ISSR AND SSR MARKERS LINKED TO POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE IN MUNGBEAN

Kitiya Arsakit

มหาวิทยาลัยเทคโนโลยีสุรนารี

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เครื่องหมาย ISSR และ SSR ที่บ่งชี้ลักษณะต้านทานโรคราแป้ง และโรคใบจุดในถั่วเขียว

นางสาวกิติยา อาษากิจ

มหาวิทยาลัยเทคโนโลยีสุรนารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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มหาวิทยาลัยเทคโนโลยีสุรนารี
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ISSR AND SSR MARKERS LINKED TO POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE IN MUNGBEAN

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

Thesis Examining Committee

Thitiporn Machikowa
(Asst. Prof. Dr. Thitiporn Machikowa)
Chairperson

Piyada Alisha Tantasawat
(Prof. Dr. Piyada Alisha Tantasawat)
Member (Thesis Advisor)

Kiyoshi Banno
(Prof. Dr. Kiyoshi Banno)
Member

N. Muangsan
(Assoc. Prof. Dr. Nooduan Muangsan)
Member

Teerayoot Girdthai
(Asst. Prof. Dr. Teerayoot Girdthai)
Member

(Prof. Dr. Santi Maensiri)
Vice Rector for Academic Affairs and Internationalization

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

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กิติยา อายากิจ : เครื่องหมาย ISSR และ SSR ที่บ่งชี้ลักษณะต้านทานโรคราแป้ง และโรคใบจุดในถั่วเขียว (ISSR AND SSR MARKERS LINKED TO POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE IN MUNGBEAN) อาจารย์ที่ปรึกษา : ศาสตราจารย์ ดร.ปิยะดา อลิฌาณ์ ตันตสวัสดิ์, 77 หน้า.

โรคราแป้ง (powdery mildew) เกิดจากเชื้อรา *Sphaerotheca phaseoli* พบการระบาดในช่วงฤดูแล้งและหนาว ในขณะที่โรคใบจุด (Cercospora leaf spot) ซึ่งเกิดจากเชื้อรา *Cercospora canescens* Ellis & Martin พบการระบาดในฤดูฝนของการผลิตถั่วเขียว (*Vigna radiata* L. Wilczek) โดยทั้งสองโรคนี้ทำให้ผลผลิตในพันธุ์อ่อนแอลดลง 40% และมากกว่า 50% ตามลำดับ แสดงให้เห็นถึงความต้องการของพันธุ์ต้านทานต่อโรคทั้งสองเพื่อการผลิตถั่วเขียวที่ยั่งยืน การใช้เครื่องหมายโมเลกุลช่วยในการคัดเลือกมีความสำคัญสำหรับการพัฒนาพันธุ์ต้านทานที่มียีนต้านทานหลายยีน โดยวัตถุประสงค์ของการศึกษานี้ เพื่อหาเครื่องหมาย inter simple sequence repeat (ISSR) และ simple sequence repeat (SSR) ที่เชื่อมโยงกับลักษณะต้านทานโรคใบจุดและโรคราแป้งในถั่วเขียวสายพันธุ์ V4718 โดยการศึกษาครั้งนี้ใช้ประชากร $F_{2:7}$, $F_{2:8}$ และ $F_{2:9}$ recombinant inbred line (RIL) จากคู่ผสมระหว่างพันธุ์ CN72 (พันธุ์อ่อนแอและให้ผลผลิตสูง) และสายพันธุ์ V4718 (สายพันธุ์ต้านทาน) ทำการประเมินความต้านทานต่อโรคทั้งสองในประชากร RIL ภายใต้สภาพแปลงทดลองระหว่างฤดูแล้งและหนาวของปี พ.ศ. 2556 และ 2559 สำหรับโรคราแป้ง และช่วงฤดูฝนของปี พ.ศ. 2559 และ 2560 สำหรับโรคใบจุด การวิเคราะห์การกระจายตัวแสดงให้เห็นว่าลักษณะความต้านทานโรคราแป้งและโรคใบจุดถูกควบคุมโดยยีนเดี่ยวแบบข่ม ใช้เครื่องหมาย ISSR จำนวน 20 ไพรเมอร์ และ SSR จำนวน 11 เครื่องหมาย เพื่อระบุหาเครื่องหมายที่ให้ความแตกต่างระหว่างพันธุ์/สายพันธุ์ต้านทานและอ่อนแอที่น่าจะมีความสัมพันธ์กับความต้านทานโรคใบจุดและโรคราแป้งด้วยวิธี bulk segregant analysis (BSA) เครื่องหมาย ISSR จำนวน 2 เครื่องหมาย (I13306 และ I13311) แสดงความแตกต่างและมีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับความต้านทานต่อโรคราแป้ง และเครื่องหมาย SSR จำนวน 5 เครื่องหมาย (CEDAAG002, CEDC050, CEDG084, VR108 และ VR393) แสดงความแตกต่างและมีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับความต้านทานต่อโรคใบจุด นอกจากนี้ยังพบว่าเครื่องหมาย I13306, I13311 และ VR393 มีความสัมพันธ์กับความต้านทานต่อโรคทั้งสองอย่างมีนัยสำคัญทางสถิติ แสดงให้เห็นว่ายีนที่ควบคุมลักษณะต้านทานโรคทั้งสองอาจอยู่ใกล้กัน จากเครื่องหมายเหล่านี้พบว่าเครื่องหมาย VR393 แสดงค่า LOD สำหรับโรคทั้งสองมากที่สุด (> 5.0 สำหรับโรคใบจุดและ >3.0 สำหรับโรคราแป้ง) ซึ่งช่วยยืนยันความสัมพันธ์ของเครื่องหมายนี้กับยีนต้านทานต่อโรคทั้งสอง การวิเคราะห์ด้วยวิธี multiple interval mapping (MIM) พบ QTL 1 ตำแหน่ง (*qCLSC72V18*) ที่เชื่อมโยงกับลักษณะต้านทานโรคใบจุด และ QTL 1

ตำแหน่ง (*qPMC72V18*) ที่เชื่อมโยงกับลักษณะต้านทานโรคราแป้งในประชากร RIL ของคู่ผสมระหว่างพันธุ์ CN72 และสายพันธุ์ V4718 โดย *qCLSC72V18* และ *qPMC72V18* อยู่ระหว่างเครื่องหมาย VR108 กับ VR393 ซึ่งสามารถอธิบายความแปรปรวนของคะแนนการเกิดโรคใบจุดและโรคราแป้งในทั้งสองฤดูการปลูกได้ 22.9-43.5% และ 13.9-20.2% ตามลำดับ เครื่องหมายที่มีความเชื่อมโยงกับยีนต้านทานโรคทั้งสองนี้สามารถนำไปใช้ในการคัดเลือกเพื่อรวมยีนต้านทานโรคใบจุดและโรคราแป้งในการปรับปรุงพันธุ์ถั่วเขียวในอนาคต

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา กิตติยา อาชาทัง

ปีการศึกษา 2562 ลายมือชื่ออาจารย์ที่ปรึกษา

KITIYA ARSAKIT : ISSR AND SSR MARKERS LINKED TO POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE IN MUNGBEAN. THESIS ADVISOR : PROF. PIYADA ALISHA TANTASAWAT, Ph.D. 77 PP.

BULK SEGREGANT ANALYSIS (BSA)/LINKAGE/MOLECULAR MARKER/ QUANTITATIVE TRAIT LOCI (QTL)/RESISTANCE GENE

Powdery mildew (PM) caused by the fungus *Sphaerotheca phaseoli* is a serious disease during the cool-dry season while Cercospora leaf spot (CLS) caused by the fungus *Cercospora canescens* Illis & Martin is a serious disease during the rainy season of mungbean (*Vigna radiata* L. Wilczek) production. PM and CLS diseases incur total yield loss of up to 40% and more than 50% in susceptible cultivars, respectively, suggesting the requirement of resistant cultivars to both diseases for sustainable mungbean production. Marker-assisted selection (MAS) is crucial for developing resistant cultivars with multiple resistance genes. The objective of this study was to identify inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers which are linked to CLS and PM resistance genes in V4718. An $F_{2:7}$, $F_{2:8}$ and $F_{2:9}$ recombinant inbred line (RIL) population derived from a cross between CN72 (susceptible cultivar with high yield) and V4718 (resistant line) was used in this study. Each resistance to both diseases in RIL population was evaluated under field conditions during the cool-dry seasons of 2013 and 2016 for PM resistance, and the rainy seasons of 2016 and 2017 for CLS resistance. The segregation analysis showed that each resistance to PM and CLS is controlled by single dominant genes. A total of twenty ISSR primers and eleven SSR markers were used in bulk segregant analysis (BSA) to identify polymorphic markers between resistant and

susceptible cultivar/lines possibly associated with CLS and PM resistance. Only two ISSR markers (I13306 and I13311) were polymorphic and significantly associated with the PM resistance. And only five SSR markers (CEDAAG002, CEDC050, CEDG084, VR108, and VR393) were found polymorphic and significantly associated with the CLS resistance. Moreover, I13306, I13311 and VR393 markers were significantly associated with both disease resistance, suggesting that the genes conferring resistance to these diseases may be co-localized. Among these markers, VR393 exhibited the highest LOD scores for both diseases (> 5.0 for CLS and > 3.0 for PM), confirming that it was associated with resistance genes for both diseases. The multiple interval mapping (MIM) consistently identified one QTL (*qCLSC72V18*) linked to CLS resistance and one QTL (*qPMC72V18*) linked to PM resistance in $F_{2:7}$ RIL population of the cross between CN72 and V4718. *qCLSC72V18* and *qPMC72V18* were localized between VR108 and VR393 markers and accounted for 22.9-43.5% and 13.9-20.2% of the CLS and PM disease score variation in both growing seasons, respectively. The markers that were closely linked to both resistance genes could be used in MAS for combining CLS and PM resistance in future mungbean breeding.

มหาวิทยาลัยเทคโนโลยีสุรนารี

School of Crop production Technology Student's Signature Kitiya Arsakit

Academic Year 2019 Advisor's Signature Pimda Srisuka Tulsuwan

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LIST OF ABBREVIATIONS

AVRDC	=	Asian Vegetable Research and Development Center
BSA	=	Bulk segregant analysis
CTAB	=	Cetyltrimethyl ammonium bromide
CLS	=	Cercospora leaf spot
CN72	=	Chai Nat 72
χ^2	=	Chi-square test
R^2	=	Coefficient of determination
DAP	=	Days after planting
°C	=	Degree Celsius
dNTPs	=	Deoxyribonucleotide triphosphate
DMRT	=	Duncan's Multiple Range Test
ISSR	=	Inter simple sequence repeat
LG	=	Linkage group
LOD	=	Logarithms of odds
MAS	=	Marker-assisted selection
MIM	=	Multiple interval mapping
PAGE	=	Polyacrylamide gel electrophoresis gel
PCR	=	Polymerase chain reaction
PVP	=	Polyvinylpyrrolidone

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LIST OF ABBREVIATIONS (Continued)

PM	=	Powdery mildew
QTL	=	Quantitative trait loci
RCBD	=	Randomized complete block design
RIL	=	Recombinant inbred line
R	=	Resistant
RB	=	Resistant bulk
RNase	=	Ribonuclease
SSR	=	Simple sequence repeat
S	=	Susceptible
SB	=	Susceptible bulk

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CHAPTER I

INTRODUCTION

1.1 Significance of this study

Mungbean (*Vigna radiata* L. Wilczek) is an economically important legume crop in South and Southeast Asia. More than 90% of mungbean production is recorded in South Asia, where India accounts for about 45% of the world production of mungbean (Chaitieng et al., 2002; Chankaew et al., 2011). In addition to India, China, or Pakistan, Thailand is also an important producer and is one of the major exporters in the world. Thailand has exported mungbean to several countries such as Myanmar, Australia, Kenya, Cambodia, and Canada etc. (Office of Agricultural Economic, 2016). Mungbean is an excellent source of dietary proteins and amino acids. It is mainly consumed as sprout or raw, as well as used in cosmetic. It is also used as feed and in crop rotation for maintaining soil fertility. In Thailand, mungbean is often cultivated after rice or other cereal crops, or sometimes intercropped with other field and tree crops because of its early maturity, relative drought tolerance, and ability to fix atmospheric nitrogen in association with soil Rhizobium.

The low productivity of mungbean is mainly attributed to low genetic yield potential of current varieties, poor cultural practices, and the damage from biotic and abiotic stresses. Among the biotic stresses, Cercospora leaf spot (CLS) and powdery mildew (PM) are the major foliar diseases of mungbean. The CLS is a foliar disease caused by the hemibiotrophic fungus *Cercospora canescens* Ellis & Martin (Chand et al., 2015). It

is especially devastating in the rainy season. The fungus initially causes spotting on mungbean leaves. The spots increase in number and size during flowering and the increment is most rapid at the pod-filling stage. The infection expands rapidly resulting in premature defoliation and reduction in size of pods and seeds (Grewal et al., 1980). The disease can potentially reduce mungbean yield more than 50% in susceptible cultivars, if there is no protection (AVRDC, 1984). Meanwhile, the PM is caused by the biotrophic fungus *Sphaerotheca phaseoli* which is in the section of *Podosphaera* (a genus of fungi in the Erysiphaceae family) (Meeboon et al., 2016). The disease occurs mainly in the cool-dry growing season. It can reduce the yield of mungbean by more than 40% (Fernandez and Shanmugasundaram, 1987) and 100%, particularly at the seedling stage (Reddy et al., 1994). Although these diseases can be controlled by chemicals, they often increase the cost and are very harmful to environment and health. Therefore, the development of resistant cultivars is an important strategy to increase mungbean production.

The study on inheritance of resistance to CLS and PM is important for development of resistant cultivars. CLS resistance in mungbean has been reported to be controlled by a single dominant gene (Thakur et al., 1977; Chankaew et al., 2011). While PM resistance is controlled by either a single dominant gene or quantitative genes (Young et al., 1993; Chaitieng et al., 2002; Humphry et al., 2003; Kasettranan et al., 2010; Khajudparn et al., 2010; Chankaew et al., 2013). Conventional breeding methods mostly involve with the selection of resistant lines by means of field screening which is time-consuming. Therefore, molecular markers linked to the CLS and PM resistance genes will be helpful for rapid identification of progenies carrying these genes without the disease incidence in the specific seasons. Bulk segregant

analysis (BSA) can be initially used for rapid identification of the promising markers associated with disease resistance (Bainade et al., 2014).

Several reports have identified molecular makers, which are linked to PM resistance genes in different accessions of mungbean (Young et al., 1993; Chaitieng et al., 2002, Humphry et al., 2003, Kasettranan et al., 2010; Chankaew et al., 2013; Poolsawat et al., 2017). Moreover, molecular markers linked to a CLS resistance gene in mungbean have also been identified. Chankaew et al. (2011) found one major QTL (*qCLS*) for CLS resistance on linkage group (LG) 3 in KPS1 × V4718 F_2 and BC_1F_1 populations. *qCLS* was located between CEDG117 and VR393 markers, and accounted for 65.5-80.53% of CLS resistance. However, these markers might not be significantly linked to the CLS resistance gene in other populations. In the other words, they might be monomorphic, and could not be used for molecular marker-assisted selection (MAS). QTL for PM resistance have reported to be located on LG 3, 4, 6, 7, 8, and 9 of mungbean (Young et al., 1993; Kasettranan et al., 2010; Chankaew et al., 2013). These results suggest that some of the resistance genes conferring CLS and PM resistance may be co-localized in the same LG.

Recently, the molecular markers which are developed based on microsatellites or simple sequence repeats (SSRs) are SSR and ISSR (inter-simple sequence repeat) markers. Both marker systems have become one of the choices for unraveling breeding programs for disease resistance in several plants such as potato, rice, and maize (Marczewski, 2001; Latif et al., 2013; Salah et al., 2016). ISSR and SSR markers potentially amplify the DNA products based on polymerase chain reaction (PCR) technique that helps increase the efficiency for the selection, particularly in larger population, because the selection is economical, easy, and highly reproducible in all

laboratories (Tantasawat, 2011).

The objectives of this study were to identify polymorphic ISSR and SSR markers associated with CLS and PM resistance in $F_{2:7}$ recombinant inbred line (RIL) population of the CN72×V4718 cross and to evaluate the relationships between CLS and PM resistance.

1.2 Research objectives

1.2.1 To identify the ISSR markers associated with PM resistance in RIL population of the CN72×V4718 cross.

1.2.2 To examine the previous SSR markers reported to be linked to a CLS resistance gene of the other population in RIL population of the CN72×V4718 cross.

1.2.3 To analyze the correlation between PM and CLS resistance in RIL population of the CN72×V4718 cross.

1.2.4 To study the relationship between ISSR and SSR markers and PM or CLS resistance in RIL population of the CN72×V4718 cross.

1.2.5 To construct linkage map and QTL analysis of ISSR and SSR associated with PM and CLS resistances in RIL population of the CN72×V4718 cross.

1.3 Research hypotheses

1.3.1 Molecular markers that are polymorphic and associated with PM and CLS resistance and susceptibility in $F_{2:7}$ RIL population of the CN72×V4718 cross may be linked to PM and CLS resistance genes, therefore, the markers may be used for marker-assisted selection (MAS) for improving resistance to both diseases in the future.

1.3.2 The microsatellite or SSR regions are distributed throughout the plant genome, therefore, the ISSR and SSR markers may have a chance to be linked to PM and CLS resistance in mungbean

1.3.3 The SSR markers that had been reported to be polymorphic and linked to CLS resistance in the other population (KPS1×V4718) may be linked to CLS resistance genes in $F_{2:7}$ RIL population of the CN72×V4718 cross.

1.3.4 Both PM and CLS resistance from V4718 may have correlation, therefore, the markers that linked to PM and CLS resistance may be linked to CLS and PM resistance genes, respectively in $F_{2:7}$ RIL population of the CN72×V4718 cross.

1.3.5 The polymorphic or associated markers of PM and CLS resistance may be used to construct linkage map and QTL linked to CLS and PM resistance genes in RIL population of the CN72×V4718 cross.

1.4 Research scope

This research focuses on the identification of the ISSR and SSR markers that are linked to CLS and PM resistance genes in V4718. An $F_{2:7}$ RIL population derived from a cross between CN72 (susceptible cultivar with high yield) and V4718 (resistant line) was used in this study. The PM and CLS disease severity were evaluated during environmental conditions favorable to disease incidence; cool-dry and rainy season, respectively. The 20 ISSR primers including 806, 813, 820, 822, 824, 837, 839, 845, 852, 854, 861, 863, 865, 869, 870, 874, 877, 881, 892, and 900 were analyzed in RIL population of the CN72×V4718 cross. The 11 SSR markers that had been reported to be linked to CLS resistance gene in the other population including CEDAAG002, CEDC050, CEDC031, CEDG037, CEDG084, CEDG117, CEDG150,

CEDG232, CEDG305, VR108, and VR393 were analyzed in RIL population of the CN72×V4718 cross. All polymophic ISSR primers and SSR markers were used in bulk segregant analysis (BSA) to identify which markers will be possibly associated with PM and/or CLS resistance. Finally, the linkage mapping and QTL of both disease resistances were analyzed.

