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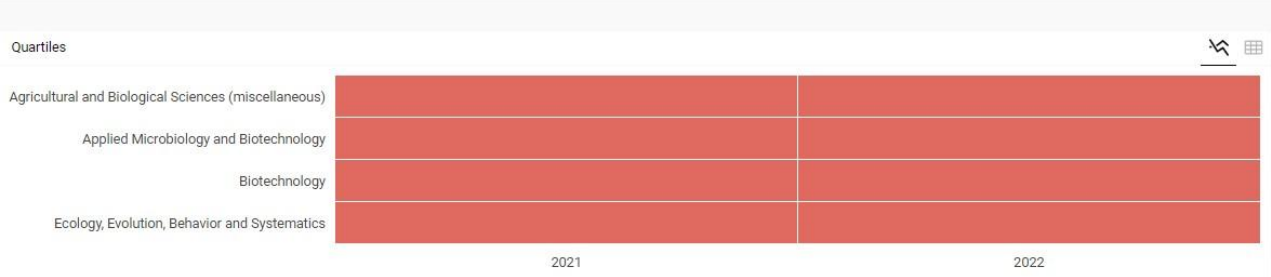

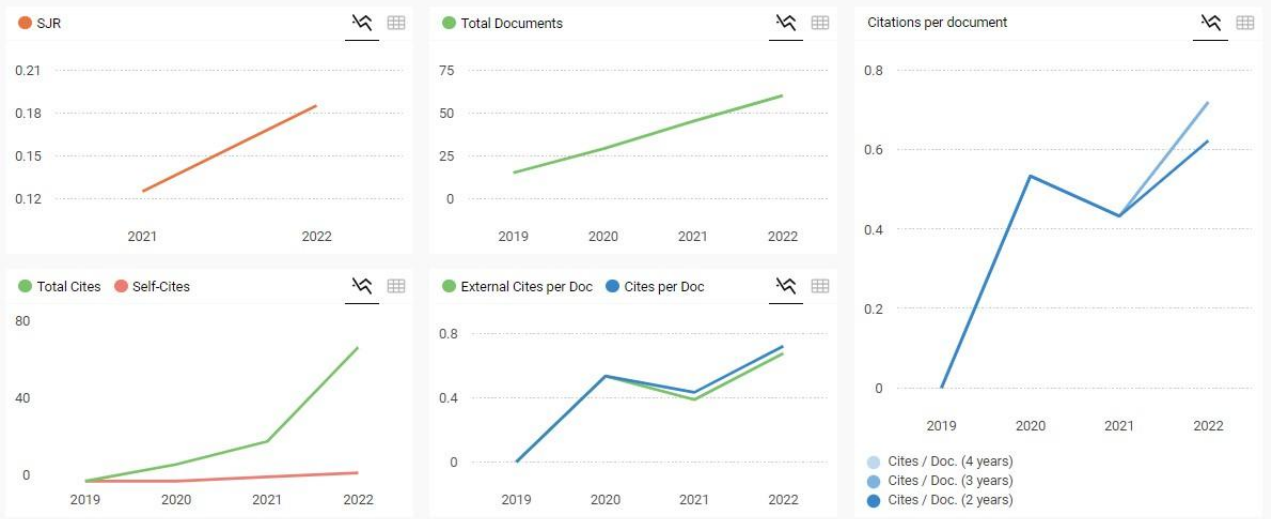

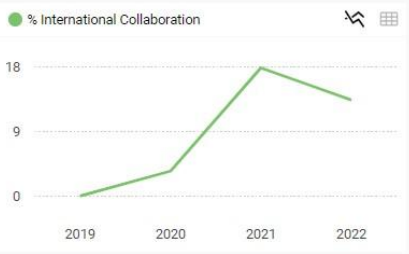

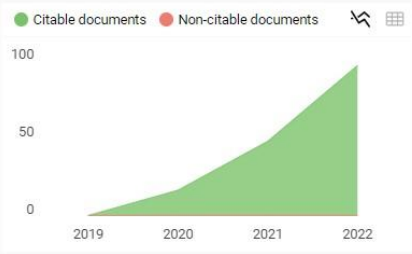

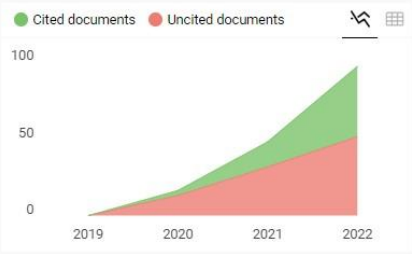

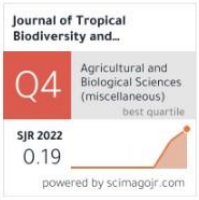

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Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on PCR-RAPD technique

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Abstract

Phaseolus lunatus L. is one of the legume plants found in some parts of Indonesia and has potential as alternative food rich in protein. This current research aimed at analysing genetic accessions of *P. lunatus* distributed in some areas in East Java, Indonesia, based on the RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) marker. 15 accessions originated from four locations were analysed. Ten primers were used and produced 68 bands out of 67 were polymorphic. The percent polymorphism was 96% to 100%. Ten unique bands were detected in eight accessions (Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6). Using the Neighbor-Joining method, a phylogenetic tree was yielded by a similarity coefficient of 64% to 100%. On the genetic similarity coefficient (GSC) of 0.6, there were two clusters: the first and second major clusters (Cluster A and B). The former contained the accessions 7, 8, 13, and 14, while the latter comprised 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region cluster in the same cluster.

Keywords: Genetic diversity; Lima bean, Phylogeny, Polymerase Chain Reaction, Random Amplified Polymorphic Deoxyribonucleic acid

1. Introduction

Phaseolus lunatus L. is categorized as a legume plant with great potential to become nutritious food. In Indonesia, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti and Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions existing in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as an alternatively functional food source (Diniyah *et al*., 2013; Diniyah *et al*., 2015; Herry *et al*., 2013; Nafi *et al*., 2015). Nowadays, Indonesia is dealing with the severe issue of protein shortage in some areas (Diana *et al*., 2017; Ickowitz *et al*., 2016; Madanijah *et al.,* 2016), so maximizing consumer consumption *P. lunatus* will be an effective solution.

With respect to elevating *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content needs actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the programs of food source enhancement (Bhanu, 2017). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a betterment scheme for the species since the beginning of systematic plant breeding (Bhanu, 2017). Furthermore, such information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, the research underpinning genetic diversity in a particular plant also leads to conservation (Carvalho *et al*., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using numerous techniques such as morphological identification, biochemical characterization, and analysis of molecular markers (Govindaraj *et al*., 2015). Related to those techniques, the selection of molecular markers is considered more appropriate and effective to avoid any bias due to environmental influence and provide eclectic information about genetic diversity in a more acceptable way (Fu, 2015). Some molecular markers are included and considered particularly promising in helping analyse genetic diversity, such as RAPD, RFLP, and SCAR.

Among those markers, RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) is the most popular marker in many research projects (AlRawashdeh and AlRawashdeh, 2015; Ben-Ari and Lavi, 2012). RAPD constitutes a PCR-based (Polymerase Chain Reaction) technique that involves a primary set with a relatively short size and can be a PCR-based technique that involves a relatively short size and can randomly amplify many DNA segments (Kumari and Thakur, 2014). This

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technique is equipped with outstanding excellence compared to others, which occupies a universal primary set without undergoing the DNA sequencing phase in its actual implementation. In addition, the RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar and Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari and Thakur, 2014), and is applicable for various laboratory situations (Kumar and Gurusubramanian, 2011). Therefore, RAPD has often been used as a genetic marker in much research on the genetic variation of various legumes.

However, analyses of the genetic diversity of *P. lunatus* in Indonesia are still rare. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in North America (Serrano-serrano *et al.*, 2010), Central America (Camacho-Pérez *et al.*, 2018), and South America (Silva *et al.*, 2019). On the one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as an alternative food source (Diniyah *et al*., 2013; Herry *et al*., 2014) alongside its essential substances (Diniyah *et al*., 2015; Praseptiangga *et al*., 2018; Sukatiningsih *et al*., 2013; Tejasari, 2016). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti and Fauzi, 2019). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on the RAPD marker.

2. Material and Methods

2.1. Collection of Samples

P. lunatus used in this present study was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. Based on the result of identification in previous research (Purwanti and Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Further, each of the accessions was planted in a polybag in which one polybag was distanced one meter long from the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agents.

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

2.2. DNA Isolation

DNA isolation was administered based on the CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue stemmed from the leaf organ of 3 mo (month-old) plants. First, leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into the buffer. The results of isolated DNA were stored under a temperature of -20 ºC.

Table 2. List of primers

Primers	Sequence 5'-3'	GC Content (%)
OPA6	GGTCCCTGAC	70
OPA8	GTGACGTAGG	60
OPA10	GTGATCGCAG	60
OPA20	GTTGCGATCC	60
OPC19	GTTGCCAGCC	70
OPD8	GTGTGCCCCA	70
OPD12	CACCGTATCC	60
OPE8	TCACCACGGT	60
OPE15	ACGCACAACC	60
OPE16	GGTGACTGTG	60

2.3. PCR-RAPD

Ten primers having 60 to 70 GC content were used in this present study (Table 2). The total volume of PCR reaction used signified 22.5 µL, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCl; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and 30 ng DNA template. The condition for PCR reaction was designed under the pre-denaturation temperature of 94 °C (in 5 min), denaturation temperature of 94 °C (in 1 min), primary attachment temperature of 36 °C (in 1 min), extension temperature of 72 °C (in 2 min), the post-extension temperature of 72 °C (in 5 min), and post PCR reaction temperature of 4 °C (in 2 min). For multiplication, the cycle of the PCR reaction was repeated 36 times.

2.4. Agarose Gel Electrophoresis

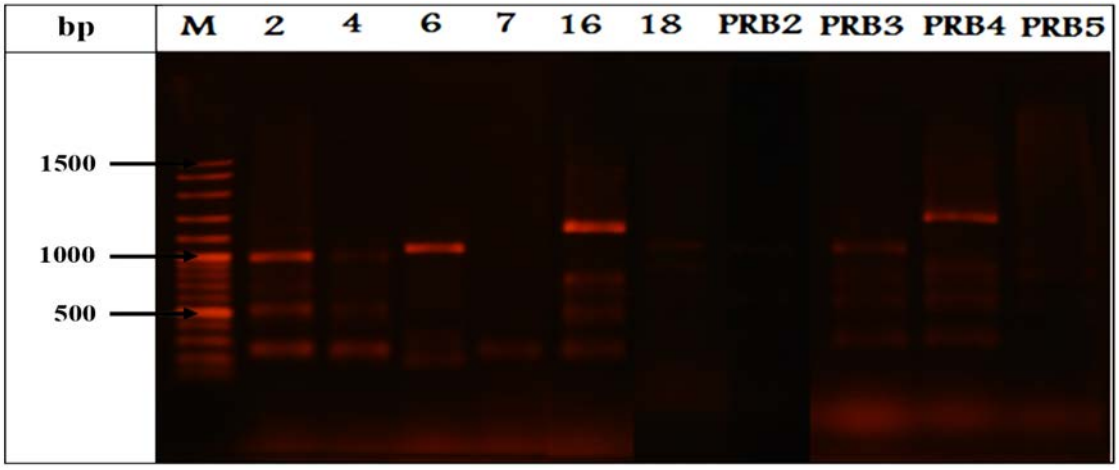
There were three stages of procedure to confirm the result of isolation process and PCR reaction after implementation. The first stage was creating agarose gel with a concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labelled electrophoresis with the electrophoresis buffer of TBE (1×), loading dye (6×) under the condition of 60 V, 400 mA within 45 min. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

2.5. Analysis on the DNA Bands Yielded from RAPD

Data analysis was performed by observing the pattern of visible bands from the electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data (0 and 1), indicating the existence and inexistence of bands typifying specific sizes. Afterward, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and create a phylogenetic tree by creating a phylogenetic tree with Popgen software version 3.1 (Yeh and Boyle, 1997).

3. Results and Discussion

In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, as a result of PCR–RAPD amplification at those genotypes (Figure 1), the bands were assessed based on the binary data, with the description of 1 for the amplified band and 0 for unamplified. The following Table 3 showed the record of the number of loci found.



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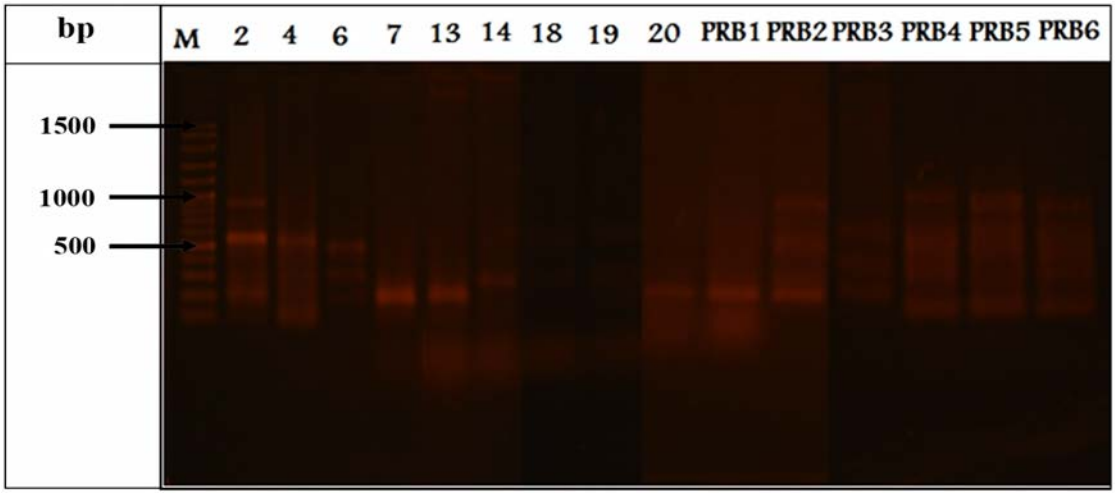

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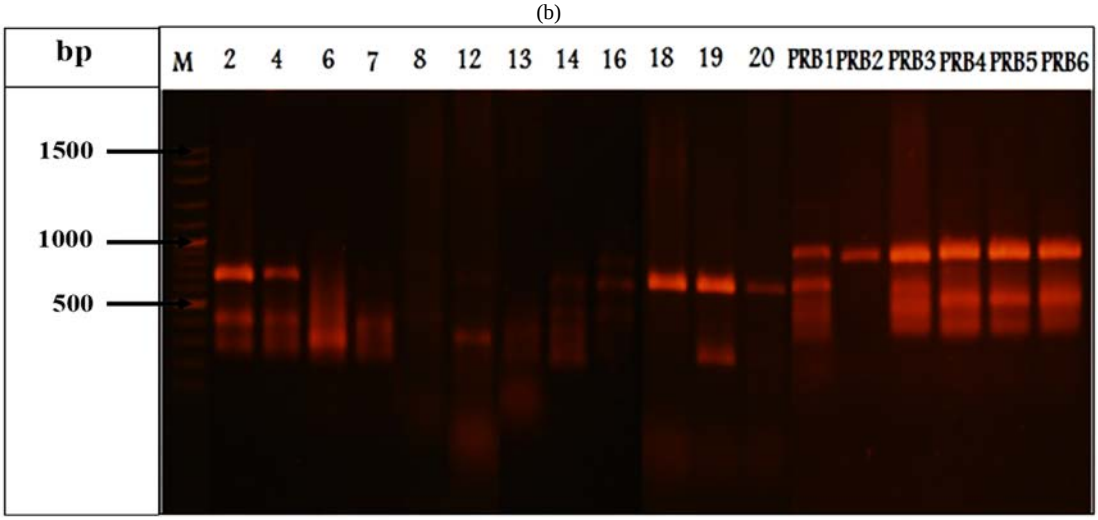

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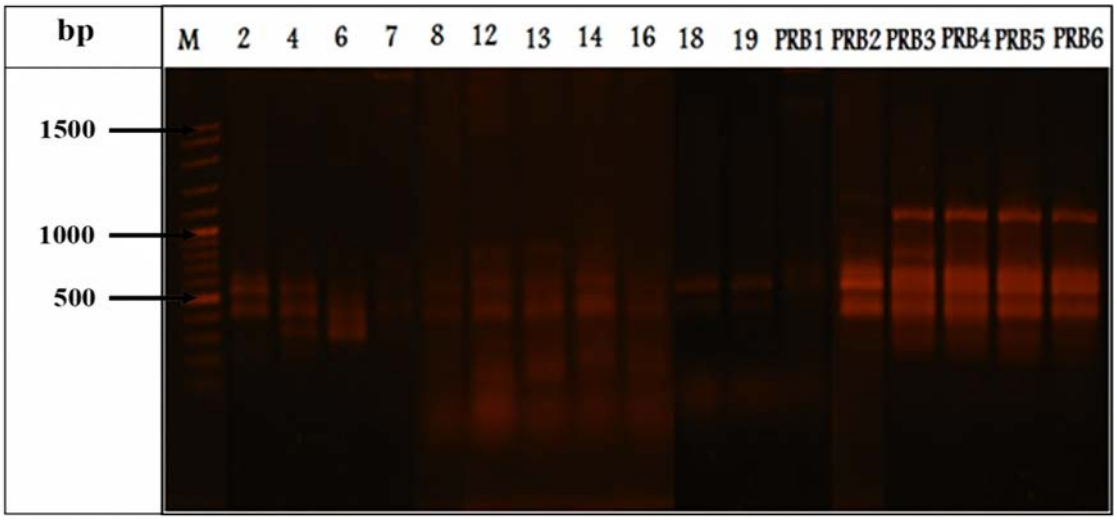

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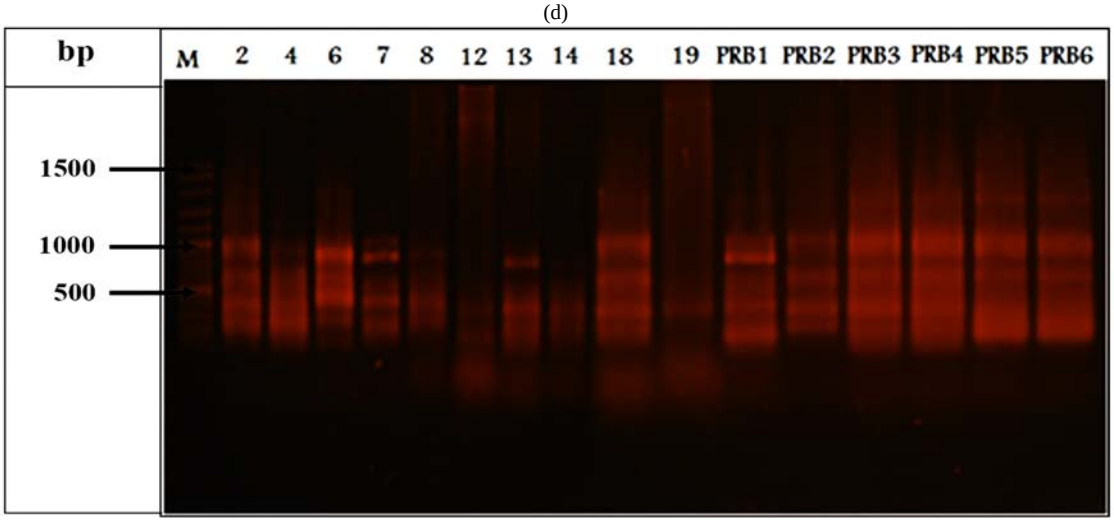

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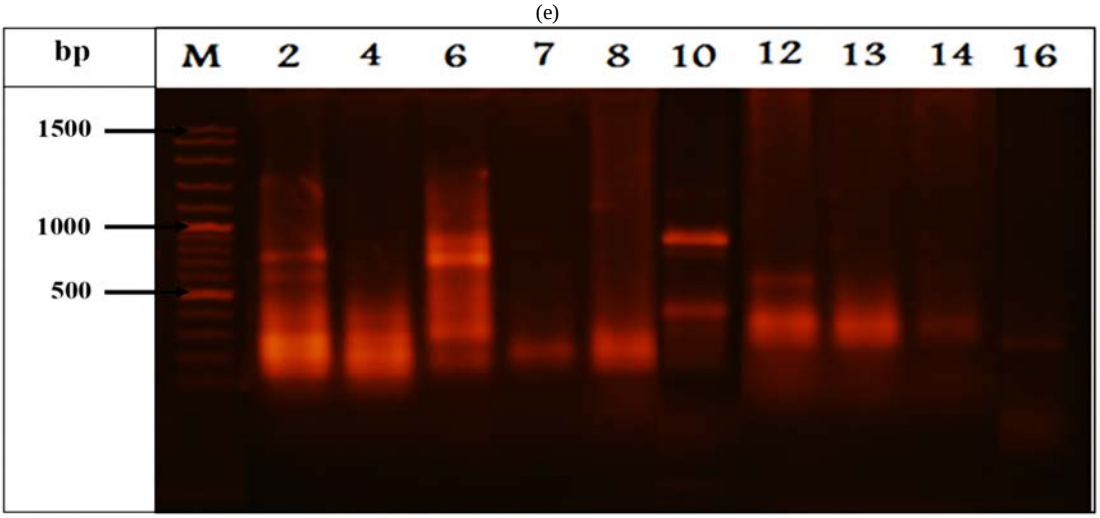
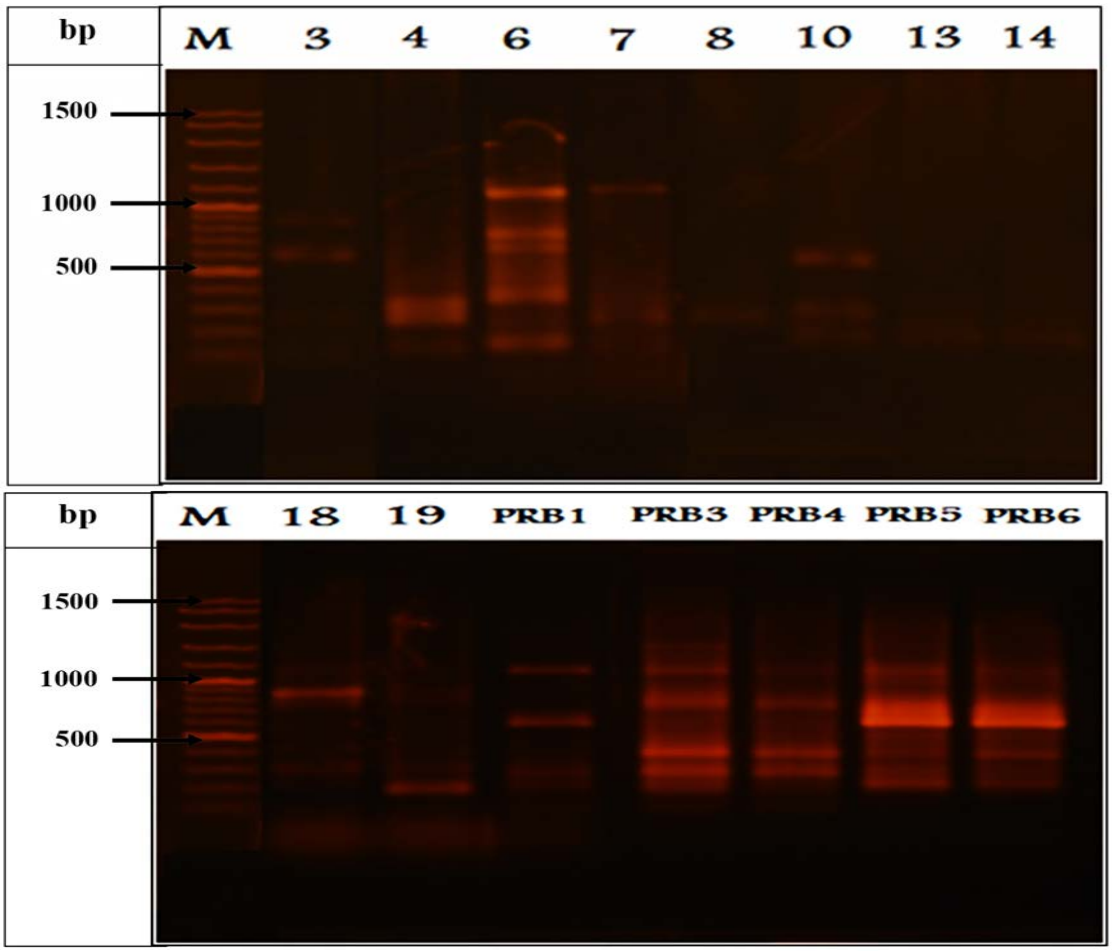
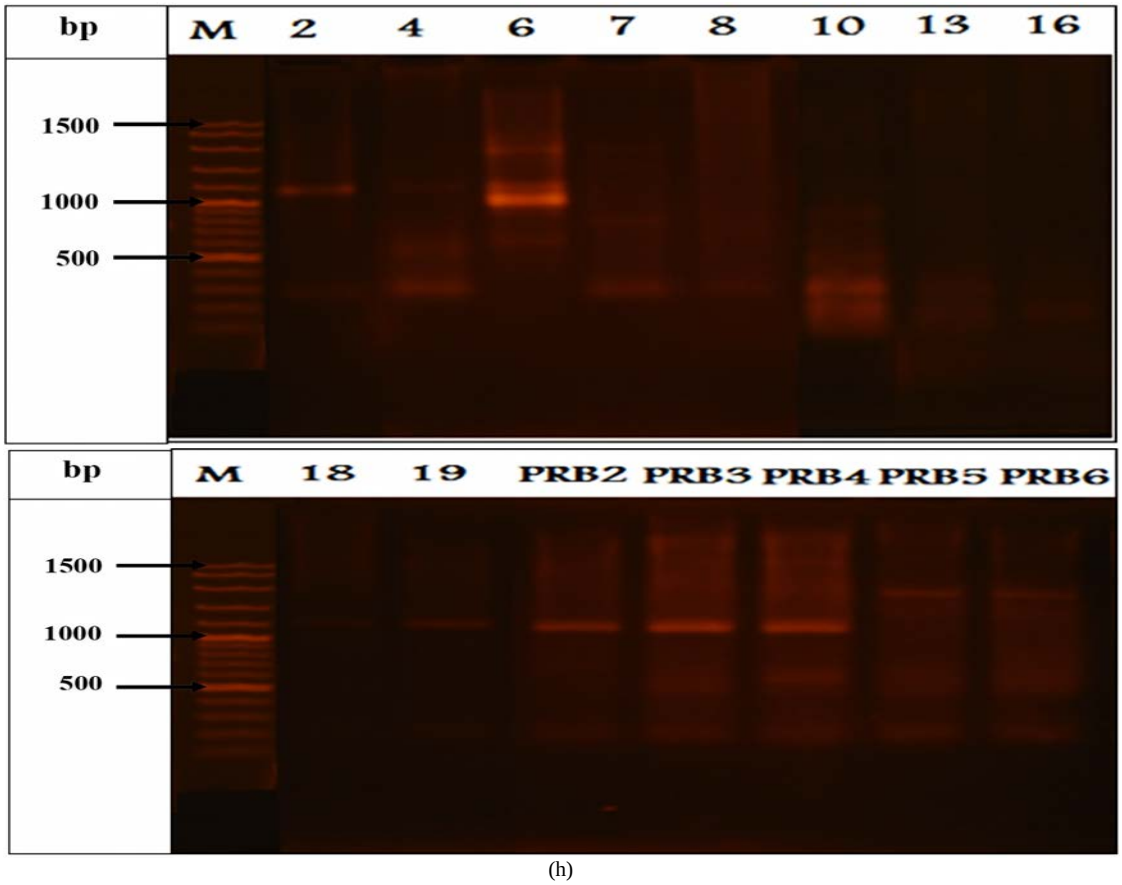


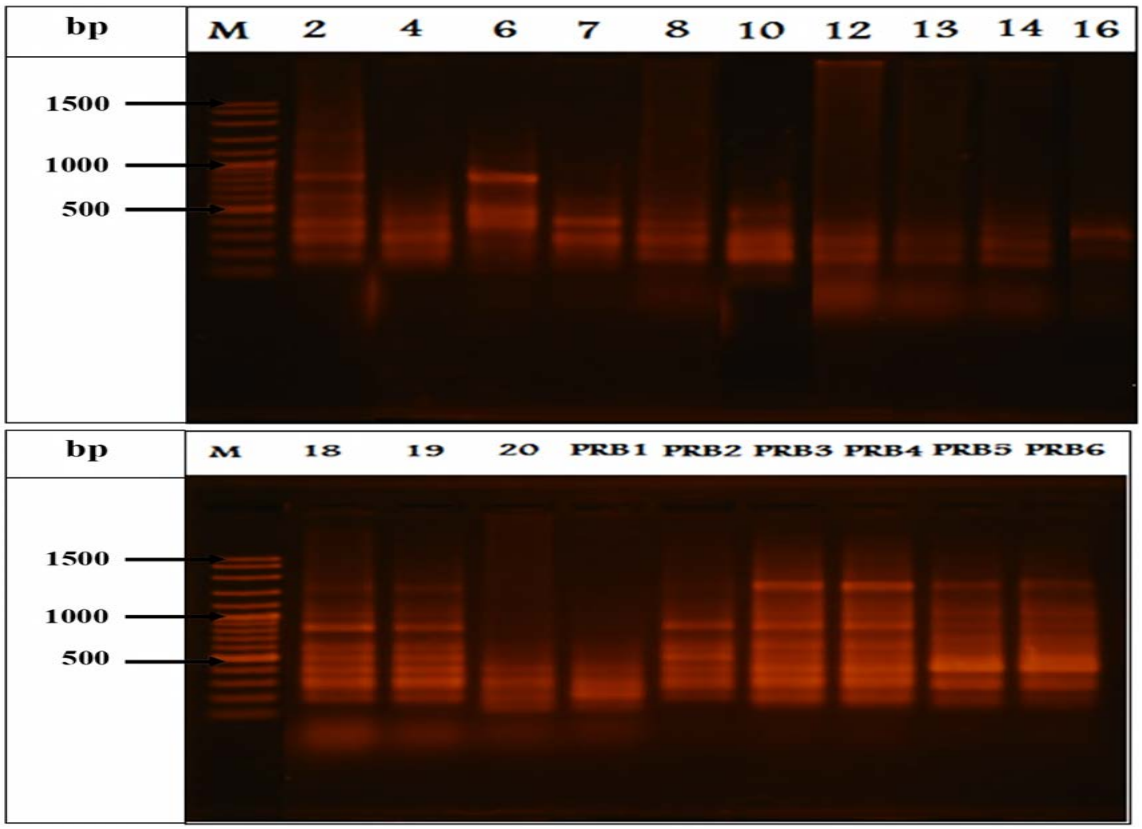
(f)


bp
M 3 4 6 7 8 10 13 14
1500
1000
500
bp
M 18 19 PRB1 PRB3 PRB4 PRB5 PRB6
1500
1000
500

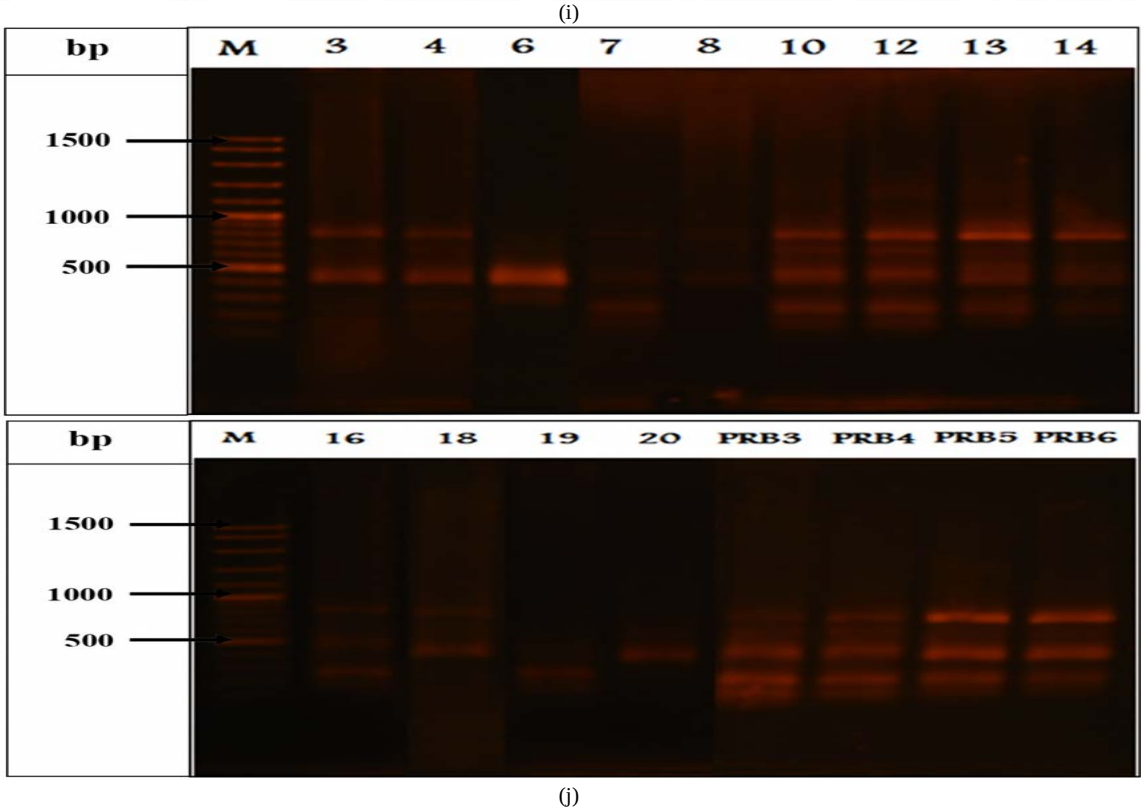
(g)


bp
M 2 4 6 7 8 10 13 16
1500
1000
500
bp
M 18 19 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(h)



(i)



(j)

Figure 1. Gel electrophoresis profiles for PCR–RAPD results using (a) OPA6, (b) OPA8, (c) OPA10, (d) OPA20, (e) OPA19, (f) OPD8, (g) OPD12, (h) OPE8, (i) OPE15, (j) OPE16. M is the DNA size ladder

Table 3. The record of the number of locus found at 15 genotypes of *P. lunatus* using 10 random primary

Codes	Locus (bp)												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description: (1): DNA band was existent, (0): DNA band was non-existent.

