INHERITANCE OF POWDERY MILDEW RESISTANCE IN MUNGBEAN

AND DEVELOPMENT OF MOLECULAR MARKERS FOR

MARKER-ASSISTED SELECTION

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การถ่ายทอดลักษณะความต้านทานโรคราแป้งในถั่วเขียวและการพัฒนาดีเอนเอเครื่องหมายสำหรับการคัดเลือก

นางสาวบุบผา ใจเที่ยง

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MARKERS-ASSISTED SELECTION

Suranaree University of Technology Council has approved this submitted in partial fulfillment for the Degree of Doctor of Philosophy

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โรคราแป้งเป็นโรคที่มีความสำคัญของถั่วเขียว ซึ่งระบาดในฤดูที่มีอากาศเย็น และความชื้น
ต่ำ ทำให้ผลผลิตเสียหาย จึงได้ทำการศึกษาลักษณะการถ่ายทอดของโรคเพื่อเป็นข้อมูลในการปรับ
ปรุงพันธุ์ถั่วเขียวให้ต้านทานต่อโรคราแป้งรวมไปถึงการพัฒนาดีเอนเอเครื่องหมายมาช่วยในการคัด
เลือกพันธุ์ ในการศึกษาลักษณะการถ่ายทอดของโรคราแป้งนี้ ได้ทำการศึกษาจากประชากรหกชนิด
ที่ได้จากการผสมระหว่างพันธุ์ต้านทานกับพันธุ์อ่อนแอต่อโรคราแป้ง ได้แก่ สายพันธุ์อ่อนแอ (P_1)
พันธุ์ต้านทาน (P_2) ลูกผสมชั่วที่หนึ่ง (F_1) ลูกผสมชั่วที่สอง (F_2) ลูกผสมชั่วที่หนึ่งผสมกลับกับพันธุ์
อ่อนแอ (BC_1) และ ลูกผสมชั่วที่หนึ่งผสมกลับกับพันธุ์ต้านทาน (BC_2) ทำการวิเคราะห์ค่าเฉลี่ยของ
ประชากรเหล่านี้   จากการศึกษาพบว่าการแสดงออกของยีนทั้งแบบบวกและแบบข่ม ซึ่งมีผลต่อ
ลักษณะการต้านทานโรคในระดับเดียวกัน   และ ไม่พบลักษณะการข่มข้ามคู่ของยีน   และจากการ
วิเคราะห์ลักษณะการต้านทานโรคราแป้งควบคุมด้วยยีนเด่นหนึ่งคู่

การปรับปรุงพันธุ์โดยวิธีผสมกลับเป็นการย้ายยีนที่สนใจจากพันธุ์ให้ไปสู่พันธุ์รับ ในการ
ศึกษาครั้งนี้ได้ทำการปรับปรุงพันธุ์ชัยนาท 36 ซึ่งเป็นพันธุ์อ่อนแอให้ต้านทานต่อโรคราแป้ง โดยทำ
การผสมกับพันธุ์ต้านทานสองพันธุ์ คือ พันธุ์ มทส4 และ VC1210A และทำการผสมกลับกับพันธุ์
ชัยนาท 36 สามครั้ง เมล็ด BC_3F_3 ที่ได้ถูกนำมาปลูกและคัดลักษณะที่ต้องการ สายพันธุ์ที่ได้รับการ
คัดเลือกห้าสายพันธุ์ ประกอบด้วยสายพันธุ์ 105 111 132 140 และ 142 ซึ่งได้ยีนต้านทานโรคจาก
พันธุ์ มทส4. จะนำไปใช้ในการศึกษาต่อไป

ได้นำดีเอนเอจากประชากรชั่วที่สอง (F_2) ที่เกิดจากการผสมระหว่างสายพันธุ์ต้านทาน
(VC1210A) และ สายพันธุ์ที่อ่อนแอ(TC1966) มาทำการไฮบริไดเซซั่น โดยใช้ดีเอนเอติดตาม จาก
ห้องสมุดดีเอนเอ ของถั่วเขียว 42 ตัว ถั่วเหลือง 29 ตัว และ common bean 27 ตัว นอกจากนี้ยังมี ดี
เอนเอติดตามที่ได้จากวิธี amplified fragment length polymorphism: AFLP (Mac; Mungbean AFLP
clone) 4 ตัว จากการวิเคราะห์ความแปรปรวน และ การทำแผนที่ยีน พบว่า QTL ที่ตรวจพบโดย ดี
เอนเอติดตาม Mac 71a และ Mac114 ให้ค่า LOD ที่ ระดับ 20.22 อยู่บน linkage group กลุ่มใหม่
และ สามารถอธิบายความแปรปรวนของลักษณะการเกิดโรคราแป้งของประชากรนี้ได้ถึง 64.9 % ยีน
ต้านทานแสดงออกในลักษณะข่มบางส่วน และพบว่า ดีเอนเอติดตามที่พัฒนาจากการศึกษาครั้งนี้มี
ศักยภาพในการนำมาใช้ในการปรับปรุงพันธุ์ถั่วเขียวเพื่อให้ต้านทานต่อโรคราแป้ง

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ปีการศึกษา 2545   ลายมือชื่ออาจารย์ที่ปรึกษา............................

BUBPA CHAITIENG : INHERITANCE OF POWDERY MILDEW RESISTANCE IN MUNGBEAN AND DEVELOPMENT OF MOLECULAR MARKERS FOR MARKER-ASSISTED SELECTION

THESIS ADVISOR : PROF. PAISAN LAOSUWAN, Ph.D. 72 PP. ISBN 974–533–081-7

Powdery mildew (*Erysiphe polygoni* DC.) is a serious disease of mungbean [*Vigna radiata* (L.) Wilczek]. It is one of the major constraints of mungbean production. This experiment was conducted to study the inheritance of the disease, to improve mungbean varieties for resistance and to use RFLP to facilitate the selection for resistance. The inheritance of powdery mildew resistance was studied in four crosses between resistant and susceptible lines and varieties of mungbean. Six generations including P_1, P_2, F_1, F_2, BC_1 and BC_2, of each cross were subjected to generation mean analysis. Significant additive and dominant gene effects of similar magnitude were observed indicating that these two gene effects are responsible for the inheritance of the character. Interaction of genes was not found in all four crosses. Powdery mildew resistant reaction of all four crosses was found to control by single dominant gene.

Backcross breeding method is a plant breeding procedure used to transfer favorable genes from donor to recurrent parents. This study was conducted to improve susceptible cultivar, CN36, for resistant to powdery mildew. The susceptible recurrent parent, CN36, was crossed with two resistant cultivar/line, SUT4 and VC1210A, and backcrossed three times to obtained BC_3F_1. The BC_3F_1 seeds were planted and selected to produce BC_3F_2 and BC_3F_3. Five lines of no. 105, 111, 132, 140 and 142 were selected from BC_3F_3 population of SUT4 donor parent for further study.

DNA from 96 F_2 progenies from a cross between resistant line, VC1210A, and susceptible line, TC1966, were used to hybridized with 42, 29, 27 probes from libraries of mungbean, soybean and common bean and four new probes (Mac; Mungbean AFLP clone), respectively. Analysis of variance and interval mapping were used to identify QTLs associated with powdery mildew resistance. A major resistant QTL was detected at markers Mac71a and Mac114 which had a LOD score of 20.22. The new RFLP loci detected by two cloned probes from the AFLP bands associated with resistance constitute a new linkage group. A major resistance QTL was found on a new linkage group that accounted for 64.9% of the total variation for plant reaction to the disease. The resistant parent allele enhances powdery mildew resistance with partially dominant effect. One of probes developed in this study has the potential to assist in breeding for powdery mildew resistance in mungbean.

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Academic Year   Advisor……………………………….

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Bubpa Chaitieng

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Chapter I
General Introduction

Mungbean [*Vigna radiata* (L). Wilczek] is native to the Indo-Burma region with India, Burma, Thailand and Indonesia produce almost 90 per cent of the world production. Mungbeans are mainly sprouted and consumed cooked or raw. However, mungbean may also be split, boiled, roasted or ground into flour to make a variety of desserts, snacks and main dishes. Mungbean is an important source of easily-digestible protein. However, it is not a perfect protein source and should be consumed with other sources of protein which have high percentages of sulphur-containing amino acids, such as cereals and sesame. Mungbean fits well into many cropping systems in the tropics and is a cash crop grown in rotation with rice, maize, sesame and cotton. It can be divided into two groups according to its hull color, green and black.

The main production area of mungbean in Thailand is in the Northern region such as Sukhothai, Phetchabun, Nakhon Sawan, Kamphaeng Phet, Phichit, Phitsanulok and in the Central region such as Lop Buri. The total planted area in crop year 2000/2001 was 1,898,939 rai. With this area, the production of 232,861 tons was obtained giving a national average yield of 129 kg/rai (Office of Agricultural Economics, 2001). The world leading exporters are India the People's Republic of China and Taiwan (Pookpakdi, 1990) The production of mungbean in Thailand is adversely affected by many factors such as low genetic potential of current varieties, environmental stresses, diseases, insect pests, and poor cultural practices. The main foliar diseases that affect the production of mungbean are *Cercospora* leaf spot and powdery mildew. *Cercospora* leaf sot occurs in the hot wet climate of rainy season. On the other hand, powdery mildew (*Erysiphe polygoni* DC.) has a wider geographic range than *Cercospora* leaf spot (Poehlman, 1991). Severe infection of powdery mildew occurs in cool dry months and it can reduce yield of mungbean by between 20 and 40% (Soria and Quebral, 1973). The crop incurs maximum damage when powdery mildew infects plants just before the flowering stage (Poehlman, 1991). At present, most mungbean cultivars recommended to farmers in Thailand such as Chainat 36 (CN36), Chainat 60 (CN60), Kamphaeng Saen 1 (KPS1), Kamphaeng Saen 2 (KPS2) are susceptible to the diseases. Fungicide application is the only method to control the

disease which results in higher production cost. Therefore, it is necessary to develop resistant varieties to reduce the production cost and to protect the environment.

Breeding for disease resistant varieties in crop plants depends on many factors such as plant type, species, growth stage, age of plant, pathogen, interaction between plant and pathogen, environment, and so fourth (Baird *et al.*,1996). In the procedure, backcross method is very popular procedure among plant breeders who are working as breeding for resistance. The plants showing high resistance are selected and used for backcrossing into elite lines (cultivars) until resistant varieties are obtained. Visual selection of individual plants is sometimes difficult due to low incidence of the disease and the breeders may depend on indirect selection by the application of closely linked genetic markers.

The objectives of this study were to determine the mode of inheritance of powdery mildew resistance in two mungbean lines, SUT4 and VC1210A, to estimate gene effects contributable to powdery mildew resistance, to improve mungbean variety for resistance to the disease, to determine the markers linked to powdery mildew resistant genes, to determine whether a breeding line, VC1210A, resistant to races of powdery mildew in Thailand represents a gene source at new map location and to determine the potential of using this resistant line in mungbean improvement.

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Chapter II

Inheritance of Powdery Mildew Resistance in Mungbean [*Vigna radiata* (L.) Wilczek]

Abstract

Powdery mildew (*Erysiphe polygoni* DC.) is a serious disease of mungbean [*Vigna radiata* (L.) Wilczek]. It is one of the major constraints of mungbean production. This study was conducted to study the inheritance of the disease resistance to facilitate breeding of resistant varieties. The inheritance of powdery mildew resistance was studied in four crosses between resistant and susceptible lines and varieties of mungbean in 2000 and 2001. Six generations including P_1, P_2, F_1, F_2, BC_1 and BC_2, of each cross were evaluated in a randomized complete block design with three replications under field conditions and subjected to generation mean analysis. Significant additive and dominant gene effects of similar magnitude were observed indicating that these two gene effects are responsible for the inheritance of the character. Interaction of genes was not found in all four crosses. Frequency distributions for powdery mildew reaction in F_2 and BC_1 were used to analyze for segregation ratios. Powdery mildew resistant reaction of all four crosses was controlled by single dominant gene pair.

Key words: Inheritance of resistance, mungbean powdery mildew, gene action

Introduction

Mungbean [*Vigna radiata* L. Wilczek] is an important source of human protein and adapt well to the tropical environment. The production of mungbean is adversely affected by many factors such as low genetic potential of current varieties, environmental stresses, diseases and insect pests, and poor cultural practices. The main foliar diseases of mungbean are *Cercospora* leaf spot and powdery mildew. Powdery mildew (*Erysiphe polygoni* DC.) has a wider geographic range than *Cercospora* leafspot (Poehlman, 1991). Severe infection of powdery mildew occurs in cool dry months when it can reduce yield of mungbean by between 20 and 40% (Soria and Quebral, 1973). Mungbean incurs maximum damage when powdery mildew infects plants just before the flowering stage (Poehlman, 1991).

Mungbean breeders have long suspected that both qualitative and quantitative genes are responsible for resistance to powdery mildew. It was found that the resistance to the disease in two breeding lines from India, Mung Ludhiana (ML-3) and ML-5, was controlled by a single dominant gene (AVRDC, 1979). However, in RUM breeding lines by two dominant genes (Reddy *et al.*, 1994). However, different mode of inheritance has been reported to be controlled by quantitative genes (AVRDC, 1981a and b; Young *et al.*, 1993). The contradicted evidence was probably due to the difference in the plant materials and races of the pathogen used in each study. The objective of this study was to determine the inheritance of resistance to powdery mildew in Thailand.

Materials and Methods

Population development

Mungbean varieties Chainat 36 (CN36) and Kamphaeng Saen 1 (KPS1) were used as sources of susceptible parents (P_1s). Variety SUT4 from Suranaree University of Technology and line VC1210A from AVRDC were used as sources of resistance to powdery mildew (P_2s). Four crosses were made, CN36 x SUT4, CN36 x VC1210A, KPS1 x SUT4 and KPS1 x VC1210A, in 1999. The resulting F_1s were self-pollinated in greenhouse and also backcrossed to both parents to obtain F_2, BC_1 (F_1 x P_1), and BC_2 (F_1 x P_2) generations. The P_1, P_2, F_1, F_2, BC_1 and BC_2 of each cross were evaluated in the field in a randomized complete block design with three replications in 2000 and 2001 at Suranaree University of Technology research farm Nakhon Ratchasima, Northeast Thailand. Plot size varied with generations. Parental lines, F_1s, BC_1s and BC_2s were grown in single-row plots and F_2s were grown in 2-row plots with plants spaced 0.25 m within row, in 3 m row length and 0.5 m between rows. Susceptible varieties, CN36 and M5-5, were planted nearby each plot as sources of powdery mildew inoculum.

