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RESEARCH ARTICLE

Development of pyramided mung bean lines carrying resistance genes for Cercospora leaf spot disease and bruchids

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ABSTRACT

Cercospora leaf spot (CLS) disease and bruchids are the common disease and insect pest causing yield loss in mung bean (*Vigna radiata* (L.) R. Wilczek). 'KUML4' is an improved mung bean cultivar grown in Thailand having high yield, early and highly synchronous maturity, large seed size, but susceptible to CLS disease and bruchids. This study was conducted to improve 'KUML4' through introgression of *VrTAF5* for CLS resistance and *VrPGIP2* for bruchid (*Callosobruchus maculatus*) resistance from landrace mung bean accessions 'V4718' and 'V2808', respectively, by marker-assisted backcrossing (MABC). In the MABC, foreground selections were performed using *VrTAF5*- and *VrPGIP2*-specific markers. The BC_3F_3 pyramided lines with KUML4 background carrying both *VrTAF5* and *VrPGIP2* genes were developed and evaluated for agronomic and yield-related traits, CLS resistance, and bruchid resistance. Three BC_3F_3 lines, KUML4-2020-21, KUML4-2020-23, and KUML4-2020-60, exhibiting superior agronomic and yield-related traits, CLS resistance, and bruchid resistance than 'KUML4' were successfully obtained via MABC. Under the CLS outbreak, these lines showed 248.5%-331.7% higher seed yield than 'KUML4'. The lines expressed nearly perfect resistance to bruchid (< 5% seeds damaged). These lines can be developed into new resistant mung bean cultivars in the future.

Key words: Bruchid, *Callosobruchus maculatus*, Cercospora, gene pyramiding, marker-assisted backcrossing, *Vigna radiata*.

INTRODUCTION

Mung bean (*Vigna radiata* (L.) R. Wilczek) is an important legume crop widely grown in Asia. The crop is gaining popularity in several countries outside Asia. Mung bean seeds contain about 20%-25% protein and 60%-65% carbohydrate and are used widely, such as in soups, cakes, sweets, snacks, and noodles, or processed into sprouts, flour and starch (Nair and Schreinemachers, 2020). In addition, mung bean seeds are used to produced plant-based meats and egg alternatives and replacements. The demand for mung bean and its cultivation area have increased in the recent years (Nair and Schreinemachers, 2020). However, average yield of mung bean is low due partially to disease and insect pests. Cercospora leaf spot (CLS) caused by the fungus *Cercospora canescens* Illis & Martin is a common disease of mung bean that can reduce seed yield by up to 50% (Nair et al., 2019). Bruchids including azuki bean weevil (*Callosobruchus chinensis* L.) and cowpea weevil (*Callosobruchus maculatus* F.) are a serious insect pest of mung bean during storage. These insects can cause the complete loss of a seed lot within a few months (Zhang et al., 2021). Although these disease and pest can be controlled by synthetic chemicals, the practice increases farmers' production costs and has adverse effects on human health

and the environment. To solve these problems, developing resistant varieties is the most desirable strategy to manage the disease and pest in an economical and environmentally friendly way. Nowadays, farmers, consumers, and processors prefer large-seeded mung bean (Somta et al., 2024). However, although the mung bean genetic resources for CLS resistance and bruchid resistance are available and the genetics of the resistance of these biotic stresses are not complex, the progress in development of CLS- and bruchid-resistant cultivar(s) is slow (Laosatit et al., 2020). Due to the fact that CLS disease is principally widespread only in the warm-wet season, so, phenotypic selection for the CLS resistance can be conducted only one time per year. While the bruchid resistance evaluation takes at least 50-60 d. Therefore, selection time for the bruchid resistance in a single generation is relatively long, being about 130-140 d (including seed production) (Laosatit et al., 2020).

