RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Inheritance and identification of ISSR-RGA markers associated with powdery mildew resistance in mungbean for marker-assisted breeding

Piyada Alisha Tantasawat[1*], Oythip Poolsawat[1], Chitpan Kativat[1], Kitiya Arsakit[1], Pitchakon Papan[1], Witsarut Chueakhunthod[1], and Pakhawat Pookhamsak[1]

[1]Suranaree University of Technology, School of Crop Production Technology, Nakhon Ratchasima 30000, Thailand.
[*]Corresponding author (piyada@sut.ac.th).

Received: 19 July 2021; Accepted: 11 November 2021; doi:10.4067/S0718-58392022000100003

ABSTRACT

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) yield is dramatically constrained by powdery mildew (PM) caused by *Sphaerotheca phaseoli* (Z.Y. Zhao) U. Braun (1985), which is considerably prevalent in the cool-dry season of production in South, East, and Southeast Asia countries including Thailand. Exploitation of varieties resistant to the disease is crucial to meet sustainable production. A population of 64 $F_{2:9}$ and $F_{2:10}$ recombinant inbred lines (RILs) generated by hybridization of the susceptible parent 'Chai Nat 72' (CN72) with the resistant parent 'V4758' was used to assess genetic resistance and identify inter simple sequence repeat-anchored resistance gene analog (ISSR-RGA) markers linked to the PM resistance gene. The PM response in these RILs was visually scored in the field during the winter seasons, twice in 2015 and 2018, and the segregation pattern was determined by the chi-square test (χ^2). The resulting segregation ratios of 1:1 indicated a qualitative nature with a dominantly inherited resistance gene conferring PM resistance. When bulk segregant analysis (BSA) was undertaken using 378 ISSR-RGA primer combinations among both parents and DNA bulks of resistant and susceptible $F_{2:9}$ individuals, 11 of these exhibited polymorphisms, and one marker I41tP379 was closest to the PM resistance gene which revealed a highly significant correlation with PM resistance (R^2 (%) = 26; $P < 0.001$) with a logarithm of odd (LOD) score of 5.85. The closest marker I41tP379 could trace the PM resistance gene in molecular marker assisted breeding for mungbean improvement.

Key words: ISSR-RGA marker, powdery mildew (PM) resistance, *Vigna radiata*.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is a warm-season leguminous crop belonging to Fabaceae family. Green manure from mungbean can be used in crop rotation to inhibit cereal disease cycle and to improve soil fertility (Kajonphol et al., 2012; Kim et al., 2015; Mathivathana et al., 2018). Mungbean seeds are highly nutritious containing Fe, folate, and vitamin and are also vital source of easily digested proteins than any other legumes (Yi-Shen et al., 2018).

Among the biotic stress factors, powdery mildew (PM) disease causes considerable yield reduction in a broad host range (Hacquard, 2014). In mungbean, it is invoked by the biotrophic fungus *Sphaerotheca phaseoli* (Z.Y. Zhao) U. Braun (1985) encountering in the cool-dry seasons of production in South, East, and Southeast Asia countries including Thailand. This fungus is an obligate parasite infecting the upper surface of mungbean leaves and stems which gives white spotting with epiphytic mycelia and conidia covering. In susceptible varieties, yield reductions were up to 20%-40% at reproductive stage, and it can cause 100% death at seedling stage (Pooja et al., 2018). In Thailand, certified mungbean varieties which are popularly grown by Thai farmers are usually susceptible to the disease, such as 'CN3', 'CN72', 'CN84-1', 'Kampaeng Saen 1' (KPS1), and 'Suranaree University of Technology 1' (SUT1).

