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SCREENING OF SSR AND EST-SSR MARKERS FOR BACKGROUND SELECTION IN MUNGBEAN

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Abstract

This study aimed to quantify the recurrent parent genome (RPG) recovery of the new mungbean introgression lines using SSR and EST-SSR markers, and selection of backcross progenies of the crosses between a resistant line (SUPER5) and two recurrent parents (KING and H3). A total of 160 SSR and EST-SSR markers covering 11 mungbean chromosomes were applied for parental polymorphism identification. Among these, 6 polymorphic markers were linked to domestication related traits located on linkage groups (LGs) 1, 3, 7 and 10. Twenty-seven (16.9%) and 23 (14.4%) SSR and EST-SSR markers were found to be distinct and clearly polymorphic between KING and SUPER5, and H3 and SUPER5, respectively. These polymorphic markers were utilized to analyse the RPG recovery in BC_1F_1 and BC_2F_1 progenies. The BC_2F_1 selected progenies with high RPG recovery ranging from 87.2-94.7% and 92.1-97.8% in KING and H3 populations, respectively, were further used to produce BC_3F_1 seeds. These results suggest that SSR and EST-SSR markers can be used in marker-assisted selection (MAS) for background selection to accelerate backcrossing.

Key words*: marker-assisted backcross breeding, molecular markers, polymorphism, recurrent parent genome recovery, Vigna radiata (L.) Wilczek.*

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek], also known as green gram, is a type of legume that is commonly grown in Asia, Africa, and South America. It is a good source of protein, dietary fiber, and various vitamins and minerals (Hou et al., 2019). Additionally, mungbeans are often used as a cover crop, which can help to improve soil health by adding organic matter and nitrogen to the soil (Kocira et al., 2020). They are also used for forage, as a food for livestock. Overall, mungbeans play an important role in food security and sustainable agriculture. Mungbean is a significant crop in Thailand and grown primarily in the northeastern and northern regions of the country. According to the Chai Nat Field Crops Research Center, Department of Agriculture (2021), mungbean production in Thailand has been steadily increasing in recent years due to increasing demand for the crop both domestically and internationally. However, there are several limitations to mungbean production due to climate challenging because the crop is sensitive to temperatures and humidity. This can lead to reduced yields and increased susceptibility to pests and diseases. Mungbean is susceptible to a variety of pests and diseases, such as leafhoppers, thrips, Cercospora leaf spot (CLS), powdery mildew (PM), which can significantly reduce yields (Nair et al., 2019). Therefore, developing new mungbean varieties that have improved characteristics such as increased yield, improved quality, and resistance to pests and diseases is urgently needed.

Molecular markers have become an essential tool in modern plant breeding and have played an important role in the development of newly improved varieties of plants that have specific characteristics such as disease resistance, yield, and quality. Simple sequence repeat (SSR), also known as microsatellite, is a type of molecular markers that is widely used in genetics and plant breeding. SSR markers are based on the presence of short, repetitive DNA sequences that are scattered throughout the genome. These repetitive sequences can vary in number and size

among different individuals, making them useful for identifying genetic variation. Expressed sequence tag-SSR (EST-SSR) marker is a type of molecular markers that is derived from expressed genes in a genome. ESTs are short, single-pass DNA sequences that are generated from complementary DNA (cDNA) libraries, which are constructed from messenger RNA (mRNA) molecules that are transcribed from actively expressed genes. The marker-assisted backcrossing (MABC) is the backcrossing technique assisted by molecular markers which may accelerate the recovery of the recurrent parent genome (RPG) through two selection steps consisting of foreground and background selection (Ellegren, 2004; Luo et al., 2010; Kalia et al., 2011).

Therefore, the objectives of this study were to identify SSR and EST-SSR markers for background selection and to quantify the percentage of RPG recovery of the new backcross progenies in mungbean.

MATERIALS AND METHODS

Plant materials and crossing scheme

The genotypes used in this study were King originating from Australia, and SUPER5 and H3 lines developed by our research group at Suranaree University of Technology (SUT), Thailand. The seeds of SUPER5 as a donor line (F_9) were planted and the four best plants including SUPER5-1, SUPER5-2, SUPER5-3, and SUPER5-4 with no disease symptoms and growing well were selected to be the resistant source for the F_1 generation. Crosses were made between these resistant lines, which had CLS and PM resistance genes derived from the double cross [(CN72 × V4758) × (CN72 × V4718)] × [(CN72 × V4718) × (CN72 × V4785)] (Pookamsak, unpublished data), and either a susceptible variety with high yield KING or H3 the breeding line resistant to CLS but moderately resistant to PM diseases developed by Papan et al. (2022). Foreground selection was carried out for selecting the F_1 progenies that had a CLS and 2 PM resistance genes. These progenies were further used as male parents for backcrossing to generate BC_1F_1 until BC_2F_1 generations. The parental lines, the selected resistant BC_1F_1 and BC_2F_1 progenies through foreground selection and detached leaf assay were screened by marker-assisted selection (MAS) for background selection.