Marker-assisted backcrossing (MABC), a simple form of marker-assisted selection (MAS), is widely applied in molecular breeding (Lübberstedt et al., 2023). The MABC overcomes the limitations of conventional breeding and allows the pyramiding of multiple valuable genes into a single genetic background in the shortest possible time (Lübberstedt et al., 2023). Mung bean accession V4718 possessed high resistance to CLS disease and the resistance is controlled by a single major QTL, *qCLS* (Chankaew et al., 2011; Yundaeng et al., 2021). Yundaeng et al. (2021) identified *VrTAF5* encoding TATA-binding-protein associated factor 5 as the gene responsible for the CLS resistance in V4718. They also developed insertion/deletion (InDel) marker VrTAF_Indel3 to detect a 24-bp InDel polymorphism in the *VrTAF5* gene. Mung bean accession V2802 showed a nearly complete resistance to the bruchids and the resistance is controlled by a single dominant gene, *Br*, with modifiers (Zhang et al., 2021). Chotechung et al. (2016) showed that *VrPGIP2* encoding a polygalacturonase-inhibiting protein 2 (PGIP2) is the gene responsible for the bruchid resistance in V2802 and that simple sequence repeat (SSR) marker DMB-SSR158 perfectly co-segregated with the *VrPGIP2* gene. Therefore, the aim of this study was to improve the KUML4 through introgression of *VrTAF5* for CLS resistance and *VrPGIP2* for bruchid resistance from mung bean accessions 'V4718' and 'V2808', respectively, by marker-assisted backcrossing.

MATERIALS AND METHODS

Plant materials

Mung bean (*Vigna radiata* (L.) R. Wilczek) 'KUML4' and 'Chai Nat 84-1' (hereafter called 'CN84-1') were used as the recurrent parents, while the mung bean accessions V4718 and V2802 were used as donor parents for the genes *VrTAF5* and *VrPGIP2*, respectively. KUML4 and CN84-1 are commercial cultivars of Thailand that are susceptible to Cercospora leaf spot (CLS) disease and bruchids.

Marker-assisted backcrossing of 'KUML4'

Scheme for improving resistance to CLS disease and bruchids in 'KUML4' by marker-assisted backcrossing (MABC) is shown in Figure 1. Initially, two independent crosses, CN84-1 × V4718 and CN84-1 × V2802, were made to generate F_1 hybrids. The hybridity of the F_1 plants of these crosses were confirmed by the genic markers VrTAF_Indel3 (Table 1) and DMB-SSR158 (Table 1), respectively. The true F_1 plants were backcrossed with recurrent parents to generate BC_1F_1 populations. In each cross, the BC_1F_1 plants having resistance allele (*VrTAF5* or *VrPGIP2* allele) from the donor parent were selected by marker-assisted selection (MAS). Then the selected BC_1F_1 plants were backcrossed to generate BC_2F_1 populations, and this procedure was repeated till BC_4F_1 populations. After that, BC_4F_1 plants possessing *VrTAF5* or *VrPGIP2* genes were selected by MAS and intercrossed to generate F_1 hybrids. Subsequently, the F_1 plants possessing resistance alleles of both *VrTAF5* and *VrPGIP2* genes were selected and self-pollinated to generate F_2 population. The F_2 population was evaluated for CLS resistance under field conditions and was detected for the *VrTAF5* and *VrPGIP2* genes by MAS. The F_2 plants showing homozygous *VrTAF5*, homozygous or heterozygous *VrPGIP2* genes and CLS resistance under the field conditions were selected and self-pollinated to generate $F_{2:3}$ population. The $F_{2:3}$ seeds produced from each of the selected F_2 plants were evaluated bruchid resistance (Kaewwongwal et al., 2020; see also below). The $F_{2:3}$ seeds showing bruchid resistance were planted and self-pollinated to generate $F_{2:4}$ seeds. The best pyramided line expressing good agronomic characters, high yield, CLS resistance and bruchid resistance was selected and named as KUML2018-32(22) (Figure 1).

