CASSAVA VARIETIES PREFERENCE AND CASSAVA MOSAIC VIRUS TRANSMISSION CHARACTERISTICS OF THE WHITEFLY (*Bemisia tabaci* (Gennadius))

SUPAWADEE PROMBUT

มหาวิทยาลัยเทคโนโลยีสุรนารี

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การเลือกเข้าทำลายพันธุ์มันสำปะหลัง และลักษณะการถ่ายทอดเชื้อไวรัสใบด่างมันสำปะหลังของแมลงหวี่ขาว (*Bemisia tabaci* (Gennadius))

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มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2568

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

(Asst. Prof. Dr. Thitiporn Machikowa)
Chairperson

(Asst. Prof. Dr. Jariya Roddee)
Member (Thesis Advisor)

(Assoc. Prof. Dr. Jureemart Wangkeeree)
Member (Thesis Co-Advisor)

(Asst. Prof. Dr. Teerayoot Girdthai)
Member

(Dr. Wanploy Jinagool)
Member

(Assoc. Prof. Dr. Yupaporn Ruksakulpiwat)
Acting Vice Rector for Academic Affairs and Quality Assurance

(Prof. Dr. Jirawat Yongsawatdigul)
Acting Dean of Institute of Agricultural Technology

มหาวิทยาลัยเทคโนโลยีสุรนารี

สุภาวดี พรหมบุตร : การเลือกเข้าทำลายพันธุ์มันสำปะหลังและลักษณะการถ่ายทอดเชื้อไวรัสใบด่างมันสำปะหลังของแมลงหวี่ขาว (*Bemisia tabaci* (Gennadius)) (CASSAVA VARIETIES PREFERENCE AND CASSAVA MOSAIC VIRUS TRANSMISSION CHARACTERISTICS OF THE WHITEFLY (*Bemisia tabaci* (Gennadius)) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร. จริยา รอดดี, 77 หน้า.

คำสำคัญ: แมลงหวี่ขาว/โรคใบด่างมันสำปะหลัง/ขนใบ/*Bemisia tabaci*/ลักษณะการถ่ายทอดเชื้อ

มันสำปะหลัง (*Manihot esculenta* Crantz) เป็นพืชเศรษฐกิจสำคัญของประเทศไทยและทั่วโลก แต่ประสบปัญหาการระบาดของโรคใบด่างมันสำปะหลัง ซึ่งมีสาเหตุจากเชื้อไวรัส Sri Lankan cassava mosaic virus (SLCMV) และถ่ายทอดโดยแมลงหวี่ขาว (*Bemisia tabaci*) งานวิจัยนี้มุ่งศึกษาสองประเด็นที่เกี่ยวข้องกัน ได้แก่ บทบาทของความหนาแน่นของขนใบในการเข้าทำลายของแมลงหวี่ขาวในพันธุ์มันสำปะหลัง และลักษณะการถ่ายทอดเชื้อ SLCMV ของแมลงหวี่ขาว ผลการทดลองในภาคสนามและห้องปฏิบัติการพบว่า ระดับการเข้าทำลายของแมลงหวี่ขาวแตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่น 95% ระหว่างพันธุ์มันสำปะหลัง โดยพันธุ์ที่มีความหนาแน่นของขนใบสูง เช่น ห้วยบง 60 และ ระยอง 72 มีจำนวนการเข้าทำลายของแมลงหวี่ขาวต่ำกว่าพันธุ์ที่มีความหนาแน่นของขนใบต่ำ เช่น CMR 89 เกษตรศาสตร์ 50 ระยอง 4 และ พิรุณ 2 แสดงให้เห็นว่าขนใบซึ่งเป็นชนิดไม่มีกลุ่มต่อม (non-glandular trichomes) ทำหน้าที่เป็นกลไกป้องกันทางกายภาพที่สำคัญต่อการเกาะกินและการวางไข่ของแมลง การทดสอบการถ่ายทอดเชื้อไวรัสพบว่า แมลงหวี่ขาวต้องใช้เวลาในการดูดกินอย่างน้อย 24 ชั่วโมงจึงจะสามารถรับเชื้อได้อย่างมีประสิทธิภาพ และอัตราการถ่ายทอดสามารถตรวจพบได้ตั้งแต่ 24 ชั่วโมงแรก โดยเพิ่มสูงขึ้นอย่างชัดเจนหลังจาก 48 ชั่วโมง แม้ว่าพืชจะแสดงอาการไม่ชัดเจน (pre-symptomatic) ซึ่งอาจเป็นแหล่งสะสมเชื้อแฝงที่มีความเสี่ยงต่อการแพร่ระบาด ขณะที่การให้แมลงดูดกิน 48 ชั่วโมง ทำให้ต้นมันสำปะหลังแสดงอาการของโรคไวรัสใบด่างมันสำปะหลัง อย่างชัดเจนประมาณ 65 วันหลังการติดเชื้อ ผลการวิจัยชี้ให้เห็นถึงความสำคัญร่วมกันของลักษณะทางสัณฐานวิทยาของพืชและชีววิทยาการถ่ายทอดเชื้อของแมลงต่อพลวัตการระบาดของโรคไวรัสใบด่างมันสำปะหลัง การคัดเลือกพันธุ์ที่มีความหนาแน่นของขนใบสูงร่วมกับการจัดการแมลงหวี่ขาวในระยะเริ่มต้นและการตรวจวินิจฉัยการติดเชื้อแฝงจะช่วยเพิ่มประสิทธิภาพการจัดการโรคใบด่างมันสำปะหลัง และสนับสนุนการปรับปรุงพันธุ์เพื่อเสริมสร้างความทนทานของมันสำปะหลังต่อทั้งแมลงและโรคในอนาคต

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา สุภาวดี พรหมบุตร

ปีการศึกษา 2568 ลายมือชื่ออาจารย์ที่ปรึกษา Jariya Roddee

SUPAWADEE PROMBUT: CASSAVA VARIETIES PREFERENCE AND CASSAVA MOSAIC VIRUS TRANSMISSION CHARACTERISTICS OF THE WHITEFLY (*Bemisia tabaci* (Gennadius)). THESIS ADVISOR: ASST. PROF. JARIYA RODDEE, Ph.D. 77 PP.

Keywords: Whitefly/Cassava mosaic disease/*Bemisia tabaci*/Trichome density/Choice test/Transmission characteristics

Cassava (*Manihot esculenta* Crantz) is a major industrial crop in Thailand and globally, yet its productivity is severely threatened by cassava mosaic disease (CMD), caused by Sri Lankan cassava mosaic virus (SLCMV) and transmitted by the whitefly *Bemisia tabaci*. This thesis investigated two interconnected aspects of cassava–whitefly–virus interactions: (i) the role of trichome density in *B. tabaci* infestations across cassava cultivars, and (ii) the transmission dynamics (acquisition access period, latent period, and inoculation access period) of SLCMV by *B. tabaci*. Field and laboratory experiments demonstrated significant variation in whitefly colonization among cassava cultivars, strongly correlated with trichome density. Cultivars such as Huaybong 60 and Rayong 72, with higher trichome densities, exhibited reduced whitefly populations, while cultivars with lower trichome densities were more susceptible. Non-glandular trichomes acted as physical barriers, restricting insect landing, feeding, and oviposition, with density emerging as the dominant resistance factor. Transmission assays revealed that SLCMV acquisition by *B. tabaci* required at least 24 hours, with efficiency increasing substantially thereafter. The experimental results did not show that insects had a latent period. Inoculation followed similar time-dependent trends of acquisition, the presence of SLCMV was detected in cassava plants after a 24-hour inoculation access period by *B. tabaci*. Furthermore, cassava plants inoculated with SLCMV by *B. tabaci* for a 48-hour inoculation access period developed cassava mosaic disease symptoms at 65 days post-inoculation. These findings underscore the epidemiological significance of asymptomatic infections as hidden reservoirs for virus spread. Integrating these results highlights the dual importance of host morphological defenses and vector–virus biology in CMD epidemiology. High trichome density confers resistance by reducing vector colonization, while extended feeding durations remain essential for efficient SLCMV transmission. From a management perspective, breeding

for high-trichome cultivars, combined with early vector control and improved diagnostics for asymptomatic infections, offers promising avenues for integrated CMD management.

มหาวิทยาลัยเทคโนโลยีสุรนารี

School of Crop Production Technology Student's Signature Supawadee Promboot

Academic Year 2025 Advisor's Signature Jariya Roddee

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LIST OF ABBREVIATIONS

AAP	Acquisition Access Period
ACMV	African cassava mosaic virus
ANOVA	Analysis of variance
CBSV	Cassava brown streak virus
CCYD	Cucurbit chlorotic yellows disease
CCYV	Cucurbit chlorotic yellows virus
CIAT	International Center for Tropical Agriculture
CMD	Cassava mosaic disease
CRD	Completely randomized design
cm	Centimeter
FISH	Fluorescence in situ hybridization
g	Gram
IAP	Inoculation Access Period
ICMV	Indian cassava mosaic virus
LP	Latent Period
LSD	Least significant difference
m	Meter
MYMD	Mung bean yellow mosaic disease
MYMIV	Mung bean yellow mosaic India virus
MYMV	Mung bean yellow mosaic virus
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PeVYV	Pepper vein yellows virus
p.m.	Post meridiem
PYVMV	Pumpkin yellow vein mosaic virus
SCWL	Sugarcane white leaf
SEA	Southeast Asia

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LIST OF ABBREVIATIONS (Continued)

SEM	Scanning electron microscope
SLCMV	Sri Lankan cassava mosaic virus
SLCV	Squash leaf curl virus
ToCV	Tomato chlorosis virus
ToLCBV	Tomato leaf curl Bangalore virus
ToLCV	Tomato leaf curl virus
ToYVSV	Tomato yellow vein streak virus
TRV	Tobacco rattle virus
TYLCD	Tomato yellow leaf curl disease
TYLCV	Tomato yellow leaf curl virus
TYLCSV	Tomato yellow leaf curl Sardinia virus
TYVSV	Tomato yellow vein streak virus
t	Ton
µL	Microliter
µm²	Square micrometer
V	Volt
%	Percent

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CHAPTER I

INTRODUCTION

1.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy root crop native to South America that has become one of the most important tropical crops worldwide, both as a food and an industrial crop. Its ability to grow in nutrient-poor soils and under drought-prone conditions has made cassava a staple food in many developing countries. In Thailand alone, cassava holds major economic significance: in 2024, the country exported over 6.3 million tons of cassava and related products, generating over 100 billion Thai Baht in revenue (Office of Agricultural Economics, 2025). Despite its importance, cassava production is threatened by several pests and viral diseases, among which cassava mosaic disease (CMD) is the most destructive.

In Southeast Asia (SEA), CMD outbreaks have been reported in Vietnam, Thailand, and China (Wang et al., 2019). The predominant causal agent in the region is the Sri Lankan cassava mosaic virus (SLCMV) (Saunders et al., 2002). The virus spreads primarily using infected cassava stem cuttings and by the whitefly vector *Bemisia tabaci* (*B. tabaci*) (Legg and Fauquet, 2004). Infected plants typically display chlorosis, mosaic, and mottled leaf patterns, stunting, and significant reductions in plant size compared to healthy plants (Chikoti, 2011). Yield losses caused by CMD are substantial, ranging from 40% to complete crop failure under severe epidemic conditions (Legg and Fauquet, 2004). Current CMD management strategies rely on insecticides and healthy planting material. However, a promising complementary approach is to reduce virus transmission by improving understanding of the host selection behavior of *B. tabaci* (Navas-Castillo et al., 2011). Plants have evolved diverse defense mechanisms against herbivores, including tolerance, antibiosis, and antixenosis (Punnuri et al., 2022). These plant defenses can directly influence the host selection and feeding behavior of insect vectors, thereby reducing pathogen transmission. Among these traits, morphological defenses such as trichome density and leaf surface characteristics play a critical role

in deterring pests (Avery et al., 2015; Heinz and Parrella, 1994; McAuslane, 1996).

Whiteflies, like other hemipteran vectors, rely on a sequence of probing and feeding behaviors to assess host plant suitability. Once a plant is identified as suitable, it initiates sustained phloem feeding, a critical step for virus acquisition and transmission (Fereres and Moreno, 2009). Trichomes, hair-like epidermal structures, can interfere with vector behavior by acting as physical and sometimes chemical deterrents, thereby influencing insect landing, movement, and feeding (Skoczek et al., 2017). However, the relationship between trichome traits and whitefly infestation in cassava cultivars remains poorly understood, limiting the identification and recommendation of less susceptible varieties.

Virus transmission efficiency by insect vectors is also governed by three key biological phases: the acquisition access period (AAP), the latent period (LP), and the inoculation access period (IAP) (Purcell, 1982). For example, transmission of the Indian cassava mosaic virus (ICMV) by cassava-biotype *B. tabaci* requires an AAP of 48 hours (Antony et al., 2006), while the transmission of tomato yellow vein streak virus (ToYVSV) by *B. tabaci* involves an LP of 16 hours and an IAP as short as 10 minutes (Firmino et al., 2009). Such information highlights the critical role of vector feeding biology in determining the spread of viruses. Yet, the transmission characteristics of cassava mosaic virus (CMV) and the host preferences of *B. tabaci* on cassava cultivars in Thailand remain largely unstudied.

Therefore, the present study aims to investigate the transmission characteristics of *B. tabaci* as a vector of CMV and examine the influence of trichome density and size on whitefly host selection among cassava cultivars. A better understanding of these factors will not only contribute to improved whitefly and CMD management strategies but also provide insights into plant vector pathogen interactions that can inform the development of resistant cultivars, ultimately enhancing cassava productivity.

1.2 Objectives

1.2.1 To study the role of trichome density in *B. tabaci* infestations across cassava cultivars

1.2.2 To study the transmission characteristics of the *B. tabaci* vector of the SLCMV.

1.3 Scope and limitation of the research

Study transmission characteristics of cassava mosaic virus (CMV) transmission by whitefly (*B. tabaci*), including the time of AAP, LP, and IAP by using Polymerase chain reaction (PCR). The fluorescence in situ hybridization (FISH) technique is used to localize the virus within the whitefly (*B. tabaci*) body. Moreover, study host preference of the whitefly (*B. tabaci*) in 6 cassava varieties as follows: Rayong 4, Rayong 72, Kasetsart 50, Huai Bong 60, CMR 89, and Pirun 2 by the choice test experiment.

1.4 Location of thesis work

Entomology Laboratory, Suranaree University of Technology, Suranaree Subdistrict, Mueang District, Nakhon Ratchasima, Thailand

1.5 Anticipated outcomes

1.5.1 Improved understanding of the role of trichome density in *B. tabaci* infestations across cassava cultivars.

1.5.2 Characterization of the transmission characteristics of *B. tabaci* as a vector of SLCMV.

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CHAPTER II

LITERATURE REVIEW

2.1 Cassava and its importance

Cassava (Figure 1) is a perennial shrub belonging to the family Euphorbiaceae, which is characterized by the prominent development of lactiferous vessels composed of secretory cells known as laticifers. These cells produce the characteristic milky secretion, or “latex,” that typifies members of this family. Cassava is a monoecious species, bearing both male and female flowers on the same plant, but spatially separated. The leaves are conspicuous and palmately lobed, typically divided into five to nine deep segments.

The storage root of cassava is long and tapered, with a rough, brown periderm. Plant height ranges from 1 to 5 meters, although most cultivars do not exceed 3 meters in height. Morphological variation among cultivars is considerable, ranging from low-growing, highly branched shrubs to slender, unbranched trees.

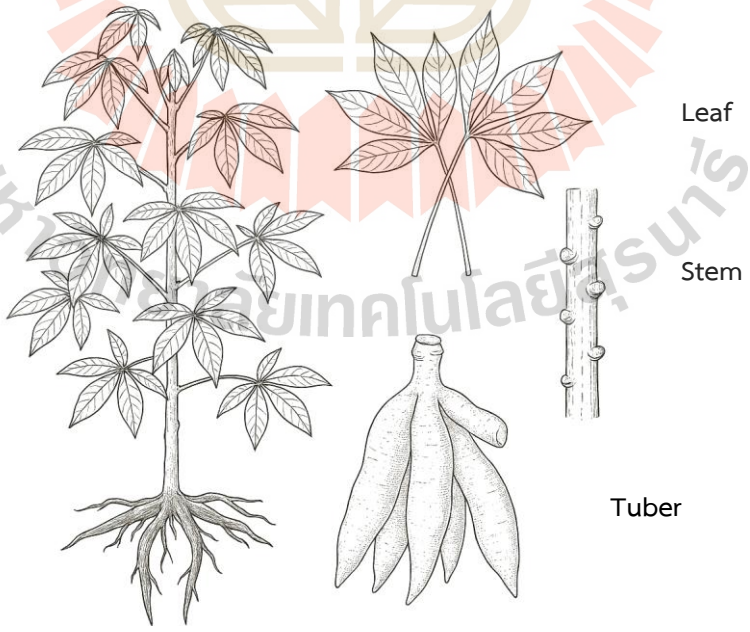


Figure 2.1 The leaves, stem, and tubers of cassava

Source: Adapted from Otálora et al. (2024)

Cassava is recognized as one of the most drought-tolerant crops and can thrive on marginal soils. Under optimal growing conditions, cassava has a yield potential of approximately 90 tons of fresh tubers per hectare and 30 tons of dry matter per hectare (El-Sharkawy, 2004). Although cassava is native to South America, it is now widely cultivated as an annual crop in tropical and subtropical regions. Its starchy, tuberous root constitutes a major source of carbohydrates and plays a critical role in food security. Cassava serves as the basis for a wide range of products (Figure 2).

