RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 10: 1164-1171 (October 2021)

Association of ISSR and ISSR-RGA Markers with Powdery Mildew Resistance Gene in Mungbean

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10.18805/LR-606

ABSTRACT

Background: Powdery mildew (PM) possesses a wide range of host plants, including mungbean that causes severe yield reductions. Using resistant varieties is an economically and environmentally effective approach in controlling the disease.

Methods: In this study, the genetic basis of inheritance and marker loci for PM resistance were investigated using 126 $F_{2:9}$ recombinant inbred lines (RILs) raised from resistant and susceptible parents, V4785 and CN72, respectively. PM reaction in these RILs was visually scored from natural infection in the field and the segregation pattern was determined by the chi-square test (χ^2).

Result: The results revealed the segregation ration of 1:1, indicative of a dominantly inherited resistance gene controlling resistance against PM in V4785. Observation of potential polymorphism in two parents and two different bulks, each containing 10 RIL individuals showing either the highest resistance or susceptibility, identified 2 and 37 putatively linked inter-simple sequence repeat (ISSR) and ISSR-anchored resistance gene analog (ISSR-RGA) markers, respectively. Of these, 1 ISSR and 3 ISSR-RGA markers were stably inherited and linked to PM resistance (P<0.01). These linked marker systems may prove useful for facilitating the improvement of a durable resistant variety through marker-assisted selection in mungbean breeding programs.

Key words: Inter-simple sequence repeat (ISSR), ISSR-anchored resistance gene analog (ISSR-RGA), Powdery mildew (PM) resistance, *Vigna radiata* (L.) Wilczek.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] serves as an important food legume crop due to its highly nutritious benefits for human health and it is grown over 7 million hectares, mostly in temperate, tropical and subtropical zones of East, South and Southeast Asia (Yu *et al.,* 2011; Nair *et al.,* 2019). In general, its yield potential ranges from 2.5-3.0 t/ha, whereas the total production is low, being about 0.5 t/ha (Nair *et al.,* 2019). Powdery mildew (PM) constitutes one of the major constraints to global mungbean production. *Sphaerotheca phaseoli* is severe biotrophic parasite causing PM disease on the lower and upper leaf surfaces of mungbean, particularly widespread in the cool dry season. Although there has been an attempt to control the disease by fungicides, it is not only responsible for increased costs, but it is also severely harmful to human health and the environment. Alternatively, there has long been interest in developing PM resistant varieties for sustainable management of the disease.

Understandably, plant resistance genes (*R*) are either qualitative or quantitative in nature. Qualitative resistance is monogenic by a single gene or a few genes and typically is race specific, while quantitative resistance is polygenic and race non-specific (Barakat *et al.,* 2008). Previously, our observations revealed that resistance to PM in three Indian mungbean accessions from the World Vegetable Center, Taiwan, namely V4718, V4758 and V4785 showing highly and stable resistance to PM resulted from a single dominant gene with independent segregation and these monogenic resistance genes could be pyramided to develop a resistant variety with broad spectrum resistance (Khajudparn *et al.,* 2010).

Molecular markers tightly linked to any gene of interest which are regarded as steady landmarks have long been reported to be an effective tool for plant breeders to indirectly select promising plants, particularly those obtained from pyramiding multiple resistance genes (Kour *et al.,* 2011). In particular, inter-simple sequence repeat (ISSR) markers which implicate the use of a single SSR-containing primer for direct amplification of sequences at multiple loci are highly efficient, easy, economical and rapid. In many reports, ISSR markers have been more accurate and consistently

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How to cite this article: Tantasawat, P.A., Poolsawat, O., Kativat, C., Arsakit, K., Papan, P., Chueakhunthod, W. and Pookhamsak, P. (2021). Association of ISSR and ISSR-RGA Markers with Powdery Mildew Resistance Gene in Mungbean. Legume Research. 44(10): 1164-1171. DOI: 10.18805/LR-606.

