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POPULATION STRUCTURE AND GENETIC DIVERSITY ANALYSIS OF INDONESIAN YARDLONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis*)

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SUMMARY

The identification of population structure and genetic diversity is an important step toward enabling the effective and efficient genetic improvement of crop species. We report the analysis of population structure and genetic diversity of Indonesian yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*) genotypes based on inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism markers (REMAP). Results showed that 52% (11 out of 21) of the markers that were applied in this study were useful for the identification of DNA polymorphism in yardlong bean. STRUCTURE analysis revealed two subpopulations, i.e., P1 and P2. Principal coordinate analysis and neighbor-joining cluster analysis were performed to investigate subpopulation differentiation. Significant differentiation was detected in P2, whereas no significant differentiation was observed in P1. The results corroborated the findings of STRUCTURE analysis. Although broad morphological diversity was present across genotypes, narrow genetic diversity was observed at the DNA level. These findings suggested that increasing genetic diversity to obtain favorable genotypes should be emphasized in the genetic improvement of yardlong bean.

Keywords: Population structure, genetic diversity, inter-retrotransposon amplified polymorphism, retrotransposon microsatellite amplified polymorphism markers, yardlong bean

Key findings: IRAP and REMAP markers are useful for dissecting the population structure of yardlong bean. Yardlong bean had narrow genetic diversity at the DNA level.

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INTRODUCTION

Yardlong bean (*V*igna *unguiculata* subsp. *sesquipedalis* L. Verdc.) is considered as one of the most important vegetable legumes in Asia. This plant has ranked third in terms of total cultivation areas in Asia (Ali *et al*., 2001). Compared with

other vegetables, such as spinach, watercress, and bean sprouts, the immature pods of yardlong bean are rich in vitamins and minerals, as well as protein and total dietary fiber (Wills *et al*., 1984). The adaptability of yardlong bean to various environmental conditions and their short growth period render this plant a popular cash crop among farmers (Pidigam *et al*., 2019). However, several constraints challenge the cultivation of yardlong bean; these constraints include nutritional content, visual quality, pests and disease, and low yield (Duangsong *et al*., 2018, Merin *et al*., 2018, Vidya *et al*., 2002,). With the growth of the human population and the effect of global climate change in the coming years, crop production, including yardlong bean production, is projected to be threatened. The genetic improvement of yarldong bean through breeding is needed to overcome this problem.

Crop breeding consists of several steps that are initiated with the assembly of genetic diversity. The comprehensive analyses of population structure and genetic diversity are important for the efficient exploration, utilization, and management of crop genetic resources in breeding programs (Basak *et al*., 2019). Today, a biotechnological tool, namely, molecular markers, can be used to evaluate crop diversity efficiently (Shahabzadeh *et al*., 2020). Abundant types of molecular markers are available for the analysis of crop diversity; they range from markers based on polymerase chain reaction (PCR) to those based on next-generation sequencing (Ramesh *et al.,* 2020). Inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism (REMAP) markers are retrotransposon-based molecular markers that have the potential to elucidate the population structure and genetic diversity of crops due to their coverage, abundance, occurrence in the genome of organism, and transferability across species (Kalendar *et al*., 1999, Roy *et al*., 2015).

The analysis of population structure and genetic diversity based on IRAP and REMAP has been reported for various crops, such as flax (*Linum usitatissimum* L.) (Holasou *et al*., 2016), bread wheat (*Triticum aestivum* L.) (Holasou *et al*., 2019), grapefruit (*Vitis vinifera* L.) (Strioto *et al.* 2019), and citrus (*Citrus sinensis* L.) (El Zayat *et al.,* 2021). Thus, such a procedure has potential application for other crop species, including yardlong bean. We report the analysis of the population structure and genetic diversity of Indonesian yardlong bean genotypes based on IRAP and REMAP markers. This study aimed to provide information on the marker polymorphism, population structure, and genetic diversity of Indonesian yardlong bean. Potential markers for diversity analysis, population structure, genetic diversity, and breeding strategies for yardlong bean genetic improvement are discussed.

