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THESIS

IDENTIFICATION OF SSR MARKERS TAGGED TO YELLOW MOSAIC VIRUS RESISTANCE IN MUNGBEAN (*Vigna radiata* (L.) Wilczek)

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The study was conducted to evaluate mungbean recombinant inbred lines (RILs) against *mungbean yellow mosaic virus* (MYMV). A recombinant inbred line (RIL) mapping population was generated in Thailand from a cross between NM10-12-1 (MYMV resistance) and KPS2 (MYMV susceptible). One hundred and twenty-two mungbean RILs in an F_8 series were evaluated in a field in comparison with resistant parent NM-10-12-1 and susceptible parent KPS2 during summer 2008 under high inoculum pressure. A large number of RILs (60) were found susceptible, 49 were highly susceptible, 10 were tolerant and 3 were highly resistant. Overall screening results showed that three RILs, viz. line no. 30, 100 and 101, were good sources of resistance to MYMV in spite of high disease pressure and can therefore be used directly as varieties to control the MYMV disease in Thailand. This study reported for the first time mapping of quantitative trait locus (QTL) for MYMV resistance in mungbean. A genetic linkage map was developed from the RIL population using simple sequence repeat (SSR) markers. Single marker analysis of QTL identified 9 markers on linkage group (LG) 5 and 10 associated with the MYMV resistance. Composite interval mapping identified a single QTL, *qMYMV*, on LG 5 controlling the resistance. *qMYMV* explained 47.43% of disease variation. Markers DMB-SSR121and MB-SSR248 flanking the *qMYMV* at the 5.2 cM interval are useful for marker-assisted selection of MYMV-resistance cultivars.

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Ratanakorn Kitsanachandee

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Appendix Figure

LIST OF ABBREVIATIONS

AFLP	=	amplified fragment length polymorphisms
BC	=	backcross
bp	=	base pairs
cM	=	centiMorgan
CIM	=	composite interval mapping
CTAB	=	cetyltrimetyl ammonium bromide
DPA	=	days after planting
dNTP	=	deoxynucleotide triphosphate
ISSR	=	inter simple sequence repeats
LG	=	linkage group
MYMV	=	*mungbean yellow mosaic virus*
MYMD	=	mungbean yellow mosaic disease
MYMIV	=	*mungbean yellow mosaic India virus*
PCR	=	polymerase chain reaction
QTL	=	quantitative trait loci
RAPD	=	random amplified polymorphic DNA
RFLP	=	restriction fragment length polymorphisms
RGA	=	resistance gene analog
RIL	=	recombinant inbred line
SCAR	=	sequence characterized amplified region
SIM	=	simple interval mapping
SSR	=	simple sequence repeats
YMD	=	yellow mosaic disease
µl	=	microliter

IDENTIFICATION OF SSR MARKERS TAGGED TO YELLOW MOSAIC VIRUS RESISTANCE IN MUNGBEAN (*Vigna radiata* (L.) Wilczek)

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an important legume crop of South and Southeast Asia. The total planting area of mungbean is about 6 M ha. Mungbean is relatively tolerant to drought and can be harvested within 60-75 days (short duration). So it can be grown as a sole crop or as a component in various cropping systems for its dry seeds which are rich in proteins, amino acids and minerals. Several kinds of food are made from mungbean seeds. Apart from the seeds, mungbean sprout is very popular in Asian cuisine. However, economic yield of mungbean is low due to various biotic and abiotic constraints and diseases are the major impediments to production (Malik and Bashir, 1992). When grown in the field, mungbean is exceedingly prone to various viral diseases, which are caused by *Mungbean yellow mosaic virus* (MYMV), *Urdbean leaf crinkle virus* (ULCV), *Cucumber mosaic virus* (CMV), *Bean yellow mosaic virus* (BYMV) and *Alfalfa mosaic virus* (AMV), some of which can cause significant economic losses (Aftab *et al*., 1993; Bashir *et al*., 1991; Bashir *et al*., 2006; Malik, 1991). One of the most destructive and important disease of mungbean is mungbean yellow mosaic disease (MYMD). The disease can reduce up to 100% of mungbean seed yield or even kill the plant infected at early vegetative stage (Verma and Singh, 1986). MYMD is the major threat to mungbean production in India, Sri Lanka, Pakistan, Bangladesh, Papu New Guinea, Philippines and Thailand (Honda *et al*., 1983; Jones, 2003) and inflicts on heavy yields losses annually.

Mungbean yellow mosaic disease (MYMD) is caused by *Mungbean yellow mosaic virus* belonging to genus *Begomovirus*, family Geminiviridae. Its viral particles are isometric and geminate having 18 to 30 nm in size with two single stranded DNA molecules (DNA A and DNA B) of 2726 and 2775 nucleotides,

respectively (Bos, 1999; Hull, 2004; Morinaga *et al*., 1990, 1993). This virus is transmitted by whitefly (*Bemisia tabaci*) and through grafting but not through seed, sap and soil (Bashir, 2003). The MYMV also infects blackgram (Mandal *et al*., 1997), cowpea (Rouhibakhsh and Malathi, 2005), and soybean (Girish and Usha, 2005). Disease reactions after MYMV infection depend on host species and susceptibility of each plant. Initially the disease appears as small yellow spots along the veins on young leaves and then spread over the leaves. Under severe infection, the entire leaf can show yellowing or chlorosis on the whole plant followed by necrosis, shortening of internode, severe stunting of plants with no yield or few flowers and deformed pods producing small, immature and shriveled seeds (Akhtar and Haq, 2003; Bashir *et al*., 1991; Bashir *et al*., 2006; Malik, 1991).

Chemical control of whitefly vector, is ineffective and is negative to environments. Using resistant cultivar(s) is the best option to manage MYMV. Improvement of resistance to MYMV is now the major goal in mungbean breeding programs in several mungbean production countries. Resistance to MYMV in mungbean was reported to be controlled by single major recessive gene with modifiers (Thakur *et al*., 1997, Khattak *et al*., 2000), or two recessive genes (Singh, 1981) or complementary recessive genes (Shukla and Pandya, 1985). However, a major difficulty for breeding MYMV resistance mungbean is field evaluation of the disease which is hampered by non uniform outbreak of whitefly population that is only prevailing in summer season.

Marker-assisted selection (MAS) is a useful tool for genetic improvement of crops. Selection based on molecular marker(s) associated with the target trait helps decreasing number of phenotypic evaluations, and thus reducing time and cost, and increasing gain from selection. Up until now there are only a few reports of DNA markers for MYMV resistance in mungbean. Lambrides et al. (2000) reported that RAPD marker $OPAJ_{20}$ distantly linked with the resistance gene. Selvi et al. (2006) identified RAPD marker $OPS\ 7_{900}$ associated with YMV. Maiti et al. (2011) reported that resistance gene analog (RGA) markers, YR4 and CYR1, associated with resistance to MYMIV in blackgram (*Vigna mungo* (L.) Hepper). CYR1 cosegregated

with the resistance in F_2 and F_3 populations from the cross between resistant and susceptible blackgram, and completely linked with MYMIV-resistant blackgram germplasm. They further demonstrated that marker CYR1 was also associated with the resistance in mungbean germplasm.

OBJECTIVES

1. To evaluate the reaction to MYMV in a recombinant inbred line population derived from the cross between the resistant cultivar NM10-12-1 and the susceptible cultivar Kamphaeng Saen 2

2. To locate QTL for MYMV resistance

LITERATURE REVIEW

Mungbean yellow mosaic virus (MYMV)

Mungbean yellow mosaic virus (MYMV) was first reported by Nariani (1960) in mungbean (*Vigna radiata* (L.) Wilczek) from Delhi, India. Later, MYMV was found in Bangladesh, Pakistan, Sri Lanka and Thailand. In Thailand, MYMV was first reported in 1977 (Goodman *et al*., 1977; Thongmeearkom *et al*., 1981). This virus severely outbroke in mungbean grown in lower northern Thailand and caused total yield losses. The first complete DNA sequence of MYMV was isolated from mungbean originating from Thailand (Morinaga *et al*., 1990, 1993).