DNA isolation and PCR amplification

Fresh young leaves of seedlings were collected from parents (SUPER5-1, SUPER5-2, SUPER5-3, and SUPER5-4, KING and H3) and selected resistant lines in BC_1F_1 and BC_2F_1 generations, and the genomic DNA of each genotype was extracted by the cetyl trimethyl ammonium bromide (CTAB) method as described by Lodhi et al. (1994). For use in PCR analysis, the DNA concentration and purity were determined using an ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) at the A_{260} and A_{280} and adjusted to a final concentration of 150 ng/µl. The polymerase chain reaction (PCR) of SSR and EST-SSR markers were performed in a 20 µl reaction mix containing 150 ng of genomic DNA template, 1 × buffer (50 mM KCL, 10 mM Tris-HCL, pH 9.1, 0.01% Triton^{TM} X-100), 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 1 unit of *Taq* DNA polymerase, and 0.5 µM each of forward and reverse primers. The PCR reactions were subjected to amplification with initial denaturation at 95°C for 5 min; 35 cycles of denaturing at 95°C for 1 min, annealing at 50-60°C for 1 min, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. This protocol was described by Chen et al. (2015), Arsakit et al. (2017), Poolsawat et al. (2017) and Papan et al. (2021). The PCR products of these markers were electrophoresed on 6.0% PAGE at 200 V for 40-70 min depending on the specificity of primers, and stained with the silver nitrate method according to Sambrook and Russell (2001). All amplifications were repeated at least twice, and only clear bands were considered for analysis.

SSR and EST-SSR analysis

One hundred and sixty SSR and EST-SSR primers that have been reported by Isemura et al. (2012) were used in this study for identifying polymorphism between the parents SUPER5, KING and H3. These primers were associated with domestication traits, putative protein functions and unknown functions in mungbean (Table 1). Out of these 160 markers, 63 markers were linked to the domestication related traits such as 100-seed weight, pod length, stem

length, days to first flower, days to maturity of first pod etc. The polymorphic markers between SUPER5 (SUPER5-2 and SUPER5-4) and KING, and SUPER5 (SUPER5-1 and SUPER5-3) and H3 were then used for background selection of the BC_1F_1 and BC_2F_1 progenies in the KING×SUPER5 (SUPER5-2 and SUPER5-4) and H3×SUPER5 (SUPER5-1 and SUPER5-3) populations, respectively.

Data scoring and analysis

The clearly amplified alleles of each primer were coded using different combinations of 0 and 1. The polymorphic markers were further analyzed for similarity coefficients between various genotypes, in a pair-wise comparison using Jaccard's coefficient, and the similarity matrix was analyzed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm; the computations were carried out using NTSYSpc version 2.0 (Rohlf, 2000).

RESULTS AND DISCUSSIONS

Identification of polymorphic markers for background selection

Out of 160 markers, 27 (16.9%) and 23 (14.4%) polymorphic markers were found in KING and H3 populations, respectively. The number of markers/linkage group, length, and the primer name of polymorphic markers were showed in Tables 2 and 3 for KING × SUPER5 and H3 × SUPER5 crosses, respectively. These polymorphic markers were distributed on various linkage groups (Figure 1).