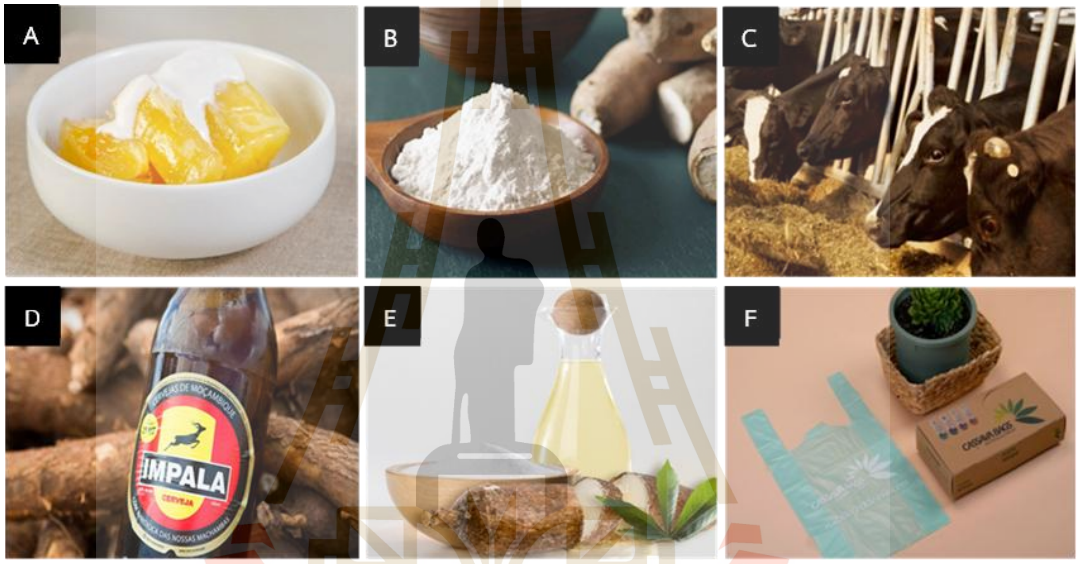


Figure 2.2 Cassava products, including direct consumption as food (A), processed flour (B), animal feed (C), alcohol (D), sweeteners (E), and biodegradable materials (F). Source: Kubala and Lang, (2022)

Thailand is a major global producer and exporter of cassava products, consistently ranking among the top exporters by volume and generating substantial economic value. In 2023-2024, Thai cassava exports alone totaled approximately 150 billion baht. Cassava is not only a crucial economic crop for millions of Thai farmers but also a primary raw material for a diverse range of downstream industries. These include processed food, beverages, pharmaceuticals, cosmetics, chemicals, and ethanol, and are also a key ingredient in the biofuel and animal feed sectors. The adaptability of the Thai cassava industry, particularly its numerous processing factories, enables it to produce a wide range of products and to respond effectively to shifts in global market demand. (Food and Agriculture Organization of the United Nations, 2024).

Thailand has a total cassava plantation area of 10 million rai, with the province with the most planted area being Nakhon Ratchasima Province with a planting area of 1,676,987 rai, followed by Chaiyaphum Province with a planting area of 894,588 rai and Kamphaeng Phet Province with a planting area of 792,322 rai (Office of Agricultural Economics, 2022).

The cassava varieties used in this study include Huai Bong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2, which are among the most widely cultivated commercial varieties in Thailand due to their high starch content and adaptability to different agro-ecological zones.

Huai Bong 60, a hybrid of Kasetsart 50 and Rayong 5, resembles Kasetsart 50 in appearance but has lighter purple shoots, more frequent branching, and first branching at 90–140 cm. Its low soluble sugar content enhances starch accumulation, making it highly suitable for industrial processing.

CMR 89, developed under the Kasetsart University and International Center for Tropical Agriculture (CIAT) breeding program, yields approximately 15 t/rai of fresh roots with 22.5% starch. It is characterized by green shoots, silver-green stems, and two levels of branching, making it a high-yielding variety.

Kasetsart 50, a cross between Rayong 1 and Rayong 90, is one of the most widely cultivated varieties in Thailand due to its high adaptability, good germination, and ease of harvesting. It produces ~23.3% starch and ~3.67 t/rai of fresh roots, with purple young shoots, sparse trichomes, and 0–3 branching levels.

Rayong 72, obtained from Rayong 1 × Rayong 5, contains ~21% starch and ~5.5 t/rai fresh roots. Its drought tolerance makes it particularly suitable for cultivation in the Northeast, and it is identified by dark purple shoots, red petioles, and 0–1 branching levels.

Rayong 4, an early-released breeding line, has moderate starch content (~20–21%) and is commonly used as a reference in breeding trials due to its stable yield performance. Morphologically, it has green shoots, red petioles, and 1–2 levels of branching.

Pirun 2, a hybrid between HB60 and Hanatee, is a newly developed high-yielding variety with ~24.7% starch and ~5.8 t/rai of fresh roots. It is a fast-growing plant with upright, light-brown stems, light-green shoots, and no branches. It grows best in red clay

soils, making it suitable for specific agro-ecological zones (Department of Agricultural Extension, 2017).

2.2 Cassava mosaic disease

CMD is one of the most critical constraints affecting cassava yield. CMD is caused by cassava mosaic viruses belonging to the family *Geminiviridae* and the genus *Begomovirus*. The disease was first reported in the Usambara Mountains of north-eastern Tanzania in 1894 (Warburg, 1894), and it was suggested to be of viral origin in the early twentieth century. The first confirmed outbreak of CMD in Southeast Asia occurred in Cambodia in 2015, followed by subsequent reports from Vietnam, Laos, Thailand, and southern China (Legg et al., 2015; Wang et al., 2019; Uke et al., 2021). Among the virus species identified in CMD-infected cassava from this region, SLCMV is the predominant species. SLCMV is genetically distinct from ACMV and ICMV, yet it induces similar symptoms, including leaf mosaic patterns, leaf distortion, and stunted growth (Wang et al., 2019; Hareesh et al., 2023).

In Thailand, the first CMD outbreaks were reported in the eastern provinces of Prachinburi and Sa Kaeo, later spreading to Buriram, Sisaket, and Surin between 2018 and 2019. Field surveys in affected areas recorded CMD incidence rates of up to 80% in Prachinburi and 43% in Sa Kaeo (Saokham et al., 2021). The primary mode of pathogen transmission is through virus-infected cassava cuttings, with *B. tabaci* serving as a vector (Calatayud et al., 1994). Typical CMD symptoms include leaf lamina distortion, chlorotic mosaic patterns, mottling, and reduced plant size (Figure 10) (Chikoti, 2011).



Figure 2.3 Cassava mosaic disease reduces overall plant size and causes chlorotic

mosaic symptoms.

2.3 Insect vector of cassava mosaic disease

B. tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a major insect vector of CMD (Legg et al., 2011). Among whiteflies, *B. tabaci* and *Trialeurodes vaporariorum* (*T. vaporariorum*) are considered the most economically important species (Agrios, 2008). Both species are pests of crops in open fields and greenhouses. *T. vaporariorum*, commonly known as the glasshouse or greenhouse whitefly, has long been a concern in protected cultivation, whereas *B. tabaci*, originally referred to as the tobacco whitefly due to its prevalence on open-field tobacco, has become a major pest in a variety of field crops, including tomatoes.

Patel et al. (2022) examined the morphology and developmental biology of these two whitefly species. The total developmental duration from egg to adult did not differ significantly between *B. tabaci* (Asia II 5) and *T. vaporariorum*. However, *T. vaporariorum* exhibited greater adult longevity, higher fecundity, and higher adult emergence rates than *B. tabaci* (Asia II 5). Similarly, egg hatching success and immature survival rates were higher in *T. vaporariorum* than in *B. tabaci* (Asia II 5). Morphologically, immature stages of *T. vaporariorum* were larger than those of *B. tabaci* (Asia II 5). Adult males and females of both species could be distinguished by size, wing posture, eye morphology, and antennae, with *T. vaporariorum* being generally larger. Notably, the wings of *T. vaporariorum* rest flat in a triangular shape, whereas those of *B. tabaci* (Asia II 5) are held at an acute angle in a linear fashion, confirming previous descriptions by Ribak et al. (2016). Globally, over 1,500 whitefly species have been recorded, with *B. tabaci* ranking among the most economically significant agricultural pests. *B. tabaci* was first described by Gennadius (1889) as *Aleurodes tabaci*, a tobacco pest in Greece. The genus *Bemisia* was later established by Takahashi (1936), who reclassified *A. tabaci* as *B. tabaci*.

2.3.1 Biology of whitefly *B. tabaci*

Whiteflies are plant feeders with sucking mouthparts. Additionally, adults of both sexes have four membranous wings. Finally, members of the family undergo incomplete metamorphosis. The lifecycle of whiteflies is composed of 6 stages: eggs,

four stages of nymph, and adults. The life cycle is about 42-45 days, eggs 7-10 days, nymphs 17-20 days, and adults 14-16 days (Figure 4). The female lays eggs (approximately 119 eggs/female) singly on the undersurface of leaves. The egg is 2 mm long, pear-shaped, and light yellowish, upright on leaves. The first stage of the nymph is flattened and translucent. The second stage of nymph is expanded and opaque-white with dorsal and lateral waxy, spinelike processes. The third stage of a nymph, when the pharate adult form is present, has red eyes and a yellow body. The last stage is characterized by yellowing and hair around the body. Whiteflies are distinctive in all life stages, except the egg, and can produce extra cuticular waxes that cover the body (Perring et al., 2018).

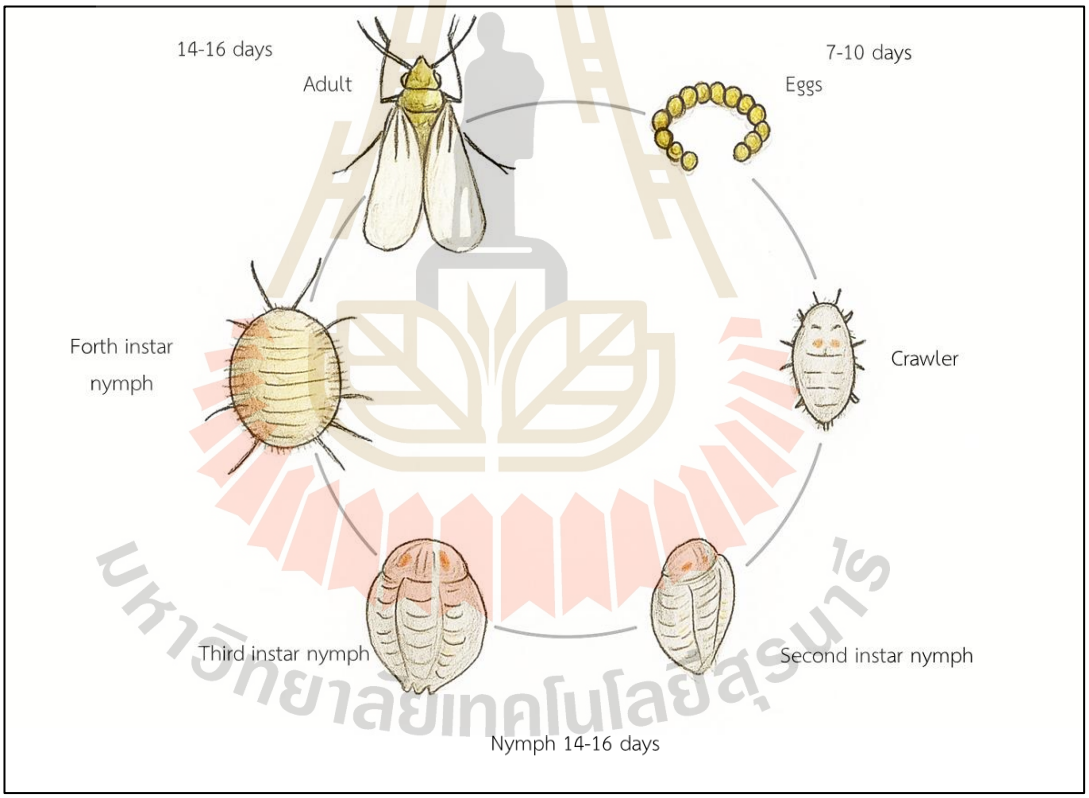


Figure 2.4 Life cycle of the whitefly *B. tabaci*. Source: Syngenta Ornamentals United Kingdom, (2020)

2.3.2 Damage by whitefly *B. tabaci*

Whiteflies damage plants in two primary ways: by directly sucking plant sap and by indirectly excreting honeydew on plant surfaces. Direct damage is inflicted by both adults and nymphs, which insert their piercing–sucking mouthparts, particularly into young plants, to extract phloem sap from sieve tubes (Gangwar and Charu, 2018). This nutrient depletion reduces photosynthetic capacity, leading to leaf yellowing, premature defoliation, plant weakening, and, in severe cases, the death of young plants. Indirect damage results from honeydew secreted by *B. tabaci*, which facilitates the growth of sooty molds on leaves and fruits. These molds inhibit photosynthetic activity and can adversely affect the quality and marketability of agricultural produce (Kunjwal and Srivastava, 2018).

Indirect damage also arises from whiteflies' remarkable role as vectors of numerous plant viruses. To date, *B. tabaci* has been reported to transmit over 200 plant virus species (Lu et al., 2019). The majority belong to the genus *Begomovirus* (family *Geminiviridae*), though whiteflies also transmit *Criniviruses*, *Ipomoviruses*, *Torradoviruses*, and *Carlaviruses* (Navas-Castillo et al., 2011). Crops highly susceptible to these viruses include cassava, cucumber, cotton, cowpea, crucifers, eggplant, tobacco, tomato, potato, soybean, sweet potato, okra, lettuce, pea, bean, pepper, poinsettia, and chrysanthemum (Jones, 2003). Several major viral diseases are transmitted by whiteflies. Cucurbit chlorotic yellows disease (CCYD), caused by the cucurbit chlorotic yellows virus (CCYV, genus Crinivirus), infects a broad range of cucurbits, including melon, cucumber, watermelon, loofah, and pumpkin. Typical symptoms include chlorotic leaf spots and progressive leaf yellowing (Okuda et al., 2010). In cotton, cotton leaf curl disease—caused by cotton leaf curl Multan virus (*CLCuMuV*, genus *Begomovirus*)—remains a serious constraint on production. Infected plants exhibit leaf margin curling (upward or downward), vein thickening, and enations (Tang et al., 2015; Pan et al., 2018).

Tomato yellow leaf curl disease (TYLCD), caused by tomato yellow leaf curl Sardinia virus (*TYLCSV*, genus *Begomovirus*), is one of the most devastating viral diseases of tomato worldwide, in tropical and temperate regions. Infected plants show marginal chlorosis, upward curling or cupping of leaf margins, reduced apical leaf size, chlorosis of older leaves, shortened internodes, stunted growth, bushy appearance, and floral abortion, often resulting in severe yield losses (Navas-Castillo et al., 2011).

In mung beans, mung bean yellow mosaic disease (MYMD) is caused by mung bean yellow mosaic virus (*MYMV*) and mung bean yellow mosaic India virus (*MYMIV*) (both genus *Begomovirus*), which are transmitted by *B. tabaci*. MYMV is prevalent in Thailand, Pakistan, and western and southern India, while *MYMIV* occurs in northern, eastern, and central India, as well as in Pakistan, Nepal, and Bangladesh. Symptoms begin with scattered yellow spots on young leaves, which expand into a yellow mosaic pattern, eventually causing complete yellowing, drying, and withering. Infected plants also produce smaller pods, and reduced photosynthetic efficiency leads to significant yield losses (Malathi and John, 2009).