After PCR was conducted, DNA fragments of diverse sizes (polymorphic) were produced (Table 4). A total of 68 amplified bands were obtained, out of 67 were polymorphic. The percent polymorphism was 96% to 100%. The total number of amplified bands varied between 5 (OPA10 and OPA20) to 9 (OPA12), with an average of 6.8 bands per primer. Ten unique bands were detected in several accessions. The size of the unique band ranged from 200 (Mdr6) to 1 300 bp (Prb5).

Table 4. RAPD primers used for diversity analysis of *P. lunatus*.

No.	**Markers (100 bp–2000 bp)**	**∑ Band**	**Polymorphic**	**% Polymorphic**	**Unique band**		
					Total	**Locus**	**Accession**
1	OPA 6	8	8	100%	2	700	Mdr16
						200	Mdr6
2	OPA 8	7	7	100%	0	-	-
3	OPA 10	5	5	100%	0	-	-
4	OPA 20	5	5	100%	1	300	Mdr4
5	OPC 19	6	5	96%	0	-	-
6	OPD 8	8	8	100%	1	500	Prb1
7	OPD 12	9	9	100%	2	1 200	Prb3
						900	Mdr19
8	OPE 8	7	7	100%	2	1 300	Prb5
						500	Mdr4
9	OPE 15	7	7	100%	0	-	-
10	OPE 16	6	6	100%	2	700	Mdr12
						500	Mdr16
	Total	68	67		10		

The cluster analysis upon the 68 RAPD bands was administered. The phylogenetic tree using Neighbor-Joining method was produced, equipped with similarity coefficient that ranged from 64% to 100%, or there was genetic variation with the range of 0% to 36% (Figure 2). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close.

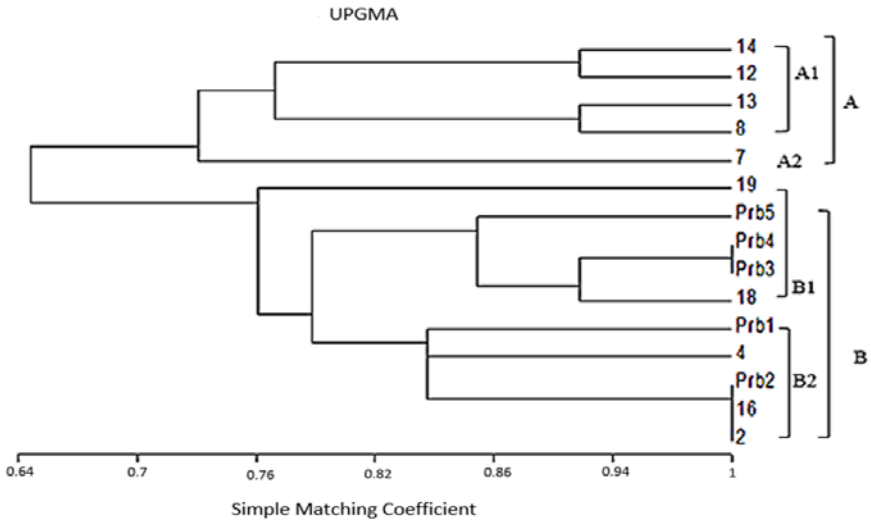


Figure 2. The dendrogram showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96% to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez *et al*., 2018; Chacón-Sánchez and Martínez-Castillo, 2017). The existing high polymorphism had indicated the vast range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes a series of parameters to define genetic diversity on particular species (Singh and Kulathinal, 2013). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of the gene pool. According to the previous study, the 15 accessions of *P. lunatus* included in the current research were originated from five different gene pools, name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti and Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The ecological condition was closely interconnected with the agro-climatic zone in which it was grown. The origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity considered high had brought about an urgent implication in terms of crop improvement attempt, including the breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not effective as the main food resource (Doria *et al*., 2012). Consequently, the process of maturation in *P. lunatus* seeds needed proper and acceptable process; thus it could reduce the anti-nutritional substances (Sukatiningsih *et al*., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factors. In addition, the breeding procedure could be designed that way to yield the generation of *P. lunatus* with highly pest-resistant performance and a shorter period of maturation phase.

Next, concerning the phylogenetic tree formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width, and pod width compared to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti and Fauzi, 2019).

Despite this fact, cluster analysis also indicated the existence of accessions of which pod lengths were quite different but still grouped in the same cluster. In contrast, when the pod length was not significantly different, cluster analysis classified those accessions in different clusters. This was probably due to DNA related to the RAPD marker used in this current research being unrelated to the character set. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, the use of RAPD was not only affordable but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. However, there were some studies of genetic variation on germplasm of beans that were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al*., 2015), South Africa (Adesoye and Ojobo, 2012), Turkey (Ince and Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al*., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al*., 2018) and SNP (Chacón-Sánchez and Martínez-Castillo,

2017). In short, this current research has advocated the credibility of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

4. Conclusion

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analysed. The result of RAPD using ten primers resulted in 68 bands in which nine primers possessed 100% level of polymorphism, and one primary was equipped with 96% level of polymorphism. In addition, ten unique bands were detected in eight accessions, i.e. Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6. Based on cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13, and 14, while the latter comprised the 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

References

Adesoye 0A and Ojobo OA. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv*. **4 (13)**: 453–460.

AlRawashdeh IM and AlRawashdeh NQ. 2015. Evaluating the genetic relatedness within *Lupinus pilosus* L. species based on RAPD analysis. *Jordan J. Biol. Sci*. **8** (1): 61–64**.**

Basheer-Salimia R, Shtaya M, Awad M, Abdallah J and Hamdam Y. 2013. Genetic diversity of Palestine landraces of Faba bean (*Vicia faba*) based on RAPD markers. *Genet. Mol. Res*. **12 (3)**: 3314–3323.

Ben-Ari G and Lavi U. 2012. Marker-assisted selection in plant breeding. In: Chapter 11, **Plant Biotechnology and Agriculture**. Academic Press. Elsevier. USA.pp 163–184

Bhanu AN. 2017. Assessment of genetic diversity in crop plants - An overview. *Adv. Plants Agric. Res*. **7 (3)**: 279–286.

Bukhari A, Bhat MA, Ahmad M and Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol*. **14** (**6)**:451–458.

Carvalho YGS, Vitorino LC, de Souza UJB and Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*. **11 (4)**: 1–21.

Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, Nugraha GI, Gibson RS and Houghton L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLoS One*. **12 (4)**: 1–17.

Diniyah N, Windarti WS and Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagai bahan pangan alternatif pensubtitusi kedelai [Development of food technology based on koro-koroan as an alternative food material to substitute soybeans] Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi. UPN Veteran, Surabaya, Indonesia.

Diniyah N, Windrati WS and Riady S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (*Phaseolus lunatus* L.) dengan perlakuan lama perendaman [The functional properties of black, red and white koro kratok flour (*Phaseolus lunatus* L.) with long soaking treatment. *Jurnal Hasil Penelitian Industri*. **28 (2)**:70–77.

Doria E, Campion B, Sparvoli F, Tava A and Nielsen E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (*Phaseolus vulgaris* L. and *Phaseolus lunatus* L.) landraces cultivated in southern Italy. *J Food Compost Anal*. **26** (1–2): 72–80.

Doyle J J. and Doyle JL. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*. **19**: 11–15.

Fu YB. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet*. **128** (11): 2131–2142.

Govindaraj M, Vetriventhan M. and Srinivasan M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. **ID 431487**: 1–14

Herry B, Windarti WS and Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein [The potential of koro-koroan as a source of local ingredients for the manufacture of various processed protein products] Prosiding Seminar Nasional. Universitas Muhammadiyah Jember, Jember, Indonesia.

Huang W, Zhao X, Zhao X, Li Y and Lian J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. Ecol. Evol. **6 (22)**: 8256–8266.

Ickowitz A, Rowland D, Powell B and Salim MA. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLoS One* **11 (5)**: 1–15.

Ince AG and Karaca M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics*. **4** (4): 220–227.

Kalaminasih D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (*Canavalia ensiformis* L.) terhadap mutu organoleptik tempe koro [Effect of the proportion of *Phaseolus lunatus* and *Canavalia ensiformis* L.) on the organoleptic quality of tempe koro]. *E-Journal Boga*. **2** (3): 104–113.

Kumar NS and Gurusubramanian G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis.* **11** (3): 116–124.

Kumari N and Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. *Am J Anim Vet Sci*. **9**(1): 6–13.

Lim TK. 2012. *Lablab purpureus*. In: **Edible Medicinal And Non-Medicinal Plants, Vol. 2, Fruits.** Springer Netherlands. pp. 730–741.

Madanijah S, Briawan D, Rimbawan R, Zulaikhah Z, Andarwulan N, Nuraida L, Sundjaya T, Murti L, Shah Priyali, Bindels J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: A cross-sectional dietary and nutrient intake study. *Br J Nutr*. **116**: 1–10.

Maftuchah and Zainuddin. 2006. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (*Jathropa curcas* L.) [Development of genomic DNA isolation methods in *Jathropa curcas* L.]. *Humanity*. **2 (1)**: 63–69.

Nafi A, Diniyah N. and Hastuti, FT. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (*Phaseolus lunatus* L.) termodifikasi yang diproduksi secara fermentasi spontan [Physicochemical and technical functional characteristics of modified *Phaseolus lunatus* L. flour produced by spontaneous fermentation]. *Agrointek*, **9 (1)**: 24–32.

Praseptiangga D, Tryas A A, Affandi DR, Atmaka W, Ariyantoro AR and Minardi S. 2018. Physical and chemical characterization of composite flour from Canna flour (*Canna edulis*) and Lima bean flour (*Phaseolus lunatus*). *AIP Conf Proc*. **1927 (030020)**: 1–6.

Purwanti E and Fauzi A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. *IOP Conf. Ser. Earth Environ. Sci*. **276** (012017):1–10

Serrano-serrano ML, Hernández-torres J, Castillo-villamizar G, Debouck DG and Chacón, MI. 2010. Molecular phylogenetics and evolution gene pools in wild Lima bean (*Phaseolus lunatus* L.) from the Americas: Evidences for an Andean origin and past migrations. *Mol. Phylogenet. Evol*. **54** (1): 76–87.

Silva RNO, Lopes ACA, Gomes RLF, Pádua JG and Burle ML. 2019. High diversity of cultivated Lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. *Genet. Mol. Res.* **18** (4): 1–15.

Singh RS and Kulathinal RJ. 2013. Polymorphism. In: Maloy S and Hughes K (Eds.). **Brenner's Encyclopedia of Genetics 2nd Edition.** Academic Press - Elsevier. Cambridge, Massachusetts, USA, pp. 398–399.

Sukatiningsih, Yustian AM and Windarti SW. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet] [Addition of soy protein isolate and toxic sucrose to the sprouts of Phaseolus lunatus (L) sweet. *Agritrop Jurnal Ilmu-Ilmu Pertanian.* **11 (1)**: 1–7.

Syuaib MF. 2016. Sustainable agriculture in Indonesia: Facts and challenges to keep growing in harmony with environment. *Agri Eng Int: CIGR J.* **18 (2)**:170–184.

Yeh, F.C., and T.J.B. Boyle. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian J. Bot.* **129**:157–163.

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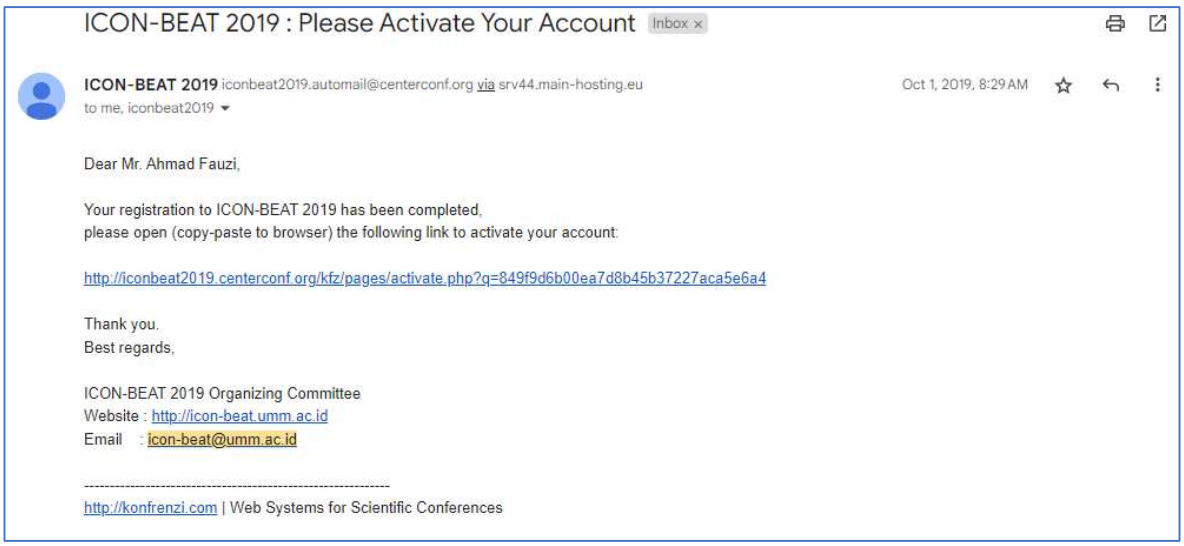

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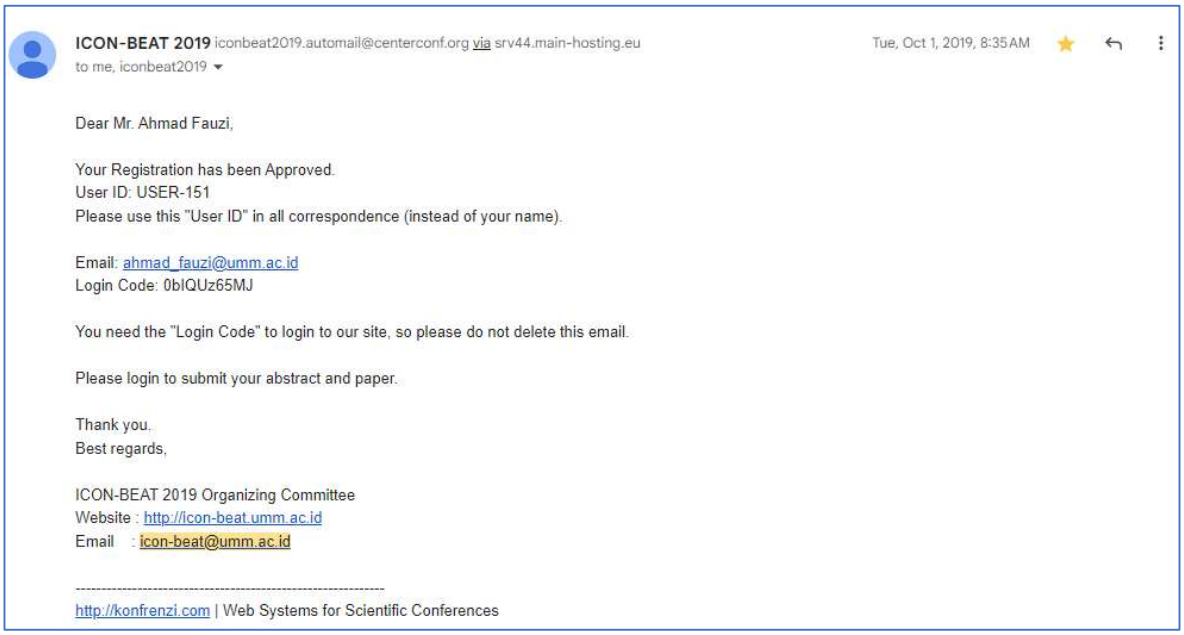

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Title:

Genetic Diversity of *Phaseolus lunatus* L. in East Java based on RAPD Marker

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GENETIC DIVERSITY OF *Phaseolus lunatus* L. IN EAST JAVA BASED ON RAPD MARKER

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ABSTRACT

Phaseolus lunatus L. is one of legume plants that can be found at some parts of Indonesia and is potential for alternative food rich of protein. This current research aimed at analyzing genetic accessions of *P. lunatus* distributed at some areas in East Java, Indonesia based on RAPD marker. A total of 15 accessions originated from Tulungagung, Kediri, Malang, Probolinggo, and Madura were analyzed. In addition, there were 10 primers used, i.e. OPA6, OPA8, OPA10, OPA20, OPC19, OPD8, OPA10, OPA20, OPC19, OPD8, OPE15, and OPE16 generating 68 lines of DNA band typified with different sizes. Further, there were 9 primers in the possession of 100% level of polymorphism in which only Primary OPC19 yielded 96% level of polymorphism. By means of Neighbor-Joining method, a phylogenic tree was yielded by similarity coefficient of 64-100%. On the genetic similarity coefficient of 0.6 in *P. lunatus* accessions, there were two groups, the first and second major clusters, Cluster A and B. The former contained the accessions 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

Keywords: Genetic diversity, *Phaseolus lunatus,* RAPD.

INTRODUCTION

Phaseolus lunatus L. is categorized as a legume plant with its great potential to become nutritious food. This kind of plant is also known as *koro* beans or lima beans and is identical with its vital nutritious contents, from protein (Chel-Guerreoro *et al.,* 2002; Betancur-Ancona *et al.,* 2003; Bello-p *et al.,* 2007; Tejasari, 2016) to fiber (Betancur-Ancona *et al.,* 2003; Segura-Campos & Betancur-Ancona. 2010; Betancur-Ancona *et al.,* 2004; Ancona *et al.*, 2011; Novelo-cen & Betancur-Ancona, 2005). In addition, this plant also contains extra nutrition stored at its stipule or stalk (Heil, 2004; Kost & Heil. 2005). In addition to its richness of nutritional contents, *P. lunauts* L. is also reported to contain a number of anti-oxidant compounds (Betancur-ancona *et al.,* 2004; Da *et al.,* 2009) and other substances that are effective to optimize human's health (Bello-p *et al.,* 2007; Da *et al.,* 2009; Wong & Ng, 2005; Al *et al.,* 2007). For that reason, not only is *P. lunatus* L. known as protein-sourced food in many countries (Almeida & Pedrosa-Harand, 2011; Giami, 2001), it is also reported to be effective in preventing various illnesses (Bello-p *et al.,* 2007; Da *et al.,* 2009; Wong & Ng, 2005; Al *et al.,* 2007).

In addition to its benefits, *P. lunatus* L. can grow in some patterns of conditions (Lim, 2012). Therefore, it is common if *P. lunatus* can be found in many parts of countries as it has been domesticated there (Chacón-Sánchez & Martínez-Castillo, 2017; Félix *et al.,* 2014; Camacho-Pérez *et al.,* 2018). In Indonesia, to be specific, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti & Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions to exist in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as alternatively functional food source (Nafi *et al.,* 2015; Nafi *et al.,* 2006; Kalaminasih, 2013; Diniyah *et al.,* 2015; Herry *et al.,* 2014; Diniyah *et al.,*2013). Now that Indonesia is dealing with serious issue of protein shortage in some particular areas (Ickowitz *et al.,* 2016; Fatmah, 2005; Diana *et al.,* 2017; Madanijah *et al.,* 2016), maximizing the consumption of *P. lunatus* will be of effectiveness for the solution.

With respect to the attempt of elevating the use of *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content is in need of actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential

foundation to run all the program of food source enhancement (Bhanu, 2017; Mohammadi & Prasanna, 2003). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a scheme of betterment for the species since the beginning of the period of systematic plant breeding (Bhanu, 2017). Furthermore, such kinds of information can be used to reach a phase of sustainable crop production (Fu, 2015). What is more, a research underpinning genetic diversity in particular plant also leads to an attempt of conservation (Carvalho *et al.,* 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using such numerous techniques as morphological identification, biochemical characterization, and analysis of DNA marker (Govindaraj *et al.*, 2015).

With reference to the existence of those techniques, the selection of molecular marker is considered more appropriate and effective, not only to avoid any bias due to environmental influence but also to provide eclectic information related to genetic diversity in more acceptable way (Fu, 2015). There are some molecular markers included and considered particularly promising in helping analyse genetic diversity, namely RAPD, RFLP, and SCAR.

Amongst those markers, RAPD is defined as the most popular marker in many researches (Ben-Ari & Lavi, 2012). RAPD constitutes PCR-based technique which involves primary set with relatively short size and is able to randomly amplify many DNA segments (Kumari & Thakur, 2014). This technique is equipped with notable excellence in comparison with other techniques, which is that the technique occupies a universal primary set without undergoing DNA sequencing phase in its real implementation (Ali *et al.,* 2004). In addition, RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar & Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari & Thakur, 2014), and is applicable for various laboratory situations (Kumar & Gurusubramanian, 2011). RAPD, therefore, was used in this current research in order to study genetic variation of various legumes, from *P. vulgaris* to *P. lunatus.*

With respect to the aforementioned rationale, analyses of genetic diversity of *P. lunatus* in Indonesia are still of rarity. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in Africa (Fofana *et al.,* 2001), Europe (Sparvoli *et al.*, 2001), North America (Fofana *et al.,* 2001; Serrano *et al.,* 2010), Central America (Bi *et al.,* 2005) Latin America (Fofana *et al.,* 2001; Serrano *et al.,* 2010) and Caribbean countries (Fofana *et al.,* 2001). On one hand, researches in Indonesia are still limited to study on the potential of *P. lunatus* as alternative food source (Herry *et al.,* 2014; Diniyah *et al.,*2013) alongside its essential substances (Tejasari, 2016; Nafi *et al*., 2006 ; Diniyah *et al*., 2015; Praseptiangga *et al.,* 2018; Sukatiningsih *et al.*, 2013). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti & Fauzi, 2019; Waluyo *et al.,*2016). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on RAPD marker.

MATERIAL AND METHODS

Plant material

P. lunatus used in this research was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. On the basis of the result of identification in previous research (Purwanti & Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Each of the accessions, further, was planted in a polybag in which one polybag distanced one meter long to the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agent.

Table 1. List of accessions to analyze

DNA Isolation

DNA isolation was administered based on CTAB method of Doyle & Doyle (1984), which was modified by Maftuchah & Zainuddin (2010). The used tissue was stemmed from leaf organ of 3-month-old plant. The followings were the procedures of DNA isolation: **1)** leaves were cut out into 2-3 pieces with the length of 1-2 cm; **2)** 0.3-gram leaves and liquid nitrogen were crushed using mortar and pestle; **3)** 0.002-gram Natrium bisulfite was weighed for each of 12 samples; **4)** 0.02-gram Na bisulfite was dissolved into 9,600-µl buffer (labelled Buffer I); **5)** label was given to each 1.5-ml tube based on the name of the sample; **6)** Buffer I mixed with 800-µl Natrium bisulfite was poured into each tube; **7)** the crushed sample was added to each tube; **8)** the tube was shaken until the crushed leaves were submersed into the buffer; **9)** the sample was incubated under the ideal temperature with the span of ± 5 minutes; **10)** the sample was centrifuged under the speed of 12.000 rpm, the temperature of 4ºC with the span of 10 minutes; **11)** supernatant was eliminated; **12)** 500-µl buffer II was added to the natant placed in the tube; **13)** the tube was vortexed; **14)** the sample was incubated under the temperature of 65ºC with the span of 30 minutes while flipping over the tube for every 10 minutes during the incubation; **15)** chloroform was added: alcohol isoamyl (24:1) with the volume of ±500µl; **16)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **17)** the supernatant was taken and put into new tube; **18)** absolute ethanol with the volume of ± 800µl was added along with natrium acetate with the volume of 80µl while doing homogenization through flipping over the tube all around; **19)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **20)** the supernatant was eliminated; **21)** 70%-concentrated ethanol with the volume of ± 800 µl was added; **22)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **23)** the DNA pellet was dried out; **24)** Tris-EDTA

(TE) with the volume of 60 µl was added into tube; **25)** the results of isolated DNA were stored under the temperature of -20^{0}C.

PCR-RAPD

In the reaction of PCR, the isolated DNAs of *P. lunatus* L. were used as the mold. There were 10 kinds of primers used such as OPA6, OPA8, OPA10, OPA20, OPC19, OPD8, OPD12, OPE8, OPE15, and OPE16. The total volume of PCR reaction used signified 22.5 µl, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCI; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and molded DNA. The condition for PCR reaction was designed under pre-denaturation temperature of 94°C (in 5 minutes), denaturation temperature of 94°C (in 1 minute), primary attachment temperature of 36°C (in 1 minute), extension temperature of 72°C (in 2 minutes), post-extension temperature of 72°C (in 5 minutes), and post PCR reaction temperature of 4°C (in 2 minutes). For multiplication, the cycle of PCR reaction was repeated 36 times.

Electrophoresis

There were three stages of procedure to confirm the result of isolation process as well as PCR reaction after implementation. The first stage was the creation of agarose gel with the concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labeled electrophoresis with the electrophoresis buffer of TBE (1x), loading dye (6x) under the condition of 60 V, 400 mA within 45 minutes. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

Analysis on the DNA bands yielded from RAPD

Data analysis was performed by observing the pattern of visible bands from electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data indicating the existence and inexistence of bands typifying certain sizes. Afterwards, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and to create a phylogenetic tree by the assistance of Popgen software version 3.1.

RESULTS AND DISCUSSION

In this current research, as many as 10 primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, the bands, as a result of PCR–RAPD amplification at those genotypes (originated from East Java), were assessed based on the binary data, with the description of: 1 for amplified band and 0 for unamplified. The following Table 2 shows the record of the number of locus found.

Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

10 primers used to amplify the DNA at 15 genotypes of *P. lunatus* L. could reproduce DNA fragments with diverse sizes (polymorphic) despite its capability of yielding 68 DNA band lines (Table 3).

Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

Variation of genotype diversity at 15 genotypes resulted in 68 RAPD band assessed from 10 primary oligonucleotide with the means of 6.8 bands per primary. Out of the 10, 68 bands were yielded with various sizes of DNA fragments. The result of amplification showed that almost all bands indicated similar pattern of DNA band, polymorphic. The primary with the smallest number of bands was primary OPA10 and OPA20; while the one with the highest number of bands was OPD12. Moreover, there were 9 primers with 100% level of polymorphism. To be specific, OPC19 was only effective to result in 96% level of polymorphism.

Next, cluster analysis upon the 68 RAPD bands was administered. This phase yielded a phylogenetic tree by the assistance of Neighbor-Joining method, equipped with similarity coefficient that ranged from 64%-100%, or there was genetic variation with the range of 0-36% (Figure 1). In genetic similarity coefficient of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with genetic similarity coefficient of 0.932 (including 8, 12, 13, 14 genotypes), A2 with genetic similarity coefficient of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). With respect to that with genetic similarity coefficient of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close. In addition, a phylogenetic tree constructed on the basis of RAPD analysis is shown in Figure 1.

Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* under the study was relatively high (ranging from 96 to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Chacón-Sánchez & Martínez-Castillo, 2017; Camacho-Pérez, *et al.,* 2018) , South America, and Afrika (Fofana, *et al.*,1997). The existing high polymorphism had indicated the huge range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes one of a series of parameters to define genetic diversity on particular species (Snustad & Simmons, 2012). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of gene pool. According to previous study, the 15 accessions of *P. lunatus* included in current research were originated from 5 different gene pool, to name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti & Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The different ecological difference was closely interconnected with agroclimatic zone difference in which the origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity which was considered high had brought about an urgent implication in terms of crop improvement attempt, to include breeding procedure for quality improvement (Bhanu, 2017; Mohammadi & Prasanna, 2003; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not really effective as main food resource (Doria *et al.,* 2012; Offei *et al.,* 2003; Adeparusi, 2001). Consequently, the process of maturation in *P. lunatus* seeds was in need of proper and acceptable process, thus it could reduce the anti-nutritional substances (Adeparusi, 2001; Granito *et al.,*2007; Sukatiningsih *et al.*, 2013). Further, by making use of data about genetic variety in breeding procedure, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factor. In addition, breeding procedure could be designed that way so as to yield the generation of *P. lunatus* with highly pest-resistant performance and shorter period of maturation phase.

Next, with reference to the phylogenetic tree that was formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior.

Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width and pod width in comparison to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti & Fauzi, 2019). This demonstration was in line with phylogenetic tree resulted in this current research in which Probolinggo accessions as well as Accession 4 were clustered altogether in Cluster B.

Despite the fact, the result of cluster analysis also indicated the existence of accessions of which pod lengths were quite different, but still grouped in the same cluster. In contrast, when the pod length was not significantly different, the result of cluster analysis classified those accessions in different cluster. This was probably due to DNA related to RAPD marker used in this current research was unrelated to the character setting. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, moreover, the use of RAPD was not only affordable, but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. In spite of it, there were some studies of genetic variation on germplasm of beans which were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al.,* 2015), South Africa (Adesoye & Ojobo, 2012), Turkey (Ince & Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al.,* 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al.,* 2018) and SNP (Chacón-Sánchez & Martínez-Castillo, 2017). In short, this current research has advocated the credibility of the use of RAPD in the study of genetic variation in beans, such as in *P. lunatus.*

CONCLUSION

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analyzed. The result of RAPD utilizing 10 primers resulted in 68 bands in which 9 primers possessed 100% level of polymorphism and one primary was equipped with 96% level of polymorphism. On the basis of cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

REFERENCES

Chel-Guerreoro, L., Perez-Florez, V., Betancur-Ancona, D., & Davila-Ortiz, G. 2002. Functional properties of flours and protein isolates from Phaseolus lunatus and Canavalia ensiformis seeds. *J. Agric. Food Chem.*, 50(3): 584–591, 2002.