Genetic analysis of backcross progenies derived from KING × SUPER5 cross

In this study, we evaluated genetic profiles of 4 BC_1F_1 progenies derived from crossing between recurrent parent KING and donor parent SUPER5-2 and 3 BC_1F_1 progenies derived from crossing between recurrent parent KING and donor parent SUPER5-4. All of these 7 progenies had one CLS resistance gene and two PM resistance genes identified by using 6 linked marker loci including I16274, I85420, I42PL222, I27R565, VrTAF5_Indel, and VrMLO12_Indel3 markers which were reported in previous studies (Poolsawat et al., 2017; Yundaeng et al., 2020; Tantasawat et al., 2021). One hundred and sixty SSR and EST-SSR primers unlinked to these resistance genes were screened in both parents and 27 were found to be polymorphic (16.9%). The pairwise genetic similarity between all mungbean genotypes studied varied from 0.526 (SUPER5-2 vs K30, K147, K171 and SUPER5-4 vs K30, K147, K171) to 1.000 (SUPER5-2 vs SUPER5-4). The Mantel test in the materials studied with a cophenetic correlation coefficient value of 0.98, indicated that data in the similarity matrix were relatively well presented by dendrogram (Figure 2). The UPGMA dendrogram separated all genotypes into 2 major clusters. Cluster I included KING and all BC_1F_1 progenies including K9, K41, K30, K20, K147, K150, and K171, while cluster II consisted of the 2 donor lines, SUPER5-2 and SUPER5-4. In the cluster I, there were 2 subclusters including IA, IB and one individual, KING. Subcluster IA contained all 4 BC_1F_1 progenies derived from KING × SUPER5-2, while Subcluster IB contained all 3 BC_1F_1 progenies derived from KING × SUPER5-4. In the population of KING × SUPER5-2, the percentage of RPG recovery ranged from 68.8 to 75.0%. The high genetic similarity of 75.0% with KING was observed in one of the four progenies from this cross, K9. In the population of KING × SUPER5-4, one backcross progeny, K147, was found to be most genetically related to KING with the genetic similarity of 77.9% and the percentage of RPG recovery ranged from 75.8 to 77.9%, while the genetic similarity between donor lines (SUPER5-2 and SUPER-4) and KING were distantly related (57.1%) (Figure 2). All of these 7 BC_1F_1 were backcrossed to KING to produce BC_2F_1.

In the BC_2F_1 generation, 15 progenies generated from 7 BC_1F_1 progenies had 3 CLS and PM resistance genes, and were evaluated for their genetic profiles compared with their parents. The pairwise genetic similarity varied from 0.526 (SUPER5-2 vs K9-17, K9-32, K30-239 and SUPER5-4 vs K9-32, K30-239) to 1.000 (SUPER5-2 vs SUPER5-4 and K9-60 vs K150-374). The Mantel test with a cophenetic correlation coefficient value of 0.98, indicated that data in the similarity matrix were relatively well presented by dendrogram (Figure 3).

Table 1. SSR and EST-SSR primers used for screening background selection

Primer name	Linkage groups	Primer name	Linkage groups	Primer name	Linkage groups	Primer name	Linkage groups
CEDCAA001	1	CEDG117	3	cp09781	6	CEDG070	9
CEDG051	1	CEDG176	3	GMES0294	6	CEDG166	9
CEDG074	1	CEDG186	3	GMES0659	6	CEDG259	9
CEDG141	1	CEDG305	3	GMES1156	6	CEDG267	9
CEDG214	1	CEDGAT008	3	GMES3316	6	CEDG290	9
CEDG220	1	GMES0214	3	MBSSR021	6	GATS11	9
CEDG241	1	GMES0294	3	CEDG041	7	GMES0206	9
CEDG256	1	GMES0963	3	CEDG064	7	GMES1216	9
cp04220	1	GMES6583	3	CEDG085	7	GMES3893	9
cp05137	1	VR108	3	CEDG174	7	VCEDG056	9
cp06173	1	CEDC055	4	CEDG176	7	VM27	9
GMES0294	1	CEDG074	4	CEDG186	7	CEDG026	10
GMES1216	1	CEDG088	4	CEDG218	7	CEDG075	10
GMES1604	1	CEDG107	4	CEDG295	7	CEDG097	10
GMES2320	1	CEDG154	4	cp00228	7	CEDG113	10
GMES3316	1	CEDG232	4	cp05941	7	CEDG116	10
GMES4400	1	CEDG269	4	cp06427	7	CEDG150	10
VrD1	1	cp00416	4	cp07863	7	CEDG198	10
CEDAAG002	2	cp00674	4	GMES0856	7	cp02585	10
CEDC050	2	DMBSSR199	4	GMES4101	7	cp05325	10
CEDG006	2	GMES0216	4	GMES6625	7	cp05914	10
CEDG026	2	GMES1124	4	MBSSR008	7	BM149	11
CEDG050	2	GMES1156	4	CEDC031	8	CEDG002	11
CEDG168	2	GMES1216	4	CEDG030	8	CEDG013	11
cp00228	2	GMES3316	4	CEDG040	8	CEDG044	11
GMES0214	2	MBSSR015	4	CEDG059	8	CEDG072	11
GMES0216	2	BM170	5	CEDG071	8	CEDG075	11
GMES0477	2	CEDG008	5	CEDG099	8	CEDG076	11
GMES0856	2	CEDG132	5	CEDG247	8	CEDG098	11
GMES1156	2	CEDG171	5	CEDG257	8	CEDG168	11
GMES3316	2	CEDG184	5	CEDG269	8	CEDG281	11
GMES4137	2	CEDCAA001	6	CEDG271	8	cp00464	11
MBSSR015	2	CEDG037	6	CEDG302	8	cp05096	11
VR0200	2	CEDG041	6	cp06108	8	cp08695	11
CEDAAG004	3	CEDG121	6	GMES0206	8	cp10667	11
CEDC008	3	CEDG146	6	GMES0856	8	GMES0216	11
CEDG010	3	CEDG169	6	GMES5301	8	GMES3893	11
CEDG043	3	CEDG191	6	VM37	8	GMES5007	11
CEDG063	3	CEDG245	6	VR0255	8	GMES5575	11
CEDG084	3	CEDG282	6	CEDG024	9	GMES6098	11