Field screening

Individual plants were scored for powdery mildew response at 55 days after germination using the scoring system described by Young *et al.*(1993) as follows: 1; no visible mycelial growth, 2; 1-25% foliage area covered by fungus, 3; 26-50% foliage covered, 4; 51-75% foliage covered, and 5; 76-100% foliage covered.

Data analysis

Generation mean analyses were carried out on the original scale to determine the gene effects of powdery mildew resistance. The mean observation of each character consists of components of generation means proposed by Hayman (1958) and Jinks and Jones (1958) to include six parameters, m= mean, [d] = additive effects, [h] = dominance effects, [i] = additive x additive interactions, [j] = additive x dominance interactions, [l] = dominance x dominance interactions. In this study, a joint-scaling test was performed using data from parents, F_1, F_2, BC_1 and BC_2 to identify the model consisting of three parameters, viz, m, [d] and [h] in the absence of all interactions as described by Cavalli (1952) and Mather and Jinks (1982). In the procedure,

additive and dominance effects were estimated by the procedure of weighted least squares using as a weight the inverse of the variance of generation means. The goodness of fit was tested of the three parameter model by squaring the deviation of the observed from the expected value for each type of family, multiplying by the corresponding weight, summing the products over all six types of families and using a Chi-square test with three degrees of freedom. If the model is adequate, no further analysis is required for gene effects. The same set of data was also used to estimate broad-sense heritability (Warner, 1952) and number of genes controlling the inheritance of resistance (Sinnot *et al.*, 1953) using the following respective formulae:

$$\text{Broad-sense heritability } (h_b^2) \;=\; \frac{V_{F_2} - V_e}{V_{F_2}}$$

$$\text{The estimate of the environmental variance } (V_e) \;=\; \frac{(V_{P_1} + V_{P_2} + V_{F_1})}{3}$$

$$\text{Minimum number of genes } (k) \;=\; \frac{(\overline{P_1} - \overline{P_2})^2}{8(V_{F_2} - V_{F_1})}$$

Where $\overline{P_1}, \overline{P_2}$ are actual means of P_1 and P_2, $V_{P_1}, V_{P_2}, V_{F_1}$ and V_{F_2} are variances of the P_1, P_2, F_1, F_2, BC_1 and BC_2 populations, respectively.

Segregation ratios of disease rating scores in F_2 and BC_1 (backcross of F_1 to susceptible parent) were re-classified as follows: plant progenies which has rating scores similar to the resistant parent and F_1 were classified as resistant. On the other hand, progenies with rating scores above F_1 or similar to susceptible parent were classified as susceptible. Chi-square tests were used to test the goodness of fit of the observed to expected ratios of the above classifications.

Results and Discussion

The mean scores for powdery mildew reaction of P_1, P_2, F_1, F_2, BC_1, and BC_2 populations of different crosses made during 2000 and 2001 are shown in Table 1. The powdery mildew susceptible lines (P_1), CN36 and KPS1, had the disease scores from 3.94-4.16 and the resistant lines (P_2), SUT4 and VC1210A, had consistently low scores from 1.89-2.06. The F_1 and F_2 mean scores of disease of all crosses in two years were lower than the midparent [MP = (P_1 + P_2) / 2] and tended towards resistant parents. BC_1 population had lower disease scores than P_1 but the scores were closed to midparent. BC_2 populations had mean scores of disease closed to P_2's scores.

Generation mean analysis was performed on sets of original data to estimates the genetic effects of powdery mildew reaction. The additive-dominance model is adequate for the analysis of the variation in all sets of data given in Table 2. The estimates of genetic effects of three parameters, m, [d], and [h] for all crosses were significant (P < 0.01) and are shown in Table 2. The χ^2 value of all crosses was not significant indicating a good fit of the model. The low disease score rating is more resistant to the disease; therefore, the negative estimates of dominance gene action indicate the resistance to powdery mildew. Significant additive and negative dominance effects were found in all crosses and were of similar magnitude. Therefore, the variation among generation means for resistance to powdery mildew was sufficiently explained by additive-dominance model.

Number of genes controlling the resistance and broad-sense heritability estimates are shown in Table 3. The estimates of the minimum number of genes controlling powdery mildew resistance were in the range of 0.60 to 0.91 in 2000 and 0.67 to 1.05 in 2001. The estimates are lower than one indicating only a single major gene is responsible for the inheritance of resistance to powdery mildew in variety SUT4 and line VC1210A.

Broad-sense heritability values (h_b^2) calculated from variance components of all crosses and environments varied from 0.71 to 0.89. The highest estimates found in this study showed that the high proportion of variation was under gene control. However, the low narrow-sense heritability (not shown) indicated the low additive variance. This suggests that conventional procedures such as pedigree would not be effective in improving this character. Thus, the backcross method is recommended to develop powdery mildew resistant lines.

Table 1. Means and standard errors for disease reaction of different populations of mungbean obtained from susceptible x resistant crosses tested in 2000 and 2001.

Population	CN36 x SUT4		CN36 x VC1210A		KPS1 x SUT4		KPS1 x VC1210A	
	2000	2001	2000	2001	2000	2001	2000	2001
P_1	3.98± 0.09[1]	4.11±0.04	3.98±0.09	4.16±0.05	3.94±0.07	4.16±0.05	3.94±0.07	4.21±0.04
F_1	2.09±0.06	2.11±0.07	2.15±0.08	2.04±0.04	2.00±0.00	2.07±0.04	2.08±0.07	2.03±0.03
F_2	2.49±0.06	2.52±0.06	2.45±0.06	2.44±0.05	2.43±0.06	2.60±0.07	2.55±0.07	2.50±0.06
BC_1	2.90±0.10	2.96±0.12	3.01±0.1	2.95±0.11	2.96±0.12	3.22±0.14	2.86±0.12	3.20±0.13
BC_2	2.00±0.00	2.01±0.01	2.07±0.03	2.02±0.02	2.01±0.01	2.04±0.02	2.03±0.02	2.03±0.02
P_2	1.98±0.02	1.89±0.03	2.02±0.02	1.93±0.03	1.98±0.02	1.89±0.03	2.02±0.02	1.93±0.03

[1] Standard error = $\sqrt{\dfrac{s^2}{n}}$, n = number of observation for each population

Table 2. Estimates of additive and dominance gene effects (and standard errors) from the joint scale test for resistance to powdery mildew on susceptible x

resistant crosses and their P_1, P_2, F_2, BC_1 and BC_2 grown in 2000 and 2001.

Parameter[1]	CN36 x SUT4		CN36 x VC1210A		KPS1 x SUT4		KPS1 x VC1210A	
	2000	2001	2000	2001	2000	2001	2000	2001
m	2.96±0.04**	3.00±0.03**	2.97±0.04**	3.03±0.03**	2.94±0.04**	3.04±0.03**	2.97±0.03**	3.11±0.03**
[d]	0.98±0.04**	1.09±0.03**	0.95±0.04**	1.08±0.03**	0.96±0.04**	1.13±0.03**	0.96±0.03**	1.12±0.03**
[h]	-0.89±0.07**	-0.87±0.05**	-0.87±0.07**	-0.99±0.05**	-0.91±0.05**	-0.92±0.05**	-0.91±0.05**	-1.08±0.04**
$\chi^2_{(3)}$	1.42	4.03	3.06	7.43	3.11	4.62	1.93	6.88
P	0.50-0.70	0.20-0.30	0.20-0.30	0.05-0.10	0.20-0.30	0.20-0.30	0.50-0.70	0.05-0.10

*,** = significant differences at $P = 0.05$ and at $P = 0.01$, respectively.

χ^2 = Chi-square for testing the adequacy of the additive-dominance model at df = 3.

[1] = m = mid-parent effect, [d] = additive effect, [h] = dominance effect.

Table 3. Estimates of minimum number of genes (k) and broad-sense heritability (h_b^2) for powdery
mildew reaction.

Cross	k		h_b^2	
	2000	2001	2000	2001
CN36 x SUT4	0.67	0.92	0.80	0.87
CN36 x VC1210A	0.91	1.05	0.71	0.82
KPS1 x SUT4	0.96	0.67	0.88	0.86
KPS1 x VC1210A	0.60	0.93	0.89	0.87

The distribution of disease rating scores of P_1, P_2, F_1, F_2, BC_1, and BC_2 populations in 2000 and 2001 is shown in Table 4. The distribution score of F_1 and BC_2 were similar to resistant parent (P_2) in all crosses. Whereas, the distribution of BC_1 rating scores in all crosses ranges from P_1 to P_2 indicating the segregation of genes controlling powdery mildew resistance.

In the F_2 generation, the number of resistant and susceptible progenies of all crosses in 2000 and 2001 fit to a 3 :1 ratio (Table 5.). This suggests that resistance to powdery mildew is controlled by a dominant gene. The segregation of BC_1 population of all crosses was found to fit a 1 : 1 ratio providing supporting evidence of a single gene inheritance (Table 5 and Fig.1).

The results of this study show that the resistance to powdery mildew in two resistant lines, SUT4 and VC1210A, is controlled by single dominant gene. AVRDC (1979) also reported that the resistance to powdery mildew of lines ML-3 and ML-5 was controlled by a single gene pair. However, Reddy *et al.* (1994) reported that powdery mildew resistance in RUM mungbean breeding lines was controlled by two gene pairs. In other reports, the inheritance of resistance to the disease was found to be even more complex (AVRDC 1981a and b). The inconsistency of these reports may be due to the differences in the genetic background of plant materials used in the studies, different races of the pathogen. Previous researches suggested the presence of different physiological races of powdery mildew affecting mungbeans in Taiwan, India and the USA (AVRDC, 1979; AVRDC 1981a and b; Reddy *et al.*, 1994; Young *et al.*, 1993).

The resistant parent VC1210A used in this study is a useful genetic resource for resistance to both powdery mildew and *Cercospora* leafspot tested at AVRDC, Taiwan (Shanmugasundaram, 2001 personal communication). The ancestral line of VC1210A, ML-3, was resistant to powdery mildew at AVRDC but it was susceptible in India (Reddy *et al.*, 1987). This may suggest that the powdery mildew races of this pathogen in each location are different.

Table 4. Frequency distribution of number of plants based on powdery mildew disease response rating in the parents, F_1, F_2, BC_1, BC_2 generations from the crosses of two susceptible parents (P_1), CN36 and KPS1, and two resistant parents (P_2), SUT_4 and VC1210A.

Population	Year	Disease score					Total	Mean
		1	2	3	4	5		
		—— No. of plants ——						
CN36 x SUT4								
P_1 (CN36)	2000	-	-	11	31	10	52	3.98
	2001	-	-	4	94	17	114	4.11
F_1	2000	-	21	2	-	-	23	2.09
	2001	-	17	2	-	-	19	2.11
F_2	2000	-	121	8	23	8	160	2.49
	2001	-	166	15	28	15	224	2.52
BC_1	2000	-	43	10	34	-	87	2.90
	2001	-	48	6	26	9	89	2.96
BC_2	2000	-	101	-	-	-	101	2.00
	2001	-	114	1	-	-	115	2.01
P_2 (SUT4)	2000	1	55	-	-	-	56	1.98
	2001	12	100	-	-	-	112	1.89

Table. 4 (Cont.)

Population	Year	Disease score					Total	Mean
		1	2	3	4	5		
		—— No. of plants ——						
CN36x VC1210A								
P_1 (CN36)	2000	-	-	11	31	10	52	3.98
	2001	-	-	2	65	16	83	4.16
F_1	2000	-	17	3	-	-	20	2.15
	2001	-	26	1	-	-	27	2.04
F_2	2000	-	114	19	20	4	157	2.45
	2001	-	146	10	34	2	192	2.44
BC_1	2000	-	37	10	38	-	85	3.01
	2001	-	43	8	29	5	85	2.95
BC_2	2000	-	89	7	-	-	96	2.07
	2001	-	90	2	-	-	92	2.02
P_2(VC1210A)	2000	-	48	1	-	-	49	2.02
	2001	11	98	3	-	-	112	1.98

Table. 4 (Cont.)

Population	Year	Disease score					Total	Mean
		1	2	3	4	5		
		—— No. of plants ——						
KPS1 x SUT4								
P_1 (KPS1)	2000	-	-	4	28	2	34	3.94
	2001	-	-	6	81	33	110	4.16
F_1	2000	-	43	-	-	-	43	2.00
	2001	-	38	3	-	-	41	2.07
F_2	2000	-	118	25	18	3	164	2.43
	2001	-	170	18	31	21	240	2.60
BC_1	2000	-	36	9	27	3	75	2.96
	2001	-	26	9	23	9	67	3.22
BC_2	2000	-	73	1	-	-	74	2.01
	2001	-	92	4	-	-	96	2.04
P_2(SUT4)	2000	1	55	-	-	-	56	1.98
	2001	12	100	-	-	-	112	1.89

Table. 4 (Cont.)

Population	Year	Disease score					Total	Mean
		1	2	3	4	5		
		—— No. of plants ——						
KPS1 x								
VC1210A								
P_1 (KPS1)	2000	-	-	4	28	2	34	3.94
	2001	-	-	-	70	18	88	4.21
F_1	2000	-	43	-	-	-	43	2.00
	2001	-	36	1	-	-	37	2.03
F_2	2000	-	104	16	23	7	150	2.55
	2001	-	146	18	27	9	200	2.50
BC_1	2000	-	36	9	27	3	75	2.96
	2001	-	32	9	25	11	77	3.20
BC_2	2000	-	73	1	-	-	74	2.01
	2001	-	83	3	-	-	86	2.03
P_2(VC1210A)	2000	1	55	-	-	-	56	1.98
	2001	11	98	3	-	-	112	1.93

Table 5. Segregation ratios for powdery mildew disease score of F_2 and backcross populations derived from crosses of susceptible x resistant genotypes.