Betancur-Ancona, D., Lopez-Luna, J., & Chel-Guerreoro, L. 2003. Comparison of the chemical composition and functional properties of Phaseolus lunatus prime and tailing starches. *Food Chem.*, 82: 217–225.

Bello-p, L.A., Sonia, G.S., Ch, C.E., Agama-acevedo, E., & Tovar, J. 2007. Proximal composition and in vitro digestibility of starch in lima bean (Phaseolus lunatus) varieties," *J. Sci. Food Agric.*, 87: 2570–2575.

Tejasari. 2016. Protein density and quality of koro kratok (Phaseolus lunatus l. sweet) and kacang tunggak (Vigna unguiculata (L.) Walp). in *Proceeding of 1st International Conference on Medicine and Health Sciences (ICMHS)*, pp. 116–119. https://jurnal.unej.ac.id/index.php/prosiding/article/view/3850/3064

Segura-Campos, M., & Betancur-ancona, D. 2010. Effect of octenylsuccinylation on functional properties of lima bean (Phaseolus lunatus) strach. J*ournal of Food Process Engineering.* 33: 712–727. https://sci-hub.tw/10.1111/j.1745-4530.2008.00299.x

Betancur-ancona, D., Peraza-mercado, G., Moguel-ordon, Y., & Fuertes-blanco, S.2004. Physicochemical characterization of lima bean (Phaseolus lunatus) and Jack bean (Canavalia ensiformis) fibrous residues. *Food Chem.*, 84: 287–295.

Ancona, D.B., Rubi, M., Campos, S., Guerrero, L.A.C., & Da, G. 2011. Structural and some nutritional characteristics of Velvet bean (Mucuna pruriens) and Lima bean (Phaseolus lunatus) starches," *Starch/Stärke*, 63: 475–484.

Novelo-cen, L., & Betancur-ancona, D. 2005. Chemical and functional properties of Phaseolus lunatus and Manihot esculenta starch Blends. *Starch/Stärke*, 57:431–441.

Heil, M. 2004. Induction of two indirect defences benefits Lima bean (Phaseolus Fabaceae) in nature. *J. Ecol.*, 92(3):527–536.

Kost, C., & Heil, M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (Phaseolus lunatus , Fabaceae). *Basic Appl. Ecol.*, 6: 237–248.

Da, G., Torruco-uco, J., Chel-guerrero, L., Martı, A., & Betancur-ancona, D. 2009. Agiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from Phaseolus lunatus and Phaseolus vulgaris seeds. *LWT - Food Sci. Technol.*, 42: 1597–1604.

Wong, J.H., & Ng, T.B. 2005. Lunatusin , a trypsin-stable antimicrobial peptide from lima beans (Phaseolus

lunatus L .). *Peptides*, 26: 2086–2092.

Al, Q. Z. E. T., Zhao, Q., Huang, X., Lou, Y., Weber, N., & Proksch, P. 2007. Effects of ethanol extracts from adzuki bean (Phaseolus angularis Wight .) and lima bean (Phaseolus lunatus L .) on estrogen and progesterone receptor phenotypes of MCF-7 / BOS cells. *Phyther. Res.*, 21: 648–652.

Almeida, C., & Pedrosa-Harand, A. 2011. Contrasting rDNA evolution in lima bean (Phaseolus lunatus L .) and common bean (P . vulgaris L ., Fabaceae). *Cytogenet Genome Res*, 132: 212–217.

Giami, S.Y. 2001. Quality attributes of three new improved lines of Nigerian lima beans (Phaseolus lunatus L . Walp .). *Plant Foods Hum. Nutr.*, 56:325–333.

Lim, T.K. 2012. Phaseolus lunatus," in *Edible Medicinal And Non-Medicinal Plants*, Dordrecht: Springer Netherlands, pp. 804–814.

Chacón-Sánchez, M.I., & Martínez-Castillo, J. 2017. Testing domestication scenarios of Lima bean (Phaseolus lunatus L.) in mesoamerica: Insights from genome-wide genetic markers. *Front. Plant Sci.*, 8: 1–20.

Félix, D.T., Coello-Coello, J., & Martínez-Castillo, J. 2014. Wild to crop introgression and genetic diversity in Lima bean (Phaseolus lunatus L.) in traditional Mayan milpas from Mexico. *Conserv. Genet.*, 15 (6): 1315–1328.

Camacho-Pérez, L., Martínez-Castillo, J. Mijangos-Cortés, J.O., Ferrer-Ortega, M.M., Baudoin, J.P., & Andueza-Noh, R.H. 2018. Genetic structure of Lima bean (Phaseolus lunatus L.) landraces grown in the Mayan area. *Genet. Resour. Crop Evol.*, 65(1): 229–241.

Purwanti, E., & Fauzi, A. 2019. The morphological characteristics of Phaseolus lunatus L. in different areas of East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*, vol. 276, p. 012017.

Nafi, A. Diniyah, N., & Hastuti, F.T. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (Phaseolus lunatus L.) termodifikasi yang diproduksi secara fermentasi spontan. *Agrointek*, 9(1): 24–32.

Nafi, A., Susanto, T., & Subagio, A. 2006. Pengembangan tepung kaya protein (TKP) dari koro komak (Lablab purpureus (L.) sweet) dan koro kratok (Phaseolus lunatus). *Jurnal. Teknol. dan Ind. Pangan*, 17(3): 159–165.

Kalaminasih, D. 2013. Pengaruh proporsi kacang koro sayur (Phaseolus lunatus) dan kacang koro pedang (Canavalia ensiformis L) terhadap mutu organoleptik tempe koro. *e-Journal Boga*, 2(3): 104–113.

Diniyah, N., Windrati, W.S., & Riady, S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (Phaseolus lunatus l.) dengan perlakuan lama perendaman. *J. Has. Penelit. Ind.*, 28(2): 70–77.

Herry, B., Windarti, W.S., & Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein. in *Prosiding Seminar Nasional*, pp. 460–472.

Diniyah, N., Windrati, W.S., & Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai. in *Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi*, pp. 1–10.

Ickowitz, A., Rowland, D., Powell, B., & Salim, M.A. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLoS One*, 11(5): 1–15.

Fatmah, E.A.A. 2005. Baseline survey on nutritional and health status of underfive children at poor communities in DKI Jakarta , Tangerang , and Bogor year 2004. *Makara Kesehat.*, 9(2): 41–48.

A. Diana *et al.*, 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLoS One*, 12(4):1–17.

S. Madanijah *et al.*, 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: a cross-sectional dietary and nutrient intake study. *Br. J. Nutr.*, 116: 1–10.

Bhanu, A.N. 2017. Assessment of genetic diversity in crop plants - An overview," *Adv. Plants Agric. Res.*, 7(3): 279–286.

Mohammadi, S.A., & Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants—Salient statistical tools and considerations. *Crop Sci.*, 43(4): 1235–1248.

Fu, Y.B. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet.*, 128(11): 2131–2142.

Carvalho, Y.G.S.,Vitorino, L.C., de Souza, U.J.B., & Bessa, L.A.. 2019. Recent trends in research on the geetic diversity of plants: Implications for conservation. *Diversity*, 11(4): 1–21.

Govindaraj, M., Vetriventhan, M., & Srinivasan, M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int.*, vol. 2015, no. Figure 1, 2015.

Ben-Ari, G., & Lavi, U. 2012. *Marker-assisted selection in plant breeding*, First Edit. Elsevier Inc.

Kumari, N., & Thakur, S.K. 2014. Randomly amplified polymorphic DNA-A brief review. *Am. J. Anim. Vet. Sci. 9*, 9(1): 6–13.

Ali, B.A., Huang, T.H., Qin, D.N., &Wang, X.M. 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish research. *Rev. Fish Biol. Fish.*, 14(4): 443–453.

Kumar, N.S., & Gurusubramanian, G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci. Vis.*, 11(3): 116–124.

Fofana, B., du Jardin, P., & Baudoin, J.P. 2001. Genetic diversity in the Lima bean (Phaseolus lunatus L .) as revealed by chloroplast DNA (cpDNA) variations. *Genet. Resour. Crop Evol.*, pp. 1–9.

F. Sparvoli *et al.*, 2001. Lectin and lectin-related proteins in Lima bean (Phaseolus lunatus L .) seeds : biochemical and evolutionary studies. *Plant Mol. Biol.*, 45: 587–597.

Serrano-serrano, M.L., Hernández-torres, J., Castillo-villamizar, G., Debouck, D.G., & Chacón, M.I. 2010. Molecular phylogenetics and evolution gene pools in wild lima bean (Phaseolus lunatus L .) from the Americas : Evidences for an andean origin and past migrations. *Mol. Phylogenet. Evol.*, 54(1): 76–87.

Bi, I.Z., Maquet, A., & Baudoin, J. 2005. Mating system of wild *Phaseolus lunatus* L . and its relationship to population size. *Heredity (Edinb).*, 94: 153–158.

Praseptiangga, D., Tryas, A.A., Affandi, D.R., Atmaka, W., Ariyantoro, A.R., & Minardi, S. 2018. Physical and chemical characterization of composite flour from canna flour (Canna edulis) and lima bean flour (Phaseolus lunatus). in *AIP Conference Proceedings*, 1927: 1–6.

Sukatiningsih, Yustian, A.M., & Windarti, S.W. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet],” *Agritrop J. Ilmu-Ilmu Pertan.*, 11(1): 1–7.

Waluyo, B., Saptadi, D., Ardiarini, N. R., Kuswanto & Zanetta, C.U. 2016. Keragaman karakteristik fisik biji koro manis (Phaseolus lunatus L.) sebagai dasar sinkronisasi preferensi kebutuhan industri. in *Prosiding Seminar Nasional Pembangunan Pertanian 2016: Tantangan dan Arah Pembangunan Pertanian Indonesia Masa Depan,* pp. 555–560.

Doyle, J.J., & Doyle, J.L. 1984. A rapid DNA isolation procedure for small quantitiesof fresh leaf tissue. *Phytochem. Bull.*, 19: 11–15.

Maftuchah & Zainuddin. 2010. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (Jathropa curcas L.).

Fofana, B., Vekemans, X., Jardin, P., & Baudoin, J.P. 1997.Genetic diversity in Lima bean (Phaseolus lunatus L.) as revealed by RAPD markers. no. 1988, pp. 157–165.

Snustad, D.P., & Simmons, M.J. 2012. *Genetics*. New Jersey: Wiley.

Huang, W., Zhao, X., Zhao, X., Li, Y., & Lian, J. 2016. Effects of environmental factors on genetic diversity of Caragana microphylla in Horqin Sandy Land, northeast China. *Ecol. Evol.*, 6(22): 8256–8266.

Syuaib, M.F. 2016. Sustainable agriculture in indonesia: Facts and challenges to keep growing in harmony with environment. *Agric. Eng. Int. CIGR J.*, 18(20:170–184.

Doria, E., Campion, B., Sparvoli, F., Tava, A., & Nielsen, E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (Phaseolus vulgaris L . and Phaseolus lunatus L .) landraces cultivated in southern Italy. *J. Food Compos. Anal.*, 26(1–2): 72–80.

Offei, S.K., Asante, I.K., & Danquah, E.Y. 2003. Variation in size, protein, cyanide and tannin contents of lima bean (Phaseolus lunatus). *Trop. Sci.*, 43: 132–134.

Adeparusi, E.O. 2001. Effect of processing on the nutrients and anti-nutrients of lima bean (Phaseolus lunatus L .) flour. *Nahrung/Food*, vol. 45(2): 94–96.

Granito, M., Brito, Y., & Torres, A. 2007. Chemical composition , antioxidant capacity and functionality of raw and processed Phaseolus lunatus. *J. Sci. Food Agric.*, 87: 2801–2809.

Bukhari, A., Bhat, M. A., Ahmad, M., & Saleem, N. 2015. Examination of genetic diversity in common bean (Phaseolus vulgaris L.) using random amplified polymorphic DNA (RAPD) markers," *African J. Biotechnol.*, 14(6):451–458.

Adesoye, A., & Ojobo, O.A. 2012. Genetic diversity assessment of Phaseolus vulgaris L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv.*, 4(13): 453–460.

Ince, A.G., & Karaca, M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omi. J.*, 4(4): 220–227

Basheer-Salimia, R., Shtaya, M., Awad, M., Abdallah, J., & Hamdam., Y. 2013. Genetic diversity of Palestine landraces of faba bean (Vicia faba) based on RAPD markers. *Genet. Mol. Res.*, 12(3): 3314–3323

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura

Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

Codes	**Locus (bp)**												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description:

(1): DNA band was existent,

(0): DNA band was inexistent

Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

No.	Markers (100 bp–2000 bp)	∑ Band	Polymorphic	% Polymorphic
1	OPA 6	8	8	100%
2	OPA 8	7	7	100%
3	OPA 10	5	5	100%
4	OPA 20	5	5	100%
5	OPC 19	6	5	96%
6	OPD 8	8	8	100%
7	OPD 12	9	9	100%
8	OPE 8	7	7	100%
9	OPE 15	7	7	100%
10	OPE 16	6	6	100%
	Total	68	67	

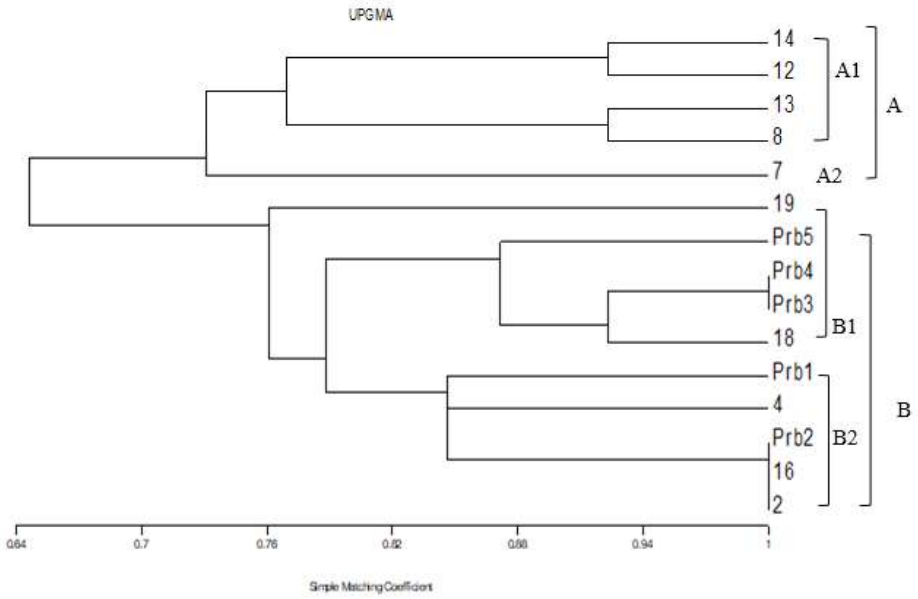

Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

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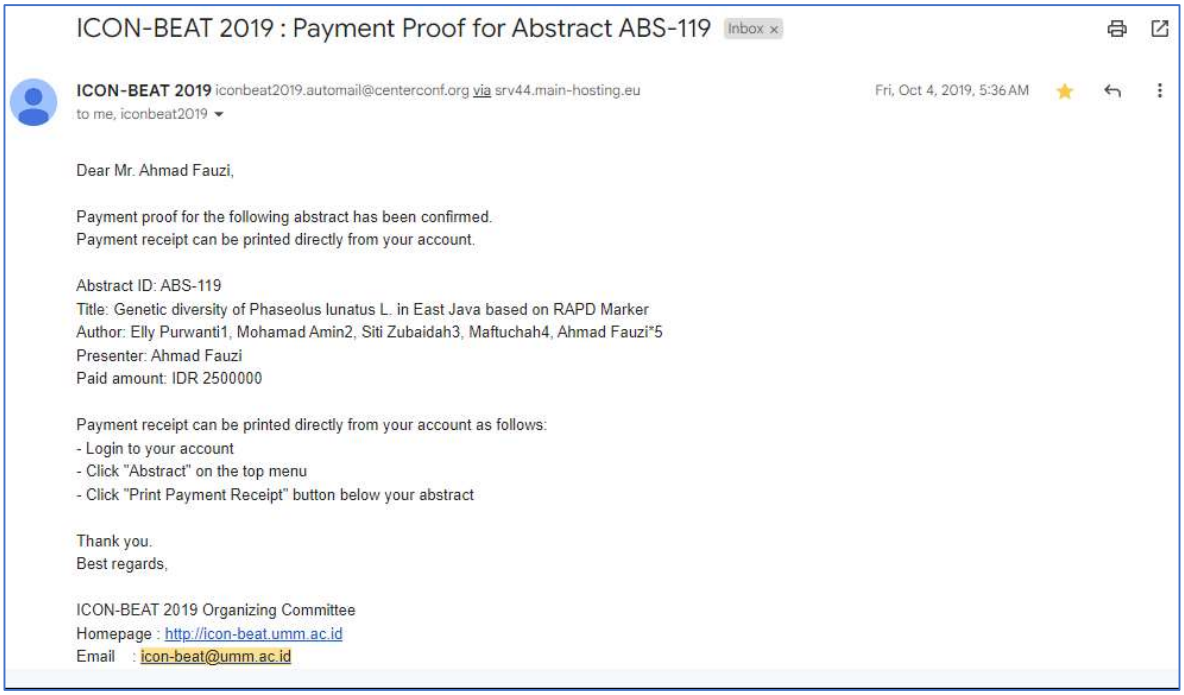

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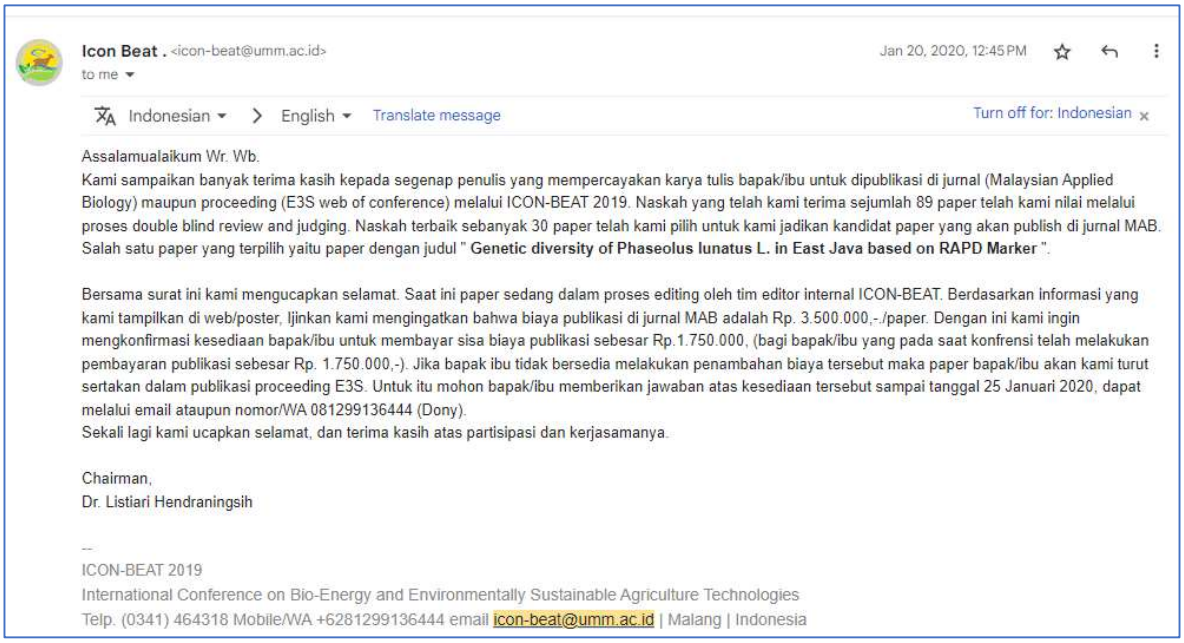

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Assalamualaikum Wr. Wb.
Kami sampaikan banyak terima kasih kepada segenap penulis yang mempercayakan karya tulis bapak/ibu untuk dipublikasi di jurnal (Malaysian Applied Biology) maupun proceeding (E3S web of conference) melalui ICON-BEAT 2019. Naskah yang telah kami terima sejumlah 89 paper telah kami nilai melalui proses double blind review and judging. Naskah terbaik sebanyak 30 paper telah kami pilih untuk kami jadikan kandidat paper yang akan publish di jurnal MAB. Salah satu paper yang terpilih yaitu paper dengan judul " **Genetic diversity of Phaseolus lunatus L. in East Java based on RAPD Marker** ".

Bersama surat ini kami mengucapkan selamat. Saat ini paper sedang dalam proses editing oleh tim editor internal ICON-BEAT. Berdasarkan informasi yang kami tampilkan di web/poster, Ijinkan kami mengingatkan bahwa biaya publikasi di jurnal MAB adalah Rp. 3.500.000,-./paper. Dengan ini kami ingin mengkonfirmasi kesediaan bapak/ibu untuk membayar sisa biaya publikasi sebesar Rp.1.750.000, (bagi bapak/ibu yang pada saat konfrensi telah melakukan pembayaran publikasi sebesar Rp. 1.750.000,-). Jika bapak ibu tidak bersedia melakukan penambahan biaya tersebut maka paper bapak/ibu akan kami turut sertakan dalam publikasi proceeding E3S. Untuk itu mohon bapak/ibu memberikan jawaban atas kesediaan tersebut sampai tanggal 25 Januari 2020, dapat melalui email ataupun nomor/WA 081299136444 (Dony).
Sekali lagi kami ucapkan selamat, dan terima kasih atas partisipasi dan kerjasamanya.

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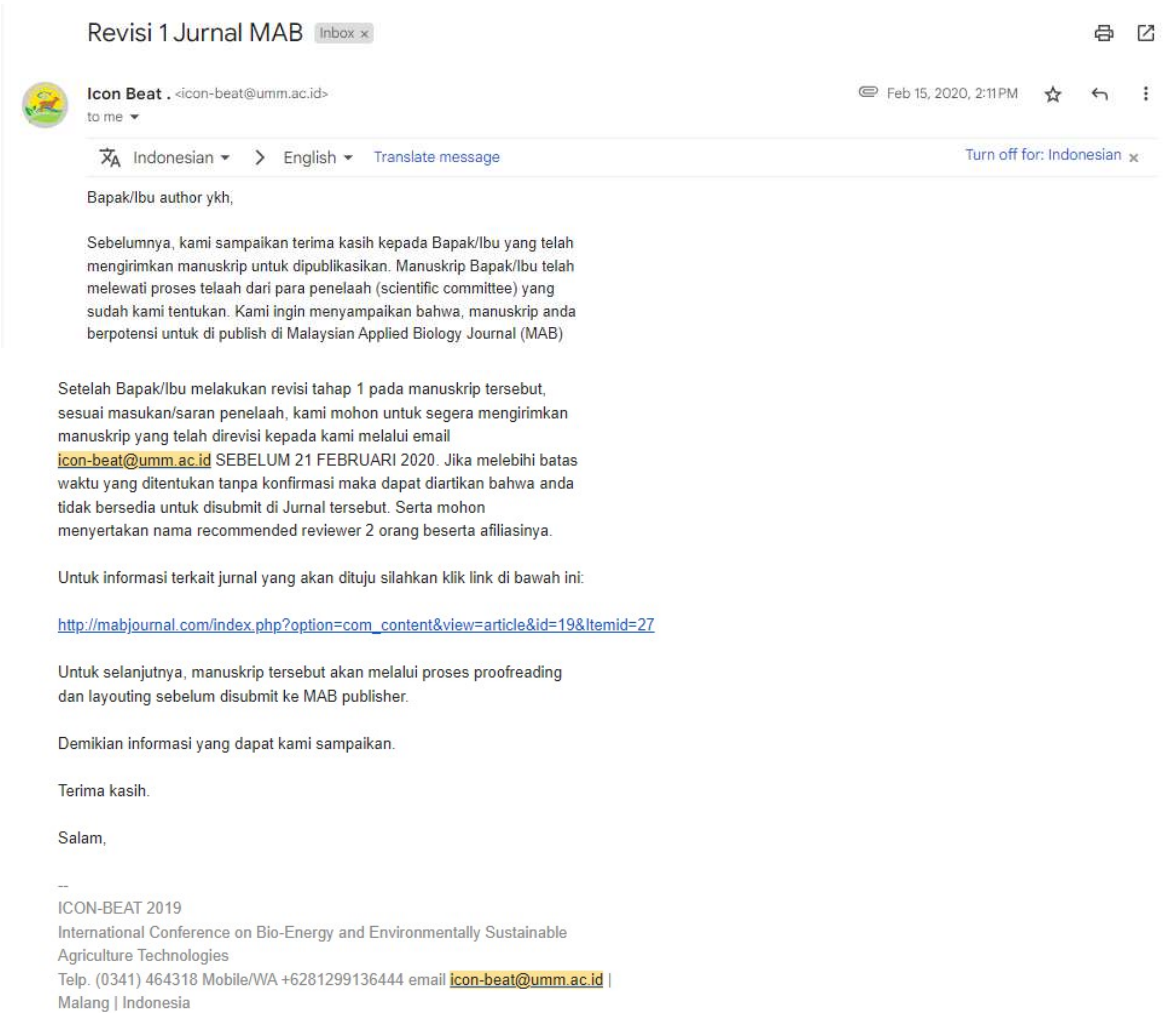

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Genetic Diversity of *Phaseolus lunatus* L. in East Java based on RAPD Marker

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GENETIC DIVERSITY OF *Phaseolus lunatus* L. IN EAST JAVA BASED ON RAPD MARKER

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ABSTRACT

Phaseolus lunatus L. is one of legume plants that can be found at some parts of Indonesia and is potential for alternative food rich of protein. This current research aimed at analyzing genetic accessions of *P. lunatus* distributed at some areas in East Java, Indonesia based on RAPD marker. A total of 15 accessions originated from Tulungagung, Kediri, Malang, Probolinggo, and Madura were analyzed. In addition, there were 10 primers used, i.e. OPA6, OPA8, OPA10, OPA20, OPC19, OPD8, OPA10, OPA20, OPC19, OPD8, OPE15, and OPE16 generating 68 lines of DNA band typified with different sizes. Further, there were 9 primers in the possession of 100% level of polymorphism in which only Primary OPC19 yielded 96% level of polymorphism. By means of Neighbor-Joining method, a phylogenic tree was yielded by similarity coefficient of 64-100%. On the genetic similarity coefficient of 0.6 in *P. lunatus* accessions, there were two groups, the first and second major clusters, Cluster A and B. The former contained the accessions 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

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Keywords: Genetic diversity, *Phaseolus lunatus,* RAPD.

INTRODUCTION

Phaseolus lunatus L. is categorized as a legume plant with its great potential to become nutritious food. This kind of plant is also known as *koro* beans or lima beans and is identical with its vital nutritious contents, from protein (Chel-Guerreoro *et al.,* 2002; Betancur-Ancona *et al.,* 2003; Bello-p *et al.,* 2007; Tejasari, 2016) to fiber (Betancur-Ancona *et al.,* 2003; Segura-Campos & Betancur-Ancona. 2010; Betancur-Ancona *et al.,* 2004; Ancona *et al.*, 2011; Novelo-cen & Betancur-Ancona, 2005). In addition, this plant also contains extra nutrition stored at its stipule or stalk (Heil, 2004; Kost & Heil. 2005). In addition to its richness of nutritional contents, *P. lunauts* L. is also reported to contain a number of anti-oxidant compounds (Betancur-ancona *et al.,* 2004; Da *et al.,* 2009) and other substances that are effective to optimize human's health (Bello-p *et al.,* 2007; Da *et al.,* 2009; Wong & Ng, 2005; Al *et al.,* 2007). For that reason, not only is *P. lunatus* L. known as protein-sourced food in many countries (Almeida & Pedrosa-Harand, 2011; Giami, 2001), it is also reported to be effective in preventing various illnesses (Bello-p *et al.,* 2007; Da *et al.,* 2009; Wong & Ng, 2005; Al *et al.,* 2007).

In addition to its benefits, *P. lunatus* L. can grow in some patterns of conditions (Lim, 2012). Therefore, it is common if *P. lunatus* can be found in many parts of countries as it has been domesticated there (Chacón-Sánchez & Martínez-Castillo, 2017; Félix *et al.,* 2014; Camacho-Pérez *et al.,* 2018). In Indonesia, to be specific, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti & Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions to exist in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as alternatively functional food source (Nafi *et al.,* 2015; Nafi *et al.,* 2006; Kalaminasih, 2013; Diniyah *et al.,* 2015; Herry *et al.,* 2014; Diniyah *et al.,*2013). Now that Indonesia is dealing with serious issue of protein shortage in some particular areas (Ickowitz *et al.,* 2016; Fatmah, 2005; Diana *et al.,* 2017; Madanijah *et al.,* 2016), maximizing the consumption of *P. lunatus* will be of effectiveness for the solution.

With respect to the attempt of elevating the use of *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content is in need of actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential

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foundation to run all the program of food source enhancement (Bhanu, 2017; Mohammadi & Prasanna, 2003). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a scheme of betterment for the species since the beginning of the period of systematic plant breeding (Bhanu, 2017). Furthermore, such kinds of information can be used to reach a phase of sustainable crop production (Fu, 2015). What is more, a research underpinning genetic diversity in particular plant also leads to an attempt of conservation (Carvalho *et al.,* 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using such numerous techniques as morphological identification, biochemical characterization, and analysis of DNA marker (Govindaraj *et al.*, 2015).

With reference to the existence of those techniques, the selection of molecular marker is considered more appropriate and effective, not only to avoid any bias due to environmental influence but also to provide eclectic information related to genetic diversity in more acceptable way (Fu, 2015). There are some molecular markers included and considered particularly promising in helping analyse genetic diversity, namely RAPD, RFLP, and SCAR.

Amongst those markers, RAPD is defined as the most popular marker in many researches (Ben-Ari & Lavi, 2012). RAPD constitutes PCR-based technique which involves primary set with relatively short size and is able to randomly amplify many DNA segments (Kumari & Thakur, 2014). This technique is equipped with notable excellence in comparison with other techniques, which is that the technique occupies a universal primary set without undergoing DNA sequencing phase in its real implementation (Ali *et al.,* 2004). In addition, RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar & Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari & Thakur, 2014), and is applicable for various laboratory situations (Kumar & Gurusubramanian, 2011). RAPD, therefore, was used in this current research in order to study genetic variation of various legumes, from *P. vulgaris* to *P. lunatus*.

With respect to the aforementioned rationale, analyses of genetic diversity of *P. lunatus* in Indonesia are still of rarity. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in Africa (Fofana *et al.,* 2001), Europe (Sparvoli *et al.*, 2001), North America (Fofana *et al.,* 2001; Serrano *et al.,* 2010), Central America (Bi *et al.,* 2005) Latin America (Fofana *et al.,* 2001; Serrano *et al.,* 2010) and Caribbean countries (Fofana *et al.,* 2001). On one hand, researches in Indonesia are still limited to study on the potential of *P. lunatus* as alternative food source (Herry *et al.,* 2014; Diniyah *et al.,*2013) alongside its essential substances (Tejasari, 2016; Nafi *et al*., 2006 ; Diniyah *et al*., 2015; Praseptiangga *et al.,* 2018; Sukatiningsih *et al.*, 2013). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti & Fauzi, 2019; Waluyo *et al.,*2016). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on RAPD marker.