Although chemical spraying can be employed, continuous use of pesticides severely impacts the environment and human health. The use of pesticides on the other hand, not only increases production costs but also results in development

of resistant strains of the pathogen. Therefore, the use of PM resistant cultivars is one of the most desirable alternatives to combat the disease. Although several mungbean resistant genotypes have been reported, such as 'M5-10', 'M5-25', 'V4189', 'V2159', 'V4207', 'V4668', 'V4990', 'V4574', 'V3912', 'V4186', 'V1104', 'V4658', 'V4631', 'V4717', 'V4662', 'V4883', 'V4718', 'V4758', and 'V4785' (Nair et al. 2019), only a few genotypes are highly resistant to PM including 'V4718', 'V4785', and 'V4758' (Khajudparn et al., 2010; Chueakhunthod et al., 2020). Many reports of genetic resistance to PM in mungbean indicated dominant or recessive, and monogenic or polygenic controls. A single dominant gene was reported in 'V4718', 'V4758', and 'V4785' by Khajudparn et al. (2010). Chankaew et al. (2013) revealed that PM resistance in 'V4718' is also governed by a single dominant gene. On the contrary, Kanwade et al. (2019) found a single recessive gene in response to PM resistance in 'BPMR-145'. While Kasettranan et al. (2009) reported that PM resistance from 'VC6468' was quantitatively inherited with predominantly additive gene action and high heritability. Moreover, Young et al. (1993) identified three quantitative trait loci (QTLs) conferring PM resistance in advanced mungbean breeding line 'VC3890'. Combining different PM resistance loci into a single variety is a useful strategy to provide a durable resistance.

Generally, conventional breeding methods are employed to develop new varieties, however they are limited in low selection efficiency and are subjected to constraints from the environment (Zhao et al., 2020). From the effectiveness of molecular approaches in accelerating breeding for disease resistance, molecular DNA markers which are the powerful genomic tools for revealing differences between individuals of species have been extensively applied to tag genes in many crops (Vidak et al., 2017). Molecular markers that are in close association with the desirable traits can be applicable for selection of the plant materials in a short time at an early stage through marker-assisted selection (MAS), thereby reducing the length of breeding time. Inter simple sequence repeats (ISSR) are DNA fragments which are oppositely oriented at microsatellite regions using microsatellite core sequences as primers. It is highly efficient, economical, and rapid. Resistance gene analog (RGA) markers which are obtained from the conserved structures of disease resistance gene, such as nucleotide binding site (NBS) and protein kinase domains have been useful for finding the position of disease resistance genes (Patil et al., 2014). Recently, we developed a new type of marker based on these conserved sequences of RGA, the ISSR-anchored resistance gene analog (ISSR-RGA) markers, to efficiently identify a PM resistance gene in another mungbean resistant line, namely 'V4718' (Poolsawat et al., 2017) and a Cercospora leaf spot (CLS) resistance gene in 'V4718' (Tantasawat et al., 2020). The ISSR-RGA markers can detect more variable genomic regions including the SSR and RGAs or *R* genes and are applicable for a large-scale genotyping due to their specificity and reliable polymerase chain reaction (PCR)-based assay. Therefore, the newly developed markers are very effective tools for identification of other resistance genes as well as combining several resistance genes into the same variety to provide more durable resistance.

Bulk segregant analysis (BSA) is a useful strategy to identify linked markers and map important QTLs (Michelmore et al., 1991). For disease resistance traits, this method which is based on constructing each of the bulked DNA samples with two distinct phenotypes (resistance and susceptible) is more convenient and rapid compared with the traditional QTL mapping method (Nie et al., 2015).

The purposes of the current study were to determine the resistance pattern against PM and to identify ISSR-RGA markers associated with the PM resistance gene using $F_{2:9}$ and $F_{2:10}$ recombinant inbred line (RIL) populations generated by crossing 'CN72' with 'V4758' for utilization in plant breeding.

MATERIALS AND METHODS

Plant materials

A recombinant inbred line (RIL) population of mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) consisting of 64 $F_{2:9}$ and $F_{2:10}$ RIL populations derived from crossing 'CN72' × 'V4758' was used. The paternal parent 'V4758' is resistant to powdery mildew (PM), while the maternal parent 'CN72' is popularly grown by Thai farmers and is susceptible to the disease.