MYMV is one of the plant viruses in the family Geminiviridae, genus *Begomovirus*. The family Geminiviridae was characterized by its twinned (germinate) icosahedral particles and circular, single stranded DNA genome in one or two components (monopartite or bipartite). This family includes the following genera 1) *Begomovirus* (formerly subgroup III; type species: *Bean golden mosaic virus*), 2) *Curtovirus* (formerly subgroup II; type species: *Beet curly top virus*), 3) *Mastrevirus* (formerly subgroup I; type species: *Maize streak virus*, and 4) *Topocuvirus* (type species: *Tomato pseudo-curly top virus*) (Gray and Banerjee, 1999; Claude and John, 2003).

Genus *Begomovirus* contains more than 100 species, differing from the other geminivirus genera in having a bipartite genome, DNA A and DNA B, which encode 6 and 2 proteins, respectively (Shivaprasad *et al*., 2005). The particles of these viruses are enveloped by nucleocaspid of 38 nm long and 15-22 nm in diameter. The particles had 2 basics but incomplete isocaherdal symmetry. There are 22 capsomeres per nucleocapsid (Mansoor *et al*., 2003; Briddon and Stanley, 2006). Both genomes are usually required for successful symptomatic infection in a host cell. DNA A has many genes that encode all factors for viral DNA replication, regulation of gene expression and encapsidation/insect transmission; the replication associated protein (Rep), DNA

helicase (Choudhury *et al*., 2006), the replication enhancer protein (REn), the transcriptional activator protein (TrAP) and the coat protein (CP). DNA B genome encodes two genes, the nuclear shuttle protein (NSP) and the movement protein (MP), which act cooperatively to move the virus cell-to-cell within plants (Qazi *et al*., 2007).

Mungbean yellow mosaic virus infects only legume crops, including mungbean, blackgram, kidney bean, azuki bean, common bean, moth bean and soybean. This virus requires the whitefly insect vector (*Bemisia tabaci*) to transmit into plants in a persistent manner (Hunter *et al*., 1998; Sinisterra *et al*., 2005). The vector is an indiscriminate feeder, encouraging rapid and efficient spread of virus from native plant species to neighboring crops. This virus could not be transmitted by mechanical inoculation; contrast, MYMV-TH (Honda *et al*., 1983). After MYMV infected plants, the disease symptoms vary depending on host species and susceptibility. Generally symptoms appeared as small yellow specks along the vein and then spread over the leaf. In case of severe infections, the entire leaf may become chlorotic, causing reduction in yield and yield components. Singh *et al.* (1978) found that yield and yield components were negatively correlated with degree of MYMV infection in mungbean. Khattak *et al.* (2000) investigated the effect of MYMV in fourteen MYMV susceptible F_3 progenies that obtained from a cross NM92×VC 1560D. They found that the decrease in yield and yield components showed no correlation with MYMV infection.

Controls of yellow mosaic disease are based mainly on preventing the establishment of whitefly vector (*Bemisia tabaci*) in the crop by application of insecticides. Other recommendations are to change in agricultural practices, such as postponing the cropping period beyond the periods of high vector incidence, especially the wet period in late summer to the times of low vector incidence in the dry season of early summer. The use of natural, host plant resistance is also effective.

Mungbean (*Vigna radiata* (L.) Wilczek)

Mungbean (*Vigna radiata* (L.) Wilczek) is the most widely cultivated *Vigna* species. It arose from the wild variety *sublobata* (Roxb.) Verdc., which is first domesticated in India. India was believed to be the center of origin of mungbean where morphological diversity, existence of wild and weedy types and archaeological remains of mungbean were found (Arora, 1985). A large number of var. *sublobata* were found in India, together with another wild type, *Vigna radiata* var. *setulos*. The wild *sublobata* showed a wide area of distribution, covering Central and East Africa, Madagascar, Southeast Asia, Northern-South America, New Guinea, and North and East Australia (IBPGR, 1985).

Mungbean is classified into the family Fabaceae, sub-family Papilionoideae, tribe Phaseoleae, sub-tribe Phaseolinae. The genus *Vigna* is divided into seven subgenera: *Vigna*, *Plectotropis*, *Ceratotropis*, *Lasiospron*, *Sigmoidotropis*, *Haydonia* and *Macrorhynchus* which each subgenus comes from different origin. The subgenus *Ceratotropis* is related to mungbean and originated in the Asiatic. It consists of mungbean/green gram (*Vigna radiata* (L.) Wilczek), black gram/urd bean (*Vigna mungo* (L.) Hepper), moth bean (*Vigna aconitifolia* (Jacq.) Maréchal), adzuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi) and rice bean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi). This group is homogeneous with highly specialized floral structures and treated as the subgenus *Ceratotropis* of the genus *Vigna* (Verdcourt, 1970; Maréchal *et al*., 1981; Ohashi, 1980; Tateishi and Ohashi, 1990). The subgenus is characterized as having peltate stipule, standard petal with a protuberance, incurved keel-petals with pocket and style-beak. From these features the subgenus is considered to be monophyletic (Maréchal *et al*., 1981; Tateishi and Ohashi, 1992). Chromosome number of *Ceratotropis* is 2n=22, 2x (Morton *et al*., 1982; Sarbhoy, 1977, 1978), except for *V. reflexo-pilosa* and *V. glabrescens* which were tetraploid (2n=44, 4x). Moreover, mungbean had a small genome ($5x10^8$ bp) with 40% repetitive DNA. Poehlman (1982) classified *V. radiata* into 3 subspecies, viz. *radiata*, *sublobata* and *glaba*. Subspecies *sublobata* is the wild ancestral form of cultivated sub species *radiata*.

Mungbean is an erect or sub-erect herb, being about 60 to 75 cm tall, having trifoliate leaves and highly branching. The pale yellow flowers are borne in clusters of 12 to 15 flowers near the top of the plant. Pods are 3 to 4 inches long, each having 10 to 15 seeds. There are several pods clustering at a leaf axil, with typically totaling 30 to 40 pods per plant. Mature pods are variable in color, from yellowish-brown, pale gray, brown, or black. Mature seed color exhibits a wide range of variation from yellow, greenish yellow, light green, shiny green, dark green, dull green, black, brown, and green with black mottle. Mungbean is a self-pollinated crop. Germination is of epigeal type with the cotyledons and stem emerging from the seedbed.

Mature mungbean seeds contain 20-25% protein, 1.0-1.2% fat, vitamins and minerals that are necessary for human body. The seeds are rich in vitamins (A, B_1, B_2, C and niacin), and minerals (K, P and Ca). Mungbean is raw materials of several food products, such as mungbean noodle, starch, low-cost high-protein foods, protein supplemented foods, bean sprouts, and several kinds of desserts and main dishes. Furthermore, protein isolated from the left-over of mungbean noodle production could be used to make textured protein for human consumption at low cost.

Sources of mungbean yellow mosaic virus resistance in mungbean

Mungbean yellow mosaic virus is an important virus causing damages to mungbean yield in South Asia. Attempts have been made to screen and breed the mungbean lines for resistant/tolerant to MYMV. MYMV-resistant/tolerant mungbean germplasm was obtained from screening of mungbean accessions, interspecific crossed with MYMV-resistant blackgram, and induce mutation by gamma ray.

A series of reports on mungbean resistant/tolerant to MYMV were given by Chhabra *et al*. (1988); Chhabra and Kooner (1980); Singh (1988); Bashir *et al*. (1988); Haq (1980); Malik (1991, 1992); Malik *et al*. (1986). The accessions included G65, LM47, LM141, LM170, LM364, M170, ML1, ML3, ML5, ML6, ML7, ML15, ML24, ML186, ML192, ML194, ML195, ML197, ML235, ML337, ML423, ML428, ML711, P131, P242, P290, P292, P293, P325, P364, T44, 191-1, 10866, 11148 and

15127. In Green *et al*. (1996) grouped MYMV resistant/tolerant mungbean in 4 South Asian countries, viz. Bangladesh, India, Pakistan and Sri Lanka with at least 498 resistance lines.