2.4 Transmission of Plant Viruses by Insect Vectors

Many major plant viruses responsible for crop diseases rely on biotic vectors for their transmission. Among these, insects represent the largest group of plant virus vectors. The best-characterized insect vectors include aphids, thrips, leafhoppers, planthoppers, and whiteflies (Bragard et al., 2013). Most insect vectors are sap-feeding species that possess piercing–sucking or chewing mouthparts, enabling them to acquire viruses while feeding on infected plants and subsequently transmit them to healthy plants through the same feeding activity (Agrios, 2008). Transmission by insect vectors generally follows a sequential process (Figure 5).

2.4.1 Acquisition Access Period (AAP)

The AAP refers to the time between the insect's feeding on an infected plant and the pathogen's successful entry into its body. The AAP may last only a few minutes, but it is typically measured in hours. In general, a longer AAP increases the likelihood of pathogen acquisition (Figure 5). Shakeel et al. (2018) demonstrated that a single whitefly *B. tabaci* was sufficient to transmit Cucurbit chlorotic yellows virus (CCYV) to healthy seedlings, requiring approximately ≈ 90 minutes of acquisition and inoculation feeding to achieve successful transmission. Similarly, in tomato crops, *B. tabaci* was reported to transmit ToYVSV with a minimum acquisition and inoculation period of 30 minutes (Firmino et al., 2009).

2.4.2 Latent Period (LP)

Following the AAP, the LP is defined as the interval between initial pathogen acquisition and the vector's ability to transmit it. This period, sometimes referred to as the incubation phase (Figure 5), may involve viral replication within the insect and systemic invasion of vector tissues prior to successful transmission. However, not all insect–virus interactions exhibit a latent period. For instance, Shakeel et al. (2018) reported that *B. tabaci* retained CCYV for up to six days post-acquisition.

2.4.3 Inoculation Access Period (IAP)

The IAP occurs after the AAP and LP. It represents the time during which insect vectors transfer viruses from their bodies to uninfected plants (Figure 5). Shakeel et al. (2018) found that a single *B. tabaci* individual could transmit CCYV to healthy seedlings, requiring at least 90 minutes of feeding for successful inoculation. Likewise, *B. tabaci* was reported to transmit ToYVSV in tomato crops with a minimum AAP and IAP of 30 minutes (Firmino et al., 2009).

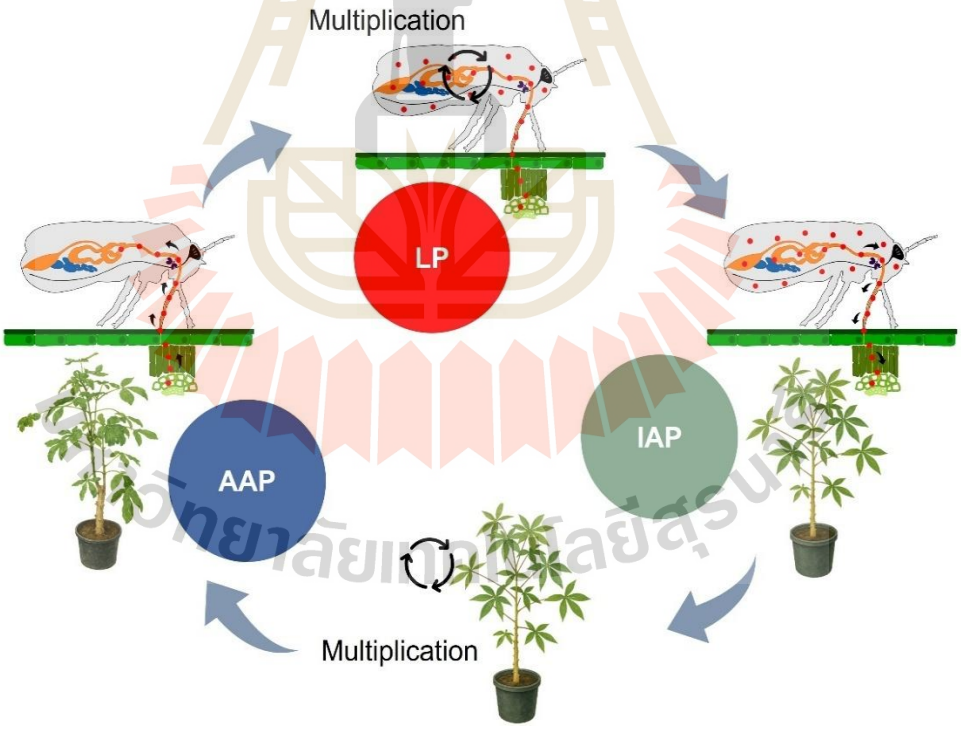


Figure 2.5 The sequential process of transmission of insect vectors

Table 2.1 The time of acquisition access period (AAP), latent period (LP), and inoculation access period (IAP) of whitefly *B. tabaci* vector in plant virus.

Plant virus transmission by whitefly *B. tabaci*	Transmission process period (hours)			**References**
	AAP	LP	IAP	
Squash leaf curl virus (SLCV)	-	24	-	Cohen et al., 1983
Tomato yellow leaf curl virus (TYLCV)	48	-	24	Rubinstein and Czosnek, 1997
Pumpkin yellow vein mosaic virus (PYVMY)	0.5	6	0.1	Muniyappa et al., 2003
Tomato leaf curl virus (ToLCV)	0.5	-	0.15	Maruthi et al., 2005
Indian cassava mosaic virus (ICMV)	48	-	48	Antony et al., 2006
African cassava mosaic virus (ACMV)	3	3	0.1	Dubern, J.,1994.
Tomato yellow vein streak virus (TYVSV)	24	16	24	Firmino et al., 2009
Tomato leaf curl bangalore virus (ToLCBV)	0.08	4	0.08	Chowda-Raddy et al., 2012
Tomato yellow leaf curl virus (TYLCV)	72	-	-	Moreno-Delafuente et al., 2013
Sri Lankan cassava mosaic virus (SLCMV)	48	-	48	Duraisamy et al. 2013
Sri Lankan cassava mosaic virus (SLCMV)	24	-	48	Kumar et al., 2014
Cassava brown streak virus (CBSV)	0.05-0.1	48	0.05-0.1	Maruthi et al., 2017
Cucurbit chlorotic yellows virus (CCYV)	1.5	-	1.5	Shakeel et al., 2018

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Plant virus transmission by whitefly *B. tabaci*	Transmission process period (hours)			References
	AAP	LP	IAP	
Tobacco rattle virus (TRV)	48	-	-	Kanakala et al., 2019
Tomato chlorosis virus (ToCV)	48	-	120	Maluta et al., 2020
Pepper vein yellows virus (PeVYV)	48	120	72	Ghosh et al., 2021
Tomato yellow leaf curl virus (TYLCV)	48	-	48	Wendy et al., 2022

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2.5 Type of transmission by the insect vector

Once an insect vector acquires a viral pathogen, the manner in which the pathogen is retained, carried, and subsequently transmitted to a new host plant may differ. These differences serve as the basis for classifying insect-mediated plant virus transmission into four main types:

2.5.1 Non-Persistent Transmission: In non-persistent transmission, viruses are retained by the vector only for a few seconds to minutes after acquisition. These viruses cannot be recovered from the insect hemolymph and are not transmissible through injection into the insect hemocoel (Hogenhout et al., 2008). Furthermore, they are lost following molting (Agrios, 2008).

2.5.2 Semi-Persistent Transmission is a form of non-circulative transmission. The viruses cannot be recovered from the insect hemolymph and cannot be transmitted to plants upon injection into the insect vector's hemocoel (Hogenhout et al., 2008). After that, the virus is lost within a few hours after acquisition from the insect body. Consequently, the insect loses the ability to transmit the virus to healthy plants and must feed on another infected plant before it can transmit it again (Webb et al., 2012).

2.5.3 Circulative, Non-Propagative Transmission In circulative, non-propagative transmission, viruses are ingested by the insect vector, traverse the gut wall into the hemolymph, and subsequently reach the salivary glands, from which they are discharged with salivary secretions. These viruses can be experimentally recovered from the hemolymph, remain transmissible after hemocoel injection, and are not lost from the vector once acquired. As a result, vectors may remain infectious for several weeks or even throughout their lifespan (Sylvester, 1980). The LP typically ranges from several hours to days (Table 2). Importantly, circulative non-propagative viruses do not replicate within their vectors and are not transmitted transovarially to the progeny of infected females (Nault, 1997).

2.5.4 Circulative, Propagative Transmission shares many features with the circulative non-propagative type. Viruses in this group also pass from the gut to the hemolymph and then to the salivary glands, where they are secreted during feeding. However, unlike non-propagative viruses, propagative viruses replicate within the insect vector’s body (Shikata, 1979; Ammar and Nault, 1985). The latent period may extend

from several days to weeks and can persist across the insect's developmental stages. Additionally, some propagative viruses are transmitted transovarially from infected females to their eggs (Agrios, 2008).

Table 2.2 Transmission characteristics and timing of plant viruses transmitted by insects.

Biological characteristic	Nonpersistent stylet-borne	Semipersistent foregut-borne	Persistent circulative	Persistent propagative
AAP and IAP	Seconds, minutes	Minutes, hours	Hours, days	Hours, days
LP	None	None	Hours, days	Days, weeks
Retention time in vector	Minutes, lost after molting	Hours, lost after molting	Days, weeks	Lifespan of an insect
Presence in the vector's hemolymph	No	No	Yes	Yes
Multiplication in vector	No	No	No	Yes
Transovarial transmission	No	No	No	Often
Insects	Aphid, Whitefly	Aphid, Whitefly	Plant hopper, Whitefly	Leafhopper

Modified from: Hogenhout et al. (2008)

When investigating interactions between plant viruses and other pathogens within insect vectors, direct visualization of the pathogens is essential. Determining their localization and abundance within the vector provides valuable insights into transmission mechanisms. Fluorescence in *situ* hybridization (FISH) is a powerful molecular technique that enables the detection and localization of specific nucleic acid sequences within tissues or whole organisms using fluorescently labeled probes.

This method allows researchers to precisely identify the location of pathogens within vectors, thereby supporting the classification of transmission types.

FISH is primarily applied for the spatial detection and quantification of nucleic acids in their cellular environment (Huber et al., 2018). The technique was originally developed to study specific regions of individual chromosomes within intact cell nuclei. Early applications involved mapping DNA sequences to specific regions of human chromosomes by hybridizing fluorescently labeled DNA probes to complementary chromosomal targets. Under a fluorescence microscope, this produces distinct fluorescent signals at the chromosomal sites of probe binding. The first single-copy human gene mapped using FISH was the thyroglobulin gene in 1985 (Landegent et al., 1985). This cytogenetic approach emerged approximately 30 years after the development of immunofluorescence-based protein detection methods (Chial, 2008).

Modern applications of FISH employ fluorescent probes that hybridize complementary sites in target DNA sequences. The emitted fluorescence provides direct evidence of the location of a specific sequence within the DNA molecule. The fundamental principle of FISH is the hybridization of fluorescent probes with complementary nucleotide sequences (O'Connor, 2008).

Using this approach, Kliot et al. (2009) investigated the transmission mode of *Tomato yellow leaf curl virus* (TYLCV; *Begomovirus*, Geminiviridae) transmitted by *B. tabaci*. The virus was localized in the hindgut of *B. tabaci*, while no virus particles were detected in the surrounding eggs. This indicates that TYLCV is not transmitted transovarially. Therefore, the transmission type of TYLCV is classified as circulative, non-propagative (Figure 7).

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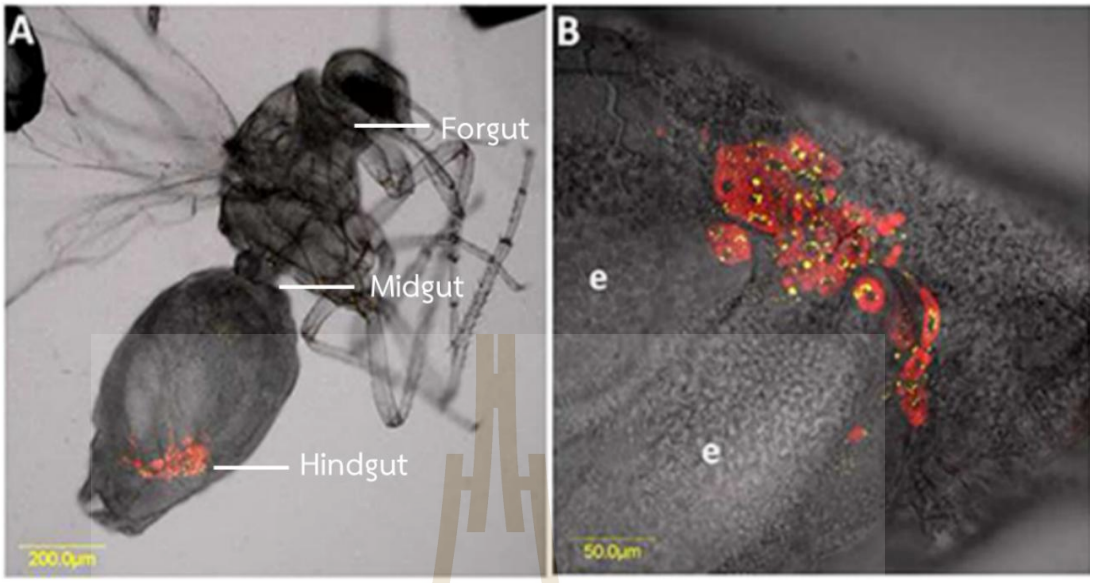


Figure 2.6 Localisation of TYLCV (red) in *B. tabaci* Q biotype adult (A) using FISH technique and zoom on the labeled area (B), e: egg.
Source: Kliot et al., (2009)

Moreover, Roddee et al. (2018a) use FISH to study the transmission type of sugarcane white leaf phytoplasma transmission by the leafhopper *Matsumuratettix hiroglyphicus.* The presence and location of sugarcane white leaf (SCWL) phytoplasma in the adult insects at days 7, 14, 21, and 28 of LP were also determined by FISH. The amount of SCWL phytoplasma increased over time at 7 and 14 days of LP (Figure 8). Therefore, the transmission type of TYLCV is circulative and propagative.

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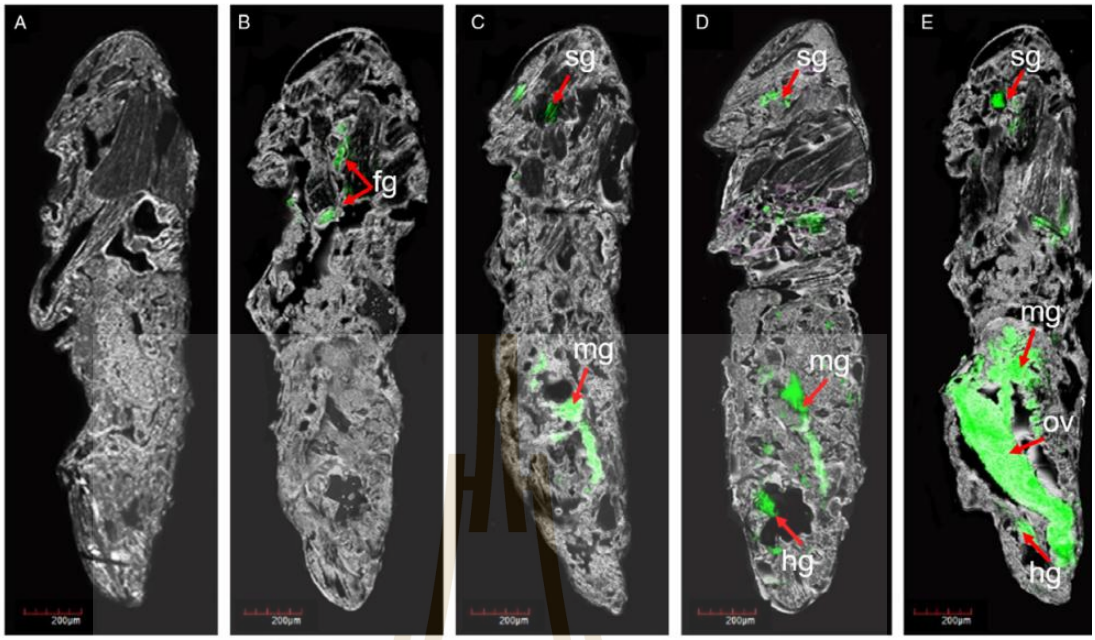


Figure 2.7 Localisation of sugarcane white leaf phytoplasma (green) in longitudinal tissue sections of *M. hiroglyphicus* using the FISH technique. fg, foregut; sg, salivary gland; mg, midgut; hg, hindgut; ov, ovary.
Source: Roddee et al. (2018a)

Kliot and Ghanim (2015) investigated the bacterial symbionts associated with the whitefly *B. tabaci*. Bacteriomes play a crucial role in transmitting symbiotic bacteria from one generation to the next. In some insects, symbiotic bacteria colonize the surface of the digestive system's epithelial cells. Many of these bacteria are obligate intracellular organisms that cannot be isolated or cultured on artificial media, making FISH microscopy an indispensable tool for their detection and for studying their functions, transmission pathways, and tissue tropism. Aphids, whiteflies, and several other insect species possess bacteriomes that harbor both primary, essential endosymbionts and secondary endosymbionts. The application of FISH probes has enabled three-dimensional visualization of these bacteriomes in aphids and across multiple developmental stages of whiteflies, including eggs, larvae, pupae, and adult males and females. With the increasing availability of genomic sequences of various endosymbiotic and pathogenic bacteria, such as spiroplasmas and phytoplasmas, more specific FISH probes have been developed. These probes facilitate the precise detection and identification of distinct bacterial species within insect hosts.