Inheritance of powdery mildew resistance

A set of the $F_{2:9}$ and $F_{2:10}$ RIL populations (64 individual lines) and their parents were evaluated for PM resistance at Suranaree University of Technology Farm (SUT Farm), Nakhon Ratchasima (14°52'39" N, 102°00'15" E, 227 m a.s.l.), north-eastern Thailand, twice in 2015 and 2018, respectively under field conditions and without spraying fungicide. The experiment used three replicates in a randomized complete block design (RCBD). In each replicate, seeds were sown in a 2 m row using a 50 cm distance between rows and 20 cm between hills with two plants per hill. 'CN72' was planted at the head and end of the plot to be a source of infection. Phenotyping of PM response was undertaken at 65 d after planting

using the severity of the disease score which was divided into two levels: 1.0-4.9 (resistant) and 5.0-9.0 (susceptible) as proposed by Khajudparn et al. (2010). Chi-square test (χ^2) was performed for studying the distribution of the PM resistance gene. We used the formula $(X + 1)^{1/2}$ to transform the scores of PM in both years from the field evaluation for analyzing the broad sense heritability (h^2_b) as followed by Khajudparn (2009). To measure the relationship of PM resistance in both years, correlation analysis was undertaken through SPSS version 14.0 (IBM, Armonk, New York, USA).

ISSR-RGA analysis

Both parental and $F_{2:9}$ RIL healthy young leaves were used for the extraction of total DNA using a modified CTAB extraction protocol (Lodhi et al., 1994). Concentration and purity of the extracted DNA were measured by means of A260/A280 ratio using a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). A total of 378 inter simple sequence repeat-anchored resistance gene analog (ISSR-RGA) primer combinations derived from 63 ISSR primers and 4 RGA primers including nucleotide binding site (NBS) (GLPLAL 1 and P-Loop; Mahanil, 2007) and kinase domains (Pto kin1; Chen et al., 1998; and LRK for; Feuillet et al., 1997) as well as two RGA primers designed from the sequences of the PM resistance gene in mungbean from the National Center for Biotechnology Information (NCBI) website, PMR1R and PMR5R were selected for the initial screening of two parents (R and S) and two distinct bulks, each containing 10 RIL informative individuals exhibiting either the highest resistance (resistant bulk; RB) or susceptibility (susceptible bulk; SB), according to bulk segregant analysis (BSA) method. PCR and gel electrophoresis for this marker system were totally carried out according to Poolsawat et al. (2017).

Data analysis

DNA patterns were compared between R and RB, S and SB to find the possible ISSR-RGA markers linked to the PM resistance gene. These putative markers related to the PM resistance gene were further used to analyze individual RILs. The association between this marker system and PM resistance was measured with a simple linear regression analysis through SPSS version 14.0 and the logarithm of odd (LOD) as described by Poolsawat et al. (2017).

RESULTS

Inheritance of powdery mildew resistance

From field evaluation at 65 d after planting during the winter seasons, the donor parent 'V4758' was immune to PM infection with the disease scores of 3.33 and 4.00 in 2015 and 2018, respectively, while the recipient parent 'CN72' was highly susceptible to the disease with the disease scores of 6.00 and 6.67 in 2015 and 2018, respectively. Each of the $F_{2:9}$ and $F_{2:10}$ RIL populations from a 'CN72 × V4758' cross observed in 2015 and 2018, respectively, was identified into two major groups (R and S). The disease scores of both years were significantly correlated (0.51; $P < 0.001$). Among the 64 $F_{2:9}$ and $F_{2:10}$ RILs, 25 progenies were resistant and 39 progenies were susceptible in 2015, and 29 progenies were resistant and 35 progenies were susceptible in 2018. Note that one progeny (55A) had a higher level of resistance to PM compared with V4758 in both years. The χ^2 test showed that the population phenotypically segregated into a ratio of 1:1 (resistant/susceptible) in both years (Table 1). The estimates of broad sense heritability for PM resistance in 2015 and 2018 were 96.4% and 82.4%, respectively. Our findings demonstrate the operation of a single gene pair inheritance in this cross.

