jump level of 5. The final linkage map was obtained by deleting ungrouped markers. The linkage map was prepared with Map Chart 2.3 (Voorrips, 2015).

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CHAPTER 4

RESULTS

1. Resistance test

Seventeen accessions were used in this research by two methods; free choice method to study the reaction of an insect to plant and no choice method to study the reaction of a plant to an insect.

1.1 Free choice method

The free choice method demonstrated the reaction of an insect to a plant as antixenosis. The resistance plants were free from thrips damage or less symptom, but the leaf deformation, curling, and silvering damage were observed in susceptible lines (Figure 12). All symptoms occurred in the first two weeks after transplanting. The results were recorded and calculated DX and AUDPC as in table 5. The damage scores were displayed by cluster dendrograms in three levels, i.e. resistance, intermediate, and susceptible (Figure 13). The accession 3553 and 3555 were shown the most resistance. On the other hand, accession 3567 is the most susceptible line in the free choice method.

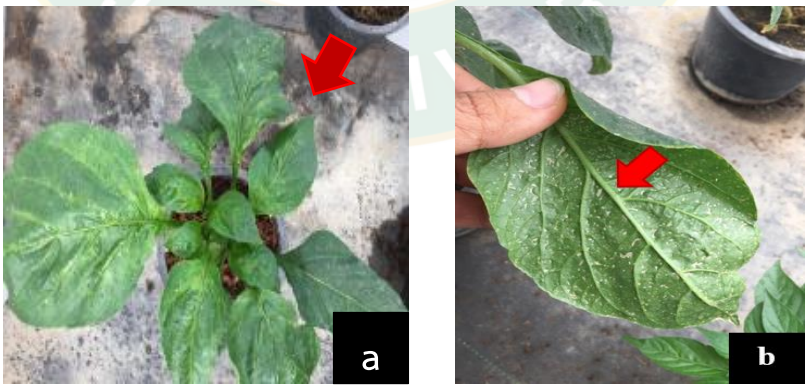

Figure 12 Damage caused by thrips.

Damage caused by thrips in free choice method. (a) leaf curling and deformation in greenhouse test on adaxial, (b) silvering symptom on abaxial.