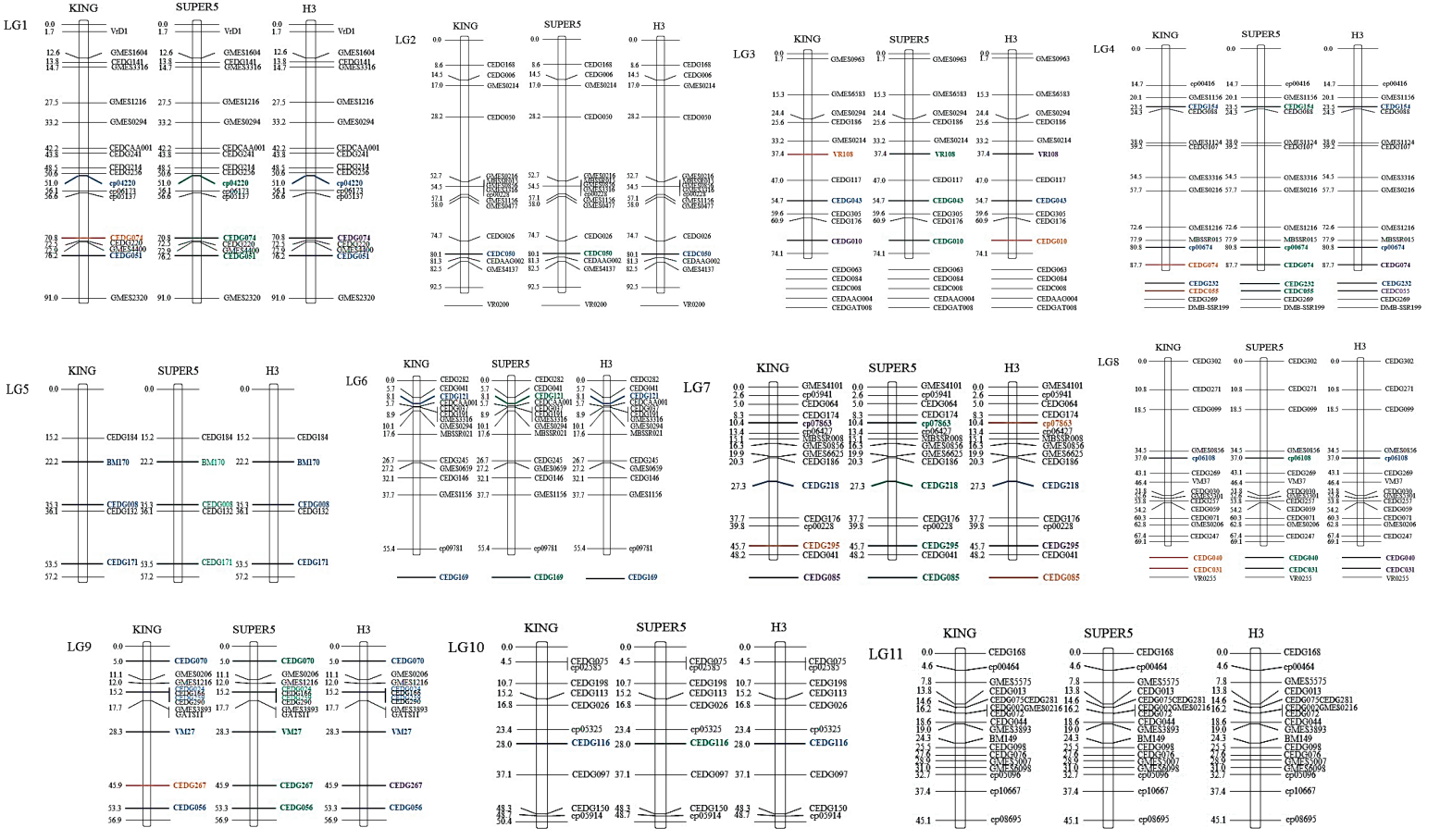

Figure 1. SSR and EST-SSR markers associated with domestication related traits and other putative protein functions or unknown functions in mungbean (Isemura et al., 2012). Different colors demonstrate polymorphism between parental variety/lines

Table 2. Polymorphic SSR and EST-SSR markers used for genetic analysis of backcross progenies derived from KING × SUPER5 cross (Isemura et al., 2012)

Linkage groups	Length (cM)	No. of total markers	No. of polymorphic markers	Primer name
1	91.0	18	3	CEDG051, CEDG074[a], cp04220[b]
2	92.5	16	1	CEDC050
3	74.1	16	2	CEDG043, VR108
4	87.7	16	5	CEDG154, CEDG232, CEDG074, CEDC055, cp00674
5	57.2	5	3	CEDG008, CEDG171, BM170
6	55.4	15	2	CEDG121, CEDG169
7	48.2	16	2	CEDG218[c], CEDG295
8	69.1	17	3	CEDG040, CEDC031, cp06108[d]
9	56.9	12	6	CEDG024, CEDG056, CEDG070, CEDG259, CEDG267, VM27
10	50.4	10	1	CEDG116[e]
11	45.1	19	0	-

[a] 100-seed weight
[b] Pod length
[c] Primary leaf width
[d] Hypocotyl plus epicotyl length
[e] Length of the internodes

Table 3. Polymorphic SSR and EST-SSR markers used for genetic analysis of backcross progenies derived from H3 × SUPER5 cross (Isemura et al., 2012)

Linkage groups	Length (cM)	No. of total markers	No. of polymorphic markers	Primer name
1	91.0	18	2	CEDG051, cp04220[a]
2	92.5	16	1	CEDC050
3	74.1	16	2	CEDG010, CEDG043
4	87.7	16	3	CEDG154, CEDG232, cp00674
5	57.2	5	3	CEDG008, CEDG171, BM170
6	55.4	15	2	CEDG121, CEDG169
7	48.2	16	3	CEDG085, CEDG218[b], cp07863[c]
8	69.1	17	1	CEDG040
9	56.9	12	5	CEDG024, CEDG056, CEDG070, CEDG259, VM27