Cross	Year	Generation	Resistant Genotype (R)	Susceptible genotype (S)	Expected ratio	χ^2	Probability
			no.	no.			
CN36 x SUT4	2000	F_2	121	39	3 : 1	0.016	0.90-0.95
		BC_1	43	36	1 : 1	0.620	0.30-0.50
	2001	F_2	166	58	3 : 1	0.090	0.70-0.80
		BC_1	48	41	1 : 1	0.560	0.30-0.50
CN36 x VC1210A	2000	F_2	114	43	3 : 1	0.480	0.30-0.50
		BC_1	37	48	1 : 1	1.420	0.20-0.30
	2001	F_2	146	46	3 : 1	0.110	0.70-0.80
		BC_1	43	42	1 : 1	0.012	0.90-0.95
KPS1 x SUT4	2000	F_2	118	46	3 : 1	0.810	0.30-0.50
		BC_1	36	39	1 : 1	0.120	0.70-0.80
	2001	F_2	170	70	3 : 1	2.230	0.10-0.20
		BC_1	26	41	1 : 1	3.360	0.05-0.10
KPS1 x VC1210A	2000	F_2	104	46	3 : 1	2.570	0.05-0.10
		BC_1	40	33	1 : 1	0.680	0.30-0.50
	2001	F_2	146	54	3 : 1	0.410	0.50-0.70
		BC_1	32	45	1 : 1	2.200	0.10-0.20

F₂ of CN36 x SUT4

BC₁ of CN36 x SUT4

F₂ of CN36 x VC1210A

BC₁ of CN36 x VC1210A

F2 of KPS1 x SUT4

BC₁ of KPS1 x SUT4

F2 of KPS1 x SUT4

BC₁ of KPS1 x SUT4

Disease rating score

Disease rating score
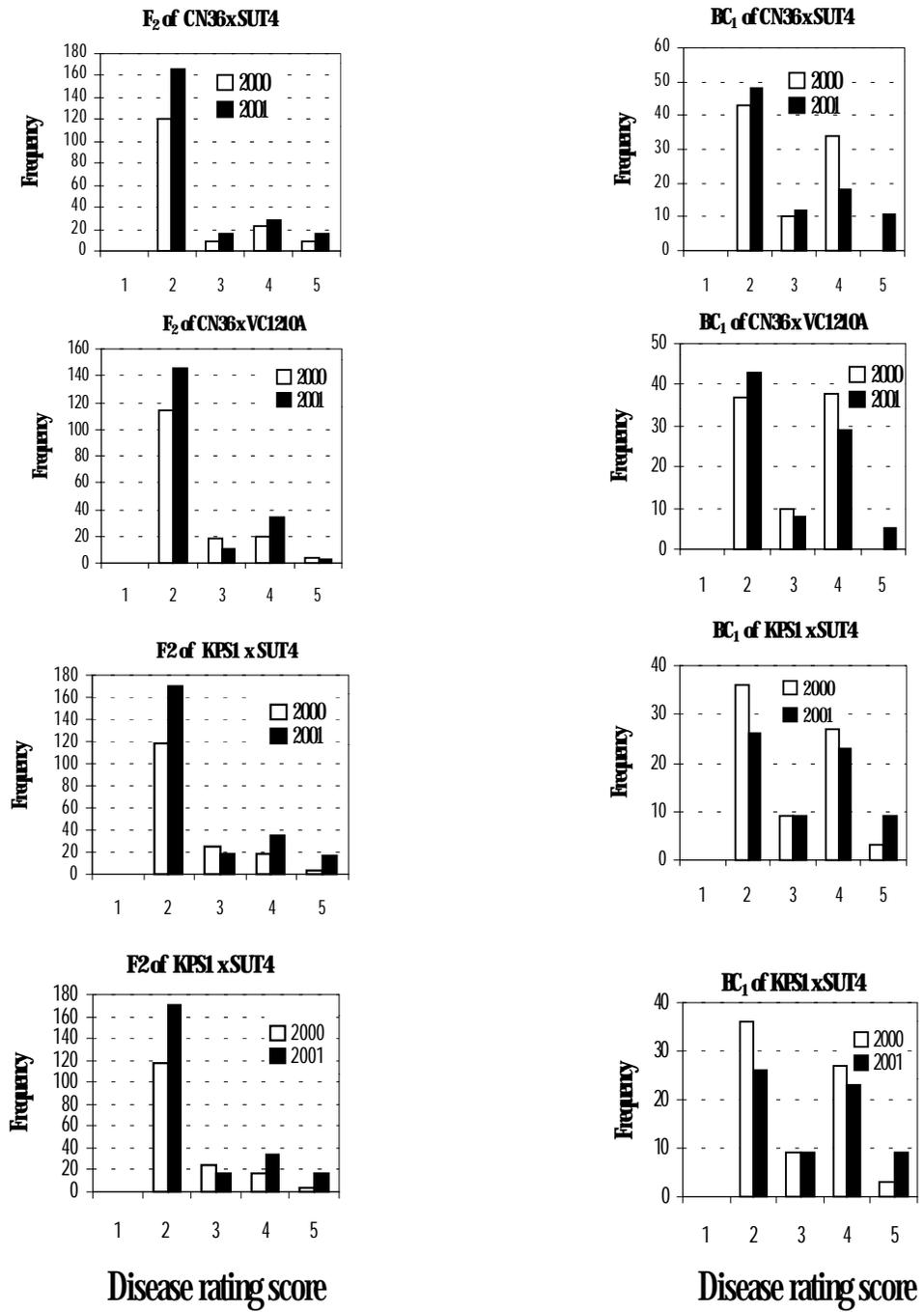
Fig. 1.   Frequency distribution of disease rating scores of F_2 and BC_1 generations in different crosses of mungbean in 2000 and 2001.

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Chapter III

Breeding for Powdery Mildew Resistance by Using Backcrossing Method

Abstract

Backcross breeding method is plant breeding procedure used in transferring favorable genes from donor to recurrent parents. At present, powdery mildew (*Erysiphe polygoni* DC.) is one of the most serious diseases of mungbean in Thailand particularity in the cool season. This study was conducted to improve susceptible cultivar, CN36, for resistance to powdery mildew. The susceptible cultivar, CN36, was crossed with two resistant cultivar/line, SUT4 and VC1210A, to produce single cross seeds. Each step was conducted following the standard backcrossing method until BC_3F_1s were obtained. The BC_3F_2 seeds were planted, selected for disease symptom-free plants and harvested separately to obtain BC_3F_3 seeds. BC_3F_3 seeds from individually selected plants and recurrent parent, CN36, were planted in a randomized complete block design with three replications. The data was analyzed for powdery mildew resistance, grain yield, 1000-seed weight and other related yield components. Line 104 (CN36 x VC1210A) gave the highest seed yield of 320 kg per rai. Line 140 (CN36 x SUT4) gave the biggest seed size of 64.10 g per 1000 seeds which is similar to CN36. Five lines were selected for further yield trial to release as varieties.

Key words: powdery mildew resistance, mungbean improvement, backcrossing

Introduction

The backcross breeding method is a plant breeding procedure used in transferring favorable genes from donor to recurrent parents. The F_1 progeny is crossed repeatedly with the recurrent parent. The procedure can be used in both self- and cross pollination crops. The success of the backcross method depends on many factors such as the ability to identify the characteristics being transferred in the successive backcrosses, heritability of the characters to be and the sufficient number of backcrosses to reconstitute the recurrent parent (Stoskopf *et al.*,1993).

The early example of backcross breeding was the development of wheat variety, Baart, resistant to bunt (Briggs, 1930 quoted in Jensen, 1988). The donor parent 'Martin' as a source of resistance was crossed with 'Baart' as a recurrent parent. Sherwood *et al.* (1967) developed the resistant line of alfalfa by transferring stem nematode resistance from a non- adapted resistant parent clone 'Lahontan' to moderately susceptible adapted clone 'DuPuits and Flamande' by backcrossing.

Mungbean breeding

Cultivated mungbean in many countries have been developed principally by selection from local strains, hybridization and mutation induction. The breeding program of mungbean was first initiated in India at the Panjab Agricultural University (PAU). The PAU program has generated a large output of disease and insect resistant breeding lines and cultivars with ML and LM designations. The Ludhiana breeding lines are used extensively in hybridization program at Asian Vegetable Research and Development Center (AVRDC) as sources of resistance to *Cercospora* leaf spot, mungbean yellow mosaic virus, bean fly, leafhopper and pod borers (Poehlman 1991).

AVRDC was organized in 1972 in Republic of China, Taiwan with the financial support from Asian countries. Mungbean was taken up as one of the crops on which to conduct research and remarkable breeding progress has been made since then. From the founding of AVRDC in 1972 up to 1993, over 6,000 *Vigna* crosses (VC) had been developed at AVRDC headquarter (Laosuwan, 1999; Srinives *et al.*, 2001)

AVRDC has improved many mungbean lines that have been named and released directly, or used as parents in mungbean breeding program of different countries. Example of such

varieties are NURI (Indonesia), PUSA-105 (India), Nm-51 (Pakistan), PSU1 (Thailand) and Er Lu No. 2 (China) (AVRDC, 2000).

Mungbean Breeding in Thailand

In Thailand, Department of Agriculture (DOA) is responsible for national mungbean research in all aspects. The main center for mungbean research is located at Chai Nat Field Crops Research Center (CFCR), Chai Nat. The major objectives of the breeding program at this center are to develop stable and high-yielding varieties and to improve cultivars for resistance to pests and diseases.

Mungbean breeding in Thailand was started in 1969 with yield trial of local and introduced cultivars or lines including an outstanding line M7A. This line was released as a variety, U-thong 1. This variety has purple color on the seedling (Laosuwan, 1999).

In mungbean breeedig for resistance to *Cercospora* leaf spot and powdery mildew was initiated at Prince of Songkla University in 1980 by crossing VC1560D that is moderately resistant to both diseases with U-Thong 1. Advanced generations were carried out using single seed descent (SSD) method. After a series of yield trials, in 1997, a variety named SUT 1 (Suranaree University of Technology 1) has been released and recommended as a moderately resistant to *Cercospora* leaf spot and powdery mildew cultivar. Simultaneously, mungebean varieties Kamphaeng Saen 1, Kamphaeng Saen 2 and PSU 1 were improved for resistance to Cercospora leaf spot. These three varieties were crossed with a resistant line, VC3689A, and backcrossed to recurrent parents for four times. Three varieties obtained from this program were named as SUT2, SUT3 and SUT4, respectively (Laosuwan *et al.*, 1997; Laosuwan, 1999).

At present, powdery mildew (*Erysiphe polygoni* DC.) is one of most serious disease of mungbean in Thailand, particularly in the cool season. Resistant cultivars released to farmers are moderately resistant. The objective of this study was to improve mungbean variety for resistance to powdery mildew.
Particularly

Materials and Methods

A susceptible mungbean cultivar, CN36, was used as recurrent parent and two resistant variety/line, SUT4 developed at Suranaree University of Technology and VC1210A introduced from AVRDC were used as donor parents. The susceptible variety was crossed with two resistant lines to produced single crosses: CN36 $\times$ SUT4 and CN36 $\times$ VC1210A. The F_1 seeds from each cross were planted and crossed with the recurrent parent to obtain BC_1F_1 seeds. In the next step, the BC_1F_1 seeds were planted and crossed with the recurrent parent. BC_2F_1 seeds were harvested individually from the resistant performance of BC_1 F_1 plants crossed with recurrent parent. This step will be repeated until BC_3F_1 was obtained and planted to produce BC_3F_2 (Fig. 1). These seeds were planted, resistant plants were identified and harvested separately. The seeds from these resistant plants may be either homozygous or heterozygous. BC_3F_3 seeds from individually selected plants and recurrent parent, CN36, were grown in a randomized complete block design with three replications. Each line was grown in single-row plot spaced 0.50 m apart. Each row was 3 m long, containing 30 plants per row with plants spaced within row at 0.25 m apart. Susceptible variety and line, CN36 and M5-5, were planted between every two plots and around the experiment as the source of powdery mildew inoculum. The non-segregated rows for powdery mildew reaction were identified as homozygous resistant lines. The data measured for each plot were analyzed as described by AVRDC (1988) with a slight modification as follows:

1. Total seed yield was adjusted for 12% moisture level using formula:

$$y = \frac{100 - x}{100 - y_s} \times \text{F.W.} \times \frac{1600}{A}$$

Where: y = yield, x = moisture level at harvesting, y_s = standard moisture at 12%, F.W = seed weight and A = harvested area (m^2)

2. 1000- seed weight = (weight of 100 randomly selected seeds x 10)

3. Mean maturity index (days) = $\dfrac{\text{(Days from planting to each harvest x yield at each harvest)}}{\text{Total yield}}$