1.5 Expected outcomes

1.5.1 The ISSR markers linked to PM and CLS resistance genes in $F_{2:7}$ RIL population of the CN72×V4718 cross will be obtained.

1.5.2 The SSR markers linked to CLS and PM resistance genes in $F_{2:7}$ RIL population of the CN72×V4718 cross will be obtained.

1.5.3 Genetic linked map and QTL linked to CLS and PM resistance genes in $F_{2:7}$ RIL population of the CN72×V4718 cross will be obtained.

1.5.4 The obtained molecular markers could be used for marker-assisted selection (MAS) in mungbean breeding programs for PM and CLS resistance.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER II

LITERATURES REVIEW

2.1 Mungbean

Mungbean (*Vigna radiata* L. Wilczek) is a warm season legume species belonging to the Fabaceae family, and has a diploid chromosome number of 2n = 2x = 22. Mungbean is an economically important legume crop in South and Southeast Asia. More than 90% of mungbean production occurs in South Asia, where India accounts for about 45% of the world production of mungbean (Chaitieng et al., 2002; Chankaew et al., 2011). In addition, Thailand is also an important producer and is one of the major exporters in the world. Thailand exports mungbeans to many countries such as Myanmar, Australia, Kenya, Cambodia, and Canada etc. (Office of Agricultural Economic, 2016). Mungbean is an excellent source of dietary proteins, carbohydrates, fibers, amino acids and contains higher levels of folate and iron than most of other legumes (Keatinge et al., 2011). It is mainly consumed as sprouted or raw, as well as used in cosmetic. Moreover, mungbean has early maturity, relative drought tolerance, and ability to fix atmospheric N_2 in association with soil Rhizobium leading to improve soil fertility and texture (Graham et al., 2003). Intercropping mungbean in rice-rice and rice-wheat systems increases the yield of the subsequent cereal crops and reduces pest incidence (Yaqub et al., 2010).

Genetic diversity information and archaeological evidence suggest that mungbean was domesticated in India (Fuller, 2007). It was selected from a wild

species of mungbean (*V. radiata* var. *sublobata*) having a wide area of distribution and stretching from Central and East Africa, Madagascar through Asia, New Guinea, to North and East Australia (Tateishi 1996). The conserved genetic resources of mungbean have more than a thousand accessions (Tomooka et al., 2002). The largest collections hold by the World Vegetable Center (formerly the Asian Vegetable Research and Development Center; AVRDC) at Taiwan.

2.2 The major diseases of mungbean

2.2.1 Cercospora leaf spot (CLS)

The CLS is a foliar disease caused by the hemibiotrophic fungus *Cercospora canescens* Illis & Martin (Chand et al., 2015). It is especially devastating disease in the rainy season. The fungus initially causes spotting on mungbean leaves. The spots increase in number and size during flowering and the increment is most rapid at the pod-filling stage. The infection expands rapidly resulting in premature defoliation and reduction in size of pods and seeds (Grewal et al., 1980). The disease can potentially reduce mungbean yield more than 50% in susceptible cultivars if there is no protection (AVRDC, 1984).

2.2.2 Powdery mildew (PM)

The PM is caused by the biotrophic fungus *Sphaerotheca phaseoli* which is in the section of *Podosphaera* (a genus of fungi in the Erysiphaceae family) (Meeboon et al., 2016). The disease occurs mainly in the cool-dry growing season. It can reduce the yield of mungbean by more than 40% (Fernandez and Shanmuga-sundaram, 1987) and 100% at the seedling stage (Reddy et al., 1994).

2.2.3 Mungbean yellow mosaic virus (MYMV)

This disease is transmitted by the vector, the whitefly (*Bemisia tabaci*). It is found to spread the begomoviruses that virus is the major hazard to the flourishing production of mungbean. The most conspicuous symptom on the foliage starts as small yellow specks along the veinlets and spreads over the lamina; the pods become thinand curl upwards. Depending on the severity of the disease infection, the yield penalty may reach up to 85% (Karthikeyan et al., 2014)

2.2.4 Seed and seedling rot

A number of fungi such as *Fusarium* sp, *Macrophomina phaseoli*, and *Rhizoctonia solani*, cause seed and seedling rot. The fungi result in poor germination. It is a serious disease and sometimes re-sowing of the crop has to be done if it is not controlled well on time.

2.3 Breeding of mungbeans

2.3.1 Conventional breeding of mungbean in Thailand

Since 1959, the comparison between each of local cultivars of mungbean in Thailand began. Then, mungbean seeds from the Philippines were used for the selection of high yield cultivars in 1969. In 1974, Department of agriculture of Thailand began to select the mungbean lines from the AVRDC and collect as well as record the characteristics of varieties. U-Thong 1, high yield cultivar was obtained from the selection and also released. This recommended cultivar has been widely cultivated in Thailand with the increase in cultivated areas of mungbean in 1976. However, U-Thong 1 has been reported to be susceptible to PM and CLS diseases (Srinives, 1995). Then, Kasetsart University performed mass selection of cultivars/lines of mungbean

derived from AVRDC for disease resistance and high yield. Two varieties, Kamphaeng Saen 1 (KPS1) and KPS2 showed more resistance to PM and CLS diseases and higher yield than U-Thong 1 and both cultivars were certified as recommended cultivars of the Department of Agriculture of Thailand. After that, several mungbean cultivars were also released such as Chai Nat 36 (CN36) and CN60 from the Department of Agriculture of Thailand, and PHU1 from Prince of Songkla University (Laosuwan, 1988). Currently, plant disease is an important factor to the production of mungbean in Thailand, especially PM and CLS diseases. Recommended cultivars such as CN36, CN60, KPS1, and KPS2 are susceptible to both diseases (Chaitieng et al., 2002). Laosuwan et al. (1997) studied the inheritance of CLS resistance in VC3689A derived from AVRDC that was also backcrossed to PHU1, KPS1, and KPS2 at Suranaee University of Technology. The BC_4F_2 lines showed the characters similar to all cultivars with high CLS resistance. Moreover, one line from BC_4F_2 of VC3689A×PHU1 called Suranaree University of Technology 4 (SUT4) showed high CLS and PM resistance. Several mungbean cultivars that the government recommends farmers for cultivation are U-Thong 1, KPS1, KPS2, PHU1, SUT1, CN36, CN60, CN72, and CN84-1 (Ngampongsai et al., 2011; Perm et al., 2019). CN72 has high yield and beanfly resistance, but this cultivar as well as those mentioned above are susceptible to PM and CLS diseases in Thailand (Ngampongsai et al., 2011). Therefore, the improvement of mungbean varieties with high resistance to both diseases is very important. However, the development of resistant lines through the conventional breeding methods such as pedigree, bulk, single seed descent, and backcross method with field screening is time-consuming and the evaluation of some diseases requires environmental conditions favorable to disease

incidence such as PM disease because the pathogen cannot be cultured on media (Bainade et al., 2014).

2.3.2 Marker-assisted selection (MAS)

Currently, the biotechnology and molecular biology methods have been used in plant breeding programs in order to obtain more efficient and overcome the limitations of conventional breeding methods. Marker assisted selection (MAS), a complementary tool for conventional breeding where a molecular marker linked to a trait, is indirectly selected. MAS depends on identifying association between genetic marker and linked quantitative traits loci (QTL). The association between marker and QTL depends on distance between markers and target traits. Many studies have showed the implication to identify and develop markers for traits such as disease and pest resistance and other abiotic stresses. Moreover, the markers should be tightly linked to target loci, preferably less than 5 cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype.

Molecular markers associated with genes are mainly useful for the identification of desirable characters, MAS, and positional cloning of genes. Molecular markers allow breeders to dissect complex traits without phenotyping, thereby reducing the need to extensive field testing over time and space (Babu et al. 1996). Moreover, molecular markers can be used for disease resistance selection. Molecular markers can be typically divided into two classes, protein markers and DNA markers.

Protein markers are based on the different isozymes from alleles. Isozymes have similar catalytic, but have different amino acid sequences, which may

have a different electric charge or molecular weights. Protein markers are codominant markers, being able to distinguish between homozygous and heterozygous. However, the number of protein markers are limited and the expression is often tissues dependent (Tantasawat, 2011).

DNA markers are molecular markers, which can be capable of detecting any variations at the DNA level (DNA polymorphisms) such as nucleotide changes; deletion, duplication, inversion and/or insertion. It can detect the differences in genetic background of any organisms. DNA markers are less affected by age, physiological condition and environmental factors. They are not tissue-specific, thus, they can be detected at any phases of organism development. Only small amount of samples is sufficient for the analysis and the physical form of the sample does not restrict the detection (Sunil, 2010).

Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. In hybridization-based markers, DNA profiles are visualized by hybridizing the restriction enzymes digested DNA to a labeled probe, which can be DNA fragment of known origin or sequence, for example, restriction fragment length polymorphism (RFLP) marker. The PCR based markers involve in vitro amplification of the particular DNA sequences or loci with the help of specifically and arbitrarily chosen primers and a DNA polymerase enzyme. The amplified fragments are electrophoretically separated and banding patterns are detected by different methods, i.e. staining and autoradiography. Various types of PCR based markers are utilized to evaluate DNA polymorphism and tag genes, such as random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism

(AFLP), simple sequence repeat (SSR), and inter simple sequence repeat (ISSR), especially the two latter of which are increasingly applied in gene mapping.

For the strategies of using DNA markers for MAS in plant breeding, the markers should be tightly-linked to target loci as a substitute or to assist phenotypic screening. By determining the allele of a DNA marker, plants that possess the particular genes or QTL may be identified by means of their genotypes rather than their phenotypes. Utilization of DNA markers with PCR technique will increase the efficiency of selection, particularly in larger populations, because the technique used is economical, easy, and stable (Tantasawat, 2011).

SSRs or microsatellites or short tandem repeats (STRs) are in general repetitions of short nucleotides motifs of 1-6 bp long, including di, tri, and tetra nucleotides that are the most common e.g. $(CA)_n$, $(AAT)_n$, and $(GATA)_n$, respectively. SSRs can be further classified into pure, compound, and interrupted repeats. Pure SSRs consist of uninterrupted repeats of single motifs, e.g. $(AT)_n$. Compound SSRs consist of two or more repeats, e.g. $(GT)_n$, $(AT)_n$. Interrupted SSRs contain interruption in repeats, e.g. $(GT)_n$, and $GG(GT)_n$ (Perkall et al., 1998). Moreover, SSRs are widely distributed throughout the genome of plants and animals (Powell et al., 1996).

SSR markers are based on microsatellites or SSRs and PCR technique. These polymorphisms are identified by constructing PCR primers for the DNA flanking the microsatellite region, which can amplify variable length of DNA fragments based on the number of repeats (Figure 2.1). The length of the repeated unit is the same for the majority of the repeats within an individual microsatellite locus. The number of repeats for a specific locus may differ, resulting in varying in length of alleles. The advantages of SSR markers are high polymorphism, reliability, co-

dominance nature, the unambiguous designation of allele, selective neutrality, high reproducibility, as well as rapid and simple genotyping. However, the SSR markers also have the limitations, for example, they can be used only in some species, because the development of SSR primer pairs must be performed by cloning of flanking DNA sequences in SSR regions. In addition, the primers may not show the differences between each individual (Tantasawat, 2011).

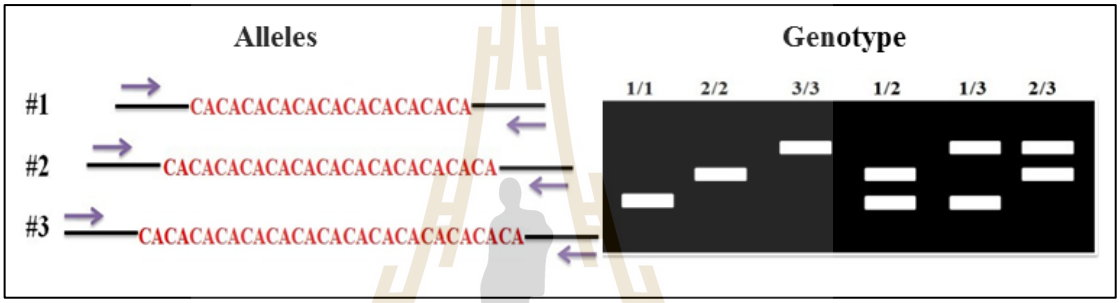

Figure 2.1 Simple sequence repeat marker (Modified from Chinese academy of fishery science (n.d.)).

ISSR markers are also based on microsatellites or SSRs and PCR technique. The markers involve amplification of DNA segments between two identical microsatellite repeat regions, which carry out in the opposite direction using primers designed from microsatellite core regions (Figure 2.2). The technique uses microsatellite primers with approximately 20 nucleotides of di-, tri-, tetra-, or penta-nucleotide repeats to target multiple genomic loci. The primers anchored at 3' or 5' with 2 to 4 degenerate bases extend into the flanking sequences (Zetkiewicz et al., 1994). ISSR primers generate polymorphism whenever any regions of the genome match the sequence repeat or have a deletion or insertion or translocation that

modifies the distance between the repeats. About 10 to 60 fragments from multiple loci are generated simultaneously, and can be separated by gel electrophoresis and then scored as the presence or absence of fragments of particular size. ISSR markers are generally considered as dominant markers according to Mendelian inheritance (Tsumura et al., 1996). The advantages of ISSR technique are simple, rapid, and less costly similar to the RAPD technique, but higher reproducibility than RAPD primers due to the longer primer length is noted. The development of ISSR markers does not need prior knowledge of the genome to be analyzed, hence, it can be used universally for plant genome analysis.

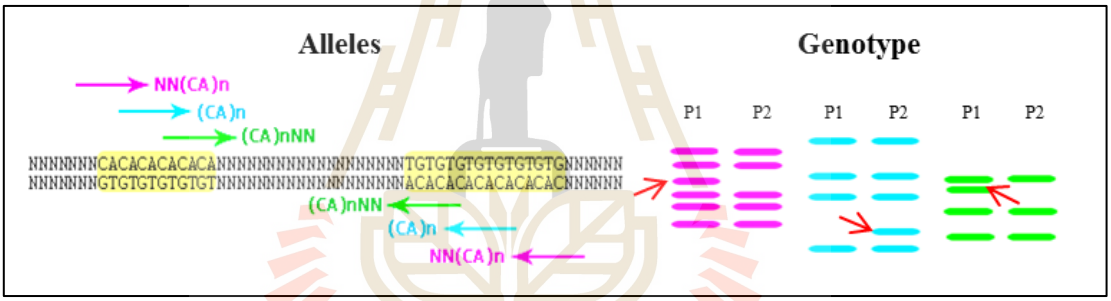

Figure 2.2 Inter simple sequence repeat markers (Modified from NCBI (n.d.)).

In recent years, the molecular markers based on microsatellites or SSRs, i.e., SSR and ISSR markers have become the best choice for several plant disease resistance breeding programs, for example, Marczewski (2001) who found ISSR markers, UBC811 (660 bp) and UBC811 (950 bp) linked to the *Ns* resistance gene, responsible for a resistance of potato (*Solanum tuberosum* L.) to potato virus *S* (PVS). Latif et al. (2013) found seven SSR markers and five ISSR markers associated with resistance to rice tungro virus. Salah et al. (2016) found the RAPD, ISSR, SSR, and

sequence tagged site (STS) markers linked to maize stalk rot disease resistance (*Fusarium moniliforme*) in maize.

ISSR markers have been also employed for genetic diversity analysis in *Vigna* species including mungbean (Ajibade et al. 2000; Souframanien and Gopalakrisna, 2004; Tantasawat et al., 2010). Moreover, mungbean has reported to contain many microsatellites distributed throughout the genome such as $(AT)_n$ or $(ATT)_n$ repeats which are found in every 34.6 kb of genomic DNA (Wang et al., 1994). Therefore, SSR and ISSR markers are the promising markers for the identification of the disease resistance genes in mungbean. However, the identification of PM and CLS resistance genes in mungbean has been rarely reported elsewhere.

For PM resistance, Young et al. (1993) found three RFLP loci associated with the PM resistance gene in a cross between VC3980A (resistant) and TC1966 (susceptible). Three RFLP loci, including sgK472, mgM208, and mgQ39 are located on linked group (LG) 3, 7, and 8, respectively. A total of three genomic regions were found to have an effect on PM response explaining 58% of the total variation. Chaitieng et al. (2002) mapped a new source of resistance to PM in F_2 population derived from a cross between a moderately resistant breeding line VC1210A and a susceptible wild relative (*V. radiata* var. *sublobata*, accession TC1966) using RFLP and AFLP analyses. The major QTL (*PMR1*) was found on new LG (not agree with Young et al. (1993)) accounting for 64.9% of the variation in resistance to PM. Humphry et al. (2003) used RFLP markers in a cross between Berken, a highly susceptible variety, and ATF 3640, a highly resistant line, for the identification of a major locus conferring resistance to PM. Fifty one probes generated 52 mapped loci, which were used to construct a linkage map spanning 350 cM of the

mungbean genome over 10 LGs. Using these markers, a single locus was identified explaining up to a maximum of 86% of the total variation in the resistance response to the pathogen. Zhang et al. (2008) developed SSR markers from RFLP marker (VrCS65) closely linked to one of PM resistance loci. Four of the new PCR markers co-segregated with the original RFLP marker VrCS65 and another SSR marker (VrCS SSR2) was located 0.5 cM away from PM resistance loci in the population of Berken×ATF3640. Kasettranan et al. (2010) identified two QTLs on two LGs, *qPMR-1* and *qPMR-2* accounting for 20.10 and 57.81% of the total variation of PM resistance, respectively in F_7 RILs of the KPS1×VC6468-11-1A cross. The SSR markers closely flanked and linked to *qPMR-1*(CEDG282 and CEDG191) and *qPMR-2* (MB-SSR238 and CEDG166) are useful for MAS for mungbean resistance to PM. Chankaew et al. (2011a) found the SSR markers (VA259 and DMB130) linked to PM resistance accounting for 16.42 and 19.74% of the total variation for PM resistance, respectively in F_1 and $F_{2:3}$ of the KPS1 (susceptible cultivar)×V4718 (highly resistant line) cross. Bainade et al. (2014) reported an ISSR marker amplified by the ISSR 834 primer possibly associated with the PM resistance gene in the bulk segregant analysis (BSA) of the Kopargaon×BPMR-48 cross. Chankaew et al. (2013) mapped QTL for PM resistance in two crosses by using SSR markers. It was found that one major and two minor QTL in KPS1×V4718 cross and two major and one minor QTL in a cross between CN60 (susceptible) and RUM5 (resistant) for controlling PM resistance. Recently, Poolsawat et al. (2017) identified a major QTL associated with PM resistance in mungbean using ISSR and ISSR-RGA markers in $F_{2:7}$ and $F_{2:8}$ RIL populations derived from a cross between CN72 (susceptible cultivar in Thailand) and V4718 (resistant line from Asian Vegetable Research and Development Center;

AVRDC). The major QTL, *qPMC72V18-1* explained up to 92.4% of the phenotypic variation. Moreover, the QTL was flanked by I42PL229 and I85420 markers at the distance of 4 and 9 cM, respectively.

