Screening of Oil Palm Varieties Resistant against the Leaf Spot Disease Caused by *Curvularia oryzae* Bugnic.

Jittra Kittimorakul

A Thesis Submitted in Partial Fulfillment of the Requirements for the Doctor of Philosophy in Plant Science
Prince of Songkla University
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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Candidate

ชื่อวิทยานิพนธ์ การคัดเลือกพันธุ์ปาล์มน้ำมันเพื่อต้านทานต่อโรคใบจุดที่เกิดจากเชื้อรา *Curvularia oryzae* Bugnic.

ผู้เขียน นางสาวจิตรา กิตติโมรากุล

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บทคัดย่อ

โรคใบจุดของปาล์มน้ำมันที่เกิดจากเชื้อรา *Curvularia oryzae* สามารถสร้างความเสียหายอย่างมากทั้งในด้านคุณภาพและปริมาณของต้นกล้าปาล์มน้ำมันในประเทศไทย การใช้สารเคมีกำจัดเชื้อราเป็นวิธีที่ได้รับความนิยมเพื่อควบคุมการแพร่กระจายของเชื้อสาเหตุโรค แต่เมื่อใช้สารเคมีกำจัดเชื้อราติดต่อกันเป็นระยะเวลานาน เชื้อสาเหตุโรคสามารถพัฒนาตัวเองขึ้นเพื่อต้านทานต่อสารเคมี ดังนั้นวิธีการควบคุมโรคใบจุดอีกทางหนึ่งที่มีความปลอดภัยต่อเกษตรกรและสิ่งแวดล้อม คือ การคัดเลือกพันธุ์ปาล์มน้ำมันที่ต้านทานต่อโรคใบจุด ในการศึกษาครั้งนี้ได้ทำการทดสอบและคัดเลือกจากแม่พันธุ์ปาล์มน้ำมันจำนวน 122 หมายเลข พ่อพันธุ์ 2 หมายเลข และ 4 พันธุ์การค้า (A B C และ ทรัพย์ มอ.1) ด้วยวิธี detached leaf หลังการปลูกเชื้อเป็นเวลา 2 สัปดาห์ แม่พันธุ์ปาล์มน้ำมันจำนวน 13 หมายเลข ต้านทานโรคใบจุดได้ในระดับสูงมาก (0% ระดับการปรากฏของโรค) ในขณะที่หมายเลข 129 และพันธุ์การค้า B อ่อนแอต่อโรคมากที่สุด (100% ระดับการปรากฏของโรค) จากแม่พันธุ์ปาล์มน้ำมันที่ต้านทานโรคได้ในระดับสูง 13 หมายเลข ทำการทดสอบการถ่ายทอดความต้านทานโรคใบจุดสู่ปาล์มน้ำมันพันธุ์ลูกผสมเทเนอรา บนต้นกล้าปาล์มน้ำมันภายในเรือนทดลองด้วยวิธี pathogenicity test จำนวน 9 หมายเลข เปรียบเทียบกับหมายเลขที่อ่อนแอต่อโรคใบจุด จากการทดลองพบว่า ปาล์มน้ำมันพันธุ์ลูกผสมหมายเลข 187 ต้านทานต่อโรคได้สูงที่สุด โดยเริ่มแสดงอาการของโรคใบจุดที่ 12 วัน หลังการปลูกเชื้อ มีระดับการเกิดโรค ที่ 0.25 ระดับความรุนแรงของโรคที่ 5% หลังการปลูกเชื้อ 20 วัน และมีกิจกรรมของ PR-proteins (chitinase และ β-1,3-glucanase) ที่ 48 ชั่วโมงหลังการปลูกเชื้อสูงที่สุด ที่ 17.84 ± 1.46 และ 14.23 ± 1.3 U mL^{-1} ตามลำดับ ซึ่งแตกต่างอย่างมีนัยสำคัญกับหมายเลขอ่อนแอ (หมายเลข 129) ที่พบอาการเกิดโรคที่ 3 วัน หลังการปลูกเชื้อ มีระดับการเกิดโรค ที่ 4.55 ระดับความรุนแรงของโรคถึง 91% หลังการปลูกเชื้อ 20 วัน และมีกิจกรรม

ของ PR-proteins ที่ 48 ชั่วโมงหลังการปลูกเชื้อต่ำที่สุด ที่ 3.27 ± 0.27 และ 2.05 ± 0.70 U mL^{-1} ดังนั้นเพื่อจำแนกพันธุ์ลูกผสมเป็นสายพันธุ์อ่อนแอและสายพันธุ์ต้านทาน จึงคัดเลือกปาล์มน้ำมันพันธุ์ลูกผสมหมายเลข 129 และ 187 มาทดสอบเปรียบเทียบกิจกรรมของ PR-proteins ทั้ง 2 ชนิด โดยวัดกิจกรรมของเอนไซม์ทุก ๆ 24 ชม. ตั้งแต่ 0 - 168 ชม. พบว่า หมายเลข 187 มีกิจกรรมของ PR-proteins ทั้ง 2 ชนิดได้สูงกว่าหมายเลข 129 ตั้งแต่ 24 - 168 ชม. โดยหลังการปลูกเชื้อ 48 ชั่วโมง หมายเลข 187 มีกิจกรรมของเอนไซม์ chitinase และ β-1,3-glucanase ได้สูงที่สุดที่ 14.03 ± 0.87 และ 13.51 ± 1.04 U mL^{-1} ในขณะที่หมายเลข 129 มีกิจกรรมของเอนไซม์ chitinase และ β-1,3-glucanase ที่ 3.76 ± 0.41 และ 4.31 ± 0.83 U mL-1 เมื่อวิเคราะห์การสะสมของเอนไซม์ด้วยวิธี SDS-PAGE พบว่าหมายเลข 187 พบน้ำหนักโมเลกุล PR-proteins ที่ 22 25 และ 33 kDa ในขณะที่ในหมายเลข 129 ไม่มีการปรากฏน้ำหนักโมเลกุลของ PR-proteins นอกจากนี้เมื่อทดสอบต้นกล้าปาล์มน้ำมันพันธุ์ลูกผสมเทเนอราจากหมายเลขต้านทาน 3 หมายเลข (138 187 และ 203) เพื่อทดสอบความต้านทานต่อโรคใบจุดภายในสภาพแปลงปลูกของเกษตรกรในอำเภอเหนือคลอง จังหวัดกระบี่ และอำเภอย่านตาขาว จังหวัดตรัง ตั้งแต่เดือนกุมภาพันธ์ 2559 ถึง เดือนตุลาคม 2559 พบว่าทั้ง 3 หมายเลขสามารถต้านทานต่อโรคใบจุดได้ดีกว่าพันธุ์การค้าของเกษตรกรโดยเฉพาะอย่างยิ่งในเดือนกรกฎาคม 2559 (การเก็บข้อมูลครั้งที่ 2) ซึ่งเป็นฤดูฝนที่มีการระบาดอย่างหนักของโรคใบจุดในต้นกล้าปาล์มน้ำมัน ดังนั้นปาล์มน้ำมันทั้ง 3 สายพันธุ์ คือ 138 187 และ 203 จึงสามารถนำไปใช้เป็นข้อมูลพื้นฐานต่อการปรับปรุงและพัฒนาปาล์มน้ำมันสายพันธุ์ใหม่ที่สามารถต้านทานต่อโรคใบจุดของปาล์มน้ำมันได้

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Academic Year	2018

ABSTRACT

Curvularia leaf spot (CLS) is one of the diseases caused by *Curvularia oryzae* that can devastatingly damage both quality and quantity of oil palm seedlings in Thailand. Chemical fungicides have been intensively applied to control transmission of this disease. However, pathogen gains resistance to fungicide and causes low effectively. An alternative or complementary cost-effective and environmentally friendly approach is to find resistant varieties for disease management. In this study, a total of 122 lines of Dura female plant, 2 lines of Pisifera male plant, and 4 Tenera commercial varieties (A, B, C, and SUP-PSU1) were screened by *Curvularia* inoculation using detached leaf method. Two weeks after inoculation, the results showed 13 Dura lines were highly resistant to CLS (0% disease incidence), whereas line 129 and the commercial variety B were highly susceptible (100% disease incidence). From 13 Dura highly resistant lines, nine Tenera hybrid lines were selected to test against CLS under greenhouse conditions by pathogenicity test. Among nine Tenera hybrid lines, disease symptom was found to delay up to 12 days after inoculation in line 187. The disease score was 0.25 and disease incidence was 5% at 20 days after inoculation. Enzyme activity at 48 h of chitinase and β-1,3-glucanase after inoculations were 17.84 ± 1.46 and 14.23 ± 1.31 U mL^{-1}, respectively, higher than that of susceptible lines. CLS disease was found at 3 days after inoculation at the susceptible line 129. The study found that disease score was 4.55, disease incidence was 91% at 20 days after inoculation, and the lowest PR-proteins at 48 h after inoculation at 3.27 ± 0.27 and 2.05 ± 0.70 U mL^{-1}. To classify hybrid crosses as susceptible and resistant variety, the hybrid line 129 and line 187 were selected to test

chitinase and β-1,3-glucanase activities every 24 h from 0 to 168 h. The results showed enzyme assay of line 187 could express both of PR-proteins higher than line 129 from 24 to 168 h. At 48 h after inoculation, line 187 could express the highest chitinase and β-1,3-glucanase activities at 14.03 ± 0.87 and 13.51 ± 1.04 U mL^{-1} while line 129 could express chitinase and β-1,3-glucanase activities at 3.76 ± 0.41 and 4.31 ± 0.83 U mL^{-1}. The SDS-PAGE showed accumulation of 22, 25, and 33 kDa. chitinase and β-1,3-glucanase proteins in inoculated line 187, whereas no chitinase and β-1,3-glucanase proteins were observed in line 129. In addition, 3 oil palm Tenera hybrid genotypes (line 138, line 187, and line 203) were tested for field conditions at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province from February 2016 to October 2016. The results showed the selected Tenera hybrid genotypes had more resistance than commercial varieties especially during a raining season in July (2^{nd} data recorded) that was severe CLS disease in oil palm nursery. This study suggested that 3 oil palm Tenera hybrid genotypes line 138, line 187, and line 203 were candidates to be a database and useful for breeding and developing new oil palm variety resistant to CLS disease.

ACKNOWLEDGEMENT

I would like to express my sincere thanks to my thesis advisor, Professor Dr. Theera Eksomtramage for his invaluable help and constant encouragement throughout the course of this research. I am most grateful for his teaching and advice for not only the research methodologies but also many other methodologies in life. And also, this expression of deep appreciation goes to my co-advisor, Associate Professor Dr. Anurag Sunpapao for encouragement, advice, and support during my study. My thanks also go to Dr. Saowapa Duangpan, Associate Professor Dr. Vasun Petcharat for serving as an examining committee supervisor and Assistant Professor Dr. Prawat Wettayaprasit for correcting all grammars of my thesis. This thesis would not have been completed without all the support that I have always received.

I am grateful to PSU Ph.D. scholarship, PSU Graduated School, Oil Palm Agronomical Research Center: Phase 2, and Center of Excellence in Agricultural and Natural Resources Biotechnology, Prince of Songkla University for partial financial support.

In addition, I greatly appreciate for Dr. Natthapach Srihanant, Assistant Professor Dr. Chaninun Pornsuriya, Assistant Professor Dr. Thanunchanok Chairin and Assistant Professor Dr. Narit Thaochan for all suggestions, encouragement, supporting, and teaching all technical skills in my laboratory work and my publications.

Finally, I would like to thank my friends, seniors, and juniors in the Oil Palm Breeding Laboratory, Plant Science Graduate Club in the Department of Plant Science and Department of Pest Management for their experimenting facilitation of my thesis. I especially appreciate my family and lovely friends for their support, love, encouragement, and serving throughout the period of this research.

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LIST OF ABBREVIATIONS

CLS	*Curvularia* leaf spot
DW	Distilled water
µm	Micrometer
PR-protein	Pathogenesis-related protein
cm	Centimeter
mm	Millimeter
HR	Highly resistant
R	Resistant
MR	Moderately resistant
MS	Moderately susceptible
S	Susceptible
HS	Highly susceptible
ml	Milliliter
µl	Microliter
mM	Millimole
DNS	Dinitrosalicylic acid solution
U mL^{-1}	Unit per milliliter
BSA	Bovine serum albumin
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
V	Voltage
nm	Nanometer
LSD	Least significant difference
CRD	Completely randomized design

CHAPTER 1

INTRODUCTION

1. Background

Oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous and an important economic crop with the highest oil production compared with the other vegetable oil plants (Murphy, 2014). Oil palm is an eco-friendly plant because ecology areas damaged from deforestation will be recovered after planting oil palms for several years. Moreover, the production of oil palm can be used for consumption, food industry, the human cosmetics industry, and renewable energy.

The main factors that should be considered for supporting oil palm production include plantation area, diseases, agronomic methods, environment, and high-performance variety. Recently, *Curvularia* leaf spot (CLS) disease caused by *Curvularia oryzae* Bugnic. has become a problematic issue of producing oil palm with high-performance variety seedling at the nursery stage (Sunpapao *et al.*, 2014). Turner (1981) mentioned that the disease could primarily affect seedlings up to 3 months old or seedlings which had recently been transplanted. The outbreak of this disease usually occurs in the raining season. Once the outbreak occurs, the disease will spread rapidly throughout the whole oil palm nurseries. The only measure that the farmers have been used to control the disease is the application of fungicides including antracol, captan, mancozeb, and prochloraz. However, the results have not yet satisfied as the *C. oryzae* becomes resistant to these fungicides. Also, chemicals used can contaminate and have a negative effect on environmental pollution, agricultural ecology, human health, and animal health.

Normally, integrated plant disease management must be managed by many methods to succeed in plant disease control such as mechanical control, chemical control, biological control, and resistant variety (Khoury and Makkouk, 2010). In every plant breeding program, screening and selection of plant segregating population are important data to develop and to get the homogeneity variety. Oil palm breeding program for high-performance variety was conducted for breeding between Dura (mother) plant and Pisifera (father) for the purpose of Tenera hybrid variety which will have better performance than a parent. Oil palm Tenera hybrid variety SUP-PSU1 was crossed and developed under the Oil Palm Breeding Project at the Faculty of Natural Resources, Prince of Songkla University, Thailand since 1997 (Eksomtramage *et al.,* 2009). This variety showed high performance with high yield and stability in the South of Thailand and has already been recommended for farmers (Eksomtramage, 2011). However, oil palm Tenera hybrid variety that has resistance for CLS disease has never been reported before. Thus, the resistance to CLS disease of this oil palm SUP-PSU germplasm should be considered to increase good characteristic for breeding new variety with quality and quantity characteristic. Moreover, the pathogenesis-related (PR) proteins that respond to pathogen attack in oil palm resistant for CLS disease are studied also for understanding and will be compared with commercial variety under both greenhouse and farmer's nurseries conditions.