ISSR-RGA analysis

Out of 378 ISSR-RGA primer combinations used to screen parental polymorphism between 'CN72' and 'V4758' using BSA analysis, only 338 ISSR-RGA primer combinations (89.42%) amplified clear DNA bands in all the mungbean DNA extracted. It was found that 248 out of 338 ISSR-RGA primer combinations (73.37%) showed polymorphic DNA

Table 1. Segregation in reaction to powdery mildew in $F_{2:9}$ and $F_{2:10}$ recombinant inbred line (RIL) populations derived from 'CN72 × V4758' cross.

Populations	Years	Lines	Resistant: susceptible lines	Chi-square test: Expected ratio R:S	Chi-square test: χ^2 value	Heritability
		Nr	Nr			%
$F_{2:9}$	2015	64	25:39	1:1	3.06^{ns}	96.4
$F_{2:10}$	2018	64	29:35	1:1	0.56^{ns}	82.4

The χ^2 value was calculated for goodness of fit against 1:1 ratio for RILs.
R: Resistant RIL; S: susceptible RIL; ^{ns}: nonsignificant.

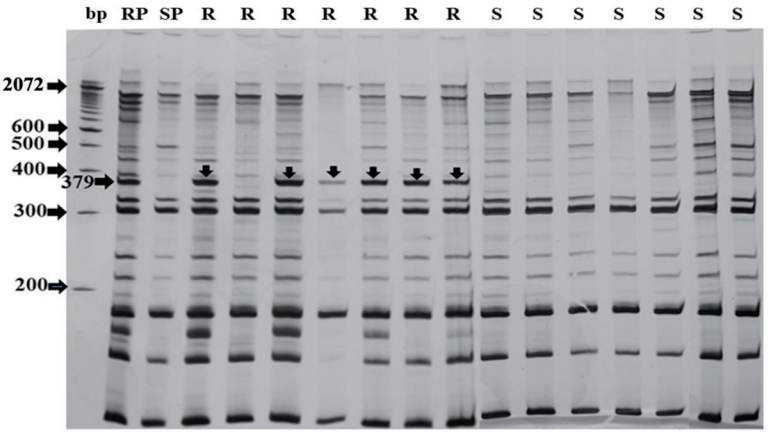
bands between both parents, however, only 11 ISSR-RGA primer combinations (3.25%) amplified DNA bands that were putatively associated with the PM resistance gene. These 11 primer combinations amplified a total of 288 scorable DNA bands with an average of 26.18 bands/primer pair. When we considered the polymorphic bands between male and female parents of these 11 ISSR-RGA primer combinations, the results revealed that all of these generated 39 polymorphic bands with percentages of the polymorphic bands between male and female parents of 3.70%-26.09% (average 13.60%). The highest percentage of the polymorphic bands between male and female parents was observed in marker I15PL457 (26.09%), while the lowest was observed in marker I84PMR1R600 (3.70%). Each of the primer combination produced one specific DNA band in BSA, indicating possible association of these ISSR-RGA markers with the PM resistance gene. From a simple linear regression analysis to evaluate the relationship between these markers and the PM resistance phenotypes, the results showed that five markers (I41P252, I90PMR1R400, I40R211, I41tP379, and I84PMR1R600) were significantly correlated with PM resistance (R^2 = 21%, 21%, 27%, 27%, and 30%, respectively; $P < 0.05$) (Table 2). The results revealed that LOD score of the I41tP379 marker was higher than those of the other markers. Black arrows in Figure 1 show the position of the I41tP379 marker putatively linked to the PM resistance gene. This marker was further observed with individuals of the 64 $F_{2:9}$ RIL population and found to be highly significantly correlated with the PM resistance (R^2 = 26%; $P < 0.001$) with a LOD score of 5.85.