MATERIALS AND METHODS

Genetic material and DNA isolation

The plant material consisted of 33 yardlong bean genotypes that comprised landrace, cultivar, and breeding lines and two cowpea (*Vigna unguiculata* subsp. *unguiculata*) genotypes. Detailed information about the genotypes used in this study, as well as their notable morphological features, i.e., growth habit, pod length, seed color, and seed shape, are presented in Table 1. The genetic materials were provided by the Center of Agro Innovation Technology, Universitas Gadjah Mada, Indonesia. Genomic DNA was extracted from fully expanded leaves (±0.1 g) from seedlings aged 2 weeks after sowing by using the protocols described by Murray and Thompson (1988). The quantity and quality of extracted genomic DNA were examined with NanoDropTM (Thermo Fisher) and through 1.5% agarose gel electrophoresis (w/v).

Table 1. Detailed information about the yardlong bean genotypes used in this study.

No.	ID	Species	Status	Origin/Remarks	Growth habit	Pod length	Seed color (primary, secondary)	Seed shape
1	KT.657	Cowpea	Landrace	Gunung Kidul, Special Region of Yogyakarta	Erect	Short	Brown	Elliptic
2	KT.659	Cowpea	Landrace	Gunung Kidul, Special Region of Yogyakarta	Erect	Short	Brown	Elliptic
3	KP.OS	Yardlong bean	Cultivar	Oriental Seed Co.	Twining	Long	Black, white mottled	Kidney-shaped
4	KP.PT	Yardlong bean	Cultivar	Parade Tavi (East West Seed Co.)	Twining	Medium	Reddish brown, white mottled	Kidney-shaped
5	KP.26	Yardlong bean	Landrace	Surabaya, East Java, Indonesia	Twining	Medium	White, mottled black	Kidney-shaped
6	KP.111	Yardlong bean	Landrace	Sleman, Special Region of Yogyakarta	Twining	Long	Black	Curved
7	KP.137	Yardlong bean	Landrace	KacangCabut	Erect	Short	Yellow	Kidney-shaped
8	KP.139	Yardlong bean	Landrace	Pekalongan, Central Java	Twining	Long	Reddish brown	Kidney-shaped
9	KP.140	Yardlong bean	Landrace	Unknown	Erect	Short	Purplish brown	Elliptic
10	KP.141	Yardlong bean	Landrace	Gresik, East Java	Erect	Medium	Purplish brown	Elliptic
11	KP.TJ	Yardlong bean	Cultivar	Tunas Jaya Seeds Co.	Twining	Long	White, mottled black	Kidney-shaped
12	KP.143	Yardlong bean	Landrace	Unknown	Twining	Long	Reddish brown	Kidney-shaped
13	KP.144	Yardlong bean	Landrace	Unknown	Erect	Short	Reddish brown	Kidney-shaped
14	KP.145	Yardlong bean	Landrace	White Seed	Twining	Short	Light yellow	Kidney-shaped
15	KP.146	Yardlong bean	Landrace	Unknown	Twining	Long	Brown	Kidney-shaped
16	KP.150	Yardlong bean	Landrace	JangkangPutih	Erect	Short	Purplish brown	Elliptic
17	KP.151	Yardlong bean	Landrace	Unknown	Twining	Long	Black, white mottled	Kidney-shaped
18	KP.153	Yardlong bean	Landrace	Blitar, East Java	Twining	Long	Light yellow, red mottled	Kidney-shaped
19	KP.156	Yardlong bean	Landrace	JembatanKembar	Erect	Short	Purplish brown	Kidney-shaped
20	KP.PA	Yardlong bean	Cultivar	Pangeran Anvi (Agri Makmur Pertiwi co.)	Twining	Long	Light yellow, red mottled	Kidney-shaped
21	KP.158	Yardlong bean	Landrace	Solo, Central Java	Erect	Short	Light yellow	Elliptic
22	KP.159	Yardlong bean	Landrace	Red Seed	Erect	Long	Brown	Elliptic
23	KP.160	Yardlong bean	Landrace	Subang, West Java	Twining	Long	Reddish brown	Kidney-shaped
24	KP.163	Yardlong bean	Landrace	Pontianak, West Kalimantan	Twining	Medium	Brown	Kidney-shaped
25	KP.PS	Yardlong bean	Cultivar	Putih Super (Chia Tai Seed Co.)	Twining	Medium	Reddish brown	Kidney-shaped
26	KP.Fagiola	Yardlong bean	Cultivar	IPB University, Bogor.	Twining	Medium	Brown	Kidney-shaped
27	KP.HS	Yardlong bean	Cultivar	Hijau Super' (Chia Tai Seed Co.)	Twining	Long	Reddish brown	Kidney-shaped
28	KP.169	Yardlong bean	Landrace	Unknown	Twining	Long	Brown	Kidney-shaped
29	KP.MGS	Yardlong bean	Cultivar	Kacang Panjang Merah (Mix Garden Seed Co.)	Twining	Medium	Brown	Kidney-shaped
30	KP.276	Yardlong bean	Breeding Line	IVEGRI	Twining	Medium	Reddish brown	Kidney-shaped
31	KP.277	Yardlong bean	Breeding Line	IVEGRI	Twining	Long	Brown	Kidney-shaped
32	KP.280	Yardlong bean	Breeding Line	IVEGRI	Twining	Medium	Brown	Kidney-shaped
33	KP.375	Yardlong bean	Landrace	Prambanan, Special Region of Yogyakarta	Twining	Long	Reddish Brown	Kidney-shaped
34	KP.PYB	Yardlong bean	Cultivar	Purple Yardlong Bean (Thailand)	Twining	Long	Reddish brown	Elliptic
35	KP.GD	Yardlong bean	Cultivar	Golden Dragon (Thailand)	Twining	Medium	Black	Elliptic