4. Plant height (cm) measured from the cotyledonary node to the tip of the plant.

5. Mean number of pods counted on ten randomly selected plants.

6. First harvest percentage = $\dfrac{\text{First harvest yield}}{\text{Total yield}}$ x 100

7. Pod length (cm) was the mean of measurement made on 10 randomly selected pods.

8. Seeds per pod was the mean of count made on ten randomly selected pods.
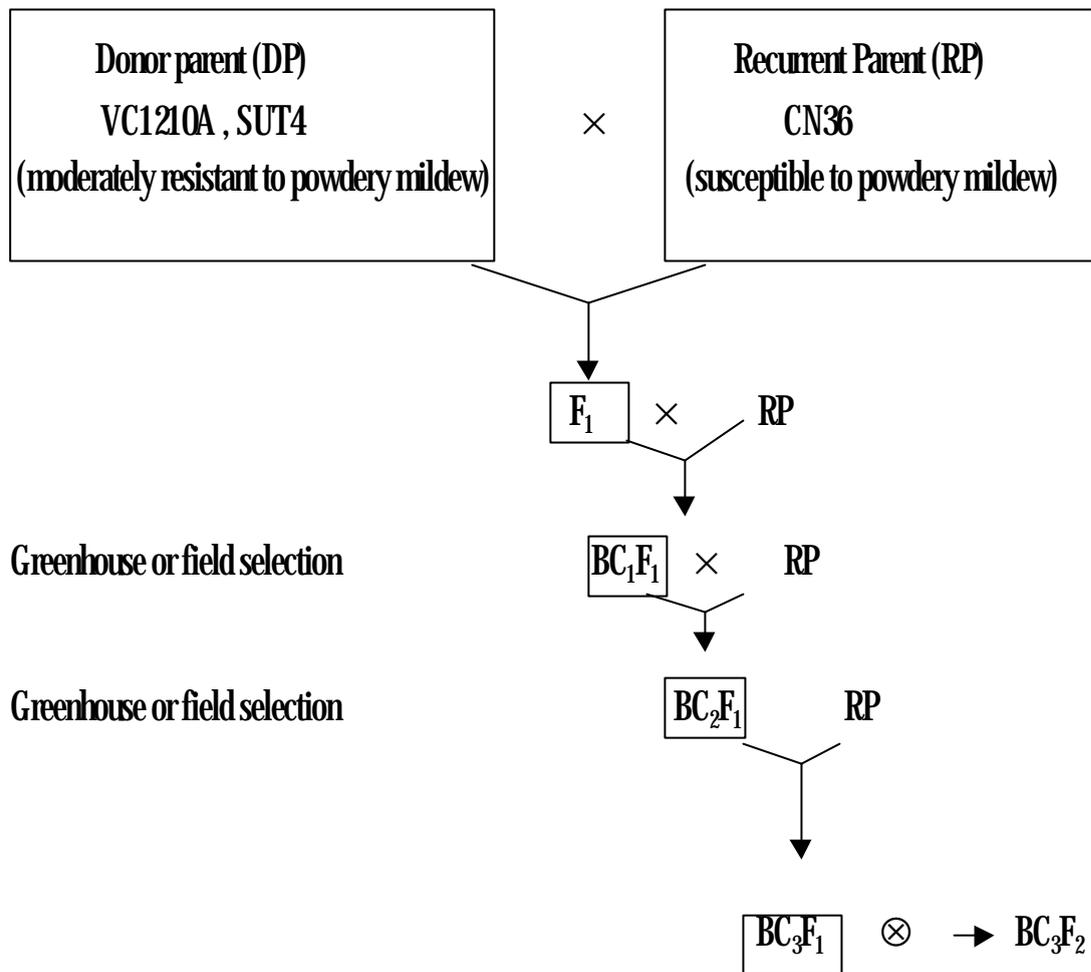
Fig. 1.   Schematic diagram of backcross breeding and selection for disease resistance.

Results and Discussion

The F_1s from crosses CN36 x SUT4 and CN36 x VC1210A were produced in February-April, 2000. Ten F_1 plants from each cross were backcrossed to recurrent parent, CN36, to produce 80 BC_1F_1 lines per cross in June-August, 2000. All BC_1F_1 seeds were planted in greenhouse in October-December, 2000. The vigorous and resistant plants of each BC_1F_1 lines were selected individually. Thirty-five BC_1F_1 plants from CN36 x SUT4 and 43 BC_1F_1 plants from CN36 x VC1210A were selected and backcrossed to recurrent parent to produce BC_2F_1 seeds. All steps were repeated until BC_3F_1 seeds were produced in January-march, 2001. Twenty-one BC_3F_2 plants from CN36 x SUT4 and 29 BC_3F_2 plants from CN36 x VC1210A were selected for resistance to the disease and planted in October-December, 2001 to produce BC_3F_3 seeds. In January-March, 2002, 51 individual plants of BC_3F_3 lines and recurrent parent, CN36 were planted in a randomized complete block design as described above. After sowing for 55 days, 51 BC_3F_3 lines were evaluated for disease response. The BC_3F_3 lines showing no sign of powdery mildew on all plants in the three replications were selected as homozygous for disease resistance (Fig. 2). Ten lines including line no. 104, 105, 108, 111, 124, 132, 138, 140, 142 and 145 were selected as homozygous and harvested for further testing.

Results from analysis of variance of ten selected lines plus check parent are shown in Table 1. The significant difference was found among lines for grain yield, pods per plant, seed weight, plant height and mean maturity but not for first harvest percentage, pod length and seeds per pod.

Seed yield

Means for yield and other characters of mungbean lines and variety CN36, the check, are given in Table 2. Line no. 104 gave the highest seed yield of 320 kg/rai. There were only two lines including no. 104 and no. 132 yielded significantly higher than the check. Other lines gave similar yield to the check or even lower. Theoretically, the performance of these lines should be similar to each other and to the check as they have similar genetic background. Therefore, any difference should be attributable to the resistance to the disease. In breeding for resistance to Cercospora leaf spot in three mungbean varieties, after backcrosses, Laosuwan *et al.* (1997) found that yield of all backcrosses was about 10 % higher than their respective recurrent parents. This was concluded to be due to the vigorous performance of resistant lines.

Seed size

Seed size of mungbean lines ranged from 52.50 to 64.10 g/1000 seeds. Lines no. 104, 108, 138 and 145 gave smaller seed size than the check. These lines were of the VC1210A donor parent. On the other hand, lines no. 105, 111, 132, 140 and 142 gave seed sized similar to CN36. These lines were backcross progenies using SUT4 as the donor parent. Selection for seed size can be practiced in each cycle of backcross. However, high seed weight can be dominant (Malhotra, 1983) or recessive (Malik et al., 1988).

Other character

Variations in other characters were observed including mean maturity index, plant height and pods per plant (Table 2). These variations will be reduced along with the successive backcrosses.

The result of this study confirm that backcross breeding is an efficient method in improving mungbean varieties for resistance to powdery mildew. Resistance lines selected at this stage may be released as a variety after a minor selection or subjected to further backcrosses.

Fig. 2. Difference between resistant (A) and susceptible (B) lines in responses to powdery mildew.

Table 1. Analysis of variance of grain yield and other characters of mungbean lines selected from backcross progenies for resistance to powdery mildew [1]

Source of variation	Degrees of freedom	Mean square							
		yield	SW	MM	H	PN	FHP	PL	SP
Replications	2	267.75^{ns}	0.10^{ns}	70.36^{ns}	606.08^{**}	0.73^{ns}	154.30^{ns}	0.01^{ns}	0.65^{ns}
Varieties	10	8903.00	70.00	36.56^{ns}	119.96^{*}	18.47^{*}	82.40^{ns}	0.32^{ns}	0.77^{ns}
error	20	1109.57	4.83	28.27	50.89	7.67	64.77	0.21	0.38

[1]SW = 1000-seed weight, MM = mean maturity index, H = height, PN = number of pods per plant, FHP = first harvest percentage, PL = pod length and SP = seeds per pod.

Table. 2 Grain yield and other characters of mungbean lines selected from backcross progenies
for resistance to powdery mildew. [1]

Line no.	Grain yield	SW	MM	Height	PN	FHP	PL	SP
	(kg/rai)	(g)	(day)	(cm)	(no.)	%	(cm)	(no.)
104 (CN36 x VC1210A)	320**	52.77**	60.90	67.07	21.83*	91.60	8.67	11
105 (CN36 x SUT4)	216	62.03	55.30	61.53	18.10	82.30	9.20	10
108 (CN36 x VC1210A)	245	56.23**	65.90	70.37	19.70	98.00	8.67	11
111 (CN36 x SUT4)	195	61.97	60.00	54.93*	16.20	92.30	9.10	11
121 (CN36)	232	64.53	53.90	69.67	16.30	80.33	9.40	11
124 (CN36 x VC1210A)	268	54.70**	63.80	76.53	22.40*	95.33	8.95	10
132 (CN36 x SUT4)	309*	62.47	61.60	67.23	21.90*	91.68	9.07	10
138 (CN36 x VC1210A)	214	53.23**	61.80	73.80	19.20	92.00	8.43	11
140 (CN36 x SUT4)	165*	64.10	58.30	75.40	15.50	87.00	9.23	11
142 (CN36 x SUT4)	155*	61.63	61.70	69.23	24.30	92.00	9.30	11
145 (CN36 x VC1210A)	184	52.50**	61.70	73.03	16.90	92.00	8.57	11
Mean	227	58.74	60.44	68.98	18.71	90.42	8.96	10.77
LSD 0.05	56.73	3.74	-	12.15	4.71	-	-	-
CV %	14.6	3.7	8.8	10.3	14.8	8.9	5.0	5.8

[1]SW = 1000-seed weight, MM = mean maturity index, H = height, PN = pods per plant,
FHP = first harvest percentage, PL = pod length and SP = seeds per pod.

*, ** significantly different from the check (CN36) at 5% and 1% level, respectively.

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Chapter IV

Mapping a new source of resistance to powdery mildew in mungbean [*Vigna radiata* (L.) Wilczek]

Abstract

Powdery mildew (*Erysiphe polygoni* DC.) is a serious disease of mungbean [*Vigna radiata* (L). Wilczek]. Classical breeding using backcrossing is difficult because consistent disease reaction in the field is hard to achieve due to environmental factors. Molecular markers linked to powdery mildew resistant gene is one approach to improve selection for resistant cultivars. The objective of this study was to use restriction fragment length polymorphism (RFLP) markers to identify quantitative trait loci (QTL) conditioning resistance to powdery mildew. DNA from 96 F_2 progenies from a cross between resistant line, VC1210A, and susceptible line, TC1966, were used to hybridize with 42, 29, 27 probes from libraries of mungbean, soybean and common bean and four new probes (Mac; Mungbean AFLP clone), respectively. Analysis of variance and interval mapping were used to identify QTLs associated with powdery mildew resistance. A major resistant QTL was detected at markers Mac71a and Mac114 which had a LOD score of 20.22. The new RFLP loci detected by two cloned probes from the AFLP bands associated with resistance constitute a new linkage group. A major resistance QTL was found on a new linkage group that accounted for 64.9% of the total variation for plant reaction to the disease. The resistant parent allele enhances powdery mildew resistance with partially dominant effect. One of the probes developed in this study has the potential to assist in breeding for powdery mildew resistance in mungbean.

Introduction

Breeding for disease resistant varieties depends on many factors such as plant type, species, growth stage, age of plant, pathogen, interaction between plant and pathogen, environment, and so fourth (Baird *et al.*, 1996). Backcrossing is the main breeding method for introduction of single major resistance genes. The plants are screened against pathogens in the field by natural infection or greenhouse by inoculation. The plants showing high resistance are selected and used for backcrossing into elite lines (cultivars) until resistant varieties are produced (Briggs and Knowles, 1967). Visual selection of individual plants is sometimes difficult due to low incidence of the disease. Breeders may depend on indirect selection by the application of closely linked genetic markers. The objectives of indirect selection via markers may be:

1) to identify the resistant individuals in the early growth stage and selection for resistant plant materials prior to flowering (e.g. backcross or population improvement program):

2) to correct inaccurate direct field selection of trait expression due to many loci involved (e.g. yield) or due to uneven inoculation/infection/infestation.

Indirect methods may use morphological markers (e.g. leaf color, hypocotyl color etc.), biochemical markers (e.g. isozymes), and molecular markers (e.g. restriction fragment length polymorphism: RFLP, random amplified polymorphic DNA: RAPD, DNA amplification fingerprinting: DAF, sequence characterized amplified region: SCAR, cleaved amplified polymorphic sequences: CAPS, simple sequence repeats : SSR or microsatellites and short tandem repeats and amplified fragment length polymorphism:AFLP).

The development of the molecular marker concept offers an opportunity to apply linkage or Mendelian genetic approach for the improvement of agriculturally important species. Isozymes were the first biochemical makers used in this context. There are some limitations to the number of informative loci within many species restricted their use, but initial results in many cases were quite promising and have served to maintain interest in this approach (Helentjaris, 1992). Recently, many molecular markers have been developed and used effectively in studying plant genetics and breeding.

Restriction fragment length polymorphism (RFLP) is a marker based approach to study the variation in length of DNA fragments obtained by digestion with restriction endonucleases (Botstein *et al.*, 1980). The various lengths can be separated in agarose gels in an electric field and visualized by staining with ethidium bromide and observing with ultraviolet light. In some cases no distinct fragments can be visualized only a smear. Therefore, more complex techniques such as the use of cloned DNA probes and DNA hybridization are required to visualize differences in DNA. RFLP markers are co-dominant markers (Baird *et al.*, 1996). RFLP markers have been applied to find QTLs associated with useful traits in many crops, i.e. grain yield components in maize (Veldboom and Lee, 1994), resistance genes to **cyst nematode, Javanese root knot nematode, Southern root knot nematode, and Phytophthora root and stem rot in soybean (Concibido** *et al.***, 1997; Tamulonis** *et al.***, 1997a, Tamulonis** *et al.***, 1997b; Hegstad** *et al.***, 1998), powdery mildew in wheat (Hartl** *et al.***, 1993; Hartl** *et al.***, 1995; Ma** *et al.***, 1994; Liu** *et al.***, 2001), common bacterial blight in common bean (Yu** *et al.***, 1998; Correa** *et al.***, 2000) and aphid in cowpea (Myers** *et al.***, 1996). In addition RFLP markers have been used to analyze the size of chromosomal segments during backcross breeding in tomato (Young and Tanksley, 1989).**

Menaceo-Hautea *et al.* **(1992) developed a genetic linkage map of mungbean using RFLPs. This map has already been useful in the identification of RFLP markers associated with bruchid resistance gene that located on linkage group VIII (Young** *et al.***,1992; Kaga and Ishimoto, 1998) and powdery mildew resistance genes located on linkage group III, VII and VIII (Young** *et al.***,1993).**

There are limitations to the genetic diversity of many crops such as tomato (Rick, 1982 quoted in Weeden, 1991) and wheat (Chao *et al.*, 1990 quoted in Weeden, 1991). Despite the large number of DNA probes that can be generated from libraries, the identification of polymorphism can still be difficult. The other limitation of RFLP techniques is its high cost and complicated techniques involved. The technique requires several days and a skilled technician to obtain a marker (Weeden, 1991). So an attempt to overcome such limitations resulted in the development of other DNA markers based on polymerase chain reaction (PCR) such as RAPD, DAF , SCARs, CAPS, SSR, and AFLP.