Submitted: 28-12-2020 **Accepted:** 23-06-2021 **Online:** 05-08-2021

polymorphic than randomly amplified polymorphic DNA (RAPD) markers as well as being effective for tagging disease resistance genes (Ali *et al.,* 2011; Bainade *et al.,* 2014; Tantasawat *et al.,* 2020). In addition, resistance gene analog (RGA) markers, which were first reported by Chen *et al.* (1998) are resistance-gene based markers amplified from the highly conserved structures of genes involved in plant defense mechanism. These structures involve recognition of unique avirulence (Avr) factors and then induce signaling and programmed cell death, respectively. It is interesting to note that a large number of *R* genes, which share several conserved domains *i.e.*, leucine-rich repeat (LRR), nucleotide-binding sites (NBS) and protein kinase domain, implicate resistance against a large spectrum of pathogens. These conserved domains have been found in genes isolated from genomes of several plant species *i.e.*, tomato, eggplant and legume crops (Wei *et al.,* 2016; Dev *et al.,* 2018; Tirnaz *et al.,* 2020). Recently, we developed a new type of marker, ISSR-anchored resistance gene analog (ISSR-RGA), using ISSR primers combined with gene-targeted RGA primers to detect the SSRs dispersed at multiple loci in proximity to RGAs throughout the mungbean genome and found that the ISSR-RGA markers are more effective than ISSRs for successfully identifying the gene conferring PM resistance in the 'CN72 × V4718' cross (Poolsawat *et al.,* 2017). Therefore, we extend the uses of ISSR-RGA markers along with ISSR markers for identifying markers associated with another PM resistance gene in the 'CN72 × V4785' cross.

MATERIALS AND METHODS

Plant materials

In this study, population of 126 $F_{2:9}$ recombinant inbred lines (RILs) developed from CN72 and V4785 was used. The donor parent V4785 is highly resistant to PM at all growth stages, while the recipient parent CN72 is popularly grown by Thai farmers and is highly susceptible to PM (Khajudparn *et al.,* 2010).

Phenotypic evaluation

The $F_{2:9}$ RIL population and their parents were planted in a randomized complete block design (RCBD) with three replications during the winter season at Suranaree University of Technology farm, Nakhon Ratchasima, Northeast Thailand in 2015 and 2018. For each plot, seeds were sown in two-meter-long rows with a spacing of 0.5 and 0.2 m between rows and between plants within rows, respectively. Susceptible spreader rows were also planted as the important sources of PM inoculum. The plant reaction to PM was rated in rows at 65 days after planting using a relative scale of 1-9 according to Khajudparn *et al.* (2010). The average scores were divided into two categories (resistant and susceptible individuals with scores rating 1-4.9 and 5-9, respectively). The distribution of PM resistance was assessed with the chi-square test (χ^2). Scores of PM response in both years were transformed with the $(X+1)^{1/2}$ formula for analyzing the broad sense heritability (h^2_b) as described by Khajudparn (2009). The SPSS version 14.0 (Levesque, 2006) was used to determine the relationship of PM resistance in both years through correlation analysis.

ISSR and ISSR-RGA analysis

Young leaf samples of three-week-old plants were collected and DNA isolation was conducted using a modified CTAB extraction protocol (Lodhi *et al.,* 1994). For polymerase chain reaction (PCR) amplification, the concentration and purity of the extracted DNA were determined spectro--photometrically at A_{260} and A_{280} by Nanodrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). A total of 63 ISSR primers developed from the University of British Columbia and 241 ISSR-RGA primer combinations that were derived from 63 ISSR primers and 4 RGA primers complementary to the conserved structures of *R* genes including NBS (GLPLAL 1 and P-Loop; Mahanil *et al.,* 2007) and kinase domains (Pto kin 1; Chen *et al*., 1998 and RLK for; Feuillet *et al.,* 1997) were used to screen two parents (R and S) and two different bulks, each containing 10 RIL informative individuals showing either the highest resistance (resistant bulk; RB) or susceptibility (susceptible bulk; SB), according to bulk segregant analysis (BSA). The sequences of these primers are shown in Tables 1 and 2. PCR and DNA banding visualization for both marker systems were totally performed according to Poolsawat *et al.* (2017). Comparison of DNA banding patterns between R and S as well as RB and SB was carried out to identify putative ISSR and ISSR-RGA loci for the PM resistance.