Table 2. Polymorphism parameters of the IRAP markers used in this study.

Marker Name	IRAP									
	T_a (°C)	TAL	NPL	PPL (%)	*H*	PIC	EMR	MI	DP	RP
Nikita	40.0	3	1	33.33	0.019	0.019	2.971	0.0005	0.019	0.057
Nikita/Sukkula	42.8	10	7	70.00	0.422	0.333	6.971	0.0084	0.515	2.8
Nikita/3′ LTR	42.8	7	2	28.57	0.032	0.032	6.886	0.0009	0.032	0.229
Nikita/LTR6150	41.0	16	12	75.00	0.452	0.35	10.486	0.0085	0.571	3.714
Nikita/LTR6149	47.9	7	4	57.14	0.262	0.228	5.914	0.0063	0.287	0.971
Sukkula	43.9	13	11	84.62	0.486	0.368	7.6	0.0081	0.659	4.8
Sukkula/3′ LTR	41.0	7	4	57.14	0.284	0.244	5.8	0.0067	0.314	0.514
Sukkula/LTR6150	40.0	9	9	100.00	0.497	0.373	4.143	0.0065	0.789	2.343
Sukkula/LTR6149	41.0	11	11	100.00	0.498	0.374	5.143	0.0067	0.782	1.543
3′ LTR/LTR6150	41.0	7	5	71.43	0.424	0.334	4.171	0.0084	0.518	1.143
LTR6150	40.0	14	11	78.57	0.446	0.347	2.657	0.0085	0.560	0.8
LTR6150/LTR6149	40.0	4	2	50.00	0.446	0.346	2.66	0.0063	0.559	0.766
Average (IRAP)		9	7	67.15	0.356	0.279	5.4502	0.0063	0.467	1.64

Table 3. Polymorphism parameters of the REMAP markers used in this study.

