BIODIVERSITAS
Volume 22, Number 11, November 2021
Pages: 4880-4889

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d221121

Analysis of genetic variability in F2 interspecific hybrids of mung bean (*Vigna radiata*) using inter-retrotransposon amplified polymorphism marker system

YENI FATMAWATI[1], AGUS BUDI SETIAWAN[1,2,♥], AZIZ PURWANTORO[1,2], DYAH WENY RESPATIE[1], CHEE HOW TEO[3]

[1]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.
[2]Plant Breeding Laboratory, Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-563062, ♥email: setiawanab@ugm.ac.id
[3]Centre for Research in Biotechnology for Agriculture, Universiti Malaya. Jl. Profesor Diraja Ungku Aziz, Kuala Lumpur 50603, Malaysia

Manuscript received: 8 September 2021. Revision accepted: 19 October 2021.

Abstract. *Fatmawati Y, Setiawan AB, Purwantoro A, Respatie DW, Teo CH. 2021. Analysis of genetic variability in F2 interspecific hybrids of mung bean (*Vigna radiata*) using inter-retrotransposon amplified polymorphism marker system. Biodiversitas 22: 4880-4889.* Mung bean (*Vigna radiata* L. Wilczek) categorized as one of the pivotal annual crops of *Vigna* genera is commonly cultivated in rotation with the cereal crops during the drought season. Conversely, to ameliorate its stunted productivity, the interspecific hybridization technique has been introduced between the mung bean and the common bean to promote genetic improvement with the breeding projects in Indonesia. However, since mung bean is a self-pollinated crop and has a narrow genetic base, the selection and improvement of a specific trait using marker-assisted selection is more challenging. Hence, a precautionary investigation is imperative to evaluate the progenies resulting from interspecific hybridization using an ideal marker. This study aimed to investigate the genetic variability of the F2 population of the interspecific mung bean hybrids using retrotransposon-based markers, particularly Inter-Retrotransposon Amplified Polymorphism (IRAP) markers. In this study, we identified retrotransposon from the mung bean genome and determined the Long Terminal Repeat (LTR) sequence using the LTR Finder. The IRAP primers were designed from a conserved region of the LTR sequence. One hundred of the F2 interspecific hybrids generated from the crossing between mung bean and common bean were successfully discriminated by IRAP markers. The IRAP marker showed high heterozygosity and moderate Polymorphic Information Content (PIC) values. The IRAP markers were able to detect genetic variability in the F2 progenies resulting from the interspecific hybridization. Cluster analysis showed that 100 of the F2 progenies were grouped into three clusters. This study demonstrated that retrotransposon-based markers can offer an effective approach for evaluating the segregation in the F2 population of intercross hybrids in the mung bean.

Keywords: Interspecific hybridization, IRAP marker, genetic validation, genuine hybrids, phenotype modification

INTRODUCTION

Mung bean (*Vigna radiata* L. Wilczek) is categorized as one of the pivotal annual crops of *Vigna* genera (Mogali and Hegde 2020). Being an excellent source of nutrition such as vitamins, minerals, dietary fiber, protein and bioactive compounds (Hou et al. 2019), it is incorporated in distinct healthy food recipes of Indonesia namely mung bean porridge, bean sprouts, noodles, mung bean milk, bean paste, and sweets. Due to its short-life span, small genome size, and genetic association to other legumes (Kim et al. 2015), it is commonly cultivated in rotation with the cereal crops by the Indonesian farmers in the drought season. However, the stunted productivity (0.9 ton/ha) has been a concern to establish the mung bean breeding projects in Indonesia (Nair and Schreinemachers 2020). Currently, factors such as the narrow genetic base, plant diseases, insect pests, and abiotic stress intolerance are the main obstructions escalating the poor yield (Gayacharan et al. 2020).

Interspecific hybridization is an innovative technique that leads to genetic speciation, genome evolution, genetic diversity, and in addition improves the adaptability to a new environment and the total yield (Fukuhara et al. 2016; Goulet et al. 2017; Kikuchi et al. 2007; Yu et al. 2021; Zhang et al. 2016). With an objective to proliferate the genetic diversity and to improve the yield, the interspecific cross between mung bean and common bean (*Phaseolus vulgaris* L.) has been proposed in our breeding project for the development of the variety with copious yield and high nutritional values. However, since mung bean is a self-pollinated crop and carries a narrow genetic base, the selection and improvement of a specific trait using marker-assisted selection is more difficult and challenging (Wang et al. 2018). Hence, a precautionary investigation is imperative to evaluate the progenies generated from the interspecific hybridization with an ideal marker.

The retrotransposon is a mobile element that is prevalent in the plant genomes (Kalendar and Schulman 2014; Orozco-Arias et al. 2019). Because of the insertion and deletion events that are widely dispersed throughout the genome, it is the primary driver of variation in plants (Moghaddam et al. 2014; Setiawan et al. 2020a; Sormin et al. 2021), making it an excellent candidate to be utilized as

a molecular marker to assess the genetic diversity in a segregated population. The retrotransposons are transposable elements (TEs) categorized into Long Terminal Repeat (LTR) and non-LTR retrotransposons (Elbarbary et al. 2016; Schulman et al. 2012). The Inter-Retrotransposon Amplified Polymorphism (IRAP) marker amplified the LTRs of two retrotransposons (Kalendar and Schulman 2006). This marker system is commonly used in plant genotyping, including sunflower (Basirnia et al. 2016), potato (Sharma and Nandineni 2014), papaya (Rashid et al. 2014), date palm (Mirani et al. 2020), bamboo (Li et al. 2020), and *Bletilla* Chinese herb (Guo et al. 2018). In addition, molecular marker based retrotransposon demonstrated an extensive application in plant genetic mapping (Liang et al. 2016; Monden et al. 2015; Rey-Baños et al. 2017), genetic diversity (Mirani et al. 2020; Sormin et al. 2021; Vuorinen et al. 2018), cultivar identification (Kim et al. 2012), and the evolutionary studies (Konovalov et al. 2010).

However, the use of the IRAP marker in assessing mung bean segregated populations resulting from interspecific cross with common bean is still limited. The objective of this research is to investigate the genetic variability of the F2 population of interspecific mung bean hybrids using the retrotransposon-based markers, namely the IRAP marker system.

MATERIALS AND METHODS

Plant materials

The F1 population consisted of 12 plants resulting from interspecific crossing between mung bean landrace (Lokal Malang × common bean cultivar 'Lebat-3'. Lokal Malang is a mung bean landrace derived from Karangploso district, Malang, East Java, Indonesia. The F1 plants were self-pollinated and generated 1557 seeds of F2 population. All F2 seeds were bulked and randomly selected 100 seeds for molecular analysis using IRAP marker system in this study. The seeds were cultivated and maintained in the open field at Kebun Percobaan Banguntapan, Faculty of Agriculture, Universitas Gadjah Mada from October 2020 until April 2021.

Genomic DNA isolation

The total genomic DNA was isolated from 100 mg of mung bean leaves using CTAB isolation procedures described in Setiawan et al. (2020b), and the DNA purification was performed using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific). NanoDrop was used to measure the DNA concentration (2000c Spectrometer, Thermo Scientific). Nuclease free water (NFW) was used to dilute the DNA samples into a working solution (25 ng/µL).

LTR sequence analysis

The mung bean retrotransposon sequence (AY900121.1) was retrieved from the National Center for Biotechnology Information (NCBI) database. LTRs position of the sequence was determined with the LTR Finder (Xu and Wang 2007). The protein annotation of the conserved domain of the retrotransposon structure in the sequence was subjected to the Conserved Domain Database (CDD) of NCBI (Lu et al. 2020). The ClustalW integrated with the BioEdit was used to perform the multiple sequence alignment (MSA) of LTRs (Hall 1999).