Genetics and breeding for resistance to mungbean yellow mosaic virus in mungbean

Thakur *et al*. (1997) reported that genetic of the resistance to mungbean yellow mosaic virus (MYMV) in mungbean was controlled by a recessive gene. Singh and Patel (1977) found that the resistance was independent from seed color and maturity. On the other hand, susceptibility to MYMV is reportedly by controlled by a dominant gene in mungbean (Shukla, 1977; Shukla *et al*., 1978), blackgram (Singh, 1980) and soybean (Malick, 1976; Singh and Malick, 1978). Moreover, Singh (1981); Garcia and Mcdonald (2003); Qazi *et al*. (2007) found two recessive genes responsible for resistance in mungbean. In contrast, Khattak *et al*. (2000) reported that MYMV resistance is controlled by a single major recessive gene with modifying genes causing variation in the degree of MYMV resistance/susceptibility in the progenies. In blackgram, Singh (1980); Verma and Singh (1986) reported that the genes conferring resistance to mungbean yellow mosaic virus are two recessive genes. Later Gupta *et al*. (2005) found a single dominant gene controlling resistance to MYMV.

DNA marker techniques

DNA markers are the most widely used type of marker because they have several techniques and accurate detection. DNA markers develop from the different features of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). These mutations located in non-coding region; thus, they are suitable as selective neutral markers. Furthermore, the unique characters of DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Winter and Kahl, 1995). In plant

breeding, DNA markers assess the level of genetic diversity within germplasm and cultivar identity (Baird *et al*., 1997; Henry, 1997; Jahufer *et al*., 2003; Weising *et al*., 1995). DNA markers divided into two groups based on the method of their detection; hybridization-based and polymerase chain reaction (PCR)-based (Gupta *et al*., 1999; Jones *et al*., 1997; Joshi *et al*., 1999). DNA polymorphisms are detected on agarose gel or polyacrylamide gel, depending on types of DNA markers. The most popular DNA markers are as followed.

Restriction fragment length polymorphism (RFLP): RFLP markers were first reported in the genome revolution (Dodgson *et al*., 1997). The principle of RFLP markers is based on probes binding with specific nucleotide sequences target. DNA samples were cut by restriction endonuclease, which recognize specific 4, 5, 6, or 8 base pairs (bp) nucleotide sequences. A restriction site in DNA sequence differently indicated to occur from base substitutions, insertion, deletion or rearrangements. DNA fragments were separated using southern blot analysis (Southern, 1976), which fragment sizes can vary among individuals, population and species. These fragments were loaded on agarose gel and transferred to a membrane. DNA polymorphisms were detected by hybridization to specific probes. They showed as co-dominant markers. The advantages of RFLP marker are accuracy, repetition and transferable across population. The disadvantages of this marker are expensive to develop specific probes, take a long time to detection, large amounts of DNA required and limited polymorphism (especially in related lines) (Kochert, 1994)

Randomly amplified polymorphic DNA (RAPD) marker: RAPD markers were first developed by Welsh and McClelland (1990) and Williams *et al*., (1990). The method of RAPD markers detects the differential DNA samples by using short oligonucleotide sequences or called primers. The length of each primer is 8 to 10 bp that is not specific with the position of DNA target. RAPD markers used PCR technique for amplify the DNA target. RAPD polymorphism can occur from base substitutions at the primer binding site or insertion/deletion in the regions between the sites. The polymorphic bands were scored as presence or absence because this marker is a dominant marker. The potential advantages of RAPD markers are quick and

simple to use, inexpensive, applicable with unknown samples, and showed multiple loci from a single primer and small amounts of DNA required (Penner, 1996). The major disadvantage of this marker is reproducibility.

Amplified fragment length polymorphism (AFLP) marker: AFLP marker combines the strength of the RFLP and RAPD methods. Like RFLP marker, AFLP polymorphism comprises insertion/deletion between restriction sites and base substitutions at restriction sites. As same RAPD marker, it includes base substitution at PCR primer binding sites. The unique character of this technique is the addition of known adaptors sequence to DNA fragments generated by digestion of whole genomic DNA. The adaptors are used as primer sites for PCR amplification. The number of bases (A, T, C or G) is added to the end of primers that reduces the number of amplified fragments. One base and three beases are added to give the primers into preselective PCR amplification step and selective PCR amplification step, respectively. AFLP marker was developed by Vos *et al*., (1995). The method for analyzing AFLP marker is divided as 4 steps; (1) digestion of genomic DNA by restriction enzyme into fragments, (2) combining adapters at the end of the DNA fragments, (3) increasing DNA target by specific primers, and (4) polymorphisms of DNA detection in acrylamide gel. The inheritable of AFLP marker is dominant marker; likewise, RAPD marker (Vrieling *et al*., 1997). The major advantages of AFLP markers revealed the high levels of the polymorphism generated, multiple loci and high reproducibility; however, this marker was limited by large amounts of DNA required, complicated methodology, time-consuming and expensive cost.

Simple sequence repeat (SSR) or microsatellite marker: Microsatellite marker consists of short repeating units that range in size from 1 to 6 bp (Tautz, 1989; Litt and Luty, 1989). Microsatellite distributed in the genome on whole chromosomes and all region of the chromosome. It found inside gene coding regions and introns. The variable numbers of repeat units are based on size differences, which showed as polymorphic bands. Consequently, microsatellite is the most popular markers used to study DNA polymorphism, which occur from duplication or deletion of nucleotide sequences. The type of polymorphism is co-dominance. DNA polymorphism can be

detect on both agarose and acrylamide gel (Dakin and Avise, 2004). The advantages of SSR marker are easy technique, repetition and transferable between populations; contrast, large amounts of time and expensive cost to development primers (McCouch *et al*., 1997; Powell *et al*., 1996)

So, there have been only a few reports on molecular work on mungbean yellow mosaic virus. Most researchers used visual scores to rate different disease symptoms. Visual scores require experience of plant pathologist and MYMV epidemic duration, so molecular marker techniques can be used to help identifying the resistant plants even with no disease outbreak. Green *et al*., (1996) investigated MYMV resistant genes in mungbean germplasm for developing RFLP markers. AVRDC (2005) reported 3 AFLP markers associated with MYMV resistance genes in 84 recombinant inbred lines. Thus, there has been no report on SSR markers linking to MYMV resistant/tolerant in mungbean.

Mapping populations

The mapping population develops from two genetically divergent parents, which show clear genetic differences for one or more traits of interest. The difference between parents segregate into hybrid population. It is necessary to map or tag the interesting traits by DNA markers. The types of mapping population used in linkage mapping, including F_2, backcross (BC), double haploid (DH) and recombinant inbred line (RIL) populations. The segregation ratio of dominant and co-dominant markers is 1:1 in BC, RIL and DH. Co-dominant markers segregate 1:2:1 in F_2 populations; in contrast with dominant markers segregating at 3:1.

F_2 populations are developed by selfing (or intermating in cross-pollinated species) among F_1 hybrid derived by crossing the two parents. F_2 populations are most efficient in resolving the gene order along chromosomes because F_2 individuals are products of single meiotic cycle. The advantages of F_2 population are 1) the simplest type of mapping populations because it is easy to construct, 2) requires less time for development and 3) it can be developed with minimum efforts, when compared to

other populations. However, F_2 populations are limited to use for fine mapping because linkage construction is based on one cycle of meiosis. Quantitative traits can not be evaluated in replicated trials over locations and years.

Backcross (BC) populations are generated by crossing the F_1 with either of the parents. In genetic analysis, the recessive parent (testcross) is used to backcross because the population showed the segregation as 1:1 ratio on both dominant and co-dominant markers. In contrast, the backcross with dominant parent segregated as 1: 0 and 1:1 for dominant and co-dominant markers, respectively. As the same of F_2 population, the backcross populations require less time to be developed. However, the specific advantage of backcross populations is useful in QTL analysis, especially to estimated QTL effects (Tanksley and Nelson, 1996)

Double Haploids (DH) populations are produced by chromosome doubling of anther culture derived haploid plants from F_1 hybrid. So, every locus will be homozygous and fixed so that a DH population can be self-fertilized to generate infinite number of identical plant genotypes. The advantages of DH population are permanent mapping population which can be replicated and evaluated over locations and years. Furthermore, DH population is used for mapping both qualitative and quantitative characters and instant production of homozygous lines. However, the disadvantages of this type of population are recombination from the male side alone is accounted and the suitable culturing methods/haploid production methods are not available for number of crops and different crops differ significantly for their tissue culture response.