Marker Name	REMAP									
	T_a (°C)	TAL	NPL	PPL	*H*	PIC	EMR	MI	DP	RP
Nikita/ISSR808	49.2	5	4	80.00	0.045	0.044	4.886	0.0012	0.045	0.229
Sukkula/ISSR808	42.8	10	8	80.00	0.437	0.342	6.771	0.0085	0.542	3.714
Sukkula/ISSR826	50.0	9	3	33.33	0.288	0.247	7.429	0.0067	0.319	1.086
3′ LTR/ISSR808	51.9	9	7	77.78	0.493	0.372	5.029	0.0079	0.689	1.886
3′ LTR/ISSR826	50.0	4	2	50.00	0.406	0.324	9.314	0.0083	0.487	6.514
LTR6149/ISSR826	43.9	8	2	25.00	0.042	0.041	7.829	0.0012	0.042	0.343
Average (REMAP)		8	4	57.69	0.285	0.228	6.876	0.0056	0.354	2.295
Overall Average (IRAP+REMAP)		8	5	62.42	0.320	0.254	6.163	0.0060	0.411	1.968

Note: TAL = Total Amplified Loci, NPL = Number of Polymorphic Loci, PPL = Percentage of Polymorphic Loci, *H* = Heterozigosity, PIC = Polymorphic Information Content, MI = Marker Index, DP = Discriminating Power, and RP = Resolving Power.

Amplification of IRAP and REMAP markers

A total of 21 IRAP and REMAP markers were derived from five retrotransposon-based primers, namely, 3'LTR, LTR6149, LTR6150, Nikita, and Sukkula (Otwe *et al*., 2017), and two microsatellite primers, namely, ISSR808 and ISSR826 (Tantasawat *et al*., 2010). PCR amplification was performed in accordance with the work of Widyawan *et al*., (2020a) on a T100 Thermal Cycler (BioRad). The amplicons were electrophoresed on 1.5% (w/v) agarose gels.

Data analysis

Genotypic data were tabulated with a binary system by following the work of Krishna *et al*. (2018) to ensure the reproducibility of marker data. The parameters of genetic diversity and marker polymorphisms were calculated by applying iMEC software (Amiryousefi *et al*., 2018). Structural analysis was performed with STRUCTURE V. 2.3.4 (Pritchard *et al*., 2000) by using an admixture model with correlated allele frequencies followed by 100 000 burn-in times and Markov chain Monte Carlo replication number. The optimum number of K was estimated on the basis of the ΔK value based on Evanno method (Evanno *et al*., 2005) by utilizing Structure Harvester V0.6.94 (Earl and Von-Holdt 2012). Principal coordinate analysis (PCoA) and neighbor-joining tree cluster analysis were performed with DARwin v6 (Perrier and Jacquemoud-Collet 2006) on the basis of Jaccard's coefficient with 1000 bootstraps.

RESULTS

Genotyping and marker polymorphism

On the basis of polymorphic parameters, considerable DNA polymorphism was observed across yardlong bean genotypes in this study. The parameters of DNA polymorphism for IRAP and REMAP markers were tabulated in Tables 2 and 3, respectively. Initially, we tested 35 markers for genotyping. However, only 21 markers could be used for the genotyping assay, and the rest of the markers were not able to amplify DNA bands. Moreover, 3 out of the 21 markers, namely, 3'LTR, 3'LTR/6149, and LTR6150/ISSR826, produced monomorphic bands. IRAP markers generated a higher number of total amplified loci (TAL) (108 versus 45), number of polymorphic loci (NPL) (79 versus 26), and average percentage of polymorphic loci (PPL) than REMAP markers. TAL indicates the number of DNA bands that is amplified by a marker. TAL had an average of 8 and ranged from 3 (Nikita) to 16 (Nikita/LTR6150). The NPL value ranged from 1 (Nikita) to 12 (Nikita/LTR6150) with an average of 5. PPL had a mean of 62.42% and ranged from 25% (LTR6149/ISSR826) to 100% (Sukkula/LTR6150 and Sukkula/LTR61489). NPL and PPL showed a number of polymorphic loci that were amplified by the markers. Markers that exhibited high numbers of NPL and PPL could be useful in analyzing the genetic diversity of crop plants.

The informativeness of markers could be identified mainly by using the value of heterozigosity (H) and polymorphic information content (PIC); the higher value of these parameters, the better the informativeness (Chesnokov and Artemyeva 2015). The value of H ranged from 0.019 (Nikita) to 0.498 (Sukkula/LTR6149) with an average of 0.320. The average H value of IRAP (0.356) markers was higher than that of the REMAP markers (0.285). PIC had a mean of 0.254, the lowest value of 0.019 (Nikita), and the highest value of 0.374 (Sukkula/LTR6149). Similar to the H value, the mean PIC of IRAP (0.279) was higher than that of REMAP (0.228).