Table 2. Inter simple sequence repeat (ISSR)-anchored resistance gene analog (RGA) markers putatively associated with powdery mildew resistance in 'CN72' × 'V4758' cross.

Markers	Primers and sequences				Range of amplified products	Scorable DNA bands	Polymorphic bands (male-female parents)	Polymorphism	I-R linked[b]	P-value	R^2
	ISSR	Sequences[a] 5'-3'	RGA	Sequences 5'-3'							
					bp	Nr	Nr	%			%
I15PL457	815	$(CT)_8G$	P-Loop	$(GGI)_2GTIGGIAAIACIAC$	100-1500	23	6	6.09	1	0.060	19
I26PL250	826	$(AC)_8C$	P-Loop	$(GGI)_2GTIGGIAAIACIAC$	100-1500	38	9	23.68	1	0.752	1
I41P252	841	$(GA)_8YC$	Pto kin 1	GCATTGGAACAAGGTGAA	100-2072	30	4	13.33	1	0.049	21
I41tP379	841t	$(GA)_8TC$	Pto kin 1	GCATTGGAACAAGGTGAA	100-1500	23	5	21.74	1	0.019	27
I84PMR1R600	884	HBH $(AG)_7$	PMR1R	AGCAAGAAATCACTCCATGT	200-1500	27	1	3.70	1	0.016	30
I90PMR1R400	890	VHV $(GT)_7$	PMR1R	AGCAAGAAATCACTCCATGT	200-1500	24	1	4.17	1	0.045	21
12PMR5R160	812	$(GA)_8A$	PMR5R	TACTCTCACTGTGCGTTCTG	150-2072	31	2	6.45	1	0.220	8
I30PMR5R656	830	$(TG)_8G$	PMR5R	TACTCTCACTGTGCGTTCTG	200-2072	21	3	14.29	1	0.483	3
I40PMR5R471	840	$(GA)_8YT$	PMR5R	TACTCTCACTGTGCGTTCTG	200-1500	20	4	20.00	1	0.096	15
I40R211	840	$(GA)_8YT$	RLK for	GAYGTNAARCCIGARAA	100-2072	30	2	6.67	1	0.019	27
I59R818	859	$(TG)_8RC$	RLK for	GAYGTNAARCCIGARAA	100-1500	21	2	9.52	1	0.061	20
Total						288	39		11		
Average						26.18	3.55	13.60	1		

[a]B: C, G, T; H: A, C, T; I: inosine; N: A, G, C, T; R: A, G; V: A, C, G; Y: C, T.
[b]I-R linked: ISSR-RGA loci putatively associated with powdery mildew resistance.

Figure 1. DNA profiles from marker I41tP379 derived from ISSR 841t combined with Pto kin 1 from resistant parent (RP; 'V4758') susceptible parent (SP; 'CN72'), representative resistant recombinant inbred lines (RIL) (R) and susceptible RIL (S); M = 100 bp DNA ladder. Black arrows show markers putatively associated with powdery mildew resistance gene.