Recombinant inbred lines (RILs) populations are produced by continuous selfing or sib mating the progeny of individual member of an F_2 population until complete homozygous is achieved. Theoretically, 99% of the loci of plants in the F_8 generation are homozygous. Single seed descent method (SSD) is best suited for developing RILs. The benefits of RILs are propagated indefinitely without segregation and replicated over locations and years. They provide tightly linked genes but may be broken down when passing through several rounds of meiosis. Therefore more

accurate map distances can be estimated with RI populations (Burr and Burr, 1991). However, they require many seasons and generations to develop.

Construction of linkage maps

Linkage maps indicate the position and relative genetic distance (genes and QTLs associated with traits of interest) between markers along chromosome, which derives from two different parents (Paterson, 1996). Linkage maps are constructed from the segregating marker analysis. The segregating makers depend on the types of DNA marker and mapping population. The data of both types are used to analyze and determine linkages between markers. Linkage between markers is calculated using odds ratios. This ratio is expressed as the logarithm of the ratio, which called a logarithm of odds (LOD) value or the LOD score (Risch, 1992; Stam, 1993a). A LOD value of 3 as the minimum threshold value indicates that linkage is 1000 times more likely than no linkage (Stam, 1993a). Linked markers are grouped together into linkage groups, which represent chromosomal segments or entire chromosomes. Linkage groups are established by estimating the recombination frequencies. The frequency of recombination determined the genetic distance between markers. The minimum and maximum of the recombination value are 0 and 0.5, which 0 = complete linkage and 0.5 = free recombination (Paterson, 1996). Furthermore, the genetic distance is directly related to the number of individuals sample in the mapping population. Young (1994) suggested that the mapping populations had to consist of minimum of 50 individuals for constructing linkage maps.

Mapping functions are applied to convert recombination fractions into map unit or called centiMorgans (cM) because recombination frequency and the frequency of crossing-over are not linearly related (Hartl and Jones, 2001; Kearsey and Pooni, 1996). The popular mapping functions are Haldane (Haldane, 1931) and Kosambi (Kosambi, 1944). Haldane mapping function assumes the absent interference between crossovers in meiosis, whereas Kosambi mapping function assumes the present interference. Interference is the effect of a crossover in a certain region which reduces the probability of a crossover in the adjacent region. The statistical programs have

been developed for generating a multi-locus model such as Maximum likelihood and regression/weighted least square method and are implemented in computer packages for use in linkage map construction. Least square method attempts to minimize deviations from regression models, while the maximum likelihood method involves comparison among two or more plausible hypotheses (Staub and Felix, 1996). Several software packages are available on the internet for the construction of genetic maps such as MapMaker/Exp (Lander *et al*., 1987; Lincoln *et al*., 1993a,b), Map Manager QTX and JoinMap (Stam, 1993b).

Quantitative trait loci (QTL) analysis

Quantitative trait loci (QTL) are the location of a gene that affects a trait and are identified via statistical procedures that integrate genotypic and phenotypic data. QTL are assigned to chromosome locations based on the positions of markers on a linkage map. The principle of mapping QTL bases on the segregation of DNA markers can be used to direct and estimate the effects of linked QTL. Several methods and estimating their effects for mapping QTL have been suggested and investigated (Edwards *et al*., 1987; Haley and Knott, 1992; Jiang and Zeng, 1995; Lander and Botstein, 1989; Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). These methods involve searching for associations between the segregating DNA markers and the traits of interesting in a segregating population that identify the linkage of the marker to the QTL. The QTL method is important strategy to detect agronomic traits in plants. Furthermore, QTL information can also be used as a basis for germplasm characterization and conservation. Four methods for detecting QTLs are single-marker analysis, Simple interval mapping (SIM), Composite interval mapping (CIM) and Muliple interval mapping (MIM) (Liu, 1998; Tanksley, 1993).

Single marker analysis or point analysis: Single marker analysis is the method to detect a QTL in the proximity of a marker by studying a single genetic marker at a time. It involves comparison of the phenotypic means for each of the genotypic classes at each marker by means of a t-test or F-test, ANOVA, likelihood ratio test or simple regression. A significant value indicates a QTL which is located in the

proximity of the marker. This approach can be achieved without a complete molecular linkage map. The main disadvantage of single marker analysis is the low statistical power and confounding estimates of QTL effect and locations.

Simple interval mapping (SIM) or Interval mapping: Because of some limitations in the basic QTL analysis approach, a new method called simple interval mapping using a different approaches such as maximum likelihood and regression has been proposed (Kearsey and Hyne, 1994). Maximum likelihood searches the best approximation for quantitative trait distributions which are observed for each marker class. The map position of a QTL is determined as the maximum likelihood from the distribution of likelihood values (LOD scores). Regression method explains the association between phenotypes and marker genotypes. It estimates the probabilities from the nearest flanking markers. So, the advantage of this approach over single marker analysis is the QTL location and its effect can be more precisely determined.

Composite interval mapping (CIM): Composite interval mapping was developed for solving the two problems of the simple interval mapping which provides biased estimation of the location and effect of the QTL when other QTLs exist on the same chromosome. CIM combines together interval mapping and multiple regression analysis by including other markers in the statistical model as cofactors to control interference from both linked QTL and residual variance (Zeng, 1993). The major advantage of CIM over SIM is that the QTL interval under study is independent of external QTL effect.

Muliple interval mapping (MIM): MIM uses multiple marker intervals simultaneously to construct multiple putative QTLs in the model for QTL mapping which tends to be more powerful and precise in detecting QTL than SIM and CIM. MIM can readily search for and analyze epistatic QTL and estimate the individual genotypic value and the heritabilities of quantitative traits. On the basis of the MIM result, genetic variance components contributed by individual QTL were also estimated, and marker-assisted selection can be performed.

Consequently, these software programs can detect and determine the location and relationship between traits of interesting and QTLs. SAS program is a statistical analysis by using single marker analysis method such as ANOVA, t-test, GLM or REG. Interval mapping or simple interval mapping is analyzed by Mapmarker/QTL program. In addition to Qgene program conducts QTL mapping from single marker regression or interval regression. Both interval mapping and composite interval mapping can calculate from MQTL, PLABQTL, QTL Cartographer and MapQTL programs. MQTL program specially analyzes the composite interval mapping in multiple environments.

MATERIALS AND METHODS

Mapping population

A population of 122 recombinant inbred lines (RILs) was developed from a cross between Kamphaeng Saen2 (KPS2) (female parent) and NM10-12-1 (male parent). KPS2 is a popular high-yielding mungbean variety grown in Thailand but susceptible to MYMV, while NM10-12-1 is a breeding line from Pakistan resistant to MYMV. The RILs were developed at Kasetsart University, Kamphaeng Saen, Thailand by single seed descent method from individual F_2 plants until F_8 generation.