Oil palm Tenera hybrid, the offspring received from the Oil palm parent breeding stocks in the Oil Palm Breeding Project at the Faculty of Natural Resources, Prince of Songkla University were screened, selected, and tested for CLS disease resistant performance, and propagation. To understand the mechanism's response to CLS disease in resistant lines, Tenera resistant hybrid lines were investigated for PR-proteins (chitinase and β-1,3-glucanase) by the method of Bradford (1976) and SDS-PAGE analysis. In addition, the resistant lines were estimated and compared with the commercial varieties under farmer's nurseries condition. The information of oil palm breeding stocks, their offspring, and understanding of PR-proteins mechanism in resistant

variety defense will be evaluated for the purpose of developing new oil palm resistant variety which will then be recommended for farmers.

2. Literature Reviews

1. Classification and importance of oil palm

Elaeis guineensis is a species of palm commonly called "African oil palm" which is the principal source of palm oil. The species name *guineensis* refers to the name for the area (Guinea). Now, this plant is native in Madagascar, Sri Lanka, Malaysia, Sumatra, Central America, the West Indian, several areas in the Indian, and Pacific Oceans (Corley and Tinker, 2003). The classification of oil palm was reported as follows

Division Spermatophyta

Class Angiospermae

Order Arecales

Family Arecaceae

Genus *Elaeis*

Species *E. guineensis*

Oil palm is an important crop in Thailand. The major area of oil palm plantation is in Southern area, and the minor areas are Northeast and East. Its origin is believed to be in Africa. However, Indonesia, Malaysia, and Thailand are now the major exporters of oil palm to international trade for the food industry, cosmetics, and renewable energy.

The palm oil is one of the most widely used vegetable oils produced globally. Palm oil provides the highest yields per hectare compared to other oil crops. The oil extracted is used in many foods, (margarine, cup noodles, baked goods, and

sweets), household products (detergent and cosmetics), as well as biodiesel production (UNEP and UNESCO, 2007). The palm oil usage of global is increasing in every year. From the total of palm oil production, an estimated 74% for food products, and 26% is for industrial purposes (USDA, 2010). Since the 1990s, the areas occupied by oil palm cultivation have expanded worldwide, driven mainly by demand from India, China, and the European Union (FAO, 2015).

2. Oil palm varieties

Oil palm is a monoecious and cross-pollinated plant. The individual palms are usually highly heterozygous, and vegetative propagated clonal material cannot be made. The current classification of cultivars is mainly based on fruit structure and yield (Verheye, 2010). Figure 1 shows different varieties of oil palm.

Dura: Shell 2-8 mm thick, comprising 25-55% of fruit weight, medium mesocarp content of 35-55% by weight but up to 65% in Deli palms, less productive but hardy variety, well adapt to village gardens.

Pisifera: Shell-less, with small pea-like kernel in fertile fruit has little commercial value because of its high abortion ratio but has importance for cross-breeding commercial palms.

Tenera: Shell 0.5-3 mm thick; comprising 1-32% of the weight of fruit; medium to high mesocarp content of 60-95%, but occasionally as low as 55%. This variety is the result of a hybridization of Dura and Pisifera and has a high commercial value.

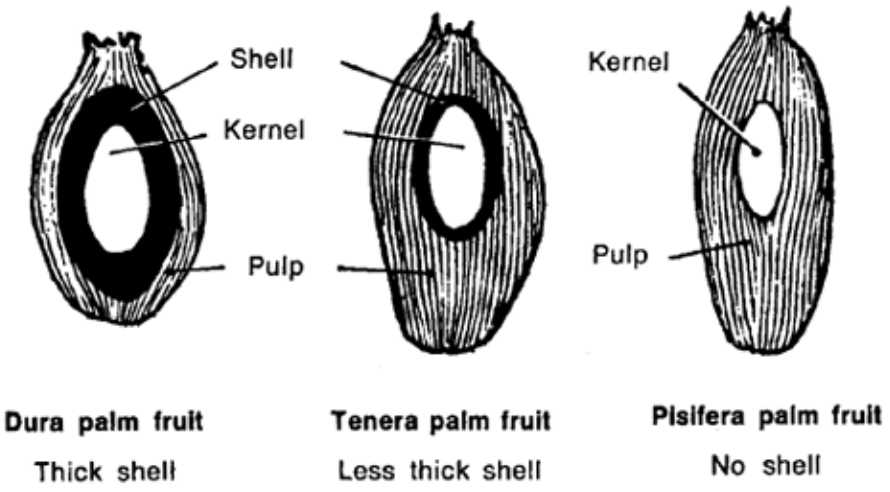

Figure 1 Different varieties of oil palm
Source: FAO (2015)

Most commercial plantations are established on the basis of Tenara palms. Oil palms may live up to 200 years, but their commercial yield rapidly decreases after 30 years of age.

3. Leaf spot disease of oil palm

Leaf spot disease has been reported for causing moderate to severe damage in wide areas of Malaysia, Sabah, Sumatra, and Thailand (Turner, 1981). In Malaysia and Indonesia, *C. lunata*, *C. affinis*, *C. maculans*, and *Colletotrichum gloeosporiodes* have been recorded as causal agents of leaf spot and leaf blight in seedling of oil palm (Englert *et al.*, 1999; Asril *et al.*, 2014). In Indonesia, the disease was the secondary problem in oil palm nursery seedlings. But in Thailand, CLS disease has been recorded as a serious disease since the year 2010 (Pornsuriya *et al.*, 2013). In 2013, the leaf spot disease caused by *Curvularia* sp. was reported at 61.01% in Southern Thailand (Kittimorakul *et al.*, 2013b). The symptomatic leaf samples were

isolated oil palm seedling, pathogenicity tested, and characterized by morphological properties as follows.

Division Ascomycota

Class Ascomycetes

Order Pleosporales

Family Pleosporaceae

Genus *Curvularia*

After identified by morphological characteristic, *Curvularia* sp. cause of oil palm leaf spot was confirmed through polymerase chain reaction analysis. The nucleotide sequences revealed that oil palm leaf spot causal organism was *C. oryzae* (Sunpapao *et al.*, 2014).

The CLS disease symptom includes the appearance of small yellow spots on leaves. The progress of symptom starts from light brown with changing to dark brown and will cover almost all parts of the leaves. Finally, the infected leaves turn black. The colony of *C. oryzae* is dark or dark brown after 7 days. Figure 2B shows the conidiophore is single with one conidium, simple or branched, straight or flexuous, and brown to dark brown. The conidium is 3-distoseptate, approximately ovoid, obclavate or almost elliptical with the second cell from the base largest and 24 - 40 × 12 - 22 µm in size (Figure 2B). The life cycle of leaf spot disease begins when the spores of *C. oryzae* land on the leaf surface. Spores germinate and penetrate through leaf stomata. Small yellow spots appear on the leaves and produce the spores within individual lesion about 3 days after the spores infected. The spores can spread by wind and rain to the other young seedlings or will be survival over the season in infected oil palm residue and will spread to young seedlings again in the new crop of next year (Figure 3).

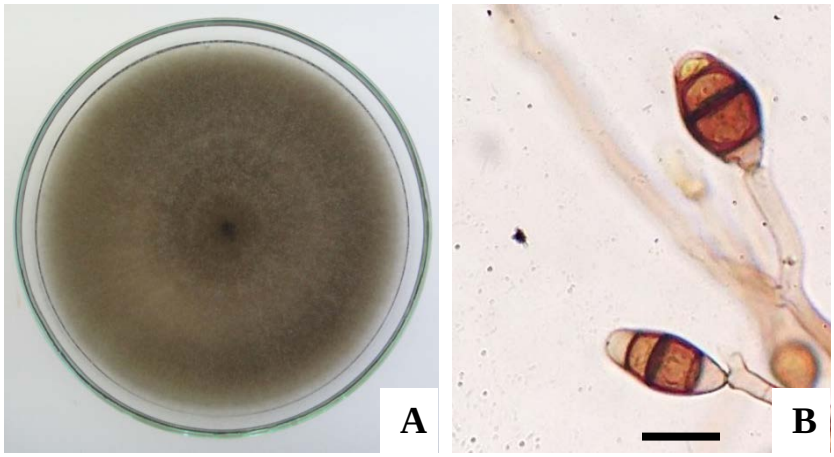

Figure 2 *Curvularia oryzae* (A) Colony of *C. oryzae* after 10 days and (B) conidiophore and conidia of *C. oryzae*, bar = 20 μm.

Source: Kittimorakul *et al.* (2013a)

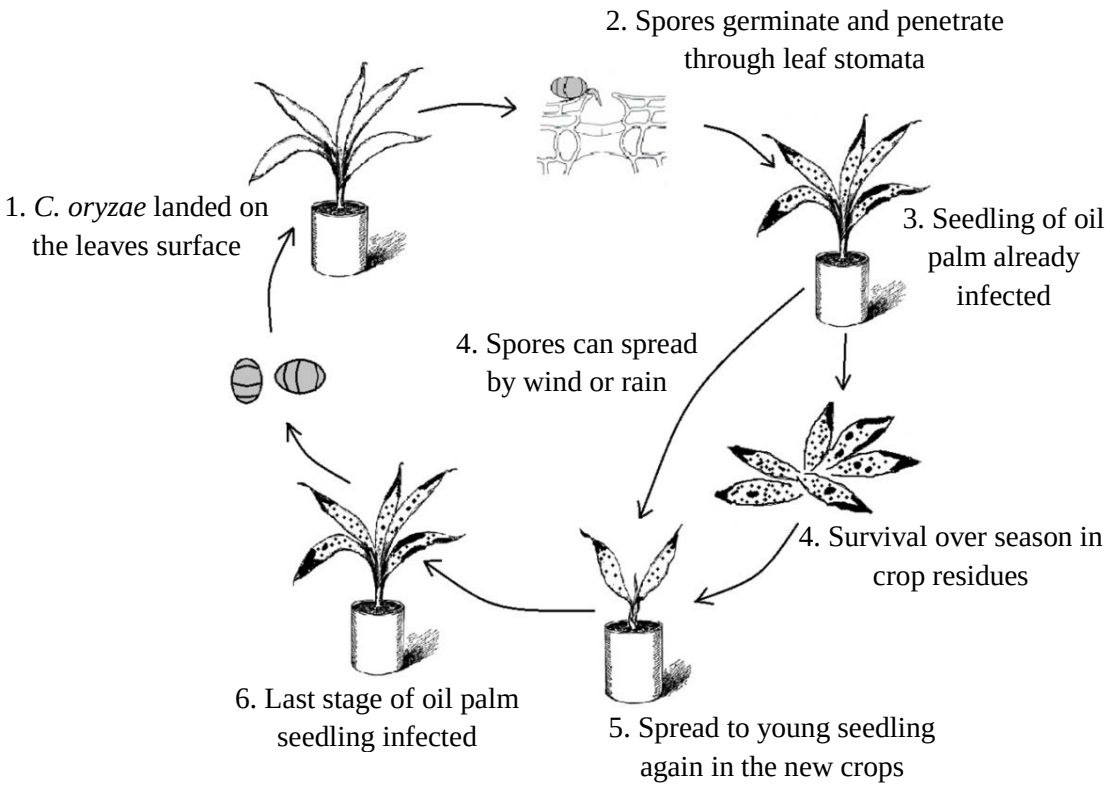

Figure 3 Life cycle of leaf spot disease caused by *Curvularia oryzae*
Source: Kittimorakul *et al.* (2013a)

C. oryzae has been reported and isolated from rice grains (de Luna *et al.*, 2002) and also causes fruit rot disease in okra (*Abelmoschus esculentus*) (Lal and Goel, 1989), but this pathogen is a minor disease of rice (Ou, 1985). However, there are a few types of research in other plants. In Thailand, this disease is also one minor disease in rice seed, but it is possible to be the major disease of oil palm seedlings because most farming areas in Southern Thailand have been changed from paddy fields to oil palm plantations. Then this pathogen must develop itself to attract new host and will be more severe when the oil palm nurseries are expanding.

The moist conditions (rainfall and irrigation) are suitable for CLS disease out breaking (Turner, 1981). This pathogen can produce a million spores which can be easily spread by wind, water, pest activity (insects), or human activity (pruning or

movement through nursery) (Elliott, 2005). Leaf moisture between 24 - 72 h can produce spores and will be critical for spore germination and infection on leaf tissue. The spacing between rows and air circulation to keep seedlings drier will help decrease pathogen infection. For minimal infection, the irrigation should be done before dawn to let leaves be wet a few hours, and the spacing between plants for air movement and full sun will help decrease germination and infection to host plants in rainy season. Moreover, leaves damaged due to water stress, sunburn, fertilizer burn, and herbicide phytotoxicity should be avoided because *C. oryzae* has been established as a saprobe (non-pathogen) on the injured tissue. Thus, leaf damage on oil palm seedlings could be the habitation of CLS disease pathogen.

4. Control of CLS disease in oil palm seedling

CLS disease usually diffuses through oil palm nursery seedling in raining season. The strategies for efficient disease control should be managed by several methods such as mechanical control, chemical control, biological control, and resistant varieties.

Mechanical control

To avoid moisture condition that supports spores germination and pathogen infection, water irrigation a few hours before dawn and the spacing area between oil palm seedlings for air movement and full sunlight should be considered. Fungal pathogens are more severe when oil palm seedlings are in less moisture condition. CLS disease should be constantly observed. The infected leaves must be pruned and destroyed to depart from nursery consistently for reducing the source of inoculum. In addition, weed control is important because most of leaf spot pathogens have plants as their hosts other than oil palm including weeds. Thus, being aware of weeds to serve as ones of CLS disease host is another concern.

Chemical control

Oil palm leaf spot disease is severe due to the favorable environment for pathogen germination caused by the spreading of raindrops and winds between neighboring nurseries during the rainy season. Most of the farmers usually spray fungicides every 7-10 days. The fungicides such as topsin, captan, thiram, prochloraz, and mancozeb have been reported for controlling *Curvularia* sp. in rice grain disease and *C. oryzae* CLS disease in oil palm seedling (Butt *et al.,* 2011; Kittimorakul *et al.,* 2013a). However, the application of fungicides not only has the effect to plant fungal pathogens but also has been known to have a negative effect on human and animal health and microorganism in agricultural soil. Furthermore, the chemical spraying in excessive dose will stimulate fungal pathogens resistance to chemicals (Whipps and Lumsden, 2001).

Biological control

Biological control aims to reduce the source of pathogen inoculum and was applied for reducing fungicides recommended for controlling oil palm leaf spot disease. This method can help to avoid the harmful effect of the chemical method by using some of microorganism called antagonists. Some antagonists (*Trichoderma* sp., *Streptomyces* spp. and *Kitasatospora nipponensis*) have been reported to test and control CLS disease of oil palm (Kittimorakul *et al.,* 2013a: Pithakkit *et al.,* 2015). Biological control was recommended to apply for protecting host plant more than controlling plant disease. The applying antagonist alternates with fungicides not only reduces chemical fungicides but also prolongs the development of fungal pathogen resistance to chemicals.