The effective multiplex ratio (EMR) value indicates the effectiveness of a marker system, whereas MI, the number of polymorphic markers per gel lane, provides an estimation of its total utility (Chesnokov and Artemyeva 2015, Nagaraju *et al*., 2001). The value of EMR

ranged from 2.971 (Nikita) to 10.486 (Nikita/LTR6150) with an average of 6.163. In contrast to that of H and PIC, the mean of EMR of IRAP markers (5.4502) was lower than that of REMAP markers (6.876). The mean MI was 0.0060. MI ranged from 0.0005 (Nikita) to 0.0085 (Nikita/LTR6150). The average MI of IRAP markers (0.0063) was higher that of REMAP markers (0.0056).

The discriminating power (DP) value indicates the possibility that randomly selected pairs of individuals exhibit different genetic profiles. Lastly, RP provides information about the capability of markers to distinguish differences in large numbers of individuals (Chesnokov and Artemyeva 2015). The lowest value of DP was 0.019 (Nikita), whereas the highest was 0.789 (Sukkula/LTR6150). The average DP was 0.368. The mean value of DP of IRAP markers (0.467) was higher than that of REMAP markers (0.354). Lastly, the average value of resolving power (RP) was 1.968. The RP ranged from 0.057 (Nikita) to 6.514 (3'LTR/ISSR826). In contrast to that of DP, the average value of RP of IRAP markers (1.64) was lower than of REMAP markers (2.295).

Population structure and genetic diversity

The Evanno method was used to determine the optimal number of subpopulations based on the ΔK value. The largest ΔK value observed in this study was K = 2 (Figure 1A), indicating that the genotypes were classified into two subpopulations, i.e., P1 and P2. The second highest value of ΔK was observed was K = 8. P1 comprised two genotypes, i.e., KT.657 and KP.141, whereas P2 consisted of 33 genotypes (Figure 1B). KP.141, which is a yardlong bean genotype, belonged to the same subpopulation as KT.657, which is a cowpea genotype. By contrast, another cowpea genotype, i.e., KT.659, belonged to the same subpopulation with the rest of the yardlong bean genotypes.

The pairwise genetic distance (F_{ST}), which is an indicator of the degree of differentiation in a population due to genetic structure, reached 0.0292 and 0.3100 for P1 and P2, respectively. Frankham *et al*. (2002) stated that on the one hand, the F_{ST} value of P1 indicates small subpopulation differentiation. On the other hand, P2 indicates significant subpopulation differentiation based on their F_{ST} value. The morphological data revealed the broad morphological diversity of yardlong bean genotypes used in this study. Morphological variation was observed in the growth habit, pod color, seed shape, and color traits. Interestingly, the genetic similarity among genotypes was high with the average of 0.81 and values ranging from 0.64 (KP.PYB vs. KT.657) to 0.91 (KP.HS vs. KP.169).

PCoA and a neighbor-joining dendrogram were generated to investigate the subpopulation differentiation pattern of P2. The biplot generated by PCoA showed the distribution of yardlong bean genotypes (Figure 2A). The total variation that could be explained by the biplot was 35.82%, with the first and second axes explaining 16.42% and 10.75% of the variation, respectively. The results of PCoA agreed with those of STRUCTURE analysis. Most of the genotypes from P2 tended to cluster together with the exception of KT.657 and KP.141, which belonged to P1 in the STRUCTURE analysis.