For CLS resistance, only one report of Chankaew et al. (2011b) found major QTL (*qCLS*) for CLS resistance on LG3 in both F_2 (KPS1×V4718) and BC_1F_1 [(KPS1×V4718)×KPS1] populations. *qCLS* was located between the SSR markers; CEDG117 and VR393. This QTL explained 65.15-78.22% of phenotypic variation depending on seasons in F_2 population and 80.53% of phenotypic variation in BC_1F_1 population.

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CHAPTER III

MATERIALS AND METHODS

The present study was conducted in the field area and Plant Breeding Laboratory of School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. This research is divided into 4 experiments.

3.1 Experiment 1

Identification of ISSR markers associated with powdery mildew (PM) resistance in recombinant inbred line (RIL) population of the CN72×V4718 cross.

3.1.1 Plant materials

A cross was made between a PM resistant line V4718 originated from India (male parent) which was obtained from Asian Vegetable Research and Development Center (AVRDC) and a susceptible cultivar with high yield Chai Nat 72 (CN72) in Thailand (female parent). F_1 plants from the cross were grown, self-fertilized, and advanced by single-seed descent method to generate RILs with highly homozygous for several loci as much as possible. The populations of 172 $F_{2:7}$ RILs and $F_{2:8}$ RILs were obtained and used in this study.

3.1.2 Evaluation for PM resistance

The $F_{2:7}$ RILs and $F_{2:8}$ RILs of the CN72×V4718 cross and their parents were planted in randomized complete block design (RCBD) with three replications in cool-dry

season (December to February) of 2013 and 2016, respectively at experimental field in Suranaree University of Technology, Nakhon Ratchasima, Thailand. Each RIL and the male and female parents were planted in a single row of 10 plants per row with spacing of 50 cm between rows and 20 cm between hills. The PM susceptible cultivar, CN72 was grown surrounding the evaluating plots as disease spreading rows (Figure 3.1).

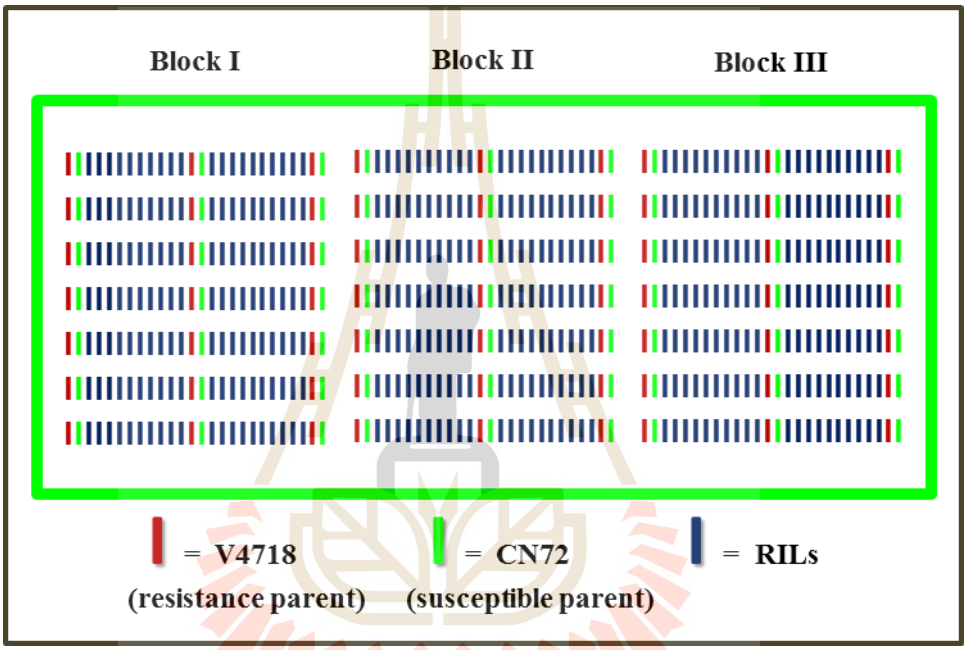

Figure 3.1 Planting plan for evaluating diseases in mungbean**.**

Disease scores were recorded at 65 days after planting (DAP) according to a standard scoring procedure with 1-9 scale (Figure 3.2) described by Khajudparn et al. (2010), as follows: 1 = no leaf symptom, 2 = 2-3 lesions on the lower part of leaves, 3 = 2-3 lesions on the lower part of leaves where spore formation can be observed, 4 = full spore formation on the lower part of leaves and a few lesions can be observed on the middle part of leaves, 5 = like number four, but chlorosis leaves and

much of spore formation can be observed, 6 = like number five, but full spore formation can be observed, 7 = spore formation on all parts of leaves and 25% dry leaves can be observed, 8 = like number seven, but 25-50% dry leaves can be observed, and 9 = like number seven, but over 50% dry leaves can be observed.

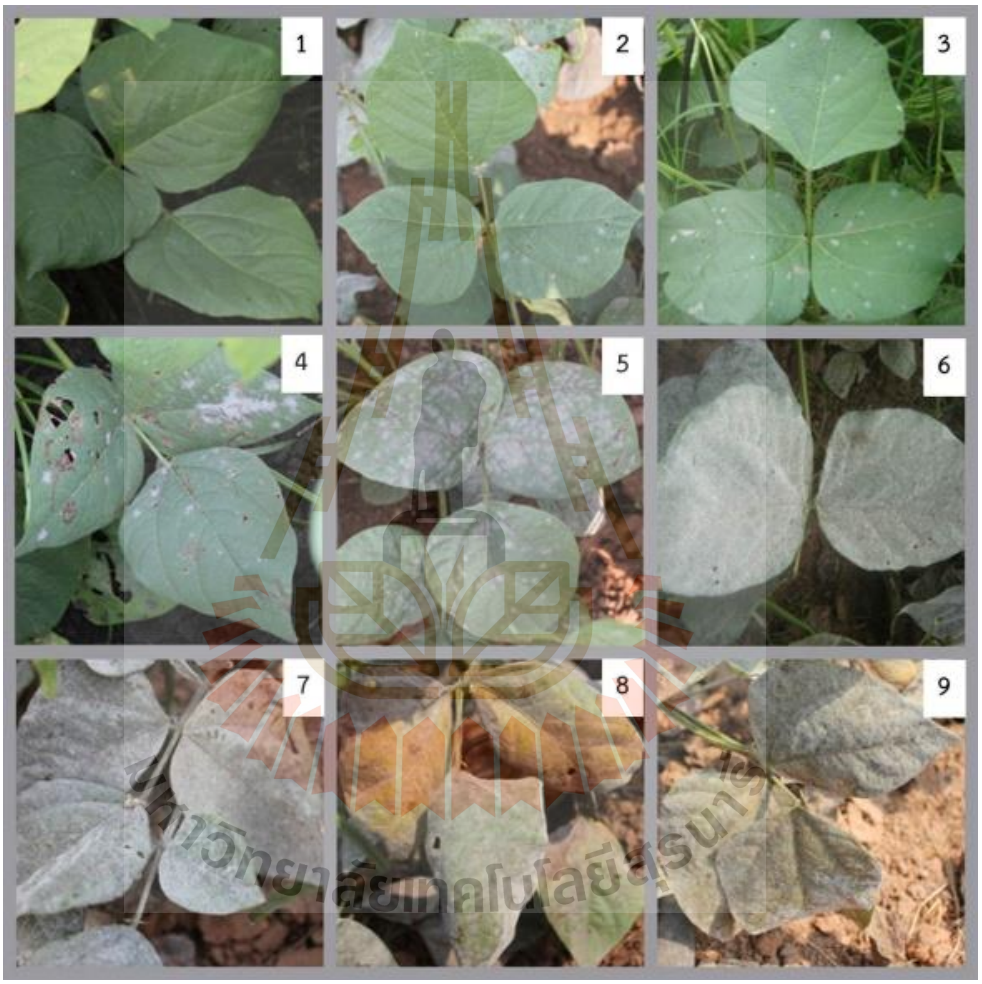

Figure 3.2 Disease symptoms in powdery mildew-infected mungbean leaves.

Based on the observed resistance levels of parents, individual RILs were categorized into two categories: resistant with score rating 1-4.9 and susceptible with score rating 5-9.

3.1.2.1 Statistical analysis

The chi-square test (χ^2) was used to study the distribution of PM resistance gene in this cross. PM scores from the field evaluation in 2013 and 2016 were transformed with $(X+1)^{1/2}$ formula when disease score data does not fit in a normal distribution curve. Then the disease scores were determined by one-way ANOVA (analysis of variance). All of the analyzed data were subjected to Duncan's Multiple Range Test (DMRT) for mean comparison at 5% level of significance analysis using SPSS version 14.0 (Levesque and SPSS Inc., 2006).

3.1.3 DNA extraction

Total genomic DNA was extracted from young leaves of CN72, V4718, and RILs following the CTAB method described by Owen (2003). The leaves were ground with 600 µL of extraction buffer (3% (w/v) cetyltrimethyl ammonium bromide (CTAB), 1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, 2% (w/v) polyvinylpyrrolidone (PVP), and 0.2% (v/v) β-mercaptoethanol, transferred into microfuge tubes and incubated for 30 min at 65 °C. Equal volume of 24 chloroform: 1 isoamyl alcohol (v/v) was added and subjected to the centrifugation at 13,000 rpm for 20 min. One volume of isopropanol (cold) and 0.5 volumes of 5M NaCl were added to the supernatant followed by centrifugation at 13,000 rpm for 15 min to pellet the DNA. The supernatant was discarded. The DNA pellet was washed with 70% and 100% ethanol (cold), and air dried. Depending upon the size of the pellet, DNA was dissolved in 100-200 µL of deionized water. Ribonuclease (RNase) was added to a concentration of 1 mg/mL, incubated for 30 min at 37 °C, and stored at -20 °C until further use. DNA was quantified by spectrophotometry using a ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) to adjust the

final concentration to 4 ng/µL for use in PCR analysis.

3.1.4 Bulk segregant analysis (BSA)

The BSA was performed as described by Michelmore et al. (1991). BSA was performed using DNA of CN72 (susceptible; S), V4718 (resistance; R), resistant bulk (RB), and susceptible bulk (SB). Genomic DNA pooled from 10 plants exhibiting the highest resistance and susceptibility to PM was selected to construct resistant bulk and susceptible bulk, respectively. Each bulk was adjusted to at a final concentration of 150 ng/µl. Banding patterns of bulks were compared with those of the parents to identify potential markers associated with the PM resistance.

3.1.5 ISSR analysis

A total of 20 ISSR primers homologus to microsatellite repeats and containing additional selective anchor nucleotides that were developed from the University of British Columbia were chosen for the initial screening. The 20 ISSR primers used in this study are the common primers that have been studied in several plants, but are newly used in mungbean for the identification of PM resistance (Table 3.1). All of the primers were used in BSA to identify polymorphisms between PM resistant parent (V4718; R) and PM susceptible parent (CN72; S) and between PM resistant bulk (RB) and PM susceptible bulk (SB).

3.1.5.1 Polymerase chain reaction (PCR) analysis

The PCR for ISSR was performed in 20-µL aliquots containing 150 ng of DNA template, 1× buffer [50 mM KCl, 10 mM Tris.HCl (pH 9.1), and 0.01% Triton^{TM} X-100], 3.5 mM $MgCl_2$, 250 µM of each deoxyribonucleotide triphosphate (dNTPs), 0.4 µM of each ISSR primer, and 1 U of Taq DNA polymerase. Amplification was carried out in an Amplitronyx^{TM} 6 Thermal Cycler (Nyx Technik,

Inc., San Diego, CA, USA) and PCR amplification conditions were subjected to amplification with initial denaturation at 95°C for 5 min; 35 cycles of denaturing at 95°C for 1 min, annealing at 45-54°C for 1 min, extension at 72°C for 1 min; and a final extension at 72°C for 10 min.

Table 3.1 List of ISSR primer sequences used in this study.

Primers	Primer sequences (5'→3')[a]	Examples of utilization in other plants	Reference
806	$(TA)_8G$	*Stipa bungeana*	Yu et al., 2014
813	$(CT)_8T$	*Triticum aestivum*	Son et al., 2013
820	$(GT)_8C$	*Triticum aestivum*	Son et al., 2013
822	$(TC)_8A$	*Triticum aestivum*	Son et al., 2013
824	$(TC)_8G$	*Thymus* spp.	Yousefi et al., 2013
837	$(TA)_8RT$	Tomato	Saravanan et al., 2013
839	$(TA)_8RG$	Tomato	Saravanan et al., 2013
845	$(CT)_8RG$	*Thymus* spp.	Yousefi et al., 2013
852	$(TC)_8RA$	*Thymus* spp.	Yousefi et al., 2013
854	$(TC)_8RG$	*Musa acuminata*	Lamare and Rao, 2013
861	$(ACC)_6$	*Aloe vera* (L.)	Bhaludra et al., 2013
863	$(AGT)_6$	Betelvine	Khadke et al., 2015
865	$(CCG)_6$	*Jatropha curcas* L.	Sunil et al., 2011
869	$(GTT)_6$	Chickpea	Gautam et al., 2016
870	$(TGC)_6$	*Melia dubia* Cav.	Rawat et al., 2016
874	$(CCCT)_4$	*Scrophularia ningpoensis*	Chen et al., 2011
877	$(TGCA)_4$	*Taxus baccata* L.	Zarek, 2016
881	$(GGGTG)_3$	*Ocimum* sp.	Chen et al., 2013
892	TAGATCTG$(AT)_2$ CTGAATTCCC	*Caragana microphylla*	Huang et al., 2015
900	ACTTCCC$(CA)_2$GG TTA$(CA)_2$	*Astragali*	Liu et al., 2016

[a] R = purines (A, G)

3.1.5.2 Resolution and visualization of PCR products on PAGE

The amplified DNA products were revealed on 6% denaturing polyacrylamide gel electrophoresis gel (PAGE) prepared in 1× TBE buffer at 200 V for 90 min. The amplified products were detected by silver nitrate according to Sambrook

and Russell (2001). Molecular weights of the DNA bands were estimated using 100 bp DNA ladder (Invitrogen, CA, USA) as standard.

3.1.5.3 DNA band scoring

Putative ISSR loci associated with the PM resistance gene were identified by comparing DNA banding patterns between R and S as well as RB and SB. The amplified bands were scored as 1 (present band) and 0 (absent band). The range of amplified products, the number of scorable DNA bands, and the number of polymorphic bands between male and female parents for each of the ISSR primer were recorded.

3.1.5.4 Statistical Analysis

The obtained polymorphic markers from BSA were further analyzed in both parents and individual plants used for BSA (10 highest resistant lines and 10 highest susceptible lines) for confirmation of the polymorphic markers associated with PM resistance in $F_{2:7}$ RIL population of the CN72×V4718 cross. The preliminary determination of markers putatively associated with PM resistance gene will be analyzed by single regression analysis and recombination will be calculated. Only markers that are significantly associated with PM resistance at $P < 0.05$ with recombination less than or equal to 30% will be used for further analysis with 100 RIL population of the cross (50 highest resistant lines and 50 highest susceptible lines) for identifying the markers associated with PM resistance in $F_{2:7}$ RIL population of the CN72×V4718 cross. A goodness of fit to the Mendelian segregation ratio was calculated using Chi-square (χ^2) analysis to examine the segregation patterns of the selected ISSR markers. Markers associated with PM resistance were preliminarily determined by the coefficient of determination (R^2). The single regression analysis was

performed using SPSS version 14.0 (Levesque and SPSS Inc. 2006). Logarithms of odds (LOD) score were calculated following Morton (1955). Recombination will be calculated as follow:

$$\text{Recombination (\%)} = \frac{\text{Number of recombinant progeny}}{\text{Total number of progeny}} \times 100$$

3.2 Experiment 2

Evaluation of SSR markers that have been reported be linked to Cercospora leaf spot (CLS) resistance gene of the other population in RIL population of the CN72×V4718 cross.

3.2.1 Plant materials

The cross was made between a CLS resistant line V4718 from India (male parent) obtained from AVRDC and a susceptible cultivar with high yield CN72 in Thailand (female parent). F_1 plants from the cross were grown, self-fertilized, and advanced by single-seed descent method. Finally, the population of 172 $F_{2:9}$ RILs was obtained and used in this study.

3.2.2 Evaluation for CLS resistance

Parents and the $F_{2:9}$ RIL of the CN72×V4718 cross were planted in a RCBD with three replications in rainy season (June to August) of 2016 and 2017 at experimental field in Suranaree University of Technology, Nakhon Ratchasima, Thailand. Each RIL and the male and female parents were planted similar to 3.1.2.

Plants were scored in rows based on percentage of leaf area covered by the disease at 65 DAP. Scoring system used was described Chankaew et al. (2011), with a scale of 1-5 (Figure 3.3): 1 = no visual disease infection, 2 = 1-25% leaf areas infected, 3 = 26-50% leaf areas infected, 4 = 51-75% leaf areas infected, and 5 = 76-100% leaf areas infected.