DISCUSSION

Several inheritance models of resistance to PM in mungbean were previously reported which included dominant or recessive, and monogenic or polygenic controls. Pandey et al. (2018) described that those markers associated with major resistance genes (*R* genes) or quantitative trait loci for PM may be potentially useful for screening of large population through marker-assisted selection (MAS). Generally, there have been limitations of developing PM resistant mungbean varieties through conventional breeding methods, especially season-dependent selection for the PM resistance since the disease is only prevalent in the cool-dry season. During selection process, MAS is employed by using tightly linked DNA markers, thereby overcoming the influence of PM occurrence. In the current study, $F_{2:9}$ and $F_{2:10}$ RIL populations from the 'CN72' × 'V4758' cross displayed a good fit with 1:1 segregation ratio for PM resistant and susceptible response in both years, indicative of dominant monogenic control for this resistance in this cross. Similarly, Khajudparn et al. (2010) and Poolsawat et al. (2017) also observed that resistance against PM in 'V4718', 'V4758', and 'V4785' was inherited as a single major gene and that these resistance genes are non-allelic. While Kanwade et al. (2019) found that the PM resistance in 'Kopergaon' × 'BPMR-145' cross of mungbean was due to a single recessive gene. Moreover, Kasettranan et al. (2009) reported that the PM resistance was quantitatively inherited with predominantly additive gene action and high heritability. Young et al. (1993) found three QTLs associated with the PM resistance. These results indicate that genetic resistance against PM is dependent on the resistant sources. The estimates of broad sense heritability for disease severity scores in the $F_{2:9}$ and $F_{2:10}$ RIL populations were high, being 96.4% and 82.4%, respectively, indicating that the environment has a low influence on this trait. Therefore, the resistance to PM in 'V4758' can be easily incorporated by conventional breeding method, such as pedigree selection, bulk selection, single seed descent or backcross method. The disease severity scores of both years were significantly correlated ($P < 0.001$), suggesting that the PM resistance in 2015 was similar to that evaluated in 2018. Moreover, the study identified one progeny (55A) with a level of resistance to PM above 'V4758' in both years and could be recommended as a donor parent through the hybridization procedure to transfer the PM resistance gene into mungbean.

The BSA analysis can be applied to markedly reduce the genotyping effort for identifying markers linked to disease resistance genes (Trick et al., 2012). Recently, Patel et al. (2018) reported that one RGA primer pair, 1F-CG/RGA1R, amplified a single 445 bp band linked to *Mungbean yellow mosaic virus* (MYMV) resistance in BSA of a 'GM-4 × Meha' cross in mungbean. In our previous works, Poolsawat et al. (2017) and Tantasawat et al. (2020) found that BSA analysis was useful for identification of ISSR and ISSR-RGA markers linked to PM and CLS resistance in another cross, 'CN72' × 'V4718', of mungbean. In this study, we found that 11 out of 338 ISSR-RGA primer combinations generated 3.70%-26.09% of polymorphic bands between male and female parents. Similarly, the report of Poolsawat et al. (2017), who studied 40 ISSR-RGA primer combinations in the 'CN72' × 'V4718' cross of mungbean, found the percentages of polymorphic bands between male and female parents of 0.00%-38.89%. In addition, these 11 ISSR-RGA primer combinations produced specific DNA fragments in BSA. Of these, the marker I41tP379 was associated with the PM resistance gene when we identified with 64 individual RILs using a simple linear regression analysis and the LOD score above three. Thus, these results indicate that the ISSR-RGA marker can facilitate the selection of PM resistant individuals in mungbean.

CONCLUSIONS

We report on genetic resistance against powdery mildew (PM) in mungbean 'V4758' which involves dominant monogenic control for the trait. The progeny 55A showed a higher level of resistance to PM compared with the resistant parent and could be useful as a new parent to introduce resistance. The marker I41tP379 was highly correlated with PM resistance and, therefore, may be useful for marker-assisted selection (MAS) of PM resistance in a mungbean breeding program.

ACKNOWLEDGEMENTS

This work was supported by Suranaree University of Technology (SUT) and by Thailand Science Research and Innovation (TSRI). The World Vegetable Center, Taiwan and Chai Nat Field Crops Research Center, Thailand are gratefully acknowledged for providing the seeds of 'V4758' and 'CN72', respectively. We also thank Mr. Peter Charles Bint for proofreading the manuscript.