10	50.4	10	1	CEDG116[d]
11	45.1	19	0	-

[a] Pod length
[b] Primary leaf width
[c] Number of twists along the length of the dehiscence pod when kept at room temperature
[d] Length of the internodes.

Based on UPGMA analysis, 2 major clusters were constructed, cluster I contained all BC_2F_1 progenies and KING and cluster II contained both donor lines, SUPER5-2 and SUPER5-4. Cluster I was further subdivided into 2 subclusters; IA (KING, K9-10, K147-335, K171-448, K9-17, K9-32, K9-66 and K9-78) and IB (K9-60, K150-374, K41-297, K150-364, K20-158, K20-163, K30-239 and K150-396). The similarity of all backcross progenies with KING ranged from 77.9% to 94.7%. Note that 5 backcross progenies including K9-10, K9-17, K9-32, K9-66 and K9-78 obtained from KING × SUPER5-2 were in subcluster IA with KING, all of them were the progenies of the BC_1F_1 K9 (showing 75.0% similarity with KING) and had similarity of 85.1% to 94.7% with KING. The maximum similarity of 94.7% with KING was observed in the K9-10. The remaining 2 progenies K147-335 and K171-448 obtained from KING × SUPER5-4 in this subcluster had similarities of 87.5% and 88.4% with KING, respectively, while other progenies in subcluster IB had similarity of 77.9% to 86.3% with KING. Meanwhile, the percentage of genetic similarity of both donor lines with KING were found at 55.7% (Figure 3). Of these 15 plants, 4 BC_2F_1 plants (K9-10, K9-17, K147-335, and K171-448) that showed high RPG recovery were backcrossed to the recurrent parent to produce BC_3F_1 seeds.