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MATERIAL AND METHODS

Plant material

P. lunatus used in this research was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. On the basis of the result of identification in previous research (Purwanti & Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Each of the accessions, further, was planted in a polybag in which one polybag distanced one meter long to the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agent.

Table 1. List of accessions to analyze

DNA Isolation

DNA isolation was administered based on CTAB method of Doyle & Doyle (1984), which was modified by Maftuchah & Zainuddin (2010). The used tissue was stemmed from leaf organ of 3-month-old plant. The followings were the procedures of DNA isolation: **1)** leaves were cut out into 2-3 pieces with the length of 1-2 cm; **2)** 0.3-gram leaves and liquid nitrogen were crushed using mortar and pestle; **3)** 0.002-gram Natrium bisulfite was weighed for each of 12 samples; **4)** 0.02-gram Na bisulfite was dissolved into 9,600-µl buffer (labelled Buffer I); **5)** label was given to each 1.5-ml tube based on the name of the sample; **6)** Buffer I mixed with 800-µl Natrium bisulfite was poured into each tube; **7)** the crushed sample was added to each tube; **8)** the tube was shaken until the crushed leaves were submersed into the buffer; **9)** the sample was incubated under the ideal temperature with the span of ± 5 minutes; **10)** the sample was centrifuged under the speed of 12.000 rpm, the temperature of 4ºC with the span of 10 minutes; **11)** supernatant was eliminated; **12)** 500-µl buffer II was added to the natant placed in the tube; **13)** the tube was vortexed; **14)** the sample was incubated under the temperature of 65ºC with the span of 30 minutes while flipping over the tube for every 10 minutes during the incubation; **15)** chloroform was added: alcohol isoamyl (24:1) with the volume of ±500µl; **16)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **17)** the supernatant was taken and put into new tube; **18)** absolute ethanol with the volume of ± 800µl was added along with natrium acetate with the volume of 80µl while doing homogenization through flipping over the tube all around; **19)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **20)** the supernatant was eliminated; **21)** 70%-concentrated ethanol with the volume of ± 800 µl was added; **22)** the sample was centrifuged with the speed of 12,000 rpm under the temperature of 4ºC during 10 minutes; **23)** the DNA pellet was dried out; **24)** Tris-EDTA

(TE) with the volume of 60 µl was added into tube; **25)** the results of isolated DNA were stored under the temperature of -20^{0}C.

PCR-RAPD

In the reaction of PCR, the isolated DNAs of *P. lunatus* L. were used as the mold. There were 10 kinds of primers used such as OPA6, OPA8, OPA10, OPA20, OPC19, OPD8, OPD12, OPE8, OPE15, and OPE16. The total volume of PCR reaction used signified 22.5 µl, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCI; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and molded DNA. The condition for PCR reaction was designed under pre-denaturation temperature of 94°C (in 5 minutes), denaturation temperature of 94°C (in 1 minute), primary attachment temperature of 36°C (in 1 minute), extension temperature of 72°C (in 2 minutes), post-extension temperature of 72°C (in 5 minutes), and post PCR reaction temperature of 4°C (in 2 minutes). For multiplication, the cycle of PCR reaction was repeated 36 times.

Electrophoresis

There were three stages of procedure to confirm the result of isolation process as well as PCR reaction after implementation. The first stage was the creation of agarose gel with the concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labeled electrophoresis with the electrophoresis buffer of TBE (1x), loading dye (6x) under the condition of 60 V, 400 mA within 45 minutes. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

Analysis on the DNA bands yielded from RAPD

Data analysis was performed by observing the pattern of visible bands from electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data indicating the existence and inexistence of bands typifying certain sizes. Afterwards, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and to create a phylogenetic tree by the assistance of Popgen software version 3.1.

RESULTS AND DISCUSSION

In this current research, as many as 10 primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, the bands, as a result of PCR–RAPD amplification at those genotypes (originated from East Java), were assessed based on the binary data, with the description of: 1 for amplified band and 0 for unamplified. The following Table 2 shows the record of the number of locus found.

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Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

10 primers used to amplify the DNA at 15 genotypes of *P. lunatus* L. could reproduce DNA fragments with diverse sizes (polymorphic) despite its capability of yielding 68 DNA band lines (Table 3).

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Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

Variation of genotype diversity at 15 genotypes resulted in 68 RAPD band assessed from 10 primary oligonucleotide with the means of 6.8 bands per primary. Out of the 10, 68 bands were yielded with various sizes of DNA fragments. The result of amplification showed that almost all bands indicated similar pattern of DNA band, polymorphic. The primary with the smallest number of bands was primary OPA10 and OPA20; while the one with the highest number of bands was OPD12. Moreover, there were 9 primers with 100% level of polymorphism. To be specific, OPC19 was only effective to result in 96% level of polymorphism.

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Next, cluster analysis upon the 68 RAPD bands was administered. This phase yielded a phylogenetic tree by the assistance of Neighbor-Joining method, equipped with similarity coefficient that ranged from 64%-100%, or there was genetic variation with the range of 0-36% (Figure 1). In genetic similarity coefficient of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with genetic similarity coefficient of 0.932 (including 8, 12, 13, 14 genotypes), A2 with genetic similarity coefficient of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). With respect to that with genetic similarity coefficient of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close. In addition, a phylogenetic tree constructed on the basis of RAPD analysis is shown in Figure 1.

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Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* under the study was relatively high (ranging from 96 to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Chacón-Sánchez & Martínez-Castillo, 2017; Camacho-Pérez, *et al.,* 2018) , South America, and Afrika (Fofana, *et al.*,1997). The existing high polymorphism had indicated the huge range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes one of a series of parameters to define genetic diversity on particular species (Snustad & Simmons, 2012). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of gene pool. According to previous study, the 15 accessions of *P. lunatus* included in current research were originated from 5 different gene pool, to name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti & Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The different ecological difference was closely interconnected with agroclimatic zone difference in which the origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity which was considered high had brought about an urgent implication in terms of crop improvement attempt, to include breeding procedure for quality improvement (Bhanu, 2017; Mohammadi & Prasanna, 2003; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not really effective as main food resource (Doria *et al.,* 2012; Offei *et al.,* 2003; Adeparusi, 2001). Consequently, the process of maturation in *P. lunatus* seeds was in need of proper and acceptable process, thus it could reduce the anti-nutritional substances (Adeparusi, 2001; Granito *et al.,*2007; Sukatiningsih *et al.*, 2013). Further, by making use of data about genetic variety in breeding procedure, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factor. In addition, breeding procedure could be designed that way so as to yield the generation of *P. lunatus* with highly pest-resistant performance and shorter period of maturation phase.

Next, with reference to the phylogenetic tree that was formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior.

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Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width and pod width in comparison to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti & Fauzi, 2019). This demonstration was in line with phylogenetic tree resulted in this current research in which Probolinggo accessions as well as Accession 4 were clustered altogether in Cluster B.

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Despite the fact, the result of cluster analysis also indicated the existence of accessions of which pod lengths were quite different, but still grouped in the same cluster. In contrast, when the pod length was not significantly different, the result of cluster analysis classified those accessions in different cluster. This was probably due to DNA related to RAPD marker used in this current research was unrelated to the character setting. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, moreover, the use of RAPD was not only affordable, but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. In spite of it, there were some studies of genetic variation on germplasm of beans which were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al.,* 2015), South Africa (Adesoye & Ojobo, 2012), Turkey (Ince & Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al.,* 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al.,* 2018) and SNP (Chacón-Sánchez & Martínez-Castillo, 2017). In short, this current research has advocated the credibility of the use of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

CONCLUSION

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analyzed. The result of RAPD utilizing 10 primers resulted in 68 bands in which 9 primers possessed 100% level of polymorphism and one primary was equipped with 96% level of polymorphism. On the basis of cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

REFERENCES

Chel-Guerreoro, L., Perez-Florez, V., Betancur-Ancona, D., & Davila-Ortiz, G. 2002. Functional properties of flours and protein isolates from Phaseolus lunatus and Canavalia ensiformis seeds. *J. Agric. Food Chem.*, 50(3): 584–591, 2002.

Betancur-Ancona, D., Lopez-Luna, J., & Chel-Guerreoro, L. 2003. Comparison of the chemical composition and functional properties of Phaseolus lunatus prime and tailing starches. *Food Chem.*, 82: 217–225.

Bello-p, L.A., Sonia, G.S., Ch, C.E., Agama-acevedo, E., & Tovar, J. 2007. Proximal composition and in vitro digestibility of starch in lima bean (Phaseolus lunatus) varieties," *J. Sci. Food Agric.*, 87: 2570–2575.

Tejasari. 2016. Protein density and quality of koro kratok (Phaseolus lunatus l. sweet) and kacang tunggak (Vigna unguiculata (L.) Walp). in *Proceeding of 1st International Conference on Medicine and Health Sciences (ICMHS)*, pp. 116–119. https://jurnal.unej.ac.id/index.php/prosiding/article/view/3850/3064

Segura-Campos, M., & Betancur-ancona, D. 2010. Effect of octenylsuccinylation on functional properties of lima bean (Phaseolus lunatus) strach. J*ournal of Food Process Engineering.* 33: 712–727. https://sci-hub.tw/10.1111/j.1745-4530.2008.00299.x

Betancur-ancona, D., Peraza-mercado, G., Moguel-ordon, Y., & Fuertes-blanco, S.2004. Physicochemical characterization of lima bean (Phaseolus lunatus) and Jack bean (Canavalia ensiformis) fibrous residues. *Food Chem.*, 84: 287–295.

Ancona, D.B., Rubi, M., Campos, S., Guerrero, L.A.C., & Da, G. 2011. Structural and some nutritional characteristics of Velvet bean (Mucuna pruriens) and Lima bean (Phaseolus lunatus) starches," *Starch/Stärke*, 63: 475–484.

Novelo-cen, L., & Betancur-ancona, D. 2005. Chemical and functional properties of Phaseolus lunatus and Manihot esculenta starch Blends. *Starch/Stärke*, 57:431–441.

Heil, M. 2004. Induction of two indirect defences benefits Lima bean (Phaseolus Fabaceae) in nature. *J. Ecol.*, 92(3):527–536.

Kost, C., & Heil, M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (Phaseolus lunatus , Fabaceae). *Basic Appl. Ecol.*, 6: 237–248.

Da, G., Torruco-uco, J., Chel-guerrero, L., Martı, A., & Betancur-ancona, D. 2009. Agiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from Phaseolus lunatus and Phaseolus vulgaris seeds. *LWT - Food Sci. Technol.*, 42: 1597–1604.

Wong, J.H., & Ng, T.B. 2005. Lunatusin , a trypsin-stable antimicrobial peptide from lima beans (Phaseolus

lunatus L .). *Peptides*, 26: 2086–2092.

Al, Q. Z. E. T., Zhao, Q., Huang, X., Lou, Y., Weber, N., & Proksch, P. 2007. Effects of ethanol extracts from adzuki bean (Phaseolus angularis Wight .) and lima bean (Phaseolus lunatus L .) on estrogen and progesterone receptor phenotypes of MCF-7 / BOS cells. *Phyther. Res.*, 21: 648–652.

Almeida, C., & Pedrosa-Harand, A. 2011. Contrasting rDNA evolution in lima bean (Phaseolus lunatus L .) and common bean (P . vulgaris L ., Fabaceae). *Cytogenet Genome Res*, 132: 212–217.

Giami, S.Y. 2001. Quality attributes of three new improved lines of Nigerian lima beans (Phaseolus lunatus L . Walp .). *Plant Foods Hum. Nutr.*, 56:325–333.

Lim, T.K. 2012. Phaseolus lunatus," in *Edible Medicinal And Non-Medicinal Plants*, Dordrecht: Springer Netherlands, pp. 804–814.

Chacón-Sánchez, M.I., & Martínez-Castillo, J. 2017. Testing domestication scenarios of Lima bean (Phaseolus lunatus L.) in mesoamerica: Insights from genome-wide genetic markers. *Front. Plant Sci.*, 8: 1–20.

Félix, D.T., Coello-Coello, J., & Martínez-Castillo, J. 2014. Wild to crop introgression and genetic diversity in Lima bean (Phaseolus lunatus L.) in traditional Mayan milpas from Mexico. *Conserv. Genet.*, 15 (6): 1315–1328.

Camacho-Pérez, L., Martínez-Castillo, J. Mijangos-Cortés, J.O., Ferrer-Ortega, M.M., Baudoin, J.P., & Andueza-Noh, R.H. 2018. Genetic structure of Lima bean (Phaseolus lunatus L.) landraces grown in the Mayan area. *Genet. Resour. Crop Evol.*, 65(1): 229–241.

Purwanti, E., & Fauzi, A. 2019. The morphological characteristics of Phaseolus lunatus L. in different areas of East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*, vol. 276, p. 012017.

Nafi, A. Diniyah, N., & Hastuti, F.T. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (Phaseolus lunatus L.) termodifikasi yang diproduksi secara fermentasi spontan. *Agrointek*, 9(1): 24–32.

Nafi, A., Susanto, T., & Subagio, A. 2006. Pengembangan tepung kaya protein (TKP) dari koro komak (Lablab purpureus (L.) sweet) dan koro kratok (Phaseolus lunatus). *Jurnal. Teknol. dan Ind. Pangan*, 17(3): 159–165.

Kalaminasih, D. 2013. Pengaruh proporsi kacang koro sayur (Phaseolus lunatus) dan kacang koro pedang (Canavalia ensiformis L) terhadap mutu organoleptik tempe koro. *e-Journal Boga*, 2(3): 104–113.

Diniyah, N., Windrati, W.S., & Riady, S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (Phaseolus lunatus l.) dengan perlakuan lama perendaman. *J. Has. Penelit. Ind.*, 28(2): 70–77.

Herry, B., Windarti, W.S., & Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein. in *Prosiding Seminar Nasional*, pp. 460–472.

Diniyah, N., Windrati, W.S., & Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai. in *Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi*, pp. 1–10.

Ickowitz, A., Rowland, D., Powell, B., & Salim, M.A. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLoS One*, 11(5): 1–15.

Fatmah, E.A.A. 2005. Baseline survey on nutritional and health status of underfive children at poor communities in DKI Jakarta , Tangerang , and Bogor year 2004. *Makara Kesehat.*, 9(2): 41–48.

A. Diana *et al.*, 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLoS One*, 12(4):1–17.

S. Madanijah *et al.*, 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: a cross-sectional dietary and nutrient intake study. *Br. J. Nutr.*, 116: 1–10.

Bhanu, A.N. 2017. Assessment of genetic diversity in crop plants - An overview," *Adv. Plants Agric. Res.*, 7(3): 279–286.

Mohammadi, S.A., & Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants—Salient statistical tools and considerations. *Crop Sci.*, 43(4): 1235–1248.

Fu, Y.B. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet.*, 128(11): 2131–2142.

Carvalho, Y.G.S.,Vitorino, L.C., de Souza, U.J.B., & Bessa, L.A.. 2019. Recent trends in research on the geetic diversity of plants: Implications for conservation. *Diversity*, 11(4): 1–21.

Govindaraj, M., Vetriventhan, M., & Srinivasan, M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int.*, vol. 2015, no. Figure 1, 2015.

Ben-Ari, G., & Lavi, U. 2012. *Marker-assisted selection in plant breeding*, First Edit. Elsevier Inc.

Kumari, N., & Thakur, S.K. 2014. Randomly amplified polymorphic DNA-A brief review. *Am. J. Anim. Vet. Sci. 9*, 9(1): 6–13.

Ali, B.A., Huang, T.H., Qin, D.N., &Wang, X.M. 2004. A review of random amplified polymorphic DNA (RAPD) markers in fish research. *Rev. Fish Biol. Fish.*, 14(4): 443–453.

Kumar, N.S., & Gurusubramanian, G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci. Vis.*, 11(3): 116–124.

Fofana, B., du Jardin, P., & Baudoin, J.P. 2001. Genetic diversity in the Lima bean (Phaseolus lunatus L .) as revealed by chloroplast DNA (cpDNA) variations. *Genet. Resour. Crop Evol.*, pp. 1–9.

F. Sparvoli *et al.*, 2001. Lectin and lectin-related proteins in Lima bean (Phaseolus lunatus L .) seeds : biochemical and evolutionary studies. *Plant Mol. Biol.*, 45: 587–597.

Serrano-serrano, M.L., Hernández-torres, J., Castillo-villamizar, G., Debouck, D.G., & Chacón, M.I. 2010. Molecular phylogenetics and evolution gene pools in wild lima bean (Phaseolus lunatus L .) from the Americas : Evidences for an andean origin and past migrations. *Mol. Phylogenet. Evol.*, 54(1): 76–87.

Bi, I.Z., Maquet, A., & Baudoin, J. 2005. Mating system of wild *Phaseolus lunatus* L . and its relationship to population size. *Heredity (Edinb).*, 94: 153–158.

Praseptiangga, D., Tryas, A.A., Affandi, D.R., Atmaka, W., Ariyantoro, A.R., & Minardi, S. 2018. Physical and chemical characterization of composite flour from canna flour (Canna edulis) and lima bean flour (Phaseolus lunatus). in *AIP Conference Proceedings*, 1927: 1–6.

Sukatiningsih, Yustian, A.M., & Windarti, S.W. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet],” *Agritrop J. Ilmu-Ilmu Pertan.*, 11(1): 1–7.

Waluyo, B., Saptadi, D., Ardiarini, N. R., Kuswanto & Zanetta, C.U. 2016. Keragaman karakteristik fisik biji koro manis (Phaseolus lunatus L.) sebagai dasar sinkronisasi preferensi kebutuhan industri. in *Prosiding Seminar Nasional Pembangunan Pertanian 2016: Tantangan dan Arah Pembangunan Pertanian Indonesia Masa Depan,* pp. 555–560.

Doyle, J.J., & Doyle, J.L. 1984. A rapid DNA isolation procedure for small quantitiesof fresh leaf tissue. *Phytochem. Bull.*, 19: 11–15.

Maftuchah & Zainuddin. 2010. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (Jathropa curcas L.).

Fofana, B., Vekemans, X., Jardin, P., & Baudoin, J.P. 1997.Genetic diversity in Lima bean (Phaseolus lunatus L.) as revealed by RAPD markers. no. 1988, pp. 157–165.

Snustad, D.P., & Simmons, M.J. 2012. *Genetics*. New Jersey: Wiley.

Huang, W., Zhao, X., Zhao, X., Li, Y., & Lian, J. 2016. Effects of environmental factors on genetic diversity of Caragana microphylla in Horqin Sandy Land, northeast China. *Ecol. Evol.*, 6(22): 8256–8266.

Syuaib, M.F. 2016. Sustainable agriculture in indonesia: Facts and challenges to keep growing in harmony with environment. *Agric. Eng. Int. CIGR J.*, 18(20:170–184.

Doria, E., Campion, B., Sparvoli, F., Tava, A., & Nielsen, E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (Phaseolus vulgaris L . and Phaseolus lunatus L .) landraces cultivated in southern Italy. *J. Food Compos. Anal.*, 26(1–2): 72–80.

Offei, S.K., Asante, I.K., & Danquah, E.Y. 2003. Variation in size, protein, cyanide and tannin contents of lima bean (Phaseolus lunatus). *Trop. Sci.*, 43: 132–134.

Adeparusi, E.O. 2001. Effect of processing on the nutrients and anti-nutrients of lima bean (Phaseolus lunatus L .) flour. *Nahrung/Food*, vol. 45(2): 94–96.

Granito, M., Brito, Y., & Torres, A. 2007. Chemical composition , antioxidant capacity and functionality of raw and processed Phaseolus lunatus. *J. Sci. Food Agric.*, 87: 2801–2809.

Bukhari, A., Bhat, M. A., Ahmad, M., & Saleem, N. 2015. Examination of genetic diversity in common bean (Phaseolus vulgaris L.) using random amplified polymorphic DNA (RAPD) markers," *African J. Biotechnol.*, 14(6):451–458.

Adesoye, A., & Ojobo, O.A. 2012. Genetic diversity assessment of Phaseolus vulgaris L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv.*, 4(13): 453–460.

Ince, A.G., & Karaca, M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omi. J.*, 4(4): 220–227

Basheer-Salimia, R., Shtaya, M., Awad, M., Abdallah, J., & Hamdam., Y. 2013. Genetic diversity of Palestine landraces of faba bean (Vicia faba) based on RAPD markers. *Genet. Mol. Res.*, 12(3): 3314–3323

Commented [D27]: 1.Harap diganti atau diperbarui referensi yang dibawah tahun 2009.
2.Beberapa referensi yang in Indonesian, jika memungkinkan harap digantikan saja dengan yang in English
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Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura

Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

Codes	**Locus (bp)**												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description:

(1): DNA band was existent,

(0): DNA band was inexistent

Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

No.	Markers (100 bp–2000 bp)	∑ Band	Polymorphic	% Polymorphic
1	OPA 6	8	8	100%
2	OPA 8	7	7	100%
3	OPA 10	5	5	100%
4	OPA 20	5	5	100%
5	OPC 19	6	5	96%
6	OPD 8	8	8	100%
7	OPD 12	9	9	100%
8	OPE 8	7	7	100%
9	OPE 15	7	7	100%
10	OPE 16	6	6	100%
	Total	68	67	

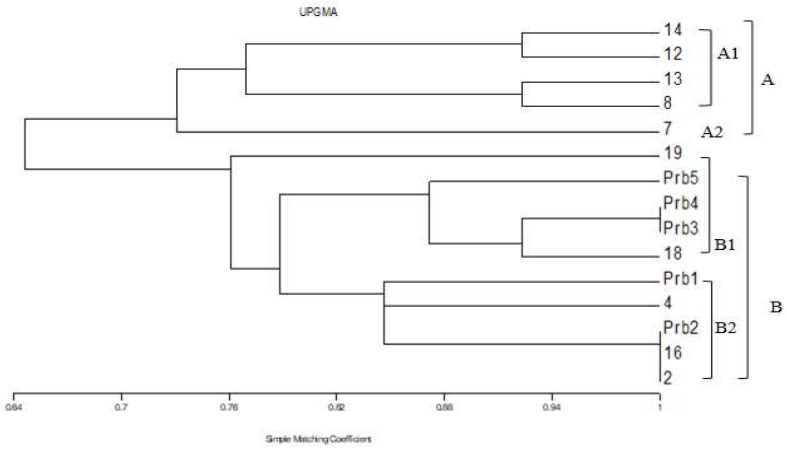

Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

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Revisi Tahap 1

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Terima kasih.
Best regards,
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Title:

Diversity of *Phaseolus lunatus* L. in East Java based on Random Amplified Polymorphic DNA Marker

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DIVERSITY OF *Phaseolus lunatus* L. IN EAST JAVA BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA MARKER

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ABSTRACT

Phaseolus lunatus L. is one of legume plants that can be found at some parts of Indonesia and is potential for alternative food rich of protein. This current research aimed at analyzing genetic accessions of *P. lunatus* distributed at some areas in East Java, Indonesia based on RAPD marker. A total of 15 accessions originated from four location were analyzed. In addition, there were 10 primers used, generating 68 lines of DNA band typified with different sizes. Further, there were 9 primers in the possession of 100% level of polymorphism in which only Primary OPC19 yielded 96% level of polymorphism. By means of Neighbor-Joining method, a phylogenetic tree was yielded by similarity coefficient of 64-100%. On the genetic similarity coefficient (GSC) of 0.6, there were two groups, the first and second major clusters, Cluster A and B. The former contained the accessions 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In

conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region tends to cluster in the same cluster.

Keywords: Genetic diversity, *Phaseolus lunatus*, phylogeny, RAPD.

INTRODUCTION

Phaseolus lunatus L. is categorized as a legume plant with its great potential to become nutritious food. This kind of plant is also known as *koro* beans or lima beans and is identical with its vital nutritious contents, from protein (Tejasari, 2016) to fiber (Ancona *et al.*, 2011; Segura-Campos & Betancur-Ancona, 2010). In addition, this plant also contains extra nutrition stored at its stipule or stalk. In addition to its richness of nutritional contents, *P. lunauts* L. is also reported to contain a number of anti-oxidant compounds (Da et al., 2009) and other substances that are effective to optimize human's health (Da et al., 2009). For that reason, not only is *P. lunatus* L. known as protein-sourced food in many countries (Almeida & Pedrosa-Harand, 2011), it is also reported to be effective in prevesnting various illnesses (Da et al., 2009).

In addition to its benefits, *P. lunatus* L. can grow in some patterns of conditions (Lim, 2012). Therefore, it is common if *P. lunatus* can be found in many parts of countries as it has been domesticated there (Camacho-Pérez et al., 2018; Chacón-Sánchez & Martínez-Castillo, 2017; Félix et al., 2014). In Indonesia, to be specific, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti & Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions to exist in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as alternatively functional food source (Diniyah et al., 2013; Diniyah et al., 2015; Herry et al., 2013; Nafi et al., 2015). Now that Indonesia is dealing with serious issue of protein shortage in some particular areas (Diana et al., 2017; Ickowitz et al., 2016; Madanijah et al., 2016), maximizing the consumption of *P. lunatus* will be of effectiveness for the solution.

With respect to the attempt of elevating the use of *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content is in need of actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the program of food source enhancement (Bhanu, 2017). Also, information that

pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a scheme of betterment for the species since the beginning of the period of systematic plant breeding (Bhanu, 2017). Furthermore, such kinds of information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, research underpinning genetic diversity in particular plant also leads to an attempt of conservation (Carvalho et al., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using such numerous techniques as morphological identification, biochemical characterization, and analysis of DNA marker (Govindaraj et al., 2015). Related to the existence of those techniques, the selection of molecular marker is considered more appropriate and effective, not only to avoid any bias due to environmental influence but also to provide eclectic information related to genetic diversity in more acceptable way (Fu, 2015). There are some molecular markers included and considered particularly promising in helping analyse genetic diversity, namely RAPD, RFLP, and SCAR.

Amongst those markers, RAPD is defined as the most popular marker in many researches (Ben-Ari & Lavi, 2012). RAPD constitutes PCR-based technique which involves primary set with relatively short size and is able to randomly amplify many DNA segments (Kumari & Thakur, 2014). This technique is equipped with notable excellence in comparison with others, which is occupies a universal primary set without undergoing DNA sequencing phase in its real implementation. In addition, RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar & Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari & Thakur, 2014), and is applicable for various laboratory situations (Kumar & Gurusubramanian, 2011). Therefore, RAPD was often used as genetic marker in many researches that studying genetic variation of various legumes.

However, analyses of genetic diversity of *P. lunatus* in Indonesia are still of rarity. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in Africa (Fofana et al., 2001), Europe (Sparvoli et al., 2001), North America (Fofana et al., 2001; Serrano-Serrano et al., 2010), Central America (Bi et al., 2005), Latin America (Fofana et al., 2001; Serrano-Serrano et al., 2010) and Caribbean countries (Fofana et al., 2001). On one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as alternative food source (Diniyah et al., 2013; Herry et al., 2014) alongside its essential substances (Diniyah et al., 2015; Praseptiangga et al., 2018; Sukatiningsih, Yustian, & Windarti, 2013; Tejasari, 2016). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti & Fauzi, 2019; Waluyo et al., 2016). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on RAPD marker.

MATERIAL AND METHODS

Plant material

P. lunatus used in this research was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. On the basis of the result of identification in previous research (Purwanti & Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Each of the accessions, further, was planted in a polybag in which one polybag distanced one meter long to the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agent.

Table 1. List of accessions to analyze

DNA Isolation

DNA isolation was administered based on CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue was stemmed from leaf organ of 3-month-old plant. Leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into buffer. After the mixture was poured into each tube, the tube was incubated under the ideal temperature with the span of ± 5 minutes. After that, the supernatant produced after centrifugation was removed and buffer II was added. Then, chloroform was added and centrifuged again. Then, the supernatant was taken and put into new tube. Absolute ethanol was added along with natrium acetate and the sample was centrifuged again. After the supernatant was eliminated; 70%-concentrated ethanol was added and the sample was centrifuged again. After that, the DNA pellet was dried out. Finally, Tris-EDTA (TE was added into tube and the results of isolated DNA were stored under the temperature of -20 ºC.

PCR-RAPD

In the reaction of PCR, the isolated DNAs of *P. lunatus* L. were used as the mold. There were 10 kinds of primers used such as OPA6, OPA8, OPA10, OPA20, OPC19, OPD8, OPD12, OPE8, OPE15, and OPE16. The total volume of PCR reaction used signified 22.5 µl, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCI; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and molded DNA. The condition for PCR reaction was designed under pre-denaturation temperature of 94°C (in 5 minutes), denaturation temperature of 94°C (in 1 minute), primary attachment temperature of 36°C (in 1 minute), extension temperature of 72°C (in 2 minutes), post-extension temperature of 72°C (in 5 minutes), and post PCR reaction temperature of 4°C (in 2 minutes). For multiplication, the cycle of PCR reaction was repeated 36 times.

Electrophoresis

There were three stages of procedure to confirm the result of isolation process as well as PCR reaction after implementation. The first stage was the creation of agarose gel with the concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labeled electrophoresis with the electrophoresis buffer of TBE (1x), loading dye (6x) under the condition of 60 V, 400 mA within 45 minutes. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

Analysis on the DNA bands yielded from RAPD

Data analysis was performed by observing the pattern of visible bands from electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data indicating the existence and inexistence of bands typifying certain sizes. Afterwards, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and to create a phylogenetic tree by the assistance of Popgen software version 3.1.

RESULTS AND DISCUSSION

In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, the bands, as a result of PCR–RAPD amplification at those genotypes (originated from East Java), were assessed based on the binary data, with the description of: 1 for amplified band and 0 for unamplified. The following Table 2 showed the record of the number of locus found.

Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

After PCR was conducted, DNA fragments with diverse sizes (polymorphic) were produced. Total, 68 DNA band lines was yielded (Table 3).

Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

Variation of genotype diversity at 15 genotypes resulted in 68 RAPD band assessed from 10 primary oligonucleotide with the means of 6.8 bands per primary. The result of amplification showed that almost all bands indicated similar pattern of DNA band, polymorphic. The primer with the smallest number of bands was OPA10 and OPA20; while the one with the highest number of bands was OPD12.

Moreover, there were 9 primers with 100% level of polymorphism. To be specific, OPC19 was only effective to result in 96% level of polymorphism.

Next, cluster analysis upon the 68 RAPD bands was administered. This phase yielded a phylogenetic tree by the assistance of Neighbor-Joining method, equipped with similarity coefficient that ranged from 64%-100%, or there was genetic variation with the range of 0-36% (Figure 1). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close. In addition, a phylogenetic tree constructed on the basis of RAPD analysis is shown in Figure 1.

Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96 to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez et al., 2018; Chacón-Sánchez & Martínez-Castillo, 2017). The existing high polymorphism had indicated the huge range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes one of a series of parameters to define genetic diversity on particular species (Snustad & Simmons, 2012). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of gene pool. According to previous study, the 15 accessions of *P. lunatus* included in current research were originated from 5 different gene pool, to name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti & Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang et al., 2016). The ecological condition was closely

interconnected with agroclimatic zone which it was grown. In the origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity which was considered high had brought about an urgent implication in terms of crop improvement attempt, to include breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not really effective as main food resource (Doria et al., 2012). Consequently, the process of maturation in *P. lunatus* seeds was in need of proper and acceptable process, thus it could reduce the anti-nutritional substances (Sukatiningsih et al., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factor. In addition, breeding procedure could be designed that way so as to yield the generation of *P. lunatus* with highly pest-resistant performance and shorter period of maturation phase.