Screening and selection resistant variety

Host-plant resistance is an effective and preferable method to manage plant diseases. The resistant characteristic of plants is the quality trait of a plant breeding program. Screening and selecting of plant segregating population are important data to develop and get new resistant variety. Oil palm variety with good yield and other desirable traits have been pervasively reported and recommended for farmers, but oil palm variety resistant to CLS disease has never been reported. The resistant variety of oil palm will be able to increase the efficacy of CLS disease management. Also, such of this variety will be safe to a human and agricultural system which is relatively inexpensive for farmers working with oil palm seedling plantation as well.

5. Mechanisms of disease resistance in plants

Multicellular plants possess a broad range of mechanisms to protect themselves against various threats from some stresses especially pathogen attacks such as fungi, bacteria, and viruses (Agrios, 1997). Normally, mechanisms of disease resistance in plants can be classified into two types: 1. Physiological defense mechanism and 2. Biochemical defense mechanism.

Physiological defense mechanism

Plants have devised different strategies to defense pathogens. Physiological change for defense mechanism is like impenetrable barrier composed of bark, waxy cuticle or cell wall, cock layers, and stomata closure (Micali *et al.,* 2011; Olori-Great and Opara, 2017). The physical barriers are developed to inhibit the pathogen from gaining entrance and spreading through the plant. However, if the layers of plant protection are breached, host plants must discharge the biochemical defense mechanisms such as phytoalexin or pathogenesis-related protein (PR-protein) that are toxicity and will inhibit the growth of pathogens.

Biochemical defense mechanism

Biochemical defense mechanism is the plant's response for more resistance from pathogen attack. The roles of response are protecting themselves and eliminating the pathogen. The plant will respond some following defense mechanisms by synthesizing and accumulating callose at fungal cell wall to inhibit fungal penetration (Lucas, 1998), the phytoalexin which has toxic property to anti-plant pathogens (Buchanan *et al.*, 2000), and PR-protein that has a response to attack pathogen or stress situation. However, phytoalexins are mainly produced by healthy cells close to damaged and necrotic cells, but PR proteins are accumulated locally in the infected and surrounding tissues, and also in distant uninfected tissues (Ebrahim *et al.*, 2011). Production of PR-proteins in the uninfected tissues of the plant will protect the affected tissues from further infection (Ryals *et al.*, 1996).

6. Pathogenesis-related proteins (PR-proteins)

PR-proteins are the secondary compound released highly after fungal pathogen attacking the host plants and have different low-molecular weights between 6 to 43 kDa which can be found in all plant organs (leaf, stem, fruit, and flower) (Van Loon, 1999). However, there is the highest PR-proteins accumulation around 5-10 % of leaf protein total at the plant leaf. Presently, PR-proteins were classified into 17 families according to their functions and properties (Sels *et al.*, 2008). All of PR-proteins, chitinase and β-1,3- glucanase are two important hydrolytic enzymes that are produced in many plant species after infection by different types of pathogens (Ebrahim *et al.*, 2011).

Chitin and glucans are widely distributed in the cell wall of most fungi. Both are polysaccharide of β-1,4-poly-N-acetyl-D-glucosamine that are highly cross-linked by hydrogen bonds. Among the PR-proteins, chitinases and glucanases presented in plants attacked by fungi are considered to limit fungal growth. This antifungal

biological function has been demonstrated *in vitro* against several fungi (Anfoka and Buchenauer, 1997; Ji and Kie, 1996; Nyochembeng and Beyl, 2015).

Normally, on part of the cell wall of different fungi including plant pathogens consists of chitin and β-1,3-, 1-6-glucan. A lot of chitinase and β-1,3-glucanase produced by the plant can inhibit fungal growth possibly by dissolving the tips of germ tubes and hyphae (Figure 4). They are known that integration of chitinase and β-1,3-glucanase shows stronger anti-fungal activity to a wider range of fungi when each of them acts alone (Mauch *et al.*, 1988).

Another indirect role of chitinase and β-1,3-glucanase is acting as an elicitor of defense reaction. There are releasing of oligosaccharides from cell walls both of fungi and plants that activate the accumulation of phytoalexins, extensins, proteinase inhibitors, and lignin in the attacked host plant (Ham *et al.*, 1991).

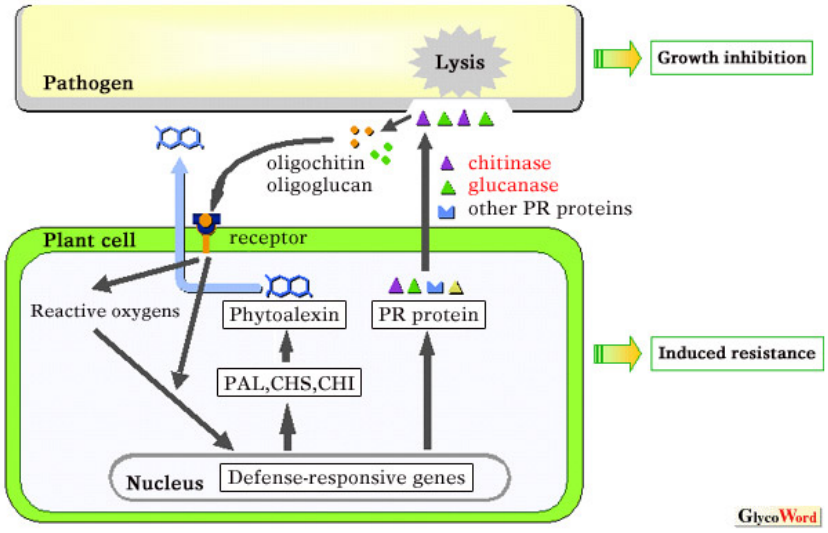

Figure 4 Reaction of plant enzymes inhibits pathogen

Source: Nishizawa (2005)

7. Field performance for disease resistance in plant

Every plant hybrid program normally consists of two phases: inbred development and hybrid testing. In the first phase, inbred development is screening populations of plants and selecting individual plants with the desired characteristics. When the first phase is succeeded or gets inbred lines, then the second phase that is a hybrid line which breeds from inbred lines must be tested for field performance for product quality traits, yield quantity performance, and variety stability before recommending to farmers. The hybrid field performance for disease resistance in plant testing should be considered as the environmental factors and plantation management related with plantation of farmers because environmental conditions and locations also have resistant influence against plant diseases. In addition, the hybrid testing process should be tested with plant age and pathogen infection. For example, CLS disease should be tested in pre-nursery and main nursery stages of oil palm to confirm the resistance of plant variety.

3. Objectives

CLS disease is the major disease of oil palm seedlings in Southern Thailand. The control strategies of this disease should be managed with multiple methods for the highest efficacy of such control. Hence, screening of oil palm variety resistance to CLS disease and understanding of mechanism underlying are needed for the development of new oil palm variety with good yield, stability, and disease resistance.

The objectives of this research are the following:

1. To screen some varieties resistant to the leaf spot disease caused by *C. oryzae* by pathogenicity test in the laboratory,
2. To study chitinase and β-1,3-glucanase on disease resistance of oil palm, and
3. To compare the leaf spot disease resistant between SUP-PSU varieties and commercial varieties.

4. Expected output

1. Identification oil palm varieties resistant against the leaf spot disease caused by *C. oryzae*.
2. Understanding the defense mechanisms of oil palm from pathogen attacks.

CHAPTER 2

RESEARCH METHODOLOGY

Experiment 1: Screening oil palm resistant varieties against the leaf spot disease

1.1 Pathogenicity test by detached leaf method

Plant material: One hundred and twenty-four oil palm lines (122 lines of female Dura variety and 2 lines of male Pisifera variety) from Oil Palm Agronomical Research Center: Phase 2, and 4 commercial variety Tenera hybrid (A, B, C, and SUP-PSU1 were used as susceptible control) at 6 - 7 years old stage were used as plant material. The leaflets on the first leaf of each oil palm genotype were cut into 20 cm in size. Leaf samples were surface sterilized with 70% alcohol for 30 sec. For each variety, three leaves were placed on a plastic tray, and the experiment followed a completely randomized design (CRD) with four replicates (4 boxes).

Fungal culture and inoculation: The fungal pathogen, *C. oryzae* NK1 (Kittimorakul *et al.*, 2014) was used as inoculum. The pathogen was cultured on corn meal agar (CMA) medium for sporulation and incubated at room temperature (25 - 28°C) for 20 days. Spore suspensions were prepared with sterilized and distilled water (DW) and adjusted at concentration 1×10^6 conidia/ml under aseptic conditions. In each line, 1.5 ml of spore suspension (1×10^6 conidia/ml) was applied onto each oil palm leaf by spraying, followed by incubation in a moist chamber box at 25 ºC for 14 days. A box sprayed with sterilized DW was served as the control.

Disease assessment and disease incidence: At the 14th day after inoculation, the disease assessment was scored with 0-5 scale (Table 1 and Figure 5). The standard disease scale of pathogenicity test was modified from a procedure described by Valent *et al.* (1991).

Table 1 Disease scale of pathogenicity test by detached leaf method

Scale	Symptoms
0	No disease symptoms
1	Some pinpoint brown spots on the leaf without any rotten tissue
2	Less than 10 spots, 1-2 mm diameter in length
3	Less than 10 spots, 3-4 mm diameter in length
4	Less than 10 spots, ≥ 5 mm diameter in length
5	More than 20 spots, ≥ 5 mm diameter in length

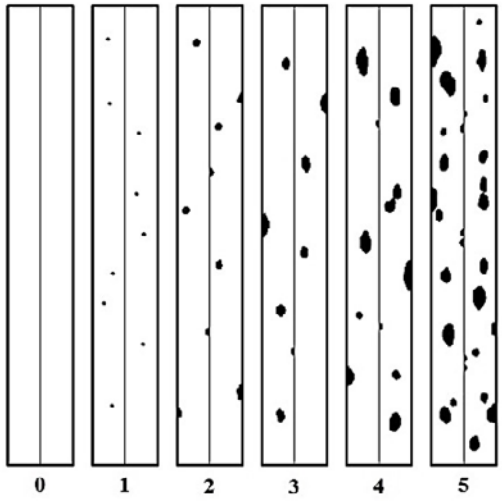

Figure 5 Standard disease assessment: 0 = no disease symptoms; 1 = some pin-point brown spots on the leaf without any rotten tissue; 2 = less than 10 spots, 1-2 mm diameter in length; 3 = less than 10 spots, 3-4 mm diameter in length; 4 = less than 10 spots, ≥ 5 mm diameter in length; 5 = more than 20 spots, ≥ 5 mm diameter in length.

The disease incidence of each line number was calculated as follows: (Modified from Monroy-Barbosa and Bosland, 2010)

$$\% \text{ Disease incidence} = \frac{\text{Mean disease score of each line number}}{\text{Highest disease score of commercial variety}} \times 100$$

The disease incidence was used to evaluate the host response in order to separate the oil palm varieties by different resistance levels. Based on disease incidence, the host responses were classified into one to six in Table 2.

Table 2 Oil palm's host responses classified from the percentage of disease incidence

% Disease incidence	Host responses
0%	Highly resistant (HR)
1-20%	Resistant (R)
21-40%	Moderately resistant (MR)
41-60%	Moderately susceptible (MS)
61-80%	Susceptible (S)
81-100%	Highly susceptible (HS).

1.2 Pathogenicity test on oil palm seedlings under greenhouse condition

Plant material: Tenera hybrid from resistant Dura variety was tested to confirm the heredity of leaf spot disease resistance. Twenty four oil palm seedlings at 4 months old stage of each line number were tested in a completely randomized design (CRD) with twenty replicates. The other four oil palm seedlings were kept and used to determine enzyme assay (chitinase and β-1,3-glucanase).

Fungal culture and inoculation: The pathogen virulent strain *C. oryzae* NK1 was used as inoculum. Twenty seedlings of oil palm were inoculated with conidial suspension (1×10^6 conidia/ml) at 50 ml per tray. The seedlings were sprayed with sterilized DW served as the control. Seedlings were subsequently covered with plastic bag and incubated for 48 h. The trays were then transferred and kept in the greenhouse with temperature varying in the range of 25-30 °C, 60 ± 5% relative humidity (RH), and 12 h photoperiod.

Disease assessment and disease incidence: Each line number, delay of disease symptom after inoculation (days) were recorded. And for 20 days after inoculation, the disease severity was scored for one of six severity levels (Table 3). The mean disease score of each line was converted to disease incidence as described previously.

Table 3 Disease scale of pathogenicity test on oil palm seedling

Scale	Symptoms
0	No lesions
1	Lesions 0–20% of the leaf area
2	Lesions 21–40% of the leaf area
3	Lesions 41–60% of the leaf area
4	lesions 61–80% of the leaf area, coalesced to 1–2 cm in size
5	75% of the leaf area covered by lesions, leaf rotten

Statistical analysis

The disease incidence and the disease severity for each variety were averaged across the samples in each box, and the statistical analyses were conducted on the box means. The data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure by SPSS software (Version 16.0). For comparisons, the varieties to the baseline commercial variety, least significant differences (LSD) were evaluated and $P \leq 0.05$ was considered significant.

Experiment 2: The activities of pathogenesis-related proteins (PR-proteins)

2.1 Enzyme assay

Plant materials and inoculation: The resistant varieties showed symptom delaying, low disease score, and disease incidence and susceptible lines were selected to compare enzyme assay. The experiments followed a completely randomized design (CRD) with four replicates (four seedlings per line number), inoculated as previous experiment 1.2 with 10 ml spore suspension while plants sprayed with sterile DW were served as controls. Seedlings were covered with plastic bags and incubated for 48 h to stimulate the pathogen infection in greenhouse condition.

Crude enzyme extraction: Forty-eight hours after inoculation, 1 gram of oil palm leaf from each plant was sampled for crude enzyme extraction. The tissue was crushed in a cool small mortar and pestle, in 5 ml of 50 mM potassium phosphate buffer (pH 7.0) for chitinase and with 50 mM acetate buffer (pH 5.0) for β-1,3-glucanase. Then leaf extracts were transferred to microcentrifuge tubes (1.5 ml) and centrifuged at 10,000 rpm for 5 min. The supernatant (crude enzyme) was transferred to a new microcentrifuge tube and stored at 2-4°C until being used in enzyme activity determinations.

Chitinase assay: The reaction mixture consisted of 250 µl sample and 250 µl of 1% colloidal chitin as a substrate in 50 mM potassium phosphate buffer at pH 7.0. The mixture was incubated at 37°C for 30 min. After incubation, 500 µl of dinitrosalicylic acid solution (DNS) was added. The enzyme activity was stopped by heating the mixture at 100°C for 15 min, and then it was cooled at room temperature. Then 4 ml of DW was added, and enzyme activity was determined by measuring the absorbance at 575 nm in an ultraviolet-visible spectrophotometer. One unit (U) of chitinase activity was defined as releasing 1 µmol of N-acetyl-D-glucosamine from the substrate per 1 min.

β-1,3-glucanase assay: β-1,3-glucanase activity was measured using laminarin as a substrate. The reaction mixture (250 µl) consisted of 125 µl sample and 125 µl of 1% laminarin in 50 mM acetic acid buffer at pH 5.0. The mixture was incubated at 37°C for 30 min. After incubation, 250 µl of DNS was added. The enzyme activity was stopped by heating the mixture at 100°C for 15 min, and then it was cooled at room temperature. Then 2 ml of distilled water was added. Enzyme activity was determined by measuring the absorbance at 550 nm in an ultraviolet-visible spectrophotometer. One unit (U) of β-1,3-glucanase activity was defined as releasing 1 µmol of glucose from the laminarin per 1 min.