Evaluation *of mungbean yellow mosaic virus*

Since Thailand currently has no outbreak of MYMV, the parents and the RIL population were evaluated for disease reaction at Nuclear Institute for Agricultural Biology (NIAB), Faisalabad, Pakistan in July 2008. The field conditions during evaluation had a large population of whiteflies with natural outbreak of MYMV. Individual F_8 lines were arranged in a randomized complete block design (RCBD) with three replications. F_8 RILs were grown together with the resistant parent (NM 10-12-1) and susceptible parent (KPS2). Each replication had 24 to 28 plants in a row of 4 m in length with 40 cm row to row distance. The parents were planted as checks after every two tested entries. Conventional agronomic practices (fertigation, irrigation, weeding, hoeing etc.) were followed to keep the crop in good condition. However, no plant protection measures were applied against the whitefly to ensured high inoculum pressure throughout the experiment. Infection and disease severity were visually scored for MYMD reactions at 24 DAP (days after planting). The plants were scored on a row basis using scales of 0 (immune) to 5 (highly susceptible) following Akhtar *et al*. (2009) as summarized in Table 1. The 1st MYMD symptom started to appear on the susceptible RILs after about 15 days after seedlings emergence. Disease symptoms were started as scattered few small yellow specks on few young leaves. After 4-5 days, most of the specks were coalesced, alternating

between yellow and green patches with irregular margins developed in the first fully formed trifoliate leaf next to the apex. Complete yellowing or chlorosis was observed within 8-10 days followed by necrosis.

Confirmation of the reaction to MYMV in Thailand

In Thailand, mungbean yellow mosaic virus has recently been found in 2008 in the northern part. MYMV was maintained and inoculated into KPS1 and KPS2 (highly susceptible cultivars) via whiteflies at Plant Virology Section, Plant Pathology Research Group, Plant Protection Research and Development Office, Department of Agriculture, Ministry of Agriculture and Cooperatives. From the F_8 population, 4 lines were chosen based on disease reaction in the field experiment in Pakistan. Highly resistant lines were no. 30, no. 100 and no. 101 while no. 114 showed highly susceptible. These RILs were evaluated for MYMV-TH symptom in a glasshouse at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom. KPS2 and NM10-12-1 were used as control checks. Each line was planted in pots, 2 seeds per pot.

Adult whiteflies were reared on tomato (*Solanum lycopersicum*) plants prior to being moved into a net box and fed with MYMV infected branches at a acquisition access period of 24 h. The viruliferous whiteflies were then fed on healthy mungbean seedling lines at 5 days after planting using aspirator and allowed to feed at the inoculation access period of 48 h. After inoculation, these lines were sprayed with 0.1% dimethoate to kill all the whiteflies. The inoculated plants were kept in a glasshouse for symptom development. The glasshouse was controlled at a constant temperature of 25°C. First disease symptom appeared as scattered yellow specks on few young leaves at 15 days after infection (DAI). Disease index was scored in the same manner as field evaluation.

Table 1 Disease scale for rating of mungbean yellow mosaic virus (MYMV) on mungbean plants*

Symptom	Score	Disease reaction
Complete absence of symptoms	0	Immune
Few small yellow specks or spots on few leaves seen after careful observations	1	Highly resistant
Bright yellow specks or spots on leaves, easily observed with some coalesced spots	2	Resistant
Mostly coalesced bright yellow specks or spots on leaves, with no or minor stunting	3	Tolerant
Plants showing coalesced bright yellow specks or spots on all leaves, with no or minor stunting and set fewer normal pods	4	susceptible
Yellowing or chlorosis of all leaves on whole plant followed by necrosis, shortening of internodes, severe stunting of plants with no yield, or few flowers and deformed produced with small, immature and shriveled seeds	5	Highly Susceptible

*Modified from Akhtar *et al*. (2009)

DNA extraction

Total genomic DNA was isolated from young leaves of the parents (NM10-12-1 and KPS2) and the RILs using CTAB method described by Lodhi *et al*. (1994) with a slight modification. The concentration of DNA was determined by comparing against a lamda DNA and diluted to 1 ng/µL for marker analysis.

DNA marker analysis

One thousand and thirty-four simple sequence repeat (SSR) primer pairs from several legumes were used to detect polymorphism between KPS2 and NM10-12-1. Among them, 547 were from mungbean (Gwag *et al*., 2006; Somta *et al*., 2008, 2009; Seehalak *et al*., 2009; Tangphatsornruang *et al*., 2009), 145 from azuki bean (*V. angularis* (Ohwi) Ohwi & Ohashi) (Wang *et al*., 2004), 226 from cowpea (*V. unguiculata* (L.) Walp.) (Li *et al*., 2001; Xu *et al*., 2011; Kongjaimun *et al*., 2012), and 116 from common bean (*Phaseolus vulgaris* L.) (Yu *et al*., 2000; Gaitán-Solís *et al*., 2002; Blair *et al*., 2003; Guerra-Sanz, 2004; Buso *et al*., 2006). Polymorphic markers were used for genotyping the RILs. PCR amplification of the markers was carried out as per Somta *et al*. (2008). In brief, PCR was performed using a total volume of 10 µl containing 1 ng of genomic DNA, 1x Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* DNA polymerase (Fermentas) and 5 pmol each of forward and reverse primers. DNA was amplified in a GeneAmp PCR System 9700 (Applied Biosystems) under the following conditions; 94º C for 2 min followed by 35 cycles of 94º C for 30 s, 50-65º C (depending on primer) for 30 s, 72º C for 1 min, with a final extension step of 72º C for 10 min. The PCR products were separated on 5% denaturing polyacrylamide gel and visualized by silver staining.

Confirmation of resistant gene analogs (RGAs) in RIL population

To confirm an association between the markers CYR1 and YR4 and MYMV resistance in mungbean, the markers were screened for polymorphism between the parents (Maiti *et al*., 2011). Analysis of the markers CYR1 and YR4 followed the same procedure mentioned above. Markers showing polymorphism between the parents were used to analyze the RIL population.

Linkage map construction

Linkage map was constructed using software JoinMap 3.0 (Van Ooijen and Voorrips, 2001). Markers were assigned to linkage groups (LGs) using a minimum

logarithm of odds (LOD) score of 4.0 and the maximum recombination frequency (*r*) of 0.5. The Kosambi mapping function (Kosambi, 1944) was used to calculate the genetic distance between markers. Linkage groups and markers orders were compared with azuki bean linkage map reported by Han *et al*., (2005).

QTL analysis

Marker(s) associated with QTLs for MYMV resistance was determined by single regression analysis at $P = 0.001$ using R-program. To locate positions of the QTLs on the linkage map, composite interval mapping (CIM) was performed with software WinQTL Cartographer 2.5 (Wang *et al*., 2007). Significant LOD threshold value for declaring QTLs was computed by a 1,000 run of a permutation test at $P = 0.001$.

RESULTS AND DISSCUSION

RESULTS

MYMV resistance in the RIL population

Resistance to MYMV in the RILs and parents were evaluated under the field condition. At 15 days after planting (DAP), MYMV symptom first appeared in the susceptible RILs and KPS2, which showed few small scattered yellow specks on young leaves. Four to five days after that most of the specks were coalesced, alternating between yellow and green patches with irregular margins developed on the first fully formed trifoliate leaf next to the apex. Complete yellowing or chlorosis was seen on susceptible plants within 8-10 days followed by necrosis. KPS2 and NM10-12-1 showed highly susceptible and highly resistant with the score of 5 and 1, respectively. Frequency distribution of the MYMD scores in the RILs showed skewness towards the susceptible parent (Figure 1). Of the 120 RILs, none of them were immune, 3 were highly resistant, 10 were tolerant, 60 were susceptible and 49 were highly susceptible (Appendix Table1).

Confirmation of the reaction to MYMV in Thailand

In the glasshouse, four RILs (no. 30, 100, 101 and 114) were chosen from the RIL population. They were infested with MYMV-TH and observed for disease reaction. The reaction was classified into 2 groups when compared with NM10-12-1 and KPS2 as in the field experiment. The disease symptom was firstly found at 15 days after infection (DAI). All tested lines showed scattered few small yellow specks on unifoliate leaves. At 30, 45 and 60 DAI, no. 30 and 101 showed limited spreading of MYMV on unifoliate leaves similar to NM10-12-1. In contrast, no.100 and 114 showed the MYMD symptom on trifoliate leaves. No. 100 showed small yellow spots and some coaleased. No.114 distinctly showed chlorosis or necrosis on the entire leaf, severe stunting of plants, deformed pods produced with small, immature and shriveled seeds. The disease scores for no. 30 and 101 were 1, no. 100 were 2 and no. 114 were

5, while NM10-12-1 and KPS2 were scored as 1 and 5, respectively. Moreover, stem lenght, pod length, number of seeds per pod and 100 seeds weight were observed in this experiment because these traits were directly affected from MYMV infection. The trait values of each line segregated between KPS2 and NM10-12-1 (Table 2). Except, no.114 showed lower 100 seed weight than KPS2.