Vos *et al.* **(1995) has developed a new PCR – based method called amplified fragment length polymorphism (AFLP). This technique can enable a molecular linkage map to be**

developed and resolved by labeling with either radioisotope or non-radioisotope. This technique produces a similar level of information to RAPD analysis in that random genomic DNA fragments are amplified and produced more polymorphisms per reaction than either RFLP and RAPD analysis, and generally amplifies smaller sized fragments. The polymorphism is due to presence/absence of a priming site, the relationship is dominance. However, AFLPs can be co-dominant markers when polymorphism is due to sequences within the amplified region. The AFLP technique is more technically complex than RAPD analysis, but fewer primers are needed to screen all possible sites (Melcher, 1999).

AFLP markers can reveal loci and alleles. They have been used to analyze genetic diversity in rice (Fuentes *et al.*, 1999; Garland *et al.*, 1999), wheat (Barrett and Kidwell, 1998), and azuki bean (Yee *et al.*, 1999; Xu *et al.*, 2000), to tag a major resistant gene to striga, a parasitic plant in cowpea (Ouedraogo *et al.*, 2001), to construct genetic linkage maps in many crops such as pines (Travis et al., 1998: Remington *et al.*, 1999), Eucalyptus (Marques *et al.*, 1998) and conifer (Nikaido *et al.*, 2000).

The molecular markers are being used to address many problems in plant breeding. DNA markers provide plant breeders and geneticists with new insights into the relationships among germplasm, DNA linked to important traits of interest to the plant breeding programs (Helenjaris, 1992). Molecular markers offer many other advantages over conventional phenotypic markers, because they are developmentally stable, detectable in all tissues, unaffected by environmental conditions, generally, insensitive to epistatic or pleiotropic effects, and provide a choice of co-dominant or dominant markers (Allen, 1994; Bostein *et al.*, 1980; Helenjaris *et al*., 1985 quoted in Baird *et al.*, 1996; Williams *et al.*, 1990)

The utility and efficient application of molecular markers to identify the most appropriate traits relies on understanding the limitation of the system and correctly identifying the critical variables. The factors must be considered before using DNA markers such as trait heritability (usually low for important trait), minimum number of markers used, the density of markers on linkage map, markers located on each side of a QTL (flanking markers), choice of optimal sample size (e.g., number of lines and replication to evaluate), and program resources (e.g., cost of each trait or marker analysis in time and money, germplasm resources, etc.) (Baird *et al.*, 1996; Dudley, 1993; Young and Tanksley, 1989).

The objectives of this study were to determine the markers linked to powdery mildew resistant genes, to determine whether a breeding line, VC1210A, resistant to races of powdery mildew in Thailand represents a gene source at new map location and to determine the potential of using this resistant line in mungbean improvement.

Materials and Methods

Plant materials

Four mungbean lines obtained from and evaluated by AVRDC, VC1210A, VC1482A, VC2273, and VC3528A, found to be resistant to powdery mildew. They were re-evaluated in this study twice, on October 20, 1999 and on December 20, 1999 at Suranaree University of Technology experimental farm (SUT), Nakhon Ratchasima, Thailand. VC1210A was found to be highly resistant and was selected as the resistant parent in this study. TC1966, a wild relative (*Vigna radiata* var. *sublobata*) and highly susceptible to the disease was used in this study. These plants were crossed to produce F_1 and F_2 generations.

Disease assay

The two parents, F_1 and F_2 progenies were planted at SUT experimental farm, Nakhon Ratchasima, Thailand on October 30, 2000. Susceptible varieties, CN36, M5-5, and TC1966, were planted around the experiment and between the plots as a source of powdery mildew inoculum. Individual plants were scored for powdery mildew response at 55 days after germination using the scoring system described by Young *et al.* (1993) as follows: 1 (no visible mycelial growth), 2 (1-25% foliage area covered by fungus), 3 (26-50% foliage covered), 4 (51-75% foliage covered), and 5 (76-100% foliage covered). Broad sense heritability was estimated following Warner's method (1952)

$$\text{Broad-sense heritability } (h_b^2) \ = \ \frac{V_{F_2} - V_e}{V_{F_2}}$$

$$\text{The estimate of the environmental variance } (V_e) \ = \ \frac{(V_{P_1} + V_{P_2} + V_{F_1})}{3}$$

DNA isolation

DNA was isolated from young leaves using a procedure based on the CTAB method (Draper and Scott, 1988). In brief, young leaves were harvested from parents and F_2 individuals, frozen in liquid nitrogen and then stored at $-80^{\circ}C$ until beginning extraction procedures. The samples, around 0.3-0.5 g, were ground into powder in liquid nitrogen, dispersed into 15 ml tubes containing 6 ml of warmed-extraction buffer (1.5 % Cetyl-tri-methylammonium bromide: CTAB, 75 mM Tris-HCl pH 8.0, 15 mM EDTA pH 8.0, 1.05 M NaCl, 0.75 % 2-mercaptoethanol), and

incubated at 65°C for 20 min with continuous gentle shaking. 6 ml of chloroform/isoamylalcohol (24:1:v/v) was added. The solution was mixed by inversion for 20 min, and centrifuged at 2000 g for 20 min. The supernatant was transferred into a new tube. 0.6 ml of 10% CTAB as added and incubated in 65°C for 5 min. 6 ml of fresh chloroform/isoamylalcohol was added and mixed for 20 min, and centrifuged following the steps described above. The supernatant was transferred into a new tube, 9 ml of CTAB precipitation buffer (1% CTAB, 50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0), was added and mixed gently to precipitate DNA. The mixture was centrifuged at 500 g for 5 min at room temperature, supernatant discarded, 5 ml of 1 M NaCl and 4 μl of RNase A (10 mg/ml) were added. The mixture was incubated while shaking gently in a water bath at 65°C until the DNA pellet was completely dissolved. The solution was incubated at 37°C for 30 min. DNA was precipitated using 10 ml of ethanol and transferred into 1.5 ml tube containing 1 ml of 70% ethanol. After incubation at room temperature for 10 min, the tube was centrifuged briefly at high speed. The supernatant was discarded completely then 100-300 μl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) was added. The extracted DNA was stored at –20°C until use.

RFLP analysis

DNA from P_1, P_2 and F_2 individuals was digested with BglII, DraI, EcoRI, EcoRV, HindIII, and XbaI restriction enzymes under conditions recommended by the manufacturer (New England BioLabs, UK). The digested DNA was separated on 1% agarose gel in 1×TAE buffer (40 mM Tris-HCl pH 8.0, 40 mM acetic acid, 1 mM EDTA pH 8.0) at 1 V/cm for 15 hr. The DNA was transferred onto Hybond N+ membrane (Amersham Pharmacia Biotech, UK) by alkaline solution (0.4 M NaOH, 1.5 M NaCl) and fixed by UV Crosslinker at 60 mJ/cm^2 (Amersham Pharmacia Biotech, UK). A RFLP linkage map for mungbean had been developed using the F_2 population of a cross between a cultivar 'VC3890' and the wild relative of mungbean 'TC1966' (Menancio-Hautea et $al.$ 1992). A revision of this linkage map available on Beangenes Database (http://beangenes.cws.ndsu.nodak.edu) was used as the basis for probe positions. Ninety-eight probes for detecting RFLPs were from a mungbean library (University of Minnesota, USA), soybean library (Iowa State University, USA) and common bean library (CIAT, Colombia), respectively. Probe DNAs were amplified from plasmid DNA by PCR using universal M13 forward and reverse primer. The PCR was carried out in a GeneAmp PCR system

9700 (Applied biosystems, USA) programmed for 60 sec at 94°C, 40 cycles of 30 sec at 94°C, 30 sec at 52°C and 60 sec at 72°C, and ending with 60 sec at 72°C. DNA hybridization was carried out by ECL direct nucleic acid labeling and detection systems according to manufacturers instructions (Amersham Pharmacia Biotech, UK). After detection, blots were placed against Fuji medical X-ray film at room temperature for 3-6 h to produce auto-radiographs. Autoradiogrammed X-ray film was used to score alleles in segregation F_2 population. A plant showing a single specific band to VC1210A and TC1966 was scored 'A' and 'B' respectively. Meanwhile, a plant showing both bands was scored 'H'.

AFLP analysis

AFLP analysis was performed according to Vos *et al.* (1995). Total genomic DNA from P_1, P_2, bulked resistance (six plants with the lowest disease score) and bulked susceptible F_2 plants (six plants with the highest disease score) were digested with *Eco*RI and *Mse*I for 3 hours at 37°C. The reaction volume was 40 μl containing 500 ng of genomic DNA, 5 unit of each restriction enzyme (New England BioLabs, UK) and 1x reaction buffer (10 mM Tris-Acetate pH7.5, 10 mM Magnesium acetate, 50 mM potassium acetate, 5 mM DTT and 2.5 μg BSA). Digested DNA fragments were ligated to *Eco*RI and *Mse*I adapters using T4 DNA ligase at 37°C, overnight. The reaction volume was 50 μl containing previous 40 μl reaction, 5 pmole *Eco*RI adapters, 50 pmole *Mse*I adapters and 1U of T4 DNA ligase (Roche Molecular Biochemicals, Germany). The restriction-ligation products were used as primary template DNA for the first PCR step (pre-amplification) with E_{oo} and M_{oo} primers with no selective nucleotides at the 3' end. The reaction volume was 20 μl containing 50 ng of restriction-ligation products, 30 ng each of E_{oo} and M_{oo} primers, 1x PCR buffer 0.2 mM dNTP, 0.25 unit ExTaq (TAKARA, Japan). PCR conditions are listed in Table 1. The PCR products were used in the second PCR (selective PCR) with 100 primer combinations (10 *Eco*RI primers and 10 *Mse*I primers) with 2 and 3 selective nucleotides at the 3' end respectively. The primer combinations are show in Table 2.

Five microliters of the PCR products were mixed with 5 μl of STR 3$\times$ loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF), denatured at 90°C 3 min and cooled on ice. Three microliters of denaturing products were run on 6% denaturing polyacrylamide gel (19:1) in 0.5x TBE buffer (44.5 mM Tris-HCl pH 8.0, 44.5 mM

Table 1. PCR conditions for AFLP analysis.

___

1. Pre-amplification.

$94^{o}C$ 60 sec

25 cycles of

denaturation	at $94^{o}C$	30 sec
annealing	at $56^{o}C$	60 sec
extension	at $72^{o}C$	60 sec
ending with	at $72^{o}C$	60 sec.

2. Selective-amplification

$94^{o}C$ 60 sec.

Step 1: 13 cycles

the first cycle	denaturation	at $94^{o}C$	30 sec
	annealing	at $65^{o}C$	30 sec
	extension	at $72^{o}C$	60 sec

Subsequent cycles the annealing temperature is reduced by $0.7^{o}C$ per cycle over 12 cycles.

Step 2: 23 cycles

denaturation	at $94^{o}C$	30 sec
annealing	at $56^{o}C$	30 sec
extension	at $72^{o}C$	60 sec

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Table 2. AFLP primer combinations used in this study.

*Eco*R*I*/*Mse*I	M-AAG	M-AAT	M-AGA	M-AGC	M-AGG	M-CAA	M-CCT	M-CGA	M-GAC	M-GTA
E-AC	17	18	19	20	21	22	23	24	25	26
E-AAC	28	29	30	31	32	33	34	35	36	37
E-AAG	39	40	41	42	43	44	45	46	47	48
E-AGA	50	51	1	52	53	54	55	56	57	58
E-AGT	110	59	60	61	62	63	64	65	66	67
E-ATC	111	68	69	70	71	72	73	74	75	76
E-CAA	112	77	109	78	79	80	81	82	83	84
E-CAC	113	85	2	86	87	88	89	90	91	92
E-CGT	114	93	3	94	95	96	97	98	99	100
E-CTG	115	101	4	102	103	104	105	106	107	108

The numbers in the table represent the name of primer combinations

Boric acid, 1 mM EDTA pH 8.0). Electrophoresis was performed at constant 70 W for 1.5 h. The products were stained according to the Silver Sequence DNA sequencing system (Promega, USA). In order to clone some important polymorphic bands, the gel containing bands were excised and squashed in micro-centrifuge tubes containing 10 μl H_2O. The suspension was centrifuged at 12,000 g for 5 min at room temperature. Five microliters of supernatant was transferred to a new tube containing 20 μl of H_2O. This solution was used as template DNA in PCR. The selective PCR condition and primer combinations producing polymorphic bands were used to re-amplify the fragments. The PCR products were used directly to insert to pGEM -T Easy plasmid vector according to protocol of the manufacturer (Promega, USA). The inserted plasmids were transformed into *E. coli* by electroporation (Sambrook and Russell 2001). The electroporation was performed using Gene pulser II (Bio-Rad, USA) to deliver an electrical pulse of 25 μF capacitance, 1.75 kV, and 200 ohm resistance. The plasmid DNA was extracted from *E. coli* by small-scale preparation method described in Maniatis *et al.* (1989) and used as template DNA for probe preparation by PCR. The PCR products were checked for the correct insert size by electrophoresis and used as probes.

Statistical analysis

One-way ANOVA was conducted to determine significant (P < 0.05) association between putative resistance- related markers and powdery mildew resistance. Chi-square tests were used to test Mendelian segregation ration (1 : 2 : 1) for codominant markers.

Linkage and QTL analysis

The genotypic data from RFLP analysis were analyzed with MAPMAKER/EXP version 3.0 program (Lander *et al.* 1987) to re-construct the RFLP linkage map of mungbean. A LOD score of 3.0 and Haldane function (Haldane and Waddington, 1931) were used. The positions of cloned fragments from AFLP analysis were determined by pairwise command against all of RFLP markers at a threshold of LOD 3.0. Then the position was determined using the compare command.