IRAP primer design and PCR

The mung bean IRAP primers were designed from a consensus of the LTR sequence (Table 1) using the FastPCR (Kalendar et al. 2017). The 12.5 µL of the PCR reaction mixture included 50 ng of gDNA, 0.2 mM dNTPs, 0.2 µM primer, 1X GoTaq® Green Master Mix (Promega, USA), 1.25 U/µL GoTaq® polymerase, and the nuclease free water. The PCR amplification was performed using the T100™ thermal cycler (Bio-Rad, USA), the amplification conditions consisted of pre-denaturation at 95°C for 2 min, 35 cycles of denaturation at 95°C for 1 min, annealing at Ta as specified in Table 2 for 1 min, with an extension at 72°C for 2 min, and the final extension at 72°C for 10 min. The amplified products of the PCR were analyzed by 1.5% agarose gel (w/v) electrophoresis using the florosafe DNA staining.

Data analysis

The binary data were analyzed using iMEC (online Marker Efficiency Calculator) (Amiryousefi et al., 2018). Parameters such as the Total Amplified Loci (TAL), Total Polymorphic Loci (TPL), Percentage of Polymorphic Loci (PPL), Heterozygosity (H), Polymorphic Information Content (PIC), Effective multiplex ratio (E), Marker Index (MI), Discriminating power (D), and the Resolving power (R) were analyzed to evaluate the polymorphism degree analysis and the profile information of the primers. Clustering and principal coordinate analysis of IRAP bands was performed using the NTSYS-PC software (Rohlf, 2009) and the GenAIEx software version 6.5 (Peakall and Smouse 2012), respectively.

Table 1. IRAP Primers used in this study

Primer name	Sequence (5'-3')	Length (bp)	GC content (%)	Tm (°C)
RTvr1-01F	CCTATGTGGAGAATAGGCCCAA	22	50.00	56.3
RTvr1-01R	TGGGCCTATTCTCCACATAGGAA	23	47.83	57.4
RTvr1-02F	YCTTCCTATGTGGARAATARRCCCA	25	44.00	56.9
RTvr1-02R	CATAGGAAGRGAGCCCARTAACC	23	52.20	56.9
RTvr1-03R	GGGCCTATTCTCCACATAGGAA	22	50.00	56.0
RTvr1-03F	GTTTGGTGCTCCTYGYAGATT	21	47.60	55.4

Table 2. Primer sequence combination of LTR retrotransposons and annealing temperature (Ta)

Primer combinations	Sequence (5'-3')	Ta (°C)
RTvr1-01F x RTvr1-01R (V1)	F: CCTATGTGGAGAATAGGCCCAA; R: TGGGCCTATTCTCCACATAGGAA	52.9
RTvr1-02F x RTvr1-02R (V2)	F: YCTTCCTATGTGGARAATARRCCCA; R: CATAGGAAGRGAGCCCARTAACC	51.4
RTvr1-01F x RTvr1-02R (V3)	F: CCTATGTGGAGAATAGGCCCAA; R: CATAGGAAGRGAGCCCARTAACC	57.4
RTvr1-03F x RTvr1-03R (V5)	F: GTTTGGTGCTCCTYGYAGATT; R: GGGCCTATTCTCCACATAGGAA	53.5
RTvr1-03F x RTvr1-01R (V6)	F: GTTTGGTGCTCCTYGYAGATT; R: TGGGCCTATTCTCCACATAGGAA	55.7
RTvr1-03F x RTvr1-02R (V7)	F: GTTTGGTGCTCCTYGYAGATT; R: CATAGGAAGRGAGCCCARTAACC	53.0
RTvr1-01F x RTvr1-03R (V8)	F: CCTATGTGGAGAATAGGCCCAA; R: GGGCCTATTCTCCACATAGGAA	55.7

RESULTS AND DISCUSSION

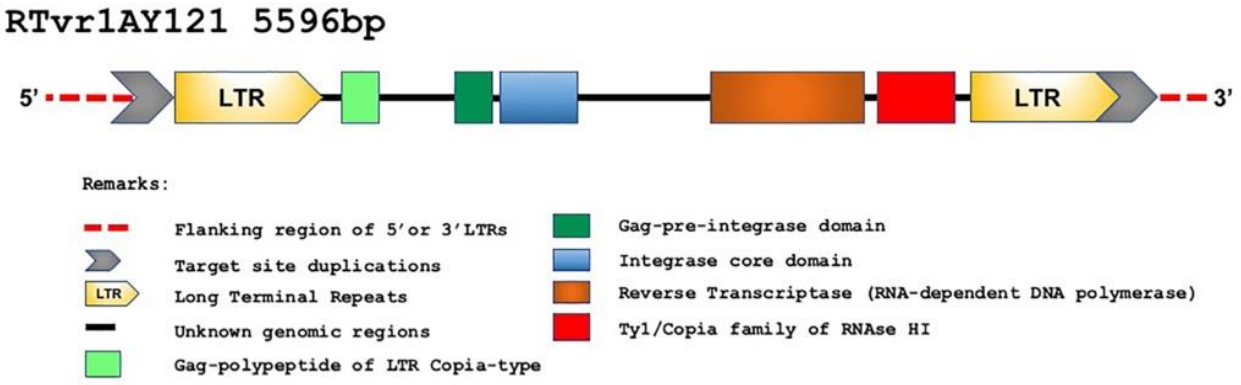
Analysis of LTR-Retrotransposon sequences and structures in the mung bean

To accelerate the genetic improvement of mung bean, it is critical to comprehend the genome structure that creates the genetic variation in the mung bean. Collectively, 50.1% of the total mung bean genome comprises the repetitive sequences, of which 25.2% is the LTR-retrotransposon (Kang et al. 2014). Therefore, an LTR retrotransposon-based marker could be used as an ideal marker to study the segregation in the F2 population of interspecific hybrids in mung beans. In this study, the retrotransposon sequence (AY900121.1) was identified from NCBI and was subjected to the LTR Finder to determine the position of the LTR sequence in the gene. Based on the LTR Finder analysis, both of 5' and 3' LTR of this retrotransposon had a sequence length of 680 bp, 95% of LTR sequence similarity and TCTTC as target site duplication at 5' and 3' end of LTR. This LTR retrotransposon is classified as Ty1/Copia Retrotransposon with 5596 bp in length based on the Conserved Domain Database (CDD) analysis. This retrotransposon consists of LTR sequences, Gag Protein, Integrase, Reverse Transcriptase, and RNAse HI domains (Figure 1.A). The organization of the polyprotein domain determines the primary difference between Ty1/Copia and Ty3/Gypsy retrotransposons. Ty1/Copia is encoded by Gag Protein, Integrase, Reverse Transcriptase, and RNAse HI, whereas Ty3/Gypsy is encoded by Gag Protein, Reverse Transcriptase, RNAse HI, and Integrase (Casacuberta and Santiago 2003; Huang et al. 2017).