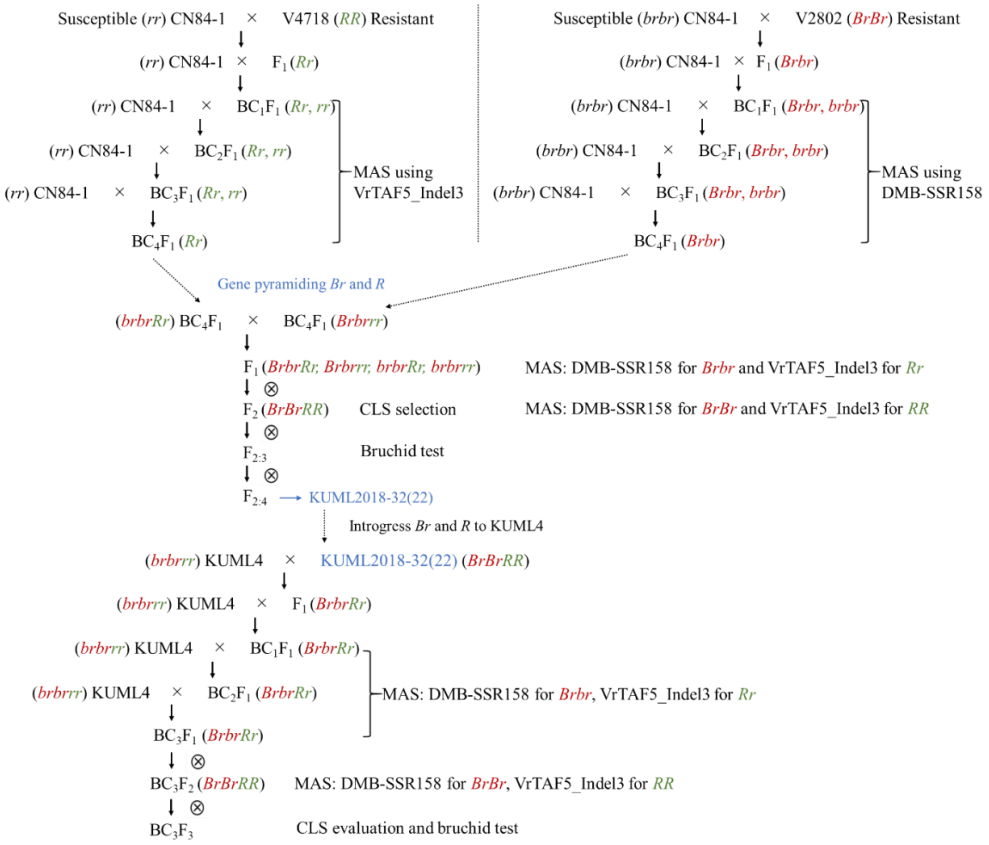

Figure 1. Breeding scheme for developing mung bean pyramided lines for bruchid and Cercospora leaf spot (CLS) resistance. *RR* and *Br* represent *VrTAF5* and *VrPGIP2* genes, respectively.

Table 1. Details of molecular markers used for mung bean foreground selection. CLS: Cercospora leaf spot.

			Sequence	
Marker name	Gene	Trait	Forward (5'-3')	Reverse (5'-3')
VrTAF5_indel3	*VrTAF5*	CLS resistance	CTCATGAAACCTGGAGAACT	CCCAGTGTACTCAGTTTGACTT
DMB-SSR158	*VrPGIP2*	Bruchids resistance	TGGAAAATTTGCAGCAGTTG	ATTGATGGAGGGCGGAAGTA

To improve the CLS resistance and bruchid resistance in 'KUML4', the line KUML2018-32(22) was used as the donor parent to transfer the *VrTAF5* and *VrPGIP2* genes into 'KUML4' (Figure 1). The line KUML2018-32(22) was crossed with 'KUML4' to create F_1 population. True F_1 plants were confirmed using the markers VrTAF5_Indel3 and DMB-SSR158 and backcrossed to 'KUML4' to generate BC_1F_1 population. The BC_1F_1 hybrids were screened for both targeted genes using markers VrTAF5_Indel3 and DMB-SSR158 and followed by background selection with polymorphic SSR markers. A BC_1F_1 plant with *VrTAF5* and *VrPGIP2* alleles and maximum genome recovery of 'KUML4' was selected and backcrossed to generate BC_2F_1, and this procedure was repeated till BC_3F_1. Then, a BC_3F_1 plant possessing both targeted genes and maximum genome recovery of 'KUML4' was selected and self-pollinated to generate BC_3F_2 population. In BC_3F_2 population, the homozygous plants for *VrTAF5* and heterozygous plants for *VrPGIP2* genes were identified through foreground selection and followed by background selection and gave a good yield-related trait then self-pollinated to generate BC_3F_3 population. The BC_3F_3 seeds produced from each of the selected BC_3F_2 were individually harvested and used to evaluate bruchid resistance. In addition, the BC_3F_3 plants were evaluated for agronomic traits, seed yield, CLS resistance and bruchid resistance under field conditions.