The mapping of QTLs was performed by the method of interval mapping (Lander and Botstein, 1989) using MAPMAKER/QTL version 1.1 (Lincoln *et al.*, 1992) based on the phenotypic and linkage map data. Scan command at threshold of LOD 3.0 was used to identify

putative QTLs in the linkage map. By fixing the strongest QTLs, others were searched. Try command was used to evaluate the genetic models. The fraction of the total phenotypic variation explained by an individual QTL was obtained by fitting the model to individual QTLs. One-way ANOVA was used to confirm the presence of QTL at the marker position.

Results

Evaluation

Of the four lines evaluated for resistance to powdery mildew, VC1210A was superior. This line exhibited rapid necrosis around a focus of powdery mildew infection that is indicative of a hypersensitive reaction. This line was selected as the resistant parent in this study. The average scores for powdery mildew resistance of P_1(VC1210A), P_2(TC1966), F_1 and F_2 are shown in Table 3. Broad sense heritability of this population is 81. The frequency distribution pattern of the F_2 population disease evaluation score is shown (Fig. 1). This frequency distribution suggests the involvement of a gene(s) with large effect(s).

QTL mapping of PM based on RFLP linkage map

P_1 and P_2 were analyzed for RFLP using three different sources of probes, mungbean, soybean, and common bean probes. Twenty-nine probes showed polymorphism and were used as probes to test for in polymorphism in 96 individuals from the F_2 population. Segregation of markers deviated significantly from the expected ratio 1 : 2 : 1 at P < 0.05 (Table 4.) A mungbean linkage map was reconstructed using segregation data from the 96 F_2 individuals. Of these only one genomic region from common bean probe, Bng065, located on linkage group 2 (Menacio-Hautea *et al.*, 1992), revealed statistically significance powdery mildew score at $P =$ 0.009 by ANOVA. The average value of resistance to powdery mildew of homozygous, VC1210A and TC1966, from this marker was 2.1 and 2.88 respectively. The value of heterozygous plants was 2.79, nearly as susceptible to powdery mildew as TC1966 (Table 5.). No significant genomic region was observed by interval mapping in spite of a relatively simple segregation pattern. Consequently, a search for additional markers linked to the resistance gene was undertaken using AFLP analysis.

AFLP analysis

The initial results of screening DNA from P_1, P_2, and bulked DNA of 6 resistant and 6 susceptible F_2 plants with 100 primer combinations showed 5,734 and 5,729 polymorphic bands from bulked resistance and bulked susceptible F_2 DNA respectively. From this results, only four

Table 3. Reaction to powdery mildew of different populations derived from a cross between P_1 (VC1210A) and P_2 (TC1966).

Population	Number of plants	Disease reaction	σ^2
P_1 (VC1210A)	31	1.45 ± 0.09	0.256
F_1	7	2 ± 0	0.000
F_2	96	2.67 ± 0.1	0.899
P_2 (TC1966)	34	3.91 ± 0.09	0.265
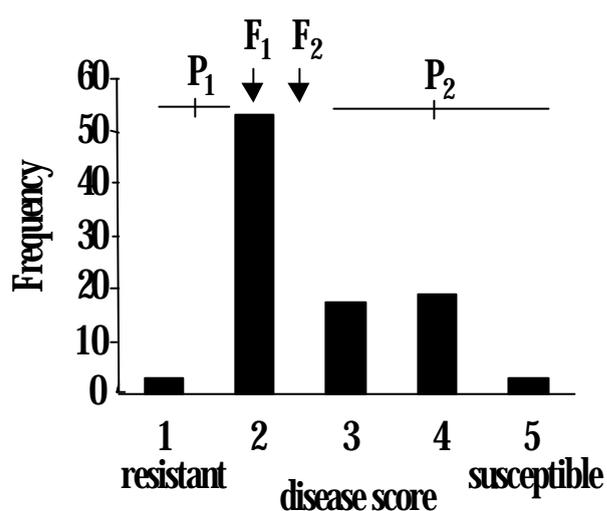
Fig. 1. Frequency distribution of F_2 population from the VC1210A ×TC1966 for the disease score of powdery mildew resistance. Disease score means for parents, the F_1 hybrid and F_2 population designated as VC1210A(P_1), TC1966(P_2), F_1 and F_2, respectively. Range for parents are indicated with bars.

Table 4. Segregations of RFLP markers in the F_2 population using mungbean, common bean, soybean and AFLP clone probes.

Marker	Restriction enzyme	No. of plants			Chi-square
		A (P_1)	H	B (P_2)	
Mungbean probes					
mc003	EcoRI	22	54	20	1.59
mc004	EcoRI	31	49	16	4.72
mgM208	BglII	18	49	19	1.74
mgM217	BglII	20	46	24	0.40
mgM244	XbaI	26	42	21	0.84
mgM247(1)	HindIII	13	60	12	14.44**
mgM247(2)	HindIII	22	47	20	0.373
mgM247(3)	HindIII	18	55	17	4.46
mgM307	HindIII	23	43	25	0.363
mgM339	XbaI	20	54	16	3.96
mgM392	EcoRV	21	53	16	3.39
mgM415	EcoRI	27	43	26	1.065
cgP137	XbaI	27	39	22	1.71
pQ062	EcoRI	24	54	18	2.25
mgQ117	EcoRI	24	41	31	3.06
Common bean probes					
Bng004	XbaI	17	57	15	7.11*
Bng025	EcoRI	38	15	43	12.07**
Bng031	XbaI	17	50	22	1.92
Bng065	HindIII	20	54	17	3.37
Bng107	EcoRI	30	47	19	2.56
Bng134	HindIII	30	34	27	6.01*

Table 4. (continued).

Marker	Restriction enzyme	No. of plants			Chi-square
		A (P$_1$)	H	B (P$_2$)	
Bng138	XbaI	23	51	16	2.69
Bng201	EcoRI	29	50	17	3.16
Soybean probe					
pA060	HindIII	22	51	16	2.71
pA106	HindIII	24	40	26	1.20
pA132	BglII	15	58	17	7.6*
pA315	EcoRI	25	50	21	0.50
pB032	BglII	23	46	23	0.31
pB069	EcoRV	18	55	17	4.46
Mungbean AFLP clone (Mac)					
Mac71(a)	XbaI	19	46	25	0.84
Mac71(b)	XbaI	17	36	33	8.03*
Mac86					
Mac95					
Mac114	BglII	19	46	25	0.84

A, B and H indicate homozygote genotype of VC1210A, TC1966 and heterozygote genotype respectively.

* and ** show markers deviated significantly from the expected ratio of 1: 2:1 (p< 0.05)

Table 5. Significant association between marker segregation and average score of phenotype (disease resistance) from F$_2$ population.

Marker	Average score of phenotype			F (2, n-2)	P	Linkage group*
	A (P$_1$)	H	B (P$_2$)			
Mungbean probes						
mc003	2.25	2.296	2.325	0.034	0.966	6
mc004	2.209	2.265	2.531	0.645	0.527	7
mgM208	2.388	2.276	2.236	0.133	0.875	7
mgM217	2.450	2.141	2.438	1.138	0.325	3
mgM244	2.615	2.155	2.190	2.103	0.128	4
mgM247(1)	2.269	2.292	2.375	0.046	0.955	7
mgM247(2)	2.523	2.138	2.450	1.566	0.215	7
mgM247(3)	2.222	2.309	2.294	0.056	0.946	7
mgM307	2.565	2.127	2.360	1.661	0.196	3
mgM339	2.225	2.259	2.469	0.352	0.704	11
mgM392	2.190	2.368	2.156	0.445	0.641	6
mgM415	2.481	2.162	2.308	0.947	0.391	3
cgP137	2.259	2.269	2.432	0.248	0.781	4
pQ062	2.333	2.222	2.444	0.399	0.672	U
mgQ117	2.438	2.268	2.210	0.410	0.665	4
Common bean probes						
Bng004	1.882	2.368	2.500	2.167	0.121	U
Bng025	2.461	2.302	1.833	2.446	0.092	5
Bng031	2.529	2.190	2.364	0.872	0.422	6
Bng065	2.100	2.796	2.882	4.937	0.009*	2

Table 5. (continued).

Marker	Average score of phenotype			F (2, n-2)	P	Linkage group*
	A (P$_1$)	H	B (P$_2$)			
Bng107	2.083	2.436	2.263	1.295	0.279	9
Bng134	2.100	2.514	2.259	1.563	0.215	9
Bng138	2.500	2.156	2.235	0.800	0.453	2
Bng201	2.362	2.310	2.117	0.373	0.689	1
Soybean probes						
pA060	2.136	2.333	2.406	0.441	0.644	11
pA106	2.354	2.238	2.385	0.216	0.806	3
pA132	1.966	2.345	2.382	1.045	0.356	2
pA315	2.200	2.250	2.500	0.671	0.513	9
pB032	2.174	2.223	2.625	1.534	0.222	10
pB069	2.556	2.209	2.265	0.905	0.408	2
Mungbean AFLP clone (Mac)						
Mac71 (a)	1.842	2.347	3.800	74.592	< 0.0001***	U*
Mac71 (b)	1.882	2.388	3.424	31.474	< 0.0001***	U
Mac86						
Mac95						
Mac114	1.842	2.347	3.800	74.592	< 0.0001***	U

A, B and H indicate homozygote genotype of VC1210A, TC1966 and heterozygote genotype, respectively.

* and ***show significant level of F- test at 0.05 and 0.0001 propability levels, respectively.

U indicates unlinked marker.

There is no band was detected with Mac86 and Mac95 showed no polymorphism.

primer combinations, 71 (E-AGT/ M-AGG), 86 (E-CAC/M-AGC), 95 (E-CGT/M-AGG), and 114 (E-CGT/M-AAC), provided four polymorphic bands corresponding to powdery mildew resistance (Table 6). The approximate sizes of each band were 200bp, 100bp, 150bp, and 180bp, respectively. Three primer combinations, 71, 86, and 95, revealed bands in the resistant parent and resistant bulk but not the susceptible parent and susceptible bulk and one primer combination, 114, showed bands in the susceptible parent and susceptible bulk and not the resistant parent and resistant bulk. The example of AFLP band patterns and co-segregating bands corresponding to disease resistance are shown (Fig. 2.). Prior to cloning, the AFLP analysis of co-segregating bands corresponding to powdery mildew was re-confirmed using four primer combinations to amplify the DNA from P_1, P_2, six individual resistant plants, and six individual susceptible plants. The results of individual plant confirmed the result using bulked DNA of resistant and susceptible plants of co-segregating patterns for the four primer combinations. The three bands in the resistant parent and one in the susceptible parent were cloned and used as probes in RFLP analysis. These probes were named after their primer combinations (Table 2) as Mac71, Mac86, Mac95 and Mac114 (Mac-Mungbean AFLP clone). In the screening of parental polymorphism, Mac95 showed no polymorphism and no band was detected with Mac86. The Mac71 revealed multiple polymorphisms. Single polymorphic band was with Mac114 (Fig.3. and Fig.4.). Four loci of Mac71 were designated as Mac71a, Mac71b, Mac71c, and Mac71d. Two of them, Mac71a and Mac71b, were co-dominant loci and the other two, Mac71c (detected in P_1) and Mac71d (detected in P_2), were dominant loci. There was no association of these loci to marker loci on the mungbean linkage map. However, linkage analysis revealed that these loci were tightly linked to each other. No recombination was found between Mac71a, Mac71d and Mac114 (Figure 5). The best order of the Mac probes was determined as Mac71b, Mac71a, Mac71d, Mac114 and Mac71d respectively by using the compare command of MAPMAKER/QTL. The distance between marker probes was calculated using the Map command.

QTL interval mapping revealed LOD score peaks at this new linkage group. A major resistance QTL was detected at marker Mac71a and Mac114 and had a LOD score of 20.22, additive value (a) = 0.98, dominant value(d) = - 0.50 and dominant to additive ratio = - 0.51. No other QTL was identified from re-scanning by fixing this QTL to the whole linkage map. (The peak of LOD was positioned on Mac71a and Mac114). This QTL accounts for 64.9 % of the

Table 6. AFLP primer combinations used, polymorphism between P_1 and P_2 and co-segregating bands corresponding to disease resistance revealed in this survey of resistance (P_1), susceptible (P_2), pooled DNA of resistance (R) and pooled DNA of susceptible (S).

Primer pair	Number of visible bands				Number of polymorphic bands	Number of co-segregating bands
	P_1	P_2	R	S		
*Eco*RI/*Mse*I						
E-AC/M-AAC (17)	112	107	117	117	6	-
E-AC/M-AAT (18)	87	95	92	92	9	-
E-AC/M-AGA (19)	100	107	106	106	9	-
E-AC/M-AGC (20)	67	63	69	69	15	-
E-AC/M-AGG (21)	96	99	100	100	19	-
E-AC/M-CAA (22)	95	95	95	95	8	-
E-AC/M-CCT (23)	71	75	76	76	11	-
E-AC/M-CGA (24)	68	70	70	70	9	-
E-AC/M-GAC (25)	53	53	58	58	7	-
E-AC/M-GTA (26)	80	79	84	84	14	-
E-AAC/M-AAC (28)	105	105	114	114	10	-
E-AAC/M-AAT (29)	115	120	125	125	13	-
E-AAC/M-AGA (30)	53	59	58	60	5	-
E-AAC/M-AGC (31)	64	64	67	66	12	-
E-AAC/M-AGG (32)	76	86	85	85	9	-
E-AAC/M-CAA (33)	82	79	80	80	6	-
E-AAC/M-CCT (34)	57	62	63	63	13	-
E-AAC/M-CGA (35)	34	31	28	28	4	-

Table 6. (continued).