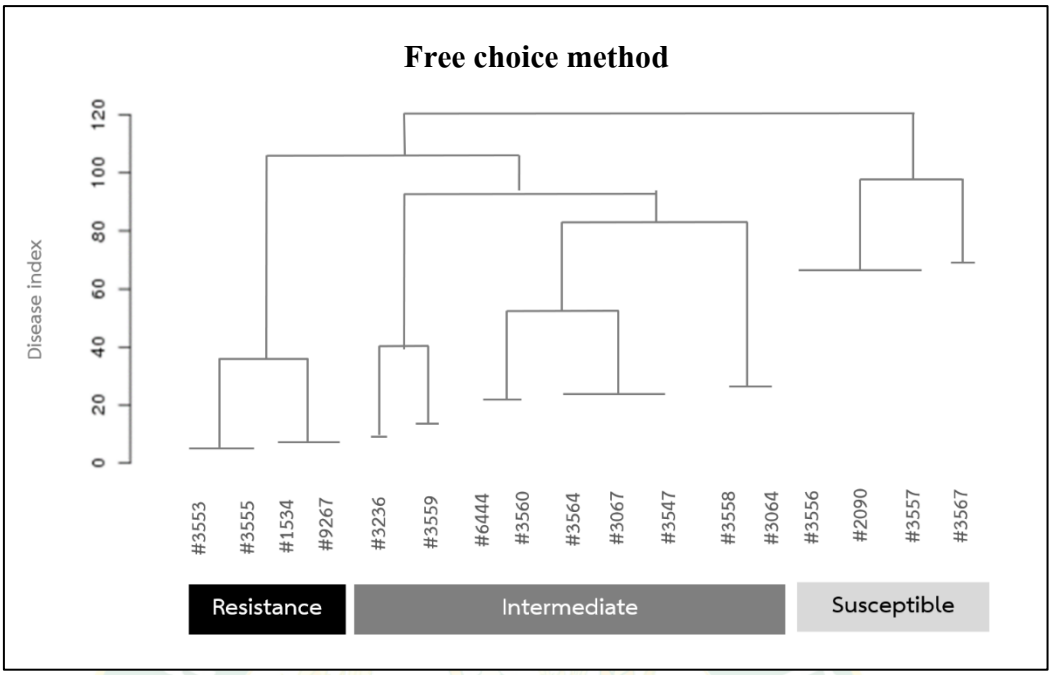

Figure 13 Cluster dendrograms classified by Disease index (DX) in three groups; resistance, intermediate and susceptible in free choice method.

Table 5 The result of Disease Index (DX) and the area under the disease progress curve (AUDPC) in free choice method.

Accession	Resistant test				Reaction
	DX		AUDPC		
#3553	4.17±0.00	e	145.83±0.00	d	Resistant
#3555 R*	4.17±2.60	e	145.80±45.40	d	Resistant
#1534 S*	9.03±5.30	cde	325.70±92.80	cd	Resistant
#9267 R*	12.50±8.30	bcde	398.60±131.50	cd	Resistant
#3236	13.89±6.40	bcde	447.23±127.40	bcd	Intermediate
#3559 R***	16.67±4.20	bcde	544.43±74.80	bcd	Intermediate
#6444	22.22±4.80	bcde	738.87±73.40	bcd	Intermediate
#3560 S*	22.22±8.70	bcde	719.43±161.30	bcd	Intermediate
#3564 S*	25.27±10.50	bcd	933.00±168.20	bc	Intermediate
#3067	27.78±6.40	bcd	952.77±127.40	bc	Intermediate
#3547	29.17±2.90	bc	962.50±30.90	bc	Intermediate
#3558 R*	32.08±13.40	bc	1,093.77±224.20	b	Intermediate
#3064	34.72±10.50	b	1,079.17±110.10	b	Intermediate
#3556 R*, **	65.56±15.00	a	2,072.80±243.60	a	Susceptible
#2090	68.05±10.90	a	2,226.40±206.00	a	Susceptible
#3557 R*	68.06±6.40	a	2,012.50±113.90	a	Susceptible
#3567	74.50±20.80	a	2,281.23±321.80	a	Susceptible

Within the same column scores followed by the same letter are not significantly different (P>0.05)

* = Resistance from Maharijaya et al., 2011 ** = Resistance from Fery and Schalk, 1991 and *** = Resistance from Eigenbrode and Trumble, 1994

1.2 No choice method

The no choice method studied the reaction of a plant to an insect as antibiosis. The resistance plants consist of a chemical that inhibits nymph development, so adult insects were reducing. As a result, living and dead nymphs were observed under a microscope (100x) 8 days after transferring L_1 stage into leaf discs. The percentage of survival nymph is between 4 % to 52 % in table 6. Survival and dead nymphs were classified by cluster dendrograms in three levels, such as resistant, intermediate, and susceptible (Figure 14). The accession 3553 and 6444 were shown the most resistance lines. On the other hand, accession 3545 and 3557 were shown the most susceptible lines in the no choice method (Table 6).

Table 6 The number of nymph survival and percentage of nymph survival after 8 days in no choice method.