Molecular marker analysis for MAS

For the MAS, total genomic DNA of each plant was extracted from the young leaves using a cetyltrimethylammonium bromide (CTAB) method. Foreground selection (*VrTAF5* and *VrPGIP2*) was conducted using VrTAF_Indel3 (Yundaeng et al., 2021) and DMB-SSR158 (Chotechung et al., 2016), respectively (Table 1). Polymerase chain reaction (PCR), gel electrophoresis, and DNA band visualization were carried out as described previously (Chotechung et al., 2016).

Evaluation of agronomic trait, yield and CLS resistance under field condition

The BC_3F_3 lines, 'KUML4', V4718, V2802, CN84-1 and 'Chai Nat 3' (hereafter called CN3) were planted in a randomized complete block design with three replicates from August to October (rainy season) in 2023 at an experimental field of Kasetsart University, Kamphaeng Saen Campus (14°1'28" N, 99°58'25" E), Nakhon Pathom, Thailand. The CN3 was included in the evaluation because it is currently popular grown by Thai farmers. Each entry was sown in a single row 3 m long with 12.5 cm intra-row and 50 cm inter-row spacing, and two plants per hill (48 plants row^{-1}). Inoculation and resistance evaluation of *Cercospora canescens* were conducted following the procedures of Yundaeng et al. (2021). In brief, the plants were inoculated by being sprayed with spore suspensions of *C. canescens* (104 spores per mL) at 20, 25, and 30 d after planting (DAP). Sixty days after planting, eight plants in each entry were randomly selected and scored for CLS reaction using a scale of 1-5, where 1 is no visual disease infection, 2 is 1%-25% of leaf area infected, 3 is 26%-50% of leaf area infected, 4 is 51%-75% of leaf area infected, and 5 is 76%-100% of leaf area infected. The average scores for each row were used for statistical analyses. The selected plants were also recorded for days to first flowering (DFF), days to first pod maturity (DFM), plant height at maturity (PH; cm), number of branches (BPP), number of pods (PPP), number of seeds per pod (SPP), 100-seed weight (100SDW; g), and seed yield per plant (SDYP; g).

Bruchid resistance evaluation

Bruchid resistance evaluation was conducted as per the procedures described by Kaewwongwal et al. (2020). In brief, a population of *Callosobruchus maculatus* was reared on seeds of 'KUML4' at 28 °C and 60% relative humidity. Fifty seeds from each plant were placed in a plastic box. Then, 10 pairs (10 males and 10 females) of newly emerged adult insects were introduced into the box, allowed to lay eggs for 7 d, and then removed. The infested seeds were maintained at 28 °C and 60% relative humidity. The numbers of seeds damaged by the bruchids (seeds with hole(s)) were counted at 60 d after insect introduction and converted into percentage of damaged seeds (PDS).

Statistical analysis

The agronomic traits, CLS disease score and PDS caused by the bruchids of the BC_3F_3 pyramided lines, parents, and controls were subjected to ANOVA using R-Program (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Mean separations were carried out by Duncan's multiple range test (DMRT) using the R-Program. Compared to the recurrent parents, improved lines with resistance to bruchid and Cercospora leaf spot disease, early maturity, large seed size, and high seed yield per plant were selected as superior lines.

RESULTS AND DISCUSSION

Pyramiding of *VrTAF5* and *VrPGIP2* in the background of 'KUML4'

An improved KUML2018-32(22) line was developed and used as the donor parent to transfer the *VrTAF5* and *VrPGIP2* genes into 'KUML4'. The background genome of the KUML2018-32(22) line was CN84-1 with introgressed *VrTAF5* and *VrPGIP2* genes from V4718 and V2802, respectively. The F_1 hybrids of the cross 'KUML4' × KUML2018-32(22) were analyzed with functional/linked molecular markers of the *VrTAF5* and *VrPGIP2* genes, where two true hybrid plants were backcrossed with 'KUML4' to generate the BC_1F_1 population. Seven out of 70 BC_1F_1 plants possessing heterozygous *VrPGIP2* and *VrTAF5* genes were identified by the markers VrTAF5_Indel3 and DMB-SSR158 and backcrossed to 'KUML4' to produce BC_2F_1. In BC_2F_1 population, 14 out of 182 plants were found to be heterozygous at the *VrTAF5* and *VrPGIP2* genes by DNA marker analysis and three plants showing highest phenotypic similarity with 'KUML4' were backcrossed to produce BC_3F_1 population. In BC_3F_1 population, 34 out of 174 plants were heterozygous at the *VrTAF5* and *VrPGIP2* genes. However, 32 plants