REFERENCES

Chankaew, S., Somta, P., Isemura, T., Tomooka, N., Kaga, A., Vaughan, D.A., et al. 2013. Quantitative trait locus mapping reveals conservation of major and minor loci for powdery mildew resistance in four sources of resistance in mungbean [*Vigna radiata* (L.) Wilczek.] Molecular Breeding 32:121-130. doi:10.1007/s11032-013-9856-6.

Chen, X.M., Line, R.F., and Leung, H. 1998. Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis. Theoretical and Applied Genetics 97(3):345-355. doi:10.1007/s001220050905.

Chueakhunthod, W., Jinagool, W., Meecharoen, K., Khwanman, R., Pattanaram, P., Jantarat, N., et al. 2020. Genetic relationship of mungbean and blackgram genotypes based on agronomic and photosynthetic performance and SRAP markers. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(4):1845-1861. doi.10.15835/nbha48412114.

Feuillet, C., Schachermayr, G., and Keller, B. 1997. Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. The Plant Journal 11(1):45-52. doi:10.1046/j.1365-313x.1997.11010045.x.

Hacquard, S. 2014. The genomics of powdery mildew fungi: past achievements, present status and future prospects. Advances in Botanical Research 70:109-142. doi:10.1016/B978-0-12-397940-7.00004-5.

Kajonphol, T., Sangsiri, C., Somta, P., Toojinda, T., and Srinives, P. 2012. SSR map construction and quantitative trait loci (QTL) identification of major agronomic traits in mungbean (*Vigna radiata* (L.) Wilczek). SABRAO Journal of Breeding and Genetics 44(1):71-86.

Kanwade, D.G., Kute, N.S., Bedis, M.R., Pawar, V.Y., Deshmukh, G.P., Jaybhaye, C.P., et al. 2019. Inheritance of powdery mildew resistance in mungbean [*Vigna radiata* (L.) R. Wilczek]. Journal of Pharmacognosy and Phytochemistry 8(3):3642-3644.

Kasettranan, W., Somta, P., and Srinives, P. 2009. Genetics of the resistance to powdery mildew disease in mungbean (*Vigna radiata* (L.) Wilczek). Journal of Crop Science and Biotechnology 12(1):37-42. doi:10.1007/s12892-008-0074-4.

Khajudparn, P. 2009. Characters associated with yield potential and development of molecular markers for powdery mildew resistance in mungbean. 121 p. PhD thesis. Suranaree University of Technology, School of Crop Production Technology, Nakhon Ratchasima, Thailand.

Khajudparn, P., Wongkaew, S., and Tantasawat, P. 2010. Identification of genes for powdery mildew resistance in mungbean. Journal of Life Sciences 4:25-29. doi:10.1111/j.1439-0523.2008.01609.x.

Kim, S.K., Nair, R.M., Lee, J., and Lee S.-H. 2015. Genomic resources in mungbean for future breeding programs. Frontiers in Plant Science 6:626-637. doi:10.3389/fpls.2015.00626.

Lodhi, M.A., Ye, G., Weeden, N.F., and Reisch, B.I. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Molecular Biology Reporter 12(1):6-13. doi:10.1007/BF02668658.

Mahanil, S. 2007. Inheritance and cloning of candidate resistance gene analogs (RGAs) for downy mildew in grapevine (*Vitis* spp.) 246 p. PhD thesis. Suranaree University of Technology, School of Crop Production Technology, Nakhon Ratchasima, Thailand.

Mathivathana, M.K., Jagadeeshselvam, N., Madhumitha, B., Karthikeyan, A., Pandiyan, M., Karthikeyan, G., et al. 2018. Screening and identification of SSR markers for genetic diversity for mungbean [*Vigna radiata* (L.) Wilczek]. International Journal of Current Microbiology and Applied Sciences 7(4):789-793. doi:10.20546/ijcmas.2018.704.088.

Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences of the United States of America 88(21):9828-9832. doi:10.1073/pnas.88.21.9828.