Accessions	No choice method			Reaction
	Total	Nymph survival	% Survival	
#6444	25	1	4.00 g	Resistance
#3553	25	1	4.00 g	Resistance
#3064	25	2	8.00 efg	Resistance
#3559 R***	25	2	8.00 efg	Resistance
#3560 S*	25	3	12.00 defg	Resistance
#3236	25	4	16.00 def	Resistance
#3555 R*	25	4	16.00 def	Resistance
#3556 R*, **	25	4	16.00 def	Resistance
#3558 R*	25	4	16.00 def	Resistance
#3067	25	6	24.00 de	Intermediate
#2090	25	7	28.00 de	Intermediate
#9267 R*	25	7	28.00 de	Intermediate
#3564 S*	25	8	32.00 bcd	Intermediate
#3567	25	10	40.00 abc	Susceptible
#3545	25	13	52.00 a	Susceptible
#3557 R*	25	13	52.00 a	Susceptible

Within the same column scores followed by the same letter are not significantly different (P>0.05)

* = Resistance from Maharijaya et al., 2011

** = Resistance from Fery and Schalk, 1991 and

*** = Resistance from Eigenbrode and Trumble, 1994

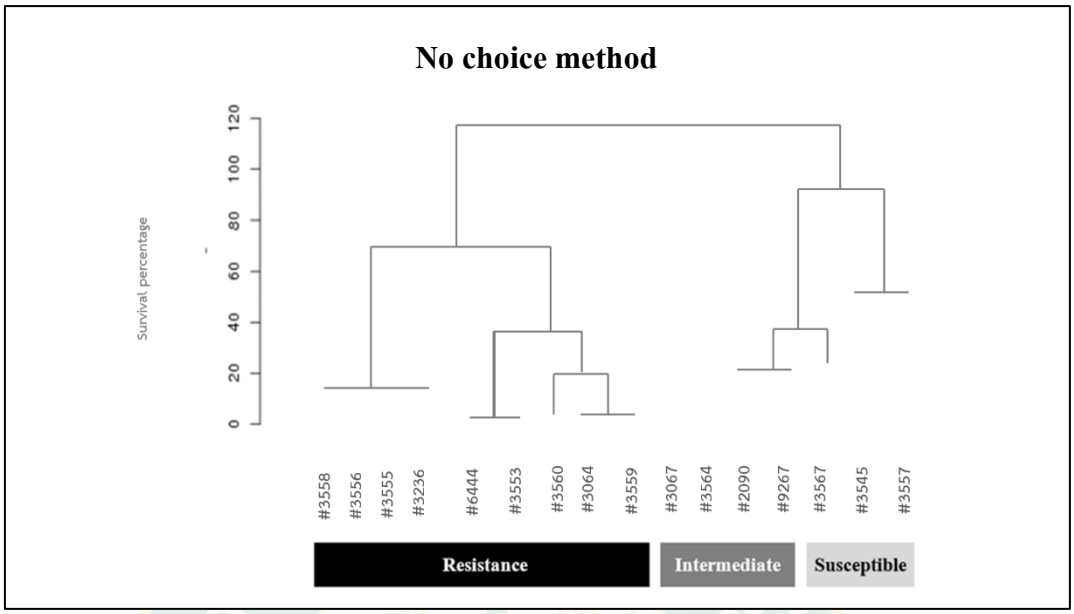

Figure 14 Cluster dendrograms classified by survival percentage in three groups; resistant, intermediate and susceptible.

The hierarchical clustering of pepper accessions was based on the test results (free choice and no choice method) with *T. Palmi* produced dendrogram (Figure 13, 14). Grouping the accessions into three clusters in both cases produced groups with resistance, intermediate and susceptible. Most of the accessions in the cluster resistant in the free choice method were also resistant in the no choice method. In contrast, accession 3556 was susceptible in the free choice method, but resistant in the no choice method (Table 6).

Two parental lines were selected from the free choice method and the no choice method. Accession 3555 was selected to resistance parent (R) and accession 3567 was selected to a susceptible parent (S), and this population was used to study gene segregation of thrips resistance in pepper and used to mapping population.

Table 7 Comparative the reaction between free choice and no choice method.