expressing greatest phenotypic resemblance to 'KUML4' were selected and self-pollinated to generate BC_3F_2 population. In the BC_3F_2 population, plants possessing the homozygous *VrTAF5* and homozygous/heterozygous *VrPGIP2* genes with phenotypic traits and seed yield at par with 'KMUL4' were selected through MAS and phenotypic selection (Figure 2). In total, 10 best BC_3F_2 plants were selected (Table 2) and self-pollinated to produce BC_3F_3 lines.

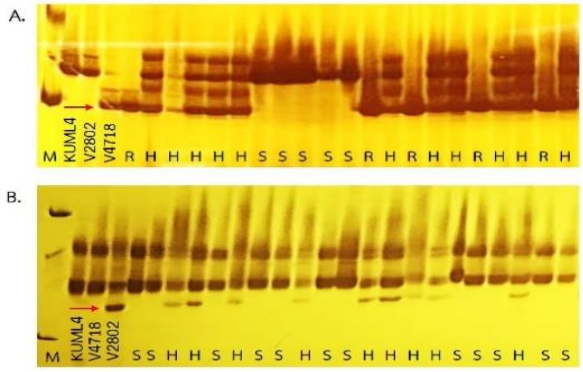

Figure 2. Foreground selection for *VrTAF5* (A) and *VrPGIP2* (B) among BC_3F_2 plants. M: 100 bp ladder; R: resistant; H: heterozygote; S: susceptible; arrow: target gene allele.

Table 2. Yield-related traits of selected pyramided lines at BC_3F_2 generation. PPP: Number of pods per plant; PL: pod length; SPP: number of seeds per pod; 100SDW: 100-seed weight; SDYP: seed yield per plant.

Line/cultivar	PPP	PL	SPP	100SDW	SDYP
	Nr plant^{-1}	cm	Nr pod^{-1}	g	g
KUML4-2020-14	29	11.33	10.7	7.60	21.60
KUML4-2020-20	14	11.81	11.9	8.10	12.90
KUML4-2020-21	26	10.37	10.9	8.44	15.48
KUML4-2020-23	27	11.01	11.2	7.60	16.57
KUML4-2020-24	32	10.66	11.0	7.36	17.00
KUML4-2020-26	47	11.03	11.5	7.50	27.46
KUML4-2020-32	21	11.23	11.8	8.00	17.47
KUML4-2020-58	32	10.41	11.3	8.58	23.78
KUML4-2020-60	27	10.66	11.4	8.34	19.38
KUML4-2020-62	20	10.47	11.3	7.04	16.38
KUML4	19.85	9.96	10.56	8.29	13.64
CN3	18.70	9.81	10.50	7.67	12.93
CN84-1	19.50	10.23	11.38	7.66	13.79
V2802	33.00	9.43	11.26	4.76	12.67
V4817	31.40	6.92	13.04	2.84	10.23