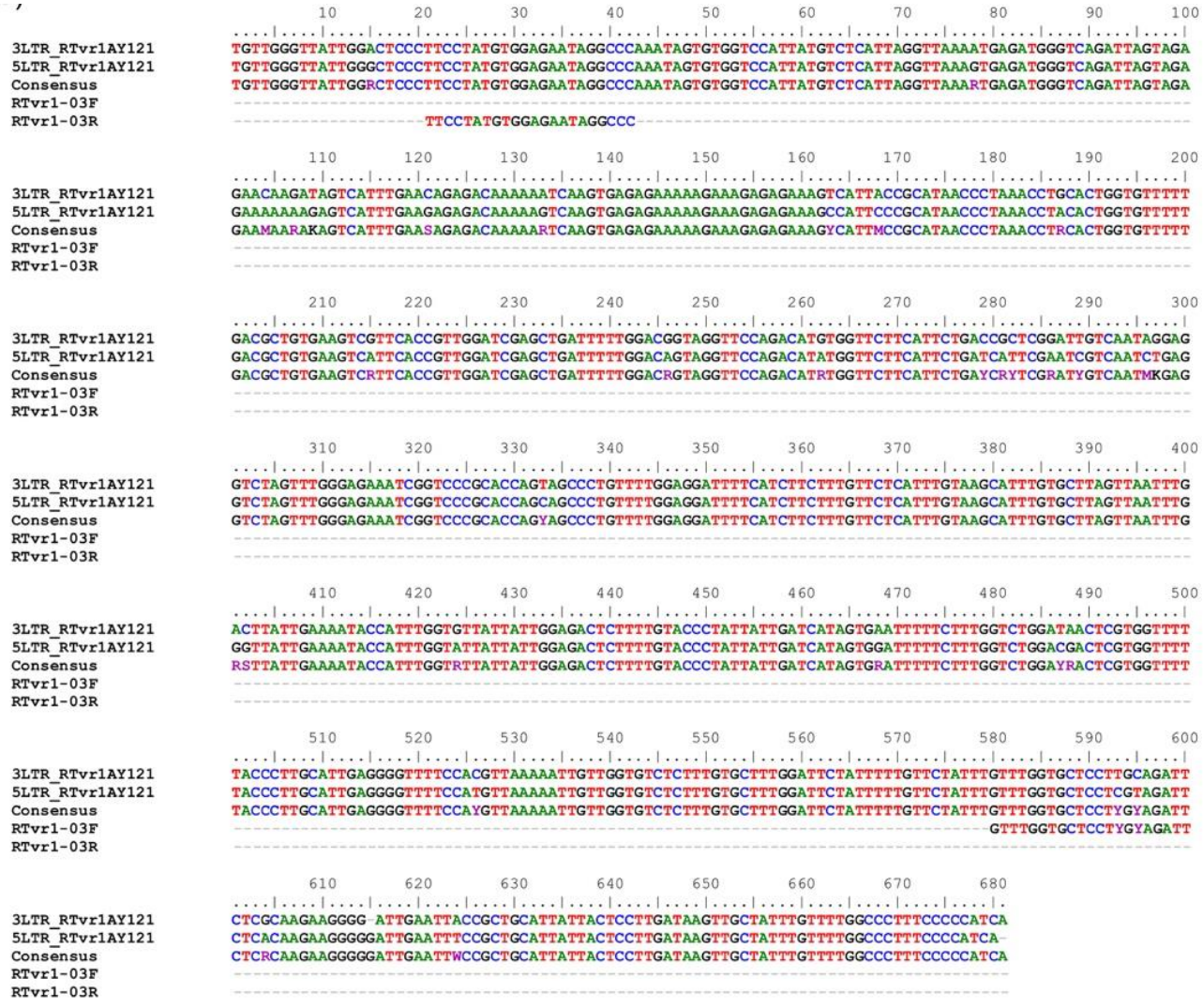
The Gag gene regulates the packaging of the RNA and the protein, while the other domains participate in the replication cycles (Zhao et al. 2016; Sanchez et al. 2017). The transposable element, particularly the LTR-retrotransposon contribute to the genetic variation in the plant because of the insertion and deletion events that are distributed extensively throughout the genome (Moghaddam et al. 2014; Setiawan et al. 2020a; Sormin et al. 2021). Therefore, this repetitive sequence is pertinent for marker development, particularly in evaluating the segregated population created from the interspecific hybridization. The identified LTR sequences were analyzed with the ClustalW program in the BioEdit. The consensus sequence of the LTR was generated and the primers were designed from the conserved regions (Figure 1.B).

Seed phenotype and marker analysis of F2 interspecific hybrid

The mung bean genome is composed of repeated sequences (50.1%), with long terminal repeat (LTR) retrotransposons accounting for 36.5% of the repeats (Kang et al. 2014), whereas in common bean, the majority of the repetitive sequences in the genome were LTR-retrotransposon, with the majority of them integrating into the genome within 2 MYA (Schmutz et al. 2014). Interspecific hybridization has an impact on plant speciation, causing substantial alterations in interspecific hybrids such as chromosomal rearrangement, tandem repeat amplification, activation of mobile elements, and gene expression changes (Glombik et al. 2020). As a result of the crossing of two distinct progenitors, phenotypic change in genuine hybrids may occur. In this study, the F1 hybrid demonstrated that it is phenotypically distinct from the female parent, with double the seed size due to heterosis effect of interspecies crosses and a brighter/shinier seed coat color than the female parent, which had dull-seeded green seeds (Figure 2). Similar research on Arabidopsis F1 hybrids revealed substantial changes in morphological and cell characteristics due to heterosis effect in which the hybrids grew bigger leaves and increased in cell size and number compared to the parents (Groszmann et al. 2014). The F2 offspring also had a bright green seed coat; both the seed size and shape of the F2 offspring are more similar to the female parent, and the bright color may have originated from the male parent, who had a shining creamy white seed coat (Figure 2). Several studies reported that insertion of retrotransposon affected the gene expression responsible for low erucid acid in *Brassica rapa* (Fukai et al. 2019) and the accumulation of anthocyanin in citrus (Butelli et al. 2012).



A



B

Figure 1. Ty1/Copia retrotransposon structure and MSA of the LTR sequences on the mung bean. A. Construction of Ty1/Copia conserved domain using the CDD consisting of the LTR, Gag Protein, Integrase, Reverse Transcriptase, and the RNAse HI. B. MSA of the LTR sequences of the mung bean retrotransposon, RTvr1-03 primers were designed from the conserved regions of the LTR

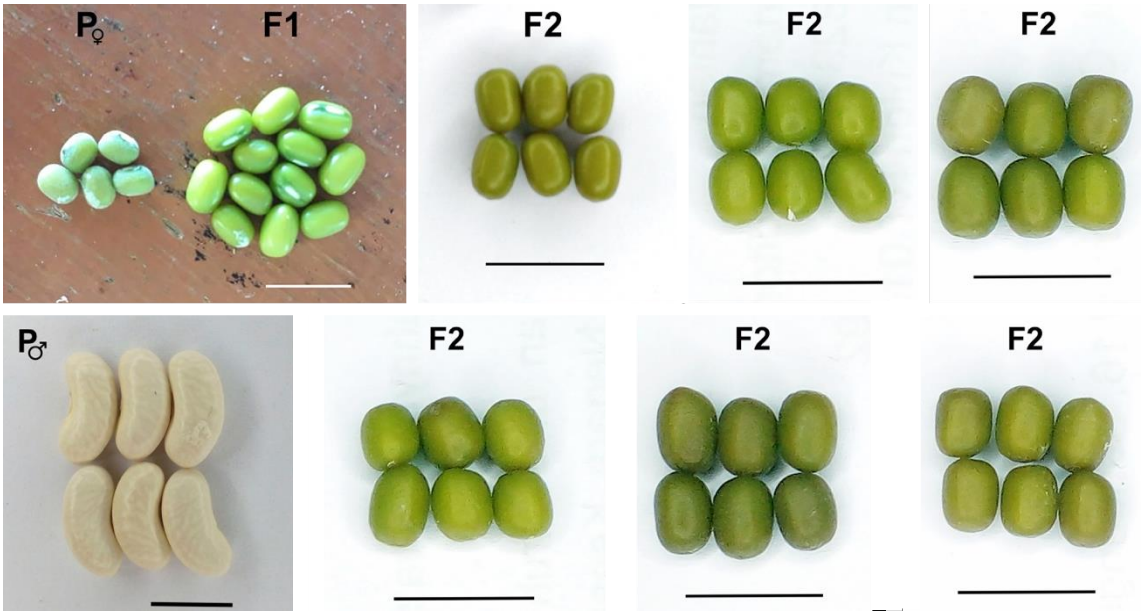


Figure 2. Seed morphology includes size, shape and coat color of the parental genotypes, F1 and F2 progenies. Scale bars = 1 cm