Accession	Description	Reaction	
		Free Choice	No Choice
#3553	904750135 - Radboud University Netherlands	Resistant	Resistant
#3555 R*	CGN16975: Capsicum annuum (AC 1979)	Resistant	Resistant
#1534 S*	CGN21557: Capsicum chinense (No.4661; PI 159236)	Resistant	N/A
#9267 R*	CGN17042	Resistant	Intermediate
#3236	MP LOCAL-15	Resistant	Resistant
#3559 R***	PBC145, Tiwari, drought-R	Resistant	Resistant
#6444	7303F	Intermediate	Resistant
#3560 S*	Miscucho Colorado; PI 152225	Intermediate	Resistant
#3564 S*	PI 315023 (Royal gold)	Intermediate	Intermediate
#3067	HPR99F	Intermediate	Intermediate
#3547	CGN21513-PI260580	Intermediate	Susceptible
#3558 R*	CGN20503: Capsicum annuum (Bisbas)	Intermediate	Resistant
#3064	Syn#F4	Intermediate	Resistant
#3556 R*, **	CGN23222: Keystone Resistant Giant	Susceptible	Resistant
#2090	Early California Wonder	Susceptible	Intermediate
#3557 R*	CGN23765: Capsicum annuum (CM 331; Criollos de Morelos)	Susceptible	Susceptible
#3567	Berceo	Susceptible	Susceptible

* = Resistance from Maharijaya et al., 2011 ** = Resistance from Fery and Schalk, 1991 and

*** = Resistance from Eigenbrode and Trumble, 1994

2. Inheritance of thrips (*T. palmi*) resistance in pepper

The segregation of thrips resistant by the free choice method was scored with DX to separate the resistance group and susceptible group by which equal or less than 50 were scored with resistance, while more than 50 were scored with susceptible. Fifty values were adjusted by DX value that showed reaction susceptible in Table 5.

Chi-square was calculated in F_2 population. The result in table 8 showed that it did not fit with the Mendelian model but fit with duplicate dominant epistasis (15:1). Frequency distributions of phenotypic data were skewed towards the resistance (Figure 15). Some plants of the recurrent parent (P2) showed resistance when separated with DX 50.

Table 8 Segregation of resistant and susceptible plants in crosses derived from parental lines in free choice method.

Population	Total plants	Observed		Expected		Ratio R:S	χ^2
		R[a]	S[b]	R	S		
R	16	16	0	16	0	-	-
S	18	5	13	0	18	-	-
F_1	16	16	0	16	0	-	-
F_2	195	183	12	146.25	48.75	3:1	36.94**
				182.8	12.2	15:1	0.004 ^{ns}

[a] Disease Index value equal or less than 50 were score with resistance.