Next, with reference to the phylogenetic tree that was formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width and pod width in comparison to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti & Fauzi, 2019).

Despite the fact, the result of cluster analysis also indicated the existence of accessions of which pod lengths were quite different, but still grouped in the same cluster. In contrast, when the pod length was not significantly different, the result of cluster analysis classified those accessions in different cluster. This was probably due to DNA related to RAPD marker used in this current research was unrelated to the character setting. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, moreover, the use of RAPD was not only affordable, but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. In spite of it, there were some studies of genetic variation on germplasm of beans which were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari et al., 2015), South Africa (Adesoye & Ojobo, 2012), Turkey (Ince & Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia et al., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic

variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez et al., 2018) and SNP (Chacón-Sánchez & Martínez-Castillo, 2017). In short, this current research has advocated the credibility of the use of RAPD in the study of genetic variation in beans, such as in *P. lunatus.*

CONCLUSION

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analyzed. The result of RAPD utilizing 10 primers resulted in 68 bands in which 9 primers possessed 100% level of polymorphism and one primary was equipped with 96% level of polymorphism. On the basis of cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

REFERENCES

Adesoye, A., & Ojobo, O. A. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *International Journal of Biodiversity and Conservation*, *4*(13): 453–460. https://doi.org/10.5897/IJBC11.216

Almeida, C., & Pedrosa-Harand, A. 2011. Contrasting rDNA evolution in lima bean (*Phaseolus lunatus* L .) and common bean (*P . vulgaris* L ., Fabaceae). *Cytogenet Genome Res*, *132*: 212–217. https://doi.org/10.1159/000321677

Ancona, D. B., Rubi, M., Campos, S., Guerrero, L. A. C., & Da, G. 2011. Structural and some nutritional characteristics of Velvet bean (Mucuna pruriens) and Lima bean (*Phaseolus lunatus*) starches. *Starch/Stärke*, *63*: 475–484. https://doi.org/10.1002/star.201000119

Basheer-Salimia, R., Shtaya, M., Awad, M., Abdallah, J., & Hamdam, Y. 2013. Genetic diversity of Palestine landraces of faba bean (*Vicia faba*) based on RAPD markers. *Genetics and Molecular Research*, *12*(3): 3314–3323. https://doi.org/10.4238/2013.September.3.8

Ben-Ari, G., & Lavi, U. 2012. *Marker-assisted selection in plant breeding*. *Plant Biotechnology and Agriculture.* 1st Ed. Elsevier Inc. https://doi.org/10.1016/B978-0-12-381466-1.00011-0

Bhanu, A. N. 2017. Assessment of genetic diversity in crop plants - An overview. *Advances in Plants & Agriculture Research*, *7*(3): 279–286. https://doi.org/10.15406/apar.2017.07.00255

Bi, I. Z., Maquet, A., & Baudoin, J. 2005. Mating system of wild *Phaseolus lunatus* L . and its relationship to population size. *Heredity*, *94*: 153–158. https://doi.org/10.1038/sj.hdy.6800527

Bukhari, A., Bhat, M. A., Ahmad, M., & Saleem, N. 2015. Examination of genetic diversity in

common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, *14*(6): 451–458. https://doi.org/10.5897/ajb2014.14281

Camacho-Pérez, L., Martínez-Castillo, J., Mijangos-Cortés, J. O., Ferrer-Ortega, M. M., Baudoin, J. P., & Andueza-Noh, R. H. 2018. Genetic structure of Lima bean (*Phaseolus lunatus* L.) landraces grown in the Mayan area. *Genetic Resources and Crop Evolution*, *65*(1): 229–241. https://doi.org/10.1007/s10722-017-0525-1

Carvalho, Y. G. S., Vitorino, L. C., de Souza, U. J. B., & Bessa, L. A. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*, *11*(4): 1–21. https://doi.org/10.3390/d11040062

Chacón-Sánchez, M. I., & Martínez-Castillo, J. 2017. Testing domestication scenarios of Lima bean (*Phaseolus lunatus* L.) in mesoamerica: Insights from genome-wide genetic markers. *Frontiers in Plant Science*, *8*(September): 1–20. https://doi.org/10.3389/fpls.2017.01551

Da, G., Torruco-uco, J., Chel-guerrero, L., Martı, A., & Betancur Ancona, D. 2009. Angiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from Phaseolus lunatus and Phaseolus vulgaris seeds. *LWT - Food Science and Technology*, *42*: 1597–1604. https://doi.org/10.1016/j.lwt.2009.06.006

Diana, A., Mallard, S. R., Haszard, J. J., Purnamasari, D. M., Nurulazmi, I., Herliani, P. D., … Houghton, L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLoS ONE*, *12*(4): 1–17. https://doi.org/10.1371/journal.pone.0175952

Diniyah, N., Windarti, W. S., & Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai. In *Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi* (pp. 1–10). Surabaya: UPN Veteran. Retrieved from https://www.researchgate.net/profile/Nurud_Diniyah/publication/273068090_Prosiding-UPN-Koro-Full/links/54f6c1de0cf27d8ed71eb914/Prosiding-UPN-Koro-Full.pdf?_sg%5B0%5D=SBY51jM0p1OyRLCl26T8CSR06PN_LDBk9BuA5zPEpUy6T2wVWeDO4nKKVz-JtSWAxNKiVzkGbCTD1epUYuKx7Q.-

Diniyah, N., Windrati, W. S., & Riady, S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (Phaseolus lunatus l.) dengan perlakuan lama perendaman. *Jurnal Hasil Penelitian Industri*, *28*(2): 70–77. Retrieved from http://download.portalgaruda.org/article.php?article=494290&val=10108&title=SIFAT FUNGSIONAL TEPUNG KORO KRATOK HITAM, MERAH DAN PUTIH (Phaseolus lunatus

L.) DENGAN PERLAKUAN LAMA PERENDAMAN (Functional Properties of Black, Red and White Lima Bean (Phaseo

Doria, E., Campion, B., Sparvoli, F., Tava, A., & Nielsen, E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (Phaseolus vulgaris L . and Phaseolus lunatus L .) landraces cultivated in southern Italy. *Journal of Food Composition and Analysis*, *26*(1–2): 72–80. https://doi.org/10.1016/j.jfca.2012.03.005

Doyle, J. J., & Doyle, J. L. (1984). A rapid DNA isolation procedure for small quantitiesof fresh leaf tissue. *Phytochem. Bull.*, *19*, 11–15.

Félix, D. T., Coello-Coello, J., & Martínez-Castillo, J. 2014. Wild to crop introgression and genetic diversity in Lima bean (Phaseolus lunatus L.) in traditional Mayan milpas from Mexico. *Conservation Genetics*, *15*(6): 1315–1328. https://doi.org/10.1007/s10592-014-0619-7

Fofana, B., Jardin, P. du, & Baudoin, J. P. 2001. Genetic diversity in the Lima bean (Phaseolus lunatus L .) as revealed by chloroplast DNA (cpDNA) variations. *Genetic Resources and Crop Evolution*, 1–9. Retrieved from http://cyber.sci-hub.tw/MTAuMTAyMy9hOjEwMTIwMTYwMzAzMTg=/10.1023%40a%3A1012016030318.pdf

Fu, Y. B. 2015. Understanding crop genetic diversity under modern plant breeding. *Theoretical and Applied Genetics*, *128*(11): 2131–2142. https://doi.org/10.1007/s00122-015-2585-y

Govindaraj, M., Vetriventhan, M., & Srinivasan, M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International*, *2015*. https://doi.org/10.1155/2015/431487

Herry, B., Windarti, W. S., & Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein. In *Prosiding Seminar Nasional* (pp. 460–472). Jember: Universitas Muhammadiyah Jember. Retrieved from https://www.researchgate.net/profile/Nurud_Diniyah/publication/273125193_Potensi_Koro-Koroan_Sebagai_Sumber_Bahan_Lokal_untuk_Pembuatan_Aneka_Produk_Olahan_Berprotein/links/54f800b80cf210398e93ca29/Potensi-Koro-Koroan-Sebagai-Sumber-Bahan-Lokal-untuk-Pemb

Huang, W., Zhao, X., Zhao, X., Li, Y., & Lian, J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. *Ecology and Evolution*, *6*(22): 8256–8266. https://doi.org/10.1002/ece3.2549

Ickowitz, A., Rowland, D., Powell, B., & Salim, M. A. (2016). Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLoS ONE*, *11*(5): 1–15.

https://doi.org/10.1371/journal.pone.0154139

Ince, A. G., & Karaca, M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics Journal*, *4*(4): 220–227. Retrieved from https://www.pomics.com/ince_4_4_2011_220_227.pdf

Kalaminasih, D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (Canavalia ensiformis L) terhadap mutu organoleptik tempe koro. *E-Journal Boga*, *2*(3): 104–113. Retrieved from https://jurnalmahasiswa.unesa.ac.id/index.php/jurnal-tata-boga/article/viewFile/4329/6731

Kumar, N. S., & Gurusubramanian, G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Science Vision*, *11*(3): 116–124. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.404.1046&rep=rep1&type=pdf

Kumari, N., & Thakur, S. K. 2014. Randomly amplified polymorphic DNA-A brief review. *American Journal of Animal and Veterinary Sciences 9*, *9*(1): 6–13. https://doi.org/10.3844/ajavssp.2014.6.13

Lim, T. K. 2012) Lablab purpureus. In *Edible Medicinal And Non-Medicinal Plants* (pp. 730–741). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-1764-0_84

Madanijah, S., Briawan, D., Rimbawan, R., Zulaikhah, Z., Andarwulan, N., Nuraida, L., … Bindels, J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: a cross-sectional dietary and nutrient intake study. *British Journal of Nutrition*, *116*(2016): 1–10. https://doi.org/10.1017/S000711451600057X

Maftuchah, & Zainuddin. 2010. *Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (Jathropa curcas L.)*. Malang.

Nafi, A., Diniyah, N., & Hastuti, F. T. (2015). Karakteristik fisikokimia dan fungsional teknis tepung koro kratok *Phaseolus lunatus* L. termodifikasi yang diproduksi secara fermentasi spontan. *Agrointek*, *9*(1): 24–32. Retrieved from http://journal.trunojoyo.ac.id/agrointek/article/download/2121/1745

Praseptiangga, D., Tryas, A. A., Affandi, D. R., Atmaka, W., Ariyantoro, A. R., & Minardi, S. 2018. Physical and chemical characterization of composite flour from canna flour (*Canna edulis*) and lima bean flour (*Phaseolus lunatus*). In I. M. Joni & C. Panatarani (Eds.), *AIP Conference Proceedings* (Vol. 1927, pp. 1–6). Jatinangor: American Institute of Physics. https://doi.org/10.1063/1.5021213

Purwanti, E., & Fauzi, A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol.

276, p. 012017). https://doi.org/10.1088/1755-1315/276/1/012017

Segura-Campos, M., & Betancur-Ancona, D. (2010). Effect of octenylsuccinylation on functional properties of lima bean *(Phaseolus lunatus*) strach. *Journal of Food Process Engineering*, *33*: 712–727. https://doi.org/10.1111/j.1745-4530.2008.00299.x

Serrano-serrano, M. L., Hernández-torres, J., Castillo-villamizar, G., Debouck, D. G., & Chacón, M. I. 2010. Molecular Phylogenetics and Evolution Gene pools in wild Lima bean (*Phaseolus lunatus* L.) from the Americas : Evidences for an Andean origin and past migrations. *Molecular Phylogenetics and Evolution*, *54*(1): 76–87. https://doi.org/10.1016/j.ympev.2009.08.028

Snustad, D. P., & Simmons, M. J. 2012. *Genetics*. New Jersey: Wiley.

Sparvoli, F., Lanave, C., Santucci, A., Bollini, R., Lioi, L., Vegetali, B., … A, V. A. 2001. Lectin and lectin-related proteins in Lima bean (*Phaseolus lunatus* L.) seeds : biochemical and evolutionary studies. *Plant Molecular Biology*, *45*, 587–597. Retrieved from http://cyber.sci-hub.tw/MTAuMTAyMy9hOjEwMTA2NDczMTAzMTE=/10.1023%40a%3A1010647310311.pdf

Sukatiningsih, Yustian, A. M., & Windarti, S. W. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet]. *Agritrop Jurnal Ilmu-Ilmu Pertanian*, *11*(1): 1–7. Retrieved from http://jurnal.unmuhjember.ac.id/index.php/AGRITROP/article/download/662/532

Syuaib, M. F. 2016. Sustainable agriculture in indonesia: Facts and challenges to keep growing in harmony with environment. *Agricultural Engineering International: CIGR Journal*, *18*(2): 170–184. Retrieved from https://www.researchgate.net/publication/305141930

Tejasari. 2016. Protein density and quality of koro kratok (*Phaseolus lunatus* L. Sweet) and kacang tunggak (*Vigna unguiculata* (L.) Walp). In *Proceeding of 1st International Conference on Medicine and Health Sciences (ICMHS)* (pp. 116–119). Jember: University of Jember. Retrieved from https://jurnal.unej.ac.id/index.php/prosiding/article/download/3850/3064/

Waluyo, B., Saptadi, D., Ardiarini, N. R., Kuswanto, & Zanetta, C. U. 2016. Keragaman karakteristik fisik biji koro manis (*Phaseolus lunatus* L.) sebagai dasar sinkronisasi preferensi kebutuhan industri. In R. Asmara & Sujarwo (Eds.), *Prosiding Seminar Nasional Pembangunan Pertanian 2016: Tantangan dan Arah Pembangunan Pertanian Indonesia Masa Depan, Malang 12 November 2016* (pp. 555–560). Malang: Badan Penerbit Fakultas Pertanian UB. Retrieved from https://www.researchgate.net/publication/311081631%0AKeragaman

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

Table 2. The record of the number of locus found at 15 genotypes of *P.lunatus* L using 10 random primary

Codes	Locus (bp)											
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300

2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description:

(1): DNA band was existent,

(0): DNA band was inexistent

Table 3. RAPD primers used for diversity analysis of *Phaseolus lunatus* L.

No.	Markers (100 bp–2000 bp)	∑ Band	Polymorphic	% Polymorphic
1	OPA 6	8	8	100%
2	OPA 8	7	7	100%
3	OPA 10	5	5	100%
4	OPA 20	5	5	100%
5	OPC 19	6	5	96%

6	OPD 8	8	8	100%
7	OPD 12	9	9	100%
8	OPE 8	7	7	100%
9	OPE 15	7	7	100%
10	OPE 16	6	6	100%
	Total	68	67	

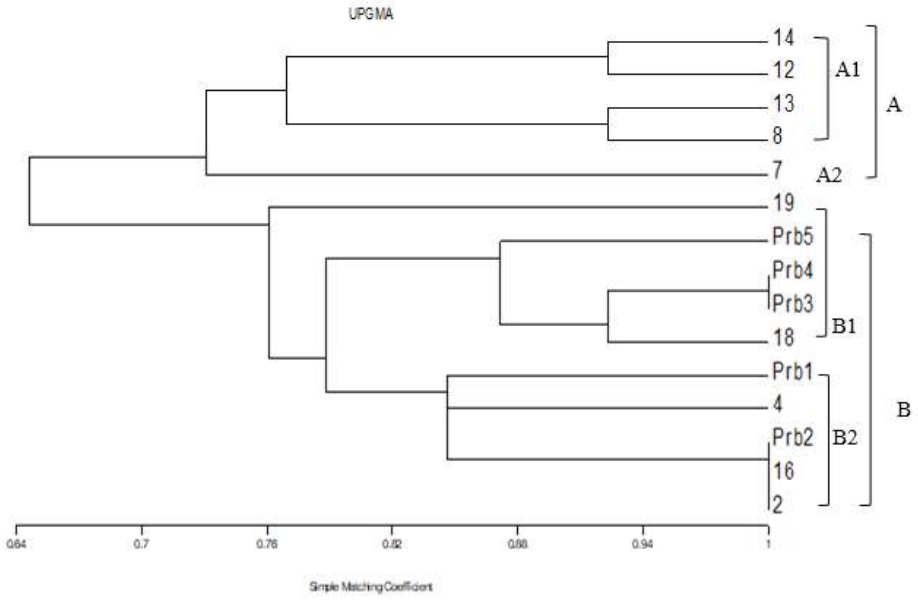

Fig. 1. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

Pengumuman pengalihan jurnal target (dari MAB ke JJBS) (5 Oktober 2020)

ICON-BEAT 2019

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Cover Letter

Manuscript title: Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on Random Amplified Polymorphic DNA Marker

Research Subject Area: Biotechnology

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Conflict of interest/sponsorship (if any):

Ethical committee approval\ Human Research Protections \ or Institutional Review Board (IRB): If Applicable, Please provide a copy of the Approval.

We affirm that the submission represents work that has not been published previously and is not currently being considered by another journal. Also, I confirm that each author has seen and approved the contents of the submitted manuscript.

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Manuscript Evaluation Report- Referee1

Manuscript ID: JJBS 36/21/R6 Due date: April 15, 2021

TITLE:

Diversity of Phaseolus lunatus L. in East Java, Indonesia based on Random Amplified Polymorphic DNA Marker

Type of Article: ☐ Review Article ☐ Research Paper ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	Is the topic of the manuscript within the scope of the journal?	2
2	Does the title clearly and sufficiently reflect its content?	2
3	Are the keywords and abstract sufficient and informative?	2
4	What is the scholarly quality of the manuscript?	1
5	Is this a new and/ or original contribution?	2
6	Is the research methodology utilized appropriate and properly administered?	2
7	Are the methods of data analysis acceptable?	2
8	Are the results and conclusions clear, adequately presented and organized in relation to rest of manuscript?	2
9	Are the illustrations and tables necessary and in acceptable format?	3
10	Are the interpretations/ conclusions sound and justified by the data?	2
11	Are the References in a proper format according to JJBS author Instruction?	
12	Is the MS written in correct and satisfactory English?	1

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3

PART B: Comments per Section of Manuscript:

Abstract	
Introduction	

Methodology	
Results	
Discussion and Conclusion	
References	

PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	
Reconsidered after Major Revision	
Reject on Ground of (Please Be Specific)	As it is (Reject) However, after substantial language editing, new resubmition

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

The manuscript needs substantial language editing. I started to do editing, but the whole manuscript is hard to follow
I recommend editing the manuscript and resubmitted it as a new submission

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Manuscript Evaluation Report- Referee2

Manuscript ID: JJBS 36/21/R6 Due date: April 15, 2021

TITLE:

Diversity of Phaseolus lunatus L. in East Java, Indonesia based on Random Amplified Polymorphic DNA Marker

Type of Article: ☐ Review Article ☐ **Research Paper** ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	**Is the topic of the manuscript within the scope of the journal?**	5
2	**Does the title clearly and sufficiently reflect its content?**	4
3	**Are the keywords and abstract sufficient and informative?**	3
4	**What is the scholarly quality of the manuscript?**	3
5	**Is this a new and/ or original contribution?**	3
6	**Is the research methodology utilized appropriate and properly administered?**	3
7	**Are the methods of data analysis acceptable?**	3
8	**Are the results and conclusions clear, adequately presented and organized in relation to rest of manuscript?**	3
9	**Are the illustrations and tables necessary and in acceptable format?**	3
10	**Are the interpretations/ conclusions sound and justified by the data?**	4
11	**Are the References in a proper format according to JJBS author Instruction?**	3
12	**Is the MS written in correct and satisfactory English?**	3

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7

PART B: Comments per Section of Manuscript:

Abstract	Add unique band results to your abstract…
Introduction	Very long…
Methodology	PCR condition and amount of material used not clear…
Results	Add agarose gel figure of DNA extraction and PCR amplification of all primers…
Discussion and Conclusion	Add unique band results to your dis. And conclusion..

References	Write it according to the journal require….

PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	
Reconsidered after Major Revision	✓
Reject on Ground of (Please be Specific)	

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

Please make all necessary correction….Best regards.

Pengiriman naskah revisi ke JJBS (24 April 2021)

Section	Reviewer Comments	Author Responses
Abstract	Add unique band results to your abstract…	We have added information related to the unique band that we get
Introduction	Very long…	We have reduced our introduction
Methodology	PCR condition and amount of material used not clear…	We have detailed the methods we used in our study
Results	Add agarose gel figure of DNA extraction and PCR amplification of all primers…	We have inserted a photo of the amplified result we obtained for ten primers we used
Discussion and Conclusion	Add unique band results to your dis. And conclusion..	We have added information regarding the number of unique bands, the length of the bands, as well as a description of the accessions where the bands appear
References	Write it according to the journal require….	We have adjusted the reference format of our manuscript with the JJBS author guideline

Title:
Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on PCR-RAPD technique

Manuscript: 36/21/R6

Keywords:

Genetic diversity, Lima bean, *phylogeny,* RAPD

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DIVERSITY OF *Phaseolus lunatus* L. IN EAST JAVA INDONESIA BASED ON PCR-RAPD TECHNIQUE

ABSTRACT

Phaseolus lunatus L. is one of the legume plants found in some parts of Indonesia and is potential for alternative food rich in protein. This current research aimed at analysing genetic accessions of *P. lunatus* distributed at some areas in East Java, Indonesia, based on the RAPD marker. A total of 15 accessions

originated from four locations were analysed. Ten primers used and produced 68 bands, out of 67 were polymorphic. The percent polymorphism was 96 to 100%. Ten unique bands were detected in eight accessions (Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6). Using the Neighbor-Joining method, a phylogenetic tree was yielded by a similarity coefficient of 64 % to 100 %. On the genetic similarity coefficient (GSC) of 0.6, there were two clusters, the first and second major clusters, Cluster A and B. The former contained the accessions 7, 8, 13, and 14, while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region tend to cluster in the same cluster.

Keywords: Genetic diversity; Lima beanPhylogeny, RAPD-PCR.

1.Introduction

Phaseolus lunatus L. is categorized as a legume plant with its great potential to become nutritious food. In Indonesia, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti and Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions exist in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as an alternatively functional food source (Diniyah *et al*., 2013; Diniyah *et al*., 2015; Herry *et al*., 2013; Nafi *et al*., 2015). Nowadays, Indonesia is dealing with the severe issue of protein shortage in some particular areas (Diana *et al*., 2017; Ickowitz *et al*., 2016; Madanijah *et al.,* 2016), maximizing the consumption of *P. lunatus* will be of effectiveness for the solution.

With respect to elevating the use of elevating *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content needs actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the program of food source enhancement (Bhanu, 2017). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a betterment scheme for the species since the beginning of the period of systematic plant

breeding (Bhanu, 2017). Furthermore, such information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, research underpinning genetic diversity in a particular plant also leads to conservation (Carvalho *et al*., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using numerous techniques such as morphological identification, biochemical characterization, and analysis of molecular markers (Govindaraj *et al*., 2015). Related to those techniques, the selection of molecular markers is considered more appropriate and effective, not only to avoid any bias due to environmental influence but also to provide eclectic information related to genetic diversity in a more acceptable way (Fu, 2015). Some molecular markers are included and considered particularly promising in helping analyse genetic diversity, such as RAPD, RFLP, and SCAR.

Among those markers, RAPD is the most popular marker in many researches (AlRawashdeh and AlRawashdeh, 2015; Ben-Ari and Lavi, 2012). RAPD constitutes a PCR-based technique that involves a primary set with a relatively short size and can be a PCR-based technique that involves a relatively short size and can randomly amplify many DNA segments (Kumari and Thakur, 2014). This technique is equipped with outstanding excellence compared to others, which occupies a universal primary set without undergoing the DNA sequencing phase in its real implementation. In addition, the RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar and Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari and Thakur, 2014), and is applicable for various laboratory situations (Kumar and Gurusubramanian, 2011). Therefore, RAPD was often used as a genetic marker in many research the genetic variation of various legumes.

However, analyses of the genetic diversity of *P. lunatus* in Indonesia are still of rarity. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in North America (Serrano-serrano *et al.,* 2010), Central America (Camacho-Pérez *et al.,* 2018), and South America (Silva *et al.,* 2019). On the one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as an alternative food source (Diniyah *et al*., 2013; Herry *et al*., 2014) alongside its essential substances (Diniyah *et al*., 2015; Praseptiangga *et al.*, 2018; Sukatiningsih *et al*., 2013; Tejasari, 2016). In addition,

researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti and Fauzi, 2019). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on the RAPD marker.

2.Material and Methods

2.1. Collection of Samples

P. lunatus used in this present study was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. On the basis of the result of identification in previous research (Purwanti and Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Further, each of the accessions, planted in a polybag in which one polybag distanced one meter long to the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agent.

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura

19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

2.2. DNA Isolation

DNA isolation was administered based on CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue was stemmed from the leaf organ of 3 month-old plants. Leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into the buffer. The results of isolated DNA were stored under the temperature of -20 ºC.

Table 2. List of primers

Primers	Sequence	GC Content (%)
OPA6	GGTCCCTGAC	70
OPA8	GTGACGTAGG	60
OPA10	GTGATCGCAG	60
OPA20	GTTGCGATCC	60
OPC19	GTTGCCAGCC	70
OPD8	GTGTGCCCCA	70
OPD12	CACCGTATCC	60
OPE8	TCACCACGGT	60
OPE15	ACGCACAACC	60

OPE16	GGTGACTGTG	60

2.3. PCR-RAPD

Ten primers having 60-70 GC content were used in this present study (Table 2). The total volume of PCR reaction used signified 22.5 µL, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCl; 15 mM $MgCI_2$; 0.01 % gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and 30 ng DNA template. The condition for PCR reaction was designed under pre-denaturation temperature of 94 °C (in 5 min), denaturation temperature of 94 °C (in 1 min), primary attachment temperature of 36 °C (in 1 min), extension temperature of 72 °C (in 2 min), post-extension temperature of 72 °C (in 5 min), and post PCR reaction temperature of 4 °C (in 2 min). For multiplication, the cycle of PCR reaction was repeated 36 times.

2.4. Agarose Gel Electrophoresis

There were three stages of procedure to confirm the result of isolation process as well as PCR reaction after implementation. The first stage was the creation of agarose gel with the concentration of 0.8 % (for isolation result) and 1 % (for PCR result) as a medium of running DNA. Next, the stage was labeled electrophoresis with the electrophoresis buffer of TBE (1×), loading dye (6×) under the condition of 60 V, 400 mA within 45 min. At last, the stage was the coloration using 10 %-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

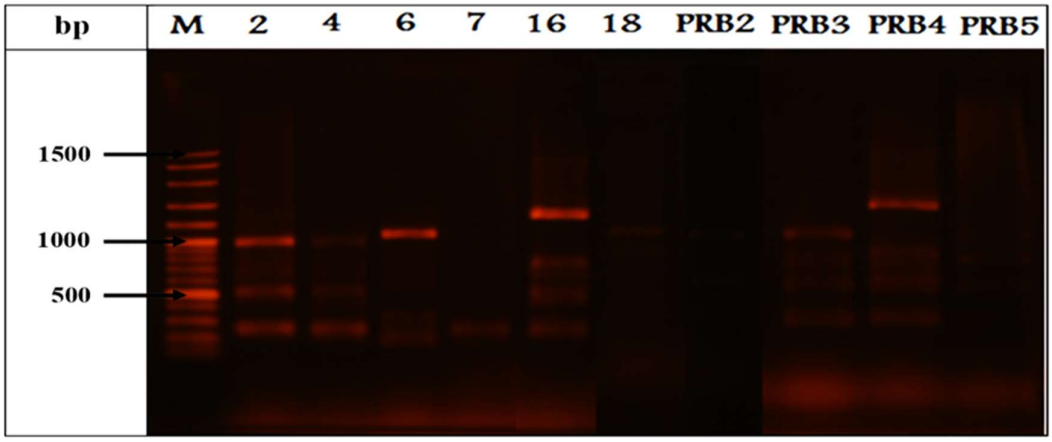
2.5. Analysis on the DNA Bands Yielded from RAPD

Data analysis was performed by observing the pattern of visible bands from electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data (0 and 1) indicating the existence and inexistence of bands typifying certain sizes. Afterwards, the existing bands were observed

to identify the percentage of polymorphic and monomorphic bands and to create a phylogenetic tree by the assistance of Popgen software version 3.1 (Yeh and Boyle, 1997).

3. Results and Discussion

In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, the bands, as a result of PCR–RAPD amplification at those genotypes (Figure 1), were assessed based on the binary data, with the description of: 1 for amplified band and 0 for unamplified. The following Table 3 showed the record of the number of locus found.