Genetic analysis of backcross progenies derived from H3 × SUPER5 cross

Among the BC_1F_1 progenies of H3 × SUPER5 population, 4 BC_1F_1 progenies derived from crossing between SUPER5-1 as a donor parent and H3 as a recurrent parent, and 4 BC_1F_1 progenies derived from crossing between SUPER5-3 and H3 were selected for PM resistance genes using VrMLO12_Indel3 marker locus (Yundaeng et al., 2020). Twenty-three out of 160 SSR and EST-SSR markers which associated with domestication traits, putative protein functions and unknown functions in mungbean were found to be polymorphic (14.4%). The pairwise genetic similarity between all mungbean genotypes in this generation varied from 0.529 (SUPER5-1 vs H218, H230 and SUPER5-3 vs H218, H230) to 1.000 (SUPER5-1 vs SUPER5-3).

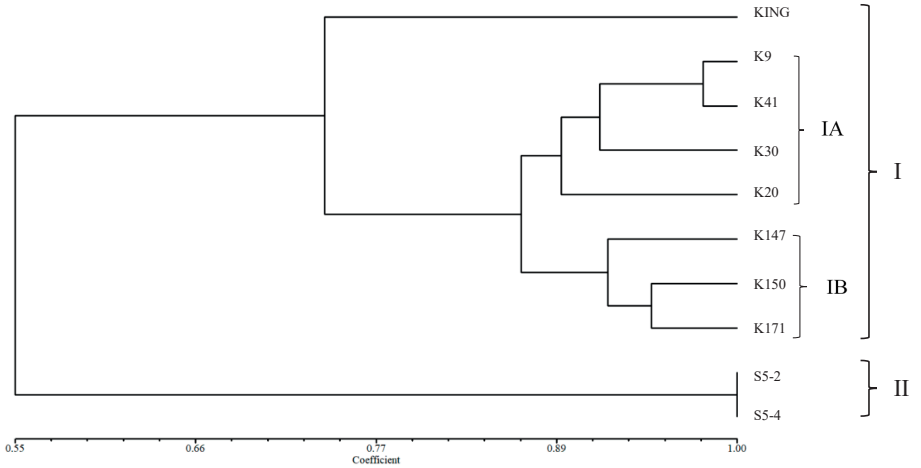

Figure 2. Dendrogram of 7 BC_1F_1 progenies and 3 parental variety/lines in KING × SUPER5 population. Dendrogram shows similarity coefficient and genetic relationships among 10 genotypes of mungbean

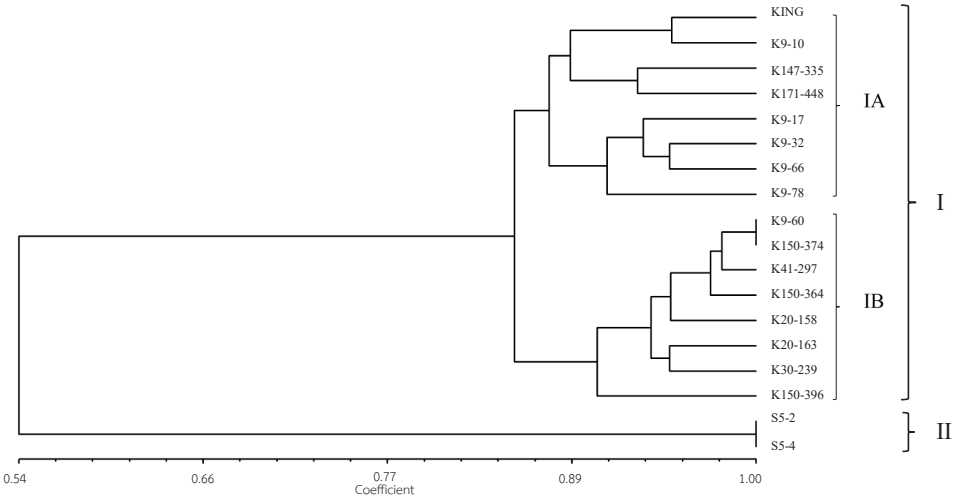

Figure 3. Dendrogram of 15 BC_2F_1 progenies and 3 parental variety/lines in KING × SUPER5 population. Dendrogram shows similarity coefficient and genetic relationships among 18 genotypes of mungbean

The UPGMA dendrogram was carried out for the Mantel test with a cophenetic correlation coefficient value of 0.97 indicating that data in the similarity matrix were relatively well presented by dendrogram (Figure 4). Eleven genotypes were separated into two major clusters by the UPGMA dendrogram. All BC_1F_1 progenies and their recurrent parent were included in Cluster I. Cluster II consisted of the two donor lines, SUPER5-1 and SUPER5-3. The similarity of all backcross progenies in this generation with H3 ranged from 69.8% to 84.1% and the highest of genetic similarity of 84.1% was observed in H207 progeny from the cross between H3 × SUPER5-1. In the cross of H3 × SUPER5-3, two BC_1F_1 progenies H230 and H247 were found to be most genetically related to H3 with the genetic similarity of 74.4%.