Chitinase and β-1,3-glucanase activities in the resistant and susceptible line: The resistant and susceptible line selected based on the previous symptom delaying, disease score, disease incidence, and enzyme assay determinations were evaluated for activity time-profiles of two PR-proteins. One gram of leaves by fresh weight was collected from the selected line at 0, 24, 48, 72, 96, 120, 144, and 168 h after fungal pathogen inoculation. The samples were extracted and centrifuged. Crude extracts were measured for the enzyme activities as the previous experiment.

Protein assay: The total protein in oil palm leaf extracts was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. The reaction mixture containing 0.1 ml of sample and 5 ml of protein reagent (Coomassie Brilliant Blue G-250 dissolved in 95% ethanol and 85% (w/v) phosphoric acid) was incubated for 5 min, and the developed color was determined at 595 nm in an ultraviolet-visible spectrophotometer.

2.2 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Crude extracts from oil palm leaves were applied to mini-gels (12% separating gel and 4% stacking gel) to determine proteins by molecular mass in the crude extracts according to the procedure of Laemmli (1970). Samples of approximately 20 µl were loaded in each well, and electrophoresis was performed at 100 V constant voltages for the stacking gel (15 min) and at 120 V for the separating gel (45 min). The proteins were fixed and stained for 15 min in Coomassie blue (R-250) staining solution. After staining the gels were washed with a destaining solution and slowly shaken on a horizontal rotator for about 10 min. The destaining solution was repeated twice. Then the samples were incubated overnight in DW. The molecular masses of resolved proteins were estimated by electrophoresis of the marker proteins (BioLabs Inc.) with molecular masses ranging within 11-245 kDa.

Statistical analysis

The enzyme activities (mean values) were subjected to analysis of variance (ANOVA) and Tukey's HSD test ($P \leq 0.05$) by SPSS software (Version 16.0).

Experiment 3: Oil palm Tenera hybrid field performance for disease resistance under farmer's commercial nursery conditions

Plant material: The oil palm Tenera hybrid genotypes with high resistant response to CLS disease by pathogenicity test were used for this experiment to test hybrid performance under farmers' commercial nurseries conditions. And farmers' nursery commercial varieties were tested as a susceptible control. Nursery management was operated (watering, fertilizer, and weed control) under farmers' condition.

Location: Two farmers' nurseries at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province.

Disease score and disease severity: Disease score and severity were scored and recorded every 3 months (1st on 30th April, 2nd on 30th July, and 3rd on 30th October 2016) following the scale shown in Table 2 in comparison with farmer's varieties.

Experimental design and statistical analysis: Twenty oil palm seedlings per varieties were used from each nursery. The experiment was designed as CRD with four replicates/time records. Placing at random and mixing with seedlings of oil palm commercial variety. The disease score and severity were subjected to analysis of variance (ANOVA) and Tukey's HSD test ($P \leq 0.05$) by SPSS software (Version 16.0).

CHAPTER 3

RESULTS AND DISCUSSION

1. Screening oil palm resistant varieties against the leaf spot disease

1.1 Pathogenicity test by detached leaf method

A total of 124 lines of oil palm SUP-PSU parent breeding storks including 4 commercial varieties were tested for the host response to CLS disease. The results showed responding differences among the lines (Table 4). Based on the detached leaf method, the Dura variety with 13 lines (31, 52, 67, 138, 172, 177, 183, 187, 188, 189, 202/6, 203, and 207) showed the highest resistance (HR) with disease score 0 and 0% disease incidence (Figure 5). A total of 49 lines of Dura variety and 2 lines of Pisifera variety were resistant (R) with mean score of 0.44 and 9.07% disease incidence, followed by 29 moderately resistant (MR) with mean score 1.56 and 32.74% disease incidence and 24 moderately susceptible Dura lines with mean score 2.25 and 47.36% disease incidence. And 3 lines (117, 118, and 155) were susceptible (S) with disease score 3.44 and 72.74% disease incidence (Figure 6a), whereas 1 line (129) was highly susceptible (HS) to leaf spot disease with disease score 4.75 and 100% disease incidence (Figure 6b).

Among the commercial varieties, SUP-PSU1 and variety C were rated as resistant (disease score of 1.13 and 1.92 with 23.83 % and 40.37% disease incidence) (Figure 7c and d), respectively. Followed by commercial variety A rated as moderately susceptible (disease score of 2.75 and 57.89% disease incidence) (Figure 7a). Commercial variety B had the highest disease score 4.75 with 100% disease incidence and was rated highly susceptible (Figure 7b) (Table 4). As described above, the detached leaf method was used for preliminary screening to select varieties resistant to leaf spot

disease, scoring from 0 to 4.5. The mean score was converted to disease incidence which was further categorized to six host response levels: HR, R, MR, MS, S, and HS.

CLS disease was usually found on young oil palm leaves. In pathogenicity test by the detached leaf method, young to middle-aged leaves should be used for accurate results because leaf susceptibility depends on age (Dhingra and Sinclair, 1995). For this experiment, the leaflets on the first leaf of oil palm at age 6 - 7 years old were collected for testing. The detached leaf method has been used to screen for a virulent fungal disease to control perennial weeds in North America (Yang *et al.*, 1991). In addition, this is the basic method to select resistant varieties of various plant species such as leaf spot resistant spring onion, *Sclerotinia* white and stalk rot resistant sunflower, foliar blight resistant soybean, and anthracnose resistant eggplant (Li *et al.*, 2014; Irani *et al.*, 2011; Rahayu, 2014; Saha *et al.*, 2010). Thus, the detached leaf method is an easy and efficient system for the preliminary screening of oil palm varieties resistant to leaf spot disease. The preliminary results require further validation under more realistic breeding conditions.

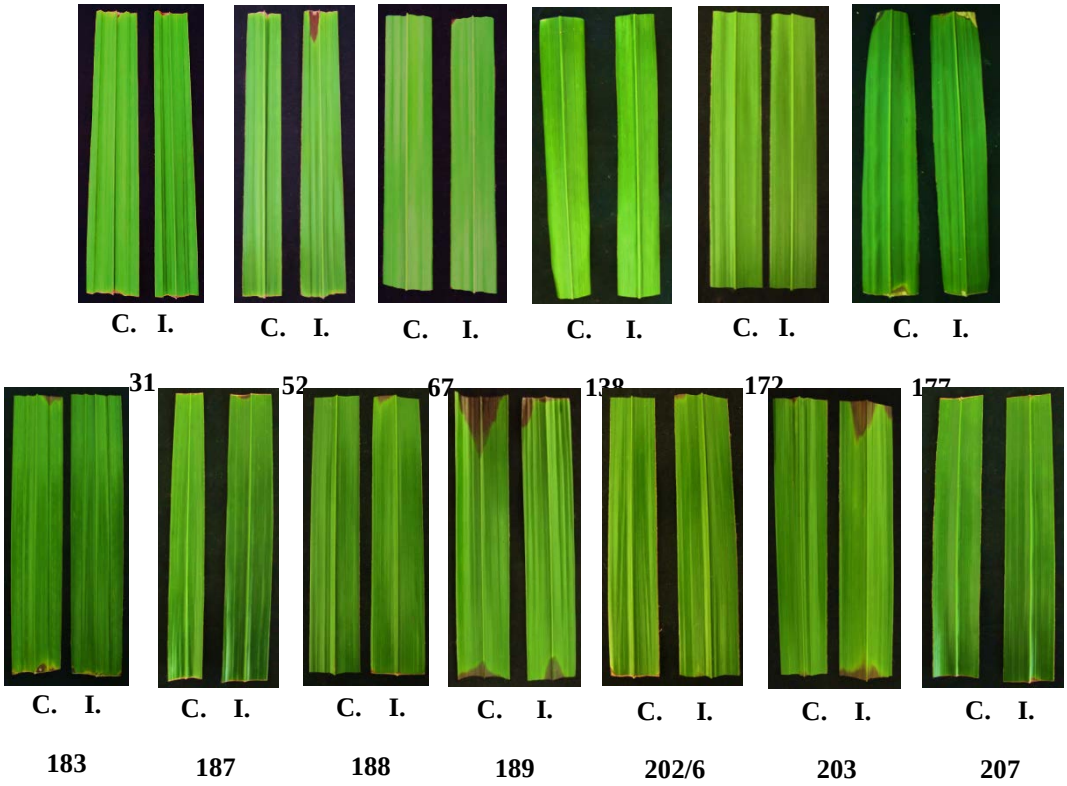

Figure 6 Thirteen lines of SUP-PSU parents breeding stock showed the highest resistant to the disease at 14 days after inoculation, C: control sprayed with sterilized DW and I: inoculated with *C. oryzae*.

Figure 7 SUP-PSU parents breeding stock (a) three lines were susceptible and (b) one line was highly susceptible at 14 days after inoculation. C: control sprayed with sterilized DW and I: inoculated with *C. oryzae*.

Figure 8 Four commercial varieties (a) commercial variety A (b) commercial variety B (c) commercial variety C and (d) SUP-PSU1 at 14 days after inoculation. C: control sprayed with sterilized DW and I: inoculated with *C. oryzae*.

Table 4 Disease score and disease incidence (%) of selected oil palm varieties including commercial varieties, 14 days after inoculation with *Curvularia oryzae* NK1

Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]	Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]
19[D]	1.00	21.00	MR	133	1.67	35.11	MR
23	2.00	42.11	MS	134	2.08	43.84	MS
27	0.75	15.84	R	136	1.92	40.37	MR
28	0.58	12.26	R	138	0	0	HR
29	0.17	3.47	R	139	1.33	28.09	MR
31	0	0	HR	141	2	42.11	MS
32	0.67	14	R	142	0.08	1.74	R
33	0.17	3.47	R	145	2	42.11	MS
35	1.25	26.32	MR	146	2.08	43.84	MS
36	0.42	8.79	R	147	2.58	54.37	MS
39	0.08	1.74	R	148	2.34	49.16	MS
41	0.25	5.21	R	149	2	42.11	MS
42	0.58	12.26	R	151	2.50	52.58	MS
43	0.92	19.32	R	155	3.58	75.37	S
44	0.58	12.26	R	157	2.00	42.11	MS
46	0.50	10.53	R	159	0.75	15.79	R
47	0.75	15.84	R	163	1.08	22.79	MR
49	0.75	15.84	R	171	1	21.05	MR
50	1.17	24.53	MR	172	0	0	HR
51	0.59	12.32	R	175	2.67	56.16	MS
52	0	0	HR	177	0	0	HR
54	0.42	8.74	R	178	2	42.11	MS
56	1.33	28.05	MR	183	0	0	HR
57	2.00	42.11	MS	184	0.25	5.21	R
58	0.08	1.74	R	185	1.59	33.37	MR
59	0.58	12.26	R	186	1.84	38.63	MR
67	0	0	HR	187	0	0	HR
70	1.33	28.05	MR	188	0	0	HR
71	1.75	36.84	MR	189	0	0	HR
73	0.4	8.74	R	196	2.75	57.89	MS
74	0.58	12.26	R	198	1.67	35.16	MR
75	1.25	26.32	MR	201	2	42.11	MS
76	0.50	10.47	R	202	0.25	5.26	R
80	0.42	8.79	R	202/6	0	0	HR
81	2.5	52.63	MS	203	0	0	HR
82	1.84	38.63	MR	205	0.25	5.21	R
83	2.17	45.58	MS	206	0.75	15.79	R
84	2.25	47.32	MS	207	0	0	HR
85	1.92	40.37	MR	212	0.08	1.74	R

Table 4 (continued) Disease score and disease incidence (%) of selected oil palm varieties including commercial varieties, 14 days after inoculation with *Curvularia oryzae* NK1

Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]	Variety line number	Disease score [x]	Disease incidence (%) [y]	Host response [z]
86	2.08	43.84	MS	213	0.08	1.74	R
90	1.84	38.63	MR	214	0.08	1.74	R
94	1.75	36.89	MR	215	0.08	1.74	R
96	0.92	19.32	R	221	0	0	HR
102	0.75	15.74	R	227	0.50	10.42	R
104	0.33	6.96	R	228	1.67	35.11	MR
105	1.08	22.79	MR	229	0.5	12.32	R
106	2.42	50.84	MS	230	0.25	5.26	R
107	1.75	36.84	MR	232	0.42	8.79	R
109	1.92	40.37	MR	235	0.33	6.95	R
110	1.58	33.32	MR	237	0.25	5.21	R
111	1.58	33.32	MR	239	0.17	3.47	R
112	2.17	45.58	MS	240	0.25	5.26	R
113	0.92	19.32	R	244	0.92	19.26	R
116	1.92	40.37	MR	246	0.08	1.74	R
117	3.00	63.16	S	248	0.25	5.26	R
118	3.75	78.95	S	255	0.17	3.53	R
119	1.75	36.89	MR	277[P]	0.92	19.32	R
123	2.84	59.68	MS	278	0.58	12.26	R
124	1.92	40.37	MR	Com. A[C]	2.75	57.89	MS
127	2.33	49.11	MS	Com. B	4.75	100	HS
128	1.42	29.84	MR	Com. C	1.92	40.37	MR
129	4.75	100	HS	SUP-PSU1	1.18	43.79	MR
130	2.25	47.37	MS				
131	2	42.11	MS	LSD (0.05)	1.13	23.83	

[D] Dura variety female plant

[P] Pisifera variety male plant

[C] Commercial variety

[x] Using standard disease score from the detached leaf method

[y] Apparent fraction of leaf area infected with pathogen (%)

[z] Categorized host response to pathogen infection: 0% = highly resistant (HR), 1-20% = resistant (R), 21-40% = moderately resistant (MR), 41-60% = moderately susceptible (MS), 61-80% = susceptible (S) and 81-100% = highly susceptible (HS)

LSD = least significant difference.

1.2 Pathogenicity test on oil palm seedlings in greenhouse conditions

In the pathogenicity test of oil palm seedlings in greenhouse conditions, a total of 12 lines Tenera F_1 hybrid variety (Dura × Pisifera) screened from resistant and susceptible Dura female plant (9 resistant, 2 susceptible, and 1 highly susceptible) were tested (Table 5). Line 187 (Figure 8b) was highly resistant with disease score of 0.25 and 5% disease incidence, and CLS symptom appeared at the latest (12 days after inoculation), followed by lines 203 (Figure 8c), 138 (Figure 8a), 183, 188, 202/6, 207, 172, and 177 (Table 5). Line 129 was highly susceptible to CLS with disease score of 4.55 and 91% disease incidence (Figure 8d) and was the first detectable disease symptoms at 3 days after inoculation (Table 5). The heredity of leaf spot resistance in oil palm may be partly a maternal effect as this is a qualitative character of oil palm.