Marker-trait analysis

For marker genotyping, the clearly resolved DNA profiles were scored manually by assigning '0' and '1' for their absence and presence of each polymorphic band, respectively. The association between the putative marker and PM resistance was analyzed through a simple linear regression using the SPSS version 14.0 (Levesque, 2006) and the logarithm of odd (LOD) analysis. Magnitude of the marker associated phenotypic effects was explained by the coefficient of determination (R^2), of which polymorphism of the marker represented the fraction of variance.

RESULTS AND DISCUSSION

Inheritance information of powdery mildew resistance

The donor parent V4785 was immune to natural PM infection with the disease scores of 1.33 and 1.00 in 2015 and 2018, respectively, while the recipient parent CN72 was highly susceptible to the disease with the disease scores of 6.33 and 6.00 in 2015 and 2018, respectively (Fig 1a and 1b). Among the $F_{2:9}$ RILs consisting of 126 individuals, 61 and 65 individuals exhibited resistant and susceptible response, respectively in 2015, while 53 and 73 individuals exhibited resistant and susceptible response, respectively in 2018.

Table 1: Information of ISSR primers used for identifying powdery mildew resistance.

Primers	Primer sequences	Range of amplified products (bp)	No. of scorable DNA bands	No. of polymorphic bands (male-female parents)	I linked
807	$(AG)_8T$	200-1,500	34	1	0
808	$(AG)_8C$	200-1,500	29	1	1
809	$(AG)_8G$	200-1,500	29	1	0
810	$(GA)_8T$	200-1,500	22	2	0
811	$(GA)_8C$	250-1,200	20	0	0
812	$(GA)_8A$	200-1,500	29	1	0
813	$(CA)_8T$	300-2,072	14	0	0
814	$(CA)_8A$	300-1,500	12	0	0
815	$(CT)_8G$	200-1,500	17	1	0
816	$(CA)_8T$	200-1,500	19	1	0
817	$(CA)_8A$	200-1,500	16	0	0
818	$(CA)_8G$	200-1,500	22	0	0
819	$(GT)_8A$	200-1,500	19	1	0
820	$(GT)_8C$	200-1,500	12	2	0
821	$(GT)_8T$	500-1,500	8	0	0
823	$(TC)_8C$	200-1,200	13	1	0
825	$(AC)_8T$	200-1,500	21	3	0
826	$(AC)_8C$	200-1,500	15	0	0
827	$(AC)_8G$	200-1,500	18	2	1
828	$(TG)_8A$	200-1,500	14	1	0
829	$(TG)_8C$	200-1,500	23	5	0
830	$(TG)_8G$	200-1,500	25	3	0
834	$(AG)_8YT$	200-1,500	22	2	0
835	$(AG)_8YC$	200-1,200	25	3	0
836	$(AG)_8YA$	200-1,500	21	0	0
840	$(GA)_8YT$	200-1,500	25	0	0
841	$(GA)_8YC$	200-1,500	22	1	0
841c	$(GA)_8CC$	200-1,500	27	2	0
841t	$(GA)_8TC$	200-1,200	19	1	0
842	$(GA)_8YG$	200-1,200	24	0	0
844	$(CT)_8RC$	500-2,072	10	0	0
846	$(CA)_8AT$	200-2,072	21	6	0
847	$(CA)_8RC$	200-2,072	18	3	0
848	$(CA)_8RG$	200-1,500	21	2	0
849	$(GT)_8YA$	200-2,072	20	2	0
850	$(GT)_8YC$	300-2,072	14	1	0
851	$(GT)_8YG$	200-1,500	15	2	0
853	$(TC)_8RT$	300-2,072	15	0	0
855	$(AC)_8YT$	200-2,072	22	0	0
856	$(AC)_8YA$	200-1,500	18	0	0
857	$(AC)_8YG$	200-1,500	24	1	0
858	$(TG)_8RT$	200-2,072	20	0	0
859	$(TG)_8RC$	200-2,072	22	1	0
860	$(TG)_8RA$	200-2,072	21	0	0
862	$(AGC)_6$	300-1,500	11	0	0
864	$(ATG)_6$	200-2,072	23	1	0
866	$(CTC)_6$	200-2,072	18	0	0
867	$(GGC)_6$	200-2,072	11	0	0
868	$(GAA)_6$	200-2,072	29	0	0

Table 1: Continue...