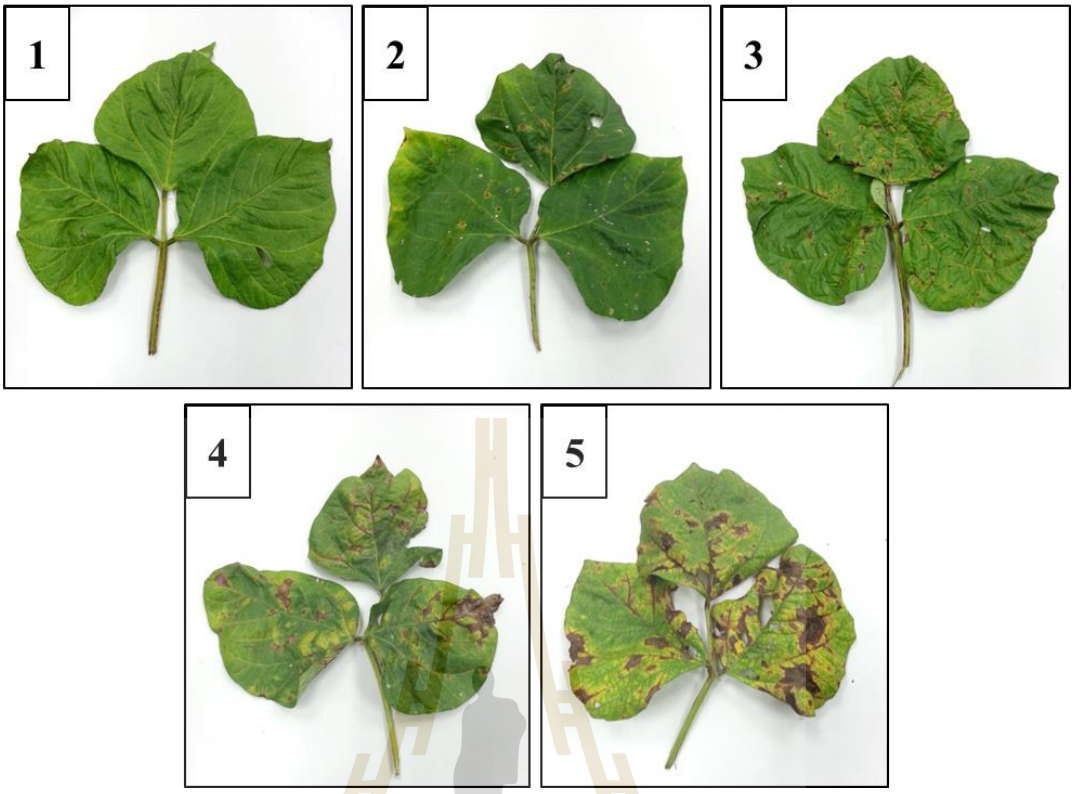

Figure 3.3 Disease symptoms in Cercospora leaf spot-infected mungbean leaves.

Disease scoring was conducted and averaged by a panel of three trained staffs. The scale of disease severity will be divided into two categories (resistant with score rating 1-2.9 and susceptible with score rating 3-5).

3.2.2.1 Statistical analysis

The chi-square test (χ^2) was used to study the distribution of CSL resistance gene in this cross. CLS scores from the field evaluation in 2016 and 2017 were transformed with $(X+1)^{1/2}$ formula and the disease scores were determined by one-way ANOVA (analysis of variance). All the analyzed data were subjected to Duncan's Multiple Range Test (DMRT) for mean comparison at 5% level of significance analysis using SPSS version 14.0 (Levesque and SPSS Inc., 2006).

3.2.3 DNA extraction

DNA extraction was performed similar to 3.1.3.

3.2.4 Bulk segregant analysis (BSA)

BSA was performed using DNA of CN72 (Susceptible; S), V4718 (Resistance; R), resistant bulk (RB), and susceptible bulk (SB). Genomic DNA pooled from 10 plants exhibiting the highest resistance and susceptibility to CLS was selected to construct resistant and susceptible bulk, respectively. Each bulk was adjusted to a final concentration of 40 ng/µl. Band patterns of bulks were compared with those of the parents to identify potential markers associated with the CLS resistance.

3.2.5 SSR analysis

A total of eleven SSR primer pairs that have been reported by Chankaew et al. (2011b) to be polymorphic and linked to CLS resistance in F_2 and BC_1F_1 populations of the KPS1×V4718 cross (Table 3.2) were used in BSA to identify polymorphisms between CLS resistant parent (V4718) and CLS susceptible parent (CN72) as well as between CLS resistant bulk and CLS susceptible bulk which consisted of 10 each of CLS resistant RILs and CLS susceptible RILs, respectively.

3.2.5.1 Polymerase chain reaction (PCR) analysis

Each 20 µL PCR mix contained 2 ng genomic DNA template, 1× buffer (75 mM Tris-HCl, pH 9.0, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 1 U Taq DNA polymerase, and 0.5 µM each of forward and reverse SSR primers. Amplification was carried out in a Techne FTCPLUS/02 TC-PLUS Series thermocyler (Bibby Scientific, Ltd, Staffordshire, UK) and PCR amplification conditions were subjected to amplification with initial denaturation at 95°C for 2 min; 35 cycles of denaturing at 95°C for 30 sec, annealing at 42-65°C for 30 sec, extension at 72°C for 1min;

and a final extension at 72℃ for 10 min.

Table 3.2 List of SSR primer pair sequences used in this study.

Primers	Primer sequences (5'→3')	
	Forward	Reverse
CEDAAG002	GCAGCAACGCACAGTTTCATGG	GCAAAACTTTTCACCGGTACGACC
CEDC050	TCCCACTTCTCCATTACCTCCAC	GAGATTATCTTCTGGGCAGCAAGG
CEDC031	GGGAATAAATAAACCTTTCC	TCTCAAATCACATTGCCAC
CEDG037	GAAGAAGAACCCTACCACAG	CACCAAAAACGTTCCCTCAG
CEDG084	ATCAACTGAGGAGCATCATCGA	CAACATTTCAACCTTGGGACAG
CEDG117	GTACACTTCCACTAATCCAAAATT	TGGTACCTTCCTTATCTGAAATTA
CEDG150	GAAGGGAATGAAAATGAAACCC	GTTCAATCCATTCAGTCTCC
CEDG232	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG
CEDG305	GCAGCTTCACATGCATAGTAC	GAACTTAACTTGGGTTGTCTGC
VR108	GCTCCAACACTCACTCACAAAC	CAGAAATGCAGGAAAAGAGAGG
VR393	TGGCACTTTCCATAACGAATAC	ATCAGCCAAAAGCTCAGAAAAC

3.2.5.2 Resolution and visualization of PCR products on PAGE

The amplified products were visualized on 6% denaturing polyacrylamide gel prepared in 1× TBE buffer at 200 V for 90 min. The amplified DNA products were detected by silver nitrate according to Sambrook and Russell (2001). Molecular weights of the DNA bands were estimated using 100 bp DNA ladder (Invitrogen, CA, USA) as standard.

3.2.5.3 DNA band scoring

The SSR alleles associated with the CLS resistance gene were identified by comparing DNA banding patterns between R and S as well as RB and SB. The segregating band from the CLS susceptible parent (CN72) was scored as 3, resistant parent (V4718) as 1 and heterozygous bands were scored as 2 in BSA.

3.2.5.4 Statistical Analysis

Then, the obtained polymorphic markers from BSA were further

analyzed in parents (CN72 and V4718) and 90 RILs of the cross (45 highest resistant lines and 45 highest susceptible lines) for identifying the markers associated with PM resistance in $F_{2:7}$ RIL population of the CN72×V4718 cross. The statistical analyses of each marker were calculated similar to 3.1.5.4.

3.3 Experiment 3

Correlation between PM and CLS resistance and the relationship between ISSR and SSR markers and PM or CLS resistance in the RIL population of the CN72×V4718 cross were evaluated.

3.3.1 Correlation between PM and CLS resistance

Coefficients of correlation between 2013 and 2016 of PM disease scores as well as 2016 and 2017 of CLS disease scores were determined by correlation analysis using SPSS version 14.0 (Levesque and SPSS Inc., 2006).

3.3.2 ISSR markers associated with CLS resistance

The obtained polymorphic ISSR markers associated with PM resistance in experiment 1 were used to identify markers associated with CLS resistance. DNA band score of each marker will be used for calculation similar to 3.1.5.3 and statistical analyses were similar to 3.1.5.4.

3.3.3 SSR markers associated with PM resistance

The obtained polymorphic SSR markers associated with CLS resistancein experiment 2 were used to identify markers associated with PM resistance. DNA band score of each marker will be used for calculation similar to 3.2.5.3 and statistical analyses were similar to 3.2.5.4.

3.4 Experiment 4

Construction of linkage map and QTL analysis of ISSR and SSR associated with PM and CLS resistance in RIL population of the CN72×V4718 cross were performed.

3.4.1 Linkage map analysis

Genetic linkage maps were constructed by MAPMAKER 3.0b (Lander et al., 1987). A threshold log likelihood ratio (LOD) of 3.0 was used to group markers into linkage groups using the Kosambi mapping function (Kosambi, 1944).

3.4.2 QTL analysis

QTL analysis was performed using the multiple interval mapping (MIM) (Kao et al. 1999) implemented in Windows QTL Cartographer 2.5 (Wang et al., 2012). The analysis was performed on the data of line means from individual experiments. Permutation tests (Churchill and Doerge, 1994) were run with 4,000 times at the significance level of P = 0.01 to determine a LOD score threshold for declaring a significant QTL.

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CHAPTER IV

RESULTS AND DISCUSSION

4.1 Experiment 1

Identification of ISSR markers associated with powdery mildew (PM) resistance in recombinant inbred line (RIL) population of the CN72×V4718 cross.

4.1.1 Evaluation for PM resistance

RILs are produced by continuous selfing the progeny of individual members of an F_2 population until complete homozygous is achieved (Poolsawat et al., unpublished data). In a practically homozygous RIL population, the $F_{2:7}$ generation and onward have percentage of homozygosity more than 98.44% according to the theory. Therefore, this experiment used $F_{2:7}$ and $F_{2:8}$ RILs of the CN72×V4718 cross for evaluated PM resistance in 2013 and 2016, respectively.

In 2013, the PM resistant line V4718 had a mean disease score of 1.76, while the susceptible variety CN72 had a score of 6.33. The disease scores were significantly different between the parents and 172 $F_{2:7}$ RILs ($P < 0.01$). Among 172 $F_{2:7}$ RILs of the CN72×V4718 cross, 25 progenies (5B, 6B, 7B, 8B, 12B, 13B, 21B, 24B, 27B, 33B, 34B, 39B, 44B, 45B, 51B, 71B, 78B, 79B, 94B, 107B, 162B, 167B, 172B, 175B, and 186B) had a mean disease score of 1.00-1.50, indicating more resistant than V4718 but not significantly different ($P > 0.05$). Twenty one progenies (4B, 18B, 31B, 52B, 53B, 59B, 70B, 83B, 92B, 93B, 102B, 104B, 114B, 125B, 126B, 127B, 128B, 136B, 141B, 159B, and 165B) had a mean disease score of 6.50-7.00, indicating susceptible than CN72 but not

significantly different (P > 0.05) (Table 4.1).

Table 4.1 Powdery mildew disease scores of CN72, V4718, and 172 RILs of the CN72×V4718 cross in 2013.

Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)
CN72	6.33 ± 0.33 a-e[1/]	35B	2.00 ± 1.00 l-q	68B	1.67 ± 0.67 n-q
V4718	1.67 ± 0.67 n-q	36B	4.00 ± 2.01 a-p	69B	1.67 ± 0.67 n-q
2B	3.67 ± 0.88 b-p	38B	4.50 ± 0.50 a-l	70B	6.50 ± 0.50 a-d
3B	2.00 ± 0.58 l-q	39B	1.00 ± 0.00 q	71B	1.33 ± 0.33 pq
4B	7.00 ± 0.00 a	40B	5.00 ± 1.00 a-j	72B	5.00 ± 1.16 a-j
5B	1.00 ± 0.00 q	41B	2.00 ± 1.00 l-q	73B	5.33 ± 1.45 a-i
6B	1.00 ± 0.00 q	42B	6.00 ± 1.00 a-f	74B	2.00 ± 0.58 l-q
7B	1.00 ± 0.00 q	43B	4.00 ± 2.01 a-p	75B	5.67 ± 1.20 a-h
8B	1.50 ± 0.50 opq	44B	1.00 ± 0.00 q	76B	2.00 ± 0.58 l-q
9B	2.50 ± 0.50 i-q	45B	1.00 ± 0.00 q	77B	1.67 ± 0.67 n-q
10B	4.00 ± 1.00 a-o	46B	5.50 ± 0.50 a-h	78B	1.00 ± 0.00 q
11B	5.50 ± 0.50 a-h	47B	2.00 ± 0.58 l-q	79B	1.33 ± 0.33 pq
12B	1.00 ± 0.00 q	48B	5.00 ± 1.53 a-k	80B	6.00 ± 1.16 a-f
13B	1.50 ± 0.50 opq	49B	2.33 ± 0.88 j-q	81B	2.00 ± 0.58 l-q
14B	2.00 ± 0.00 k-q	50B	2.00 ± 0.58 l-q	82B	2.33 ± 0.88 j-q
15B	3.50 ± 2.51 e-q	51B	1.33 ± 0.33 pq	83B	7.00 ± 0.58 ab
16B	6.00 ± 0.00 a-f	52B	6.67 ± 0.33 abc	84B	5.67 ± 0.88 a-g
17B	5.00 ± 1.00 a-j	53B	7.00 ± 1.00 ab	85B	6.00 ± 0.58 a-f
18B	6.50 ± 0.50 a-d	54B	6.33 ± 0.33 a-e	86B	1.67 ± 0.33 m-q
21B	1.50 ± 0.50 opq	55B	4.33 ± 1.20 a-n	87B	6.33 ± 0.33 a-e
22B	3.00 ± 0.00 f-q	56B	5.33 ± 1.77 a-j	88B	5.67 ± 0.33 a-g
23B	3.50 ± 0.50 c-p	57B	3.33 ± 0.88 d-q	89B	2.00 ± 0.58 l-q
24B	1.00 ± 0.00 q	58B	4.00 ± 2.01 a-p	90B	3.67 ± 0.67 a-p
25B	2.00 ± 1.00 l-q	59B	6.67 ± 0.33 abc	91B	3.67 ± 0.33 a-p
26B	3.50 ± 2.51 e-q	60B	5.67 ± 0.88 a-g	92B	7.00 ± 1.00 ab
27B	1.00 ± 0.00 q	61B	6.00 ± 0.58 a-f	93B	7.00 ± 0.58 ab
28B	5.50 ± 0.50 a-h	62B	5.67 ± 0.88 a-g	94B	1.33 ± 0.33 pq
29B	2.00 ± 1.00 l-q	63B	4.33 ± 0.88 a-m	95B	6.00 ± 0.00 a-f
31B	7.00 ± 0.00 a	64B	4.00 ± 1.16 a-o	96B	5.00 ± 0.00 a-j
32B	4.50 ± 2.51 a-n	65B	1.67 ± 0.33 m-q	97B	4.00 ± 1.00 a-o
33B	1.50 ± 0.50 opq	66B	3.33 ± 0.88 d-q	98B	5.33 ± 0.33 a-i
34B	1.00 ± 0.00 q	67B	2.33 ± 0.67 j-q	99B	6.00 ± 0.00 a-f

Table 4.1 Powdery mildew disease scores of CN72, V4718, and 172 RILs of the CN72×V4718 cross in 2013 (continued).

Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)
100B	5.33 ± 0.33 a-i	122B	5.33 ± 1.20 a-i	145B	5.00 ± 0.00 a-j
101B	6.00 ± 0.00 a-f	123B	6.00 ± 0.00 a-f	146B	6.00 ± 0.00 a-f
102B	7.00 ± 0.58 ab	124B	6.00 ± 0.58 a-f	147B	2.67 ± 0.67 g-q
103B	2.33 ± 0.67 j-q	125B	6.50 ± 0.50 a-d	148B	1.67 ± 0.67 n-q
104B	6.50 ± 0.50 a-d	126B	6.67 ± 0.33 abc	149B	3.33 ± 0.33 d-q
105B	6.00 ± 1.53 a-f	127B	6.67 ± 0.33 abc	150B	1.67 ± 0.33 m-q
106B	5.67 ± 1.33 a-h	128B	6.67 ± 0.33 abc	151B	5.67 ± 0.33 a-g
107B	1.33 ± 0.33 pq	130B	3.67 ± 0.88 b-p	152B	5.67 ± 0.33 a-g
108B	4.33 ± 1.20 a-n	131B	5.33 ± 0.88 a-i	153B	6.33 ± 0.33 a-e
109B	4.33 ± 1.20 a-n	132B	5.67 ± 0.33 a-g	155B	6.00 ± 0.58 a-f
110B	4.67 ± 1.33 a-l	133B	4.00 ± 1.16 a-o	156B	5.67 ± 0.88 a-g
111B	3.67 ± 1.20 c-p	134B	6.33 ± 0.33 a-e	157B	4.33 ± 1.20 a-n
112B	2.67 ± 0.88 h-q	135B	2.00 ± 0.00 k-q	158B	5.67 ± 0.88 a-g
113B	5.67 ± 0.88 a-g	136B	6.67 ± 0.33 abc	159B	6.50 ± 0.50 a-d
114B	7.00 ± 0.58 ab	137B	6.00 ± 0.00 a-f	161B	3.33 ± 0.67 d-q
115B	5.33 ± 0.67 a-i	138B	5.00 ± 0.58 a-j	162B	1.00 ± 0.00 q
116B	6.33 ± 0.33 a-e	139B	6.33 ± 0.67 a-e	163B	4.67 ± 1.20 a-l
117B	2.33 ± 0.88 j-q	140B	4.33 ± 0.88 a-m	164B	5.50 ± 1.50 a-i
118B	6.00 ± 0.00 a-f	141B	7.00 ± 0.58 ab	165B	6.50 ± 0.50 a-d
119B	5.50 ± 0.50 a-h	142B	5.33 ± 0.67 a-i	166B	5.00 ± 2.01 a-j
120B	6.00 ± 0.58 a-f	143B	6.33 ± 0.33 a-e	186B	1.50 ± 0.50 opq
121B	5.67 ± 0.88 a-g	144B	5.67 ± 0.33 a-g	187B	6.00 ± 0.00 a-f

[1/]Means in the same column with different letters are significantly different ($P < 0.05$) based on Duncan's multiple range test (DMRT)

In 2016, PM was more virulent to parents, particularly susceptible variety with mean disease scores of 2.67 and 6.67 for V4718 and CN72, respectively. The disease scores were significantly different between the parents and practically homozygous $F_{2:8}$ RILs ($P < 0.01$). Among 178 $F_{2:8}$ RILs of the CN72×V4718 cross, 39 progenies (2B, 5B, 6B, 7B, 8B, 13B, 14B, 21B, 24B, 26B, 27B, 29B, 33B, 34B, 35B, 41B, 45B, 51B, 55B, 63B, 64B, 65B, 66B, 67B, 68B, 71B, 76B, 77B, 79B, 82B, 86B,

90B, 91B, 94B, 107B, 135B, 150B, 162B, and 175B) had a mean disease score of 1.00-2.33, indicating more resistant than V4718 but not significantly different ($P > 0.05$). Four progenies (31B, 40B, 87B, and 158B) had a mean disease score of 7.00, indicating more susceptible than CN72 but not significantly different ($P > 0.05$) (Table 4.2).

Table 4.2 Powdery mildew disease scores of CN72, V4718, and 178 RILs of the CN72×V4718 cross in 2016.

Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)
CN72	6.67 ± 0.33 ab[1/]	27B	1.33 ± 0.33 pq	55B	1.33 ± 0.33 pq
V4718	2.67 ± 0.33 k-q	28B	6.33 ± 0.33 abc	56B	5.00 ± 1.15 a-j
2B	1.67 ± 0.33 opq	29B	2.00 ± 0.58 m-q	57B	6.33 ± 0.33 abc
3B	4.00 ± 1.15 c-m	31B	7.00 ± 0.00 a	58B	6.33 ± 0.33 abc
4B	6.33 ± 0.67 a-d	32B	5.67 ± 0.33 a-f	59B	6.33 ± 0.33 abc
5B	1.33 ± 0.33 pq	33B	1.00 ± 0.00 q	60B	6.67 ± 0.33 ab
6B	1.67 ± 0.33 opq	34B	2.33 ± 0.33 l-q	61B	6.33 ± 0.33 abc
7B	1.67 ± 0.33 opq	35B	1.67 ± 0.67 opq	62B	6.00 ± 0.58 a-e
8B	1.33 ± 0.33 pq	36B	2.67 ± 1.67 l-q	63B	2.33 ± 0.67 l-q
9B	3.00 ± 0.58 i-p	37B	3.33 ± 0.88 g-o	64B	1.33 ± 0.33 pq
10B	3.33 ± 0.33 f-o	38B	5.33 ± 1.20 a-i	65B	1.33 ± 0.33 pq
11B	4.00 ± 0.58 c-l	39B	2.67 ± 0.33 k-q	66B	1.67 ± 0.33 opq
12B	4.00 ± 1.15 c-m	40B	7.00 ± 0.00 a	67B	1.00 ± 0.00 q
13B	1.33 ± 0.33 pq	41B	1.33 ± 0.33 pq	68B	1.67 ± 0.33 opq
14B	2.00 ± 0.58 m-q	42B	5.67 ± 1.33 a-g	69B	3.00 ± 0.58 i-p
15B	5.33 ± 0.88 a-h	43B	5.67 ± 0.33 a-f	70B	5.00 ± 1.00 a-j
16B	5.00 ± 1.00 a-j	44B	1.33 ± 0.33 pq	71B	2.33 ± 0.33 l-q
17B	4.67 ± 1.20 a-k	45B	1.00 ± 0.00 q	72B	6.33 ± 0.33 abc
18B	5.67 ± 0.33 a-f	46B	6.00 ± 0.58 a-e	73B	6.00 ± 0.58 a-e
19B	3.67 ± 0.67 e-n	47B	4.00 ± 1.00 c-l	74B	4.33 ± 0.88 b-l
20B	2.67 ± 0.67 k-q	48B	3.00 ± 1.15 j-p	75B	6.00 ± 0.58 a-e
21B	1.33 ± 0.33 pq	49B	4.33 ± 0.88 b-l	76B	1.33 ± 0.33 pq
22B	4.00 ± 1.15 c-m	50B	2.67 ± 0.33 k-q	77B	2.00 ± 0.58 m-q
23B	5.33 ± 0.33 a-g	51B	1.00 ± 0.00 q	78B	4.33 ± 0.88 b-l
24B	2.00 ± 1.00 n-q	52B	5.33 ± 1.20 a-i	79B	1.67 ± 0.33 opq
25B	4.00 ± 0.58 c-l	53B	6.00 ± 0.58 a-e	80B	4.33 ± 0.88 b-l
26B	2.00 ± 0.58 m-q	54B	4.33 ± 1.33 b-l	81B	5.00 ± 0.00 a-j

Table 4.2 Powdery mildew disease scores of CN72, V4718, and 178 RILs of the CN72×V4718 cross in 2016 (continued).

Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)	Cultivars / lines	PM score (Mean ± SE)
82B	2.33 ± 0.67 l-q	115B	4.67 ± 0.67 a-k	149B	6.33 ± 0.33 abc
83B	6.33 ± 0.67 a-d	116B	6.00 ± 0.58 a-e	150B	1.00 ± 0.00 q
84B	5.00 ± 0.00 a-j	117B	5.33 ± 0.67 a-g	151B	6.33 ± 0.33 abc
85B	4.00 ± 0.58 c-l	118B	4.67 ± 0.67 a-k	152B	6.00 ± 0.58 a-e
86B	1.00 ± 0.00 q	119B	5.67 ± 0.33 a-f	153B	6.67 ± 0.33 ab
87B	7.00 ± 0.00 a	120B	6.00 ± 0.00 a-e	155B	6.00 ± 1.00 a-e
88B	6.33 ± 0.67 a-d	121B	6.00 ± 0.58 a-e	156B	5.33 ± 0.88 a-h
89B	2.67 ± 0.33 k-q	122B	4.67 ± 0.33 a-k	157B	5.00 ± 1.15 a-j
90B	1.67 ± 0.33 opq	123B	6.00 ± 0.58 a-e	158B	7.00 ± 0.00 a
91B	1.33 ± 0.33 pq	124B	5.67 ± 0.33 a-f	159B	6.67 ± 0.33 ab
92B	6.67 ± 0.33 ab	125B	5.33 ± 0.33 a-g	161B	4.33 ± 1.20 b-l
93B	4.00 ± 0.00 c-l	126B	5.33 ± 0.67 a-g	162B	2.00 ± 0.58 m-q
94B	1.67 ± 0.33 opq	127B	6.33 ± 0.33 abc	163B	6.33 ± 0.33 abc
95B	4.33 ± 0.88 b-l	128B	6.33 ± 0.33 abc	164B	6.67 ± 0.33 ab
96B	6.33 ± 0.33 abc	130B	3.00 ± 1.15 j-p	165B	5.00 ± 1.15 a-j
97B	5.67 ± 0.33 a-f	131B	6.33 ± 0.67 a-d	166B	6.67 ± 0.33 ab
98B	4.33 ± 1.33 b-l	132B	6.33 ± 0.33 abc	167B	4.33 ± 0.88 b-l
99B	5.33 ± 0.33 a-g	133B	5.00 ± 1.00 a-j	168B	4.67 ± 0.67 a-k
100B	5.67 ± 0.33 a-f	134B	6.33 ± 0.33 abc	169B	4.67 ± 0.33 a-k
101B	6.33 ± 0.33 abc	135B	2.33 ± 0.67 l-q	170B	5.33 ± 0.33 a-g
102B	6.33 ± 0.33 abc	136B	4.00 ± 1.53 d-m	172B	6.00 ± 0.58 a-e
103B	2.67 ± 0.33 k-q	137B	6.00 ± 0.58 a-e	173B	3.67 ± 0.33 e-n
104B	5.33 ± 1.20 a-i	138B	6.67 ± 0.33 ab	174B	5.67 ± 0.88 a-f
105B	6.67 ± 0.33 ab	139B	6.00 ± 1.00 a-e	175B	1.00 ± 0.00 q
106B	6.33 ± 0.33 abc	140B	6.67 ± 0.33 ab	177B	5.67 ± 0.33 a-f
107B	1.00 ± 0.00 q	141B	6.33 ± 0.33 abc	178B	6.00 ± 0.00 a-e
108B	5.00 ± 1.00 a-j	142B	6.33 ±0.33 abc	181B	6.67 ± 0.33 ab
109B	5.33 ± 1.20 a-i	143B	4.67 ± 0.33 a-k	182B	6.67 ± 0.58 ab
110B	5.00 ± 1.15 a-j	144B	6.67 ± 0.33 ab	183B	5.00 ± 0.58 a-j
111B	3.00 ± 0.00 h-p	145B	6.00 ± 0.58 a-e	184B	6.00 ± 0.58 a-e
112B	6.33 ± 0.33 abc	146B	5.67 ± 1.33 a-g	185B	6.33 ± 0.67 a-d
113B	6.67 ± 0.33 ab	147B	2.67 ± 0.33 k-q	186B	3.67 ± 0.33 e-n
114B	6.67 ± 0.33 ab	148B	2.67 ± 0.33 k-q	187B	5.67 ± 0.88 a-f

[1/]Means in the same column with different letters are significantly different ($P < 0.05$) based on Duncan's multiple range test (DMRT)

The correlation coefficient (*r*) between the disease scores of both years was 0.744 ($P < 0.05$), suggesting that they are significantly correlated. The difference may be influenced by the environment, which was more suitable for disease development in 2016. The distribution of $F_{2:7}$ and $F_{2:8}$ RILs progenies from the cross deviated from normality with skewness toward the parents (Figure 4.1). From Table 4.3, 88 were resistant and 84 were susceptible in 2013, and 83 were resistant and 95 were susceptible in 2016. The segregation ratio of 1:1 (resistant: susceptible) in both years (χ^2 = 0.09 and 0.81, respectively, $P_{0.05}$ = 3.84) was observed for this population. The results demonstrated that the resistance to PM conferred by V4718 in this cross was conditioned by a single gene. Similarly, the inheritance of resistance to PM in the breeding lines VC 1560A and ATF 3640 was controlled by single dominant gene (AVRDC, 1981; Humphry et al., 2003). Moreover, Khajudparn et al. (2007) found that the PM resistance in each of the three resistance lines (V4718, V4785, and V4758) was controlled by each single dominant gene with non-allelic interaction, suggesting that these resistance genes can be transferred into commercial varieties to provide durable resistance to PM. In addition, Khajudparn (2009) revealed that resistance to PM in six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2) of the KPS1×V4758, KPS1×V2106, and KPS2×V2106 crosses was conditioned by single major genes. However, several researchers reported that PM resistance genes were quantitatively inherited in the RUM breeding line (Raddy et al., 1994), F_7 of KPS1×VC6468-11-1A (Kasettranan et al., 2010), and $F_{2:3}$ of KPS1×V4718 (Chankaew et al., 2013). These results demonstrated that the inheritance of resistance to PM may be different in individual crosses, and should be studied individually.

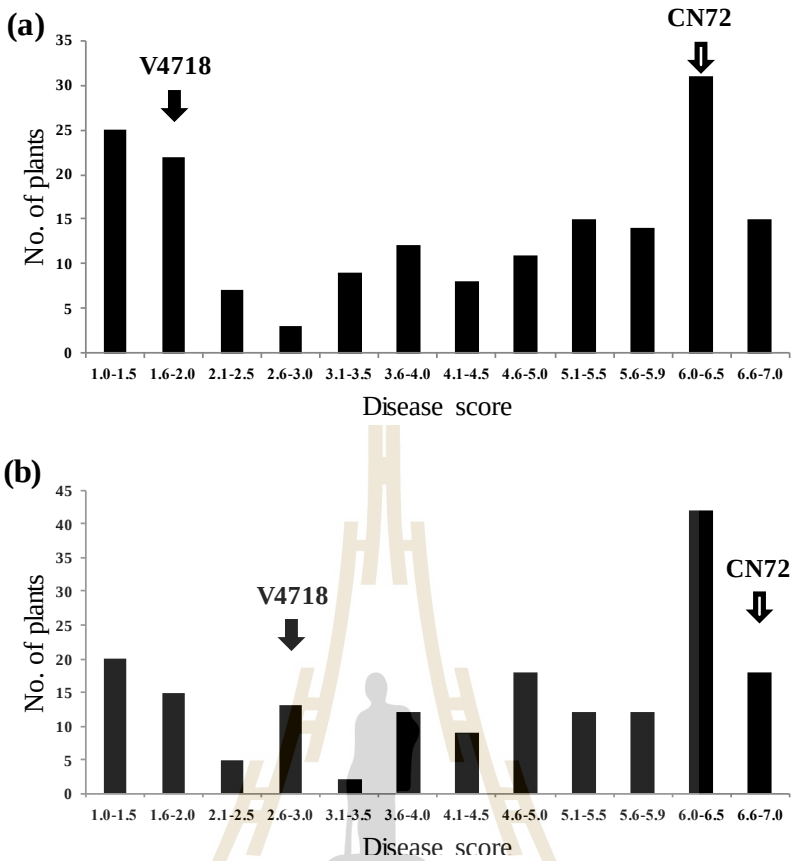

Figure 4.1 Frequency distribution of disease scores for response to powdery mildew of mungbean in the CN72×V4718 cross, (a) the $F_{2:7}$ RILs population evaluated in 2013, (b) the $F_{2:8}$ RIL population evaluated in 2016.

Table 4.3 Segregation in reaction to powdery mildew in $F_{2:7}$ and $F_{2:8}$ RIL populations derived from the CN72×V4718 cross.

Populations	Years	No. of lines	No. of resistant: susceptible lines	Chi-square test		
				Expected ratio	χ^2 value[a]	$P = 0.05$[b]
$F_{2:7}$	2013	172	88:84	1:1	0.09	3.84
$F_{2:8}$	2016	178	83:95	1:1	0.81	3.84

[a] Tha χ^2 value was tested for goodness of fit against 1:1 ratio for RILs
[b] P = 0.05, the differential levels of chi-square test for the resistance/susceptibility ratios with the probabilities of 95%

4.1.2 ISSR analysis

A total of twenty ISSR primers were used in BSA to identify polymorphic markers between CN72, V4718, PM resistant bulk, and PM susceptible bulk. The experiment is the first time to use these ISSR primers to identify PM resistance in mungbean. Nine of the ISSR primers (ISSR 806, 837, 839, 852, 863, 870, 874, 877, and 892) failed to produce consistent results and were omitted from further experiment. Ten ISSR primers (ISSR 820, 822, 824, 845, 854, 861, 865, 869, 881, and 900) were monomorphic and only one of the amplifiable primers ISSR 813 showed two polymorphic bands between male and female parents (Table 4.4). The percentage of polymorphism (%PB) generated by ISSR 813 was 10.53%. The range of amplified products of most ISSR primers was 200-2,072 bp regardless of ISSR 861 primer. The suitable annealing temperature for amplification of most ISSR primers was 50 °C regardless of two primers (ISSR 822 and 845).

Two polymorphic markers of ISSR 813 primer were further evaluated to analyze the DNA patterns of parents (CN72 and V4718) and 20 $F_{2:7}$ RIL of the cross. The polymorphic marker at 306 bp designated as I13306 with the presence of a band in resistant line (V4718) and most of the highest resistant individuals, but was absent in susceptible cultivar (CN72) and most of the highest susceptible individuals was noted. By contrast, the polymorphic marker at 311 bp designated as I13311 exhibited a band in susceptible cultivar (CN72) and most of the highest susceptible individuals, but was absent in resistant line (V4718) and most of the highest resistant individuals. Therefore, both markers from BSA may be associated with PM resistance in the population (Figure 4.2).

Table 4.4 Primer sequences, range of amplified products, number of scorable DNA bands (NB), number of polymorphic bands between male and female parents (PB), % polymorphic band between resistant and susceptible parents (% PB), and annealing temperature for each of ISSR primers in the CN72×V4718 cross.

ISSR primers	Primer sequences[a]	Range of amplified products (bp)	Number of scorable DNA bands	PB	%PB	Annealing temperature (°C)
813	$(CT)_8T$	200-2,072	19	2	10.53	50
820	$(GT)_8C$	200-2,072	17	0	0	50
822	$(TC)_8A$	200-2,072	25	0	0	54
824	$(TC)_8G$	200-2,072	13	0	0	50
845	$(CT)_8RG$	200-2,072	16	0	0	45
854	$(TC)_8RG$	200-2,072	12	0	0	50
861	$(ACC)_6$	100-2,072	18	0	0	50
865	$(CCG)_6$	200-2,072	15	0	0	50
869	$(GTT)_6$	200-2,072	17	0	0	50
881	$(GGGTG)_3$	200-2,072	20	0	0	50
900	ACTTCCC $(CA)_2$GG TTA$(CA)_2$	200-2,072	25	0	0	50

[a] R = purines (A, G)

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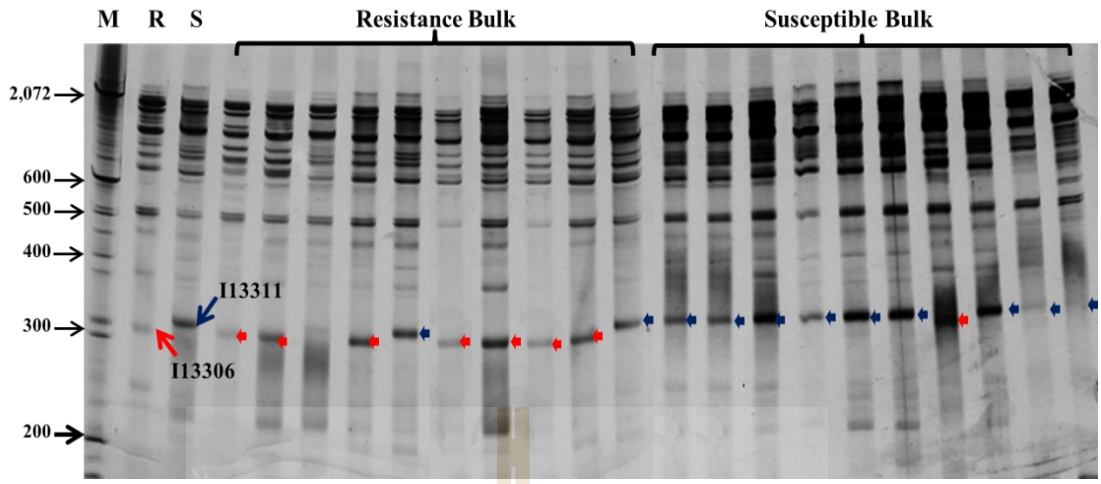

Figure 4.2 DNA pattern of I13306 and I13311 markers with powdery mildew resistance gene in $F_{2:7}$ RIL individuals of the cross CN72×V4718, where, M = 100 bp DNA ladder, R = resistant parent, S = susceptible parent, 10 individuals of resistant bulk, and 10 individuals of susceptible bulk. Blue (I13311) and red (I13306) arrows show markers putatively liked to PM resistance gene.

Single regression was calculated for each year of PM score with all polymorphic ISSR markers. The potential relationship between the marker and PM resistance score was established considering the significance of the regression coefficient. The marker which is having a strongest relationship can be judged from the coefficient of determination (R^2) value which reflects the overall percentage of variability of the particular trait that the marker can explain. Single regression analysis for PM resistance score in 2013 and 2016 of RIL population derived from the cross between CN72 and V4718 is presented in Table 4.5 and 4.6 for 20 and 100 RILs, respectively. From initial screening of 20 RIL individuals from BSA, two polymorphic markers, I13306 ($P < 0.01$) and I13311 ($P < 0.05$), were significantly

associated with PM resistance gene in both years. In 2013, the phenotypic variation of PM resistance explained (R^2) was 35.0% and 26.4% for I13306 and I13311 markers, respectively, and in 2016 showed R^2 of 44.6% and 34.3% for I13306 and I13311 markers, respectively (Table 4.5).

Table 4.5 ISSR markers associated with powdery mildew resistance in 20 RIL population derived from the cross between CN72 and V4718.

Primers	PM resistance 2013				PM resistance 2016			
	R^2 (%)[a]	Probability	LOD[b] scores	cM[c]	R^2 (%)[a]	Probability	LOD[b] scores	cM[c]
I13306	35.0	0.006	1.674	20	44.6	0.001	2.349	15
I13311	26.4	0.020	1.136	25	34.3	0.007	1.674	20

[a] R^2, coefficient of determination
[b] LOD, logarithms of odds
[c] cM, % recombination or distance between the marker and PM resistance gene

To confirm the markers linked to PM resistance, two markers were used for further evaluation with practically homozygous 100 RIL populations (50 highest resistance lines and 50 highest susceptible lines to avoid any residual heterozygosity (Table 4.6). The genotypes of two ISSR markers in 100 RIL population were shown in Appendix. The single regression analysis showed that both markers were significantly cosegregated with the PM resistance gene ($P < 0.001$) with R^2 of 23.6% (I13306) and 16.6% (I13311) and LOD score of > 3 in 2013. Also in 2016, both markers were significantly cosegregated with the PM resistance gene ($P < 0.001$) with R^2 of 26.9% (I13306) and 23.9% (I13311) and LOD score of 4.772 and 2.878, respectively.