While, the similarity between parents, H3 with SUPER5-1 and SUPER5-3 were 56.2% (Figure 4). However, all 8 promising plants were still selected to produce BC_2F_1 seeds.

In BC_2F_1 generation, the Mantel test of H3 × SUPER5 population showed a cophenetic correlation coefficient value of 0.95 indicating that data in the similarity matrix were relatively well presented by dendrogram. The pairwise genetic similarity varied from 0.512 (SUPER5-1 vs H247-552) to 1.000 (SUPER5-1 vs SUPER5-3). The UPGMA dendrogram was constructed using 18 genotypes and it separated into 2 major clusters. Cluster I was further subdivided into 2 subclusters; IA (H3, H207-519, H210-533, H218-537, H206-510, H218-536, H206-514, H210-530, H252-572, and H252-575), and IB (H207-521, H230-540, H248-561, H248-562, H230-544, and H247-552). The cluster II consisted of 2 donor lines (SUPER5-1 and SUPER5-3) that are distantly related to H3 (0.578). BC_2F_1 progenies showed the presence of 84.1-97.8% of RPG recovery in this generation. Among these, the H207-519 and H210-533 in subcluster IA showed the highest genetic similarity of 97.8% with H3. They were the progenies of the BC_1F_1, H207 and H210 (showing 84.1 and 81.8% similarity with H3, respectively) (Figure 5).

Only the BC_2F_1 plants that had higher RPG recovery and produced maximum seeds were backcrossed to the recurrent parent to produce the BC_3F_1 seeds. Among these, H207-519, H210-533, H218-536, and H218-537 were used as male parents to produce BC_3F_1 seeds.

Marker-assisted background selection is useful for obtaining information on the RPG recovery. In this study, we screened a total of 160 SSR and EST-SSR markers. Out of these, 27 (16.9%) and 23 (14.4%) of them were found polymorphic between parents in KING × SUPER5 and H3 × SUPER5 populations, respectively. Among these, 6 polymorphic markers were reported to be linked to domestication related traits i.e., 100-seed weight, pod length, length of the internodes, primary leaf width, hypocotyl plus epicotyl length and number of twists along the length of the dehiscence pod when kept at room temperature, and located on linkage groups (LGs) 1, 3, 7 and 10 (Isemura et al., 2012). When using the polymorphic markers for background selection in two populations (KING × SUPER5 and H3 × SUPER5 populations), the genetics of all BC_1F_1 and BC_2F_1 progenies were similar to their recurrent parents at these 6 loci. Interestingly, the CLS resistance gene is located in LG3 (Arsakit et al., 2017 and Yungdaeng et al., 2020), therefore the polymorphic markers linked to domestication related traits in this LG may help reduce undesirable linkage drag. In the BC_1F_1 generation, the highest RPG recovery was found to be 77.9% in K147 of the KING × SUPER5 population and 84.1% in H207 of the H3 × SUPER5 population, using polymorphic markers in those populations.

All of BC_1F_1 progenies with resistance genes were selected and backcrossed to their recurrent parents to produce the BC_2F_1 generation. When polymorphic markers were used for marker-assisted background selection in BC_2F_1 generation, some progenies of both populations showed higher RPG recovery than estimated contribution by conventional backcross of 94.7 and 88.4% (K9-10 and K171-448) and 97.8, 97.8, 93.3, and 92.1% (H207-519, H210-533, H218-536, and H218-537) in KING × SUPER5 and H3 × SUPER5 populations, respectively.

However, some BC_1F_1 and BC_2F_1 progenies revealed lower RPG recovery than the theoretical means (75 and 87.5%), which may be explained by the "pull" effect, which is an unknown mechanism described by Sundaram et al. (2008). The pull effect might be caused by the genes of interest in the research, which may have favoured the transmission of additional genetic information, resulting in % RPG recovery that is less than the theoretical mean. In the case of linkage group 11 in mungbean, the number of markers that can be identified for polymorphism may be limited due to the short length of the linkage group.