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873	$(GACA)_4$	200-1,500	17	1	0
876	$(GATA)_2(GACA)_2$	200-1,500	23	4	0
878	$(GGAT)_4$	200-2,072	16	1	0
880	$(GGAGA)_3$	200-2,072	19	0	0
881	$(GGGT)_3G$	600-2,072	10	0	0
884	HBH $(AG)_7$	200-2,072	28	3	0
885	BHB $(GA)_7$	200-2,072	25	1	0
886	VDV $(CT)_7$	200-2,072	27	3	0
887	DVD $(TC)_7$	200-2,072	21	1	0
888	BDB $(CA)_7$	200-800	22	0	0
889	DBD $(AC)_7$	200-1,500	29	1	0
890	VHV $(GT)_7$	200-1,500	26	2	0
891	HVH $(TG)_7$	200-1,500	21	2	0
900	$ACTTCCC(CA)_2GGTTAA(CA)_2$	600-2,072	7	2	0
Total			1,260	75	2
Average			20	1.19	0.03

B: C, G, T; D: A, G, T; H: A, C, T; N: A, G, C, T; R: A, G; V: A, C, G; Y: C, T.

I linked: ISSR loci associated with powdery mildew resistance.

The disease scores of both years were significantly correlated (r = 0.61, $P<0.001$). The χ^2 test revealed that the population phenotypically segregated into a ratio of 1:1 (resistant/susceptible) in both years (χ^2 = 0.13 and 3.17, respectively, $P_{0.05}$ = 3.84). The estimates of broad sense heritability for PM resistance in 2015 and 2018 were 79.57 and 89.36%, respectively. The results demonstrated that the PM resistance in V4785 was due to a single major gene. This inheritance pattern was also previously observed by Khajudparn *et al.* (2010) who reported that three mungbean resistant lines, namely V4718, V4758 and V4785 inherited PM resistance genes as single genes with nonallelic interaction. In addition, many studies also reported that dominant gene actions implicated in inheritance of PM resistance in different sources of mungbean (Reddy *et al*., 1994; Humphry *et al*., 2003). On the other hand, Kasettranan *et al*. (2010) found that PM resistance was quantitatively inherited in F_7 population of the 'KPS1 × VC6468-11-1A' cross. Therefore, it is important to note that PM resistance in various resistant sources may be inherited differently.

ISSR and ISSR-RGA analysis

To identify marker loci for the PM resistance gene, a total of 63 ISSR primers and 241 ISSR-RGA primer combinations were screened in BSA. It was found that the 63 ISSR primers amplified a total of 1,260 DNA bands with an average of 20 bands/ primer. Of these, 75 were found to be polymorphic between CN72 and V4785, giving a polymorphism percentage of 5.95. Only 40 of 63 primers gave polymorphic bands between parents with percentages of polymorphism ranging from 2.94 to 28.57% and two of these polymorphic bands from ISSR 808 and 827 were identified as the putatively linked marker loci for PM resistance (Table 1). The 63 ISSR primers were used again to combine with 4 RGA primers for identification of the PM resistance gene. The results revealed that 241 ISSR-RGA primer combinations could produce clear bands. However, only 28 out of 241 primer combinations, which amplified a total of 810 DNA bands with an average of 28.93 bands/ primer pair produced polymorphic DNA bands. In total, 96 polymorphic DNA bands were observed with an average of 3.43 bands/ primer pair and 11.85 polymorphism percentage (Table 2). Among these polymorphic loci, the ISSR and ISSR-RGA marker-based BSA revealed 2 and 37 markers, respectively, whose specific bands were exclusively present in resistant parent and resistant bulk/or susceptible parent and susceptible bulk, indicating their possible association with the PM resistance gene. BSA was proved to be a very useful tool for rapid identification of marker loci linked to any disease resistance genes (Uma *et al.,* 2016). To identify more closely linked markers, screening of more primers is required to obtain sufficient polymorphism in the target region through BSA. In this study, screening of 63 ISSR primers and 241 ISSR-RGA primer combinations was rapidly conducted to identify more closely linked markers using BSA and we found that 2 ISSR and 37 ISSR-RGA markers were putatively associated with the PM resistance. Using this analysis, Bainade *et al.* (2014) also successfully identified a marker from ISSR 834 that was associated with the PM resistance gene in the 'Kopargaon × BPMR-48' cross of mungbean.