Table 2 The average values of stem height, pod length, number of seeds per pod and 100 seeds weight of four RILs and their parents

RIL no.	STL* (cm)	Pp*	SDNPPD*	PDL* (cm)	SD100WT* (g)	Disease reaction**
30	48.7	7	9	7.37	9.51	HR
100	48.5	6	9	6.94	8.04	R
101	49.2	7	9	6.84	9.73	HR
114	41.7	5	3	1.97	6.23	HS
KPS2	35.6	6	3	2.49	7.14	HS
NM10-12-1	49.6	7	8	5.96	10.45	HR

* STL = stem height, Pp = number of pods per plant, SDNPPD = number of seeds per pod, PDL = pod length, and SD100WT = 100 seed weight

**For disease reaction, HR = highly resistant, R= resistant and HS = highly susceptible

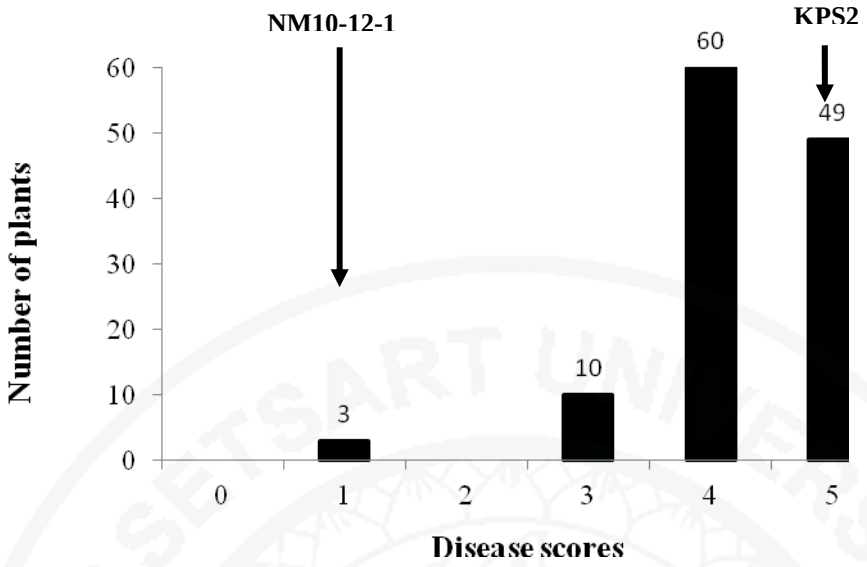

Figure 1 Frequency distribution of disease scores for response to mungbean yellow mosaic disease in the F_8 population (KPS2×NM10-12-1) evaluated in July 2008 at NIAB, Faisalabad, Pakistan

Linkage map construction

Among 1,034 SSR markers from four legume species screened for polymorphism, 693 (66.89%) amplified DNA of KPS2 and NM10-12-1. Sixty-seven (9.67 %) out of the 693 amplifiable markers showed polymorphism (Table 3). Of the 67 polymorphic SSRs, 30 were from mungbean, 21 were from azuki bean and 16 were from cowpea. None of the SSR markers from common bean showed polymorphism. When the polymorphic markers were used to construct linkage map, 66 were assigned to 11 linkage groups (LGs). Marker DMB-SSR194 was unlinked. The map spanned a total length of 524.4 cM (Figure 2) with the length of the LGs varied from 28.2 (LG8) to 66.1 cM (LG6). Number of markers per LG ranged from 4 (LG4) to 12 (LG5). The distance between the adjacent markers varied from 5.5 to 33.1 cM.

QTL conditioning MYMV

QTL analysis of single marker revealed that nine markers on LG5 and one marker on LG10 associated with MYMV resistance at P = 0.0001 (Table 4). The coefficient of determination (R^2) of these markers ranged from 12.44% for marker cp04478 (LG5) to 41.51% for marker DMB-SSR121 (LG5). CIM was employed to locate QTL for MYMV resistance onto the linkage map. The LOD score threshold for CIM determined by permutation test was 4.4. CIM identified only one QTL for the MYMV with the LOD score of 11.8. The QTL, designated as *qMYMV*, located on LG5 between markers DMB-SSR121 and MB-SSR248 (Figure 2). *qMYMV* had the additive effect of 0.47 on the rating scale and explained 47.43 % of the variation in disease reaction. It was 3.0 and 2.2 cM away from the markers DMB-SSR121 and MB-SSR248 in that order.

Table 3 Summary of amplification and polymorphism rates of SSR primer pairs from four legumes in KPS2 and NM10-12-1

SSR source	No. of SSR primer pairs		
	Screened	Amplified (%)*	Polymorphic (%)**
Mungbean	547	353 (64.30)	30 (8.49)
Azuki bean	145	107 (73.79)	21 (20.19)
Cowpea	226	171 (75.66)	16 (11.94)
Common bean	116	62 (53.45)	0 (0.00)
Total	1034	693 (66.89)	67 (9.67)

*The number of SSR primers amplified among the parental mungbeans

** SSR primers showed polymorphism in the RIL population

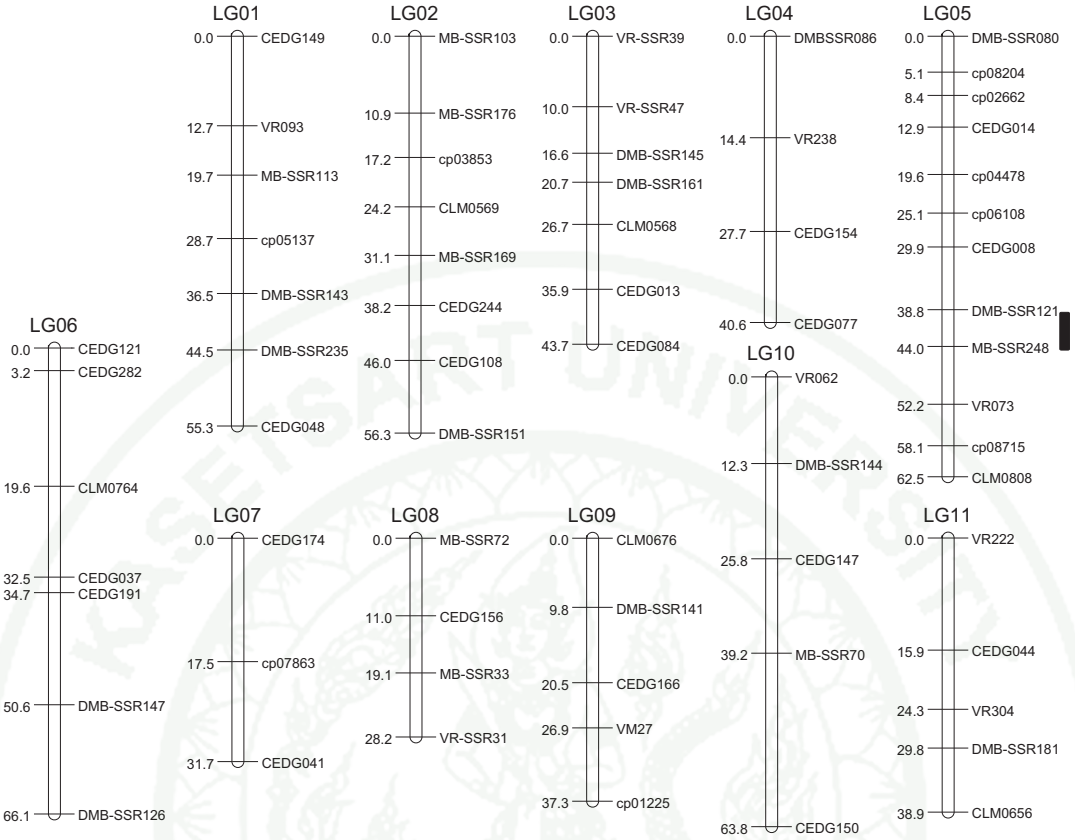

Figure 2 An SSR based linkage map of the mungbean RIL population derived from the cross KPS2×NM10-12-1 and position of the QTL conditioning mungbean yellow mosaic virus resistance (*qMYMV*) detected by composite interval mapping