The pathogenicity test of seedling will be useful for inheritance studies where there are a large number of plants per generation and crosses (Zhang *et al.,* 1997) and many oil palm disease resistant breeding programs such as vascular wilt disease (caused by *Fusarium oxysporum* f. sp. *elaeidis*) in Africa and basal stem rot (caused by *Ganoderma* spp.) in Southeast Asia using pathogenicity test method at nursery stage for testing oil palm progenies resistance against disease (Ntsefong *et al.,* 2012; Durand-Gasselin *et al.,* 2005; Rees *et al.,* 2007). Therefore, screening to select oil palm parents carrying the resistant gene by detached leaf method and tested inheritance in oil palm Tenera hybrid by pathogenicity test of seedling may be useful for developing new Tenera hybrid resistant to CLS disease varieties.

Figure 9 Disease progress on Tenera hybrid variety (4 months) in seedling trays at 20 days after inoculation. The lines are 138 (a), 187 (b), 203 (c), and 129 (d).

Table 5 Delay of disease symptom, disease score, and disease incidence for 9 highly resistant varieties and 3 susceptible varieties at 20 days after inoculation

Variety line number	Symptom post inoculation (days)	Disease score	Disease incidence (%)
117	3	3.10^{b}	62.00^{b}
129	3	4.55^{a}	91.00^{a}
138	10	0.40^{cde}	8.00^{cde}
155	3	3.70^{b}	74.00^{b}
172	9	0.95^{cd}	19.00^{cd}
177	5	1.05^{c}	21.00^{c}
183	7	0.90^{cde}	18.00^{cde}
187	12	0.25^{e}	5.00^{e}
188	10	0.65^{cde}	13.00^{cde}
202/6	7	0.60^{cde}	12.00^{cde}
203	10	0.35^{de}	7.00^{de}
207	7	0.75^{cde}	15.00^{cde}

Values are the mean, disease severity (%) caused by *Curvularia oryzae* NK1 ($P \leq 0.05$). The values in the column followed by the same letter are not significantly different according to LSD test.

2. Enzyme activities of oil palm resistant and susceptible varieties

2.1 Enzyme assay

To determine the activities of PR-proteins, DNS method was conducted with chitinase and β-1,3-glucanase. Forty-eight hours after the fungal inoculation, the oil palm lines 138 and 187 showed high chitinase activities with 14.88 ± 1.31 and 17.84 ± 1.46 U mL^{-1}, respectively, and β-1,3-glucanase activities with 12.02 ± 1.03 and 14.23 ± 1.31 U mL^{-1}, in the same order. There are no significant differences between line 138 and 187. In contrast, the lines 117, 129, and 155 presented low activities of both enzymes and were not significantly different ($P \leq 0.05$) from the non-inoculated controls for β-1,3-glucanase activity (Table 6). Based on the enzyme activity results, two candidate lines were selected. Oil palm line 187 was selected for its high enzyme activities, while line 129 was selected for its comparatively low activities. These selected oil palm lines were subjected to enzyme profiling, SDS-PAGE, and disease symptom occurrence.

Normally, chitinase and glucanase are produced in several higher plant species after induction by fungal inducers. These enzymes can inhibit fungal growth and play a role in self-defense (Ham *et al.*, 1991) because the major components of fungal cell walls are the polysaccharides, chitin, and glucan which are the substrates for chitinase and glucanase (Sela-Buurlage *et al.*, 1993). Regarding some prior studies, when coconut (*Cocos nucifera* L.) root was treated with *Pseudomonas fluorescens*, *Trichoderma viride*, and *T. harzianum* in combination with chitin, coconut root produced chitinase and β-1,3-glucanase against *Ganoderma lucidum* (Karthikeyan *et al.*, 2006). Chairin and Petcharat (2017) also reported in longkong fruit (*Aglaia dookkoo* Griff.) that chitinase and β-1,3-glucanase were detected in peel extracts after exposing to the fungus *Metarhizium guizhouense,* and these inhibited the mycelial growth of fruit rot fungi *Fusarium* sp. and *Botrytis* sp.

Table 6 Enzyme activities (mean ± standard deviation) of oil palm leaf extracts at 48 h after inoculation

Line number	Chitinase (U mL^{-1})*		β-1,3-glucanase (U mL^{-1}) *	
	Control plants	Fungal treated plants**	Control plants	Fungal treated plants**
117	2.26 ± 0.19^{B}	6.85 ± 1.40^{Adef}	2.04 ± 0.18^{A}	3.50 ± 1.07^{Afg}
129	0.41 ± 0.16^{B}	3.27 ± 0.27^{Af}	0.90 ± 0.19^{A}	2.05 ± 0.70^{Ag}
138	3.11 ± 0.74^{B}	14.88 ± 3.62^{Aab}	2.98 ± 0.09^{B}	12.02 ± 1.03^{Aab}
155	1.48 ± 0.36^{B}	4.61 ± 0.60^{Aef}	1.97 ± 0.21^{B}	4.91 ± 0.16^{Aef}
177	2.89 ± 0.79^{B}	7.16 ± 1.15^{Ade}	1.79 ± 0.33^{B}	7.04 ± 1.00^{Ade}
187	3.36 ± 0.95^{B}	17.84 ± 1.46^{Aa}	4.00 ± 0.09^{B}	14.23 ± 1.31^{Aa}
188	3.34 ± 0.80^{B}	12.56 ± 1.30^{Abc}	3.17 ± 0.61^{B}	10.09 ± 1.47^{Abc}
202/6	2.25 ± 0.50^{B}	9.76 ± 0.78^{Acd}	1.89 ± 0.11^{B}	8.18 ± 0.79^{Acd}
203	3.05 ± 0.67^{B}	13.57 ± 1.07^{Ab}	2.36 ± 0.39^{B}	11.70 ± 1.36^{Ab}
207	3.17 ± 0.15^{B}	12.51 ± 1.03^{Abc}	2.88 ± 0.97^{B}	11.17 ± 0.83^{Ab}

* Same capital superscript in a row indicates no significant difference by least significant difference test ($P \leq 0.05$).

** Same small superscript in a column indicates no significant difference by least significant difference test ($P \leq 0.05$).

The chitinase and β-1,3-glucanase activities of the selected oil palm lines were determined after fungal inoculation for every 24 hours over 7 days. The study found that the chitinase activity of line 187 was 14.03 ± 0.87 U mL^{-1} at 24 h, peaked to 16.84 U/ml at 48 h and then decreased continuously to 5.77 U mL^{-1} at 168 h. For line 129, chitinase activity 3.76 ± 0.41 U mL^{-1} was observed at 24 h, and it decreased slightly to 1.25 U mL^{-1} at 168 h after inoculation (Figure 9a). Regarding β-1,3-glucanase, the enzyme activity in oil palm line 187 at 24 h after inoculation was 13.51 ± 1.04 U mL^{-1}. The highest 15.02 U mL^{-1} activity was at 48 h, and activity then decreased continuously to 4.50 U mL^{-1} at 168 h. In contrast, the β-1,3-glucanase activity of line 129 (4.31 ± 0.83 U mL^{-1} at 24 h) was not significantly different from its non-inoculated control (Figure 9b).

Analogous observations by Pareek *et al.* (2013) showed higher chitinase and β-1,3-glucanase activities in the resistant moth bean cultivar (FMM-96) over the susceptible cultivar (RM0-40 and CZM-3). Anguelova-Merhar *et al.* (2001) found that the wheat plant resistant line (Kareee/Lr35) had higher chitinase and β-1,3-glucanase activities after inoculation with leaf rust pathogen (*Puccinia recondite* f.sp. *tritici*) than the susceptible line (Karee). In this study, PR-protein activation could be detected within 24 h after *Curvularia* inoculation, before the appearance of the disease symptoms at 72 h for the susceptible line and not before 168 h for the resistant line. The early enzyme activities significantly differed between the resistant and susceptible lines. Thus, PR-protein producing ability could be used as one factor for selecting oil palm lines resistant to CLS disease independently of disease symptom occurrence.

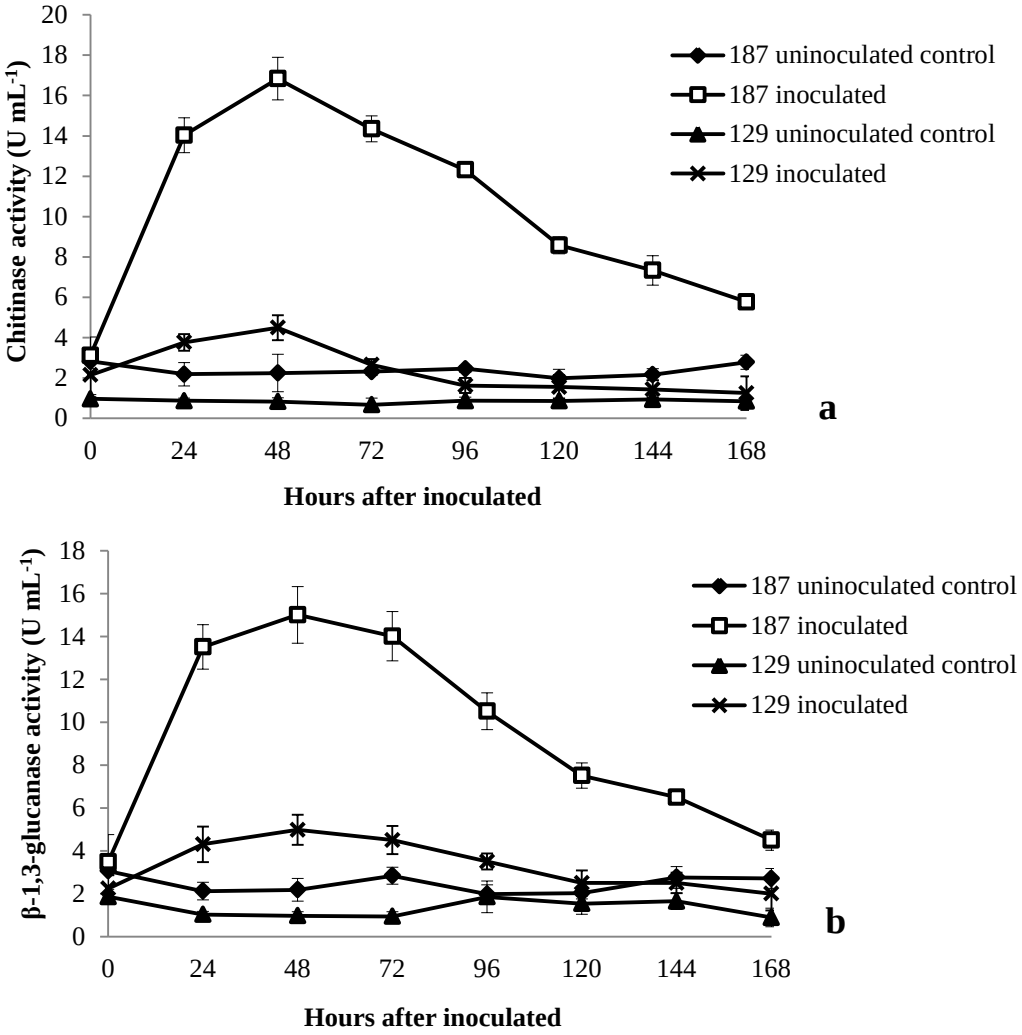

Figure 10 Enzyme activities of oil palm leaf extracts in control and CLS inoculated cases: (a) chitinase activity and (b) β-1,3-glucanase activity**.**

2.2 The SDS-PAGE analysis

The proteins in oil palm leaves were separated by SDS-PAGE and stained with Coomassie Blue R-250. The SDS-PAGE analysis showed differences in the expression of major proteins in the selected oil palm among lines 129 and 187 from their non-inoculated controls. Oil palm line 129 which produced low activities of chitinase and β-1,3-glucanase showed no visible protein bands. However, line 187 showed protein bands for 22, 25 and 33 kDa at 24 h, and the bands were dark at 48 h after fungal inoculation (Figure 11). Normally, most plant chitinases have a molecular mass in the ranges from 15 to 43 kDa, and plant glucanases have molecular masses from 33 to 44 kDa.

Hegde and Keshgond (2013) reported that most plant chitinases had a molecular mass in the ranges from 15 to 43 kDa, and plant glucanases had molecular masses from 33 to 44 kDa. Moreover, Syahanim *et al.* (2013) reported that several proteins with molecular weights less than 50 kDa were expressed in oil palm root on day 3 and day 7 after *Ganoderma boninese* infection, and one of these was glucanase. For this study, the resistant line showed molecular weights for 22, 25, and 33 kDa at 24 h and darker in 48 h. Thus, this protein bands could be confirmed that PR-proteins were produced when CLS disease pathogen had tried to infect the leaves. In addition, between resistant line 187 and susceptible line 129 were significantly different to express PR-proteins activity and protein bands. By finding and developing new resistant CLS disease variety via advance molecular technique, this study can use this data for searching resistant genes or PR-proteins genes that may be different between resistant and susceptible lines.

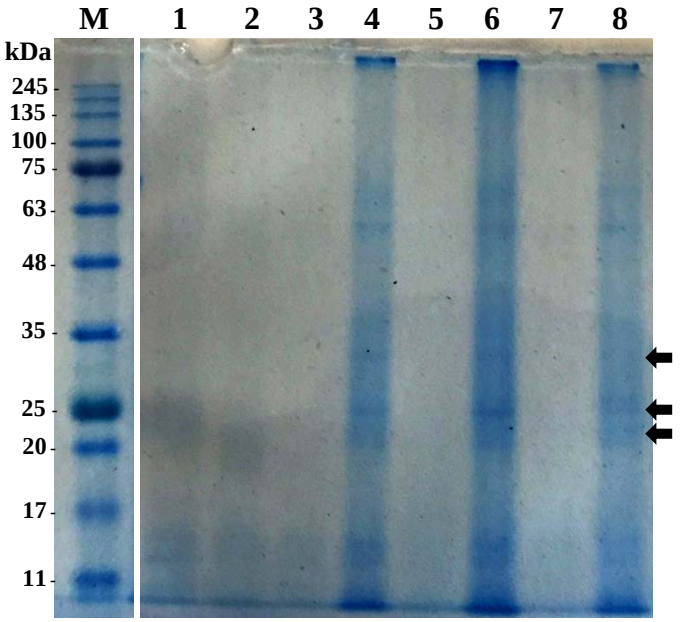

Figure 11 SDS-PAGE protein bands (chitinase and β-1,3-glucanase) of oil palm leaf extracts after CLS inoculation. Lane M: protein marker, lane 1: non-inoculated control of line 129, lane 2: non-inoculated control of line 187, lane 3, 5, and 7: line 129 at 24, 48, and 72 h, respectively, and lane 4, 6, and 8: line 187 at 24, 48, and 72 h, respectively.