Considering 2 ISSR and 37 ISSR-RGA markers as putatively associated with PM resistance from BSA, all of these markers were subsequently tested using simple linear regression and LOD analysis. One out of two ISSR markers (I27565; $P<0.01$) and three out of 37 ISSR-RGA markers (I10P321, I27R211 and I27R565; $P<0.01$) were significantly

Table 2: Information of ISSR-RGA primer combinations used for identifying powdery mildew resistance.

Primers and sequences				Range of amplified products (bp)	No. of scorable DNA bands	No. of polymorphic bands (male-female parents)	I linked
ISSR	Sequence* 5′-3′	RGA	Sequences 5′-3′				
807	$(AG)_8T$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-2,072	35	9	2
811	$(GA)_8C$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-1,200	22	3	1
827	$(AC)_8G$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-2,072	26	3	1
840	$(GA)_8YT$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-1,500	26	3	1
841	$(GA)_8YC$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-1,500	27	1	1
849	$(GT)_8YA$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-2,072	25	4	1
856	$(AC)_8YA$	GLPLAL 1	IAGIGCIAGIGGIAGICC	200-1,200	19	3	1
866	$(CTC)_6$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-2,072	35	7	1
884	HBH $(AG)_7$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-2,072	34	7	1
889	DBD $(AC)_7$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-1,500	35	2	2
891	HVH $(TG)_7$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-1,500	34	2	1
900	ACTTCCC $(CA)_2$GGTTAA$(CA)_2$	GLPLAL 1	IAGIGCIAGIGGIAGICC	150-1,500	29	2	1
810	$(GA)_8T$	Pto kin 1	GCATTGGAACAAGGTGAA	200-1,500	25	2	1
842	$(GA)_8YG$	Pto kin 1	GCATTGGAACAAGGTGAA	200-1,500	29	2	1
851	$(GT)_8YG$	Pto kin 1	GCATTGGAACAAGGTGAA	200-1,500	23	2	1
860	$(TG)_8RA$	Pto kin 1	GCATTGGAACAAGGTGAA	200-1,500	28	2	1
827	$(AC)_8G$	P-Loop	$(GGI)_2$GTIGGIAAIACIAC	150-1,500	30	4	1
834	$(AG)_8YT$	P-Loop	$(GGI)_2$GTIGGIAAIACIAC	200-1,200	25	3	1
835	$(AG)_8YC$	P-Loop	$(GGI)_2$GTIGGIAAIACIAC	200-1,200	28	3	2
856	$(AC)_8YA$	P-Loop	$(GGI)_2$GTIGGIAAIACIAC	150-1,500	27	4	1
873	$(GACA)_4$	P-Loop	$(GGI)_2$GTIGGIAAIACIAC	200-1,500	18	2	1
827	$(AC)_8G$	RLK for	GAYGTNAARCCIGARAA	150-2,072	38	3	2
828	$(TG)_8A$	RLK for	GAYGTNAARCCIGARAA	150-2,072	18	3	1
829	$(TG)_8C$	RLK for	GAYGTNAARCCIGARAA	200-2,072	31	7	1
835	$(AG)_8YC$	RLK for	GAYGTNAARCCIGARAA	150-1,500	34	5	3
840	$(GA)_8YT$	RLK for	GAYGTNAARCCIGARAA	150-1,500	36	11	2
841	$(GA)_8YC$	RLK for	GAYGTNAARCCIGARAA	150-2,072	37	6	1
857	$(AC)_8YG$	RLK for	GAYGTNAARCCIGARAA	150-2,072	36	3	3
Total					810	96	37
Average					28.93	3.43	1.32

B: C, G, T; D: A, G, T; H: A, C, T; N: A, G, C, T; R: A, G; V: A, C, G; Y: C, T.