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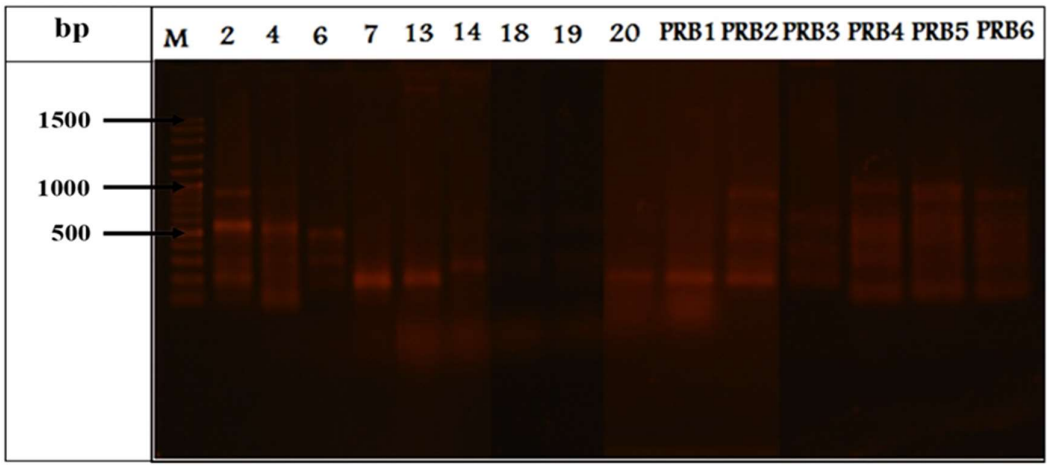

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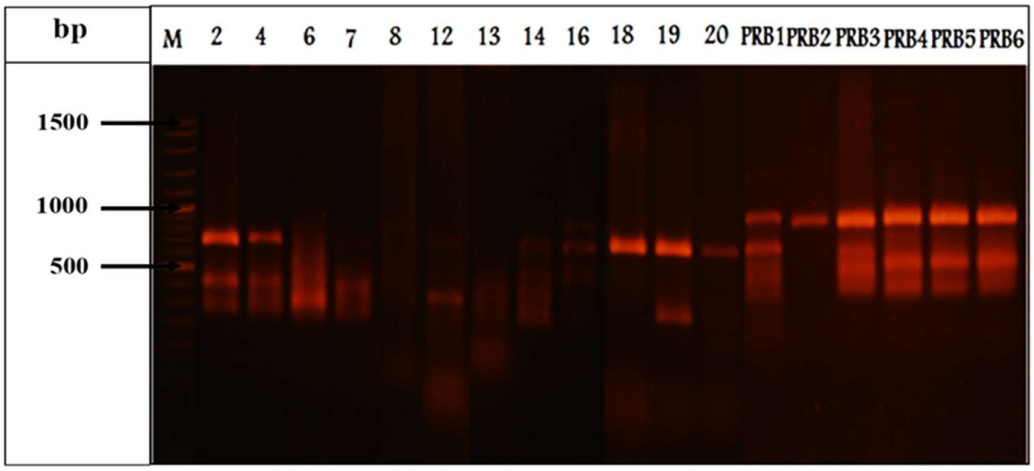
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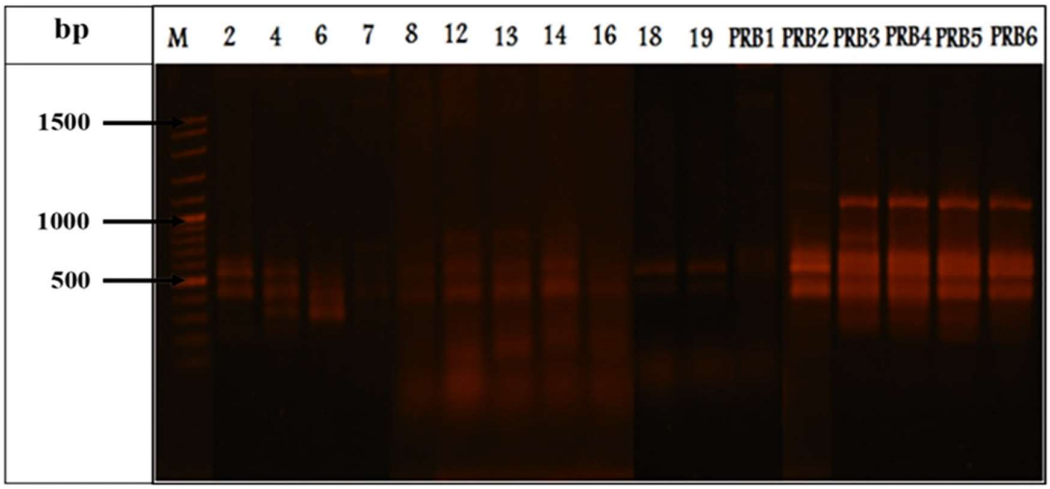
1500
1000
500

(c)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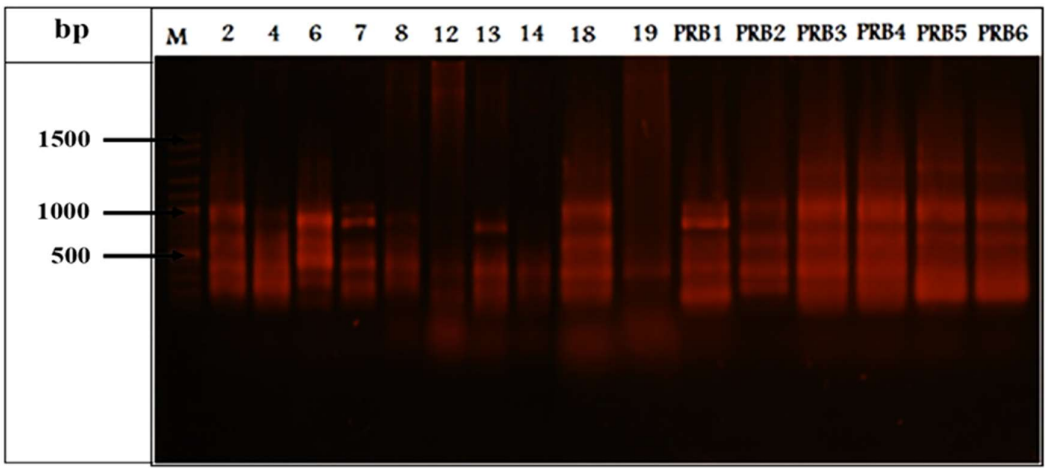
1000
500

(d)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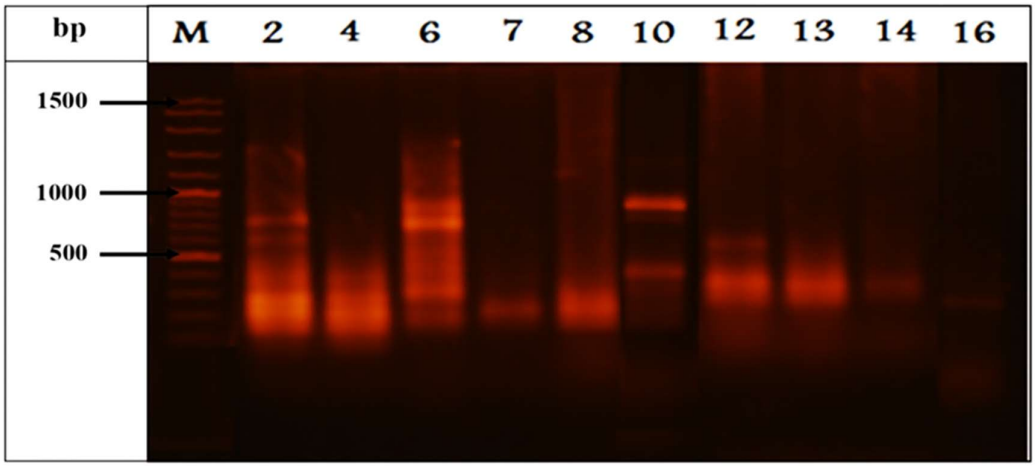
M 2 4 6 7 8 12 13 14 18 19 PRB1 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(e)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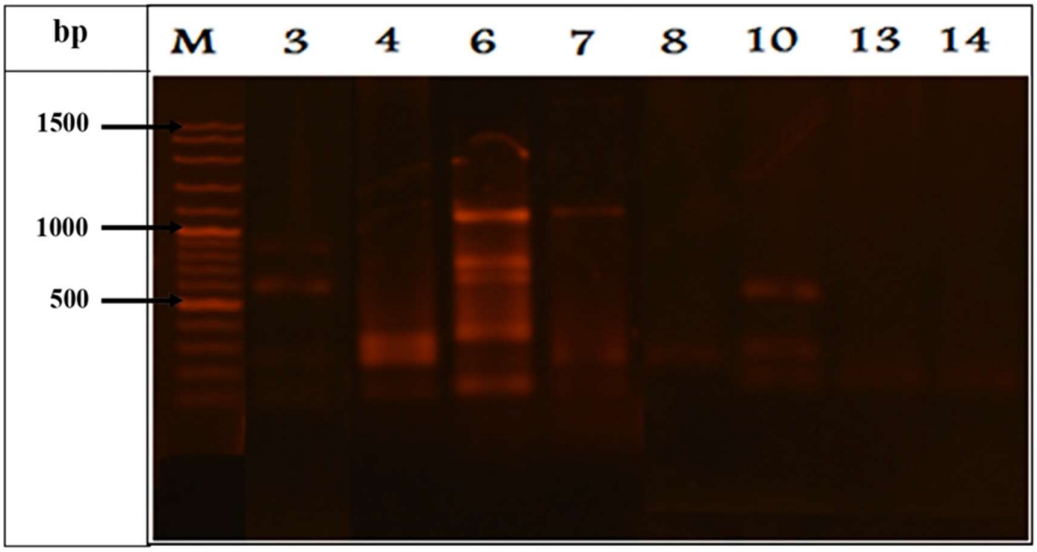
1500
1000
500

(f)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1000
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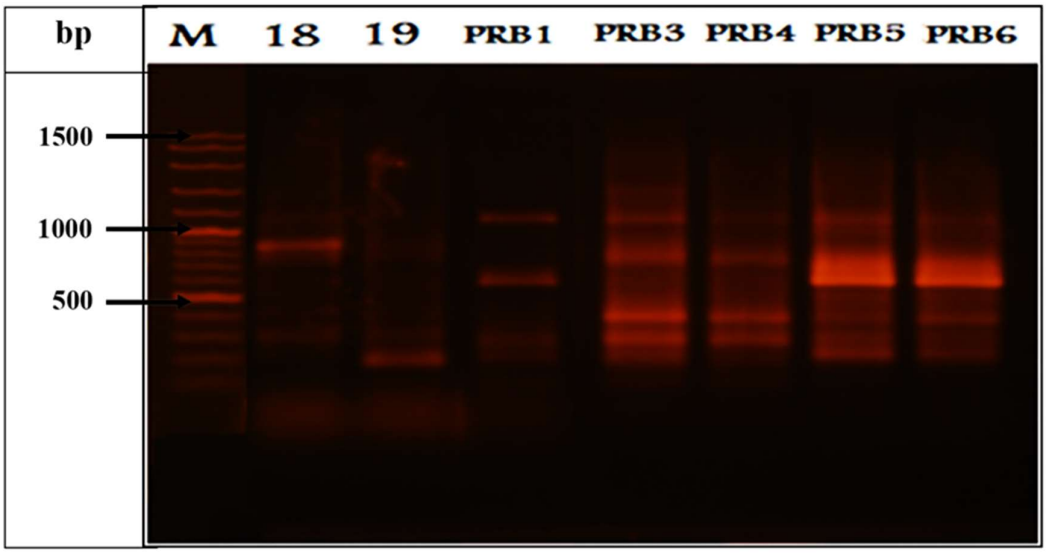
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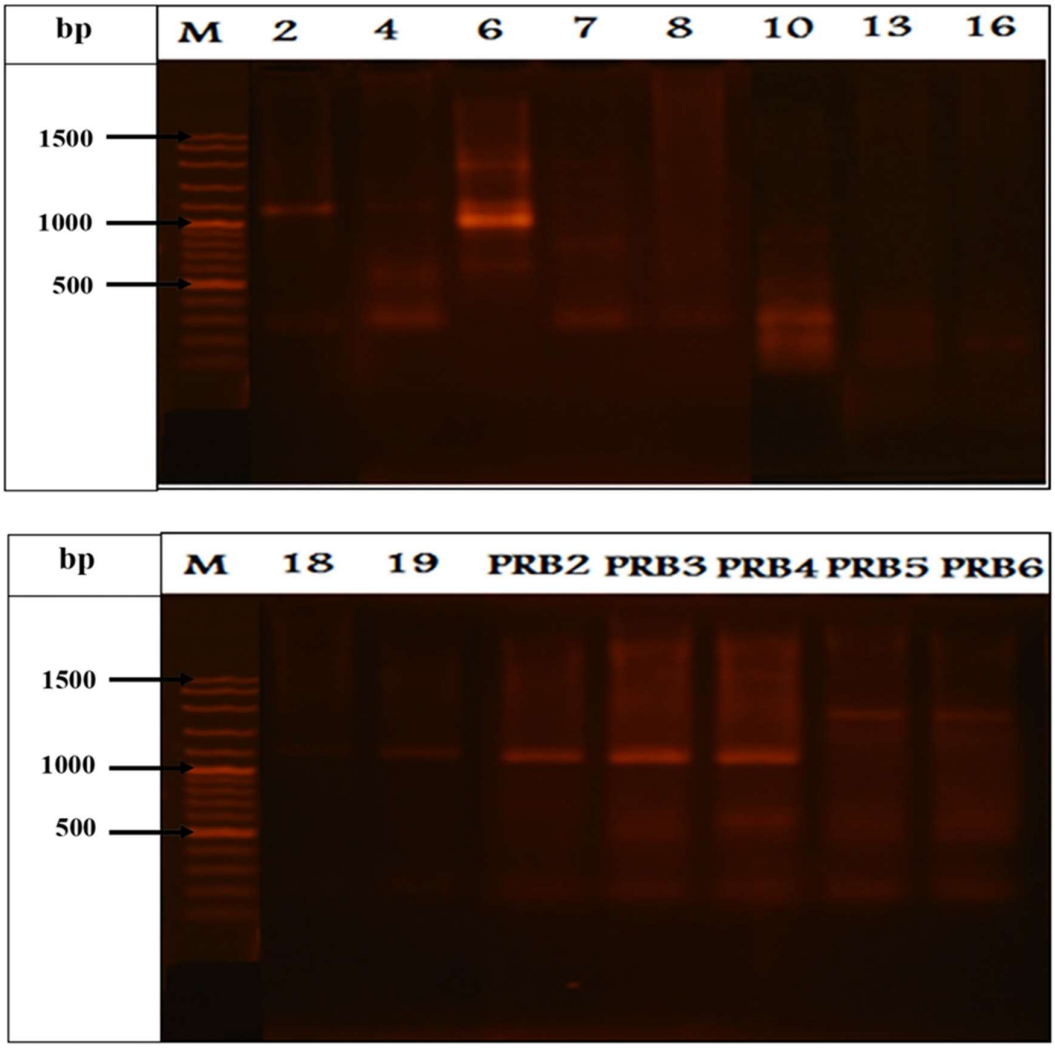
M 18 19 PRB1 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(g)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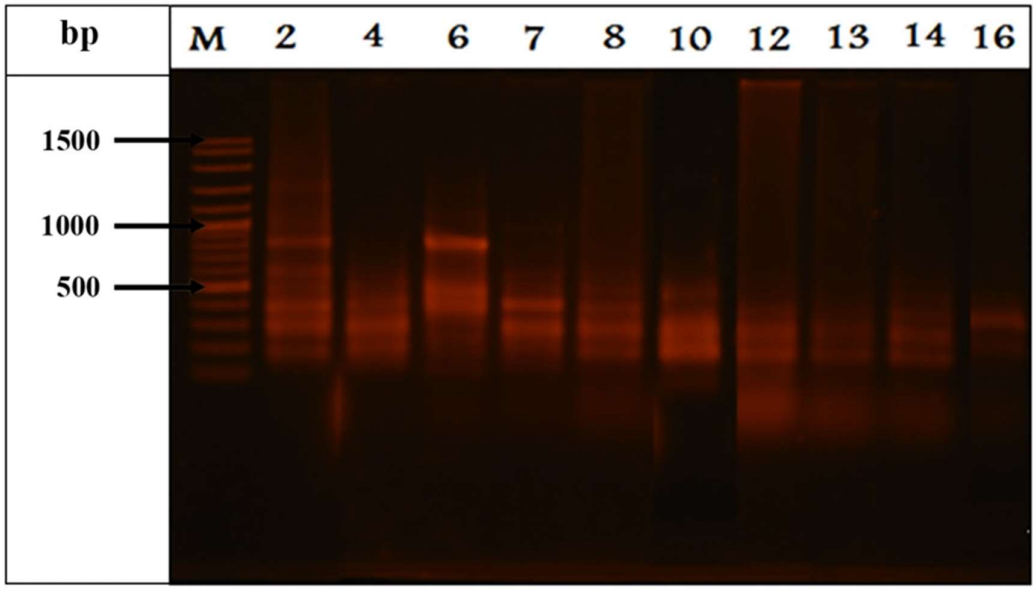
M 2 4 6 7 8 10 13 16
1500
1000
500
bp
M 18 19 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(h)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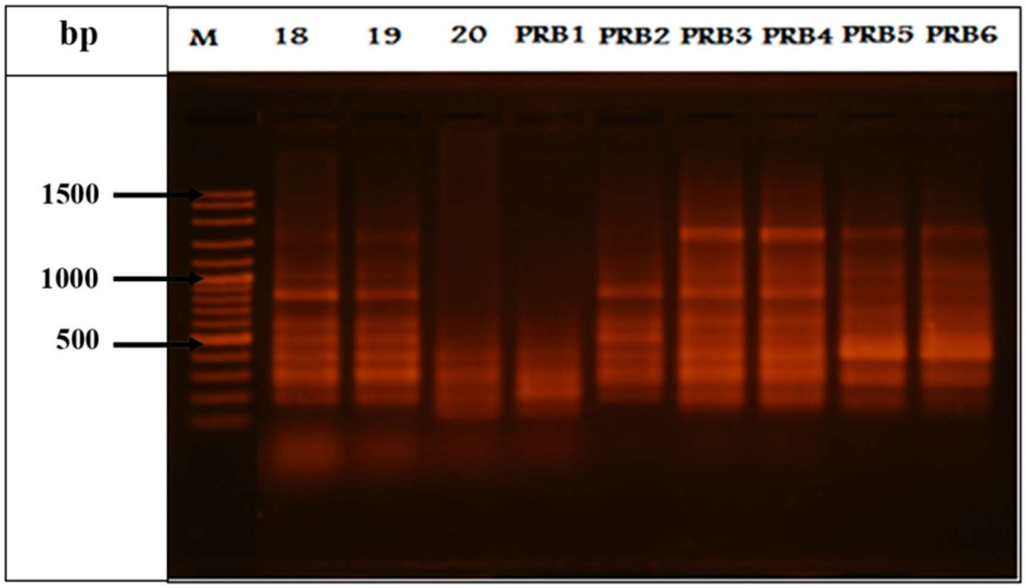
1500
1000
500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PRB3
PRB4
PRB5
PRB6
1500
1000
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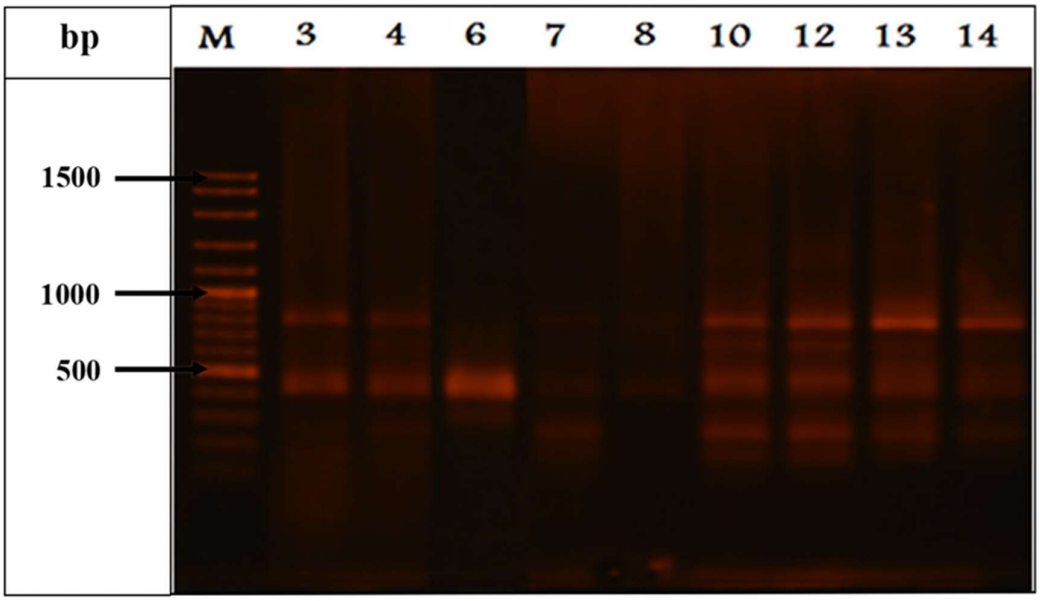
(i)


bp
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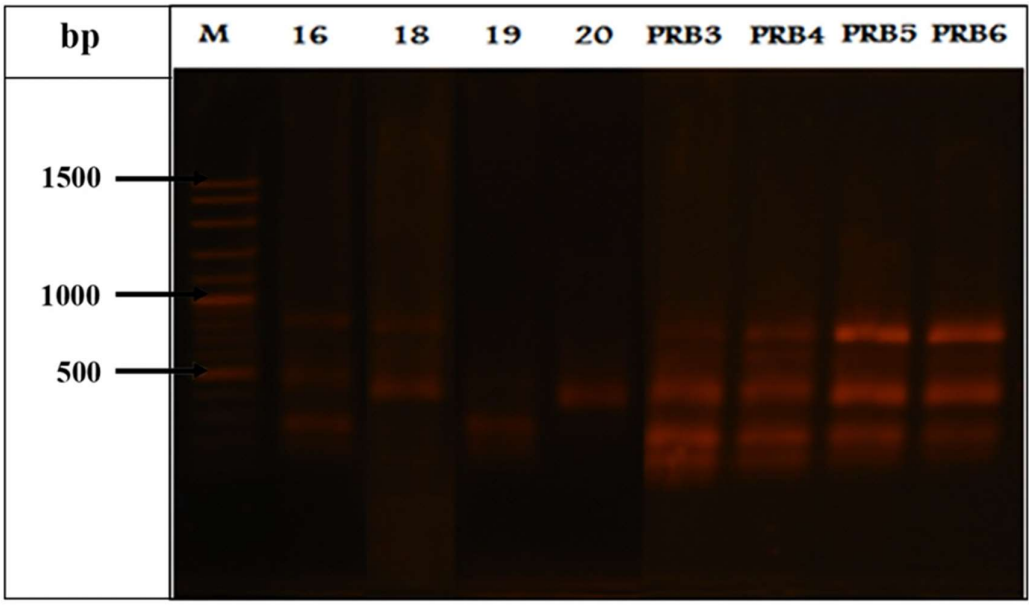

(j)

Fig. 1. PCR–RAPD results using (a) OPA6, (b) OPA8, (c) OPA10, (d) OPA20, (e) OPA19, (f) OPD8, (g) OPD12, (h) OPE8, (i) OPE15, (j) OPE16

Table 3. The record of the number of locus found at 15 genotypes of *P.lunatus* L using random primary 10

Codes	Locus (bp)												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0

18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description:

(1): DNA band was existent,

(0): DNA band was inexistent

After PCR was conducted, DNA fragments with diverse sizes (polymorphic) were produced (Table 4). A total of 68 amplified bands were obtained, out of 67 were polymorphic. The percent polymorphism was 96 to 100%. The total number of amplified bands varied betwen 5 (OPA10 and OPA20) to 9 (OPA12) with an average of 6,8 bands per primer. Ten uniques bands were detected in several accessions. The size of unique band ranged from 200 (Mdr6) to 1300 bp (Prb5).

Table 4. RAPD primers used for diversity analysis of *P. lunatus*.

No.	**Markers (100 bp–2000 bp)**	**∑ Band**	**Polymorphic**	**% Polymorphic**	**Unique band**		
					Total	**Locus**	**Accession**
1	OPA 6	8	8	100 %	2	700 200	Mdr16 Mdr6
2	OPA 8	7	7	100 %	0	-	-
3	OPA 10	5	5	100 %	0	-	-

4	OPA 20	5	5	100 %	1	300	Mdr4
5	OPC 19	6	5	96 %	0	-	-
6	OPD 8	8	8	100 %	1	500	Prb1
7	OPD 12	9	9	100 %	2	1200	Prb3
						900	Mdr19
8	OPE 8	7	7	100 %	2	1300	Prb5
						500	Mdr4
9	OPE 15	7	7	100 %	0	-	-
10	OPE 16	6	6	100 %	2	700	Mdr12
						500	Mdr16
	Total	68	67		10		

The cluster analysis upon the 68 RAPD bands was administered. The phylogenetic tree using Neighbor-Joining method was produced, equipped with similarity coefficient that ranged from 64 % to 100 %, or there was genetic variation with the range of 0 % to 36 % (Figure 2). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close.

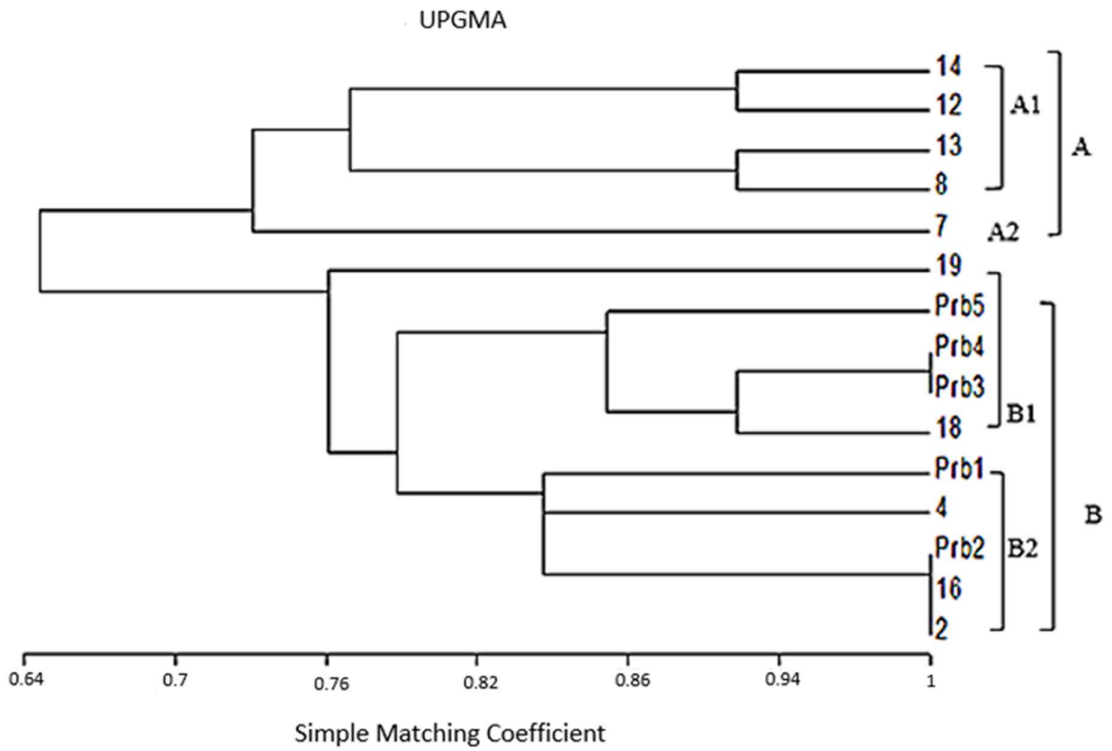

Fig. 2. The phylogenetic tree showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96 % to 100 %). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez *et al*., 2018; Chacón-Sánchez and Martínez-Castillo, 2017). The existing high polymorphism had indicated the huge range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes one of a series of parameters to define genetic diversity on particular species (Singh and Kulathinal, 2013). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of gene pool. According to previous study, the 15 accessions of *P. lunatus* included in current research were originated from five different gene pool, to name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti and Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The ecological condition was closely interconnected with agroclimatic zone which it was grown. In the origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity which was considered high had brought about an urgent implication in terms of crop improvement attempt, to include breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not really effective as main food resource (Doria *et al*., 2012). Consequently, the process of maturation in *P. lunatus* seeds was in need of proper and acceptable process, thus it could reduce the anti-nutritional substances (Sukatiningsih *et al*., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factor. In addition, breeding procedure could be designed that way so as to yield the generation of *P. lunatus* with highly pest-resistant performance and shorter period of maturation phase.

Next, with reference to the phylogenetic tree that was formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width and pod width in comparison to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti and Fauzi, 2019).

Despite the fact, the result of cluster analysis also indicated the existence of accessions of which pod lengths were quite different, but still grouped in the same cluster. In contrast, when the pod length was not significantly different, the result of cluster analysis classified those accessions in different cluster. This was probably due to DNA related to RAPD marker used in this current research was unrelated to the character setting. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, the use of RAPD was not only affordable, but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. In spite of it, there were some studies of genetic variation on germplasm of beans which were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al*., 2015), South Africa (Adesoye and Ojobo, 2012), Turkey (Ince and Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al*., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al*., 2018) and SNP (Chacón-Sánchez and Martínez-Castillo, 2017). In short, this current research has advocated the credibility of the use of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

4. Conclusion

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analysed. The result of RAPD utilizing ten primers resulted in 68 bands in which nine primers possessed 100 % level of polymorphism and one primary was equipped with 96 % level of polymorphism. Ten unique bands were detected in eight accessions, i.e. Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6. On the basis of cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13 and 14; while the latter comprised the accessions of 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

REFERENCES

Adesoye A and Ojobo OA. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv*., **4**(13): 453–460. https://doi.org/10.5897/IJBC11.216

AlRawashdeh IM and AlRawashdeh NQ. 2015. Evaluating the genetic relatedness within *Lupinus pilosus* L. species based on RAPD analysis. *Jordan J. Biol. Sci.* **8**(1): 61–64.

Basheer-Salimia R, Shtaya M, Awad M, Abdallah J and Hamdam Y. 2013. Genetic diversity of Palestine landraces of Faba bean (*Vicia faba*) based on RAPD markers. *Genet. Mol. Res*, **12**(3): 3314–3323. https://doi.org/10.4238/2013.September.3.8

Ben-Ari G and Lavi U. 2012. Marker-assisted selection in plant breeding. In: Chapter 11, **Plant Biotechnology and Agriculture**. Academic Press. Elsevier. USA.pp 163–184 https://doi.org/10.1016/B978-0-12-381466-1.00011-0

Bhanu AN. 2017. Assessment of genetic diversity in crop plants - An overview. *Adv. Plants Agric. Res*. **7**(3): 279–286. https://doi.org/10.15406/apar.2017.07.00255

Bukhari A, Bhat MA, Ahmad M and Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol*., **14**(6):451–458. https://doi.org/10.5897/ajb2014.14281

Carvalho YGS, Vitorino LC, de Souza UJB and Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*, **11**(4): 1–21. https://doi.org/10.3390/d11040062

Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, Nugraha GI, Gibson RS and Houghton L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLo S One,* **12**(4): 1–17. https://doi.org/10.1371/journal.pone.0175952

Diniyah N, Windarti WS and Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai [Development of food technology based on

koro-koroan as an alternative food material to substitute soybeans] Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi. UPN Veteran, Surabaya, Indonesia. [in Bahasa Indonesia]

Diniyah N, Windrati WS and Riady S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (*Phaseolus lunatus* L.) dengan perlakuan lama perendaman [The functional properties of black, red and white koro kratok flour (*Phaseolus lunatus* L.) with long soaking treatment. *Jurnal Hasil Penelitian Industri*, **28**(2):70–77. [in Bahasa Indonesia].

Doria E, Campion B, Sparvoli F, Tava A and Nielsen E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (*Phaseolus vulgaris* L . and *Phaseolus lunatus* L .) landraces cultivated in southern Italy. *J Food Compost Anal.* **26**(1–2): 72–80. https://doi.org/10.1016/j.jfca.2012.03.005

Doyle J J. and Doyle JL. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.***19**: 11–15.

Fu YB. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet*. **128**(11): 2131–2142. https://doi.org/10.1007/s00122-015-2585-y

Govindaraj M, Vetriventhan M. and Srinivasan M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. Article ID 431487.. https://doi.org/10.1155/2015/431487

Herry B, Windarti WS and Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein [The potential of koro-koroan as a source of local ingredients for the manufacture of various processed protein products] Prosiding Seminar Nasional. Universitas Muhammadiyah Jember, Jember, Indonesia. [in Bahasa Indonesia]

Huang W, Zhao X, Zhao X, Li Y and Lian J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. Ecol. Evol. **6**(22): 8256–8266. https://doi.org/10.1002/ece3.2549

Ickowitz A, Rowland D, Powell B and Salim MA. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLo S One* **11**(5): 1–15. https://doi.org/10.1371/journal.pone.0154139

Ince AG and Karaca M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics* **4**(4): 220–227.

Kalaminasih D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (*Canavalia ensiformis* L.) terhadap mutu organoleptik tempe koro [Effect of the proportion of *Phaseolus lunatus* and *Canavalia ensiformis* L.) on the organoleptic quality of tempe koro]. *E-Journal Boga,* **2**(3): 104–113. [in Bahasa Indonesia].

Kumar NS and Gurusubramanian G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis.* **11**(3): 116–124.

Kumari N and Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. *Am J Anim Vet Sci.* **9**(1): 6–13. https://doi.org/10.3844/ajavssp.2014.6.13

Lim TK. 2012. Lablab purpureus. In: **Edible Medicinal And Non-Medicinal Plants, Vol. 2, Fruits.** Springer Netherlands. pp. 730–741. https://doi.org/10.1007/978-94-007-1764-0_84

Madanijah S, Briawan D, Rimbawan R, Zulaikhah Z, Andarwulan N, Nuraida L, Sundjaya T, Murti L, Shah Priyali, Bindels J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: A cross-sectional dietary and nutrient intake study. Br J Nutr **116**: 1–10. https://doi.org/10.1017/S000711451600057X

Maftuchah and Zainuddin. 2006. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (*Jathropa curcas* L.) [Development of genomic DNA isolation methods in *Jathropa curcas* L.] . *Humanity* **2**(1): 63–69. [in Bahasa Indonesia].

Nafi A, Diniyah N. and Hastuti, FT. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro

kratok (*Phaseolus lunatus* L.) termodifikasi yang diproduksi secara fermentasi spontan [Physicochemical and technical functional characteristics of modified Phaseolus lunatus L.flour produced by spontaneous fermentation]. *Agrointek*, 9(1): 24–32. [in Bahasa Indonesia].