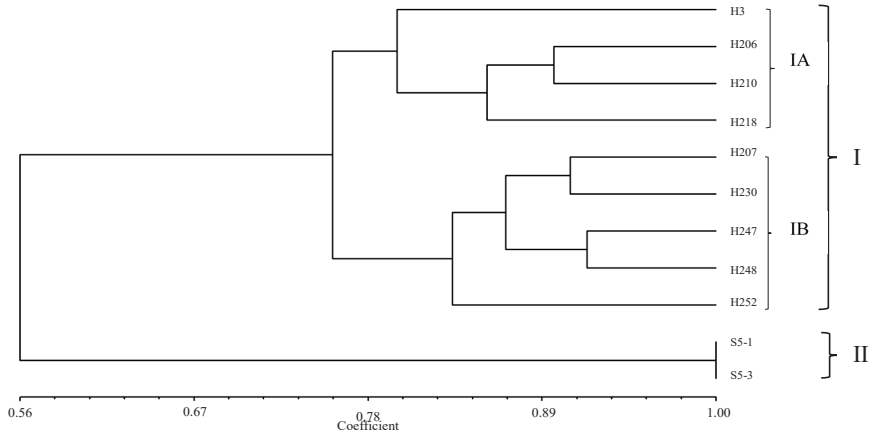

Figure 4. Dendrogram of 8 BC_1F_1 progenies and 3 parental variety/lines in H3 × SUPER5 population. Dendrogram shows similarity coefficient and genetic relationships among 11 genotypes of mungbean

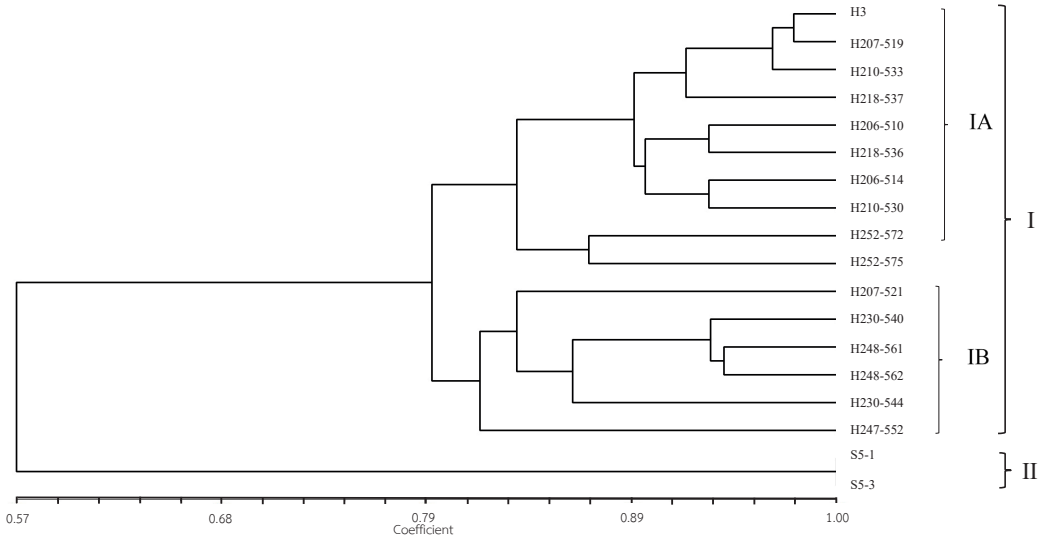

Figure 5. Dendrogram of 15 BC_2F_1 progenies and 3 parental variety/lines in H3 × SUPER5 population. Dendrogram shows similarity coefficient and genetic relationships among 18 genotypes of mungbean

However, some BC_1F_1 and BC_2F_1 progenies revealed lower RPG recovery than the theoretical means (75 and 87.5%), which may be explained by the "pull" effect, which is an unknown mechanism described by Sundaram et al. (2008). The pull effect might be caused by the genes of interest in the research, which may have favoured the transmission of additional genetic information, resulting in % RPG recovery that is less than the theoretical mean. In the case of linkage group 11 in mungbean, the number of markers that can be identified for polymorphism may be limited due to the short length of the linkage group.

CONCLUSIONS

The study found that MABC is a useful method for identifying the best BC progenies. The method effectively reduced the donor parent genome, as seen in this breeding program. The high percentage of the RPG recovery in this study suggests that using SSR and EST-SSR markers for background selection is practical in mungbean breeding. This study supports the idea that MAS has the potential to recover the genetic background of recurrent parents through accelerated backcrossing in mungbean.

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