The IRAP primer pairs were designed from the conserved region of the LTR (Figure 1.B). Seven combinations of the IRAP primer generated DNA amplicon of defined size from the genomic DNA of 100 F2 interspecific hybrids of the mung bean (Table 3 and Figure 3). Each primer combination produced a distinctive number of amplicons for each respective mung bean hybrid with an average of 7.86 amplicons per primer (Table 3). The V3 primer had the foremost number of TAL and produced 13 amplified bands. While V5 primer had the lowest number of TAL with 5 amplicons. The average percentage of polymorphic locus from 7 primer combinations is 79.69%. The degree of polymorphism was indicated by the heterozygosity value (H) and the polymorphism information index (PIC). Heterozygosity is an evaluation method to determine genetic variability (Nei and Li 1979). Heterozygosity (H) from 7 IRAP primer combinations varied from 0.0468 to 0.4673 with an average H value of 0.2455. The highest heterozygosity was shown by the V1 primer (0.4673) and the lowest by the V5 (0.0468). The average PIC value from 7 primer combinations is 0.2019, with the highest PIC value 0.3581 (V1) and the lowest PIC value 0.0458 (V5), implying that the PIC value was considered moderately informative as reported in Eltaher et al. (2018). The PIC value of the IRAP markers was also coequal as demonstrated by other researches in which the moderate IRAP marker generated the PIC value that varied between 0.20 and 0.50 with an average of 0.30 (Zein et al. 2010; Cheraghi et al. 2018; Li et al. 2020). These results indicate that both V3 and V1 exhibited a prominent value of heterozygosity and PIC. The PIC values signify the ability of a marker to form polymorphisms within populations based on detected alleles and distributed frequency (Botstein et al. 1980). Molecular markers are dominant when PIC $\leq$ 0.5 and evenly distributed within the population (Alikhani et al. 2014). High PIC values indicate that markers have the ability to detect genetic variability within tested materials and insubstantial genetic variability can sequence reduced low heterozygosity.

The average effective multiplex ratio (E) is 6.28, with the highest E value of 9.15 (V3) and the lowest E value of 4.88 (V5). The average MI is 0.001836, with the highest MI value of 0.002934 (V3) and the lowest MI value of 0.000457 (V5). Higher MI indicates better quality of molecular marker. These results imply that the increased effective multiplex ratio of the primer leads to a higher MI value. The average discriminating power (D) from 7 IRAP primer combinations is 0.29, with the highest and lowest discriminating power of 0.61 (V1) and 0.05 (V5), respectively. Discrimination power is used to illustrate the identification capabilities of two random individuals that have distinctive patterns. The average resolving power (R) is 1.35, with the highest resolving power of 3.58 (V1) and the lowest R value of 0.24 (V5). Resolving power is used to identify markers/primers that have the potential to analyze the genetic variability. These results imply that increased discriminating power of the primer leads to enhanced resolving power value of the primer. It also suggests that a highly effective multiple ratio value is evidence of improved primer efficiency. Primer combinations of V3 and V6 are more effective based on their effective multiplex ratio value. The MI estimates the usage frequency of the marker systems. MI is calculated from their polymorphism information content and effective multiple ratios (Chesnokov and Artemyeva 2015). The proliferated value of PIC, effective marker ratio, MI, discriminating power, and resolving power from the seven primer combinations reveals that these 7 distinct primer combinations are ideal candidates to be utilized for investigating the DNA profiling and the genetic variability in the F2 interspecific hybrids of the mung bean.

Common bean is closely related to mung bean and both of them belong to the Fabaceae family which diverged from the common ancestor between 4.9 and 8 MYA (Lavin

et al. 2005; Zhu et al. 2005; Wang et al. 2008; Stefanović et al. 2009). In addition, linkage groups between mung bean and common bean are highly conserved (Boutin et al. 1995; Zhu et al. 2005; McClean et al. 2010) and the synteny analysis of unigene sequences in mung bean exhibited a similar gene function with common bean (Chen et al. 2015). During the interspecific crossing, the majority of common bean cultivars (parents) exhibited failure in seed fertilization and only one male parent (Lebat-3) demonstrated crossed-compatibility with the mung bean (Lokal Malang). Of the total 100 F2 hybrids that were tested, only 8 plants of F2 generation revealed insertion and deletion DNA bands, i.e., the sample numbers 52, 53, 55, 62, 63, 65, 70, and 78 (Figure 3). These results imply that the F1 is a true hybrid. The F1 hybrid revealed a distinct seed coat color and size as compared to the female progenitor, which showed a dull-seeded green and smaller seed size than F1. This brighter seed coat might have been transmitted from the male progenitor, who had a glossy creamy white seed coat. Khajudparn et al. (2012) revealed that morphological characteristics may be used to identify F1 hybrids arising from interspecific hybridization when one of the traits has a remarkable resemblance to the male parent. In addition, both genotypes 52 and 53 of F2 population genetically exhibited four and two unique bands, which were identical to those found in common bean progenitor (Figure 3). Abbas et al. (2015) published a similar work in which recombinant hybrids emerging from interspecific hybridization between mung bean and mash bean were confirmed as genuine hybrids using molecular DNA markers.

Table 3. Marker analysis of F2 interspecific hybrid of mung bean

Primer combination	Size (bp)	TAL	TPL	PPL	H	PIC	E	MI	D	R
V1	210-1700	9	8	88.89	0.4673	0.3581	5.65	0.002934	0.61	3.58
V2	200-1000	6	4	66.67	0.2952	0.2516	4.92	0.002421	0.33	0.32
V3	200-2000	13	12	92.31	0.4169	0.3300	9.15	0.002934	0.50	1.94
V5	350-1200	5	3	60.00	0.0468	0.0458	4.88	0.000457	0.05	0.24
V6	100-3000	10	10	100.00	0.3421	0.2836	7.81	0.002672	0.39	2.42
V7	350-1200	6	4	66.67	0.0551	0.0535	5.83	0.000535	0.06	0.34
V8	350-1300	6	5	83.33	0.0950	0.0905	5.70	0.000903	0.09	0.60
Total		55	46	557.86	1.7184	1.4131	43.94	0.012855	2.03	9.44
Mean		7.86	6.57	79.69	0.2455	0.2019	6.28	0.001836	0.29	1.35

Note: TAL: Total Amplified Loci, TPL: Total Polymorphic Loci, PPL: Percentage of Polymorphic Loci, H: Heterozygosity, PIC: Polymorphic Information Content, E: Effective multiplex ratio, MI: Marker Index, D: Discriminating power, R: Resolving power