Praseptiangga D, Tryas A A, Affandi DR, Atmaka W, Ariyantoro AR and Minardi S. 2018. Physical and chemical characterization of composite flour from Canna flour (*Canna edulis*) and Lima bean flour (*Phaseolus lunatus*). *AIP Conf Proc*. 1927(030020): 1–6. https://doi.org/10.1063/1.5021213

Purwanti E and Fauzi A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. *IOP Conf. Ser. Earth Environ. Sci*. **276** (012017):1–10. https://doi.org/10.1088/1755-1315/276/1/012017

Serrano-serrano ML, Hernández-torres J, Castillo-villamizar G, Debouck DG and Chacón, MI. 2010. Molecular phylogenetics and evolution gene pools in wild Lima bean (*Phaseolus lunatus* L .) from the Americas: Evidences for an Andean origin and past migrations. Mol. Phylogenet. Evol. **54**(1): 76–87.

https://doi.org/10.1016/j.ympev.2009.08.028

Silva RNO, Lopes ACA, Gomes RLF, Pádua JG and Burle ML. 2019. High diversity of cultivated Lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. Genet. Mol. Res. **18**(4), gmr18441: 1–15. https://doi.org/10.4238/gmr18441

Singh RS and Kulathinal RJ. 2013. Polymorphism. In: Maloy S and Hughes K (Eds.). **Brenner's Encyclopedia of Genetics 2nd Edition.** Academic Press - Elsevier. Cambridge, Massachusetts, USA, pp. 398–399.

https://doi.org/10.1016/B978-0-12-374984-0.01189-X

Sukatiningsih, Yustian AM and Windarti SW. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet] [Addition of soy protein isolate

and toxic sucrose to the sprouts of Phaseolus lunatus (L) sweet. *Agritrop Jurnal Ilmu-Ilmu Pertanian,* **11**(1): 1–7.

Syuaib MF. 2016. Sustainable agriculture in Indonesia: Facts and challenges to keep growing in harmony with environment. *Agricultural Engineering International: CIGR Journal*, **18**(2):170–184.

Yeh, F.C., and T.J.B. Boyle. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian J. Bot,* **129**:157.

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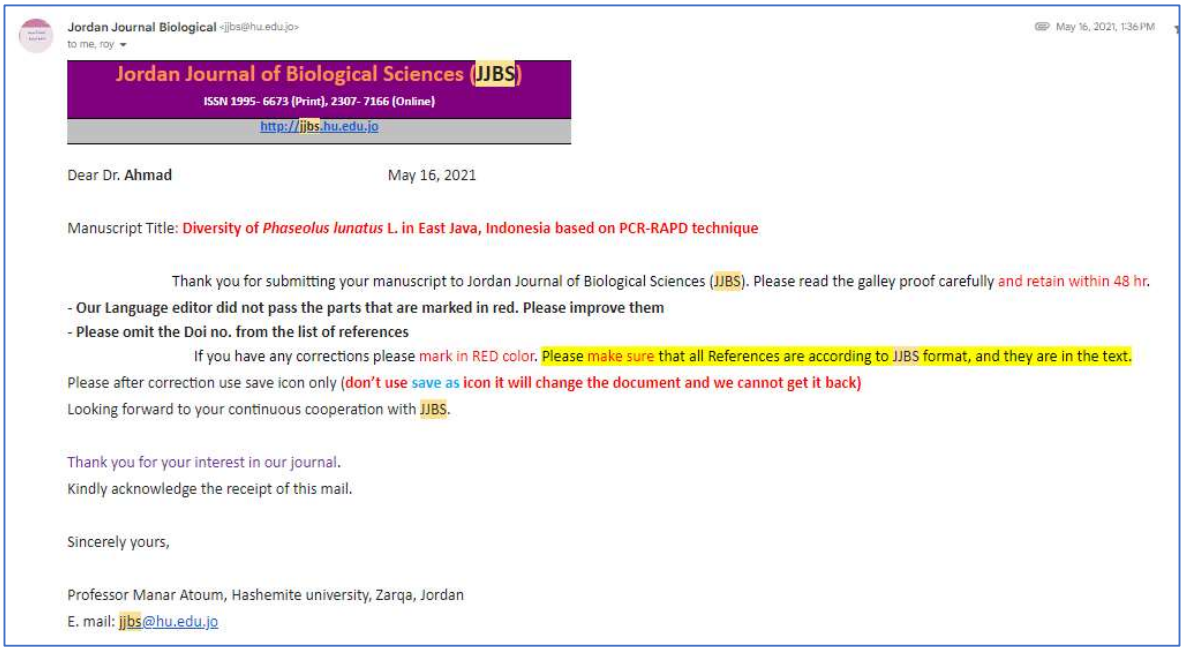

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Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on PCR-RAPD technique

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Abstract

Phaseolus lunatus L. is one of the legume plants found in some parts of Indonesia and has potential as alternative food rich in protein. This current research aimed at analysing genetic accessions of *P. lunatus* distributed in some areas in East Java, Indonesia, based on the RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) marker. 15 accessions originated from four locations were analysed. Ten primers were used and produced 68 bands out of 67 were polymorphic. The percent polymorphism was 96% to 100%. Ten unique bands were detected in eight accessions (Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6). Using the Neighbor-Joining method, a phylogenetic tree was yielded by a similarity coefficient of 64% to 100%. On the genetic similarity coefficient (GSC) of 0.6, there were two clusters: the first and second major clusters (Cluster A and B). The former contained the accessions 7, 8, 13, and 14, while the latter comprised 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region cluster in the same cluster.

Keywords: Genetic diversity; Lima bean, Phylogeny, Polymerase Chain Reaction, Random Amplified Polymorphic Deoxyribonucleic acid

1. Introduction

Phaseolus lunatus L. is categorized as a legume plant with great potential to become nutritious food. In Indonesia, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti and Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions existing in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as an alternatively functional food source (Diniyah *et al*., 2013; Diniyah *et al*., 2015; Herry *et al*., 2013; Nafi *et al*., 2015). Nowadays, Indonesia is dealing with the severe issue of protein shortage in some areas (Diana *et al*., 2017; Ickowitz *et al*., 2016; Madanijah *et al.,* 2016), so maximizing consumer consumption *P. lunatus* will be an effective solution.

With respect to elevating *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content needs actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the programs of food source enhancement (Bhanu, 2017). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a betterment scheme for the species since the beginning of systematic plant breeding (Bhanu, 2017). Furthermore, such information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, the research underpinning genetic diversity in a particular plant also leads to conservation (Carvalho *et al*., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using numerous techniques such as morphological identification, biochemical characterization, and analysis of molecular markers (Govindaraj *et al*., 2015). Related to those techniques, the selection of molecular markers is considered more appropriate and effective to avoid any bias due to environmental influence and provide eclectic information about genetic diversity in a more acceptable way (Fu, 2015). Some molecular markers are included and considered particularly promising in helping analyse genetic diversity, such as RAPD, RFLP, and SCAR.

Among those markers, RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) is the most popular marker in many research projects (AlRawashdeh and AlRawashdeh, 2015; Ben-Ari and Lavi, 2012). RAPD constitutes a PCR-based (Polymerase Chain Reaction) technique that involves a primary set with a relatively short size and can be a PCR-based technique that involves a relatively short size and can randomly amplify many DNA segments (Kumari and Thakur, 2014). This technique is equipped with outstanding excellence compared to others, which occupies a universal primary set without undergoing the DNA sequencing phase in its actual implementation. In addition, the RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar and Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari and Thakur, 2014), and is applicable for various laboratory situations (Kumar and Gurusubramanian, 2011). Therefore, RAPD has often been

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used as a genetic marker in much research on the genetic variation of various legumes.

However, analyses of the genetic diversity of *P. lunatus* in Indonesia are still rare. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in North America (Serrano-serrano *et al.,* 2010), Central America (Camacho-Pérez *et al.,* 2018), and South America (Silva *et al.,* 2019). On the one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as an alternative food source (Diniyah *et al*., 2013; Herry *et al*., 2014) alongside its essential substances (Diniyah *et al*., 2015; Praseptiangga *et al.*, 2018; Sukatiningsih *et al*., 2013; Tejasari, 2016). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti and Fauzi, 2019). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on the RAPD marker.

2. Material and Methods

2.1. Collection of Samples

P. lunatus used in this present study was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. Based on the result of identification in previous research (Purwanti and Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Further, each of the accessions was planted in a polybag in which one polybag was distanced one meter long from the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agents.

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

2.2. DNA Isolation

DNA isolation was administered based on the CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue stemmed from the leaf organ of 3 mo (month-old) plants. First, leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into the buffer. The results of isolated DNA were stored under a temperature of -20 ºC.

Table 2. List of primers

Primers	Sequence 5'-3'	GC Content (%)
OPA6	GGTCCCTGAC	70
OPA8	GTGACGTAGG	60
OPA10	GTGATCGCAG	60
OPA20	GTTGCGATCC	60
OPC19	GTTGCCAGCC	70
OPD8	GTGTGCCCCA	70
OPD12	CACCGTATCC	60
OPE8	TCACCACGGT	60
OPE15	ACGCACAACC	60
OPE16	GGTGACTGTG	60

2.3. PCR-RAPD

Ten primers having 60 to 70 GC content were used in this present study (Table 2). The total volume of PCR reaction used signified 22.5 µL, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCl; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and 30 ng DNA template. The condition for PCR reaction was designed under the pre-denaturation temperature of 94 °C (in 5 min), denaturation temperature of 94 °C (in 1 min), primary attachment temperature of 36 °C (in 1 min), extension temperature of 72 °C (in 2 min), the post-extension temperature of 72 °C (in 5 min), and post PCR reaction temperature of 4 °C (in 2 min). For multiplication, the cycle of the PCR reaction was repeated 36 times.

2.4. Agarose Gel Electrophoresis

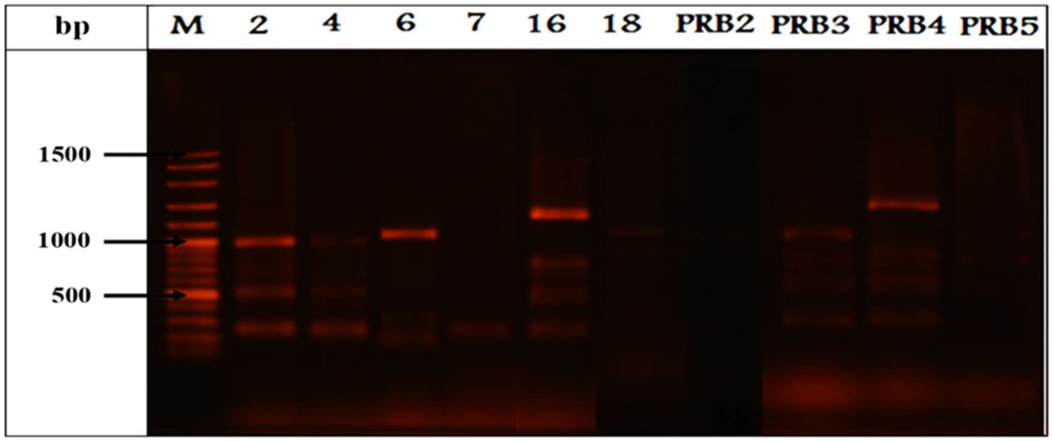
There were three stages of procedure to confirm the result of isolation process and PCR reaction after implementation. The first stage was creating agarose gel with a concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labelled electrophoresis with the electrophoresis buffer of TBE (1×), loading dye (6×) under the condition of 60 V, 400 mA within 45 min. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

2.5. Analysis on the DNA Bands Yielded from RAPD

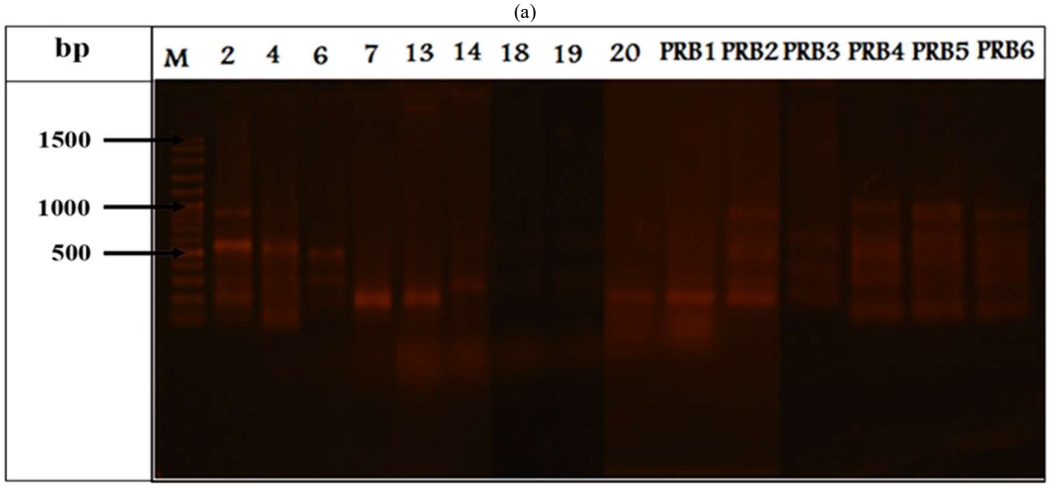
Data analysis was performed by observing the pattern of visible bands from the electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data (0 and 1), indicating the existence and inexistence of bands typifying specific sizes. Afterward, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and create a phylogenetic tree by creating a phylogenetic tree with Popgen software version 3.1 (Yeh and Boyle, 1997).

3. Results and Discussion

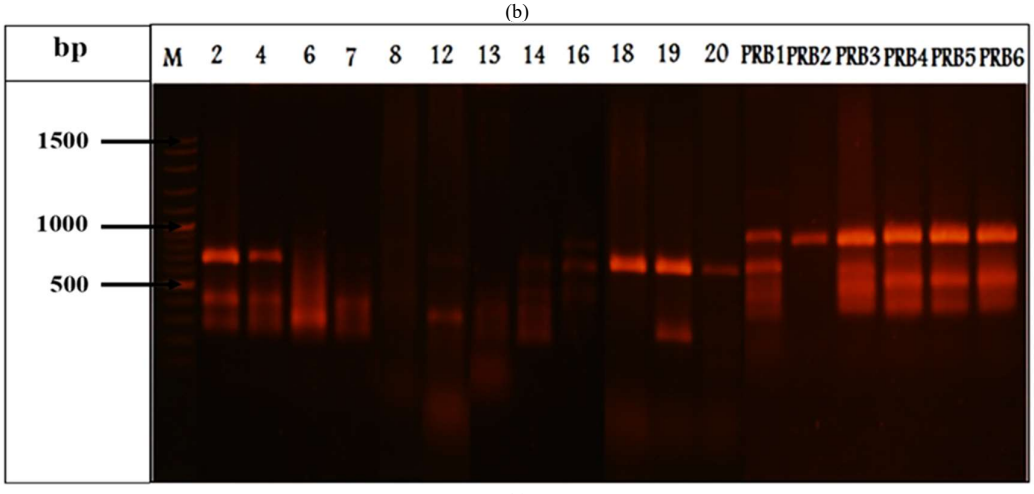
In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, as a result of PCR–RAPD amplification at those genotypes (Figure 1), the bands were assessed based on the binary data, with the description of 1 for the amplified band and 0 for unamplified. The following Table 3 showed the record of the number of loci found.



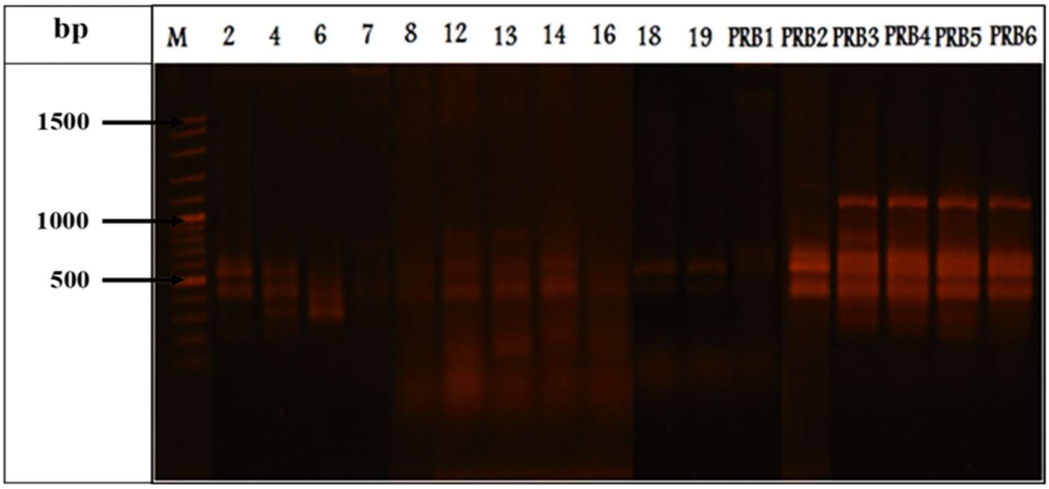
(a)



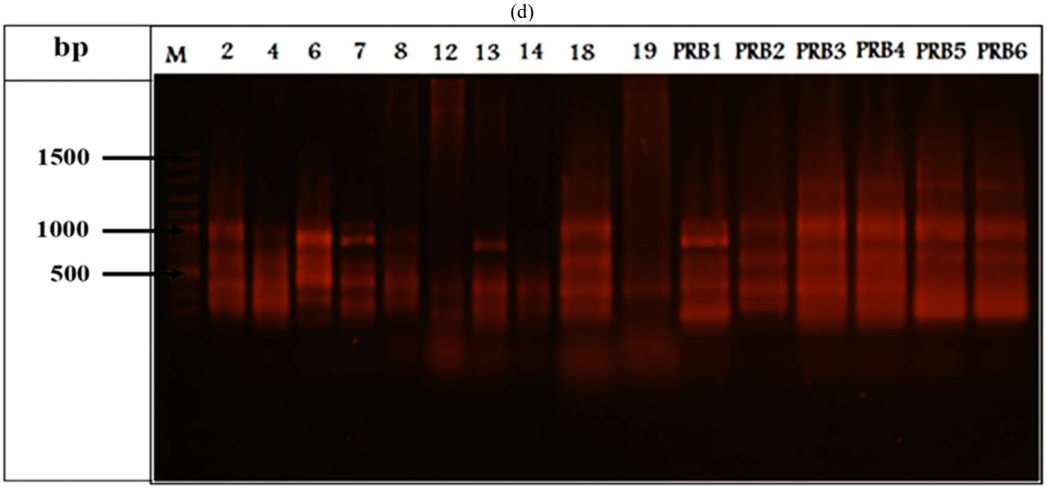
(b)



(c)


bp
M 2 4 6 7 8 12 13 14 16 18 19 PRB1 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(d)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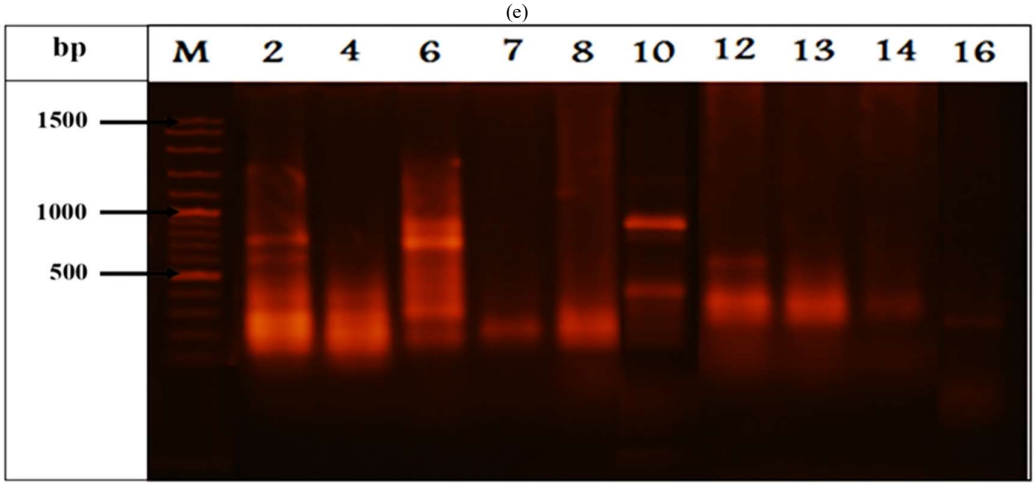
1500
1000
500

(e)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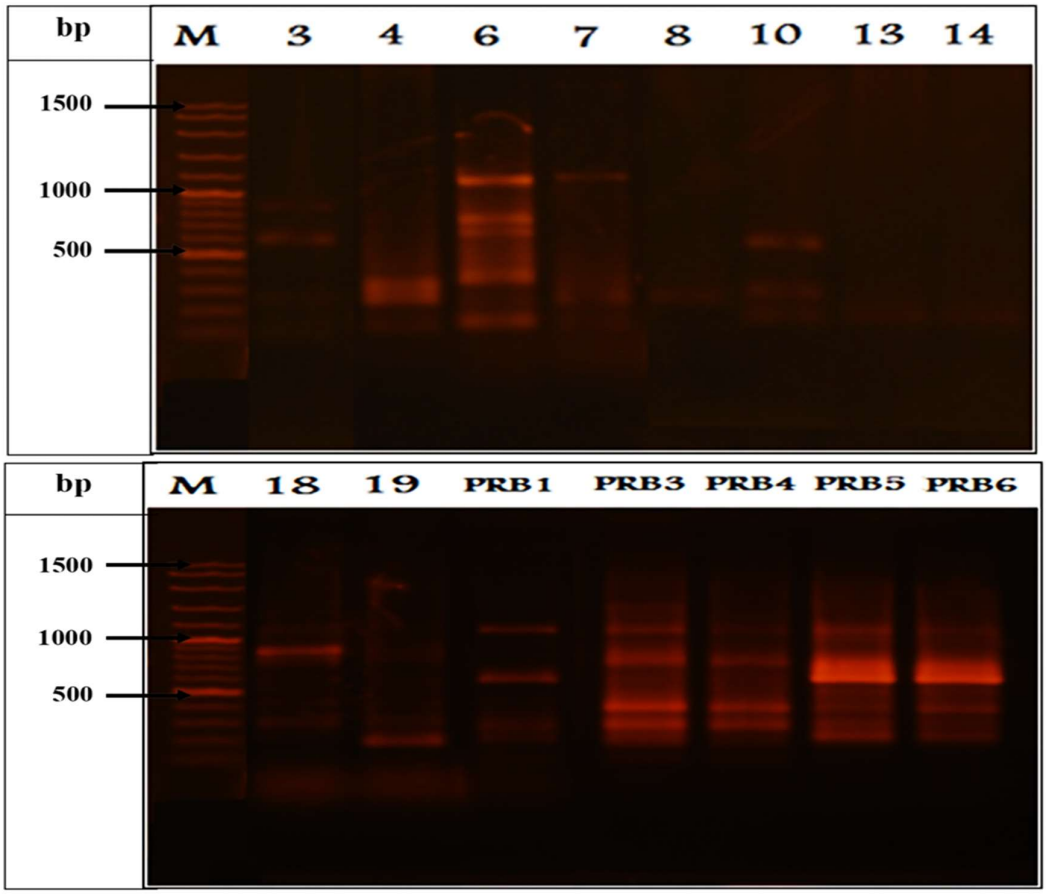
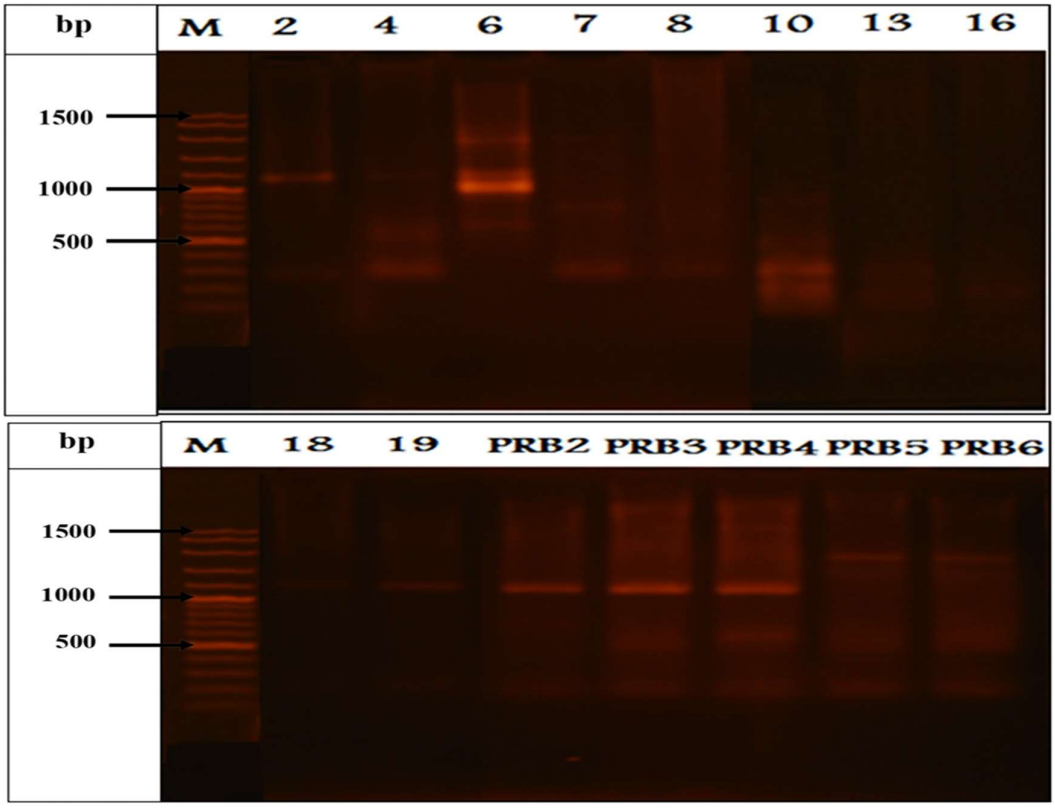
1500
1000
500

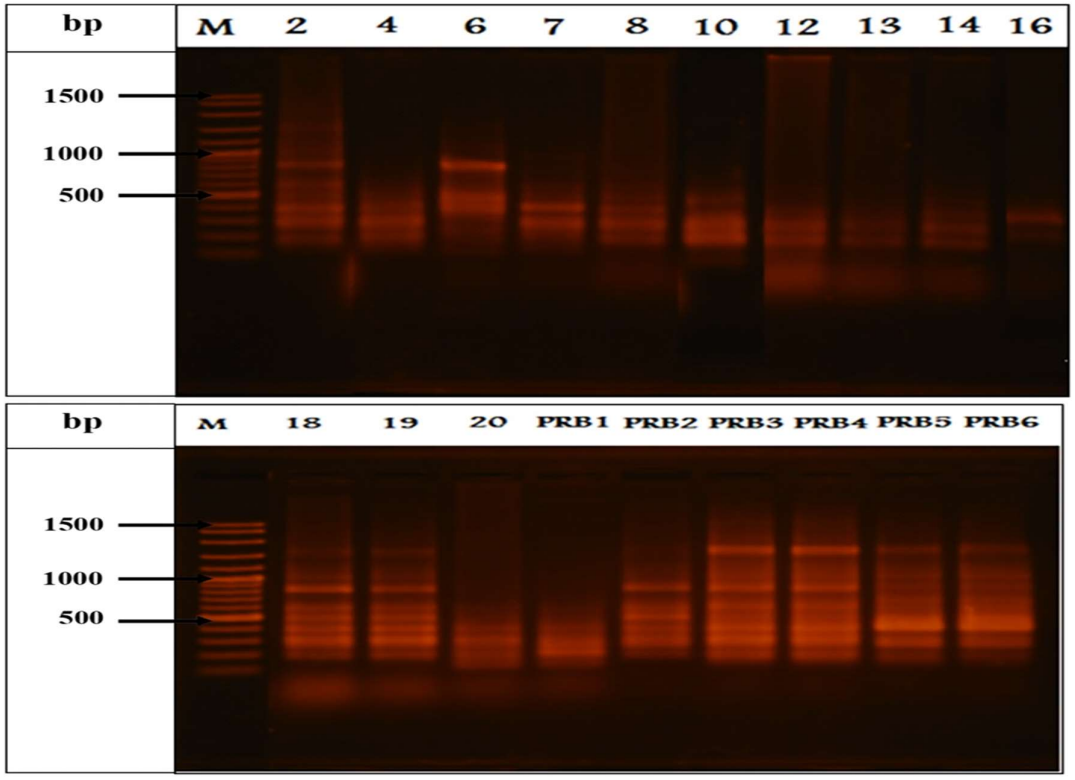
(f)


bp
M 3 4 6 7 8 10 13 14
1500
1000
500
bp
M 18 19 PRB1 PRB3 PRB4 PRB5 PRB6
1500
1000
500

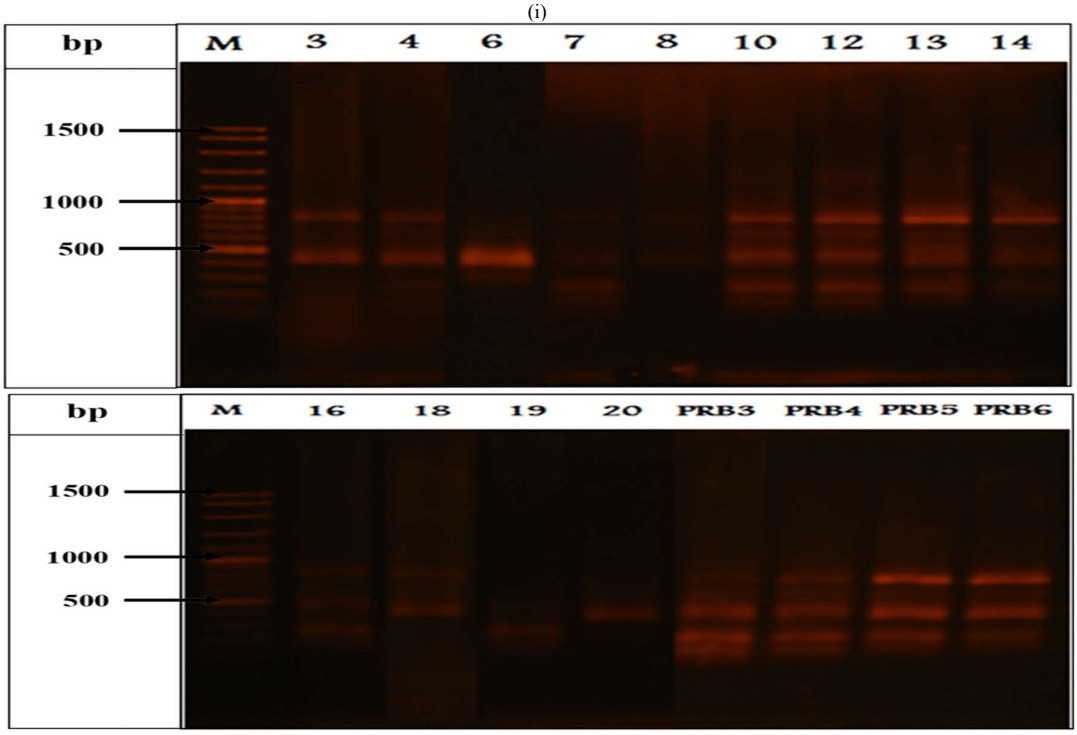
(g)


bp
M 2 4 6 7 8 10 13 16
1500
1000
500
bp
M 18 19 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(h)



(i)



(j)

Figure 1. Gel electrophoresis profiles for PCR–RAPD results using (a) OPA6, (b) OPA8, (c) OPA10, (d) OPA20, (e) OPA19, (f) OPD8, (g) OPD12, (h) OPE8, (i) OPE15, (j) OPE16. M is the DNA size ladder

Table 3. The record of the number of locus found at 15 genotypes of *P. lunatus* using 10 random primary

Codes	Locus (bp)												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description: (1): DNA band was existent, (0): DNA band was non-existent.