3. Oil palm Tenera hybrid disease resistance performance test under commercial nurseries conditions

Three oil palm Tenera hybrid genotypes (138, 187, and 203) with high resistance to CLS disease in seedling test and high activity of 2 PR-Proteins from Experiment 1.2 and 2.1 were tested for field performance for disease resistance under farmer's commercial nursery conditions compared with 3 oil palm commercial varieties (A, B, and C) at Nuea Khlong district in Krabi province and Yan Ta Khao district in Trang province. Oil palm seedlings at age 3 months old from pre-nursery were transplanted to main-nursery in 1st February 2016. Oil palm nursery management (watering, applying fertilizers, and having weed control) followed by farmer's management as presented in Table 7 except chemical or fungicide application. Data were recorded every 3 months beginning at age 6 months (30th April 2016), age 9 months (30th July 2016), and age 12 months (30th October 2016) of oil palm seedlings. Four replicates/varieties/times were records for disease score and disease severity.

Table 7 Oil palm main nursery managements (watering, applying fertilizers, and having weed control) under nursery conditions

Krabi province	Trang province
Watering by springer	**Watering by springer**
- 2 times/day (summer season)	- 1-2 times/day (summer season)
- 1-2 time/day (raining season) except raining	- 1 time/day (raining season) except raining
Applying fertilizers	**Applying fertilizers**
- 15-15-6-4 (N-P-K-Mg) alternate with 12-12-17-2 + TE (N-P-K-Mg + trace element)	- 15-15-6-4 (N-P-K-Mg) alternate with 12-12-17-2 + TE (N-P-K-Mg + trace element)
Having weed control	**Having weed control**
- 2 weeks/time	- 1-2 weeks/time

The 1st data collection on 30th April 2016 (summer season)

Two weeks after planting in main-nursery, the study found that oil palm leaves were burnt because of full sunlight. The leaves were cut off to protect weak tissues that could be crop residues and dwell of CLS disease pathogen. One month after planting, oil palm seedlings were applied with fertilizers 15-15-6-4 (N-P-K-Mg) in March 2016 alternated with 12-12-17-2 + TE (N-P-K-Mg + trace element). The first data collection on 30th April 2016 at age 6 months old of oil palm seedlings indicated no CLS disease symptom, disease scoring, and disease incidence in every oil palm genotype in both locations (Table 8). Oil palm seedlings could grow well. No CLS disease symptom appeared in every oil palm genotype until the end of the summer season in May 2016.

Table 8 Disease scoring and disease incidence of oil palm Tenera hybrid at age 6 months under farmers' commercial nurseries conditions

Locations	Lines and commercial varieties	Disease scoring	Disease incidence (%)
Nuea Khlong district, Krabi province	138	0.00[1/]	0.00
	187	0.00	0.00
	203	0.00	0.00
	Com. A	0.00	0.00
	Com. B	0.00	0.00
	Com. C	0.00	0.00
	F-test	ns	ns
Yan Ta Khao district, Trang province	138	0.00	0.00
	187	0.00	0.00
	203	0.00	0.00
	Com. A	0.00	0.00
	Com. B	0.00	0.00
	Com. C	0.00	0.00
	F-test	ns	ns

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests which ns = not significant.

The 2nd data collection on 30th July 2016 (raining season)

Nine months after planting in main-nursery, the second data collection was recorded on 30th July 2016. At Nuea Khlong district in Krabi province, 3 commercial varieties were recorded for higher disease scoring and disease incidence than resistant Tenera hybrid varieties. Commercial variety A showed the highest CLS disease scoring 4.50 and 90% disease incidence significantly different from the other treatments, followed by commercial variety C and B with disease scoring 2.00 and 1.75, and 40% and 35% disease incidence, respectively (Table 9 and Figure 12). Other two resistant varieties (187 and 203) showed disease scoring with 0.25 with 5% disease incidence, whereas line 138 showed the highest resistance with disease scoring 0 and 0% disease incidence (Table 9 and Figure 11). However, commercial variety A showed the highest susceptible genotype, and all sample tests had to be moved and destroyed to reduce the source of inoculum in order to protect the other oil palm healthy seedlings in farmer's commercial nursery.

At Yan Ta Khao district in Trang province, commercial variety C was the highest disease scoring with 0.75 and 15% disease incidence significantly different from the other commercial varieties followed by Tenera hybrid line 138 with 0.25 disease scoring and 5% disease incidence. There are no disease scoring and % disease incidence in the other Tenera hybrid resistance lines (Table 9 and Figure 12).

Table 9 Disease scoring and disease incidence of oil palm Tenera hybrid at age 9 months under farmer's commercial nurseries conditions

Locations	Lines and commercial varieties	Disease scoring	Disease incidence (%)
Nuea Khlong district, Krabi province	138	0.00c[1/]	0.00
	187	0.25c	5.00c
	203	0.25c	5.00c
	Com. A	4.50a	90.00a
	Com. B	1.75b	35.00b
	Com. C	2.00b	40.00b
	F-test	**	**
Yan Ta Khao district, Trang province	138	0.25ab	5.00ab
	187	0.00b	0.00b
	203	0.00b	0.00b
	Com. A	0.00b	0.00b
	Com. B	0.00b	0.00b
	Com. C	0.75a	15.00a
	F-test	**	**

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests with ** significantly at $P \leq 0.01$.

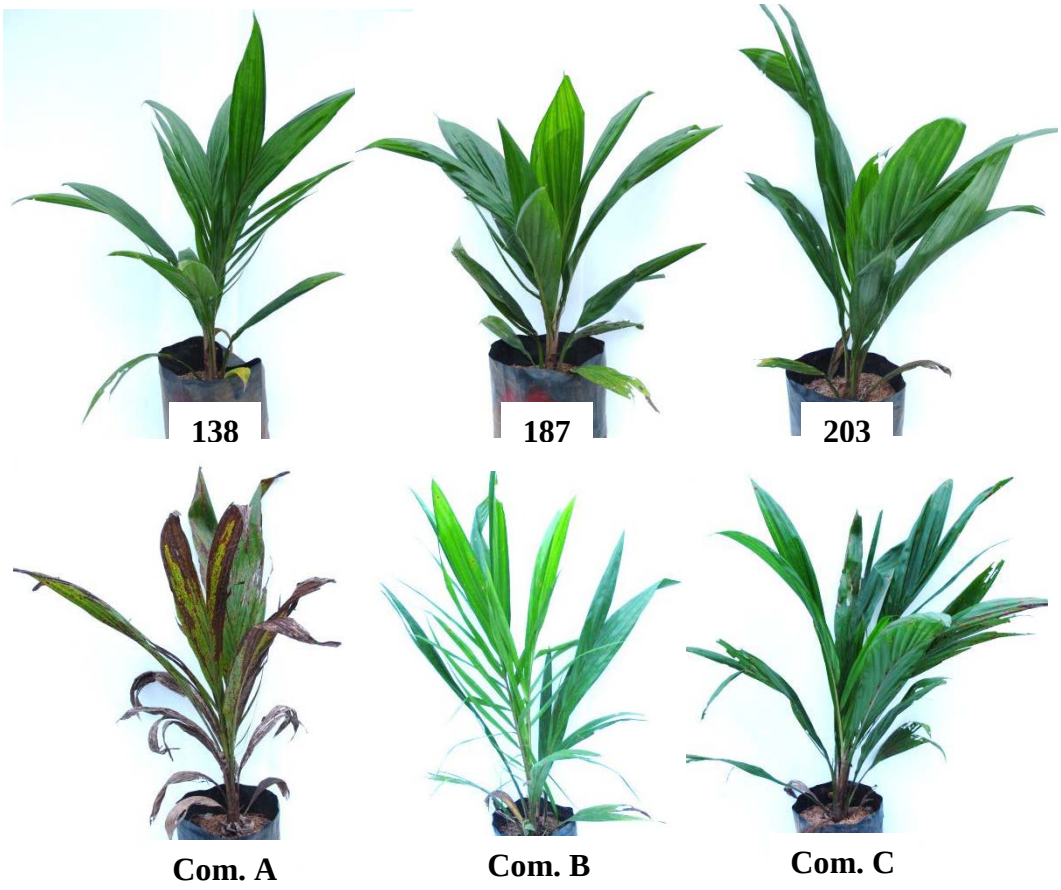

Figure 12 Six oil palm Tenera hybrid at age 9 months under farmer's nursery condition at Nuea Khlong district, Krabi province, Com.: Commercial variety (susceptible control).

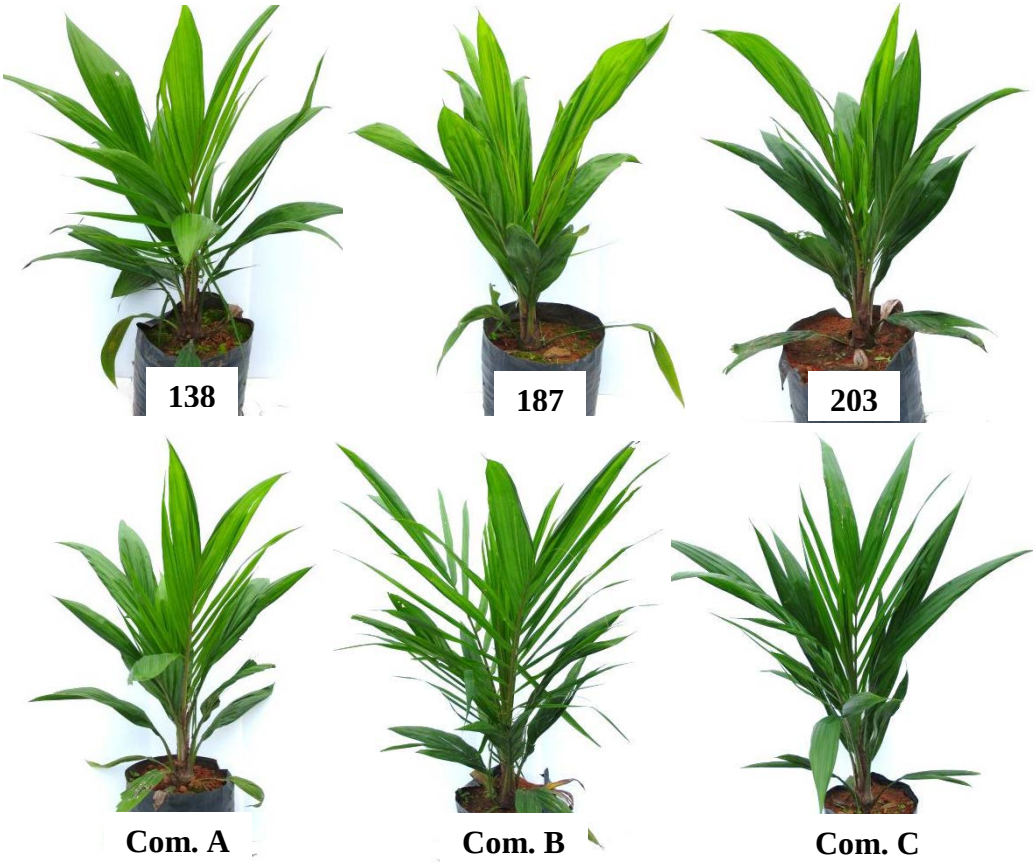

Figure 13 Six oil palm Tenera hybrid varieties at age 9 months under farmer's nursery condition at Yan Ta Khao district, Trang province, Com.: Commercial variety (susceptible control).

The 3rd data collection on 30th October 2016 (the end of the raining season)

The 3rd data collection was recorded on 30th October 2016. In Nuea Khlong district, Krabi province. Commercial variety B and C showed higher disease scoring and disease incidence than resistance Tenera hybrid varieties with the same disease scoring at 2.75 and 55% disease incidence significantly different from resistant lines. One resistant variety (203) showed disease scoring with 0.5 with 10% disease incidence, whereas other two lines (138 and 187) showed the highest resistance with disease scoring 0.25 and 5% disease incidence (Table 10, Figure 14).

At Yan Ta Khao district in Trang province, CLS disease did not show some serious effect until the end of raining season. Two resistant lines (138 and 203) were 0.25 disease scoring and 5% disease incidence while the highest resistance was line 187 with 0 disease scoring and 0% disease incidence. The commercial variety was 1.00 disease scoring and 20% disease incidence with a significant difference from the Tenera hybrid resistant line (Table 10 and Figure 15).

Table 10 Disease scoring and disease incidence of oil palm Tenera hybrid at age 12 months under farmers' commercial nursery conditions

Locations	Lines and commercial varieties	Disease scoring	Disease incidence (%)
Nuea Khlong district, Krabi province	138	0.25b[1/]	5.00b
	187	0.25b	5.00b
	203	0.50b	10.00b
	Com. A	-	-
	Com. B	2.75a	55.00a
	Com. C	2.75a	55.00a
	F-test	**	**
Yan Ta Khao district, Trang province	138	0.25b	5.00b
	187	0.00b	0.00b
	203	0.25b	5.00b
	Com. A	1.00a	20.00a
	Com. B	1.00a	20.00a
	Com. C	1.00a	20.00a
	F-test	**	**

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests with ** significantly at $P \leq 0.01$.

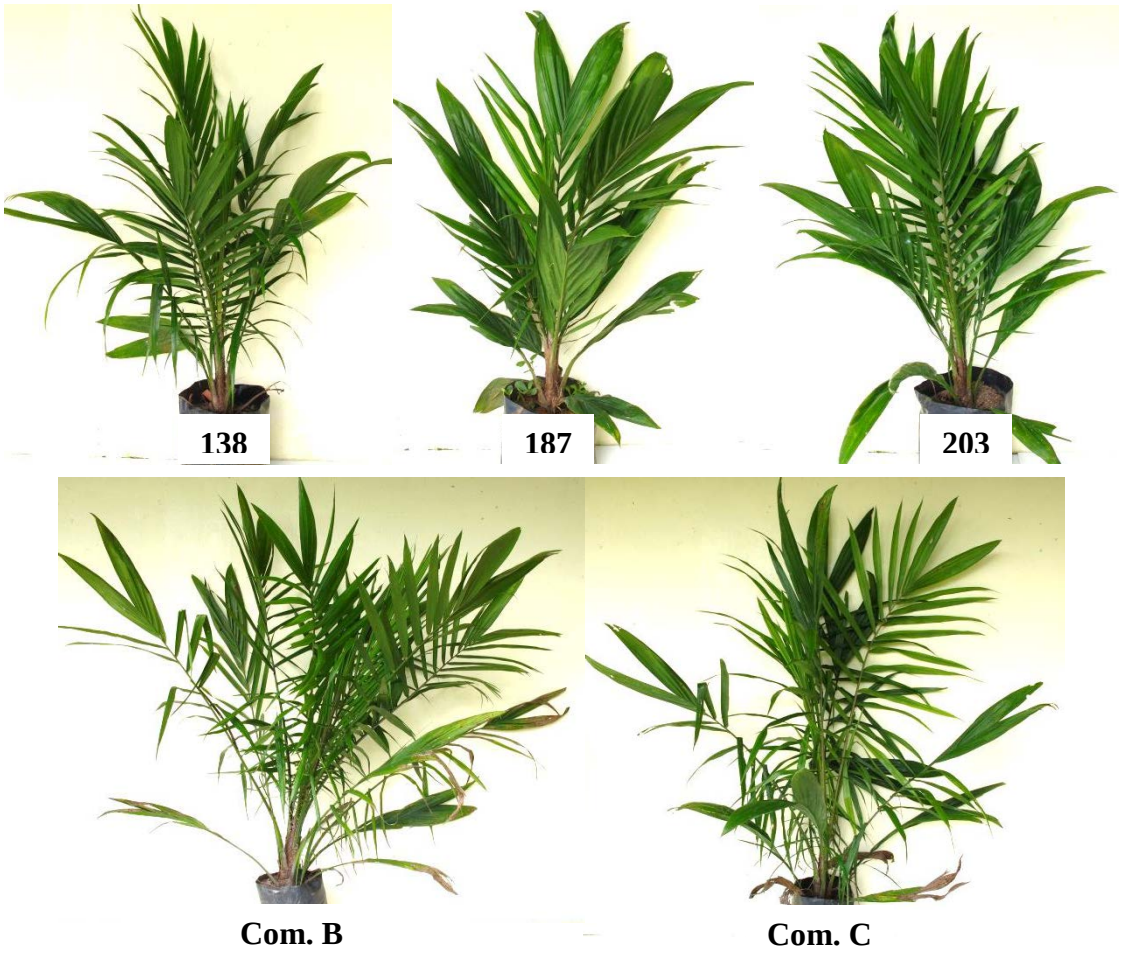

Figure 14 Five oil palm Tenera hybrid varieties at age 12 months under farmer's nursery condition at Nuea Khlong district, Krabi province, Com.: Commercial variety (susceptible control).