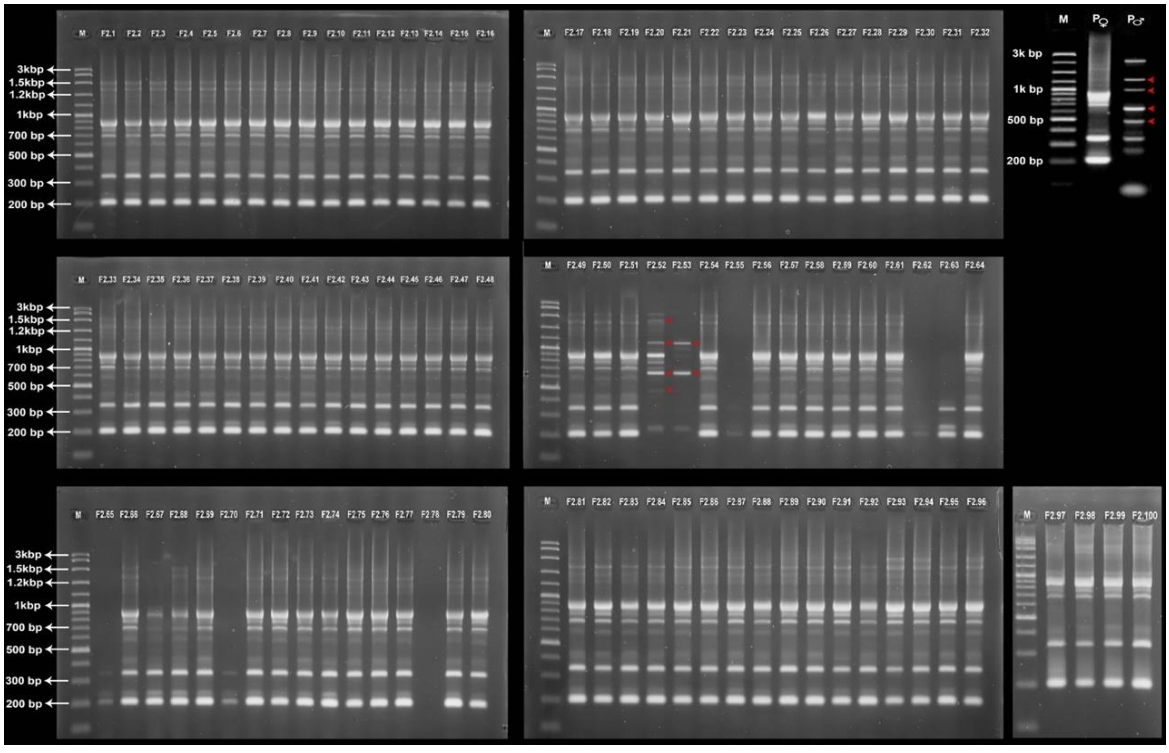
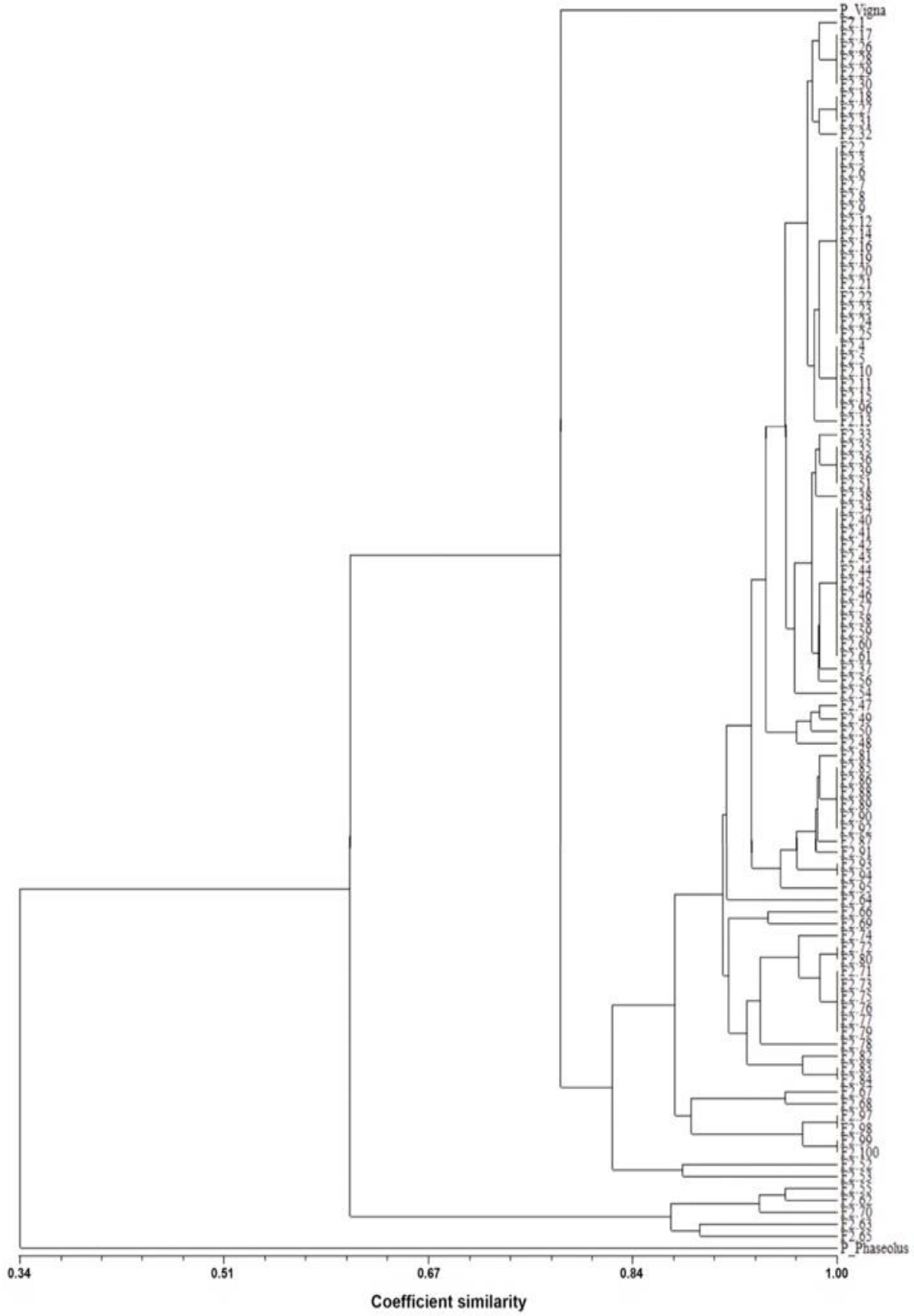
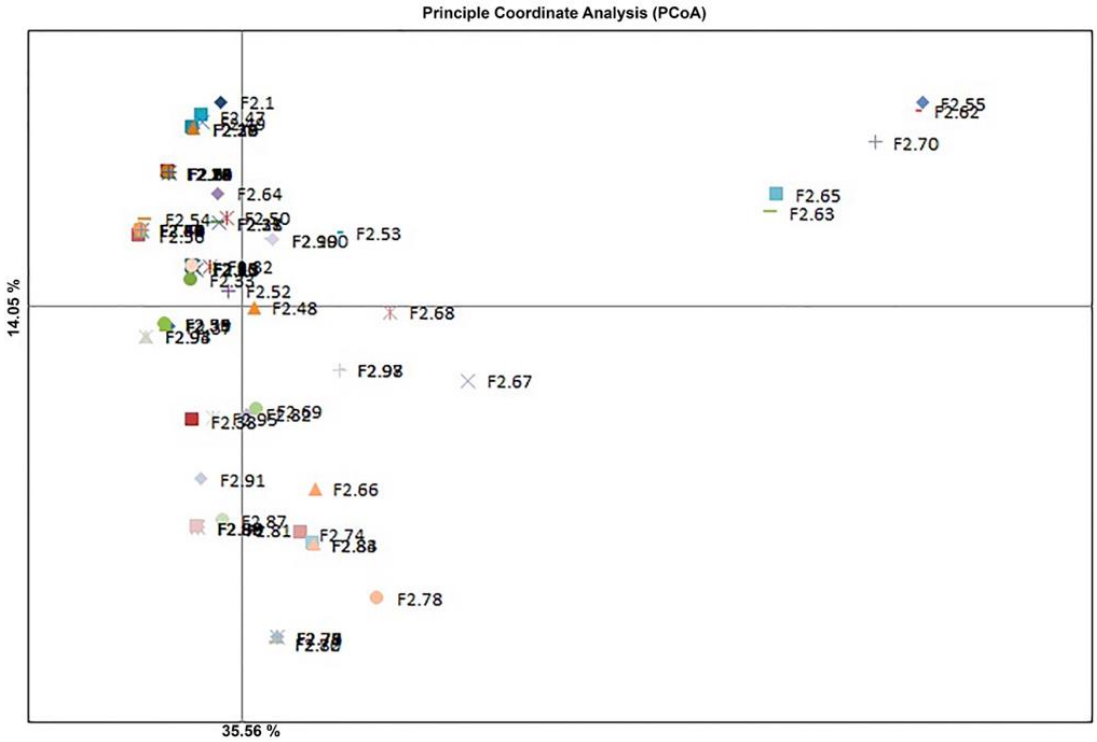