The results confirmed that BSA could be used to shorten the time needed for identification of markers associated with the PM resistance gene, and

agreed with Bainade et al. (2014) who obtained the possible linkage between an ISSR marker and PM resistance using BSA in F_3 population of Kopargaon×BPMR-48 cross. Although I13306 and I13311 markers showed association with PM resistance gene, both markers showed high percentage of recombination or distance between the markers and PM resistance gene (more than 25 % or cM; Table 4.6). These results suggest that these markers may locate on the same chromosome with PM resistance gene but the markers cannot be used for MAS in this population.

Table 4.6 ISSR markers associated with powdery mildew resistance in 100 RILs population derived from the cross between CN72 and V4718.

Primers	PM resistance 2013				PM resistance 2016			
	R^2 (%)[a]	Probability	LOD[b] scores	cM[c]	R^2 (%)[a]	Probability	LOD[b] scores	cM[c]
I13306	23.6	0.000	5.681	25	26.9	0.000	4.772	27
I13311	16.6	0.000	3.952	29	23.9	0.000	2.878	32

[a] R^2, coefficient of determination
[b] LOD, logarithms of odds
[c] cM, % recombination or distance between the marker and PM resistance gene

4.2 Experiment 2

Evaluation of previous SSR markers that were reported to be linked to Cercospora leaf spot (CLS) resistance gene of the other population to identify in RIL population of the CN72×V4718 cross.

4.2.1 Evaluation for CLS resistance

The CLS of 147 $F_{2:9}$ RILs of the CN72×V4718 cross that are practically homozygous RIL population and their parents was evaluated at 65 days after planting in 2016 and 2017, respectively. In 2016, the CLS resistant line V4718 had a mean disease score of 1.67, while the susceptible variety CN72 had a score of 4.00. The

disease scores were significantly different between the parents and 147 $F_{2:9}$ RILs ($P < 0.01$). Among 147 $F_{2:9}$ RILs of the CN72×V4718 cross, 30 progenies (5B, 10B, 11B, 15B, 21B, 22B, 28B, 30B, 35B, 36B, 43B, 68B, 70B, 85B, 90B, 91B, 94B, 95B, 97B, 98B, 123B, 140B, 155B, 156B, 166B, 168B, 169B, 170B, 174B, and 186B) had mean disease score of 1.00-1.33, indicating more resistant than V4718 but not significantly different ($P > 0.05$). Two progenies (132B and 183B) had a mean disease score of 4.67 and 4.33, respectively, indicating more susceptible than CN72 but not significantly different ($P > 0.05$) (Table 4.7).

Table 4.7 Cercospora leaf spot disease scores of CN72, V4718, and 147 RILs of the CN72×V4718 cross in 2016.

Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)
CN72	4.00 ± 0.00 abc[1/]	24B	3.00 ± 0.58 a-f	50B	3.33 ± 0.33 a-e
V4718	1.67 ± 0.33 efg	25B	3.00 ± 0.58 a-f	51B	3.00 ± 1.00 a-f
2B	2.33 ± 0.33 b-g	27B	2.00 ± 0.58 d-g	52B	3.00 ± 0.58 a-f
4B	3.33 ± 0.67 a-e	28B	1.33 ± 0.33 fg	53B	1.67 ± 0.33 efg
5B	1.33 ± 0.33 fg	29B	1.67 ± 0.33 efg	54B	2.33 ± 0.33 b-g
6B	2.00 ± 0.58 d-g	30B	1.33 ± 0.00 fg	55B	1.67 ± 0.33 efg
7B	1.67 ± 0.67 efg	31B	3.67 ± 0.67 a-d	57B	1.67 ± 0.33 efg
8B	2.33 ± 0.33 b-g	32B	4.00 ± 0.00 abc	58B	2.00 ± 0.00 d-g
10B	1.33 ± 0.33 fg	33B	2.00 ± 1.00 d-g	59B	2.00 ± 0.58 d-g
11B	1.33 ± 0.33 fg	34B	1.67 ± 0.33 efg	60B	2.33 ± 0.33 b-g
12B	2.00 ± 0.58 d-g	35B	1.33 ± 0.33 fg	61B	3.67 ± 0.88 a-d
13B	2.00 ± 0.58 d-g	36B	1.00 ± 0.00 g	62B	3.33 ± 0.67 a-e
15B	1.33 ± 0.33 fg	37B	3.67 ± 0.88 a-d	63B	3.33 ± 0.67 a-e
16B	1.67 ± 0.33 efg	42B	2.33 ± 0.33 b-g	64B	4.00 ± 0.00 abc
17B	2.33 ± 0.33 b-g	43B	1.33 ± 0.33 fg	66B	1.67 ± 0.67 efg
19B	1.67 ± 0.67 efg	44B	3.00 ± 0.58 a-f	67B	2.00 ± 0.58 d-g
20B	2.00 ± 0.58 d-g	45B	3.33 ± 0.67 a-e	68B	1.00 ± 0.00 g
21B	1.00 ± 0.00 g	46B	4.00 ± 0.00 abc	69B	4.00 ± 0.58 abc
22B	1.00 ± 0.00 g	47B	3.33 ± 0.67 a-e	70B	1.33 ± 0.33 fg
23B	3.33 ± 0.67 a-e	48B	3.00 ± 0.58 a-f	71B	2.00 ± 0.00 d-g

Table 4.7 Cercospora leaf spot disease scores of CN72, V4718, and 147 RILs of the CN72×V4718 cross in 2016 (continued).

Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)
73B	3.67 ± 0.33 a-d	109B	3.67 ± 0.33 a-d	152B	3.67 ± 0.33 a-d
74B	3.00 ± 0.58 a-f	110B	3.00 ± 0.58 a-f	153B	3.00 ± 0.58 a-f
77B	2.33 ± 0.88 c-g	111B	2.33 ± 0.33 b-g	155B	1.33 ± 0.33 fg
78B	1.67 ± 0.33 efg	112B	3.00 ± 0.58 a-f	156B	1.33 ± 0.33 fg
80B	2.33 ± 0.88 c-g	114B	3.67 ± 0.33 a-d	157B	1.67 ± 0.33 efg
81B	3.33 ± 0.67 a-e	115B	2.33 ± 0.88 c-g	158B	3.00 ± 0.58 a-f
82B	2.00 ± 0.58 d-g	118B	3.00 ± 0.58 a-f	159B	1.67 ± 0.33 efg
83B	2.00 ± 1.00 d-g	119B	3.67 ± 0.88 a-d	160B	2.00 ± 0.00 d-g
84B	1.67 ± 0.33 efg	120B	3.33 ± 0.33 a-e	161B	3.00 ± 0.58 a-f
85B	1.00 ± 0.00 g	121B	4.00 ± 0.00 abc	162B	3.67 ± 0.88 a-d
86B	2.00 ± 0.00 d-g	123B	1.33 ± 0.33 fg	164B	3.67 ± 0.33 a-d
87B	3.00 ± 0.00 a-f	124B	1.67 ± 0.33 efg	165B	3.00 ± 0.58 a-f
88B	2.00 ± 0.00 d-g	125B	2.33 ± 0.33 b-g	166B	1.00 ± 0.00 g
89B	2.33 ± 0.88 c-g	126B	3.33 ± 1.20 a-e	167B	1.67 ± 0.33 efg
90B	1.33 ± 0.33 fg	127B	1.67 ± 0.67 efg	168B	1.33 ± 0.33 fg
91B	1.33 ± 0.33 fg	130B	2.00 ± 0.58 d-g	169B	1.33 ± 0.33 fg
92B	3.33 ± 0.67 a-e	131B	3.33 ± 0.67 a-e	170B	1.00 ± 0.00 g
94B	1.00 ± 0.00 g	132B	4.67 ± 0.33 a	172B	2.33 ± 0.88 c-g
95B	1.00 ± 0.00 g	133B	4.00 ± 0.58 abc	173B	1.67 ± 0.33 efg
96B	2.00 ± 0.58 d-g	134B	2.33 ± 0.33 b-g	174B	1.33 ± 0.33 fg
97B	1.33 ± 0.33 fg	135B	3.67 ± 0.88 a-d	175B	3.00 ± 1.00 a-f
98B	1.00 ± 0.00 g	137B	3.67 ± 0.33 a-d	177B	3.33 ± 0.33 a-e
99B	3.67 ± 0.33 a-d	140B	1.33 ± 0.33 fg	181B	3.00 ± 1.00 a-f
100B	1.67 ± 0.33 efg	141B	3.33 ± 0.67 a-e	182B	3.33 ± 0.67 a-e
101B	3.33 ± 0.88 a-e	143B	3.33 ± 0.67 a-e	183B	4.33 ± 0.33 ab
102B	1.67 ± 0.33 efg	144B	4.00 ± 0.00 abc	184B	1.67 ± 0.33 efg
104B	3.33 ± 0.33 a-e	145B	3.33 ± 0.67 a-e	185B	1.67 ± 0.33 efg
105B	3.00 ± 0.58 a-f	147B	2.00 ± 0.00 d-g	186B	1.00 ± 0.00 g
106B	4.00 ± 0.00 abc	148B	2.00 ± 0.58 d-g	187B	3.33 ± 0.33 a-e
107B	3.67 ± 0.88 a-d	151B	4.00 ± 0.00 abc		

[1/]Means in the same column with different letters are significantly different ($P < 0.05$) based on Duncan's multiple range test (DMRT)

CLS resistance was also evaluated in 2017. The CLS resistant line V4718 had a mean disease score of 2.67, while the susceptible variety CN72 had a score of

3.67. The disease scores were significantly different between the parents and 147 $F_{2:9}$ RILs ($P < 0.01$). Among 147 $F_{2:9}$ RILs of the CN72×V4718 cross, 5 progenies (94B, 95B, 98B, 166B and 186B) and had mean disease score of 1.00, indicating more resistant than V4718. Twenty seven progenies (2B, 10B, 29B, 30B, 33B, 36B, 42B, 43B, 62B, 84B, 85B, 90B, 97B, 100B, 107B, 111B, 123B, 124B, 130B, 140B, 147B, 155B, 157B, 167B, 174B, 175B, and 185B) had mean disease scores of 1.33-2.00, indicating more resistant than V4718 but not significantly different ($P > 0.05$). Sixteen progenies (11B, 31B, 37B, 45B, 50B, 60B, 61B, 74B, 106B, 109B, 120B, 132B, 135B, 137B, 161B, and 182B) had mean disease scores of 3.67-4.33, respectively, indicating more susceptible than CN72 but not significantly different ($P > 0.05$) (Table 4.8).

Table 4.8 Cercospora leaf spot disease scores of CN72, V4718 and 147 RILs of the CN72×V4718 cross in 2017.

Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)
CN72	3.67 ± 0.67 a-e[1/]	20B	3.33 ± 0.33 a-e	37B	4.00 ± 0.00 ab
V4718	2.67 ± 0.33 d-i	21B	2.33 ± 0.33 d-i	42B	1.33 ± 0.33 ij
2B	1.67 ± 0.67 hij	22B	2.67 ± 0.67 b-h	43B	1.33 ± 0.88 ij
4B	3.67 ± 0.67 a-e	23B	3.33 ± 0.88 a-g	44B	3.33 ± 0.88 a-g
5B	2.50 ± 0.50 d-h	24B	2.67 ± 0.33 b-h	45B	4.33 ± 0.33 a
6B	2.50 ± 0.50 d-h	25B	3.00 ± 0.00 a-g	46B	3.67 ± 0.67 a-e
7B	3.67 ± 0.67 a-e	27B	3.33 ± 0.33 a-e	47B	3.33 ± 0.33 a-e
8B	3.67 ± 0.67 a-e	28B	2.67 ± 0.33 b-h	48B	3.33 ± 0.33 a-e
10B	2.00 ± 0.58 g-j	29B	1.33 ± 0.33 ij	50B	3.67 ± 0.33 a-d
11B	4.00 ± 0.58 abc	30B	1.33 ± 0.33 ij	51B	3.33 ± 0.33 a-e
12B	3.67 ± 0.67 a-e	31B	3.67 ± 0.33 a-d	52B	3.67 ± 0.67 a-e
13B	2.67 ± 0.33 b-h	32B	3.33 ± 0.33 a-e	53B	3.00 ± 0.58 a-g
15B	3.33 ± 0.33 a-e	33B	1.33 ± 0.33 ij	54B	2.33 ± 0.33 d-i
16B	2.67 ± 0.33 b-h	34B	3.00 ± 0.58 a-g	55B	2.67 ± 0.88 c-h
17B	2.67 ± 0.33 b-h	35B	3.33 ± 0.33 a-e	57B	2.33 ± 0.33 d-i
19B	2.50 ± 0.50 d-h	36B	1.33 ± 0.33 ij	58B	3.67 ± 0.88 a-e

Table 4.8 Cercospora leaf spot disease scores of CN72, V4718 and 147 RILs of the CN72 × V4718 cross in 2017 (continued).

Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)	Cultivars/ lines	CLS score (Mean ± SE)
59B	3.00 ± 0.00 a-g	99B	2.33 ± 0.33 d-i	145B	3.33 ± 0.33 a-e
60B	4.00 ± 0.58 abc	100B	2.00 ± 0.58 g-j	147B	2.00 ± 0.58 g-j
61B	4.00 ± 0.58 abc	101B	3.33 ± 0.88 a-g	148B	2.67 ± 0.33 b-h
62B	1.33 ± 0.33 ij	102B	3.33 ± 0.88 a-g	151B	3.67 ± 0.67 a-e
63B	3.00 ± 0.00 a-g	104B	3.33 ± 0.88 a-g	152B	3.00 ± 0.00 a-g
64B	3.00 ± 0.00 a-g	105B	3.67 ± 0.67 a-e	153B	3.67 ± 0.67 a-e
66B	3.00 ± 0.58 a-g	106B	4.00 ± 0.58 abc	155B	1.67 ± 0.33 hij
67B	3.00 ± 0.58 a-g	107B	2.00 ± 0.00 f-j	156B	2.33 ± 0.67 e-i
68B	2.67 ± 0.33 b-h	109B	4.00 ± 0.58 abc	157B	1.67 ± 0.33 hij
69B	3.33 ± 0.33 a-e	110B	3.67 ± 0.67 a-e	158B	3.33 ± 0.33 a-e
70B	2.67 ± 0.88 c-h	111B	1.33 ± 0.33 ij	159B	3.67 ± 0.67 a-e
71B	3.33 ± 0.33 a-e	112B	3.00 ± 0.58 a-g	160B	3.00 ± 0.00 a-g
73B	3.33 ± 0.88 a-g	114B	3.67 ± 0.88 a-e	161B	4.00 ± 0.58 abc
74B	3.67 ± 0.33 a-e	115B	2.33 ± 0.33 d-i	162B	3.00 ± 0.00 a-g
77B	3.67 ± 0.67 a-e	118B	2.67 ± 0.67 b-h	164B	3.00 ± 0.00 a-g
78B	2.50 ± 0.50 d-h	119B	3.33 ± 0.33 a-e	165B	3.00 ± 0.00 a-g
80B	2.67 ± 0.67 b-h	120B	3.67 ± 0.33 a-d	166B	1.00 ± 0.00 j
81B	3.67 ± 0.67 a-e	121B	3.00 ± 0.58 a-g	167B	1.67 ± 0.33 hij
82B	3.00 ± 0.00 a-g	123B	1.67 ± 0.33 hij	168B	2.67 ± 0.33 b-h
83B	3.67 ± 0.67 a-e	124B	2.00 ± 0.00 f-j	169B	2.67 ± 0.33 b-h
84B	1.33 ± 0.33 ij	125B	2.33 ± 0.67 e-i	170B	2.67 ± 0.67 b-h
85B	1.67 ± 0.33 hij	126B	3.67 ± 0.67 a-e	172B	2.67 ± 0.67 b-h
86B	2.67 ± 0.33 b-h	127B	3.33 ± 0.33 a-e	173B	2.67 ± 0.33 b-h
87B	3.67 ± 0.67 a-e	130B	2.00 ± 0.00 f-j	174B	1.67 ± 0.67 hij
88B	3.00 ± 0.00 a-g	131B	3.33 ± 0.33 a-e	175B	2.00 ± 0.58 g-j
89B	2.33 ± 0.33 d-i	132B	4.00 ± 0.58 abc	177B	3.67 ± 0.67 a-e
90B	1.67 ± 0.33 hij	133B	3.33 ± 0.67 a-f	181B	3.67 ± 0.67 a-e
91B	2.33 ± 0.67 e-i	134B	3.67 ± 0.67 a-e	182B	4.00 ± 0.58 abc
92B	3.33 ± 0.33 a-e	135B	4.00 ± 0.58 abc	183B	3.67 ± 0.67 a-e
94B	1.00 ± 0.00 j	137B	4.33 ± 0.33 a	184B	3.33 ± 0.33 a-e
95B	1.00 ± 0.00 j	140B	1.67 ± 0.33 hij	185B	1.33 ± 0.33 ij
96B	2.33 ± 0.33 d-i	141B	3.33 ± 0.88 a-g	186B	1.00 ± 0.00 j
97B	2.00 ± 0.00 f-j	143B	3.00 ± 1.00 a-g	187B	3.00 ± 0.00 a-g
98B	1.00 ± 0.00 j	144B	3.33 ± 0.33 a-e		

[1/]Means in the same column with different letters are significantly different ($P < 0.05$) based on Duncan's multiple range test (DMRT)

The correlation coefficient (*r*) between the CLS disease scores of both years was 0.605 ($P < 0.01$), suggesting that they were significantly correlated. The difference may be influenced by the environment. The distribution of $F_{2:9}$ RILs progenies from the cross deviated from normality with skewness toward the parents (Figure 4.3). From Table 4.9, 84 were resistant and 63 were susceptible in 2016, and 64 were resistant and 83 were susceptible in 2017. The segregation ratio of 1:1 (resistant:susceptible) in both years ($\chi^2 = 3.00$ and 2.46, respectively, $P_{0.05} = 3.84$) was observed for this population. The results demonstrated that the resistance to CLS conferred by V4718 in this cross was conditioned by a single dominant gene. Similarly, Thakur et al. (1977) and Chankaew et al. (2011b) reported that the CLS resistance in mungbean was controlled by a single dominant gene.

Table 4.9 Segregation in reaction to Cercospora leaf spot in $F_{2:9}$ RIL population derived from CN72×V4718 cross.