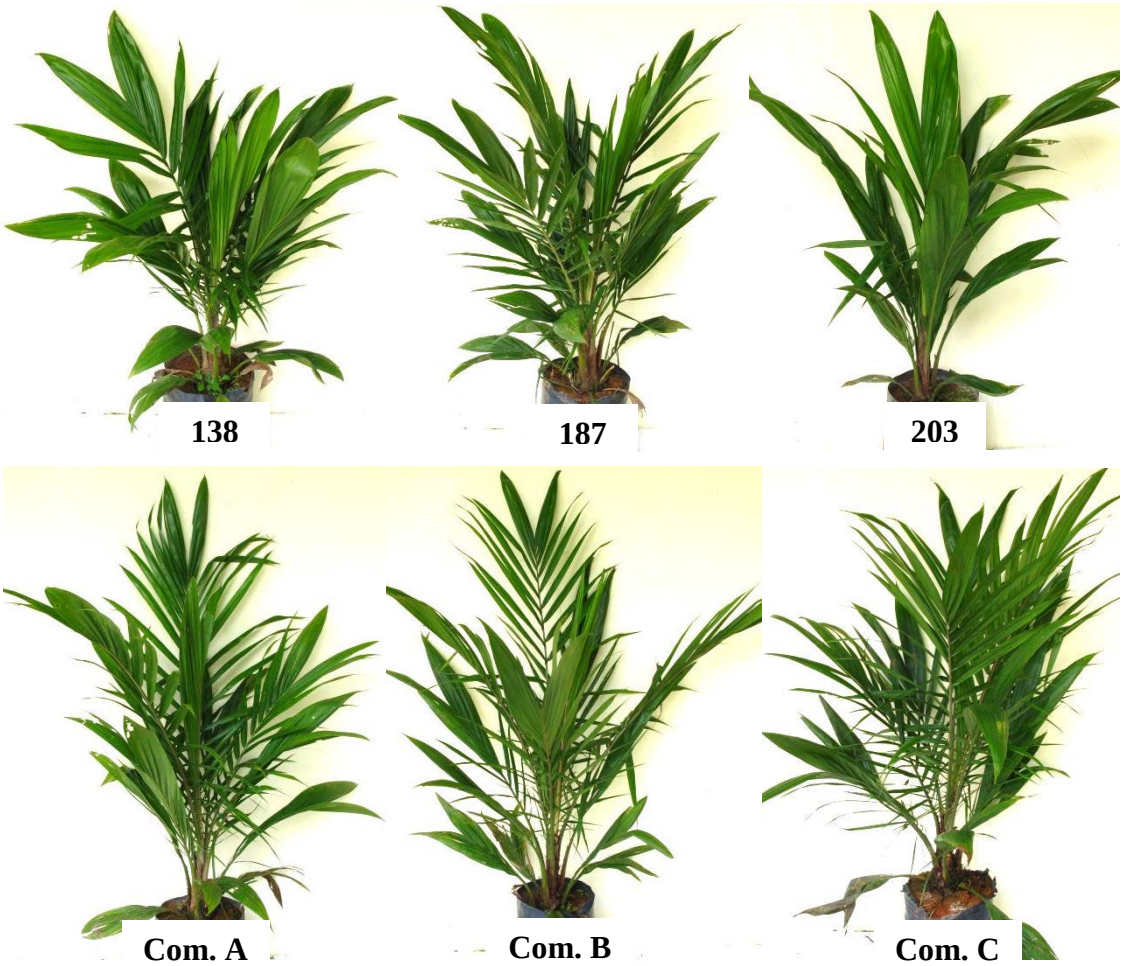

Figure 15 Six oil palm Tenera hybrid varieties at age 12 months under farmer's nursery condition at Yan Ta Khao district, Trang province, Com.: Commercial variety (susceptible control).

When passed the process to screen, to select, and to combine inbreed parents for getting the hybrid commercial variety, the values of hybrid variety in plants can be measured in characteristics such as higher yield, larger fruit or vegetable size, better disease resistance and broader environment adaptability (Hallauer, 2011). The hybrid testing process must be conducted for several years and conditions. The test results of the best management practices for growing specific crops with recommended varieties within specific areas could be suggested for farmers (Pellack and Karlen, 2017).

The oil palm SUP-PSU breeding project reported for the other high performances with good yield characteristic and sustainability of variety SUP-PSU1 in Songkhla and nearby province (Eksomtramage *et al.,* 2009; Sudanai *et al.,* 2013; Bueraheng *et al.,* 2017). Thus, the testing for the field performance of oil palm Tenera hybrid for disease resistance under farmers' commercial nursery conditions from SUP-PSU oil palm breeding stock presented some of Tenera hybrid crosses could perform resistance to CLS disease. The characteristic of CLS disease resistant will get more performance to develop SUP-PSU parent populations. For 2 different locations at Nuea Khlong district in Krabi province and Yan Ta Khao district in Trang province, the results showed different disease score, disease incidence, and also growth rate that might concern with weather of each area, the size of nursery area, farmer's plantation management, and accumulation of CLS disease pathogen. However, the selection of oil palm Tenera hybrid resistant can respond and have a good growth rate under farmers' nurseries (Appendix) from the summer season in February 2016 until the end of raining season in October 2016, respectively. Plant disease integrated with control under nursery condition is the best method that will have high efficacy for controlling plant disease and healthy oil palm seedlings since pre-nursery to main-nursery until plantation can increase income and decrease costs and time for nursery management.

Oil palm plantation areas in the South are increasing every year and have a tendency of more planting in other parts of Thailand. Most oil palm Tenera commercial varieties are breeding and importing from some other countries. Most of the oil palm breeding program has the objectives to breed and develop new oil palm variety for getting high yield performance or stable to environmental condition. Only few oil palm breeding programs attempted to find disease resistant variety. In Indonesia and Malaysia, the basal stem rot disease-resistant varieties have been studied and reported because basal stem rot disease is the major disease in the oil palm plantation while CLS disease was found as the minor disease. In Thailand, CLS disease is the major disease in oil palm nurseries seedling and has been reported not more than 10 years only the species and epidermis in oil palm plantation. Control strategy usually uses chemical fungicides that have an important effect on human and environment for controlling CLS disease. Although biological control and mechanical control are saved and cheaper than chemical control, both methods are more suitable protection than control. Screening and selecting sources of oil palm SUP-PSU parent breeding stocks resistant to CLS disease are important data to develop and to increase good characteristic for oil palm SUP-PSU breeding project. Understanding of different between resistant and susceptible lines with pathogenesis-related proteins that express when *C. oryzae* pathogen attacks may be used for finding new varieties resistant genes and breeding oil palm resistant to CLS disease. The resistant variety can solve CLS disease problem and reduces chemical fungicide in oil palm nursery system.

The screening, selecting, and PR-proteins studying of host plant resistance are important data for developing resistant variety. Presently, oil palm variety resistant to CLS disease has never been reported before. The pathogenicity test by the detached leaf method in oil palm breeding stock was used to classify a large population of oil palm high-performance breeding program. For pathogenicity test of seedling was conducted to endorse maternal resistant effect of the offspring and PR-proteins activity (chitinase and

β-1,3- glucanase) could help describe for more understanding of mechanism defense between oil palm host resistant lines and CLS disease pathogen. After that, field performance for disease resistance in the plant was also considered. The hybrid testing under multiple locations and environment factors provided more studied results for observing the potential variability. The experiment kept only the varieties that would meet consumer and grower expectations for prospective commercial variety.

CHAPTER 4

CONCLUSIONS

In this study, oil palm parents were primarily screened. Selected oil palm Tenera hybrid resistance and susceptible lines were tested by comparing PR-proteins (chitinase and β-1,3-glucanase) expression with field performance for disease resistance under farmers' commercial nursery conditions.

Oil palm SUP-PSU parent breeding stock was screened and tested by detached leaf method and pathogenicity test on oil palm seedlings in greenhouse. The results showed different resistant levels in each and some also susceptible to CLS disease. Three lines (138, 187, and 203) showed high resistance with low disease score and disease incidence but high enzyme (chitinase and β-1,3-glucanase) assay expression. However, line 129 was the susceptible line with the highest disease score, disease incidence, and the lowest enzyme assay expression. When the proteins in oil palm leaves of line 187 and 129 were separated by SDS-PAGE, line 187 expressed the protein bands with molecular weight ranges of chitinase and β-1,3-glucanase after fungal inoculation, but line 129 did not have visible protein bands. These results demonstrated that resistant and susceptible lines were not only significant with disease score and disease incidence but enzyme assay expression also can explain different resistance and susceptible line mechanism. In addition, three oil palm Tenera hybrid genotypes (138, 187, and 203) were tested for field performance to disease resistance under two farmers' commercial nursery conditions. The results showed that all Tenera hybrid resistant genotypes had more resistance than commercial varieties from February 2016 until the end of raining season in October 2016. This study suggested that 3 oil palm Tenera hybrid genotypes (138, 187, and 203) were the candidates for breeding and developing new oil palm variety resistant to CLS disease.

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APPENDIX

Growth and biomass of oil palm Tenera hybrid resistant lines and commercial variety under farmer's commercial nursery condition

Table 1 Diameter, height, leaf length, and leaves of oil palm seedling at age 6 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Cross	Diameter (cm)	Height (cm)	Leaf length (cm)	Number of leaf (number/plant)		
					Lanceolate leaf	Bifurcate leaf	Pinnate leaf
Nuea Khlong district, Krabi province	138	1.91 ± 0.16b[1/]	7.17 ± 0.43b	27.02 ± 1.10b	4.50 ± 0.58	2.50 ± 0.58b	0.00
	187	1.54 ± 0.24b	6.48 ± 1.48b	23.22 ± 1.78b	3.50 ± 0.58	1.75 ± 0.96b	0.00
	203	1.63 ± 0.32b	6.00 ± 0.39b	20.72 ± 3.20b	4.00 ± 1.15	2.00 ± 0.82b	0.00
	Com. A	1.86 ± 0.18b	8.10 ± 0.62b	26.70 ± 2.82b	4.50 ± 0.58	2.25 ± 0.50b	0.00
	Com. B	3.30 ± 0.20a	12.00 ± 0.91a	46.75 ± 5.29a	3.75 ± 0.50	5.50 ± 0.58a	0.00
	Com. C	2.94 ± 0.76a	11.62 ± 1.80a	49.10 ± 6.58a	3.75 ± 0.50	5.00 ± 2.20a	0.00
	F-test	**	**	**	ns	**	ns
Yan Ta Khao district, Trang province	138	1.68 ± 0.26b	6.55 ± 0.54b	22.28 ± 3.59b	4.50 ± 0.58a	1.75 ± 0.96b	0.00
	187	1.75 ± 0.20b	6.48 ± 1.74b	21.40 ± 3.02b	4.25 ± 0.50a	2.50 ± 1.00b	0.00
	203	1.65 ± 0.23b	5.66 ± 0.66b	19.68 ± 1.92b	3.50 ± 1.00ab	2.75 ± 0.96b	0.00
	Com. A	1.61 ± 0.16b	5.88 ± 1.03b	21.48 ± 2.73b	4.75 ± 0.50a	2.25 ± 0.50b	0.00
	Com. B	2.79 ± 0.31a	10.00 ± 1.47a	33.27 ± 4.79a	3.75 ± 0.50ab	6.50 ± 0.58a	0.00
	Com. C	3.07 ± 0.15a	11.88 ± 1.31a	36.87 ± 6.12a	2.75 ± 0.50b	7.50 ± 1.29a	0.00
	F-test	**	**	**	**	**	ns

[1/] Means with in a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$, ns = not significant.

Table 2 Leaf fresh weight (LFW), stem fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and root/soot ratio of oil palm seedling at age 6 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Crosses	LFW (g)	SFW(g)	RFW (g)	TFW (g)	LDW (g)	SDW (g)	RDW (g)	TDW (g)	Root/shoot ratio
Nuea Khlong district, Krabi province	138	14.56 ± 2.62b[1/]	10.39 ± 0.87b	13.72 ± 2.11b	38.67 ± 4.92b	3.93 ± 0.73b	2.23 ± 0.08b	2.70 ± 0.26b	8.86 ± 0.99b	0.70 ± 0.10
	187	9.33 ± 1.40b	6.37 ± 2.72b	10.58 ± 5.16b	26.28 ± 8.46b	2.51 ± 0.33b	1.35 ± 0.45b	1.90 ± 0.86b	5.76 ± 1.41b	0.74 ± 0.32
	203	8.35 ± 3.08b	6.02 ± 2.12b	9.05 ± 4.58b	23.41 ± 9.53b	2.24 ± 0.86b	1.23 ± 0.52b	1.55 ± 0.92b	5.01 ± 2.22b	0.65 ± 0.16
	Com. A	12.63 ± 1.51b	9.80 ± 1.73b	15.79 ± 2.84b	38.23 ± 3.74b	3.47 ± 0.41b	2.08 ± 0.36b	2.92 ± 0.52b	8.48 ± 0.78b	0.84 ± 0.18
	Com. B	48.16 ± 9.59a	39.65 ± 5.50a	48.15 ± 9.20a	135.97 ± 20.93a	13.34 ± 2.63a	10.34 ± 1.08a	9.18 ± 2.07a	32.87 ± 5.82a	0.69 ± 0.15
	Com. C	43.77 ± 17.42a	39.93 ± 12.66a	33.88 ± 14.80a	117.58 ± 42.50a	11.93 ± 4.83a	10.36 ± 4.05a	7.08 ± 3.14a	29.37 ± 11.42a	0.62 ± 0.20
	F-test	**	**	**	**	**	**	**	**	ns
Yan Ta Khao district, Trang province	138	8.86 ± 4.07b	6.53 ± 2.63b	5.79 ± 3.09b	21.19 ± 9.49b	2.32 ± 1.06b	1.22 ± 0.55b	1.28 ± 0.54b	4.81 ± 2.13b	0.57 ± 0.15
	187	11.80 ± 3.84b	8.79 ± 3.50b	9.25 ± 3.07b	29.84 ± 8.88b	3.18 ± 0.96b	1.89 ± 0.63b	2.07 ± 0.66b	7.13 ± 1.99b	0.67 ± 0.18
	203	8.93 ± 1.80b	6.76 ± 1.87b	9.15 ± 2.18b	24.85 ± 5.36b	2.44 ± 0.50b	1.34 ± 0.38b	1.76 ± 0.38b	5.54 ± 1.17b	0.72 ± 0.12
	Com. A	9.10 ± 1.51b	7.02 ± 1.83b	9.70 ± 2.06b	25.82 ± 3.79b	2.53 ± 0.51b	1.43 ± 0.38b	1.93 ± 0.26b	5.91 ± 0.93b	0.79 ± 0.23
	Com. B	30.07 ± 8.13a	33.78 ± 5.20a	31.34 ± 6.91a	104.19 ± 17.38a	9.15 ± 2.35a	6.68 ± 2.27a	5.51 ± 1.87a	21.35 ± 6.47a	0.59 ± 0.04
	Com. C	31.01 ± 7.84a	26.82 ± 9.19a	23.82 ± 6.36a	81.67 ± 23.20a	11.85 ± 2.47a	8.84 ± 1.60a	7.52 ± 1.90a	28.21 ± 5.37a	0.64 ± 0.10
	F-test	**	**	**	**	**	**	**	**	ns

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$, ns = not significant.