Primer pair	Number of visible bands				Number of polymorphic bands	Number of co-segregating bands
	P_1	P_2	R	S		
*Eco*RI/*Mse*I						
E-AAC/M-GAC (36)	42	42	43	43	10	-
E-AAC/M-GTA (37)	38	42	43	43	16	-
E-AAG/M-AAC (39)	80	87	90	90	11	-
E-AAG/M-AAT (40)	68	76	76	76	11	-
E-AAG/M-AGA (41)	55	54	58	58	10	-
E-AAG/M-AGC (42)	52	46	52	52	11	-
E-AAG/M-AGG (43)	56	64	60	60	14	-
E-AAG/M-CAA (44)	88	89	89	89	8	-
E-AAG/M-CCT (45)	77	82	87	87	11	-
E-AAG/M-CGA (46)	60	60	66	66	11	-
E-AAG/M-GAC (47)	51	57	58	58	7	-
E-AAG/M-GTA (48)	55	52	56	56	8	-
E-AGA/M-AAC (50)	68	74	77	77	11	-
E-AGA/M-AAT (51)	58	59	66	65	16	-
E-AGA/M-AGA (1)	41	48	48	48	5	-
E-AGA/M-AGC (52)	36	38	37	37	10	-
E-AGA/M-AGG (53)	63	53	61	61	9	-
E-AGA/M-CAA (54)	86	90	97	97	15	-
E-AGA/M-CCT (55)	56	56	64	64	13	-
E-AGA/M-CGA (56)	41	39	43	43	10	-
E-AGA/M-GAC (57)	48	45	48	48	12	-

Table 6. (continued).

Primer pair	Number of visible bands				Number of polymorphic bands	Number of co-segregating bands
	P_1	P_2	R	S		
*Eco*RI/*Mse*I						
E-AGA/M-GTA (58)	37	40	39	39	11	-
E-CAA/M-AAC (110)	92	88	87	87	18	-
E-CAA/M-AAT (59)	47	45	39	39	3	-
E-CAA/M-AGA (60)	57	65	68	68	14	-
E-CAA/M-AGC (61)	48	51	51	51	7	-
E-CAA/M-AGG (62)	80	88	87	87	13	-
E-CAA/M-CAA (63)	75	82	80	80	13	-
E-CAA/M-CCT (64)	58	59	61	61	14	-
E-CAA/M-CGA (65)	19	18	21	21	8	-
E-CAA/M-GAC (66)	51	51	52	52	8	-
E-CAA/M-GTA (67)	54	59	60	60	15	-
E-AGT/M-AAC (111)	74	82	86	86	15	-
E-AGT/M-AAT (68)	76	78	80	80	18	-
E-AGT/M-AGA (69)	50	47	48	48	5	-
E-AGT/M-AGC (70)	47	52	53	53	15	-
E-AGT/M-AGG (71)	48	46	49	48	15	1
E-AGT/M-CAA (72)	58	60	63	63	13	-
E-AGT/M-CCT (73)	33	28	27	27	10	-
E-AGT/M-CGA (74)	12	12	12	12	-	-
E-AGT/M-GAC (75)	17	19	17	17	3	-
E-AGT/M-GTA (76)	41	44	45	45	5	-

Table 6. (continued).

Primer pair	Number of visible bands				Number of polymorphic bands	Number of co-segregating bands
	P_1	P_2	R	S		
*Eco*RI/*Mse*I						
E-ATC/M-AAC (112)	83	84	87	87	13	-
E-ATC/M-AAT (77)	117	113	120	120	11	-
E-ATC/M-AGA (109)	87	92	101	101	17	-
E-ATC/M-AGC (78)	43	43	46	46	7	-
E-ATC/M-AGG (79)	66	78	78	78	12	-
E-ATC/M-CAA (80)	81	84	78	78	10	-
E-ATC/M-CCT (81)	58	64	63	63	6	-
E-ATC/M-CGA (82)	46	46	43	43	5	-
E-ATC/M-GAC (83)	40	43	41	41	3	-
E-ATC/M-GTA (84)	61	68	71	71	18	-
E-CAC/M-AAC (113)	43	53	52	52	10	-
E-CAC/M-AAT (85)	65	69	70	70	15	-
E-CAC/M-AGA (2)	50	50	53	53	3	-
E-CAC/M-AGC (86)	36	30	38	36	13	2
E-CAC/M-AGG (87)	57	66	65	65	12	-
E-CAC/M-CAA (88)	47	51	52	52	12	-
E-CAC/M-CCT (89)	36	47	43	44	10	-
E-CAC/M-CGA (90)	23	23	23	23	2	-
E-CAC/M-GAC (91)	32	36	37	37	12	-
E-CAC/M-GTA (92)	37	36	36	36	6	-
E-CGT/M-AAC (114)	71	68	69	70	6	1

Table 6. (continued).

Primer pair	Number of visible bands				Number of polymorphic bands	Number of co-segregating bands
	P_1	P_2	R	S		
*Eco*RI/*Mse*I						
E-CGT/M-AAT (93)	12	12	12	12	-	-
E-CGT/M-AGA (3)	19	18	24	24	9	-
E-CGT/M-AGC (94)	12	8	8	8	5	-
E-CGT/M-AGG (95)	12	7	10	8	4	2
E-CGT/M-CAA (96)	20	19	22	22	6	-
E-CGT/M-CCT (97)	22	22	16	16	4	-
E-CGT/M-CGA (98)	21	32	35	35	12	-
E-CGT/M-GAC (99)	28	26	29	29	3	-
E-CGT/M-GTA (100)	33	27	36	34	7	-
E-CTG/M-AAC (115)	77	76	81	81	13	-
E-CTG/M-AAT (101)	43	44	47	47	10	-
E-CTG/M-AGA (4)	30	37	36	36	11	-
E-CTG/M-AGC (102)	17	17	16	16	4	-
E-CTG/M-AGG (103)	28	29	32	32	5	-
E-CTG/M-CAA (104)	30	30	35	35	13	-
E-CTG/M-CCT (105)	22	15	15	15	4	-
E-CTG/M-CGA (106)	17	27	26	26	3	-
E-CTG/M-GAC (107)	24	27	26	26	3	-
E-CTG/M-GTA (108)	32	33	31	31	13	-
Total	5386	5566	5734	5729		
Range	12-117	7-120	10-125	8-125		

Numbers in parentheses, ( ), indicated the primer combinations
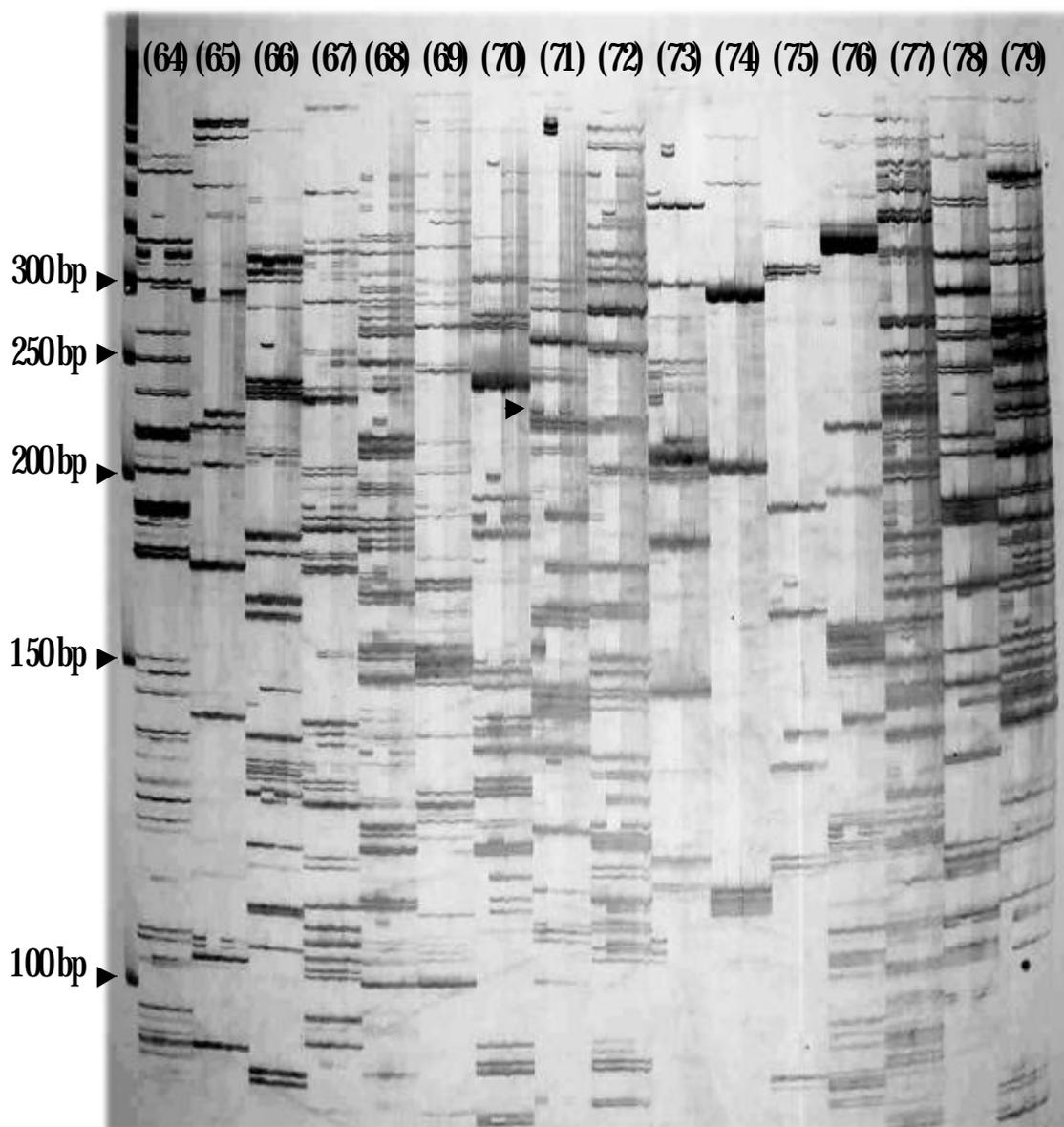
Fig. 2. A photograph of gel of AFLP bands from 15 primer combinations. Numbers in parentheses, ( ), refer to the name primer combinations as shown in table 2. The first lane from the left is 50 bp marker bands. All other lanes represent randomly selected bands for each primer pairs within samples of P1, P2 , R (bulked resistance) and S (bulked susceptible) respectively. Co-segregating bands corresponding to powdery mildew resistance are indicated by arrow.
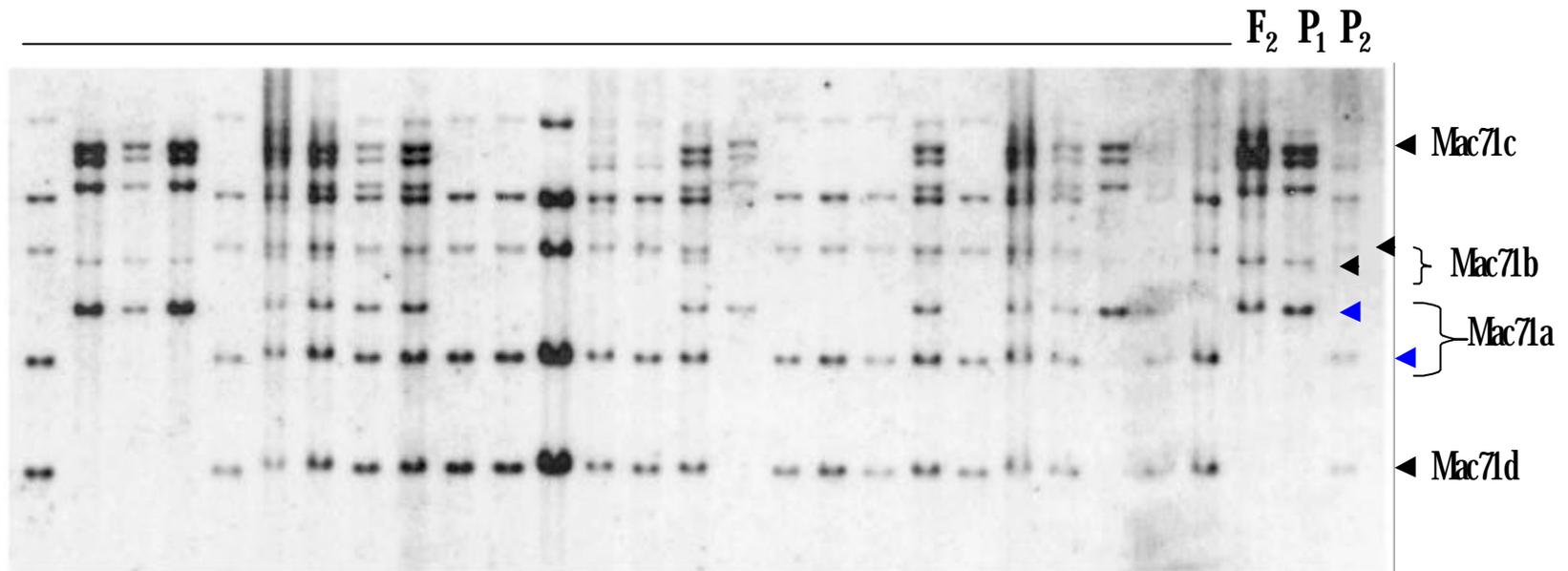
Fig. 3. Genomic DNA polymorphism among patents and F_2 population of VC1210A × TC1966. DNA was digested with *Xba* I restriction endonuclease and hybridized with Mungbean AFLP cloned probe : Mac71. P_1 and P_2 are VC1210A and TC1966. Polymorphic bands indicated by arrows.
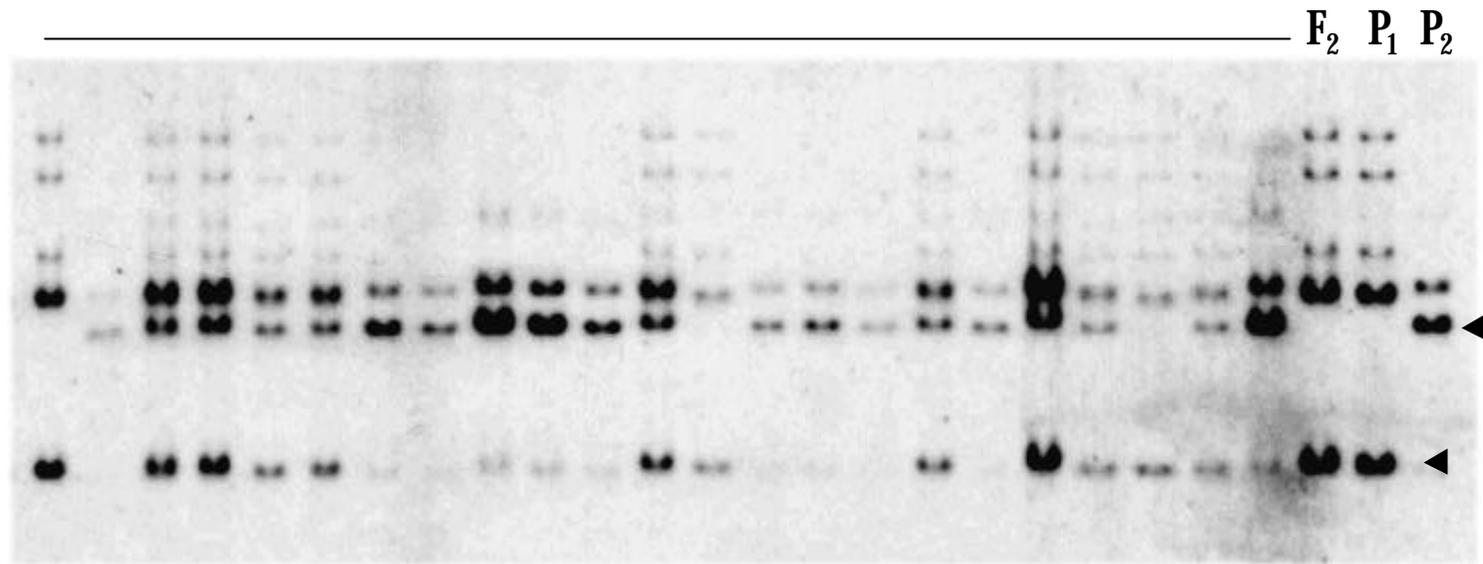
Fig. 4. Genomic DNA polymorphism among patents and F_2 population of VC1210A × TC1966. DNA was digested with BglII restriction endonuclease and hybridized with Mungbean AFLP cloned probe : Mac114. P_1 and P_2 are VC1210A and TC1966. Polymorphic bands indicated by arrows.
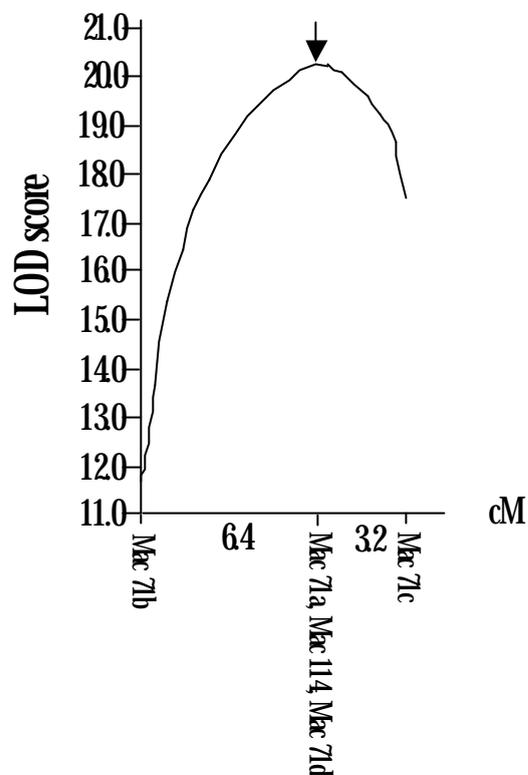
Fig. 5. QTL likelihood plot showing LOD scores for mean score of powdery mildew resistance from F_2 population on clone Mac71a. The most likely position of QTL peak is indicated with arrow. Bar on the top of QTL map corresponds to a 2 LOD support interval from the peak LOD of QTL.