One notable case is the secondary symbiont *Rickettsia* in *B. tabaci*. FISH analysis revealed that *Rickettsia* is distributed throughout the body across different developmental stages, including eggs, nymphs, and adults. This wide localization indicates that the transmission mode of *Rickettsia* in *B. tabaci* is circulative and propagative, involving transovarial transmission from females to their offspring.

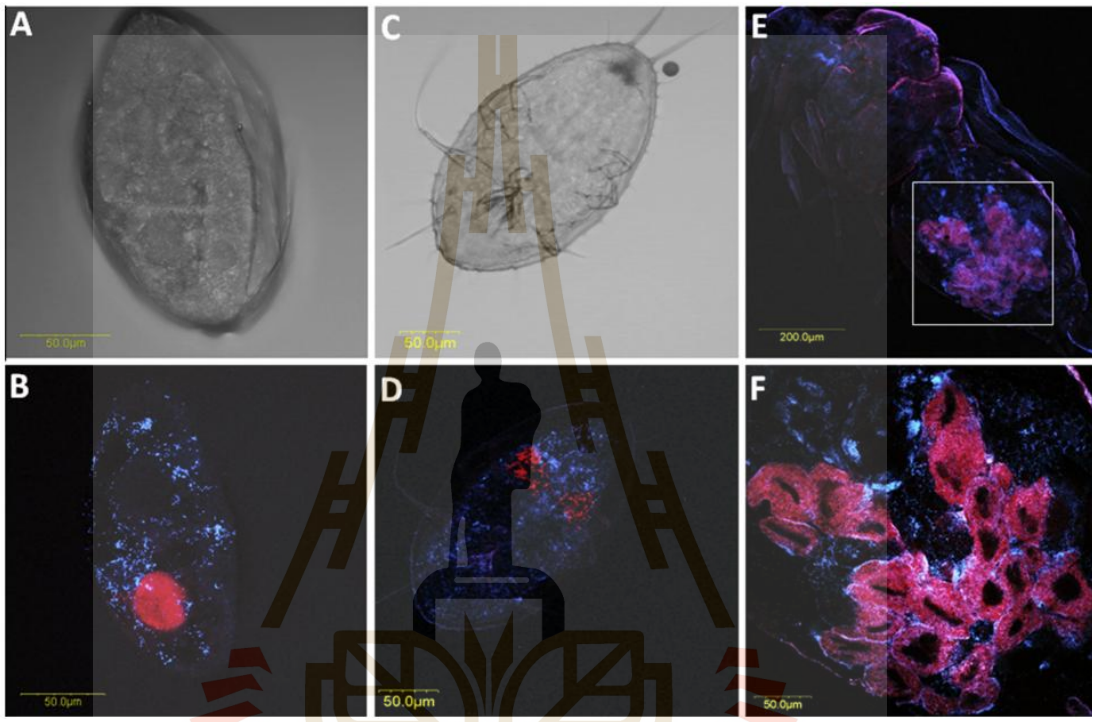


Figure 2.8 FISH on egg, nymph, and adult of *B. tabaci* showing the localization of the primary symbiont Rickettsia (blue). Source: Kliot and Ghanim (2015)

2.6 Management Strategies for cassava mosaic disease

A variety of strategies have been implemented to manage CMD. The use of virus-free planting material through clean seed systems helps prevent the introduction of the virus into new fields, although it does not stop secondary spread by vectors (Ntawuruhunga et al., 2017). Chemical control of whiteflies with insecticides has also been widely adopted. However, concerns about environmental safety, cost, and the development of resistance make this strategy less sustainable in the long term (Ghosh et al., 2022). In addition to insecticides, physical trapping methods such as yellow sticky traps have been used as an eco-friendly, low-cost tool for monitoring and partially controlling whitefly populations. These traps exploit the whiteflies' attraction

to yellow wavelengths and can reduce adult density when deployed strategically across the field, especially during early infestation stages (Reddy et al., 2023). While not sufficient as a standalone solution, sticky traps contribute to integrated pest management (IPM) systems by reducing whitefly pressure and helping to delay the spread of viruses. Moreover, breeding programs have focused on developing CMD-resistant cassava varieties using both conventional and molecular approaches. While African breeding programs have made progress, resistance to *SLCMV* in Asian cassava varieties remains limited (Wolfe et al., 2016).

However, resistance to SLCMV in Asian cassava cultivars is still under development and not yet widely available. Additionally, host selection is the process by which insect vectors identify, evaluate, and select suitable host plants for feeding and egg laying (oviposition). In the case of *B. tabaci*, host selection is influenced by a combination of visual, olfactory, and tactile cues, including leaf color, shape, surface chemistry, and trichome characteristics. This behavioral preference has significant epidemiological implications: plants that are more attractive to whiteflies are more likely to serve as virus sources, thereby facilitating the rapid spread of disease. Conversely, less preferred plants may act as a form of natural barrier, reducing vector contact and subsequent transmission (Finch and Collier, 2003; Bernays and Chapman, 2007).

2.7 Host plant preference of whitefly *B. tabaci*

The preference for host plants is a fundamental factor influencing the development and survival of herbivorous insects. Host selection, oviposition behavior, and the nutritional demands of offspring are strongly interrelated (Carrasco et al., 2015). Evolutionary perspectives on host plant choice suggest that natural selection favors a close correspondence between female oviposition preferences and offspring performance, commonly referred to as the preference–performance or “mother knows best” hypothesis (Jaenike,1978). Nevertheless, female choice and larval survival are not determined solely by dietary requirements but are also shaped by additional factors, including predation, parasitism, and both intra- and interspecific competition. Moreover, interactions with pathogenic and beneficial organisms have been shown to significantly influence insect host plant preferences (Mauck et al., 2010). In certain

species, larvae can compensate for maternal choice by leaving the initially selected plant and establishing on alternative hosts (Soler et al., 2012; Gamberale-Stille et al., 2014). Therefore, models of optimal foraging should integrate multiple ecological factors that may cause deviations from a strict association between oviposition preference and host plant quality (Figure 10).

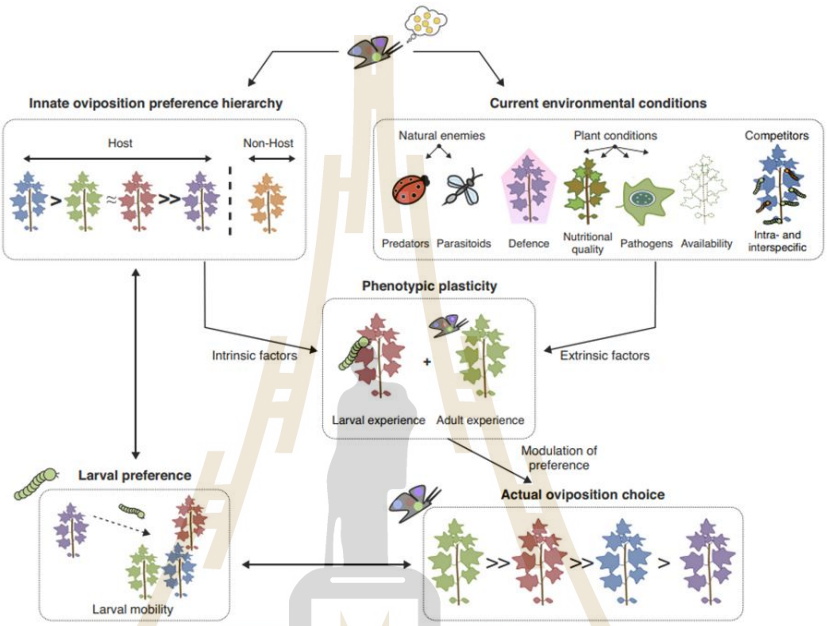


Figure 2.9 Concept of the factors influencing host plant choice in herbivorous insects. Source: Carrasco et al. (2015)

However, the ultimate host plant suitability is also strongly determined by plant resistance mechanisms, which are broadly classified into tolerance, antibiosis, and antixenosis.

Tolerance refers to a plant's ability to withstand or recover from insect damage without significantly reducing yield or growth (Smith, 2005). While tolerance does not directly affect *B. tabaci* preference, it reduces the pest's overall impact on the crop. Antibiosis involves plant traits that negatively affect insect biology, such as delayed development, reduced survival, or lower fecundity. For *B. tabaci*, antibiosis has been reported in tomato genotypes carrying resistance genes, where nymphal survival is reduced and development is prolonged (Jiang et al., 2001; Firdaus et al., 2012).

Antixenosis, or non-preference, plays the most direct role in host plant selection and preference. Plants exhibiting antixenosis possess traits that deter insect

colonization, oviposition, or feeding. For *B. tabaci*, antixenosis may arise from morphological barriers such as high trichome density, thick cuticles, or epicuticular wax composition (Berlinger, 1986; Toscano et al., 2002). Volatile compounds can also contribute to antixenosis by repelling whiteflies or interfering with host location (Bleeker et al., 2009). For instance, tomato lines with glandular trichomes not only impede settling but also reduce oviposition, demonstrating a strong antixenotic effect (Simmons et al., 2003). In cassava, differences in leaf surface characteristics have been shown to influence whitefly landing rates, further highlighting the role of antixenosis in shaping host preference (Saínz et al., 2019).

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CHAPTER III

EVALUATING THE IMPACT OF LEAF TRICHOME ON WHITEFLY *Bemisia tabaci* (Hemiptera: Aleyrodidae) DYNAMICS IN CASSAVA

3.1 Abstract

Cassava (*Manihot esculenta*) is an economically important crop in Thailand, but its productivity is severely limited by CMD, caused by the SLCMV and transmitted by whiteflies (*B. tabaci*). Understanding host plant resistance is therefore essential for effective disease management. Antixenosis, a resistance mechanism associated with plant morphological traits such as trichome density, may reduce whitefly colonization. This study evaluated the relationship between trichome density and whitefly infestation in six cassava cultivars: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2. Whitefly populations were monitored over time, and trichome density was assessed using scanning electron microscopy. Significant differences in whitefly infestation were observed among cultivars. CMR 89, Pirun 2, Rayong 4, and Kasetsart 50 showed higher infestation levels, whereas Huaybong 60 and Rayong 72 consistently exhibited lower whitefly populations. A strong negative correlation between trichome density and whitefly infestation was detected, indicating that increased trichome density effectively restricts whitefly colonization. These findings suggest that Huaybong 60 and Rayong 72 possess promising antixenosis-based resistance and may serve as valuable genetic resources for breeding cassava cultivars resistant to whiteflies and CMD.

3.2 Introduction

Cassava is a starchy, nutty root vegetable native to South America. This versatile crop serves as the foundation for a wide range of products, including food, flour, animal feed, alcohol, starches for paper and textiles, sweeteners, prepared foods, and biodegradable materials. However, a significant challenge in enhancing cassava productivity is the threat posed by diseases and pests. One of the most critical diseases

affecting cassava globally is CMD, which is particularly prevalent in Africa, India, Sri Lanka, and SEA (Legg et al., 2015; Wang et al., 2019). In SEA, CMD has been reported in countries such as Vietnam, Thailand, and China, with the SLCMV identified as the primary pathogen (Wang et al., 2019; Saunders et al., 2002). The disease is predominantly spread through infected cassava cuttings and the whitefly vector *B. tabaci* (Calatayud et al., 1994). Symptoms of CMD include distorted leaves, chlorotic mosaic patterns, mottling, and stunted plant growth (Chikoti, 2011), which can potentially reduce cassava yields by up to 40% or, in severe cases, by 100% (Legg and Fauquet, 2004).

Whitefly (*B. tabaci*, Hemiptera: Aleyrodidae) is a significant insect vector responsible for transmitting CMD (Legg et al., 2011) and over 200 other plant viruses, most of which belong to the *Begomovirus* genus family Geminiviridae (Navas-Castillo et al., 2011; Lu et al., 2019), the causative agents of CMD, the cassava brown streak disease, and SLCMV (Maruthi et al., 2005; Legg et al., 2011; Saunders et al., 2002). CMD can be effectively managed by propagating and distributing disease-free stem cuttings. However, breeding cassava resistant to the SLCMV via conventional methods remains a significant challenge due to the high heterozygosity and susceptibility to inbreeding depression in elite cultivars (Ntui et al., 2015). Furthermore, heavy reliance on chemical pesticides for whitefly management disrupts ecosystems and increases production costs, making it economically unviable for small-scale farmers (Abubakar et al., 2022). In response to insect pests like whiteflies, plants have evolved various defense mechanisms, including tolerance, antibiosis, and antixenosis (Punnuri et al., 2022). Tolerance enables plants to sustain growth and reproduction despite insect damage. Antibiosis directly impacts pest development by reducing fertility, size, and lifespan (Tefera et al., 2016). Antixenosis, in contrast, involves plant traits that deter pest attacks, often through the production of volatile organic compounds (VOCs) such as (E)-β-farnesene, (Z)-3-hexenal, and (E)-2-hexenal (Peterson et al., 2017).

As a result, developing host-plant resistance against insect vectors has emerged as a highly effective strategy for managing vector-borne viral diseases (Fereres and Moreno, 2009). Plant defenses, such as trichome density and leaf surface characteristics, are crucial in deterring pests (Avery et al., 2015; Heinz and Parrella,

1994; Mcauslane, 1996). As hemipteran vectors of plant viruses, whiteflies rely on a sequence of behaviors to identify and select suitable host plants. Once a plant is deemed appropriate, it initiates sustained phloem-sap feeding (Fereres and Moreno, 2009). Trichomes—small hair-like structures on plant surfaces—play a critical role in defense against insect pests by providing both toxic and deterrent effects (Skoczek et al., 2017). Increased trichome density has been shown to negatively impact insect behaviors, including oviposition, feeding, and larval development (War et al., 2012). However, the relationship between trichome density and whitefly infestation in cassava cultivars remains unclear. This knowledge gap hinders the identification and recommendation of less susceptible cultivars to whitefly damage. Therefore, this study aims to investigate the influence of trichome density and size on whitefly infestation across different cassava cultivars.

3.3 Materials and methods

3.3.1 Plant Materials

Six cassava cultivars: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2—were sourced from Nakhon Ratchasima, Thailand. DNA was extracted from the auxiliary buds at both ends of 10 cassava plants per cultivar to determine their disease-free or infected status using PCR with specific SLCMV primers. DNA extraction was performed according to the protocols described by Kollar et al. (1990) and Nakashima et al. (1993). The quality and quantity of the extracted DNA were evaluated using a spectrophotometer (Thermo Scientific™ NanoDrop™ 2000). The presence of SLCMV was detected through PCR, employing AV1 gene-specific primers (Saokham et al., 2021): AV1 forward (5’-GTT GAA GGT ACT TAT TCC C-3’) and AV1 reverse (5’-TAT TAA TAC GGT TGT AAA CGC-3’). PCR amplification was conducted in a 25-µl reaction volume, containing 1X PCR buffer (PCR Biosystems), 0.2 µM of each primer, and approximately 50 ng of DNA template. The thermal cycling conditions were as follows: an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 40 seconds, with a final elongation at 72 °C for 5 minutes. The reactions were performed using a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR amplification results were analyzed via DNA gel electrophoresis to confirm the disease-

free or infected status of the cassava samples (Supplementary data, S1). Only disease-free cassava plants were used for propagation.