Nair, R.M., Pandey, A.K., War, A.R., Hanumantharao, B., Shwe, T., Alam, A.K.M.M., et al. 2019. Biotic and abiotic constraints in mungbean production-progress in genetic improvement. Frontiers in Plant Science 10:1340-1363. doi:10.3389/fpls.2019.01340.

Nie, J., He, H., Peng, J., Yang, X., Bie, B., Zhao, J., et al. 2015. Identification and fine mapping of *pm5.1*: a recessive gene for powdery mildew resistance in cucumber (*Cucumis sativus* L.) Molecular Breeding 35(1):7-19. doi:10.1007/s11032-015-0206-8.

Pandey, A.K., Burlakoti, R.R., Kenyon, L., and Nair, R.M. 2018. Perspectives and challenges for sustainable management of fungal diseases of mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*]: a review. Frontiers in Environmental Science 6:53-67. doi:10.3389/fenvs.2018.00053.

Patel, P., Modha, K., Kapadia, C., Vadodariya, G., and Patel, R. 2018. Validation of DNA markers linked to MYMV resistance in mungbean (*Vigna radiata* (L.) R. Wilczek). International Journal of Pure & Applied Bioscience 6(4):340-346. doi:10.18782/2320-7051.6820.

Patil, P.G., Datta, S., Agbagwa, I.O., Singh, I.P., Soren, K.R., Das, A., et al. 2014. Using AFLP-RGA markers to assess genetic diversity among pigeon pea (*Cajanus cajan*) genotypes in relation to major diseases. Acta Botanica Brasilica 28(2):198-205. doi:10.1590/S0102-33062014000200007.

Pooja, S.K., Sarkale, A.P., and Bhat, S. 2018. Validation of molecular marker associated with powdery mildew resistance in mungbean. Indian Journal of Genetics and Plant Breeding 78(3):386-388. doi:10.31742/IJGPB.78.3.17.

Poolsawat, O., Kativat, C., Arsakit, K., and Tantasawat, P.A. 2017. Identification of quantitative trait loci associated with powdery mildew resistance in mungbean using ISSR and ISSR-RGA markers. Molecular Breeding 37(12):150-161. doi:10.1007/s11032-017-0753-2.

Tantasawat, P.A., Poolsawat, O., Arsakit, K., and Papan, P. 2020. Identification of ISSR, ISSR-RGA and SSR markers associated with Cercospora leaf spot resistance gene in mungbean. International Journal of Agriculture and Biology 23:447-453. doi:10.17957/IJAB/15.1308.

Trick, M., Adamski, N.M., Mugford, S.G., Jiang, C.C., Febrer, M., and Uauy, C. 2012. Combining SNP discovery from next-generation sequencing data with bulked segregant analysis (BSA) to fine-map genes in polyploid wheat. BMC Plant Biology 12(1):14-30. doi:10.1186/1471-2229-12-14.

Vidak, M., Carović-Stanko, K., Barešić, A., Grdiša, M., Šatović, Z., and Liber, Z. 2017. Microsatellite markers in common bean (*Phaseolus vulgaris* L.) Journal of Central European Agriculture 18(4):902-917. doi:10.5513/JCEA01/18.4.1983.

Yi-Shen, Z., Shuai, S., and FitzGerald, R. 2018. Mung bean proteins and peptides: nutritional, functional and bioactive properties. Food and Nutrition Research 62:1290-1300. doi:10.29219/fnr.v62.1290.

Young, N.D., Danesh, D., Menancio-Hautea, D., and Kumar, L. 1993. Mapping oligogenic resistance to powdery mildew in mungbean with RFLPs. Theoretical and Applied Genetics 87:243-249. doi:10.1007/BF00223772.

Zhao, F., Li, D., Tian, X., and Du, W. 2020. QTL mapping for the resistance to yellow rust race CYR34 in triticale. Chilean Journal of Agricultural Research 80:405-412. doi:10.4067/S0718-58392020000300405.