Cluster analysis was performed to obtain additional information on the classification of yardlong bean genotypes and confirm subpopulation differentiation. The results of cluster analysis shared similar information with the findings of STRUCTURE analysis and PCoA (Figure 2B). Genotypes that belonged to P1 clustered together with the addition of another genotype, i.e., KP.150. According to the morphological record, similar to KP.657 and KP.141, KP.150 exhibits erect growth and short pod type. Moreover, cluster analysis revealed detailed subpopulation differentiation in P2 as suggested by the F_{ST} value in STRUCTURE

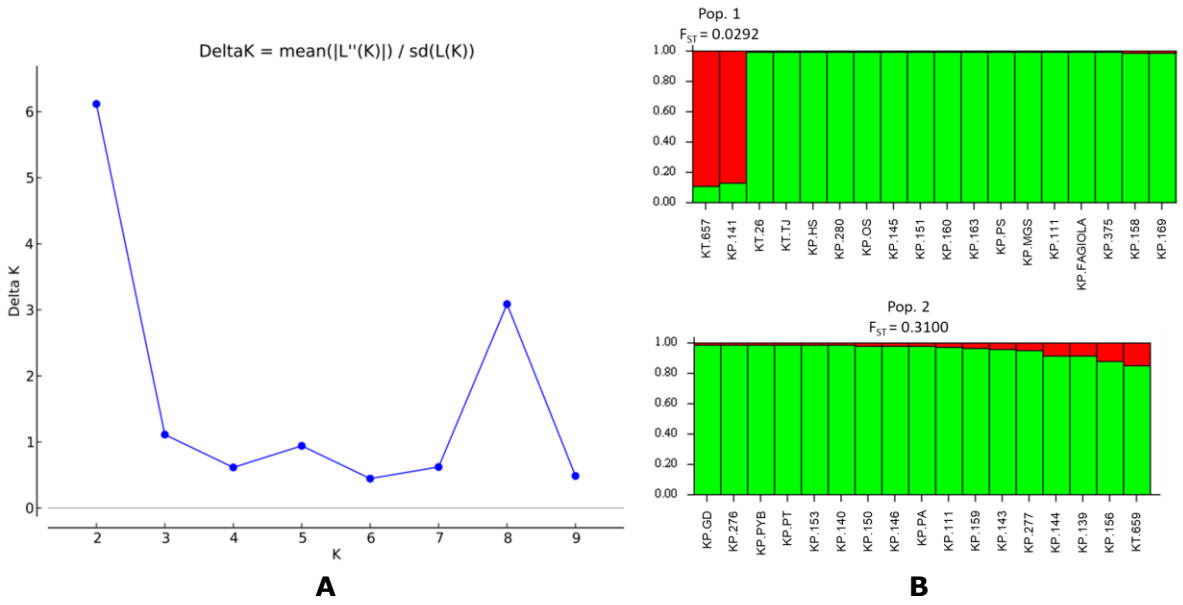

Figure 1. (A) Delta *K* (Δ*K*) for different numbers of subpopulations (*K*); (B) estimated population structure of 33 yardlong bean genotypes and two cowpea genotypes.