Evaluation of pyramided lines for agronomic and yield-related traits

Ten selected BC_3F_3 lines together with 'KUML4', V4718, V2802, CN84-1, and CN3 were evaluated for agronomic traits, yield and yield-related traits, CLS resistance and bruchid resistance. The ANOVA revealed that these entries were significantly different for all of the traits except number of branches per plant (Table 3). However, in most cases, the BC_3F_3 lines performed better than its recurrent parent ('KUML4') and the improved cultivars (CN84-1 and CN3). The days to first flowering of the BC_3F_3 lines ranged from 38.33 to 41.00 which was not significantly different from 'KUML4' and the improved cultivars CN84-1 and CN3 (Table 3). Similarly, days to first pod maturity of all the BC_3F_3 lines except the line KUML4-2020-23 was not significantly different from 'KUML4' and other improved cultivars (Table 3). Although most of the BC_3F_3 lines showed similar flowering time and maturity time with 'KUML4', all of them except KUML4-2020-26 were significantly taller than 'KUML4', CN84-1 and CN3 (Table 3). All the BC_3F_3 lines showed significantly higher number of pods per plant and number of seeds per pod than 'KUML4' with the values ranging between 8.17-11.87 and 9.89-11.27, respectively (Table 3). While most of the BC_3F_3 had nonsignificant difference in seed weight with 'KUML4', two lines including KUML4-2020-26 and KUML4-2020-58 showed significantly higher seed weight than 'KUML4' (Table 3). In addition, the line KUML4-2020-26 also showed significantly greater seed weight than CN84-1 and CN3. The seed yield per plant of the BC_3F_3 lines ranged from 4.15 to 7.51 g which was significantly greater than all the released cultivars (KUML4, CN84-1 and CN3), but significantly lower than V4718 (Table 3). The greater seed yield of the BC_3F_3 lines is due to the better resistance CLS (see below).

Table 3. Agronomic and yield-related traits of mung bean selected pyramided lines at BC_3F_3 generation under Cercospora leaf spot disease outbreaks. DFF: Days to 50% flowering; DFM: days to 50% maturity; PH: plant height; BPP: number of branches per plants; SPP: number of seeds per pod; PPP: number of pods per plant; 100SDW: 100-seed weight; SDYP: seed yield per plant; CLS: CLS disease score; PDS: percentage of damage seeds by bruchids (*Callosobruchus maculatus*); ^{ns}nonsignificant, **significant at 0.01 level, mean with the same letter(s) are not significantly different at the 0.05 level.

Line/cultivar	DFF	DFM	PH	BPP	SPP	PPP	100SDW	SDYP	CLS	PDS
	d	d	cm	Nr plant^{-1}	Nr pod^{-1}	Nr plant^{-1}	g	g		%
KUML4-2020-14	39.00^{b}	56.67^{bc}	86.43^{ab}	2.00	9.89^{d}	10.93^{bcd}	5.72^{abc}	5.27^{bcd}	2.49^{ef}	46.00^{b}
KUML4-2020-20	39.00^{b}	56.33^{bc}	86.97^{ab}	2.13	11.04^{bcd}	11.87^{b}	5.50^{abc}	6.53^{ab}	2.37^{efg}	32.67^{c}
KUML4-2020-21	39.67^{b}	57.00^{bc}	89.70^{ab}	1.97	10.5^{bcd}	9.52^{bcd}	5.46^{bc}	4.15^{cd}	2.82^{c}	1.33^{e}
KUML4-2020-23	41.00^{b}	58.33^{b}	95.17^{a}	1.77	10.44^{bcd}	8.83^{bcd}	5.25^{bc}	4.27^{cd}	2.33^{efg}	1.33^{e}
KUML4-2020-24	39.00^{b}	56.00^{bc}	86.90^{ab}	1.67	10.53^{bcd}	10.03^{bcd}	5.45^{bc}	4.99^{bcd}	2.48^{ef}	36.67^{c}
KUML4-2020-26	39.67^{b}	57.00^{bc}	76.90^{cd}	2.53	9.89^{d}	9.09^{bcd}	6.11^{a}	4.62^{bcd}	2.53^{de}	60.00^{b}
KUML4-2020-32	38.33^{b}	56.00^{bc}	91.40^{ab}	1.90	11.24^{bc}	10.73^{bcd}	5.72^{abc}	5.99^{abc}	2.22^{fg}	38.00^{bc}
KUML4-2020-58	40.00^{b}	56.67^{bc}	91.13^{ab}	2.20	11.27^{bc}	11.13^{bc}	5.82^{ab}	7.51^{a}	2.17^{g}	51.00^{b}
KUML4-2020-60	39.00^{b}	56.67^{bc}	89.27^{ab}	1.83	10.51^{bcd}	8.17^{bcd}	5.09^{c}	5.54^{abcd}	2.78^{cd}	4.00^{e}
KUML4-2020-62	38.33^{b}	55.00^{c}	89.07^{ab}	2.43	11.06^{bcd}	11.63^{b}	5.30^{bc}	6.02^{abc}	2.44^{efg}	18.67^{d}
CN3	38.33^{b}	54.33^{c}	75.90^{d}	1.50	10.26^{cd}	6.87^{de}	5.34^{bc}	3.36^{de}	4.91^{a}	100.00^{a}
CN84-1	38.33^{b}	55.00^{c}	84.33^{bc}	1.33	11.32^{bc}	7.30^{cde}	5.16^{bc}	3.89^{cd}	4.78^{ab}	100.00^{a}
KUML4	39.00^{b}	55.00^{c}	70.90^{d}	1.90	8.23^{e}	4.02^{e}	5.10^{c}	1.67^{e}	4.86^{a}	100.00^{a}
V2802	41.33^{b}	57.33^{bc}	86.37^{ab}	2.37	11.65^{b}	11.90^{b}	3.30^{d}	4.09^{cd}	4.52^{b}	7.33^{de}
V4718	51.33^{a}	66.33^{a}	89.13^{ab}	2.73	13.36^{a}	20.27^{a}	2.42^{e}	5.42^{abcd}	1.84^{h}	100.00^{a}
F-test	12.70**	9.19**	5.63**	1.40^{ns}	8.99**	8.42**	23.90**	4.60**	141.16**	75.71**