Figure 3. DNA fingerprints of 100 F2 interspecific hybrids of mung bean and their parents amplified using combination of V3 IRAP markers with 3000 bp of ladder. Red arrowheads depict the new bands that only presents in 52 and 53 genotypes similar to DNA profile of male parent. M: Ladder, P♂: Male parent (Common bean ′Lebat-3′), P♀: Female parent (Mung bean ′Lokal Malang′)


P_Vigna
P_Phaseolus
0.34
0.51
0.67
0.84
1.00
Coefficient similarity

A



B

Figure 4. Cluster analysis of parents and 100 F2 interspecific hybrids of mung bean. A. Dendrogram of parents and 100 F2 interspecific hybrids based on IRAP markers. B. A PCoA biplot shows distribution of 100 F2 interspecific hybrids of mung bean based on IRAP markers

Cluster analysis of the IRAP marker system

Cluster analysis was performed using the Unweighted Paired Group Method (UPGMA) based on the genetic similarity coefficients. The similarity coefficient of 100 F2 interspecific hybrids of mung bean ranged between 0.34 – 1.00, with an average similarity coefficient of 0.67 (Figure 4.A). The UPGMA dendrogram separated 100 F2 interspecific hybrids of mung bean into three major clusters i.e., cluster I, II and III. The first cluster (I) is made up of female parents and 95 mung bean genotypes, whereas the second cluster (II) is made up of 5 genotypes that are closely linked to the common bean (cluster III). In addition, a PCoA biplot also confirmed the clustering analysis result (Figure 4.B). Five out of 100 genotypes were categorized under one cluster. This result implies that the IRAP marker can clearly distinguish the genetic variation of the interspecific hybrids, which is supported by seed morphology, including seed size, shape, and color, in which some F2 plant accessions with specific traits are more closely related to one of the parental species, while others are genetically closer to the other species. Therefore, the IRAP marker system developed can be applied as MAS in interspecific mung bean breeding projects. Another study revealed that IRAP is a substantive marker for plant genotyping and identification of hybrid plants (Basirnia et al. 2016; Ghonaim et al. 2020; Li et al. 2020; Mirani et al. 2020).

The designed IRAP primers offer valuable genetic information about the variation in the F2 population of mung bean resulting from the interspecific hybridization between mung bean and common bean. From the pattern of IRAP polymorphism, we conclude that because of their abundance in the mung bean and common bean genomes, Ty1/copia element significantly identified polymorphism among the 100 of F2 interspecific genotypes (Figure 3). The IRAP marker system used in this study depicted polymorphism between the parents and the offspring. Furthermore, seed size and shape in F2 hybrids are more identical to the female parent, whereas the bright seed coat color may be inherited from the male parent. Due to the heterosis effect of interspecies crossings, the F1 hybrid also had a larger seed size than the female parent. The genetic basis of the F2 population genetically revealed distinct bands that were identical to those observed in mung bean and common bean progenitors, suggesting that the F1 interspecific hybrid is genuine. The LTR-retrotransposon-based markers were effectively used to identify heterospecific alleles in interspecific BC_1 hybrids of *Iris fulva* and *I. brevicaulis* (Bouck et al. 2005). Other investigations have found that IRAP markers may be utilized to detect novel recombinants due to somaclonal variation produced from tissue culture (Lightbourn et al. 2007; Teo et al. 2005; Mirani et al. 2020). In conclusion, our current study demonstrates that retrotransposon based-marker can offer an effective approach for evaluating the segregation in the F2 population of intercross hybrids in the mung bean. The IRAP marker successfully identified genetic variability among the 100 of the F2 interspecific genotypes.

ACKNOWLEDGEMENTS

The authors would like to show our gratitude to Rector of Universitas Gadjah Mada who supported this research project through Rekognisi Tugas Akhir (RTA) scheme (1185/UN1.P.III/SK/HUKOR/2021) and Badan Penerbit dan Publikasi (BPP) UGM for English editing services.

REFERENCES

Abbas G, Hameed A, Rizwan M, Ahsan M, Asghar MJ, Iqbal N. 2015. Genetic confirmation of mung bean (*Vigna radiata*) and mashbean (*Vigna mungo*) interspecific recombinants using molecular markers. Front Plant Sci 6: 1107. DOI: 10.3389/fpls.2015.01107.

Alikhani L, Rahmani M-S, Shabanian N, Badakhshan H, Khadivi-Khub A. 2014. Genetic variability and structure of *Quercus brantii* assessed by ISSR, IRAP and SCoT markers. Gene 552: 176-183. DOI: 10.1016/j.gene.2014.09.034.

Amiryousefi A, Hyvönen J, Poczai P. 2018. iMEC: Online marker efficiency calculator. Appl Plant Sci 6: 4-7. DOI: 10.1002/aps3.1159.

Basirnia A, Darvishzadeh R, Abdollahi Mandoulakani B. 2016. Retrotransposon insertional polymorphism in sunflower (*Helianthus annuus* L.) lines revealed by IRAP and REMAP markers. Plant Biosyst 150: 641-652. DOI: 10.1080/11263504.2014.970595.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314-331.

Bouck A, Peeler R, Arnold ML, Wessler, SR. 2005. Genetic mapping of species boundaries in louisiana irises using IRRE retrotransposon display markers. Genetics 171: 1289-1303. DOI: 10.1534/genetics.105.044552.

Boutin SR, Young ND, Olson TC, Yu ZH, Shoemaker RC, Vallejos CE. 1995. Genome conservation among three legume genera detected with DNA markers. Genome 38: 928-937. DOI: 10.1139/g95-122.

Butelli E, Licciardello C, Zhang Y, Liu J, Mackay S, Bailey P, Reforgiato-Recupero G, Martin C. 2012. Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24: 1242-1255. DOI: 10.1105/tpc.111.095232.

Casacuberta JM, Santiago N. 2003. Plant LTR-retrotransposons and MITEs: Control of transposition and impact on the evolution of plant genes and genomes. Gene 311: 1-11. DOI:10.1016/S0378-1119(03)00557-2.

Chen H, Wang L, Wang S, Liu C, Blair MW, Cheng, X. 2015. Transcriptome sequencing of mung bean (*Vigna radiata* L.) genes and the identification of EST-SSR markers. PLoS One 10: e0120273. DOI: 10.1371/journal.pone.0120273.