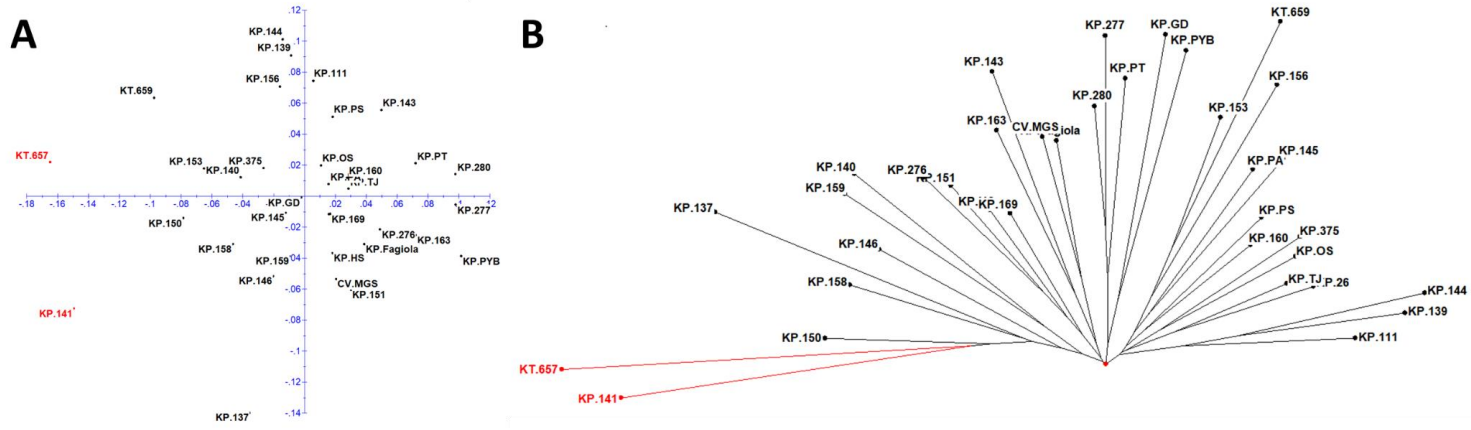

Figure 2. (A) Biplot of PCoA; (B) neighbor-joining dendrogram of 33 yardlong bean genotypes and two cowpea genotypes. Note: The color of genotypes indicates their subpopulation based on STRUCTURE analysis.

analysis. The dendrogram showed that genotypes in P2 could be classified into four different subpopulations on the basis of cluster analysis.

DISCUSSION

The monomorphic DNA bands that were observed in this study suggested the presence of highly conserved DNA regions flanked by markers (Van de Velde *et al*., 2016). Although the REMAP markers were expected to be highly polymorphic (Kalendar *et al*., 1999, Mandoulakani *et al*., 2012), they were outperformed by IRAP markers for most polymorphism parameters (except EMR). The fewer numbers of REMAP than IRAP markers used in this study might have reduced the overall polymorphism parameters of REMAP markers. Most of the markers that contained 3'LTR produced low numbers of polymorphisms in this study as similarly reported for cowpeas (Otwe *et al*., 2017), suggesting that the target DNA regions were absent in the yardlong bean genome.

The H and PIC in this study were higher than those in another retrotransposon-based genetic diversity analysis on yardlong bean reported by Widyawan *et al*. (2020a) and intersimple sequence repeat reported by Tantasawat *et al*. (2010). Compared with the study by Widyawan *et al.* (2020), our study utilized more genotypes with broader morphological diversity and a larger number of markers. However, other studies that applied different marker systems, such as random amplified polymorphic DNA (RAPD) in yardlong bean (Pidigam *et al*., 2019), showed a higher value of PIC. Although RAPD can produce high levels of polymorphism, several drawbacks, such as spurious DNA bands and low reproducibility, reduce its reliability (Govindaraj *et al*., 2015).

The other polymorphism parameters calculated in this study, such as EMR, MI, DP, and RP, provided additional insights into IRAP and REMAP markers that are useful for the genetic diversity analysis of yardlong bean. In general, high values of polymorphism parameters indicate that the markers are useful for genetic diversity analysis. The value of polymorphism parameters suggested that several markers, i.e., Nikita/Sukkula, Sukkula, Sukkula/LTR6150, Sukkula/LTR6149, 3'LTR/LTR6150, 3'LTR/6149, LTR6150, LTR6150/LTR6149, Sukkula/ISSR808, 3'LTR/ISSR808, and 3'LTR/ISSR826, are useful for detecting DNA polymorphism in yardlong bean.

STRUCTURE analysis revealed two subpopulations with considerable differentiation in P2. This finding indicated that during domestication in Asia, the yardlong bean founder was subjected to extensive selection that resulted in diverse morphological features. This condition might account for the inability of cluster analysis to separate the genotypes from P1 and P2 distinctly. Phansak *et al*. (2005), Tantasawat *et al*. (2010), and Widyawan *et al*. (2020a) also reported a low level of genetic diversity among yardlong bean genotypes based on DNA markers. Pasquet (2000) suggested that this phenomenon may be caused by a single domestication event from cowpea, the self-pollinating nature of yardlong bean, and the double genetic bottleneck effect. Xu *et al*. (2016) proposed that the founder cowpea underwent extensive selection for pod length, cooking properties, yield, and adaptation to the local agroecosystem during domestication.