[b] Disease Index value more than 50 were score with susceptible

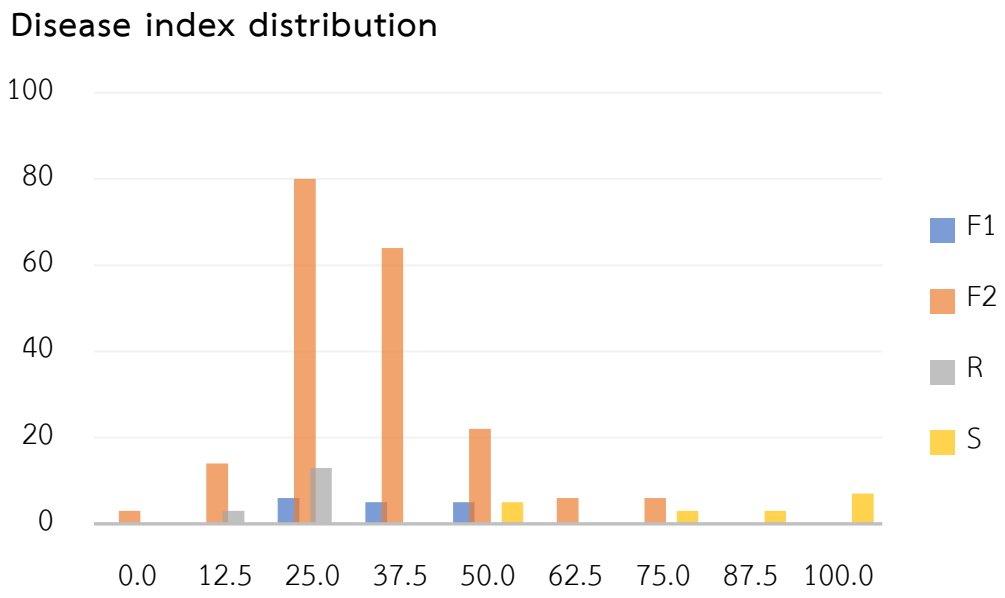

Figure 15 Free choice method disease index distribution in F_2 population.

The segregation of thrips resistant by the choice method was scored with a nymph survival percentage for separate the resistance group. The percentage of survival nymph more than 0.40 was scored with resistance, while equal or less than 0.40 was scored with susceptible. 0.40 value was adjusted by % nymph survival that showed reaction susceptible in Table 6. Chi-square was calculated in F_2 population. The result in table 9 showed that it did not fit with the Mendelian model but fit with duplicate recessive epistasis (9:7). Some plants of the recurrent parents (P2) showed resistance when separated with nymph survival percentage.

Table 9 Segregation of resistant and susceptible plants in crosses derived from parental lines by no choice method.

Population	Total	Observed		Expected		Ratio R:S	χ^2
		R[a]	S[b]	R	S		
R	10	10	0	10	0	-	-
S	9	3	6	0	9	-	-
F_1	0	0	0	0	0	-	-
F_2	220	121	99	165	55	3:1	46.93**
				123.75	96.25	9:7	0.14^{ns}

[a] % Nymphs survival more than 0.40 were scored with resistance.

[b] % Nymphs survival equal or less than 0.40 were scored with susceptible.

3. SNP markers development

3.1 Construct linkage map

SNP markers were chosen from multiple sources. Twenty-four SNP markers designed from Maharijaya et al. (2015) were used to screen both parental lines. Nine polymorphic SNP markers were used to screen F_2 population and not found any significant on Chromosome 6. After that, 549 SNP markers in house were screened from East West Seed (R&D), Chiang Mai, Thailand to survey polymorphic SNP markers. Then, 143 polymorphic SNP markers were used to screen F_2 population and construct a linkage map. MapQTL was done with the linkage map, phenotyping, and genotyping data and found highly significant on LG1. So fine mapping on that region had a total of 23 SNPs markers. Nine SNPs markers showed polymorphism. Total of 161 SNP markers were screened with F_2 population for free choice method and no choice method. The analysis is revealed no significant linkage map was found in no choice method. Then MapQTL was not done in no choice method. Linkage groups were assigned to pepper chromosomes based on 'CM334' *C. annuum* v.1.55

reference genome sequence. Four linkage groups (LG1-LG4) were reported which located on chromosome 3, 12, 6 and 7 respectively (Table 10 and Figure 16).

Table 10 Relate between linkage groups, chromosome, distance, LOD score and % explanation for thrips (*T. palmi*) resistance in free choice method.

LG	Chr.	Distance (cM)	LOD	% expl
1	Chr.3	146.349	5.38	12.9
2	Chr.12	90.613	3.25	8.0
3	Chr.6	102.515	2.00	4.9
4	Chr.7	43.766	2.01	5.0