The cassava stem cuttings, each measuring 20–25 cm in length, were prepared with 20 plants allocated per cultivar. The cuttings were initially planted in pots and cultivated under greenhouse conditions without the application of chemical treatments for insect pests or disease control. After one month of growth, the cassava plants were transferred to field conditions for further study. This experimental design enabled a natural assessment of plant-pest interactions, specifically evaluating the susceptibility of different cassava cultivars to whitefly infestation.

3.3.2 Whitefly *B. tabaci* settling preference assay in the field

The choice test experiment employed a completely randomized design (CRD) to assess the preference of whitefly adults (*B. tabaci*) for six cassava cultivars: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2. One-month-old cassava plants were individually potted at Suranaree University of Technology, with a spacing of 30 cm between rows and pots. The experiment was designed with 10 replications per cassava cultivar. Whiteflies were allowed to infest the cassava plants naturally, and the number of adult whiteflies on all cassava leaves was counted at every five-day intervals until the plants reached four months of age. Host preference was assessed based on the mean number of *B. tabaci* observed on each cassava cultivar. The study was conducted in field settings during December 2023 to January 2024 at Suranaree University of Technology (14°58’14.38” N, 102°06’7.06” E), Nakhon Ratchasima, Thailand.

3.3.3 Whitefly *B. tabaci* settling preference assay in the laboratory

The insect choice test study was conducted using a CRD. This experiment consisted of six replications, each with 120 adult *B. tabaci*. The preference of six cassava varieties by adult *B. tabaci* was quantified using a laboratory-based choice test. The six cassava cultivars: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2. One-month-old cassava plants were placed at different corners of a nylon-mesh cage (dimensions: 0.6 × 1.2 × 0.6 m), with one pot per cassava variety. The experiment was conducted under controlled conditions at 28 ± 2 °C and 50–60%

relative humidity (Erdogan et al., 2024) in the entomology laboratory at Suranaree University of Technology. Adult *B. tabaci* 2–3-day-old were released into the cage using an aspirator. The net opening was unrolled and directed toward one side of the cage during release, after which the cage was immediately sealed by tying the net opening.

The number of adult whiteflies present on each cassava variety was recorded at 24 and 48 hours after the release. The test was repeated six times, with the positions of the cassava varieties rotated clockwise and counterclockwise in each replication to minimize positional bias. Host preference was evaluated by comparing the mean number of *B. tabaci* adults found on each cassava variety.

3.3.4 Trichome size and density

The morphology of the abaxial surface of three-month-old cassava leaves was analyzed to explore its correlation with whitefly feeding preferences. Each measuring 5 mm² leaf samples were soaked in 37% formaldehyde for 24 hours to preserve tissue structure. Following fixation, the specimens were dehydrated in a graded ethanol series (70%, 80%, 90%, and 100%) according to the protocol outlined by Schneeberg et al. (2017). The dehydrated specimens were then dried at the critical point and coated with gold using a Neo Coater. The leaf surface morphology of the samples was examined using a scanning electron microscope (SEM; JEOL JSM-6010LV) at the SEM Laboratory of Suranaree University of Technology, with 10 surface area replicates for each cassava cultivar. Trichome density and size were assessed by measuring 10 µm² per surface area, 10 times per replication.

3.3.5 Statistical Analysis

All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Inc., Cary, NC). Data on whitefly settling preferences, trichome size, and density were analyzed using Tukey's post hoc test at a significant level of 0.05, with all analyses performed using SAS® OnDemand for Academics.

For correlation analysis (Pearson correlation), similar studies by Ghaffer et al. (2011) were referenced to assess the relationships between the mean values of settling preference and biophysical factors. The mean number of whiteflies in six cassava cultivars, density, and size of trichomes were analyzed using the Pearson correlation. Differences were considered statistically significant at the 5% level. Principal Component Analysis (PCA) was employed to evaluate the relationships among the six

cassava cultivars. As a multivariate statistical method, PCA facilitated the identification of patterns among the cultivars based on multiple parameters, including settling preference and biophysical characteristics. PCA was performed using Origin Pro 2024 software (OriginLab Corporation, Northampton, MA, USA) to visualize and interpret relationships among the cassava cultivars (Moberly et al., 2018).

3.4 Results

3.4.1 Whitefly *B. tabaci* settling preference in the field

Analysis of variance (ANOVA) was performed to assess the infestation levels of *B. tabaci* on six cassava cultivars over a 95-day period. The results revealed significant differences in whitefly abundance among the cultivars at several observation times ($P < 0.05$), specifically at 40, 55, 60, 65, and 95 days.

At 40 days, significant differences were detected among cultivars ($P < 0.001$), with CMR 89 exhibiting a high infestation level of 4.5 ± 0.39 whiteflies per plant. Similarly, at 55 days ($P = 0.027$), Pirun 2 showed the highest infestation level, reaching 5.9 ± 1.62 whiteflies per plant, whereas Rayong 72 exhibited a comparatively low infestation of 1.6 ± 0.45 whiteflies per plant. At 60 days ($P = 0.026$) and 65 days ($P = 0.018$), higher infestation levels were consistently observed on CMR 89, Kasetsart 50, Rayong 4, and Pirun 2, while significantly lower numbers of whiteflies were recorded on Huaybong 60 and Rayong 72. At 90 days, no significant differences among cultivars were detected ($P = 0.081$). However, at 95 days, significant differences re-emerged ($P = 0.019$), with CMR 89 and Pirun 2 showing relatively high infestation levels of approximately 3.0 whiteflies per plant. In contrast, Rayong 72 maintained a low infestation level, with only 1.1 ± 0.31 whiteflies per plant (Figure 1). According to Palumbo (2021), whitefly populations should not be allowed to exceed three adults per leaf, as this action threshold in IPM programs for spring melons is associated with an increased risk of whitefly infestation and resultant crop damage.

The mean number of *B. tabaci* whiteflies recorded over the 95-day observation period differed significantly among the six cassava cultivars ($F = 5.243$; $df = 5$; $P < 0.01$). The highest mean infestation levels (mean ± SE) were observed on CMR 89 (45.6 ± 2.40), followed by Pirun 2 (42.4 ± 2.23), Rayong 4 (40.9 ± 2.14), and Kasetsart 50 (38.0 ± 2.01). In contrast, significantly lower infestation levels were recorded on

Huaybong 60 (29.8 ± 1.57) and Rayong 72 (23.0 ± 1.21). Detailed infestation levels among cultivars are presented in Figure 2.

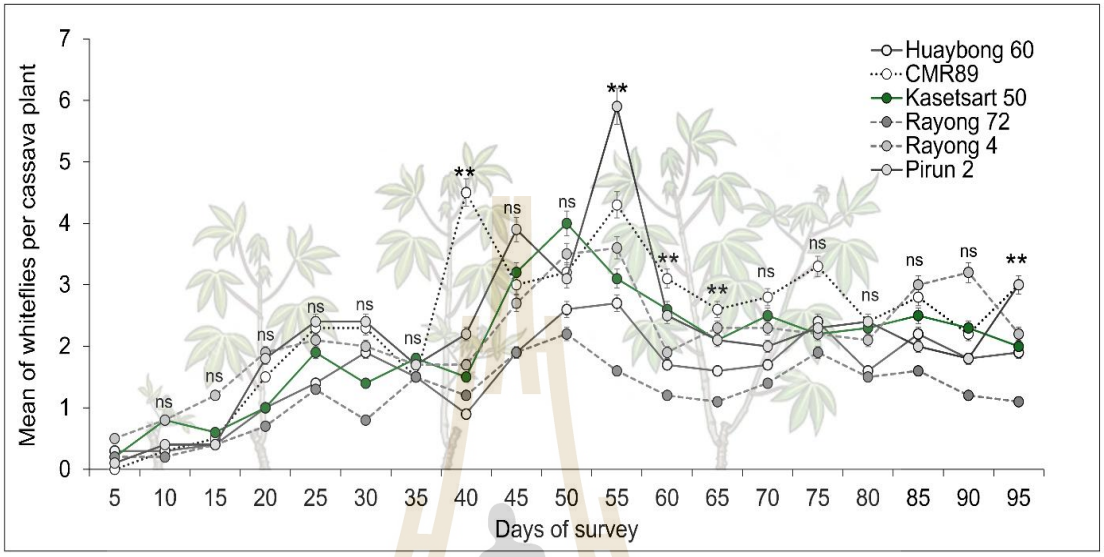


Figure 3.1 The mean number of *B. tabaci* whiteflies infesting six cassava cultivars over a 95-day survey period.

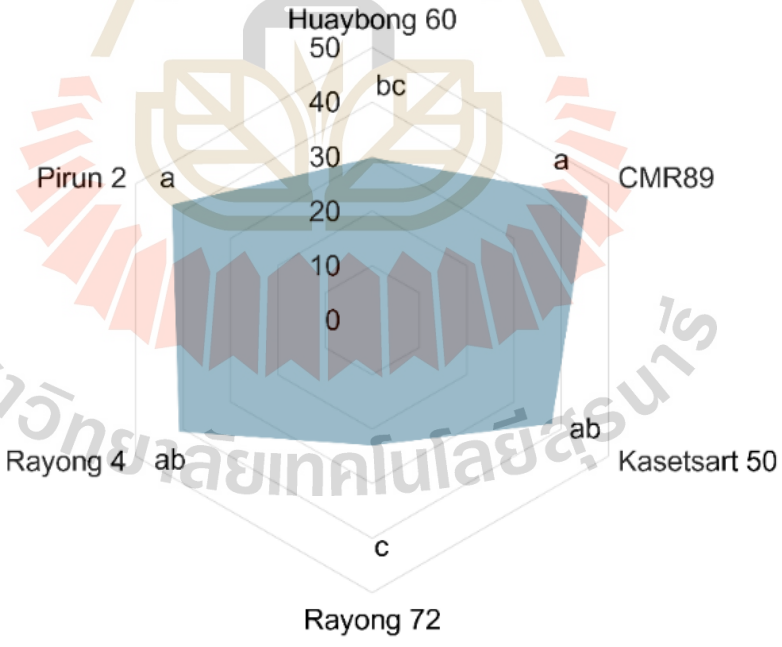


Figure 3.2 The mean number of *B. tabaci* whiteflies infesting six cassava cultivars over a 95-day period.

3.4.2 Whitefly *B. tabaci* settling preference in the laboratory

Analysis of variance (ANOVA) was conducted to evaluate the infestation levels of *B. tabaci* whiteflies on six cassava cultivars after releasing whiteflies in the cage at 24 and 48 hours. The results indicated significant differences in the number of *B. tabaci* among the cultivars at specific time points ($P < 0.05$) at 24 and 48 hours. The mean number of whiteflies per cassava plant was recorded at 24 and 48 hours after release for six cassava varieties: Huaybong 60, CMR 89, Kasetsart 50, Rayong 72, Rayong 4, and Pirun 2 (Figure 3).

At 24 hours, CMR 89 had the highest number of whiteflies (2.00 ± 0.24), followed by Pirun 2 (1.83 ± 0.28), Kasetsart 50 (1.33 ± 0.30), Rayong 4 (1.17 ± 0.28), Rayong 72 (0.17 ± 0.15), and Huaybong 60 (0.17 ± 0.15). Statistical analysis revealed that CMR89 differed significantly from Rayong 72 and Huaybong 60 ($p < 0.05$).

At 48 hours, the same trend was observed. CMR 89 again had the highest number of whiteflies (2.5 ± 0.06), followed by Kasetsart 50 (1.17 ± 0.12), Rayong 4 (1.17 ± 0.11), and Pirun 2 (1.17 ± 0.11), while Rayong 72 (0.17 ± 0.06) and Huaybong 60 (0.17 ± 0.06) remained the lowest. CMR 89 was significantly different from RY72 and HB60, as at 24 hours.

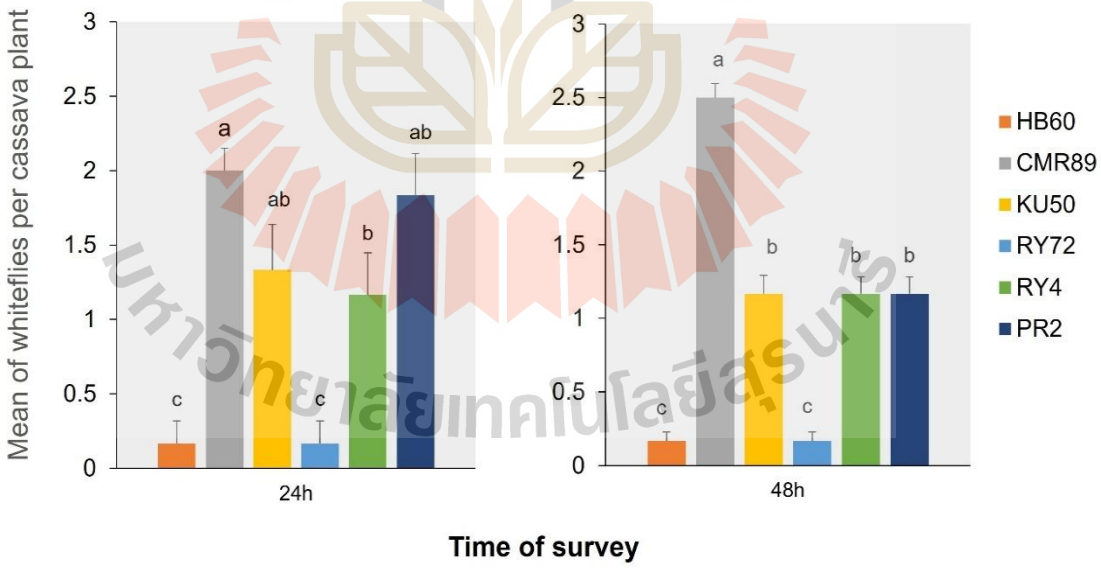


Figure 3.3 The mean number of *B. tabaci* whiteflies infesting six cassava cultivars at 24 and 48-hour surveys.

3.4.3 Trichome Density and Size

The three-month-old leaf morphology of the six cassava cultivars was examined using SEM (Figure 4), revealing the presence of non-glandular trichomes as a characteristic feature across all cultivars. The number of trichomes per 10 µm length scale varied significantly among the cassava cultivars ($F = 49.914$; $df = 5$; $P < 0.01$). The Huaybong 60 cultivar exhibited the highest trichome density (79.30 ± 2.42), followed by Rayong 72 (66.00 ± 1.90) and Pirun 2 (65.50 ± 3.56). In contrast, lower trichome densities were observed in Rayong 4 (46.40 ± 3.29) and Kasetsart 50 (44.20 ± 5.17) (Table 1).

The trichome size also varied significantly among the cassava cultivars ($F = 11.211$; $df = 5$; $P < 0.01$). The Pirun 2 cultivar had the largest trichome size (8.18 ± 0.30), followed by Kasetsart 50 (7.42 ± 0.33), Rayong 72 (7.31 ± 0.31), Huaybong 60 (7.26 ± 0.10), and CMR 89 (7.19 ± 0.34). The smallest trichome size was recorded in Rayong 4 (6.63 ± 0.34; Table 1).

Table 3.1 Mean trichome density and trichome size of six cassava cultivars

Cassava cultivars	Trichome density per 10 (µm^2)	Size of trichome (µm^2)
Huaybong 60	79.30 ± 2.42 a	7.26 ± 0.10 b
CMR 89	60.90 ± 1.90 b	7.19 ± 0.34 b
Kasetsart 50	44.20 ± 5.17 c	7.42 ± 0.33 b
Rayong 72	66.00 ± 6.03 b	7.31 ± 0.31 b
Rayong 4	46.40 ± 3.29 c	6.63 ± 0.34 c
Pirun 2	65.50 ± 3.56 b	8.18 ± 0.30 a

Values in the same column followed by different letters are significantly different ($p < 0.05$) according to one-way ANOVA followed by Tukey's post hoc test.