I linked: ISSR-RGA loci associated with powdery mildew resistance.

associated with the PM resistance gene that a coefficient of determination (R^2) of 0.560 for the I27565 marker and 0.479, 0.723 and 0.560 for the I10P321, I27R211 and I27R565 markers, respectively was found (Table 3). However, the I27565 and I27R565 markers localized on the same locus and all four markers (I27565, I10P321, I27R211 and I27R565) resided on the same side relative to the PM resistance gene. Three of these markers had LOD scores

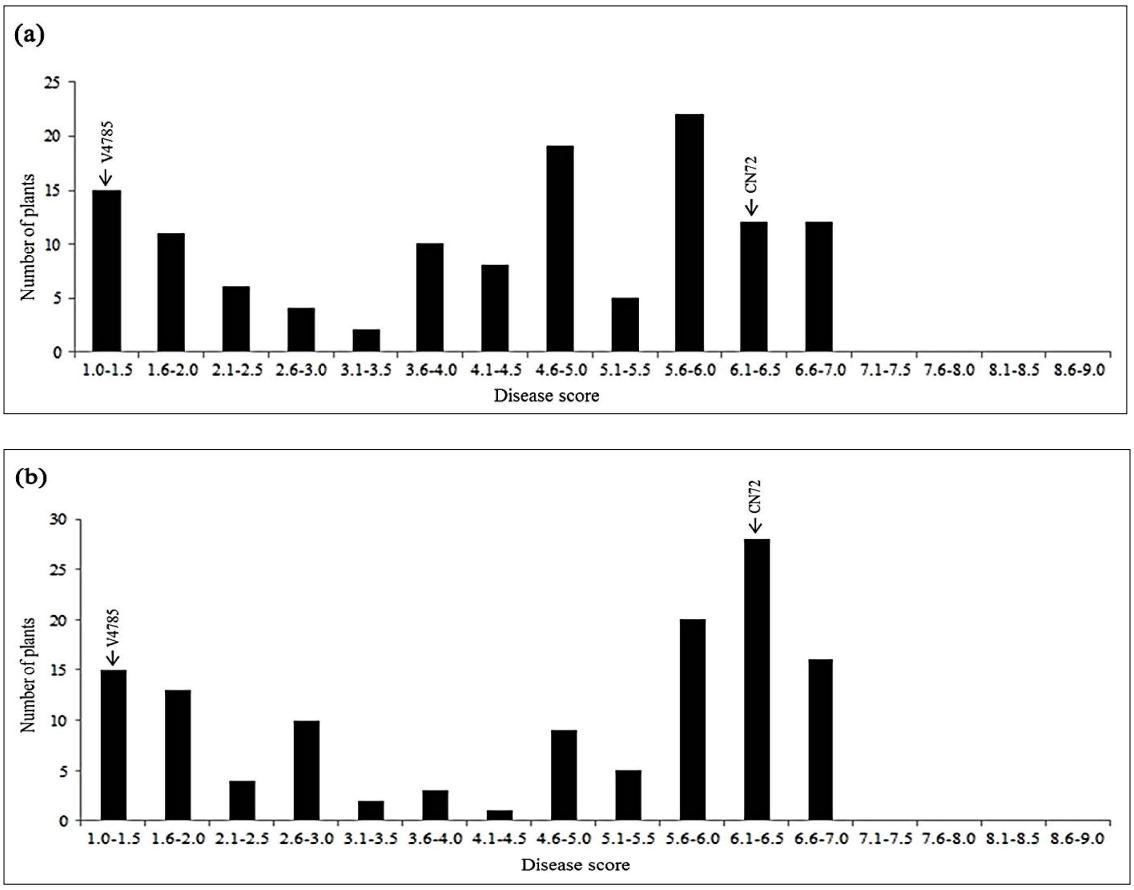

Fig 1: Frequency distribution of disease scores for response to powdery mildew of mungbean in the $F_{2:9}$ RILs population of 'CN72 × V4785' cross, evaluated in 2015 (a) and 2018 (b).