Table 3 Diameter, height, leaf length, and leaves of oil palm seedling at age 9 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Cross	Diameter (cm)	Height (cm)	Leaf length (cm)	Number of leaf (number/plant)		
					Lanceolate leaf	Bifurcate leaf	Pinnate leaf
Nuea Khlong district, Krabi province	138	3.64 ± 0.44ab[1/]	12.55 ± 1.25b	53.10 ± 5.01b	3.00 ± 0.81ab	3.25 ± 0.50ab	3.25 ± 0.50abc
	187	3.66 ± 0.27ab	12.15 ± 0.65b	51.67 ± 4.65b	2.75 ± 0.95b	4.75 ± 0.95a	1.75 ± 1.25c
	203	3.33 ± 0.62b	12.62 ± 0.76b	50.90 ± 6.41b	4.75 ± 0.50a	4.50 ± 0.58a	1.50 ± 1.29c
	Com. A	2.73 ± 0.23b	12.15 ± 0.70b	44.82 ± 8.15b	1.25 ± 0.95b	2.00 ± 1.82b	2.25 ± 1.70bc
	Com. B	4.78 ± 0.67a	17.32 ± 1.02a	82.22 ± 13.90a	2.75 ± 1.25b	3.50 ± 1.29ab	5.75 ± 1.50a
	Com. C	3.48 ± 0.83b	15.02 ± 2.83ab	98.40 ± 15.07a	2.50 ± 0.57b	3.75 ± 0.95ab	4.75 ± 0.50ab
	F-test	**	**	**	**	**	**
Yan Ta Khao district, Trang province	138	3.95 ± 1.44b	12.10 ± 1.02c	41.60 ± 4.62b	3.75 ± 0.95a	3.00 ± 0.81b	4.00 ± 0.81ab
	187	3.71 ± 1.85b	12.25 ± 1.61c	43.97 ± 4.33b	3.50 ± 0.57a	4.50 ± 1.00ab	3.50 ± 0.57ab
	203	3.67 ± 0.82b	12.47 ± 1.87c	44.20 ± 3.95b	3.00 ± 0.82a	3.75 ± 0.95b	3.50 ± 1.00ab
	Com. A	3.89 ± 4.50b	13.25 ± 1.12bc	42.27 ± 5.47b	3.25 ± 0.95a	5.50 ± 2.38ab	2.75 ± 2.12b
	Com. B	4.90 ± 5.30a	16.52 ± 1.42ab	66.05 ± 2.89a	2.75 ± 0.95a	5.75 ± 0.95ab	5.00 ± 0.00ab
	Com. C	5.04 ± 4.06a	16.95 ± 2.40a	75.50 ± 11.54a	0.50 ± 0.58b	7.00 ± 0.81a	5.50 ± 0.57a
	F-test	**	**	**	**	**	**

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$

Table 4 Leaf fresh weight (LFW), stem fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and root/soot ratio of oil palm seedling at age 9 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Crosses	LFW (g)	SFW(g)	RFW (g)	TFW (g)	LDW (g)	SDW (g)	RDW (g)	TDW (g)	Root/shoot ratio
Nuea Khlong district, Krabi province	138	67.59 ± 18.11bc[1/]	52.28 ± 17.07bc	49.63 ± 12.58b	169.51 ± 44.34bc	19.10 ± 5.34b	13.47 ± 3.95b	11.13 ± 2.21b	43.70 ± 10.78b	0.60 ± 0.10ab
	187	62.25 ± 15.16bc	45.90 ± 8.81c	52.92 ± 10.01b	161.08 ± 28.84bc	17.09 ± 4.04b	11.55 ± 2.18b	12.52 ± 2.26b	41.16 ± 6.80b	0.76 ± 0.20a
	203	57.94 ± 12.16c	48.18 ± 12.83c	29.12 ± 4.34b	135.25 ± 25.51c	16.41 ± 4.09b	12.16 ± 3.94b	6.81 ± 1.29b	35.39 ± 8.80b	0.42 ± 0.09b
	Com. A	31. 83 ± 12.62c	28.97 ± 11.51c	30.50 ± 10.73b	91. 31 ± 33.19c	11.80 ± 5.16b	9.05 ± 4.04b	6.43 ± 2.06b	27.29 ± 7.17b	0.57 ± 0.15ab
	Com. B	167.88 ± 52.99bc	110.49 ± 29.70a	93.97 ± 24.54a	372.35 ± 98.48a	45.05 ± 14.89a	34.30 ± 15.63a	21.81 ± 5.71a	101.17 ± 36.06a	0.49 ± 0.04ab
	Com. C	124.17 ± 33.53a	93.96 ± 30.43ab	54.82 ± 8.58b	272.95 ± 70.31ab	30.03 ± 12.97ab	26.11 ± 8.16ab	12.06 ± 2.22b	68.21 ± 21.31ab	0.44 ± 0.15b
	F-test	**	**	**	**	**	**	**	**	**
Yan Ta Khao district, Trang province	138	60.73 ± 9.76b	56.13 ± 7.87b	62.88 ± 13.75	179.76 ± 30.36b	31.10 ± 28.72	16.05 ± 2.44b	12.90 ± 3.21b	60.06 ± 32.64ab	0.58 ± 0.26ab
	187	68.84 ± 9.62b	58.20 ± 5.41b	106.40 ± 56.86	233.44 ± 67.05b	20.53 ± 3.13	17.01 ± 2.01ab	19.61 ± 4.63ab	57.15 ± 8.83b	0.95 ± 0.16ab
	203	58.92 ± 6.92b	54.15 ± 4.85b	68.77 ± 20.23	181.84 ± 26.27b	17.14 ± 1.82	16.77 ± 2.69b	15.15 ± 5.33ab	49.07 ± 6.98b	0.87 ± 0.27ab
	Com. A	64.74 ± 8.87b	59.16 ± 10.14b	89.67 ± 24.29	213.58 ± 35.93b	19.04 ± 3.07	16.43 ± 3.79b	19.10 ± 6.03ab	54.58 ± 11.12b	0.99 ± 0.22a
	Com. B	140.85 ± 14.91a	127.14 ± 19.92a	112.02 ± 37.76	380.01 ± 62.30a	38.63 ± 4.53	36.00 ± 8.48a	25.40 ± 6.07a	100.04 ± 16.15a	0.65 ± 0.13ab
	Com. C	148.22 ± 32.19a	135.49 ± 37.11a	94.23 ± 1.64	377.95 ± 68.51a	44.35 ± 10.89	32.46 ± 18.26ab	23.17 ± 5.48ab	99.99 ± 24.11a	0.52 ± 0.05b
	F-test	**	**	ns	**	ns	**	**	**	ns

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$, ns = not significant.

Table 5 Diameter, height, leaf length, and leaves of oil palm seedling at age 12 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Cross	Diameter (cm)	Height (cm)	Leaf length (cm)	Number of leaf (number/plant)		
					Lanceolate leaf	Bifurcate leaf	Pinnate leaf
Nuea Khlong district, Krabi province	138	5.69 ± 6.17ab[1/]	21.05 ± 0.28ab	83.22 ± 10.23b	0.25 ± 0.50	3.00 ± 1.63	7.50 ± 0.57ab
	187	4.86 ± 8.36ab	17.85 ± 1.75b	66.60 ± 2.97b	0.50 ± 1.00	4.50 ± 0.57	4.50 ± 0.57c
	203	4.37 ± 13.15b	18.40 ± 3.61b	68.47 ± 7.50b	0.25 ± 0.50	4.50 ± 0.57	5.00 ± 1.15c
	Com. A	-	-	-	-	-	-
	Com. B	6.84 ± 8.15a	28.12 ± 6.26a	116.82 ± 18.63a	0.50 ± 0.57	2.00 ± 0.00	9.50 ± 0.57a
	Com. C	4.75 ± 9.82ab	22.10 ± 5.06ab	119.52 ± 5.22a	0.25 ± 0.50	3.50 ± 1.00	8.50 ± 0.57a
	F-test	**	**	**	ns	ns	**
Yan Ta Khao district, Trang province	138	4.97 ± 0.44	18.55 ± 2.04	63.00 ± 10.41	0.50 ± 0.57	4.25 ± 1.70ab	6.50 ± 0.57
	187	5.31 ± 0.66	17.32 ± 1.22	64.32 ± 3.51	0.25 ± 0.50	5.00 ± 1.15ab	6.75 ± 1.70
	203	4.94 ± 0.47	16.50 ± 0.87	64.67 ± 7.26	1.25 ± 0.50	3.00 ± 0.81b	6.25 ± 2.06
	Com. A	5.27 ± 0.56	17.15 ± 2.76	61.07 ± 8.42	1.25 ± 0.50	3.00 ± 0.81b	6.25 ± 2.06
	Com. B	6.27 ± 0.88	20.00 ± 1.07	78.02 ± 18.96	0.25 ± 0.50	5.75 ± 1.50ab	7.75 ± 2.21
	Com. C	5.62 ± 0.76	21.15 ± 3.31	79.20 ± 14.86	0.25 ± 0.50	6.00 ± 1.41b	6.50 ± 2.51
	F-test	ns	ns	ns	ns	**	ns

[1/]Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$, ns = not significant.

Table 6 Leaf fresh weight (LFW), stem fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and root/soot ratio of oil palm seedling at age 12 months in 2 commercial nursery seedling at Nuea Khlong district, Krabi province and Yan Ta Khao district, Trang province

Locations	Crosses	LFW (g)	SFW(g)	RFW (g)	TFW (g)	LDW (g)	SDW (g)	RDW (g)	TDW (g)	Root/shoot ratio
Nuea Khlong district, Krabi province	138	267.77 ± 98.29b[1/]	231.37 ± 87.10b	97.86 ± 14.29b	597.01 ± 190.70bc	72.06 ± 36.85ab	69.24 ± 30.48b	26.52 ± 4.93b	167.83 ± 68.89bc	0.47 ± 0.33
	187	156.93 ± 60.51b	143.14 ± 33.36b	112.82 ± 19.35b	412.90 ± 92.34bc	48.17 ± 11.54b	41.74 ± 10.91b	24.55 ± 3.86b	114.46 ± 24.75bc	0.51 ± 0.07
	203	156.46 ± 35.23b	131.81 ± 41.40b	78.00 ± 16.12b	366.27 ± 89.50c	44.31 ± 9.98b	34.65 ± 8.82b	18.11 ± 2.66b	97.08 ± 20.00c	0.41 ± 0.04
	Com. A	-	-	-	-	-	-	-	-	-
	Com. B	470.95 ± 117.03a	424.87 ± 117.78ab	219.59 ± 54.93a	1115.42 ± 245.50a	115.59 ± 49.15a	142.29 ± 44.36a	55.01 ± 26.08a	312.90 ± 92.44a	0.47 ± 0.09
	Com. C	332.38 ± 84.47ab	285.76 ± 60.66a	134.45 ± 25.70b	752.59 ± 154.19ab	99.51 ± 18.44ab	90.73 ± 19.02ab	36.24 ± 6.15ab	226.48 ± 41.02bc	0.36 ± 0.05
	F-test	**	**	**	**	**	**	ns	**	ns
Yan Ta Khao district, Trang province	138	152.32 ± 47.46b	134.57 ± 33.89b	75.53 ± 18.07b	362.43 ± 97.86c	42.33 ± 13.99c	37.58 ± 10.53c	17.41 ± 4.50c	97.33 ± 28.51c	0.42 ± 0.04
	187	164.31 ± 28.37b	155.99 ± 25.37 b	102.14 ± 14.52ab	422.55 ± 61.25bc	48.98 ±8.93c	50.22 ± 8.43bc	25.92 ± 5.57bc	125.13 ± 20.41bc	0.52 ± 0.04
	203	138.06 ± 19.26b	114.14 ± 18.38b	76.95 ± 13.44b	329.15 ± 46.63c	40.52 ± 5.66c	31.21 ± 5.30c	17.17 ± 3.21c	88.90 ± 12.70c	0.42 ± 0.05
	Com. A	152.99 ± 39.69b	133.44 ± 38.73b	90.26 ± 20.12ab	376.69 ± 97.81c	54.78 ± 15.16bc	43.98 ± 13.35bc	23.14 ± 6.81c	121.91 ± 32.31c	0.43 ± 0.10
	Com. B	290.64 ± 64.69a	282.84 ± 70.44a	133.11 ± 31.25a	706.60 ± 156.02a	107.60 ± 22.10a	115.60 ± 35.26a	44.27 ± 9.15a	267.48 ± 63.06a	0.41 ± 0.03
	Com. C	266.72 ± 32.22a	211.48 ± 66.92ab	130.11 ± 22.67a	608.92 ±112.96ab	81.26 ± 9.00ab	77.99 ± 7.92ab	38.36 ±6.86ab	197.62 ± 16.26ab	0.47 ± 0.03
	F-test	**	**	**	**	**	**	**	**	ns

[1/] Means within a column followed by the same letters are significantly different ($P \leq 0.05$) according to Turkey's comparison tests, ** significantly at $P < 0.01$, ns = not significant

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List of Publication and Proceeding

Kittimorakul, J., Sunpapao, A., Duangpan, S., Anothai, J. and Eksomtramage, T. 2018. Screening oil palm (*Elaeis guineensis* Jacq.) varieties for resistance to *Curvularia* leaf spot disease. Australian Journal of Crop Science. Indexed in Scopus.

Kittimorakul, J., Eksomtramage, T. Sunpapao, A. and Chairin, T. 2018. Indication of oil palm (*Elaeis guineensis* Jacq.) resistance to *Curvularia* leaf spot disease by PR-proteins producing ability (Preparing for submission).