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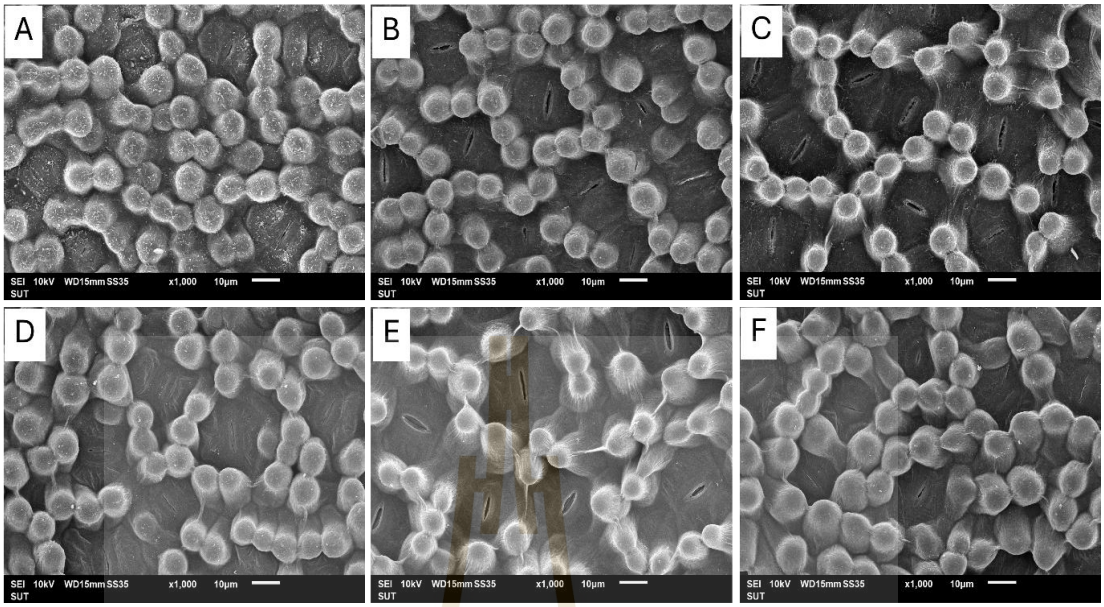


Figure 3.4 Trichomes of six cassava cultivars were observed through scanning electron microscopy (SEM) at 1000x magnification. Images show the leaf surface of (A) Huaybong 60, (B) CMR 89, (C) Kasetsart 50, (D) Rayong 72, (E) Rayong 4, and (F) Pirun 2.

3.4.4 Correlation Analysis (Pearson Correlation)

The number of whitefly infestations at various time intervals demonstrated strong negative correlations with trichome density at three months old, indicating an inverse relationship between the two variables. Specifically, at 10, 15, 40, 55, and 90 days, strong negative correlations (dark blue color) with trichome density per 10 µm² were observed: r = -0.86, r = -0.70, r = -0.75, r = -0.82, and r = -0.70, respectively (Figure 5). Moreover, the trichome density negatively correlated with the number of whitefly infestations at 5, 20, 25, 60, 65, 80, and 85 days of the observed period. (blue color). The result suggests that higher trichome density on cassava leaves is associated with lower whitefly infestations.

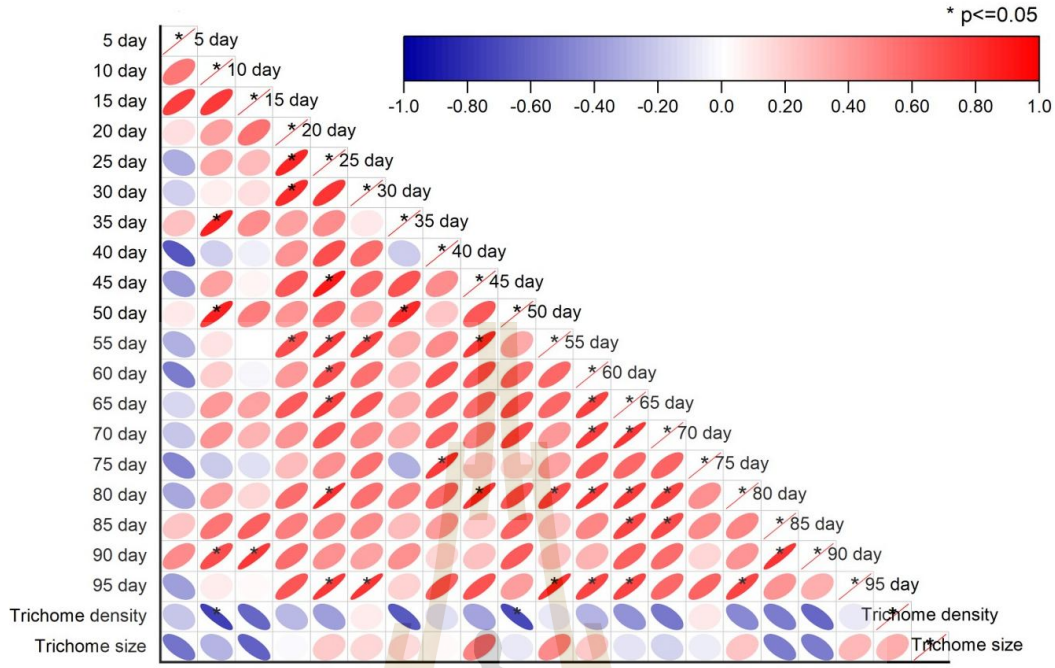


Figure 3.5 Correlation analysis of the number of whiteflies infested in a 5 to 95-day-old cassava plant with trichome density and size. (Blue means negative correlation, and red indicates positive correlation).

3.4.5 Principal Component Analysis

The first two principal components (PCs) accounted for 94.01% of the total variability in the data, with PC1 contributing 64.34% and PC2 contributing 29.67%. Through PCA, cassava cultivars were classified into two distinct groups. Group A included CMR 89, Kasetsart 50, Rayong 4, and Pirun 2. Group B comprised Huaybong 60 and Rayong 72 cultivars (Figure 6). Group A has a lower trichome density than Group B, which also experienced fewer whitefly infestations.

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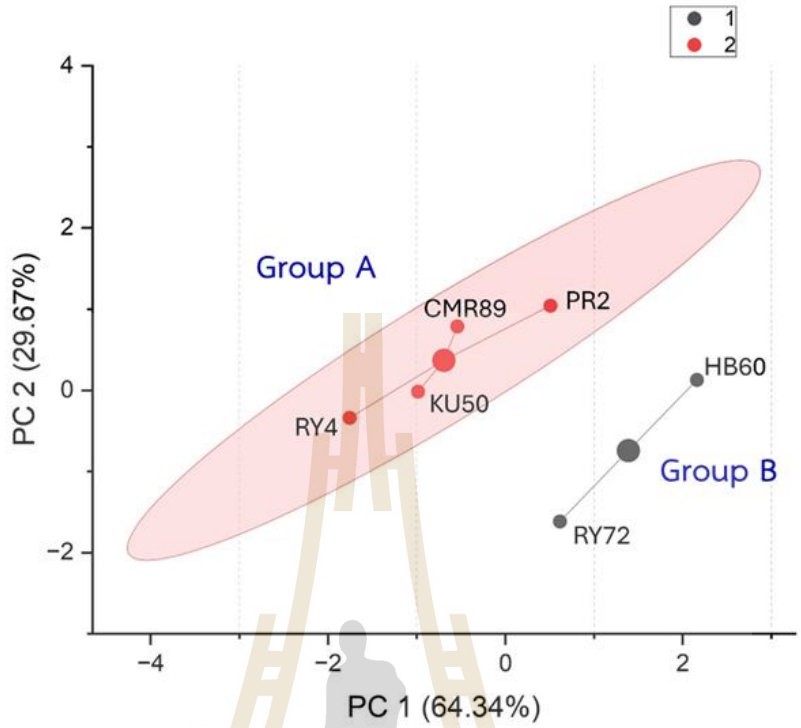


Figure 3.6 PCA (PC1 and PC2) of the number of whiteflies infested at different times with trichome density and size from six cassava cultivars.

3.5 Discussion

Whitefly infestations differed significantly among the six cassava cultivars, as demonstrated by ANOVA, and these differences were consistent with the PCA results, which categorized the cultivars into two distinct groups. Cultivars CMR 89, Kasetsart 50, Rayong 4, and Pirun 2 were associated with higher whitefly infestations, whereas Huaybong 60 and Rayong 72 exhibited comparatively lower infestation levels. Correlation analysis revealed both positive and strong negative relationships between whitefly abundance and trichome density. In cultivars showing a positive relationship, increases in trichome density were accompanied by higher whitefly infestation, whereas in cultivars exhibiting a negative relationship, higher trichome density corresponded with reduced whitefly numbers. These contrasting correlations highlight the complex role of trichomes in plant–insect interactions and underscore their importance in contributing to plant resistance mechanisms.

Trichome development is primarily under genetic control rather than being influenced by plant age. In *Arabidopsis thaliana*, trichome initiation and patterning are

regulated by key transcription factors such as GLABRA1 (GL1), which interacts with GLABRA3 (GL3) and TRANSPARENT TESTA GLABRA1 (TTG1) to form a regulatory complex that determines trichome formation (Pattanaik et al., 2014). This genetic regulation suggests that trichome density is a stable trait that can be exploited in breeding programs to enhance resistance to insect pests.

Trichomes function as physical barriers that restrict whitefly movement, feeding, and oviposition. Due to their small, delicate bodies, whiteflies have difficulty navigating leaf surfaces with dense trichome coverage, which limits their access to feeding sites and suitable oviposition areas (Lambert and Shah, 1999). In Brazilian cassava cultivars, reduced susceptibility to *Bemisia tuberculata* has been associated with morphological traits, including trichome density, as well as optical properties such as light reflectance and chroma values (Pastório et al., 2023), suggesting that resistance may result from multiple interacting plant traits.

Consistent evidence supporting the role of trichomes in whitefly resistance has been reported in tomato plants. Several studies have demonstrated that increased trichome density significantly reduces colonization and population development of *B. tabaci*. Simmons et al. (2003) reported that tomato plants with higher trichome densities exhibited lower whitefly infestations, as trichomes physically blocked whiteflies from establishing feeding sites, regardless of trichome size. Similarly, Escobar-Bravo et al. (2016) showed that increasing trichome density enhanced resistance to whiteflies and suggested that this trait could be introduced into cultivated tomato varieties through breeding programs. Furthermore, Andrade et al. (2017) demonstrated that crossing Solanum lycopersicum with the wild species S. galapagense produced progeny with high densities of type IV glandular trichomes, conferring resistance levels comparable to those of the resistant wild accession. Together, these studies indicate that trichome density is a heritable and effective trait for improving whitefly resistance in tomatoes.

Similar patterns have been observed in other plant species. In geraniums and cotton, higher trichome densities were correlated with reduced whitefly oviposition and lower pest populations (Lambert and Shah, 1999). In tobacco, a negative correlation between trichome density and whitefly resistance has also been reported (Li et al., 2014). In soybean, increased trichome density was associated with higher

mortality rates of whitefly nymphs, contributing to a reduction in overall infestation pressure (Heinz and Zalom, 1996). Moreover, high-density trichomes have been shown to impede feeding efficiency and reproductive success of whiteflies, resulting in fewer mature individuals and population decline over time (McAuslane, 1996).

While trichome density strongly influenced whitefly infestation, trichome size exhibited both positive and negative correlations with infestation levels in this study. Previous research suggests that trichome density is generally more influential than trichome size in reducing insect infestations. For instance, studies in *Arabidopsis thaliana* indicated that dense trichome coverage was more effective in impeding insect movement and feeding than trichome size alone (Mauricio and Rausher, 1997). Similar conclusions were drawn from studies on cotton, in which trichome density was more critical than trichome size in reducing infestations of cotton bollworms (Lu and Adang, 1996).

The six cassava cultivars examined in this study possessed non-glandular trichomes, which primarily function as a mechanical defense by limiting insect access and delaying damage to the epidermal layer (Kariyat et al., 2019). This physical defense mechanism contributes to cassava resistance by hindering whitefly colonization and feeding. Although PCA classified the cultivars into two main groups—Group A (CMR 89, Kasetsart 50, Rayong 4, Pirun 2, and CMR 89) and Group B (Huaybong 60 and Rayong 72)—Rayong 72 did not differ significantly from CMR 89 in trichome density or size. This suggests that factors beyond trichome traits, potentially including secondary metabolites or allelochemicals, may also play important roles in shaping plant–insect interactions (Ibanez et al., 2012; Tlak Gajger and Dar, 2021).

However, the experiment of choice, tested in both the field and the laboratory, found that temperature did not significantly affect the host preference of *B. tabaci* on cassava cultivars, as both temperature-controlled and uncontrolled choice tests showed consistent varietal preferences.

The temperature and photoperiod influenced whitefly population density in the field. During days 5–40 (December–January, Thai winter), when temperatures were lower and day length was shorter, whitefly numbers remained low. In contrast, during days 45–95 (February–March, hot season), the population increased markedly as both temperature and daylight hours rose.

Photoperiod is known to affect insect development and reproduction, with short-day conditions often suppressing population growth (Kaur et al., 2019) . Additionally, previous studies have reported that the optimal temperature for *B. tabaci* reproduction lies between 28 and 33 °C (Curnutte et al., 2014; Sohani et al., 2007). The increased whitefly numbers during longer-day, warmer periods in this study support previous findings that temperature and light duration play key roles in whitefly population dynamics (Muñiz and Nombela, 2001; Ghosh et al., 2022).

3.6 Conclusion

In conclusion, trichome density is vital for cassava's defense against whitefly infestations, with higher trichome densities providing excellent protection against pest pressures. Trichome density is crucial for defending cassava cultivars against whitefly infestations. Cultivars such as Huaybong 60 and Rayong 72, which possess high-density trichomes, demonstrate enhanced resistance to whiteflies by obstructing their movement. Consequently, cassava cultivars with higher trichome densities experience significantly lower whitefly infestations. This highlights the significance of trichome density as a crucial trait for breeding insect-resistant cassava cultivars. Enhancing trichome density can improve crop resilience against whitefly pests, contributing to more sustainable and resilient cassava cultivation. Moreover, the temperature did not affect whiteflies' preference behavior; it played a key role—along with photoperiod—in influencing whitefly population growth, which is important for developing effective seasonal pest management strategies.

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CHAPTER IV

CASSAVA MOSAIC VIRUS TRANSMISSION CHARACTERISTICS OF THE WHITEFLY (*Bemisia tabaci* (Gennadius))

4.1 Abstract

SLCMV is transmitted by the whitefly *B. tabaci* and represents a major threat to cassava production. This study investigated the transmission characteristics of SLCMV by *B. tabaci*, focusing on the AAP, LP, and IAP. Transmission assays revealed that SLCMV acquisition by *B. tabaci* required a minimum of 24 h, with transmission efficiency increasing substantially after 48 h. The experimental results did not indicate the presence of a latent period. Inoculation followed a similar time-dependent pattern to acquisition, with SLCMV detected in cassava plants after a 24 h inoculation access period. Furthermore, cassava plants inoculated with SLCMV by *B. tabaci* during a 48 h inoculation access period developed CMD symptoms at 65 days post-inoculation. These findings demonstrate that successful transmission of SLCMV by *B. tabaci* depends on extended acquisition and inoculation periods and provide essential information for understanding SLCMV epidemiology and improving disease management strategies.

4.2 Introduction

Cassava is a starchy, nutty-flavored root crop native to South America and is now widely cultivated across the tropics. It is notable for its ability to thrive in poor soil conditions and withstand drought, making it a vital food and industrial crop in many developing regions. Cassava is processed into diverse products, including food, flour, animal feed, starches for paper and textile industries, sweeteners, alcohol, and biodegradable materials. Despite its economic and nutritional importance, cassava productivity is seriously constrained by pests and viral diseases, particularly CMD.

CMD is recognized as one of the most devastating diseases of cassava, with widespread impacts reported in Africa, India, Sri Lanka, and SEA (Legg et al., 2015; Wang

et al., 2019). Within SEA, CMD has been documented in Vietnam, Thailand, and China (Wang et al., 2019). The causal agent in CMD-affected samples from the region has been identified as SLCMV (Saunders et al., 2002). The virus spreads through infected stem cuttings and via whitefly vectors, particularly *B. tabaci* (Calatayud et al., 1994a). CMD symptoms include leaf distortion, chlorotic mosaic, mottling, and severe stunting of plant growth compared with healthy plants (Chikoti, 2011). Yield reductions may reach 40%, and under severe epidemics, total crop failure can occur (Legg and Fauquet, 2004).

The whitefly *B. tabaci* (Hemiptera: Aleyrodidae) is a principal vector of CMD (Legg et al., 2011) and is also responsible for transmitting more than 200 plant viruses, most of which belong to the genus *Begomovirus* within the family Geminiviridae (Navas-Castillo et al., 2011; Lu et al., 2019). Economically important crops affected by *B. tabaci*-transmitted viruses include cassava, tomato, cucumber, cotton, cowpea, crucifers, eggplant, tobacco, potato, soybean, sweet potato, okra, lettuce, pea, bean, pepper, poinsettia, and chrysanthemum (Jones, 2003). Examples include CCYV, which causes CCYD (Okuda et al., 2010); TYLCSV, which causes TYLCD (Navas-Castillo et al., 2011); and MYMV, the causal agent of MYMD (Malathi and John, 2009).