variance for plant reaction to the disease. The main effect of the QTL at Mac71a and Mac114 predicted the mean of the disease score per plant when the marker was homozygous for disease resistance, VC1210A (A/A) = 1.854, heterozygous (A/B) = 2.334, and homozygous for susceptible to disease, TC1966 (B/B), = 3.814. Results from analysis of variance were similar to results found by MAPMAKER-QTL analysis (Table 7).

Table 7. RFLP markers significantly (P ≤ 0.009) associated with powder mildew (*E. polygoni* DC) based on analysis of variance

RFLP Markers	P	R^2	Marker mean		
			A/A[*]	A/B	B/B[**]
			Disease score		
Cloned71a	P = 1.35E-19	63.16	1.842	2.35	3.80
Bng065	P = 0.009	10.00	2.1	2.79	2.88

[*] A = VC1210A

[**] B = TC1966

Discussion

Resistance gene

Although several researchers have confirmed resistance to powdery mildew in mungbean genetic resources in field studies only a few resistance genes have been reported. Two independent dominant genes *Pm-1* and *Pm-2* are well characterized under controlled environmental conditions using field isolates of the pathogen (Reddy *et al.*, 1994). Powdery mildew infection and severity in the field are largely affected by environmental factors. Molecular markers are valuable in genome mapping, QTL mapping and gene tagging (Ma *et al.*, 1994). QTL mapping can identify traits controlled by a small number of major genes that have a quantitative effect and are associated with large environmental variance. QTL mapping can provide a good understanding of inheritance (Dudley, 1993). The disease score of F_2 progenies in the cross between VC1210A and TC1966 showed a continuous distribution (Fig. 1). Thus segregation for field resistance in this cross was treated as a quantitative trait. However, high heritability, bimodal distribution, low disease score of the F_1, suggest that resistance is controlled by major gene(s) in a dominant manner. Previously, three putative field resistance QTL for powdery mildew were identified in the cross between VC3890 and TC1966 by QTL mapping (Young *et al.*, 1993). These three markers, sgK472, mgM208 and mgQ039, are located on linkage group 3, 7 and 8 respectively. These results were done in the USA in which the race of the powdery mildew is considered to be different from the race in Thailand. The studies reported here were conducted in Thailand using native inoculum.

In this study, the markers located across all linkage groups of the current mungbean linkage map were checked to identify resistant QTLs. Although the involvement of a gene with a large effect was anticipated, only one marker, Bng065, located on linkage group 2, showed significant association to powdery mildew resistance by ANOVA, but not MAPMAKER/QTL analysis. The genetic variation explained by the locus linked to Bgn065 was low. No other nearby markers showed association with powdery mildew resistance.

The haploid chromosome number of mungbean is 11, however, the number of linkage groups is currently 13 and many markers are unlinked (Menancio-Hautea *et al.*, 1992). The mungbean linkage map construction is incomplete with many large gaps in the current 13 linkage

groups. Consequently, the major locus associated with powdery mildew resistance in this cross is believed to be in a genomic region not yet covered by the linkage map.

DNA pooling strategies based on phenotypic information can be reliably used to tag occasional QTL of large effect (Wang and Paterson, 1994). By AFLP analysis many bands can be screened. Using a DNA pooling strategy combined with AFLP analysis we attempted to efficiently find markers linked to powdery mildew resistance. Approximately 5700 polymorphic fragments were visualized by AFLP analysis. Among them, four polymorphic fragments were confirmed to co-segregate with powdery mildew resistance among individuals that constituted the pooled DNA. These four polymorphic fragments were cloned to develop probes named Mac71, Mac85, Mac95 and Mac114. Using these probes Mac 71 and Mac114 identified five loci linked to powdery mildew resistance. These five loci were not associated with any linkage group on the mungbean linkage map and constitute a new linkage group. Both MAPMAKER/QTL and ANOVA indicated the small genomic region containing the putative QTL can account for 64.9% of the variance for the plant reaction to powdery mildew. The partial dominant gene effect of the QTL supports the observations of high heritability, bimodal distribution and disease score of the F_1. Since the phenotypic data contains environmental effects, experimental error based on field evaluation of the disease, whether this putative QTL is a single partial dominant gene requires additional study. However, the results of this study strongly suggest that a single major gene controls the resistance to powdery mildew in VC1210A

Pathogen

The relationship between the putative QTL identified here and physiological races of powdery mildew (*Erisiphe polygoni* DC.) will be crucial in determining its value in mungbean improvement. Currently systematic studies of *E. polygoni* in relation to mungbean are lacking. *E. polygoni* can infect not only mungbean but many other crops such as clover (*Trifolium* spp.), pea (*Pisum sativum* L.), and cabbage (*Brassica oleracea* L). Several races of powdery mildew have been reported only in red clover (*Trifolium pretense* L.) (Yarwood, 1936). Research suggests the presence of different physiological races of powdery mildew affects mungbeans in India, Taiwan and the USA. Resistant parent VC1210A used in this study is a useful genetic resource for resistance to both powdery mildew and *Cercospora* leafspot during tests in Taiwan (S. Shanmugasundaram, personal communication). The ancestral line, ML-3, was resistant to

powdery mildew in Taiwan but susceptible in India (Reddy *et al.*, 1987) suggesting that the powdery mildew races in each location are different. In gene for gene interaction, host defense response, the induction of plant defense response is initiated by plant recognition of specific signal molecules produced by a particular pathogen. These signal molecules are encoded directly or indirectly by virulence gene (*Avr*) and *R* genes are thought to encode receptors to recognize these elicitors (Staskawicz *et al.*, 1995). Race specific response to the powdery mildew in *Hordeum* involves the same interaction. Resistance is triggered by R genes (*Mlx*) and is associated with the activation of a hypersensitive response (HR) at sites of attempted fungal ingress (Schulze-Lefert and Vogel, 2000). The VC1210A shows a different response and a high level of resistance to powdery mildew compared to the other powdery mildew resistant lines in northeast Thailand. HR to *E. polygoni* has not been previously reported in mungbean, the resistance response in the resistant parent used here, VC1210A, exhibited a HR with localized cell death in the host plant around the site of infection, to this pathogen. The QTL identified in this study might be a R gene that can activate the HR in response to signal molecules from the pathogen.

Marker-assisted selection

The advantage of tightly linked markers to genes of interest are useful in breeding programs for marker assisted selection and gene cloning called map-based cloning (Christiansen and Giese, 1990; Hartl *et al.*, 1993; Ma *et al.*, 1994; Hu *et al.*, 1997; Tanksley *et al.*, 1989). Selection for powdery mildew resistance in mungbean is difficult because a simple technique for propagating the disease in not available. Field screening is affected by amount and distribution of pathogen inoculum and severity of mungbean infection. In such circumstances marker assisted selection may be useful by shortening the time needed to fix resistance to powdery mildew in segregating populations.

Progress in mapping agriculturally important genes using DNA markers has been made in many crops. Markers from a high density map increases the efficiency and accuracy of selection. The accuracy of markers-assisted selection for bacterial blight in rice was higher than 90% using a single marker tightly linked to the target genes (Sanchez *et al.*, 2000). Young and Tanksley (1989) used 9 RFLP markers linked to *Tm-2*, conferring resistance to tobacco mosaic

virus, in a breeding program. A high density map can be used to estimate the size of introgressed segments rapidly and accurately.

The putative QTL for powdery mildew resistance reported here was positioned in the center of a new small linkage group. No flanking markers to this linkage group are currently available. Two additional fragments from AFLP analysis (86 and 95) that show no polymorphism based on RFLP analysis should be re-analyzed.

It remains to be determined whether the markers linked to the new putative QTL will reveal polymorphism between breeding lines. However, the probe Mac71 has the potential to assist in breeding for powdery mildew resistance in mungbean and to identify more markers because it is possible to detect recombination at three positions surrounding the QTL at the same time.

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Chapter V

General Conclusion

Inheritance of Powdery Mildew Resistance in Mungbean [*Vigna radata* (L.) Wilczek]

Inheritance study of powdery mildew resistance in cultivar SUT4 and line VC210A was found to be controlled by single dominance gene. The generation mean analysis performed on sets of original data showed that the additive – dominance model was adequate to explain the variation occurring in the population. The significant additive and dominance effects were found in all crosses indicating that the variation among generation means for resistance to powdery mildew was sufficiently explained by additive – dominance model. No interaction between effects was observed for this trait in these plant materials.

Broad sense heritability for resistance to powdery mildew varied from 0.71-0.89. The high estimates found in this study showed that the high proportion of variation was under the genetic control.

Breeding for Powdery Mildew Resistance by Using Backcrossing Method

The backcross method used to transfer resistant genes from donor to recurrent parents was effective. Resistant progenies were identified and will be tested further for release to farmer as resistant varieties. However, degree of resemblance between these progenies and the recurrent was varied due to the low number of backcross cycles. These materials may be subjected to further backcrosses or released as it is if accepted by farmers.

Mapping a new source of resistance to powdery mildew in mungbean [*Vigna radata* (L.) Wilczek]

A major QTL was detected at markers Mac71a and Mac114 which had a LOD score of 20.22. These two probes were from cloned probes of AFLP bands associated with resistance phenotype. These two probes contributed to a new linkage group. A major QTL found on a new linkage group accounted for 64.9% of the total variation of the disease reaction. This indicates that it was tightly linked with these markers. The putative QTL for powdery mildew resistance

reported here was positioned in the center of a new small linkage group. Probe Mac71 has the potential to assist in breeding for powdery mildew resistance in mungbean. It can also be used to identify more markers since it is possible to detect recombination at three positions surrounding the QTL (figure 3 in chapter 4). The results of this study strongly suggest that one major QTL control the resistance to powdery mildew in line VC1210A.

In conclusion, we have shown that inheritance of powdery mildew resistance in mungnean variety SUT4 and line VC1210A is controlled by one gene pair with additive and dominance effects. Powdery mildew resistance in mungbean can be bred using the backcross method. And finally genetic marker for assisting breeding for powdery mildew resistance has been identified.

BIBLIOGRAPHY

Miss Bubpa Chaitieng was born on September 22,1963 in Samut Prakam. She received Bachelor degree in B.Sc. (Plant Science) in 1986 from Khon Kaen University and worked as a plant breeder in a private company for 5 years. After that she came back to study and received a Master degree (Horticulture) in 1994 from Khon Kaen University. Her present position is a lecturer at Department of Horticulture Faculty of Agriculture, Ubon Ratchathani University Warinchamrab, Ubon Ratchathani. Her experiences in oversea training:

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