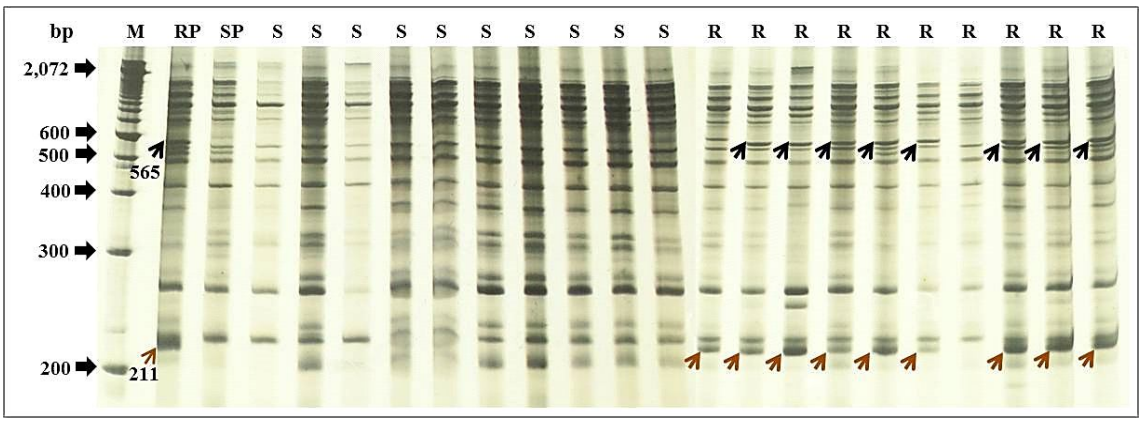

Fig 2: DNA profiles from ISSR 827 combined with RLK for amplification of resistant parent (RP; V4785), susceptible parent (SP; CN72), susceptible RIL (S) and resistant RIL (R); M = 100 bp DNA ladder. Black and brown arrows show I27R565 and I27R211 markers putatively linked to powdery mildew resistance gene, respectively.

Table 3: Simple linear regression, recombination and LOD score of ISSR and ISSR-RGA marker loci linked to powdery mildew resistance.

Markers	Beta	*t*-value	*P*-value	R^2	Position (cM)	LOD
I27565	-0.748	-4.786	0.000	0.560	10	3.197
I10P321	-0.692	-4.070	0.001	0.479	15	2.349
I27R211	-0.850	-6.664	0.000	0.723	5	4.018
I27R565	-0.748	-4.786	0.000	0.560	10	3.197

R^2: Coefficient of determination explained by the simple linear regression.
LOD: Log of odd score.

of above 3, passing threshold after permutation. The I27R211 and I27R565 markers in Fig 2, which were amplified by the same primer pair (ISSR827 combined with RLK for) and were scored simultaneously were closest to the PM resistance gene (5 and 10 cM, respectively). The position of both markers (I27R211 and I27R565) in the current 'CN72 × V4785' cross studied was different from those of I42PL229 and I85420 markers flanking the PM resistance gene in the 'CN72 × V4718' cross of mungbean (Poolsawat *et al.,* 2017), confirming the nonallelic nature of the PM resistance genes in V4718 and V4785. These results suggest the possibility of developing a variety with broad spectrum resistance to PM through marker-assisted gene pyramiding in mungbean breeding programs.

CONCLUSION

The donor resistant line V4785 has conferred a qualitative resistance for PM through a single dominant gene. The I27R211 and I27R565 ISSR-RGA markers closest to this resistance gene will be applicable for MAS of a PM resistant mungbean that allow pyramiding of multiple PM resistance genes to provide more durable and sustainable resistance in the future. Moreover, we also verify the usefulness of ISSR-RGA marker system for tagging a disease resistance gene.

ACKNOWLEDGEMENT

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 V4785 and CN72, respectively. We are also thankful to Mr. Peter C. Bint for proofreading the manuscript.