Successful virus transmission by insect vectors depends on three critical phases: the AAP, the LP, and IAP (Purcell, 1982). For instance, transmission of ICMV by cassava-biotype *B. tabaci* requires both an AAP and an IAP of 48 hours, as confirmed by serological and PCR assays (Antony et al., 2006). In contrast, ToLCBV transmission occurs within 15–30 minutes of an AAP, with transmission efficiency reaching 100% when the AAP exceeds 16 hours (Chowda-Reddy et al., 2012). Similarly, transmission of ToYVSV involves an LP of 16 hours and an IAP as short as 10 minutes in *B. tabaci* (Firmino et al., 2009). These parameters illustrate how vector-borne biology strongly influences viral epidemiology.

Despite the global significance of CMD, information regarding the transmission characteristics of CMV and the host preference of *B. tabaci* for cassava cultivars in Thailand remains scarce. Such knowledge is crucial for understanding vector–pathogen–host interactions and for designing effective disease management strategies. Therefore, this study was conducted to (i) determine the transmission characteristics

of *B. tabaci* as a vector of CMV and (ii) evaluate the host selection behavior of *B. tabaci* on cassava cultivars. Insights from this research will support the development of targeted control strategies for *B. tabaci*, help reduce CMD incidence, and ultimately improve cassava productivity.

4.3 Materials and methods

4.3.1 Insect colonies

Adult *B. tabaci* were collected from a cassava field at Suranaree University of Technology, Nakhon Ratchasima Province, Thailand, using an aspirator between 09:00 and 16:00 h. After collection, the insects were transferred to cucumber plants (*Cucumis sativus*), a suitable host for *B. tabaci*, and maintained in plastic mass-rearing cages (5.5 inches in diameter × 30 inches in height). The whitefly colony was mass-reared under controlled conditions at 28 ± 2 °C and 65–73% relative humidity in the Entomology Laboratory, Suranaree University of Technology.

To ensure the genetic purity of the *B. tabaci* colony and to confirm the absence of *Sri Lankan cassava mosaic virus* (SLCMV), PCR screening was conducted every 3–5 generations using AV1 gene-specific primers, following the method described by Saokham et al. (2021). The primers used were AV1 forward (5′-GTT GAA GGT ACT TAT TCC C-3′) and AV1 reverse (5′-TAT TAA TAC GGT TGT AAA CGC-3′).

4.3.2 Maintenance of plants

The cassava cultivar CMR 89 was used for maintaining SLCMV isolates and for virus transmission experiments. Cassava stem cuttings used in this study were collected from a field in Nakhon Ratchasima Province, Thailand. Thirty-day-old cassava plants propagated from these stem cuttings were selected for experimental use. The presence of SLCMV was confirmed by PCR using AV1 gene-specific primers, following the method described by Saokham et al. (2021).

Following PCR confirmation of SLCMV infection, cassava plants were classified into infected and non-infected groups. Healthy and infected cassava plants were individually transplanted into plastic pots and enclosed in plastic mass-rearing cages (5.5 inches in diameter × 30 inches in height) to prevent unintended transmission of viruses by whiteflies. All plants were maintained under semi-greenhouse conditions

at the Entomology Laboratory of Suranaree University of Technology, for subsequent experimental use.

4.3.3 Acquisition Access Period (AAP) Study

A CRD was employed for the AAP experiment. Six acquisition time treatments (0, 4, 6, 12, 24, and 48 h) were evaluated, with each treatment consisting of 10 replications. Each replication included 10 adult *B. tabaci* (2–3 days old), resulting in a total of 600 whiteflies. The whiteflies were allowed to feed on 60 SLCMV-infected cassava plants, with 10 whiteflies confined to each plant per acquisition period. Whiteflies fed on non-infected cassava plants served as the control.

Following the acquisition period, whiteflies from each replication were collected and placed individually into tubes, preserved in acetone, and stored at −20 °C until further analysis. PCR was performed to detect the presence of SLCMV in both whiteflies and cassava plants. The number of PCR-positive samples was recorded and subjected to statistical analysis using the least significant difference (LSD) test at the 0.05 significance level. All statistical analyses were conducted using SAS® OnDemand for Academics.

4.3.4 Latent Period (LP) Study

The LP experiment was also conducted under a CRD with five treatments, 10 replications, and 10 whiteflies per replication (total of 500 adult whiteflies *B. tabaci*). The whiteflies were fed on 50 infected cassava plants at the optimal AAP time. After acquiring the virus, the whiteflies were transferred to non-infected cassava plants to determine the latent period. Then, 500 adult whiteflies were released onto non-infected cassava (1 cassava per 5 whiteflies) for 0, 1, 3, 5, and 7 days. At the end of each period, the insects were divided into two groups: one was preserved in acetone and stored at -20 °C for PCR detection of SLCMV. The second group (5 whiteflies per treatment) was transferred to cages containing non-infected cassava (1 plant per 5 whiteflies). After one month, symptoms of CMD were observed, and leaf tissue was collected and stored at -20 °C for PCR analysis. Data from both whiteflies and cassava were analyzed using LSD at the 0.05 significance level. Statistical analyses were conducted using SAS® OnDemand for Academics.

4.3.4.1 Fluorescent In *Situ* Hybridization (FISH) in whitefly

This experiment included five treatments, three replications, and 10 whiteflies per replication. A total of 150 adult *B. tabaci* were fed on 15 infected cassava plants (10 per plant) at the optimal AAP time. After feeding, insects were sampled at 0, 1, 3, 5, and 7 days of LP and preserved in acetone.

Whiteflies were then immersed in PBS, and the abdomens were pricked under a stereo microscope to facilitate solution infiltration. Fixation was performed using Carnoy's solution (ethanol:chloroform : acetic acid, 6:3:1) overnight on a shaker. Autofluorescence was removed by immersing samples in 6% hydrogen peroxide in 100% ethanol in the dark for 1–3 weeks.

Subsequently, the samples were washed with 100% ethanol, PBST, and hybridization buffer for three rounds (each for 10 minutes). Hybridization with CY5-labeled probes against SLCMV was conducted overnight at 42 °C. After hybridization, samples were washed with PBST and mounted on slides using Prolong antifade reagent. The slides were stored overnight in the dark at room temperature and then analyzed using a confocal laser scanning microscope (Roddee et al, 2018a).

4.3.5 Inoculation Access Period (IAP) Study

This experiment was conducted using a completely randomized design (CRD) with six transmission time treatments (0, 4, 6, 12, 24, and 48 hours), each comprising 10 replications with 10 adult *B. tabaci* per replication. A total of 600 adult whiteflies were initially allowed to feed on 60 SLCMV-infected cassava plants, with 10 whiteflies per plant, under the optimal acquisition and latent period conditions determined from previous experiments (AAP = 24 hours; LP = 0 hours).

After the acquisition phase, the whiteflies were transferred to non-infected cassava plants at a density of one plant per 10 whiteflies and allowed to feed for the designated transmission periods (0, 4, 6, 12, 24, and 48 hours). Disease symptoms on cassava plants were assessed 1 month after inoculation. Leaf tissue samples were subsequently collected and stored at −20 °C for the detection of SLCMV using PCR.

4.3.6 Insect DNA Extraction

Ten whiteflies (whole body) were placed in 1.5 µl tubes, and DNA extraction buffer was added. The insects were ground, then incubated at 37 °C for 24 hours in a shaker. A phenol: chloroform: isoamyl alcohol mixture was added and mixed, followed by chloroform: isoamyl alcohol. Tubes were centrifuged at 13,000 rpm, 4 °C for 5 minutes.

Sodium acetate (3M) was added, and the mixture was stored at -20 °C for 30 minutes. DNA was precipitated using isopropanol and stored at 4 °C for 2–3 hours. The pellet was washed with 70% ethanol, centrifuged, and air-dried. TE-RNase buffer was added, and tubes were incubated at 37 °C overnight. DNA concentrations were measured for PCR analysis.

4.3.7 Cassava DNA Extraction

Leaf tissues were cut into small pieces, washed with 4% Clorox, rinsed with DI water, and ground into powder. About 0.1 g of tissue was transferred into a microtube containing 600 µL of 2% CTAB buffer. The mixture was incubated at 65 °C for 1 hour, then cooled to room temperature and mixed with 500 µL of chloroform: isoamyl alcohol (24:1).

After centrifugation (13,000 rpm, 10 minutes, 4 °C), the aqueous phase was transferred to a new tube, and 350 µL of cold isopropanol was added. DNA was precipitated, washed with 70% ethanol, and air-dried. TE-RNase buffer (100 µL) was added, and the tubes were incubated at 37 °C for 24 hours. They were then stored at -20 °C for future PCR use.

4.3.8 PCR amplification for insect and cassava tissue

PCR amplification was carried out in 20 µl reaction volumes containing 1× PCR buffer, 0.2 µM of each primer, and ~50 ng of DNA template. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation (94 °C, 40 s), annealing (55 °C, 40 s), and extension (72 °C, 40 s), with a final extension at 72 °C for 5 minutes. A 1% agarose gel was prepared by dissolving 1 g of agarose in 100 mL of TAE buffer. The solution was microwaved, mixed with Safe-Red (4 µl), and then poured into a gel box, where it was allowed to solidify.

After gel casting, the PCR products, mixed with loading dye, were loaded into the wells. Electrophoresis was performed at 100 V for 40 minutes. Gels were then visualized.

4.4 Results

4.4.1 Acquisition access period (AAP) study

The different AAPs for the percentage of virus-infected whiteflies were evaluated. As shown in Table 1, no whiteflies were found to be infected after 0, 4, 6, or 12 hours of AAP (0/100; 0%). In contrast, 40 out of 100 whiteflies (40%) were infected after 24 hours of virus acquisition, and this number increased to 80 out of 100 whiteflies (80%) after 48 hours of exposure.

Table 4.1 Transmission of SLCMV by *B. tabaci* after different AAP

Acquisition Access Period (Hours)	Whiteflies infected/ Whiteflies tested (No.)	Whiteflies infected (%)
0	0/100	0
4	0/100	0
6	0/100	0
12	0/100	0
24	40/100	40
48	80/100	80

4.4.2 Latent Period (LP) study

The latent period (LP) of *B. tabaci*, as assessed by PCR, showed no increase in the detectable amount of SLCMV at 0, 1-, 3-, 5-, and 7-days post-acquisition. In addition, the fluorescence in *situ* hybridization (FISH) assay failed to detect the presence of the virus in the whitefly samples at any of the tested time points.

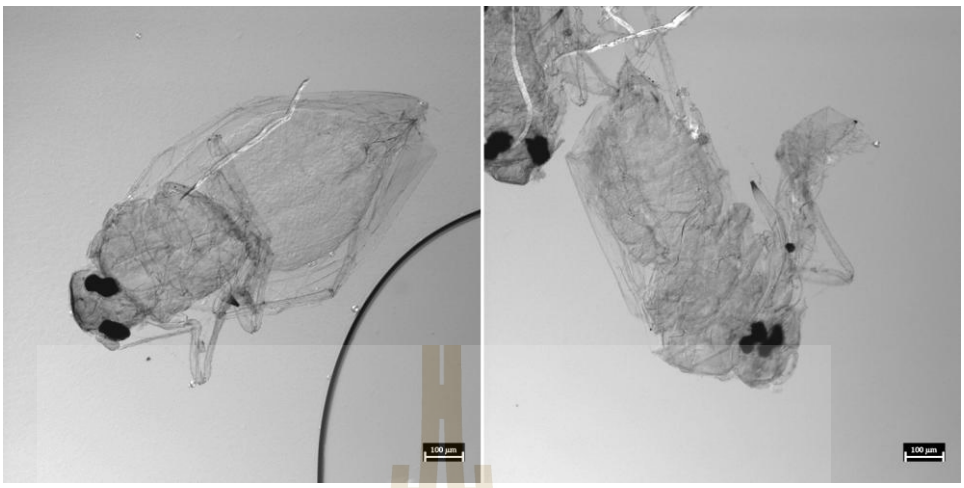


Figure 4.1 Whiteflies by using the Fluorescent in *Situ* Hybridization (FISH) technique

4.4.1 Inoculation Access Period (IAP) Study

The effect of the IAP on SLCMV transmission to cassava plants is summarized in Table 2. No infections were detected when whiteflies were given IAPs of 0, 4, 6, or 12 hours. In contrast, virus transmission was observed when the IAP was extended to 24 and 48 hours. After 24 hours, 2 out of 10 cassava plants became infected, representing a 20% transmission rate. A higher infection rate was recorded at 48 hours, with 6 out of 10 plants infected, corresponding to 60%.

Typical SLCMV symptoms were only observed in cassava plants subjected to the 48-hour IAP. The first visible signs, including mosaic leaf patterns and lamina deformation (Figure 2), appeared approximately 65 days after inoculation. These symptoms progressively intensified over time, confirming systemic infection and the establishment of viral replication in the host. In contrast, cassava plants exposed to IAP of 0, 4, 6, 12, and 24 hours remained symptom-free throughout the observation period.

Table 4.2 Transmission of SLCMV by *B. tabaci* after different inoculation access period (IAP)

Inoculation Access Period (Hours)	Cassava infected/ Cassava tested (No.)	Cassava infected (%)
0	0/10	0
4	0/10	0
6	0/10	0
12	0/10	0
24	2/10	20
48	6/10	60



Figure 4.2 The cassava shows disease symptoms after being inoculated

4.5 Discussion

The present study demonstrates that the successful acquisition and transmission of SLCMV by *B. tabaci* requires extended feeding periods, with transmission efficiency markedly increasing after 48 hours. Specifically, no infections were detected at shorter IAPs, while a 20% infection rate was recorded at 24 hours and a 60% rate at 48 hours. These results underscore the importance of prolonged acquisition and inoculation access periods in facilitating SLCMV transmission. This finding is consistent with previous studies on cassava mosaic begomoviruses. Antony et al. (2006) reported that Indian cassava mosaic virus (ICMV) required 48 hours of both AAP and IAP for effective transmission. Similarly, Duraisamy et al. (2013) and Kumar et

al. (2014) showed that SLCMV acquisition is most efficient within the 24–48-hour range. In contrast, ACMV can be acquired within as little as three hours, although higher transmission efficiency is achieved after longer feeding durations (Legg, 2008). Taken together, these findings highlight the central role of sufficient acquisition and inoculation time in the epidemiology of cassava mosaic begomoviruses.

Interestingly, the LP of *B. tabaci* in relation to SLCMV transmission revealed no detectable increase in viral load over a 0–7-day period based on PCR assays, and no viral signals were observed in FISH analyses. This suggests that, unlike viruses with short or negligible latent periods, SLCMV does not replicate or accumulate detectably within *B. tabaci*. A similar pattern has been described for other plant viruses. For example, cassava brown streak virus (CBSV) has an LP of 5–10 minutes (Maruthi et al., 2005), whereas squash vein yellowing virus (SqVYV) requires approximately 3 hours (Wintermantel et al., 2008). Semi-persistent viruses, such as criniviruses (e.g., lettuce infectious yellows virus), typically exhibit no latent period at all, as viral particles are retained in the foregut or stylets and can be transmitted almost immediately (Ng and Falk, 2006). The absence of a detectable LP in this study indicates that SLCMV may share features with persistently transmitted begomoviruses in which the virus requires prolonged acquisition but does not multiply within the vector.

Our inoculation data also align with previous reports of extended IAP requirements for begomoviruses. Firmino et al. (2009) demonstrated that tomato yellow vein streak virus (TYVSV) required an incubation period of at least 24 hours for successful inoculation. Likewise, both ICMV and SLCMV have been shown to require 24–48 hours for efficient inoculation (Antony et al., 2006; Kumar et al., 2014). In the current study, while 20% of cassava plants exposed to a 24-hour IAP tested positive for SLCMV, none developed CMD symptoms during the observation period. This suggests that infections initiated under shorter IAPs may remain asymptomatic due to low initial viral titers or delayed systemic movement. Similar symptomless infections have been described in earlier studies, in which latent or low-titer infections went undetected solely on the basis of symptom expression (Thresh et al., 1994; Legg and Fauquet, 2004; Maruthi et al., 2017). The detection of such pre-symptomatic infections emphasizes the risk of “silent carriers,” which may act as hidden reservoirs for further disease spread (Legg et al., 2011; Patil and Fauquet, 2009).