Population	Year	No. of lines	No. of resistant: susceptible lines	Chi-square test		
				Expected ratio	χ^2 value[a]	$P = 0.05$[b]
$F_{2:9}$	2016	147	84:63	1:1	3.00	3.84
$F_{2:9}$	2017	147	64:83	1:1	2.46	3.84

[a] The χ^2 value was tested for goodness of fit against 1:1 ratio for RILs
[b] $P = 0.05$, the differential level of chi-square test for the resistance/susceptibility ratios with the probabilities of 95%

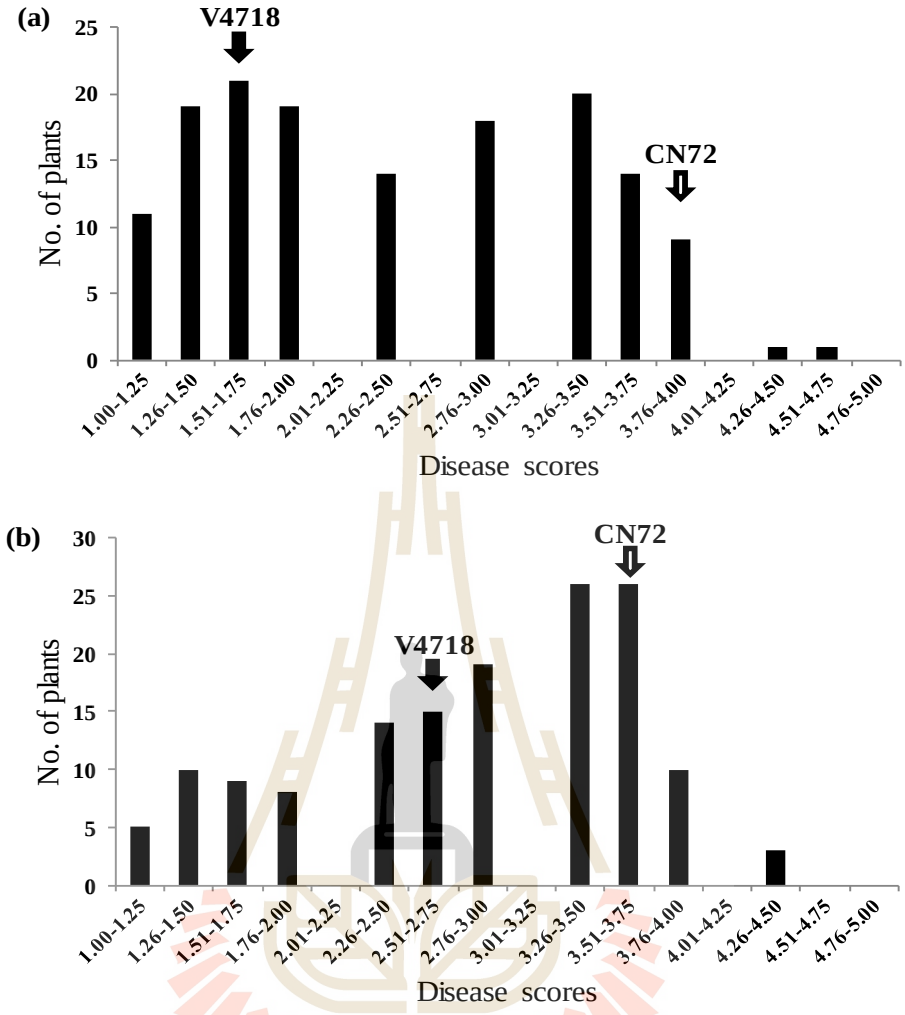

Figure 4.3 Frequency distribution of disease scores for response to Cercospora leaf spot of mungbean in CN72×V4718 cross, (a) the $F_{2:9}$ RILs population evaluated in 2016, (b) the $F_{2:9}$ RILs population evaluated in 2017.

4.2.2 SSR analysis

A total of eleven SSR markers that have been reported to be polymorphic and linked to CLS resistance in other populations (F_2 and BC_1F_1 populations of the KPS1×V4718 cross; Chankaew et al., 2011b) were used in BSA to identify polymorphic markers between resistant and susceptible parents and the

markers possibly linked to CLS resistance in our population ($F_{2:7}$ RIL population of the CN72×V4718 cross). Because the CLS resistance source (V4718) is the same in both populations, the position of CLS resistance gene is likely the same, enabling possible transfer of these CLS-linked markers to be used for MAS in our cross. However, because the maternal parents in the two populations are different, the polymorphic nature of markers as well as the genetic distance between the markers and between markers and the desirable genes may differ between populations. Therefore, these markers were needed to be evaluated before MAS. Suitable annealing temperature for amplification of each primer was determined and reported in Table 4.10.

Table 4.10 Suitable annealing temperature for each SSR primer pair.

Primers	Annealing temperature (°C)	Primers	Annealing temperature (°C)	Primers	Annealing temperature (°C)
CEDAAG002	65	CEDG084	50	CEDG305	55
CEDC050	60	CEDG117	42	VR108	55
CEDC031	50	CEDG150	50	VR393	50
CEDG037	50	CEDG232	50		

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The BSA was performed using CN72, V4718, CLS resistant bulk and CLS susceptible bulk. Three of the SSR markers (CEDC031, CEDG117 and CEDG232) failed to produce consistent results and were omitted from further experiment. CEDG037, CEDG150 and CEDG305 were monomorphic and only five of the amplifiable markers showed polymorphisms between the parents namely CEDAAG002, CEDC050, CEDG084, VR108, and VR393, and the range of amplified products of most SSR primers were 100-300 bp (Figure 4.4).

Single regression analysis of the 90 RIL derived from the cross between CN72 and V4718 using CLS disease scores identified all these five SSR markers as significantly associated with the CLS resistance in 2016 and 2017 ($P < 0.01$) (Table 4.11). The genotypes data of 90 RIL population using five SSR markers were shown in Appendix. In 2016, the R^2 and LOD scores of these markers ranged from 7.9% (CEDC050) to 34.0% (VR393) and 1.474 (CEDAAG002) to 8.151 (VR393), respectively. In 2017, the R^2 and LOD scores of these markers ranged from 12.1% (CEDAAG002) to 15.9% (CEDG084) and 3.000 (CEDAAG002) to 5.858 (VR393), respectively. The results suggest that five SSR markers can be used to construct genetic mapping and may be linked to CLS resistance gene in the RIL population of CN72×V4718 cross.

Table 4.11 SSR markers associated with Cercospora leaf spot resistance in 90 RIL derived from the CN72×V4718 cross.

Primers	CLS resistance 2016			CLS resistance 2017		
	R^2 (%)[a]	Probability	LOD[b] scores	R^2 (%)[a]	Probability	LOD[b] scores
CEDAAG002	8.5	0.006	1.474	12.1	0.001	3.000
CEDC050	7.9	0.009	1.734	12.1	0.001	3.375
CEDG084	30.3	<0.001	7.534	15.9	<0.001	5.355
VR108	26.9	<0.001	5.858	14.3	<0.001	3.999
VR393	34.0	<0.001	8.151	15.1	<0.001	5.858

[a] R^2, coefficient of determination
[b] LOD, logarithms of odds

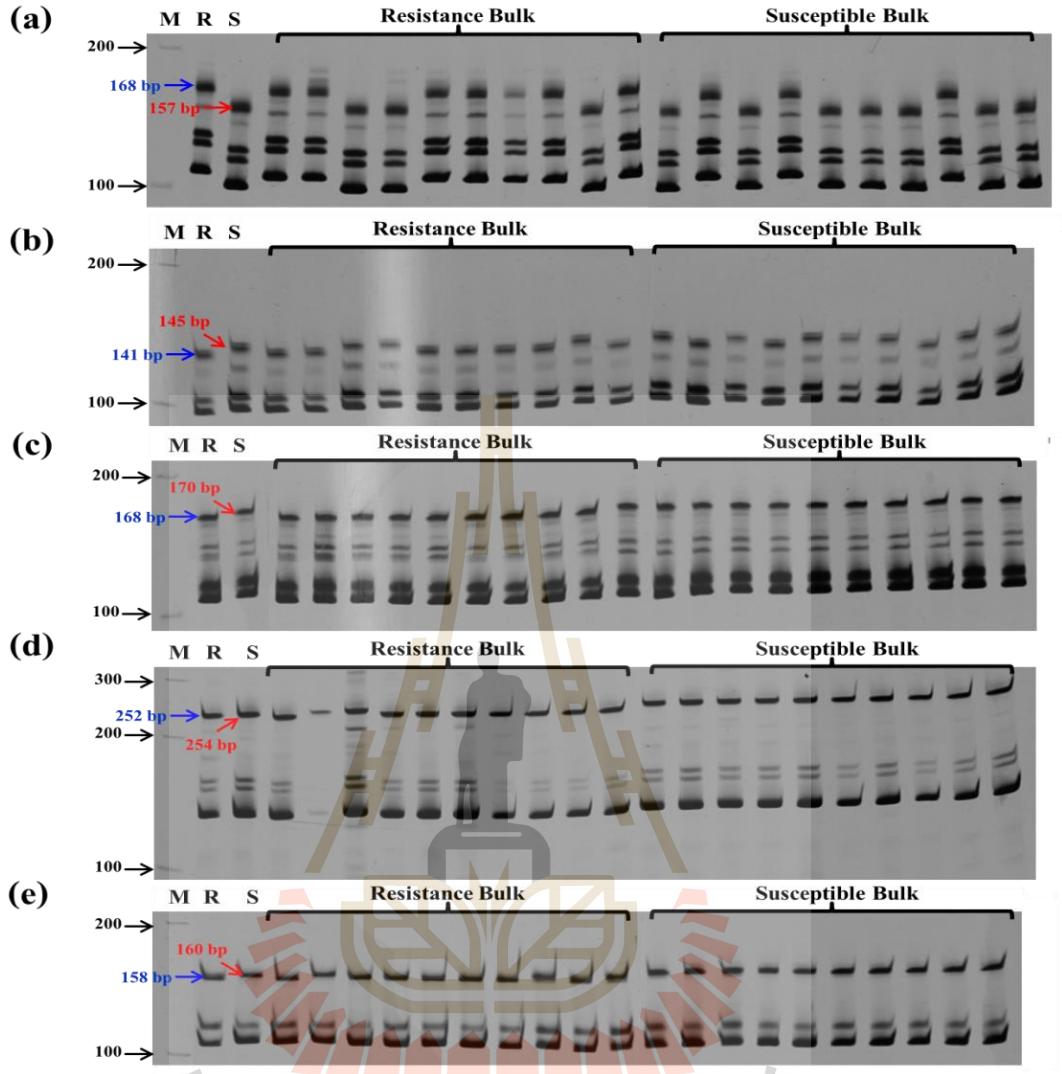

Figure 4.4 DNA patterns of CEDAAG002 (a), CEDC050 (b), CEDG084 (c), VR108 (d), and VR393 (e) products amplified from the resistant parent (R; V4718), susceptible parent (S; CN72), 10 individuals of resistant bulk, and 10 individuals of susceptible bulk of mungbean in the CN72×V4718 cross; M = 100 bp DNA ladder.

4.3 Experiment 3

Correlation between PM and CLS resistance and the relationships between ISSR and SSR markers and PM or CLS resistance in the RIL population of the CN72×V4718 cross were evaluated.

4.3.1 Correlation between PM and CLS resistance

V4718 is the resistant source of both CLS and PM. It has also been used to develop resistant cultivars and to map CLS and PM resistance genes in F_2 and BC_1F_1 populations (Chankaew et al., 2011b) and in $F_{2:3}$ and $F_{2:4}$ populations (Chankaew et al., 2013) of a cross between KPS1 and V4718. However, the correlation between CLS and PM resistance derived from V4718 has not been studied yet. The coefficient of correlation between each year of PM and CLS disease scores was performed in this study. It was found that CLS disease scores in 2016 were significantly correlated with those of PM in 2013 (r = 0.360; P < 0.001) and in 2016 (r = 0.365; P < 0.001). The CLS disease scores in 2017 were also significantly correlated with those of PM in 2013 (r = 0.216; P < 0.05) and in 2016 (r = 0.245; P < 0.05). The results suggest that the resistance genes of these two diseases may be linked or the resistance to these two diseases may be indirectly associated via certain characteristics of V4718 which provide resistance to both diseases. Therefore, the ISSR markers associated with PM in experiment 1 may be associated with CLS resistance. Also, the SSR markers associated with CLS resistance in experiment 2 may be associated with PM resistance.

4.3.2 ISSR markers associated with CLS resistance

Two ISSR markers (I13306 and I13311) were evaluated for correlation between markers and CLS resistance in 2016 and 2017 by single regression and LOD analysis (Table 4.12). I13306 and I13311 markers were significantly associated with

CLS resistance ($P < 0.01$) in 2016 and 2017. In 2016, they showed R^2 of 14.0% and 8.1% with LOD score 1.725 and 0.874 for I13306 and I13311 markers, respectively. In 2017 they showed R^2 of 10.6% and 7.7% with LOD score of 3.952 and 3.216 for I13306 and I13311 markers, respectively. The results demonstrate that the I13306 and I13311 markers linked to PM resistance were also partly linked to CLS resistance in RIL population of the CN72×V4718 cross.

Table 4.12 ISSR markers associated with Cercospora leaf spot resistance in population of 100 RILs derived from the CN72×V4718 cross.

Primers	CLS resistance 2016			CLS resistance 2017		
	R^2 (%)[a]	Probability	LOD[b] scores	R^2 (%)[a]	Probability	LOD[b] scores
I13306	14.0	0.000	1.725	10.6	0.001	3.952
I13311	8.1	0.004	0.874	7.7	0.005	3.216

[a] R^2, coefficient of determination
[b] LOD, logarithms of odd

4.3.3 SSR markers associated with PM resistance

Five SSR markers namely CEDAAG002, CEDC050, CEDG084, VR108, and VR393 that significantly associated with the CLS resistance were evaluated for correlation between markers and PM resistance in 2013 and 2016 by single regression and LOD analysis (Table 4.13). Only one SSR marker (VR393) was significantly associated with PM resistance gene ($P < 0.001$) with the R^2 of 18.9% and 14.0% with LOD score of 3.216 and 4.426 in 2013 and 2016, respectively. The results demonstrate that the VR393 markers linked to CLS resistance was also partly linked to PM resistance in RIL population of the CN72×V4718 cross.

Table 4.13 SSR markers associated with powdery mildew resistance in 90 RILs derived from the CN72×V4718 cross.

Primers	PM resistance 2013			PM resistance 2016		
	R^2 (%)[a]	Probability[b]	LOD[c] scores	R^2 (%)[a]	Probability[b]	LOD[c] scores
CEDAAG002	1.3	0.303[ns]	0.824	2.1	0.184[ns]	0.253
CEDC050	0.8	0.418[ns]	0.640	2.2	0.178[ns]	0.430
CEDG084	2.5	0.148[ns]	0.475	2.7	0.122[ns]	1.407
VR108	4.4	0.055[ns]	0.621	2.6	0.128[ns]	0.787
VR393	18.9	<0.001	3.216	14.0	<0.001	4.426

[a] R^2, coefficient of determination
[b] ns, non-significant
[c] LOD, logarithms of odd

Interestingly, two ISSR markers (I13306 and I13311) and one SSR marker (VR393) were also significantly associated with the CLS and PM resistance, respectively (Table 4.12 and 4.13), implying that the PM and CLS resistance genes may be co-localized in the same chromosomal region. Several reports found the QTLs linked to PM resistance locate on LG 3, 4, 6, 7, 8, and 9 (Young et al., 1993; Kasettranan et al., 2010; Chankaew et al., 2013). While a major QTL for CLS resistance was located on LG 3 (Chankaew et al., 2011b). Noteworthy, the QTLs linked to both disease resistance genes may co-localized in the same LG. Therefore, the genetic linkage map and QTL analysis are needed to confirm the hypothesis.

4.4 Experiment 4

Construction of linkage map and QTL analysis of ISSR and SSR associated with PM and CLS resistances in RIL population of the CN72×V4718 cross.

4.4.1 Linkage map analysis

A total of 7 markers associated with PM and CLS resistances were used to

construct genetic linkage map of RIL population of the CN72×V4718 cross. The linkage map consisted of 3 markers (CEDG084, VR108, and VR393) ordered in 1 linkage group (LOD > 3) with a total genetic distance of 18.0 cM (Figure 4.5). However, four markers (I13306, I13311, CEDAAG002, and CEDC050) could not be used to construct genetic linkage map of RIL population of the CN72×V4718 cross because they might be too far from other markers. However, these results need to be verified by larger number of molecular markers in future experiment. In 2017, we confirmed that the I13306 marker could be used to construct the genetic linkage map and was located on the same LG with PM resistance gene in RIL population of the CN72 × V4718 cross (Poolsawat et al., 2017).

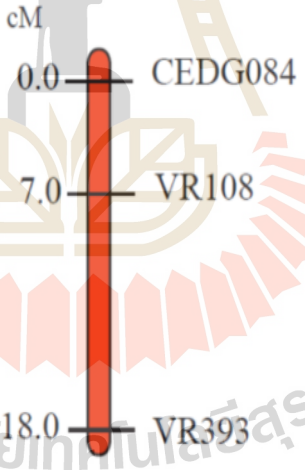

Figure 4.5 An SSR linkage map of mungbean constructed from $F_{2:7}$ RIL population from the CN72×V4718 cross.

4.4.2 QTL analysis

Permutation analyses (4,000 runs at P = 0.01) for 2016 and 2017 CLS resistance data of the $F_{2:7}$ RIL population revealed that LOD score threshold for QTL

was 3. In both years, multiple interval mapping (MIM) consistently identified one QTL for CLS resistance (Table 4.14). The QTL was designated as *qCLSC72V18* for CLS resistance. It was located between VR108 and VR393 markers on this LG at 15.0 and 13.0 cM using the 2016 and 2017 data, respectively. The *qCLSC72V18* showed a LOD score of 8.8901 and 3.9547 and explained 43.10 and 22.67% of the phenotypic variation in the 2016 and 2017 CLS resistance data, respectively. Additive effects of the *qCLSC72V18* were -0.67 and -0.41 in 2016 and 2017, indicating that the allele from V4718 contributed to the reduction in disease score (increasing resistance), which confirmed that these markers were linked to CLS resistance gene. These results are in agreement with those of previous report showing that one major QTL (*qCLS*) for CLS resistance was located between CEDG117 and VR393 markers, and accounted for 65.5-80.53% of the disease score variation depending on seasons and populations (Chankaew et al., 2011b).

QTL for PM resistance was also identified in the $F_{2:7}$ RIL population of the CN72×V4718 cross. The LOD score threshold for QTL as determined by permutation was 3. MIM detected one QTL designated *qPMC72V18* on the same LG between VR108 and VR393 markers, which overlapped the *qCLSC72V18* (Table 4.14). The QTL at 17.0 cM exhibited a LOD score of 3.7565 and 2.7648 and accounted for 19.97 and 13.76% of the 2013 and 2016 PM disease variation. The additive effects of the *qPMC72V18* were -0.86 and -0.68 in 2013 and 2016, demonstrating that the allele from V4718 decreased the PM disease score. These results suggest that it conferred PM resistance.