REFERENCES

Ali, Z., Xu, Z.L., Zhang, D.Y., He, X.L., Bahadur, S. and Yi, J.X. (2011). Molecular diversity analysis of eggplant (*Solanum melongena*) genetic resources. Genetics and Molecular Research. 10(2): 1141-55.

Bainade, P.S., Kale, A.A., Kumbhar, S.D. and Deshmukh, S.G. (2014). Inter simple sequence repeats (ISSR) based polymorphism for powdery mildew resistance in green gram [*Vigna radiata* (L.) Wilczek]. Journal of Cell and Tissue Research. 14(3): 4547-50.

Barakat, M.N., Milad, S.I., El-Shafei, A.M. and Khatab, S.A. (2008). Genetic analysis and identification of RAPD markers linked to northern corn leaf blight disease resistance in a white maize population. Journal of King Abdulaziz University-Meteorology Environment and Arid Land Agriculture Sciences. 20(1): 45-61.

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: 345-55.

Dev, S.S., Poornima, P. and Venu, A. (2018). Molecular characterization and phylogenetic analysis of *NBS-LRR* genes in wild relatives of eggplant (*Solanum melongina* L.). Indian Journal of Agricultural Research. 52: 167-71.

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: 45-52.

Humphry, S.M.E., Magner, T., McIntyre, C.L., Aitken, E.A. and Liu, C.L. (2003). Identification of major locus conferring resistance to powdery mildew (*Erysiphe polygoni* D.C.) in mungbean [*Vigna radiata* (L.) Wiczek] by QTL analysis. Genome. 46, 738-44.

Kasettranan, W., Somta, P. and Srinives, P. (2010). Mapping of quantitative trait loci controlling powdery mildew resistance in mungbean [*Vigna radiata* (L.) Wilczek]. Journal of Crop Science and Biotechnology. 13(3): 155-61.

Khajudparn, P. (2009). Characters associated with yield potential and development of molecular markers for powdery mildew resistance in mungbean. Ph.D. Thesis, Suranaree University of Technology, 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.

Kour, G., Bakshi, P., Wali, V.K. and Jastotia, A. (2011). Role of molecular markers in some perennial crops-brief review. Agricultural Reviews. 32(4): 256-67.

Levesque, R. and SPSS Inc. (2006). SPSS Programming and Data Management, 3rd Edn. SPSS Institute, Somers, New York.

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. 12: 6-13.

Mahanil, S., Reisch, B.I., Owens, C.L., Thipyapong, P. and Laosuwan, P. (2007). Resistance gene analogs from *Vitis cinerea*, *Vitis rupestris* and *Vitis* hybrid Horizon. American Journal of Enology and Viticulture. 58: 484-93.

Nair, R.M., Pandey, A.K., War, A.R., Rao, B.H., Shwe, T., Alam A., Pratap, A., *et al.* (2019). Biotic and abiotic constraints in mungbean production-progress in genetic improvement. Frontiers in Plant Science. 10: 1340-63.

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(150): 150-61.

Reddy, K.S., Pawar, S.E. and Bhatia, C.R. (1994). Inheritance of powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean [*Vigna radiata* (L.) Wilczek]. Theoretical and Applied Genetics. 88: 945-48.

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: 447-53.

Tirnaz, S., Zhang, Y. and Batley, J. (2020). Genome-wide mining of disease resistance gene analogs using conserved domains. Methods and Protocols. 2107: 365-75.

Uma, M.S., Hegde, N. and Hittalmani, S. (2016). Identification of SSR marker associated with rust resistance in cowpea (*Vigna unguiculata* L.) using bulk segregant analysis. Legume Research. 39: 39-42.

Wei, C., Chen, J. and Kuang, H. (2016). Dramatic number variation of *R* genes in Solanaceae species accounted for by a few *R* gene subfamilies. PLoS ONE. 11(2): e0148708.

Yu, S.C., Lin, P.C. and Lin, J. (2011). Effects of Extrusion Processing Condition on the Physic-chemical Properties of Mungbean Extrudates. In: Book of Proceedings of the 12th Asian Food Conference 2011, Bangkok. pp 524-526.