In contrast, cassava plants subjected to a 48-hour IAP not only tested positive for SLCMV but also developed typical CMD symptoms approximately 65 days post-inoculation. Although this incubation period is longer than the commonly reported 3–8 weeks (Thresh et al., 1994; Fargette et al., 2006; Duraisamy et al., 2012; Maruthi et al., 2017), it remains within the plausible biological range. The extended symptom onset may reflect a low initial inoculum load or inherent host tolerance. Moreover, the gradual intensification of symptoms confirmed systemic infection and viral establishment. Such variability in incubation periods has also been reported for cassava-infecting viruses; for example, Anitha et al. (2020) observed SLCMV symptom expression ranging from 6 to 49 days, depending on host genotype. This variability highlights the complex interplay between virus load, host susceptibility, and environmental factors in determining disease outcomes (Legg et al., 2015; Kumar et al., 2014).

Overall, the results of this study confirm that SLCMV transmission by *B. tabaci* depends on extended acquisition and inoculation periods, whereas latent period dynamics differ from those of semi-persistent viruses. Importantly, asymptomatic infections detected at shorter IAPs underscore the limitations of symptom-based surveillance and the potential role of molecular diagnostics in CMD management. These findings not only advance our understanding of SLCMV–vector interactions but also provide insights essential for developing integrated disease management strategies to reduce the spread of CMD and improve cassava productivity.

4.6 Conclusion

This study confirms that the acquisition and transmission of SLCMV by *B. tabaci* require extended feeding and inoculation periods. A minimum of 24 hours is necessary for both acquisition and inoculation, with transmission efficiency substantially increasing after 48 hours. Notably, plants exposed for 24 hours tested positive for SLCMV without developing symptoms, suggesting pre-symptomatic or low-titer infections that may serve as hidden inoculum sources. No latent period was detected, further emphasizing the virus's rapid transmission potential once acquired. Symptom expressions in plants subjected to a 48-hour IAP emerged approximately 65 days post-

inoculation, confirming systemic infection within a biologically plausible timeframe. Collectively, these findings underscore the critical role of adequate feeding duration in CMD epidemiology and provide valuable insights for designing more effective vector- and disease-management strategies for cassava production.

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CHAPTER V

GENERAL DISCUSSION AND CONCLUSION

5.1 General discussion and conclusion

This thesis investigated two interconnected aspects of cassava–whitefly–virus interactions: (i) the role of trichome density across cassava cultivars in influencing *B. tabaci* infestations, and (ii) the transmission characteristics of SLCMV by *B. tabaci*. Together, these findings provide an integrated perspective on how plant morphological defenses and vector–virus biology shape cassava health and the epidemiology of CMD.

Whitefly infestation levels differed significantly among cassava cultivars and were strongly associated with trichome density. Cultivars such as Huaybong 60 and Rayong 72, which exhibited higher trichome densities, supported fewer whiteflies, while cultivars with lower trichome densities (e.g., CMR 89, Kasetsart 50, Rayong 4, Pirun 2) were more susceptible. Trichomes act as physical barriers, restricting whitefly landing, feeding, and oviposition, thereby reducing colonization and reproduction. This pattern is consistent with previous research on other crops, in which dense trichomes suppress whitefly survival and oviposition (Simmons et al., 2003; Lambert and Shah, 1999; Andrade et al., 2017). Although trichome size showed variable effects, density consistently emerged as the dominant resistance factor. The non-glandular trichomes observed in all cultivars further reinforce this physical barrier, underscoring the importance of structural traits as a first line of plant defense.

The investigation of SLCMV transmission dynamics revealed that successful virus acquisition by *B. tabaci* required at least 24 hours of feeding, with efficiency increasing markedly after 48 hours. Inoculation followed a similar trend: infections were detectable after 24 hours of exposure, although these plants often remained detectable after 24 hours of exposure, although these plants often remained symptomless, indicating pre-symptomatic or low-titer infections. Such “silent carriers” pose a significant epidemiological risk, as they may act as hidden reservoirs for virus spread. By contrast, plants exposed to a 48-hour IAP developed visible CMD symptoms

viruses. These findings align with earlier reports on Indian and African cassava mosaic viruses, which also require extended feeding durations for efficient transmission (Antony et al., 2006; Kumar et al., 2014; Legg, 2008).

Integrating these results highlights the dual importance of host plant traits and vector–virus interactions in cassava disease dynamics. On the one hand, high trichome density reduces whitefly colonization and, indirectly, limits opportunities for virus transmission. On the other hand, the efficiency of SLCMV acquisition and inoculation underscores that even moderate whitefly infestations may contribute significantly to CMD spread when feeding durations are sufficiently long. Environmental factors, including temperature and photoperiod, further modulate whitefly population growth in the field, with warmer and longer-day conditions favoring outbreaks and amplifying the risk of virus transmission.

From a management perspective, these findings offer valuable implications for breeding and integrated pest management. Incorporating high trichome density into elite cassava cultivars presents a promising avenue for developing durable resistance to whiteflies and reducing transmission pressure. At the same time, the time-dependent nature of SLCMV acquisition and inoculation emphasizes the need for early intervention to minimize feeding opportunities, particularly during peak vector population periods. Monitoring asymptomatic infections is also critical, as reliance on visible symptoms alone may underestimate disease incidence.

In conclusion, this thesis demonstrates that trichome density is a key morphological defense trait conferring resistance to *B. tabaci*, while extended feeding periods are essential for efficient SLCMV transmission and systemic infection. The integration of plant resistance traits with insights into vector–virus transmission biology underscores the necessity of a multifaceted approach to CMD management. Future research should prioritize breeding for high-trichome cultivars, refining diagnostic tools to detect asymptomatic infections, and implementing integrated pest management strategies that account for seasonal vector dynamics. Collectively, these approaches will enhance cassava resilience, safeguard productivity, and reduce the burden of CMD in cassava-growing regions.

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APPENDIX

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Reagents

I Reagents for genomic DNA preparation

1. Insect DNA extraction buffer

1M Tris pH 8.0:	100 µl
0.5 M EDTA pH:	25 µl
1M NaCl:	25 µl
10% SDS:	25 µl
Proteinase K (2 mg/ml):	25 µl

Add sterile distilled water to the final volume of 500 ml

2. Cassava DNA extraction buffer (2X CTAB buffer)

1 M Tris pH 8.0:	50 ml
0.5 EDTA pH 8.0:	20 ml
NaCl:	40.91 g
CTAB:	10 g

Add distilled water to reach a final volume of 490 ml, then autoclave. Put the buffer at room temperature until it reaches 60 °C, then add 2% CTAB and bring the final volume to 500 ml. Add 0.02% mercaptoethanol before use.

3. 1 M Tris pH 8.0

Tris (hydroxymethyl) aminomethane: 121.14 g

Dissolve 121.4 g in 800 mL of distilled water, then adjust the pH to 8.0 with the appropriate volume of concentrated HCl. Bring the final volume to 1 L with distilled water. Autoclave and then store at room temperature.

4. 0.5 EDTA pH 8.0

Ethylenediaminetetraacetic acid disodium salt $2H_2O$

Dissolve 186.1 g in 800 mL of distilled water and approximately 20 g of NaOH pellets while stirring to adjust the pH to 8.0. Bring the final volume to 1 L with distilled water. Autoclave and then store at room temperature.

5. 1M NaCl

Sodium chloride: 5.88 g

Dissolve 5.88 g in 80 mL of distilled water, then add distilled water to a final volume of 100 mL. Autoclave and store at room temperature.

6. 3M Sodium acetate pH 5.2

Sodium acetate trihydrate: 40.8 g

Dissolve 40.8 g in 80 mL of distilled water, adjust the pH to 5.2, and then add distilled water to a final volume of 100 mL. Autoclave and store at room temperature.

7. 10% SDS

Sodium dodecyl sulfate: 10 g

Dissolve 10 g in 80 mL of sterile, deionized water, and then add sterile, deionized water to a final volume of 100 mL. Store at room temperature.

8. 1X TE buffer

10 mM Tris pH 8.0

0.1 mM EDTA pH 8.0

Add distilled water to the final volume of 100 ml, autoclave, and then store at room temperature.

9. Proteinase K (20 mg/ml), Store -20 °C

10. RNase A (10 mg/ml), Store -20 °C

11. Chloroform/Isoamyl alcohol (24:1)

Chloroform: 24 ml

Isoamyl alcohol: 1 ml

Store at room temperature

12. Phenol/Chloroform/Isoamyl alcohol (25:24:1)

Phenol:	25 ml
Chloroform:	24 ml
Isoamyl alcohol:	1 ml

Store at 4 °C

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II Reagents for Fluorescent in situ hybridization (FISH)

1. 6% H_2O_2 (Hydrogen peroxide) in 100% Ethanol

Ethanol: 94 ml

H_2O_2: 6 ml

2. PBST (Phosphate buffer saline with Tween 20)

1X PBS	50 ml
0.02% Tween20	1 ml

Store at room temperature

3. Hybridization buffer 500 µl

Stock reagent	Volume (µl)	Final concentration
5M NaCl	90	900 mM
1M Tris	10	20 mM
Formamide	150	30%
5% SDS	0.5	0.01%
H_2O	249.5	

4. Hybridization buffer and probe 500 µl

Stock reagent	Volume (µl)	Final concentration
5M NaCl	90	900 mM
1M TRIS	10	20 mM
Formamide	150	30%
10 µM Probe	5	100 nM
5% SDS	0.5	0.01%
Distilled water	244.5	

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III Reagents for PCR

1. Primers were used in PCR amplification

No.	Primer name	Sequence (5'-3')
1	AV1 (Forward)	GTTGAAGGTACTTATTCCC
2	AV1 (Reverse)	TATTAATACGGTTGTAAACGC

2. PCR reaction mixture

Components	Volume (µl)	Final concentration
10X PCR buffer, Minus Magnesium Chloride	2	1X
10 mM dNTP mixture	0.4	0.2 mM each
50 mM Magnesium Chloride	0.6	1.5 mM
10 µM forward primer	0.5	0.5 µM
10 µM reverse primer	0.5	0.5 µM
Template DNA	2	
Taq DNA Polymerase	0.1	
Sterile Distilled water	13.9	

3. PCR condition

PCR program	Temperature (°C)	Time (min)	
Initial denaturation	94	5	
Denaturation	94	0.4	35 cycles
Annealing	55	0.4	
Extension	72	0.4	
Final extension	72	5	

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Gel electrophoresis of cassava and whitefly

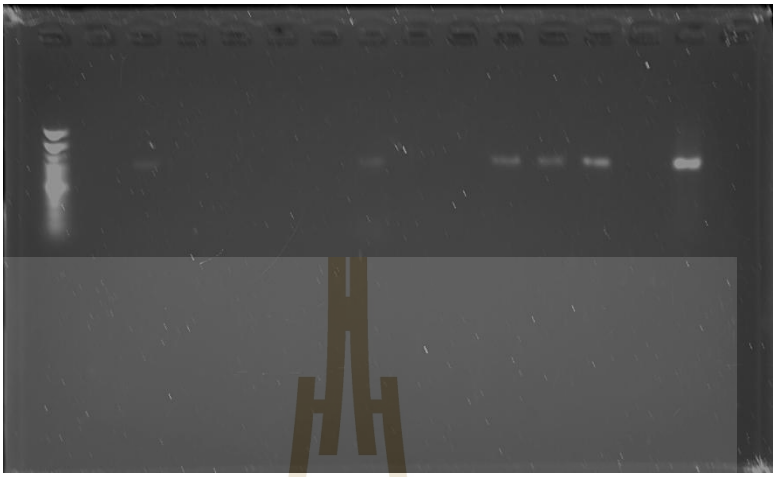


Figure 1. Positive and negative results of SLCMV virus testing in cassava.



Figure 2. Positive and negative results of SLCMV virus testing in whiteflies.

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Trichomes of six cassava cultivars

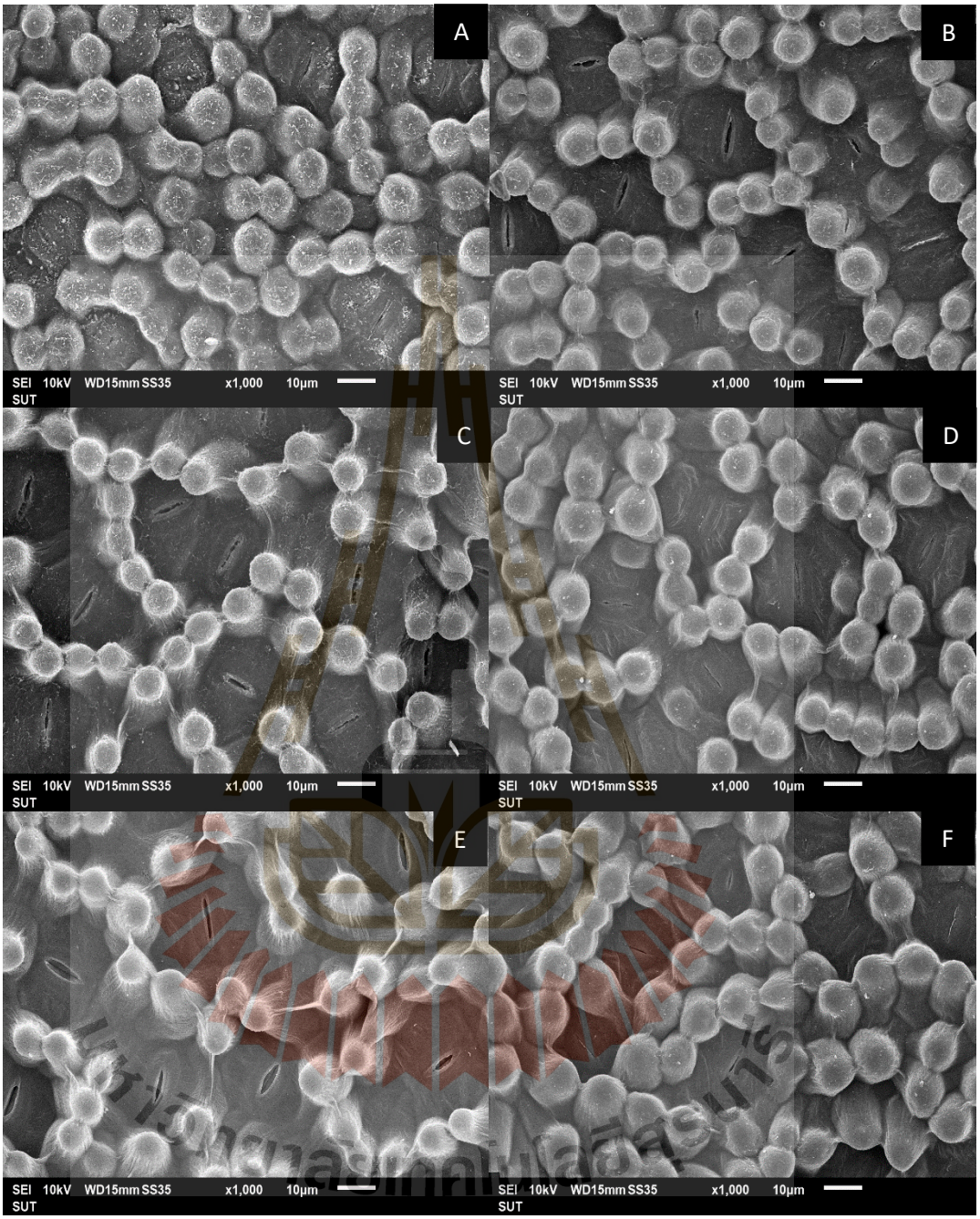


Figure 3. Trichomes of six cassava cultivars were observed through scanning electron microscopy (SEM) at 1000x magnification. Images show the leaf surface of (A) Huaybong 60, (B) CMR 89, (C) Kasetsart 50, (D) Rayong 72, (E) Rayong 4, and (F) Pirun 2

BIOGRAPHY

Ms. Supawadee Prombut was born in Thailand. She received a Bachelor of Science degree in Crop Production Technology from Suranaree University of Technology, Nakhon Ratchasima, Thailand, in 2021. During her undergraduate studies, she was awarded the Sakayabundit Scholarship, which supported her tuition fees. She later pursued a Master of Science degree in Entomology at Suranaree University of Technology from 2022 to 2025, supported by the OROG Scholarship. During her graduate studies, she conducted research in the field of entomology with a focus on insect–plant interactions, particularly whitefly (*B. tabaci*) behavior and cassava resistance traits. She has published research articles in national and international peer-reviewed journals, including Khon Kaen Agriculture Journal, Plants, and Food Agricultural Sciences and Technology (FAST). In addition, she presented her research at the International Conference on Biodiversity, Science, and Technology, held on January 16–17, 2025, in Pattaya, Thailand, where she delivered an oral presentation entitled "Trichome Density of Cassava Impact on Whitefly (*Bemisia tabaci*) Infestation."

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