The BC_3F_3 lines together 'KUML4', CN84-1, V4718, V2802, and CN were also evaluated for the resistance to the CLS disease and bruchid infestation. For the CLS resistance, V4718, the gene source for the CLS resistance, showed the highest resistance with the disease score of 1.84, while 'KUML4', CN84-1, CN3 and V2802 showed

highly susceptible to the disease with the scores of no less than 4.52 (Table 3). The BC_3F_3 lines expressed highly resistance to the CLS disease with the scores varying from 2.17 to 2.82 which were significantly lower than 'KUML4', CN84-1, CN3 and V2802 (Table 3 and Figure 3). However, the CLS disease score of the BC_3F_3 lines was significantly higher than that of V4718. Nonetheless, these results indicate that the marker VrTAF5_indel3 can be utilized for MAS of the *VrTAF-5* gene for the CLS resistance with 100% precision.

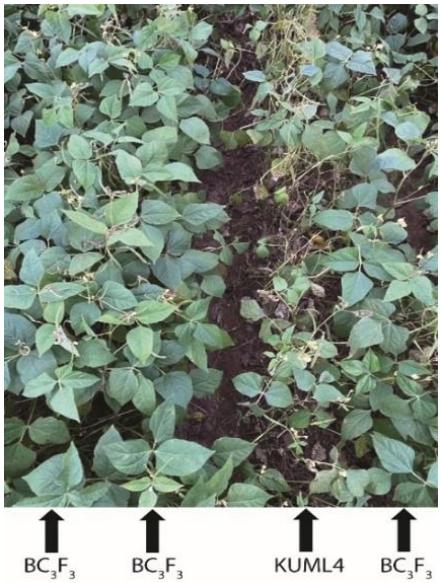

Figure 3. Resistance to Cercospora leaf spot disease in some BC_3F_3 pyramided lines compared with 'KUML4' mung bean.

In the case of bruchid resistance, bulked seeds of the BC_3F_3 lines and all the other cultivars/accessions were evaluated for the resistance to *C. maculatus*. The V2808, the gene source for the bruchid resistance, expressed a nearly perfect resistance to *C. maculatus* (7.33% percentage of damaged seeds, PDS), whereas 'KUML4', CN84-1, V4718 and CN3 expressed a complete susceptibility to *C. maculatus* disease (100% PDS) (Table 3). Among the 10 BC_3F_3 lines, three lines including KUML4-2020-21, KUML4-2020-23 and KUML4-2020-60 (Figure 4) expressed a nearly complete resistance to *C. maculatus* (PDS = 1.33%, 1.33% and 4.00%, respectively), indicating that the *VrPGIP2* gene in these lines was in the homozygous state, while the rest of lines showed moderate resistance (PDS = 18.67%-60.00%), indicating that the *VrPGIP2* gene in these lines was in the heterozygous state. Nonetheless, these results indicate that the dominant marker DMB-SSR158 can be used for MAS with 100% precision for the bruchid resistance gene *VrPGIP2*. Our results are in line with that of Chotechung et al. (2016), Liu et al. (2018), and Wu et al. (2022), who reported that DMB-SSR158 is a perfect marker for MAS of the bruchid resistance in the mung bean using V2802 as the gene source for the resistance, albeit the marker is dominant in nature. Nonetheless, based on agronomic and yield-related traits, CLS resistance, and bruchid resistance, the lines KUML4-2020-21, KUML4-2020-23, and KUML4-2020-60, exhibiting superior than 'KUML4' were selected.