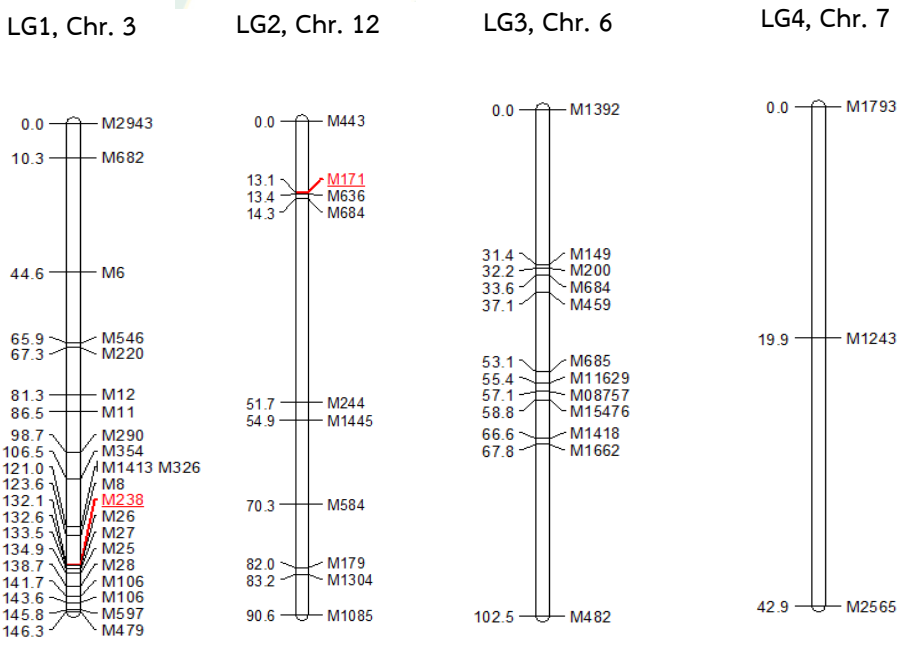

Figure 16 Linkage group and chromosome number of free choice method that LOD score > 2.00.

3.2 QTL mapping

Regarding the interval mapping of the free choice method from DX and AUDPC, was found significant in LG1 (Chromosome 3) and LG2 (Chromosome 12). The highly significant in LG1 was located on M238, but it was found that the second peak was located on M106. The LOD scores were 5.38 and 4.41, with an explained phenotypic variance of 12.9 % and 10.7 %, respectively. The highly significant in LG2 was located on M171, but the second peak was located on M1445. The LOD scores were 3.90 and 3.16, with an explained phenotypic variance of 9.5 % and 7.8 %, respectively (Table 11). For MQM Mapping, we found the same pattern and marker located in LG1 and LG2. (Figure 17).

Table 11 QTL effects for resistance-related traits (Disease index and AUDPC) in pepper.

QTL	Chr.	Position [a]	Significant marker	LOD	R^2 (%) [b]
QTL1	3	123.160-132.059	M238	5.38	12.9
QTL1	3	141.735-143.579	M106	4.41	10.7
QTL2	12	2.563-13.128	M171	3.90	9.5
QTL2	12	51.676-54.915	M1445	3.16	7.8