Cheraghi A, Rahmani F, Hassanzadeh-Ghorttapeh A. 2018. IRAP and REMAP based genetic diversity among varieties of *Lallemantia iberica*. Mol Biol Res Commun 7: 125-132. DOI: 10.22099/mbrc.2018.29924.1327.

Chesnokov YV, Artemyeva AM. 2015. Evaluation of the measure of polymorphism information of genetic diversity. Agric Biol 50: 571-578. DOI: 10.15389/agrobiology.2015.5.571rus.

Elbarbary RA, Lucas BA, Maquat LE. 2016. Retrotransposons as regulators of gene expression. Science 351: 1-18. DOI: 10.1126/science.aac7247.

Eltaher S, Sallam A, Belamkar V, Emara HA, Nower AA, Salem KFM, Poland J, Baenziger PS. 2018. Genetic diversity and population structure of F3:6 Nebraska Winter wheat genotypes using genotyping-by-sequencing. Front Genet 9: 76. DOI: 10.3389/fgene.2018.00076.

Fukai E, Karim MM, Shea DJ, Tonu NN, Falk KC, Funaki T, Okazaki K. 2019. An LTR retrotransposon insertion was the cause of world's first low erucic acid *Brassica rapa* oilseed cultivar. Mol Breed 39: 15. DOI: 10.1007/s11032-018-0916-9.

Fukuhara S, Muakrong N, Kikuchi S, Tanya P, Sassa H, Koba T, Srinives P. 2016. Cytological characterization of an interspecific hybrid in *Jatropha* and its progeny reveals preferential uniparental chromosome transmission and interspecific translocation. Breed Sci 66: 838-844. DOI: 10.1270/jsbbs.16069.

Gayacharan, Archak S, Gupta K, Gupta V, Tyagi V, Singh K. 2020. Mung bean genetic resources and utilization. In: Nair RM, Schafleitner R, Lee S-H (Eds). The Mung bean Genome. Springer International Publishing, Cham.

Ghonaim M, Kalendar R, Barakat H, Elsherif N, Ashry N, Schulman AH. 2020. High-throughput retrotransposon-based genetic diversity of maize germplasm assessment and analysis. Mol Biol Rep 47: 1589-1603. DOI: 10.1007/s11033-020-05246-4.

Glombik M, Bačovský V, Hobza R, Kopecký D. 2020. Competition of Parental Genomes in Plant Hybrids. Front Plant Sci 11: 200. DOI: 10.3389/fpls.2020.00200.

Goulet BE, Roda F, Hopkins R. 2017. Hybridization in plants: Old ideas, new techniques. Plant Physiol 173: 65-78. DOI: 10.1104/pp.16.01340.

Groszmann M, Gonzalez-Bayon R, Greaves IK, Wang L, Huen AK, James Peacock W, Dennis ES. 2014. Intraspecific arabidopsis hybrids show different patterns of heterosis despite the close relatedness of the parental genomes. Plant Physiol 166: 265-280. DOI: 10.1104/pp.114.243998.

Guo Y, Zhai L, Long H, Chen N, Gao C, Ding Z, Jin B. 2018. Genetic diversity of *Bletilla striata* assessed by SCoT and IRAP markers. Hereditas 155: 35. DOI: 10.1186/s41065-018-0074-4.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.

Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, Feng N, Shen Q. 2019. Mung bean (*Vigna radiata* L.): Bioactive polyphenols, polysaccharides, peptides, and health benefits. Nutrients 11: 1-28. DOI: 10.3390/nu11061238.

Huang Y, Luo L, Hu X, Yu F, Yang Y, Deng Z, Wu J, Chen R, Zhang M. 2017. Characterization, genomic organization, abundance, and chromosomal distribution of Ty1-copia retrotransposons in *Erianthus arundinaceus*. Front Plant Sci 8: 924. DOI: 10.3389/fpls.2017.00924.

Kalendar R, Khassenov B, Ramankulov Y, Samuilova O, Ivanov KI. 2017. FastPCR: An in silico tool for fast primer and probe design and advanced sequence analysis. Genomics 109: 312-319. DOI: 10.1016/j.ygeno.2017.05.005.

Kalendar R, Schulman AH. 2006. IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. Nat Protoc 1: 2478-2484. DOI: 10.1038/nprot.2006.377.

Kalendar R, Schulman AH. 2014. Transposon-Based Tagging: IRAP, REMAP, and iPBS. In: Besse P (Ed). Molecular Plant Taxonomy. Vol. 1115. Humana Press, Totowa.

Kang YJ, Kim SK, Kim MY, Lestari P, Kim KH, Ha BK, Jun TH, Hwang WJ, Lee T, Lee J, et al. 2014. Genome sequence of mung bean and insights into evolution within *Vigna* species. Nat Commun 5: 5443. DOI: 10.1038/ncomms6443.

Khajudparn P, Prajongjai T, Poolsawat O, Tantasawat PA. 2012. Application of ISSR markers for verification of F1 hybrids in mung bean (*Vigna radiata*). Genet Mol Res 11: 3329-3338. DOI: 10.4238/2012.September.17.3.

Kikuchi S, Kino H, Tanaka H, Tsujimoto H. 2007. Pollen tube growth in cross combinations between torenia fournieri and fourteen related species. Breed Sci 57: 117-122. DOI: 10.1270/jsbbs.57.117.

Kim H, Terakami S, Nishitani C, Kurita K, Kanamori H, Katayose Y, Sawamura Y, Saito T, Yamamoto T. 2012. Development of cultivar-specific DNA markers based on retrotransposon-based insertional polymorphism in Japanese pear. Breed Sci 62: 53-62. DOI: 10.1270/jsbbs.62.53.

Kim SK, Nair RM, Lee J, Lee SH. 2015. Genomic resources in mung bean for future breeding programs. Front Plant Sci 6: 626. DOI: 10.3389/fpls.2015.00626.

Konovalov FA, Goncharov NP, Goryunova S, Shaturova A, Proshlyakova T, Kudryavtsev A. 2010. Molecular markers based on LTR retrotransposons BARE-1 and Jeli uncover different strata of evolutionary relationships in diploid wheats. Mol Genet Genomics 283: 551-563. DOI:10.1007/s00438-010-0539-2.

Lavin M, Herendeen PS, Wojciechowski MF. 2005. Evolutionary rates analysis of leguminosae implicates a rapid diversification of lineages during the tertiary. Syst Biol 54: 575-594. DOI: 110.1080/10635150590947131.

Li S, Ramakrishnan M, Vinod KK, Kalendar R, Yrjälä K, Zhou M. 2020. Development and deployment of high-throughput retrotransposon-based markers reveal genetic diversity and population structure of asian bamboo. Forests 11: 31. DOI: 10.3390/f11010031.