Although mung bean was among the legume crops subjected to genome research at the early age of plant genome research about 30 yr ago, the progress in genome research and molecular breeding in mung bean is slow (Somta et al., 2022). Only recently, there were a few reports on molecular breeding of insect (Chotechung et al., 2016; Kaewwongwal et al., 2017; 2020) and disease resistance (Yundaeng et al., 2020; 2021; Tantasawat et al., 2022; Waengwan et al., 2024) in the mung bean. Papan et al. (2021) pyramided a CLS resistance gene and two PM resistance genes into a susceptible 'KING' through MABC. In addition, Papan et al. (2023) pyramided two PM resistance genes into the Thai-certified variety, Suranaree University of Technology 1 using MABC. Wu et al. (2022) introgressed the bruchid resistance gene *VrPGIP2* from V2802 into 'Kamphaeng Saen 1'. However,

our results are the first report on pyramiding the insect resistance gene and disease resistance genes into mung bean cultivars and substantiate the usefulness of the MAS in transferring multiple genes for the biotic stress resistance in this crop. Nonetheless, the superior lines developed in this our study can be further developed into new mung bean varieties and used as parents in breeding of new mung bean varieties with resistance to bruchid and Cercospora leaf spot disease. Such resistant varieties will be useful for sustainable production of the mung bean by reducing use of synthetic chemicals to control the bruchid and Cercospora leaf spot disease.

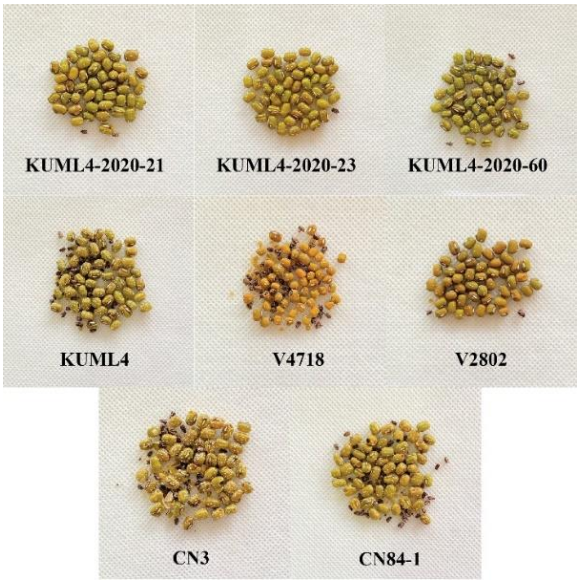

Figure 4. Resistance to bruchid (*Callosobruchus maculatus*) in pyramided line (KUML4-2020-21, KUML4-2020-23 and KUML4-2020-60) compared with susceptible mung bean control (CN3, CN84-1, V4718 and 'KUML4')

CONCLUSIONS

In this study, we report introgression of *VrTAF5* and *VrPGIP2* into KUML4, a high yield and large seed mung bean cultivar, by applying marker-assisted backcrossing. Three pyramided lines showed high resistance to Cercospora leaf spot (CLS) and bruchids with superior agronomic and yield-related traits than 'KUML4' under CLS outbreak condition.

Author contribution

Conceptualization: P.S., K.L. Methodology: P.S., K.L. Validation: K.L., M.S. Formal analysis: K.L., M.S. Investigation: T.Y., A.K., K.A., M.S., K.L., O.T., R.M. Resources: P.S. Data curation: K.L., T.Y. Writing-original draft preparation: K.L. Writing-review and editing: K.L., P.S. Visualization: K.L. Supervision: P.S. Project administration: K.L. Funding acquisition: K.L., P.S. All authors have read and agreed to the published final version of the manuscript.

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