Table 4 SSR markers associated with mungbean yellow mosaic disease in the RIL mungbean population as revealed by single regression analysis

Marker	Linkage group	R^2 (%)*	Probability
cp04478	5	12.44	<0.0001
cp06108	5	14.64	<0.0001
CEDG008	5	27.77	<0.0001
DMB-SSR121	5	41.51	<0.0001
MB-SSR248	5	40.68	<0.0001
VR073	5	35.98	<0.0001
cp08715	5	31.85	<0.0001
CLM0808	5	16.51	<0.0001
CEDG150	10	13.46	<0.0001

*Coefficient of determination

DISCUSSION

Identification of reliable sources of resistance against the destructive MYMD is an important aspect of mungbean breeding. Accurate measurement of plant disease is crucial in all studies related to disease severity, disease losses and subsequent management tactics (Horsfall and Cowling, 1978; Akhtar and Khan, 2002). In all the present MYMD assessment systems the percentage of diseased plants are used for the expression of varietal response (resistance/susceptible) (Ahmad, 1975; Bashir, 2005; Bashir *et al*., 2006; Khattak *et al*., 2008). However, it is not a suitable method for quantitative assessment of varietal resistance or susceptibility. Existing mungbean cultivars show a great variation in response level, which have not been properly discussed in these assessment systems. It means a disease assessment method is needed which can properly describe different severity levels for proper rating of an individual plant or cultivar under high inoculation pressure. Use of resistant variety is an important aspect of integrated disease management program as well as for a successful breeding program. A reliable screening method is a prerequisite (Akhtar and Haq, 2003). As the disease is vectored by whitefly, field screening is the most commonly used method for evaluation of resistance and susceptibility. To exert maximum inoculum pressure, the crop was sown late, about 10 days after the sowing of other surrounding field with mungbean experiments to receive maximum inoculum. Our strategy was successful and we got 100% infection in all the test material when the crop was about 30-35 days old. The high inoculum pressure together with the disease severity scale resulted in three RILs as highly resistance, 10 RILs as tolerant, 60 RILs as susceptible, and 49 RILs as highly susceptible. Our findings showed that these RILs have good resistance and can be used to manage the disease in the areas with high incidence of MYMD. Additionally, the results showed harmony with earlier findings that the resistance in mungbean against MYMD is rare (Ahmad, 1975; Pandya *et al*., 1977; Gill *et al*., 1983; Naqvi *et al*., 1995; Singh *et al*., 1996; Saleem *et al*., 1998; Bashir, 2005; Shad *et al*., 2006).

In northern part of Thailand, mungbean yellow mosaic disease (MYMD) occurred in 2008, which rapidly outbroke in many mungbean production areas.

Mungbean cultivars showed severe disease symptom and then were collected as MYMV stocks. Present by, MYMD disappeared from the areas, in the same maner as previously outbroke in 1977 (Thongmeearkom *et al*., 1981). This may be due elimination of whitefly population, as well as eradication of alternate hosts of MYMV. The climatic change may also affect MYMV life cycle and epidemic. The virus was maintained in healthy mungbean plants but was not enough to test in all the RILs. So, the RIL population was sent for testing in Pakistan. The RILs showing highly resistant (no. 30, no. 100 and no. 101) and highly susceptible (no. 114), as well as the parents, NM10-12-1 and KPS2, were used to evaluate against MYMV-TH. These lines were inoculated with MYMV-TH via whiteflies in the glasshouse (Honda *et al*., 1983). No. 100 was found resistant, in contrast with the result from Pakistan field. Thus it is possible that MYMD in Thailand and Pakistan may not be the same strain. No. 30, no. 101 and no.114 showed corresponding reaction with that in Pakistan. Based on the observation on stem length, pod length, number of seeds per pod and 100 seed weight together with the effect of MYMV to growth and development, RILs no. 30 and 101 were identified as resistant lines suitable for planting in the disease outbreak areas of Thailand.

More than 80% of host plant resistance to viral diseases is monogenically controlled (Kang *et al*., 2005). This is the first report of gene mapping for MYMV resistance in mungbean. CIM identified a major QTL (PVE > 45%) for the resistance to MYMV, while single marker analysis suggested that there possibly is a minor gene on LG10 involves in the resistance. This is in line with the results of Malik *et al*. (1986), Thakur *et al*. (1997), and Khattak *et al*. (2000) who reported that resistance to MYMV is controlled by a single gene with modifiers. In SSR analysis, low marker polymorphism between the mapping parents result in low effeiency in taging modified genes in this study. Therefore it is possible that other gene loci with moderate and/or minor effects associated with the resistance were not detectable, if any existed. Since SSR markers DMB-SSR121 (F:5'-AGCTATTGGTGCATAGGTTC-3',R:5'-GATAT GATGAGTATGGTGTAG-3') and MB-SSR248 (F:5'-GGTACAACATTCTTC TAT TTG-3', R:5'GGCTTATGA GTTTATCTTATC-3') are respectively only 3.0 and 2.2

cM from the *qMYMV*, they can be used to aid the selection for resistant mungbean genotypes. However, it is advisable to confirm the *qMYMV* in the selected population.

In blackgram, resistance gene analog markers YR4 and CYR1 were reported to completely link with resistance to mungbean yellow mosaic India virus (MYMIV) (Maiti *et al*., 2011). Marker CYR1 also associated with the resistance in mungbean (amplifies DNA band in resistant mungbean but no DNA band in susceptible mungbean). CYR1 is proposed as a part of the candidate disease resistance (*R*) gene (Maiti *et al*., 2011). Recently, the *R* gene *CYR1* was fully isolated from black gram (Maiti *et al*., 2012). Protein CYR1 may act as a signaling molecule to protect *V. mungo* plants from MYMIV and involve in recognizing effector molecule of the pathosystem to contribute in incompatible interaction (Maiti *et al*., 2012). In the present study, markers YR4 and CYR1 were used in polymorphism survey in the parents, but none of them showed polymorphism. Although CRY1 showed no polymorphism between KPS2 and NM10-12-1, the association between *CYR1* and the MYMV resistance in mungbean cannot be ruled out. The contrast between our result and that of Maiti *et al*. (2011) is possibly due to the difference in genotypes of mungbean as well as strains/species of the virus causing MYMV. DNA B of a MYMV isolate is an important determinant of host range between mungbean and blackgram. Balaji *et al*. (2004) showed that agroinoculation of mungbean and blackgram with two different DNA B components together with DNA A of a blackgram isolate of MYMV-*Vig* resulted in distinctly different symptoms between the two crops. Additional research is required to clarify whether the *CYR1* involves in the MYMV resistance in mungbean.

CONCLUSIONS AND RECOMMENDATION

Conclusion

An F_8 RIL population was evaluated for MYMD in a field in Nuclear Institute for Agricultural Biology, Pakistan during high inoculum pressure. They were compared with KPS2 (highly susceptible to MYMV) and NM10-12-1 (highly resistant to MYMV). The RILs can be divided based on their disease symptoms that there are 3 lines with highly resistant (DI=1), 10 lines with tolerant (DI=3), 60 lines with susceptible (DI=4) and 49 lines with highly susceptible (DI=5). In Thailand, lines no. 30, 101 and 114 showed the same disease reaction as the phenotypic data in Pakistan. Only line no. 100 showed different disease reaction from theirs. Map construction of this population spanned 524.4 cM, which composed of 66 SSR markers. These markers located on each 11 linkage groups, showing QTL linked MYMV resistant trait on LG5. The QTL was designated as *qMYMV*. The *qMYMV* located between DMB-SSR121 and MB-SSR248 and away from 3.0 and 2.2 cM, respectively. LOD score of *qMYMV* was 11.8 and explained 47.43 % of the phenotypic variation, respectively. The additive effect of the *qMYMV* was 0.47 of the scoring unit.