The results show that at least one QTL for resistance to each of the two diseases was flanked by the similar SSR markers, suggesting that they may be

localized in the same chromosomal region. The linkage map of mungbean in this study was compared with previous linkage maps developed for another mungbean breeding population using common SSR markers (Chankaew et al., 2011b; Figure 4.6). The comparison revealed that in general the linkage groups and orders of the markers among the mungbean population were the same or highly similar in LG 3 of KPS1×V4718 F_2 population. The results show that both QTLs linked to the CLS resistance gene of each population were located between VR108 and VR393 markers, indicating that the LG in this study likely located on LG 3 of mungbean based on the positions of SSR markers. Furthermore, QTL linked to PM resistance gene (*qPMC72V18*) in this result has a possibility that the QTL may also be located on LG 3. Young et al. (1993) found the minor QTL of PM resistance gene located on LG3 in VC3980A×TC1966 population, supporting that *qPMC72V18* may be located on LG3 in RILs of CN72×V4718 cross.

In this study, VR393 was closest to both resistance genes with the distance of 3-5 and 1 cM from the QTLs of CLS and PM resistance, respectively. VR108 was also close to both resistance genes with the distance of 6-8 and 10 cM from the QTLs of CLS and PM resistance, respectively. However, if using only a single marker for MAS, VR393 has 3-5% and 1% of the recombination with CLS and PM resistance gene, respectively, while the chance of recombination of VR108 is 6-8% with CLS resistances gene and 10% with PM resistance gene. Therefore, using both markers (VR108 and VR393) flanking both resistance genes in MAS, the chance of recombination decrease to only 0.48-0.60% for the selection of CLS resistance gene and 0.20% for PM resistance gene, which are much lower than using only either single marker. Chueakhunthod (unpublished data) successfully used SSR markers

flanked this CLS resistance in marker assisted backcross breeding (MABB) of two different populations using SUT1 and KING as recurrent parents. Therefore, both markers could be used in MAS for combining CLS and PM resistance in mungbean breeding.

Table 4.14 The location and effects of QTL controlling resistance to CLS and PM in $F_{2:7}$ RIL population derived from the CN72×V4718 cross, as detected by multiple interval mapping (MIM).

Diseases	Year	QTL[a] name	Position (cM)	LOD[b] score	Interval markers	PVE[c] (%)	Additive effect
CLS	2016	*qCLSC72V18*	15.0	8.88	VR108-VR393	43.5	-0.67
	2017	*qCLSC72V18*	13.0	3.95	VR108-VR393	22.9	-0.41
PM	2013	*qPMC72V18*	17.0	3.75	VR108-VR393	20.2	-0.86
	2016	*qPMC72V18*	17.0	2.76	VR108-VR393	13.9	-0.68

[a] QTL, quantitative trait loci
[b] LOD, logarithms of odds;
[c] PVE, phenotypic variance explained by the QTL

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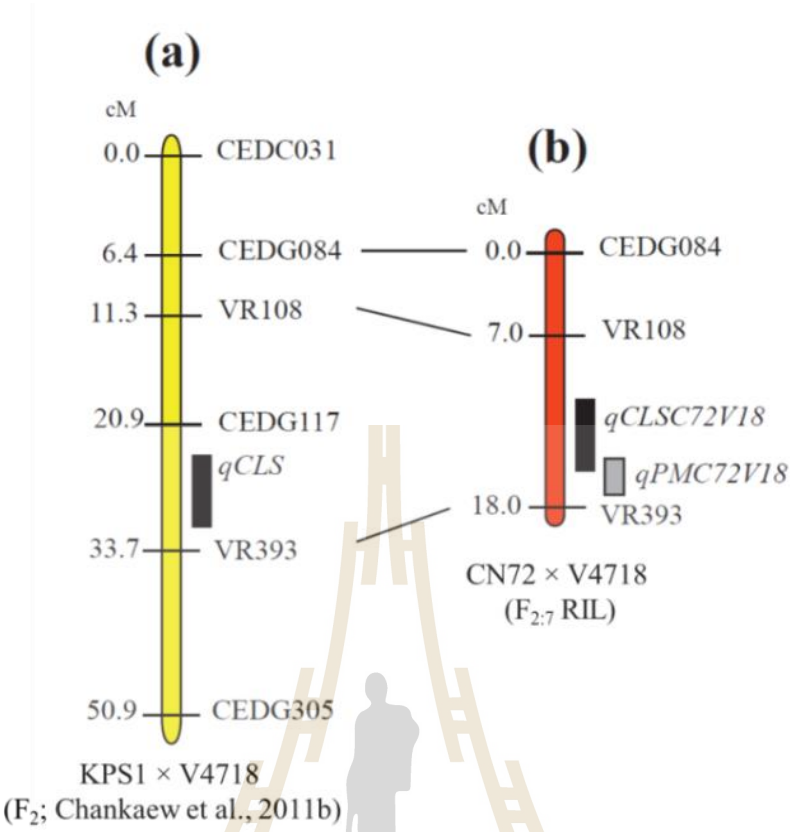

Figure 4.6 Comparative linkage group showing conservation of markers and QTL on LG3 for Cercospora leaf spot (*qCLSC72V18*; black bar) detected in RILs of the CN72×V4718 cross in this study (**b**) compared to those in another population of V4718 (**a**; Chankaew et al., 2011). Position of QTL for powdery mildew (*qPMC72V18*) was detected in RILs of the CN72×V4718 cross as shown on gray bar.

CHAPTER V

CONCLUSION

In Thailand, powdery mildew (PM) and Cercospora leaf spot (CLS) were serious diseases of mungbean in rainy season and cool-dry season, respectively. CLS and PM diseases incur total yield loss of up to 50% and more than 40% in susceptible cultivars, respectively, implicating the requirement of resistant cultivars to both diseases for sustainable mungbean production. Moreover, the conventional breeding for resistance to these diseases can be accomplished only in specific seasons. Therefore, selection of mungbean progenies with resistance to both diseases by field screening is laborious and time-consuming. Marker assisted selection (MAS) using linked markers may help overcome these limitations.

In this study, we found the segregation of 1:1 for resistant and susceptible progenies of PM and CLS disease score data in different years in $F_{2:7}$ RIL population derived from the CN72×V4718 cross, confirming that resistance to both diseases was controlled by single dominant genes. Therefore, both disease resistance genes may be pyramided into a single variety for more durable resistance.

Markers associated with PM and/or CLS resistances were preliminary determined by single regression analysis. Among twenty ISSR primers, it was found that only two ISSR markers were significantly associated with the PM resistance ($P < 0.001$) with a coefficient of determination (R^2) of 23.6-26.9% (I13306) and 16.6-13.9% (I13311) and LOD score of 2.878-5.681 in both years. Five SSR markers were

significantly associated with the CLS resistance ($P < 0.01$) with R^2 of 8.5-12.1% (CEDAAG002), 7.9-12.1% (CEDC050), 15.9-30.3% (CEDG084), 14.3-26.9% (VR108), and 15.1-34.0% (VR393) and LOD score of 1.474-8.1513 in both years.

The significant correlation between PM and CLS resistance in each year ($r = 0.216$-0.365; $P < 0.001$) suggests that the resistance genes of these two diseases may be linked or the resistance to these diseases may be indirectly associated via certain characteristics of V4718 which provide resistance to both diseases. Moreover, I13306, I13311, and VR393 markers were also significantly associated with the PM resistance, suggesting that some of the genes conferring resistance to CLS and PM may be co-localized.

Only three markers namely CEDG084, VR108 and VR393 could be used to construct genetic linkage maps of $F_{2:7}$ RILs population of the CN72×V4718 cross with a total genetic distance of 18.0 cM.

Multiple interval mapping (MIM) consistently identified one QTL (*qCLSC72V18*) for CLS resistance and one QTL (*qPM C72V18*) for PM resistance in the $F_{2:7}$ RIL population derived from the cross between CN72 and V4718. *qCLSC72V18* and *qPMC72V18* were localized between VR108 and VR393 markers accounting for 22.67-43.10% and 13.76-19.97% of the CLS and PM disease score variation in different years, respectively.

In addition, VR108 and VR393 were closest to QTL for resistance to CLS at the distance of 6-8 and 3-5 cM, and closest to QTL for resistance to PM at the distance of 10 and 1 cM, respectively. The markers that were closely linked to both resistance genes could be used in MAS for combining CLS and PM resistance in mungbean breeding.

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APPENDIX

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table A.1 Genotypic data of RIL population of the CN72×V4718 cross using ISSR and SSR markers.

Cultivars/ lines	ISSR[a] markers		SSR[b] markers				
	I13311	I13306	CEDAAG002	CEDC050	CEDG084	VR108	VR393
V4718	A_	B_	CC	DD	EE	FF	GG
CN72	aa	bb	cc	dd	ee	ff	gg
2B	A_	B_	cc	dd	-	-	-
4B	aa	bb	cc	dd	ee	ff	gg
5B	A_	B_	-	-	EE	FF	GG
6B	A_	B_	CC	DD	EE	FF	GG
7B	A_	B_	cc	dd	EE	FF	GG
8B	aa	bb	-	-	ee	FF	GG
9B	aa	bb	-	-	-	-	-
11B	-	-	CC	DD	ee	ff	gg
12B	aa	B_	-	-	-	-	-
13B	A_	B_	-	-	-	-	-
14B	A_	B_	-	-	-	-	-
16B	aa	bb	-	-	EE	FF	GG
17B	-	-	-	-	EE	FF	GG
18B	aa	bb	-	-	-	-	-
19B	-	-	-	-	EE	FF	GG
20B	-	-	-	-	EE	FF	GG
21B	A_	B_	CC	DD	ee	ff	gg
22B	-	-	cc	dd	EE	ff	GG
23B	-	-	CC	DD	-	-	-
24B	A_	B_	-	-	-	-	-
25B	aa	bb	-	-	-	-	-
26B	A_	B_	-	-	-	-	-
27B	A_	B_	-	-	-	-	-
28B	-	-	CC	DD	-	-	-
29B	aa	B_	cc	dd	ee	FF	GG
30B	-	-	cc	dd	-	-	-
31B	aa	bb	-	-	-	-	-
32B	-	-	cc	dd	ee	ff	aa
33B	aa	bb	cc	dd	EE	FF	GG
34B	A_	B_	-	-	-	-	-
35B	A_	B_	cc	dd	EE	FF	GG
36B	-	-	CC	-	-	-	-
37B	-	-	CC	DD	ee	ff	gg
39B	aa	bb	-	-	-	-	-
41B	aa	bb	-	-	-	-	-
42B	aa	bb	-	-	EE	FF	GG

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table A.1 Genotypic data of RIL population of the CN72×V4718 cross using ISSR and SSR markers (continued).

Cultivars/ lines	ISSR[a] markers		SSR[b] markers				
	I13311	I13306	CEDAAG002	CEDC050	CEDG084	VR108	VR393
43B	-	-	CC	DD	EE	FF	GG
44B	aa	bb	-	-	-	-	-
45B	aa	bb	-	-	-	-	-
46B	aa	bb	cc	dd	ee	ff	gg
47B	aa	bb	cc	dd	-	-	-
49B	A_	B_	-	-	-	-	-
50B	aa	bb	CC	DD	ee	ff	GG
51B	A_	B_	-	-	-	-	-
53B	aa	bb	-	-	-	-	-
54B	-	-	-	-	EE	FF	GG
55B	-	-	-	-	EE	FF	GG
56B	aa	bb	-	-	-	-	-
59B	aa	bb	CC	DD	EE	ff	GG
60B	-	-	-	-	EE	FF	gg
61B	aa	bb	CC	DD	-	-	-
62B	-	-	CC	DD	-	-	-
63B	-	-	CC	DD	ee	ff	gg
64B	-	-	CC	DD	ee	ff	gg
65B	aa	bb	-	-	-	-	-
66B	aa	bb	cc	dd	-	-	-
67B	A_	B_	cc	dd	-	-	-
68B	aa	B_	-	-	-	-	-
71B	A_	B_	-	-	-	-	-
72B	aa	bb	-	-	-	-	-
73B	aa	bb	cc	dd	ee	ff	gg
76B	aa	bb	CC	DD	-	-	-
77B	A_	B_	-	-	EE	FF	GG
78B	aa	B_	cc	dd	ee	ff	GG
79B	A_	B_	-	-	-	-	-
80B	aa	bb	cc	DD	EE	ff	GG
82B	-	-	cc	dd	EE	ff	GG
83B	aa	bb	-	-	-	-	-
84B	A_	B_	-	-	-	-	-
85B	aa	bb	CC	DD	EE	FF	GG
86B	A_	B_	-	-	-	-	-
87B	aa	bb	cc	dd	ee	ff	gg
88B	aa	bb	-	-	-	-	-
89B	A_	B_	-	-	-	-	-

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table A.1 Genotypic data of RIL population of the CN72×V4718 cross using ISSR and SSR markers (continued).

Cultivars/ lines	ISSR[a] markers		SSR[b] markers				
	I13311	I13306	CEDAAG002	CEDC050	CEDG084	VR108	VR393
90B	-	-	CC	DD	ee	ff	gg
91B	-	-	CC	DD	ee	ff	gg
92B	aa	bb	cc	dd	ee	ff	gg
93B	A_	B_	-	-	-	-	-
94B	aa	bb	-	-	-	-	-
95B	aa	bb	CC	DD	EE	FF	GG
96B	-	-	CC	DD	EE	FF	GG
97B	-	-	CC	DD	EE	FF	GG
98B	-	-	CC	DD	EE	FF	GG
99B	aa	bb	CC	DD	EE	FF	GG
100B	-	-	CC	DD	EE	FF	GG
101B	aa	bb	cc	dd	ee	ff	gg
102B	aa	bb	-	-	-	-	-
104B	-	-	CC	DD	ee	ff	gg
105B	aa	bb	cc	dd	ee	ff	gg
107B	A_	B_	CC	DD	ee	ff	gg
109B	-	-	CC	DD	ee	ff	gg
110B	-	-	cc	dd	ee	FF	GG
111B	A_	B_	-	-	-	-	-
114B	aa	bb	cc	dd	aa	ff	gg
115B	-	-	-	-	EE	FF	gg
119B	-	-	CC	DD	ee	ff	gg
120B	aa	bb	cc	dd	ee	ff	gg
121B	aa	bb	cc	dd	ee	ff	gg
122B	A_	B_	-	-	-	-	-
123B	aa	bb	-	-	-	-	-
124B	A_	B_	-	-	-	-	-
125B	A_	B_	-	-	-	-	-
126B	aa	bb	cc	dd	ee	ff	aa
127B	aa	B_	-	-	-	-	-
128B	aa	bb	-	-	-	-	-
130B	A_	B_			EE	ff	GG
131B	-	-	cc	dd	EE	FF	gg
132B	-	-	cc	dd	ee	ff	gg
133B	-	-	CC	DD	ee	ff	gg
134B	aa	bb	-	-	ee	FF	gg
135B	aa	bb	CC	DD	ee	ff	gg
136B	aa	bb	-	-	-	-	-

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table A.1 Genotypic data of RIL population of the CN72×V4718 cross using ISSR and SSR markers (continued).

Cultivars/ lines	ISSR[a] markers		SSR[b] markers				
	I13311	I13306	CEDAAG002	CEDC050	CEDG084	VR108	VR393
137B	aa	bb	cc	dd	ee	ff	gg
139B	aa	bb	-	-	-	-	-
141B	aa	bb	cc	dd	ee	ff	gg
143B	aa	bb	cc	dd	ee	ff	gg
144B	aa	bb	cc	dd	ee	ff	gg
145B	-	-	cc	dd	ee	ff	gg
146B	aa	bb	-	-	-	-	-
147B	A_	B_	cc	dd	EE	ff	gg
148B	A_	B_	-	-	ee	ff	GG
150B	A_	B_	cc	dd	-	-	-
151B	aa	bb	cc	dd	ee	ff	gg
152B	aa	bb	cc	dd	ee	ff	gg
153B	aa	bb	cc	dd	ee	ff	gg
155B	-	-	CC	DD	ee	ff	gg
156B	-	-	CC	DD	ee	ff	gg
157B	-	-	CC	DD	ee	ff	gg
158B	-	-	cc	dd	ee	ff	gg
159B	aa	bb	-	-	-	-	-
161B	-	-	cc	dd	EE	FF	GG
162B	aa	bb	cc	dd	ee	ff	gg
164B	-	-	cc	dd	ee	ff	gg
165B	A_	B_	cc	dd	EE	FF	gg
166B			CC	DD	EE	FF	gg
167B	aa	bb	CC	DD	ee	ff	GG
168B	aa	bb	CC	DD	EE	FF	GG
169B	-	-	cc	dd	EE	FF	GG
170B	-	-	cc	dd	EE	FF	GG
173B	-	-	cc	dd	EE	FF	GG
175B	aa	B_	-	-	-	-	-
177B	aa	bb	-	DD	ee	ff	gg
177B	-	-	cc	dd	ee	ff	gg
181B	-	-	cc	dd	EE	FF	gg
186B	A_	B_	CC	DD	ee	FF	GG
187B	aa	bb	cc	dd	ee	ff	gg

[a]ISSR marker showing either homozygous or heterozygous alleles (A_, B_)
[b]SSR marker showing homozygous alleles

มหาวิทยาลัยเทคโนโลยีสุรนารี

BIOGRAPHY

Ms. Kitiya Arsakit was born on May 13, 1990 at Samut Sakhon, Thailand. She graduated with the Bachelor of Science degree in Crop Production Technology, Silpakorn University in 2011. Then, in the same year, she had worked as a research assistant in Plant Breeding Laboratory for 4 years at Suranaree University of Technology, Nakhon Ratchasima, Thailand. In 2015, she decided to further study for master degree in School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. During her study, she received scholarship from Suranaree University of Technology to support her tuition and fee. Her research topic was the development of molecular markers linked to Cercospora leaf spot and powdery mildew resistance in mungbeans under supervision of Professor Dr. Piyada Alisha Tantasawat. The results from some part of this study have been presented in the International Forum-Agriculture, Biology and Life Science 2017 (IFABL 2017), June 27-29, 2017, Kyoto, Japan (Poster presentation in "Simple sequence repeat markers associated with Cercospora leaf spot and powdery mildew resistance in mungbean (*Vigna radiata* L. Wilczek)"). After that, she studied Double Master's Program in Department of Agriculture at the Graduate School of Science and Technology, Shinshu University, Japan. Her research topic was Background of low acidity in red-fleshed apple and utilization of marker-assisted selection. She has many opportunities to learn and exchange her knowledge about pomology from Professor Dr. Kiyoshi Banno.