[a] Position of the QTL, in cM, referred to the linkage group

[b] Percentage of phenotypic variance explained by each QTL

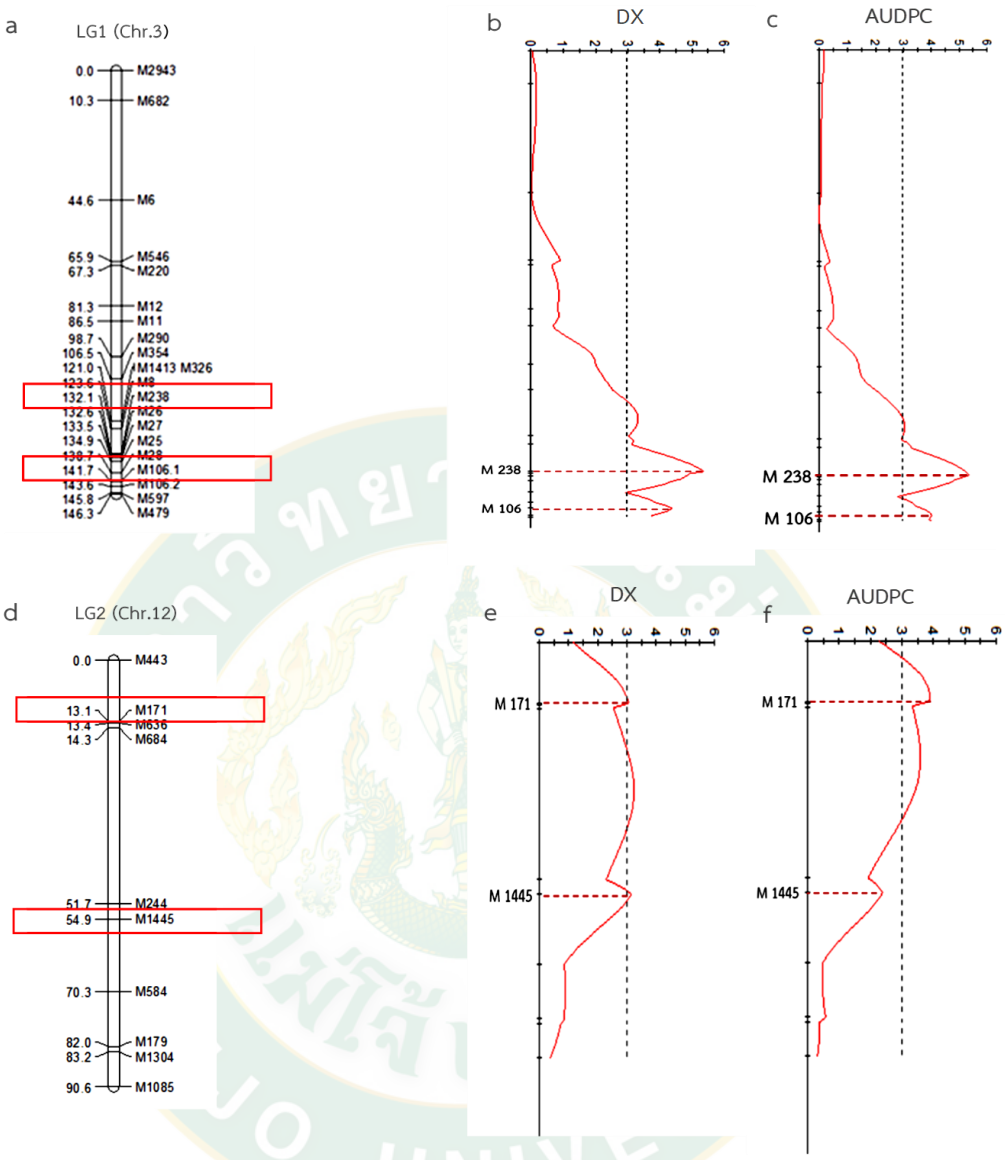

Figure 17 LOD profiles and LG1 (Chr.3) and LG2 (Chr.12)
support intervals for resistance QTL on Chromosome 3 (a) and Chromosome 12 (d). b and c are the same pattern of disease index (DX) and AUDPC on Chr.3 but e is the pattern of disease index and f is the pattern of AUDPC.

CHAPTER 5
CONCLUSIONS AND DISCUSSIONS

Different methods showed almost similar results in peppers

According to the results from the free choice and no choice method (Table 7), the most resistant in both methods are related to different resistance tests for thrips which showed very similar results in pepper (Maharijaya et al., 2011). A large variation in thrips resistant was found in pepper germplasm. The Keystone Resistant Giant was reported as the most resistant accession in the studies from Fery and Schalk's (1991) and Maharijaya et al., (2011) but it showed susceptible in this research by the free choice method. Moreover, *C. annuum* CM 331 (Accession 3557) showed a big contrast in this research: *C. annuum* CM 331 was reported as good source of resistance but was identified as susceptible in both methods. The Miscucho Colorado; PI 152225 was reported as susceptible, but was identified as intermediate in the free choice method and resistance in the no choice method.