Recommendation

Result of the glasshouse testing against MYMV Thai isolate showed that RIL, no.100 had resistant reaction, contrasted to the reaction in Pakistan. Thus MYMV in Thailand and Pakistan may not be the same strain. The Thai MYMV should tested against all RILs to confirm this speculation.

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APPENDIX

Appendix Table 1 Screening of F_8 recombinant inbred lines population (KPS 2 x NM 10-12-1) against mungbean yellow mosaic virus in a field under high inoculum pressure at NIAB, Pakistan during summer 2008

No.	Lines	Disease score	Disease reaction
1	KPS 2 (Parent)	5	Highly susceptible
2	NM 10-12-1 (Parent)	1	Highly resistant
3	Line 1	5	Highly susceptible
4	Line 2	5	Highly susceptible
5	Line 3	4	Susceptible
6	Line 4	5	Highly susceptible
7	Line 5	4	Susceptible
8	Line 6	4	Susceptible
9	Line 7	4	Susceptible
10	Line 8	4	Susceptible
11	Line 9	4	Susceptible
12	Line 10	3	Tolerant
13	Line 11	4	Susceptible
14	Line 12	5	Highly susceptible
15	Line 13	5	Highly susceptible
16	Line 14	4	Susceptible
17	Line 15	5	Highly susceptible
18	Line 16	4	Susceptible
19	Line 17	5	Highly susceptible
20	Line 18	4	Susceptible
21	Line 19	5	Highly susceptible
22	Line 20	4	Susceptible
23	Line 21	5	Highly susceptible
24	Line 22	4	Susceptible
25	Line 23	5	Highly susceptible
26	Line 24	4	Susceptible
27	Line 25	4	Susceptible
28	Line 26	5	Highly susceptible
29	Line 27	3	Tolerant
30	Line 28	5	Highly susceptible
31	Line 29	4	Susceptible
32	Line 30	1	Highly resistant
33	Line 31	4	Susceptible
34	Line 32	4	Susceptible
35	Line 33	4	Susceptible
36	Line 34	4	Susceptible

Appendix Table 1 (Continued)

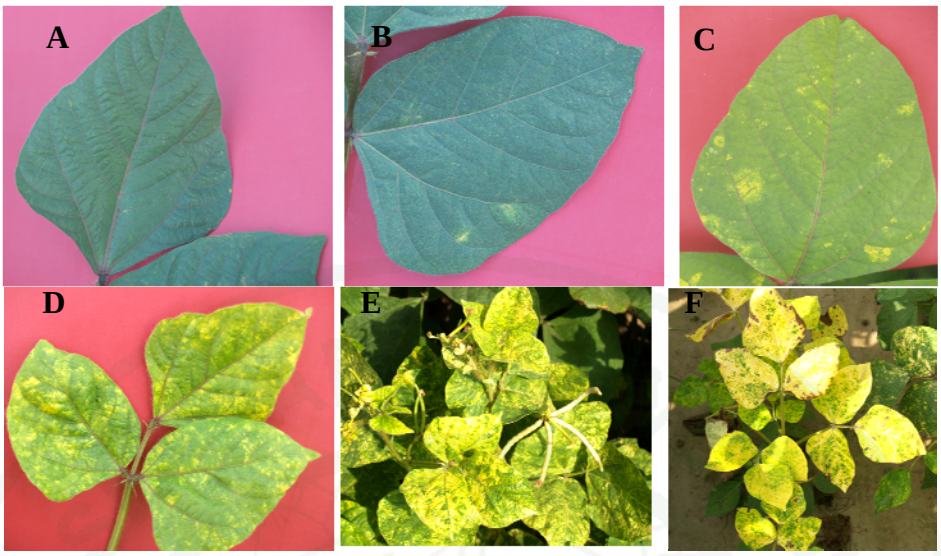
No.	Lines	Disease score	Disease reaction
37	Line 35	4	Susceptible
38	Line 36	3	Tolerant
39	Line 37	5	Highly susceptible
40	Line 38	4	Susceptible
41	Line 39	4	Susceptible
42	Line 40	5	Highly susceptible
43	Line 41	5	Highly susceptible
44	Line 42	4	Susceptible
45	Line 43	5	Highly susceptible
46	Line 44	4	Susceptible
47	Line 45	5	Highly susceptible
48	Line 46	4	Susceptible
49	Line 47	4	Susceptible
50	Line 48	3	Tolerant
51	Line 49	5	Highly susceptible
52	Line 50	5	Highly susceptible
53	Line 51	5	Highly susceptible
54	Line 52	5	Highly susceptible
55	Line 53	5	Highly susceptible
56	Line 54	5	Highly susceptible
57	Line 55	4	Susceptible
58	Line 56	4	Susceptible
59	Line 57	4	Susceptible
60	Line 58	4	Susceptible
61	Line 59	5	Highly susceptible
62	Line 60	5	Highly susceptible
63	Line 61	5	Highly susceptible
64	Line 62	4	Susceptible
65	Line 63	5	Highly susceptible
66	Line 64	4	Susceptible
67	Line 65	4	Susceptible
68	Line 66	4	Susceptible
69	Line 67	5	Highly susceptible
70	Line 68	4	Susceptible
71	Line 69	5	Highly susceptible
72	Line 70	5	Highly susceptible
73	Line 71	4	Susceptible
74	Line 72	3	Tolerant
75	Line 73	5	Highly susceptible
76	Line 74	4	Susceptible
77	Line 75	4	Susceptible

Appendix Table 1 (Continued)

No.	Lines	Disease score	Disease reaction
78	Line 76	4	Susceptible
79	Line 77	5	Highly susceptible
80	Line 78	5	Highly susceptible
81	Line 79	5	Highly susceptible
82	Line 80	4	Susceptible
83	Line 81	3	Tolerant
84	Line 82	3	Tolerant
85	Line 83	4	Susceptible
86	Line 84	4	Susceptible
87	Line 85	4	Susceptible
88	Line 86	4	Susceptible
89	Line 87	4	Susceptible
90	Line 88	5	Highly susceptible
91	Line 89	3	Tolerant
92	Line 90	4	Susceptible
93	Line 91	5	Highly susceptible
94	Line 92	5	Highly susceptible
95	Line 93	4	Susceptible
96	Line 94	5	Highly susceptible
97	Line 95	5	Highly susceptible
98	Line 96	4	Susceptible
99	Line 97	4	Susceptible
100	Line 98	5	Highly susceptible
101	Line 99	5	Highly susceptible
102	Line 100	1	Highly resistant
103	Line 101	1	Highly resistant
104	Line 102	3	Tolerant
105	Line 103	3	Tolerant
106	Line 104	5	Highly susceptible
107	Line 105	5	Highly susceptible
108	Line 106	5	Highly susceptible
109	Line 107	4	Susceptible
110	Line 108	4	Susceptible
111	Line 109	4	Susceptible
112	Line 110	4	Susceptible
113	Line 111	5	Highly susceptible
114	Line 112	4	Susceptible
115	Line 113	4	Susceptible
116	Line 114	5	Highly susceptible
117	Line 115	4	Susceptible
118	Line 116	4	Susceptible

Appendix Table 1 (Continued)

No.	Lines	Disease score	Disease reaction
119	Line 117	4	Susceptible
120	Line 118	4	Susceptible
121	Line 119	4	Susceptible
122	Line 120	5	Highly susceptible
123	Line 121	5	Highly susceptible
124	Line 122	5	Highly susceptible



Appendix Figure 1 Disease scale of mungbean yellow mosaic disease until 0-5, where 0= immune (A), 1= highly resistant (B), 2= resistant (C), 3= tolerant (D), 4= susceptible (E), and 5= highly susceptible (F).

Appendix Figure 2 Band patterns of parents (NM10-12-1 and KPS2) and individual F_8 RILs that were screened by CEDG108 primers.

CIRRICULUM VITAE

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