Although yardlong bean exhibited low genetic variation at the DNA level, considerable variations were observed among its various morphological traits, such as growth habit, pod characteristics, seed characteristics, flowering time, and nutritional content (Pidigam *et al*., 2019, Tantasawat *et al*., 2010, Widyawan *et al*., 2020b). Vegetables must meet specific consumer preferences that depend on various factors, such as shape, size, color, taste, and nutrition (Yang *et al*., 2021). In the case of yardlong bean, every country, such as Thailand and Indonesia, has a specific preference (Benchasri and Bairaman 2010, Simarmata *et al*., 2015).

Specific consumer preferences might have contributed to the broad morphological diversity of yardlong bean during its evolution from the primitive group variety (Xu *et al*., 2016).

Methods, such as hybridization and mutation, can be considered to increase the genetic diversity of yardlong bean. Considering the low genetic diversity of yardlong bean, the utilization of its relatives could be beneficial in the effort to broaden its genetic diversity. Some of the cowpea genotypes have been identified as sources of insect, disease, and abiotic stress resistance (Boukar *et al*., 2020). Hybridization between yardlong bean and cowpea is possible because they belong to the same species and share high genetic similarity. The utilization of yardlong bean × cowpea hybrids has been reported in the genetic improvement program of cowpea (Edematie *et al*., 2021), as well as in the generation of genetic mapping populations (Kongjaimun *et al*., 2012, Watcharatpong *et al*., 2020). Moreover, the wild relatives of *Vigna*, i.e., *Vigna vexillate*, *Vigna vexillata macrosperma*, *Vigna luteola, Vigna oblongifolia*, *Vigna unguiculata dekindtiana*, *Vigna racemosa*, *Vigna reticulata*, and *Vigna ambacensis*, could also be potentially used as donors. However, additional treatment, such as embryo rescue, should be applied to obtain hybrids.

Although transgenic methods have been reported for cowpea (Kumar *et al*., 2017, Solleto *et al*., 2008), no study has reported the utilization of this method in yardlong bean. Jahan *et al*., (2015) reported in vitro propagation methods for yardlong bean with high survival rates that can facilitate the development of transgenic methods. Hybridization may result in the emergence of novel superior phenotypes through inter- and intra-allelic interaction or transgressive segregation (Goulet *et al*., 2017). Mutation offers an opportunity to produce mutants with novel alleles for favorable phenotypes that may be nonexistent in nature (Nurmansyah *et al*., 2019).

The genotypes used in this study did not cluster properly on the basis of their geographic origin. Similar results have been reported for yardlong bean germplasm from South East Asia, India, and Indonesia (Phansak *et al*., 2005, Pidigam *et al*., 2019, Widyawan *et al*., 2020a), and for the other crops, such as alfalfa (Mandoulakani *et al*., 2015), einkorn wheat (Taheri *et al*., 2018), bread wheat (Gupta *et al*., 2003), and finger millet (Kumar *et al*., 2016). Anthropogenic factors, such as seed migration by people or trade, may explain this phenomenon (Basak *et al*., 2019). Clustering is more likely to be affected by the morphological features of each genotype rather than geographical origins (Berdugo-Cely *et al*., 2017, Mohammed *et al*., 2019). For example, morphological data records revealed that KP.141, KT.657, and KP.150 used to exhibit short pods, erect growth habit, and seed shape and belonged to the same cluster. Given that the results of marker analysis reflect the morphological features of yardlong bean genotypes, they can potentially reduce the cost and labor needed for screening large numbers of germplasms (Widyawan *et al*., 2020c).

Several IRAP and REMAP markers used in this study showed high polymorphism parameters. These markers can be applied to obtain reliable information on the genetic diversity of yardlong bean genotypes at the DNA level. Information on the genetic diversity of germplasm is important because it determines the management and strategies of breeding programs. Despite its broad morphological diversity, yardlong bean has narrow genetic diversity as revealed by STRUCTURE analysis and PCoA. The results suggested that the genetic improvement of yardlong bean should focus on broadening genetic diversity through hybridization to obtain novel favorable phenotypes and transgressive segregation. These findings will provide an opportunity to obtain yardlong bean cultivars with improved agronomic characters and which are suitable to consumer preference.

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