Most germplasms selected from the literature were identified in the same manner as reported, but some of the germplasms were identified in a different way because they were identified as resistance or susceptible against *T. parvispinus* and *F. Occidentalis* in the literature, but it was different in this research (*T. palmi*). *C. annuum* AC 1979 was identified as resistance in the literature, and so as this research. Therefore, *C. annuum* AC 1979 was selected as donor lines to make a population for marker development.

No choice method (Nymph survival) development

The first step in the production of a mapping population is to select two genetically divergent parents that show apparent genetic differences for one or more traits of interest. The parents should be genetically divergent enough to exhibit sufficient polymorphism and, at the same time, should not be too genetically distant so as to a) Cause sterility of the progenies and/or b) Display very high levels of segregation distortion during linkage analysis. According to the no choice method

(nymph survival) in the recurrent parent, the intermediate percent survival of the nymph did not show a clear genetic difference. The no choice method (nymph survival) should be developed to increase the number of replicated per accession and reduce the time to observe from 8 days, but 3 days 3 times for additional observation data. In this research, the no choice of method data was not strong to use for the development of marker, so the development of the marker was done by the free choice method data.

Inheritance of thrips (*T. palmi*) resistance in pepper

The segregation of thrips resistant gene in the free choice method was studied by DX value, by equal or less than 50 scored with resistance, while the no choice method was adjusted by percentage of nymph survival, by more than 40 % scored with resistance. The segregation of F_2 population did not fit with the Mendelian ration, so thrips resistance gene was controlled by QTLs. The result was not clearly shown in the susceptible line in both methods as opposed to the identified germplasm result because the research was done in different seasons, which means the difference population of thrips. However, the population of thrips should be controlled in every experiment.

QTL mapping

In the free choice method, 4 linkage groups were constructed by 161 polymorphic SNP markers. The two resistances in our test: DX and AUDPC were highly correlated that the QTLs found that those two parameters co-localized near the same markers which were M238 and M106 on chromosome 3 and M171 and M1445 on chromosome 12. Four QTLs were detected for two parameters, even when using these markers as a cofactor in a multiple-QTL mapping (MQM) approach, only two QTLs were detected. These QTLs explained about 20 % of the genetic variation for the two parameters. The major QTL described by Maharijaya et al. (2015) on chromosome 6 was not detected in our study as the same resistant parent (*C. annuum* AC 1979), but a different susceptible parent. Another possibility would be that the chromosome 6 QTL is effective exclusively against larvae, but our

experiment detected in a bioassay using the damage score in the greenhouse and chromosome 6 reported to be resistant to two different thrips species (*F. occodentalis* and *T. parvispinus*). However, our experiment used *T. palmi* as they became the main insect in Thailand.

The QTLs detected on chromosome 3 (Major QTL) and Chromosome 12 (Minor QTL) are important factors affecting thrips resistance in pepper. The resistance line

(*C. annuum* AC1979) is a good donor source because resistance exists in different thrips species and belongs to *C. annuum,* which is the dominant pepper crop species that support the breeder to introgression in the breeding program. M238 and M171, these two QTLs explained about 20 % of the genetic variation. Most of the genes examined from these 2 QTLs on *C. annuum* 334 genome are 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, an apyrase-like protein that mediates biotic and abiotic stress responses and class III peroxidases that are involved in plant defense reactions. These genes play a role in *T. palmi* resistance in *C. annuum*.

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CURRICULUM VITAE

NAME	Areerat Saeko
DATE OF BIRTH	28 December 1980
EDUCATION	High school: Wattanothai Payap School Bachelor's degree: Plant science, Faculty of Agricultural Production, Maejo University
WORK EXPERIENCE	Work with Hortigenetics Research (S.E. Asia) Company Limited in 2004. Now still worked in Breeder.