Liang Y, Lenz RR, Dai W. 2016. Development of retrotransposon-based molecular markers and their application in genetic mapping in

chokecherry (*Prunus virginiana* L.). Mol Breed 36: 109. DOI: 10.1007/s11032-016-0535-2.
Lightbourn GJ, Jelesko JG, Veilleux RE. 2007. Retrotransposon-based markers from potato monoploids used in somatic hybridization. Genome 50: 492-501. DOI: 10.1139/G07-026.
Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS, et al. 2020. CDD/SPARCLE: The conserved domain database in 2020. Nucleic Acids Res 48: D265-D268. DOI: 10.1093/nar/gkz991.
McClean PE, Mamidi S, McConnell M, Chikara S, Lee R. 2010. Synteny mapping between common bean and soybean reveals extensive blocks of shared loci. BMC Genomics 11: 184. DOI: 10.1186/1471-2164-11-184.
Mirani AA, Teo CH, Markhand GS, Abul-Soad AA, Harikrishna JA. 2020. Detection of somaclonal variations in tissue cultured date palm (*Phoenix dactylifera* L.) using transposable element-based markers. Plant Cell Tissue Organ Cult 141: 119-130. DOI: 10.1007/s11240-020-01772-y.
Mogali SC, Hegde GM. 2020. Recent advances in mung bean breeding: A perspective. In: Gosal SS, Wani SH (Eds). Accelerated Plant Breeding. Springer International Publishing, Cham.
Moghaddam SM, Song Q, Mamidi S, Schmutz J, Lee R, Cregan P, Osorno JM, McClean PE. 2014. Developing market class specific InDel markers from next generation sequence data in *Phaseolus vulgaris* L. Front Plant Sci 5: 185. DOI: 10.3389/fpls.2014.00185.
Monden Y, Hara T, Okada Y, Jahana O, Kobayashi A, Tabuchi H, Onaga S, Tahara M. 2015. Construction of a linkage map based on retrotransposon insertion polymorphisms in sweetpotato via high-throughput sequencing. Breed Sci 65: 145-153. DOI: 10.1270/jsbbs.65.145.
Nair R, Schreinemachers P. 2020. Global status and economic importance of mung bean. In: Nair RM, Schafleitner R, Lee SH (Eds). The Mung bean Genome. Springer International Publishing, Cham.
Nei M, Li W-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci 76: 5269-5273.
Orozco-Arias S, Isaza G, Guyot R. 2019. Retrotransposons in plant genomes: Structure, identification, and classification through bioinformatics and machine learning. Int J Mol Sci 20: 3837. DOI: 10.3390/ijms20153837.
Peakall R, Smouse PE. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539. DOI: 10.1093/bioinformatics/bts460.
Rashid K, Othman RY, Ali BSBKS, Yusof YM, Nezhadahmadi A. 2014. The application of irap markers in the breeding of papaya (*Carica Papaya* L.). Indian J Sci Technol 7: 1720-1728. DOI: 10.17485/ijst/2014/v7i11.1
Rey-Baños R, Sáenz De Miera LE, García P, De La Vega MP. 2017. Obtaining retrotransposon sequences, analysis of their genomic distribution and use of retrotransposon-derived genetic markers in lentil (*Lens culinaris* Medik.). PLoS One 12: e0176728. DOI: 10.1371/journal.pone.0176728.
Rohlf FJ. 2009. NTSYSpc. Numerical Taxonomy and Multivariate Analysis: Version 2.2. Exeter Software Setauket, New York.
Sanchez DH, Gaubert H, Drost HG, Zabet NR, Paszkowski J. 2017. High-frequency recombination between members of an LTR retrotransposon family during transposition bursts. Nat Commun 8: 1283. DOI: 10.1038/s41467-017-01374-x.
Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, Jenkins J, Shu S, Song Q, Chavarro C, et al. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46: 707-713. DOI: 10.1038/ng.3008.
Schulman AH, Flavell AJ, Paux E, Ellis THN. 2012. The application of LTR retrotransposons as molecular markers in plants. In: Bigot Y (Ed). Mobile Genetics Element: Protocols and Genomic Applications, Method in Molecular Biology. Humana Press, London.
Setiawan AB, Teo CH, Kikuchi S, Sassa H, Kato K, Koba T. 2020a. Chromosomal locations of a non-LTR retrotransposon, *Menolird18*, in *Cucumis melo* and *Cucumis sativus*, and its implication on genome evolution of *Cucumis* species. Cytogenet Genome Res 160: 554-564. DOI: 10.1159/000511119.
Setiawan AB, Teo CH, Kikuchi S, Sassa H, Kato K, Koba T. 2020b. Centromeres of *Cucumis melo* L. comprise Cmcent and two novel repeats, CmSat162 and CmSat189. PLoS One 15: e0227578. DOI: 10.1371/journal.pone.0227578.
Sharma V, Nandineni MR. 2014. Assessment of genetic diversity among Indian potato (*Solanum tuberosum* L.) collection using microsatellite and retrotransposon based marker systems. Mol Phylogenet Evol 73: 10-17. DOI: 10.1016/j.ympev.2014.01.003.
Sormin SYM, Purwantoro A, Setiawan AB, Teo CH. 2021. Application of inter-SINE amplified polymorphism (ISAP) markers for genotyping of *Cucumis melo* accessions and its transferability in *Coleus* spp. Biodiversitas 22: 2918-2929. DOI: 10.13057/biodiv/d220557.
Stefanović S, Pfeil BE, Palmer JD, Doyle JJ. 2009. Relationships among phaseoloid legumes based on sequences from eight chloroplast regions. Syst Bot 34: 115-128. DOI: 10.1600/036364409787602221.
Teo CH, Tan SH, Ho CL, Faridah QZ, Othman YR, Heslop-harrison JS, Kalendar R, Schulman AH. 2005. Genome constitution and classification using retrotransposon-based markers in the orphan crop banana. J Plant Biol 48: 96-105. DOI: 10.1007/BF03030568
Vuorinen AL, Kalendar R, Fahima T, Korpelainen H, Nevo E, Schulman AH. 2018. Retrotransposon-based genetic diversity assessment in wild emmer wheat (*Triticum turgidum* ssp. dicoccoides). Agronomy 8: 107. DOI: 10.3390/agronomy8070107.
Wang L, Bai P, Yuan X, Chen H, Wang S, Chen X, Cheng X. 2018. Genetic diversity assessment of a set of introduced mung bean accessions (*Vigna radiata* L.). Crop J 6: 207-213. DOI: 10.1016/j.cj.2017.08.004.
Wang ML, Barkley NA, Gillaspie GA, Pederson GA. 2008. Phylogenetic relationships and genetic diversity of the USDA *Vigna* germplasm collection revealed by gene-derived markers and sequencing. Genet Res 90: 467-480. DOI: 10.1017/S0016672308009889.
Xu Z, Wang H. 2007. LTR-FINDER: An efficient tool for the prediction of full-length LTR retrotransposons. Nucleic Acids Res 35: 265-268. DOI: 10.1093/nar/gkm286.
Yu X, Zhai Y, Wang P, Cheng C, Li J, Lou Q, Chen J. 2021. Morphological, anatomical and photosynthetic consequences of artificial allopolyploidization in *Cucumis*. Euphytica 217: 5. DOI: 10.1007/s10681-020-02735-2.
Zein I, Jawhar M, Arabi MIE. 2010. Efficiency of IRAP and ITS-RFLP marker systems in accessing genetic variation of *Pyrenophora graminea*. Genet Mol Biol 33: 328-332. DOI: 10.1590/S1415-47572010005000041.
Zhang X, Liu T, Li X, Duan M, Wang J, Qiu Y, Wang H, Song J, Shen D. 2016. Interspecific hybridization, polyploidization, and backcross of *Brassica oleracea* var. *alboglabra* with *B. rapa* var. *purpurea* morphologically recapitulate the evolution of *Brassica* vegetables. Sci Rep 6: 18618. DOI: 10.1038/srep18618.
Zhao D, Ferguson AA, Jiang N. 2016. What makes up plant genomes: The vanishing line between transposable elements and genes. Biochim Biophys Acta-Gene Regul Mech 1859: 366-380. DOI: 10.1016/j.bbagrm.2015.12.005.
Zhu H, Choi HK, Cook DR, Shoemaker RC. 2005. Bridging model and crop legumes through comparative genomics. Plant Physiol 137: 1189-1196. DOI: 10.1104/pp.104.058891.