After PCR was conducted, DNA fragments of diverse sizes (polymorphic) were produced (Table 4). A total of 68 amplified bands were obtained, out of 67 were polymorphic. The percent polymorphism was 96% to 100%. The total number of amplified bands varied between 5 (OPA10 and OPA20) to 9 (OPA12), with an average of 6.8 bands per primer. Ten unique bands were detected in several accessions. The size of the unique band ranged from 200 (Mdr6) to 1 300 bp (Prb5).

Table 4. RAPD primers used for diversity analysis of *P. lunatus*.

No.	Markers (100 bp–2000 bp)	∑ Band	Polymorphic	% Polymorphic	Unique band		
					Total	Locus	Accession
1	OPA 6	8	8	100%	2	700	Mdr16
						200	Mdr6
2	OPA 8	7	7	100%	0	-	-
3	OPA 10	5	5	100%	0	-	-
4	OPA 20	5	5	100%	1	300	Mdr4
5	OPC 19	6	5	96%	0	-	-
6	OPD 8	8	8	100%	1	500	Prb1
7	OPD 12	9	9	100%	2	1 200	Prb3
						900	Mdr19
8	OPE 8	7	7	100%	2	1 300	Prb5
						500	Mdr4
9	OPE 15	7	7	100%	0	-	-
10	OPE 16	6	6	100%	2	700	Mdr12
						500	Mdr16
	Total	68	67		10		

The cluster analysis upon the 68 RAPD bands was administered. The phylogenetic tree using Neighbor-Joining method was produced, equipped with similarity coefficient that ranged from 64% to 100%, or there was genetic variation with the range of 0% to 36% (Figure 2). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close.

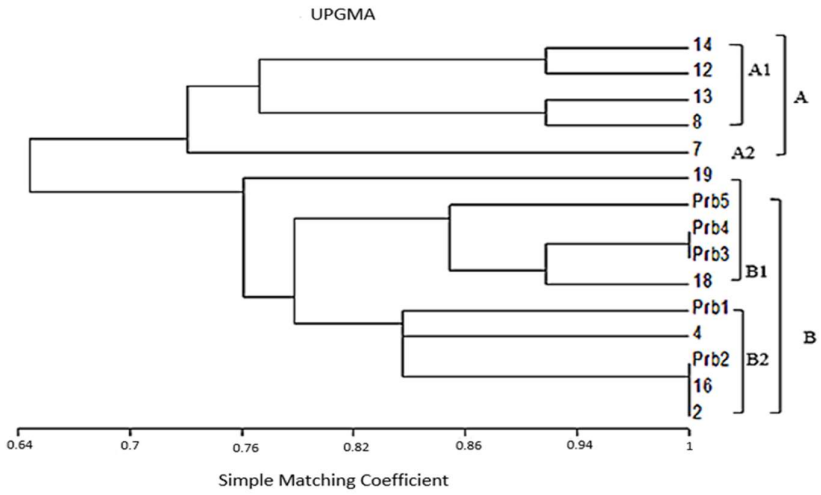


Figure 2. The dendrogram showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96% to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez *et al*., 2018; Chacón-Sánchez and Martínez-Castillo, 2017). The existing high polymorphism had indicated the vast range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes a series of parameters to define genetic diversity on particular species (Singh and Kulathinal, 2013). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of the gene pool. According to the previous study, the 15 accessions of *P. lunatus* included in the current research were originated from five different gene pools, name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti and Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The ecological condition was closely interconnected with the agro-climatic zone in which it was grown. The origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity considered high had brought about an urgent implication in terms of crop improvement attempt, including the breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not effective as the main food resource (Doria *et al*., 2012). Consequently, the process of maturation in *P. lunatus* seeds needed proper and acceptable process; thus it could reduce the anti-nutritional substances (Sukatiningsih *et al*., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factors. In addition, the breeding procedure could be designed that way to yield the generation of *P. lunatus* with highly pest-resistant performance and a shorter period of maturation phase.

Next, concerning the phylogenetic tree formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width, and pod width compared to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti and Fauzi, 2019).

Despite this fact, cluster analysis also indicated the existence of accessions of which pod lengths were quite different but still grouped in the same cluster. In contrast, when the pod length was not significantly different, cluster analysis classified those accessions in different clusters. This was probably due to DNA related to the RAPD marker used in this current research being unrelated to the character set. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, the use of RAPD was not only affordable but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. However, there were some studies of genetic variation on germplasm of beans that were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al*., 2015), South Africa (Adesoye and Ojobo, 2012), Turkey (Ince and Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al*., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al*., 2018) and SNP (Chacón-Sánchez and Martínez-Castillo, 2017). In

short, this current research has advocated the credibility of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

4. Conclusion

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analysed. The result of RAPD using ten primers resulted in 68 bands in which nine primers possessed 100% level of polymorphism, and one primary was equipped with 96% level of polymorphism. In addition, ten unique bands were detected in eight accessions, i.e. Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6. Based on cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13, and 14, while the latter comprised the 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

References

Adesoye 0A and Ojobo OA. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv*., **4** (13): 453–460. https://doi.org/10.5897/IJBC11.216

AlRawashdeh IM and AlRawashdeh NQ. 2015. Evaluating the genetic relatedness within *Lupinus pilosus* L. species based on RAPD analysis. *Jordan J. Biol. Sci.* 8 (1): 61–64.

Basheer-Salimia R, Shtaya M, Awad M, Abdallah J and Hamdam Y. 2013. Genetic diversity of Palestine landraces of Faba bean (*Vicia faba*) based on RAPD markers. *Genet. Mol. Res*, **12** (3): 3314–3323. https://doi.org/10.4238/2013.September.3.8

Ben-Ari G and Lavi U. 2012. Marker-assisted selection in plant breeding. In: Chapter 11, **Plant Biotechnology and Agriculture**. Academic Press. Elsevier. USA.pp 163–184 https://doi.org/10.1016/B978-0-12-381466-1.00011-0

Bhanu AN. 2017. Assessment of genetic diversity in crop plants - An overview. *Adv. Plants Agric. Res.* **7** (3): 279–286. https://doi.org/10.15406/apar.2017.07.00255

Bukhari A, Bhat MA, Ahmad M and Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol.*, **14** (6):451–458. https://doi.org/10.5897/ajb2014.14281

Carvalho YGS, Vitorino LC, de Souza UJB and Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*, **11** (4): 1–21. https://doi.org/10.3390/d11040062

Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, Nugraha GI, Gibson RS and Houghton L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLo S One,* **12** (4): 1–17. https://doi.org/10.1371/journal.pone.0175952

Diniyah N, Windarti WS and Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai [Development of food technology based on koro-koroan as an alternative food material to substitute soybeans] Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi. UPN Veteran, Surabaya, Indonesia. [in Bahasa Indonesia]

Diniyah N, Windrati WS and Riady S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (*Phaseolus lunatus* L.) dengan perlakuan lama perendaman [The functional properties of black, red and white koro kratok flour (*Phaseolus lunatus* L.) with long soaking treatment. *Jurnal Hasil Penelitian Industri*, **28** (2):70–77. [in Bahasa Indonesia].

Doria E, Campion B, Sparvoli F, Tava A and Nielsen E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (*Phaseolus vulgaris* L. and *Phaseolus lunatus* L.) landraces cultivated in southern Italy. *J Food Compost Anal.* **26** (1–2): 72–80. https://doi.org/10.1016/j.jfca.2012.03.005

Doyle J J. and Doyle JL. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.***19**: 11–15.

Fu YB. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet*. **128** (11): 2131–2142. https://doi.org/10.1007/s00122-015-2585-y

Govindaraj M, Vetriventhan M. and Srinivasan M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. Article ID 431487.. https://doi.org/10.1155/2015/431487

Herry B, Windarti WS and Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein [The potential of koro-koroan as a source of local ingredients for the manufacture of various processed protein products] Prosiding Seminar Nasional. Universitas Muhammadiyah Jember, Jember, Indonesia. [in Bahasa Indonesia]

Huang W, Zhao X, Zhao X, Li Y and Lian J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. Ecol. Evol. **6** (22): 8256–8266. https://doi.org/10.1002/ece3.2549

Ickowitz A, Rowland D, Powell B and Salim MA. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLo S One* **11** (5): 1–15. https://doi.org/10.1371/journal.pone.0154139

Ince AG and Karaca M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics* **4** (4): 220–227.

Kalaminasih D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (*Canavalia ensiformis* L.) terhadap mutu organoleptik tempe koro [Effect of the proportion of *Phaseolus lunatus* and *Canavalia ensiformis* L.) on the organoleptic quality of tempe koro]. *E-Journal Boga,* **2** (3): 104–113. [in Bahasa Indonesia].

Kumar NS and Gurusubramanian G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis.* **11** (3): 116–124.

Kumari N and Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. *Am J Anim Vet Sci.* **9** (1): 6–13. https://doi.org/10.3844/ajavssp.2014.6.13

Lim TK. 2012. Lablab purpureus. In: **Edible Medicinal And Non-Medicinal Plants, Vol. 2, Fruits.** Springer Netherlands. pp. 730–741. https://doi.org/10.1007/978-94-007-1764-0_84

Madanijah S, Briawan D, Rimbawan R, Zulaikhah Z, Andarwulan N, Nuraida L, Sundjaya T, Murti L, Shah Priyali, Bindels J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: A cross-sectional dietary and nutrient intake study. Br J Nutr **116**: 1–10. https://doi.org/10.1017/S000711451600057X

Maftuchah and Zainuddin. 2006. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (*Jathropa curcas* L.) [Development of genomic DNA isolation methods in *Jathropa curcas* L.]. *Humanity* **2** (1): 63–69. [in Bahasa Indonesia].

Nafi A, Diniyah N. and Hastuti, FT. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (*Phaseolus lunatus* L.) termodifikasi yang diproduksi secara fermentasi spontan [Physicochemical and technical functional characteristics of modified Phaseolus lunatus L. flour produced by spontaneous fermentation]. *Agrointek*, 9 (1): 24–32. [in Bahasa Indonesia].

Praseptiangga D, Tryas A A, Affandi DR, Atmaka W, Ariyantoro AR and Minardi S. 2018. Physical and chemical characterization of composite flour from Canna flour (*Canna edulis*) and Lima bean flour (*Phaseolus lunatus*). *AIP Conf Proc.* 1927 (030020): 1–6. https://doi.org/10.1063/1.5021213

Purwanti E and Fauzi A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. *IOP Conf. Ser. Earth Environ. Sci*. **276** (012017):1–10 https://doi.org/10.1088/1755-1315/276/1/012017

Serrano-serrano ML, Hernández-torres J, Castillo-villamizar G, Debouck DG and Chacón, MI. 2010. Molecular phylogenetics and evolution gene pools in wild Lima bean (*Phaseolus lunatus* L.) from the Americas: Evidences for an Andean origin and past migrations. Mol. Phylogenet. Evol. **54** (1): 76–87. https://doi.org/10.1016/j.ympev.2009.08.028

Silva RNO, Lopes ACA, Gomes RLF, Pádua JG and Burle ML. 2019. High diversity of cultivated Lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. Genet. Mol. Res. **18** (4). gmr18441: 1–15. https://doi.org/10.4238/gmr18441

Singh RS and Kulathinal RJ. 2013. Polymorphism. In: Maloy S and Hughes K (Eds.). **Brenner's Encyclopedia of Genetics 2nd Edition.** Academic Press - Elsevier. Cambridge, Massachusetts, USA, pp. 398–399. https://doi.org/10.1016/B978-0-12-374984-0.01189-X

Sukatiningsih, Yustian AM and Windarti SW. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet] [Addition of soy protein isolate and toxic sucrose to the sprouts of Phaseolus lunatus (L) sweet. *Agritrop Jurnal Ilmu-Ilmu Pertanian,* **11** (1): 1–7.

Syuaib MF. 2016. Sustainable agriculture in Indonesia: Facts and challenges to keep growing in harmony with environment. *Agricultural Engineering International: CIGR Journal*, **18** (2):170–184.

Yeh, F.C., and T.J.B. Boyle. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian J. Bot,* **129**:157–16

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Section	Notes	Changes
Authors name	The fourth author's name is written twice	Maftuchah Maftuchah becomes Maftuchah
Authors affiliation	Our university name needs to be changed in Indonesian	University of Muhammadiyah Malang becomes Universitas Muhammadiyah Malang
Abstract	None	None
Introduction	None	None
Methodology	None	None
Results	None	None
Discussion and Conclusion	None	None
References	The Editor said “Please omit the Doi no. from the list of references”	We've removed DOIs from every reference

Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on PCR-RAPD technique

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Abstract

Phaseolus lunatus L. is one of the legume plants found in some parts of Indonesia and has potential as alternative food rich in protein. This current research aimed at analysing genetic accessions of *P. lunatus* distributed in some areas in East Java, Indonesia, based on the RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) marker. 15 accessions originated from four locations were analysed. Ten primers were used and produced 68 bands out of 67 were polymorphic. The percent polymorphism was 96% to 100%. Ten unique bands were detected in eight accessions (Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6). Using the Neighbor-Joining method, a phylogenetic tree was yielded by a similarity coefficient of 64% to 100%. On the genetic similarity coefficient (GSC) of 0.6, there were two clusters: the first and second major clusters (Cluster A and B). The former contained the accessions 7, 8, 13, and 14, while the latter comprised 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region cluster in the same cluster.

Keywords: Genetic diversity; Lima bean, Phylogeny, Polymerase Chain Reaction, Random Amplified Polymorphic Deoxyribonucleic acid

5. Introduction

Phaseolus lunatus L. is categorized as a legume plant with great potential to become nutritious food. In Indonesia, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti and Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions existing in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as an alternatively functional food source (Diniyah *et al*., 2013; Diniyah *et al*., 2015; Herry *et al*., 2013; Nafi *et al*., 2015). Nowadays, Indonesia is dealing with the severe issue of protein shortage in some areas (Diana *et al*., 2017; Ickowitz *et al*., 2016; Madanijah *et al.,* 2016), so maximizing consumer consumption *P. lunatus* will be an effective solution.

With respect to elevating *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content needs actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the programs of food source enhancement (Bhanu, 2017). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a betterment scheme for the species since the beginning of systematic plant breeding (Bhanu, 2017). Furthermore, such information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, the research underpinning genetic diversity in a particular plant also leads to conservation (Carvalho *et al*., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using numerous techniques such as morphological identification, biochemical characterization, and analysis of molecular markers (Govindaraj *et al*., 2015). Related to those techniques, the selection of molecular markers is considered more appropriate and effective to avoid any bias due to environmental influence and provide eclectic information about genetic diversity in a more acceptable way (Fu, 2015). Some molecular markers are included and considered particularly promising in helping analyse genetic diversity, such as RAPD, RFLP, and SCAR.

Among those markers, RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) is the most popular marker in many research projects (AlRawashdeh and AlRawashdeh, 2015; Ben-Ari and Lavi, 2012). RAPD constitutes a PCR-based (Polymerase Chain Reaction) technique that involves a primary set with a relatively short size and can be a PCR-based technique that involves a relatively short size and can randomly amplify many DNA segments (Kumari and Thakur, 2014). This technique is equipped with outstanding excellence compared to others, which occupies a universal primary set without undergoing the DNA sequencing phase in its actual implementation. In addition, the RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar and Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari and Thakur, 2014), and is applicable for various laboratory situations (Kumar and Gurusubramanian, 2011). Therefore, RAPD has often been

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used as a genetic marker in much research on the genetic variation of various legumes.

However, analyses of the genetic diversity of *P. lunatus* in Indonesia are still rare. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in North America (Serrano-serrano *et al.,* 2010), Central America (Camacho-Pérez *et al.,* 2018), and South America (Silva *et al.,* 2019). On the one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as an alternative food source (Diniyah *et al*., 2013; Herry *et al*., 2014) alongside its essential substances (Diniyah *et al*., 2015; Praseptiangga *et al.*, 2018; Sukatiningsih *et al*., 2013; Tejasari, 2016). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti and Fauzi, 2019). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on the RAPD marker.

6. Material and Methods

6.1. Collection of Samples

P. lunatus used in this present study was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. Based on the result of identification in previous research (Purwanti and Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Further, each of the accessions was planted in a polybag in which one polybag was distanced one meter long from the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agents.

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

6.2. DNA Isolation

DNA isolation was administered based on the CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue stemmed from the leaf organ of 3 mo (month-old) plants. First, leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into the buffer. The results of isolated DNA were stored under a temperature of -20 ºC.

Table 2. List of primers

Primers	Sequence 5'-3'	GC Content (%)
OPA6	GGTCCCTGAC	70
OPA8	GTGACGTAGG	60
OPA10	GTGATCGCAG	60
OPA20	GTTGCGATCC	60
OPC19	GTTGCCAGCC	70
OPD8	GTGTGCCCCA	70
OPD12	CACCGTATCC	60
OPE8	TCACCACGGT	60
OPE15	ACGCACAACC	60
OPE16	GGTGACTGTG	60

6.3. PCR-RAPD

Ten primers having 60 to 70 GC content were used in this present study (Table 2). The total volume of PCR reaction used signified 22.5 µL, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCl; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and 30 ng DNA template. The condition for PCR reaction was designed under the pre-denaturation temperature of 94 °C (in 5 min), denaturation temperature of 94 °C (in 1 min), primary attachment temperature of 36 °C (in 1 min), extension temperature of 72 °C (in 2 min), the post-extension temperature of 72 °C (in 5 min), and post PCR reaction temperature of 4 °C (in 2 min). For multiplication, the cycle of the PCR reaction was repeated 36 times.

6.4. Agarose Gel Electrophoresis

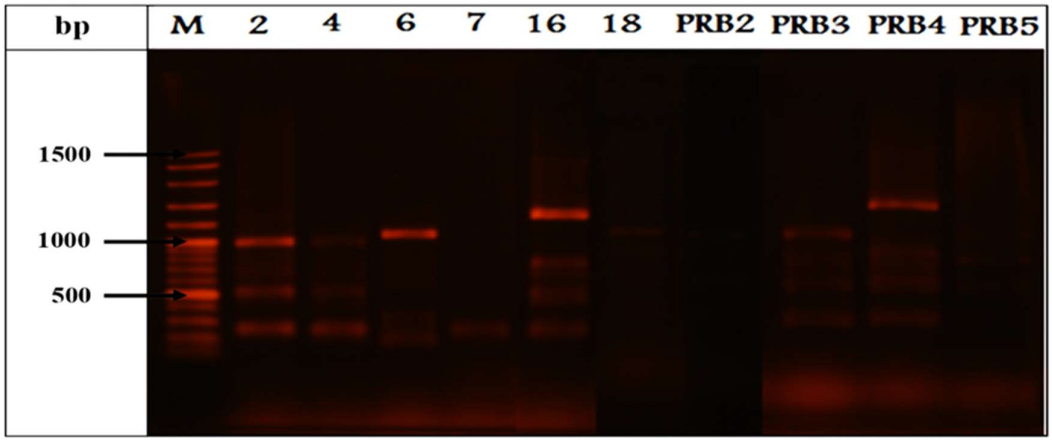
There were three stages of procedure to confirm the result of isolation process and PCR reaction after implementation. The first stage was creating agarose gel with a concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labelled electrophoresis with the electrophoresis buffer of TBE (1×), loading dye (6×) under the condition of 60 V, 400 mA within 45 min. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

6.5. Analysis on the DNA Bands Yielded from RAPD

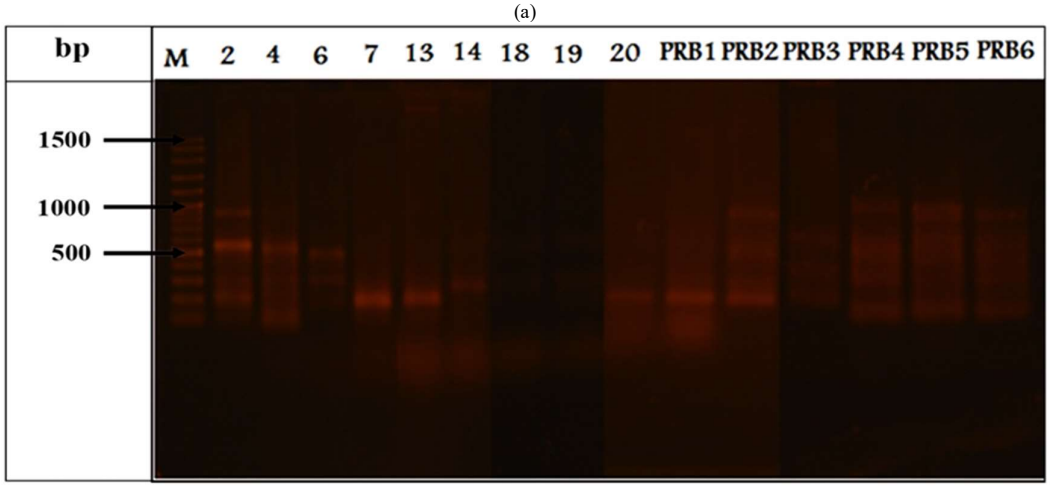
Data analysis was performed by observing the pattern of visible bands from the electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data (0 and 1), indicating the existence and inexistence of bands typifying specific sizes. Afterward, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and create a phylogenetic tree by creating a phylogenetic tree with Popgen software version 3.1 (Yeh and Boyle, 1997).

7. Results and Discussion

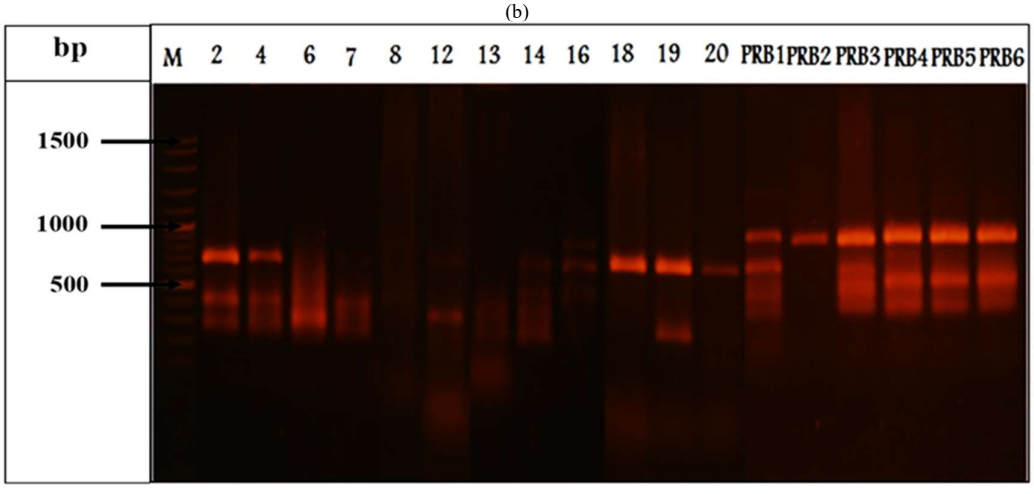
In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, as a result of PCR–RAPD amplification at those genotypes (Figure 1), the bands were assessed based on the binary data, with the description of 1 for the amplified band and 0 for unamplified. The following Table 3 showed the record of the number of loci found.



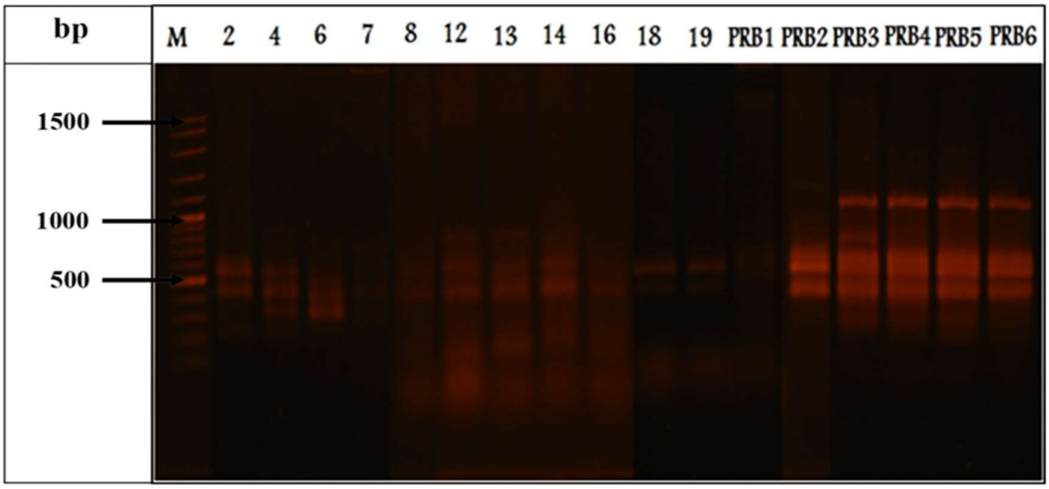
(a)



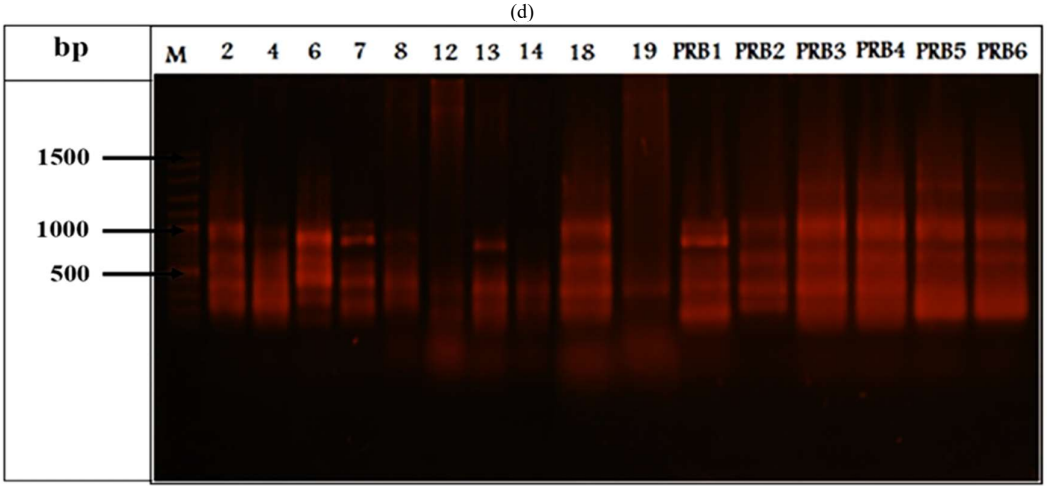
(b)



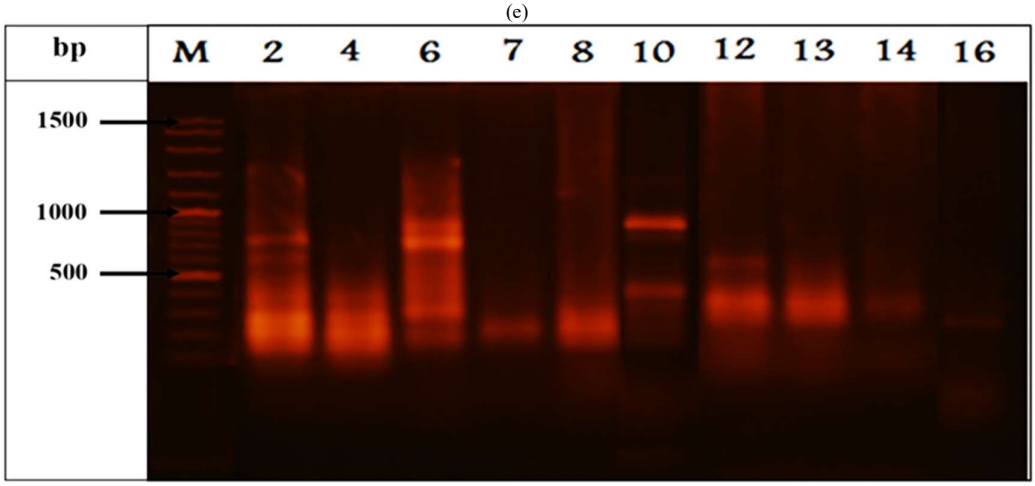
(c)


bp
M 2 4 6 7 8 12 13 14 16 18 19 PRB1 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

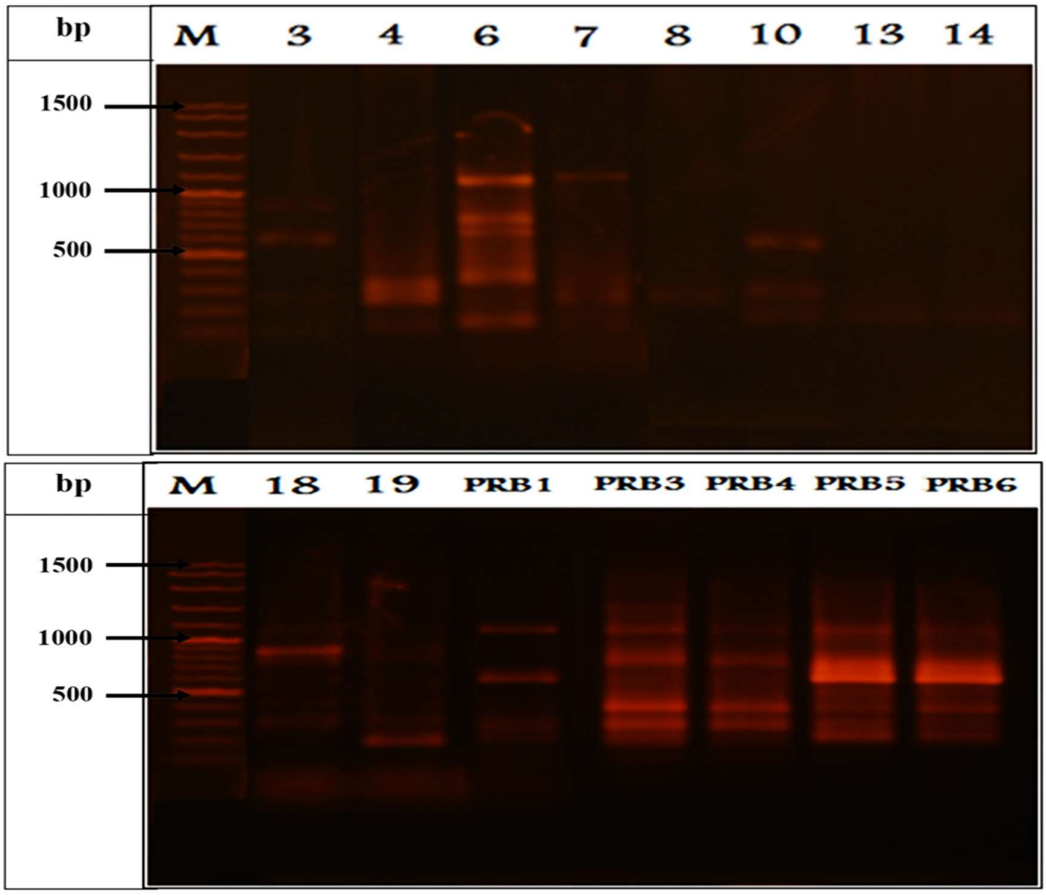
(d)


bp
M 2 4 6 7 8 12 13 14 18 19 PRB1 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

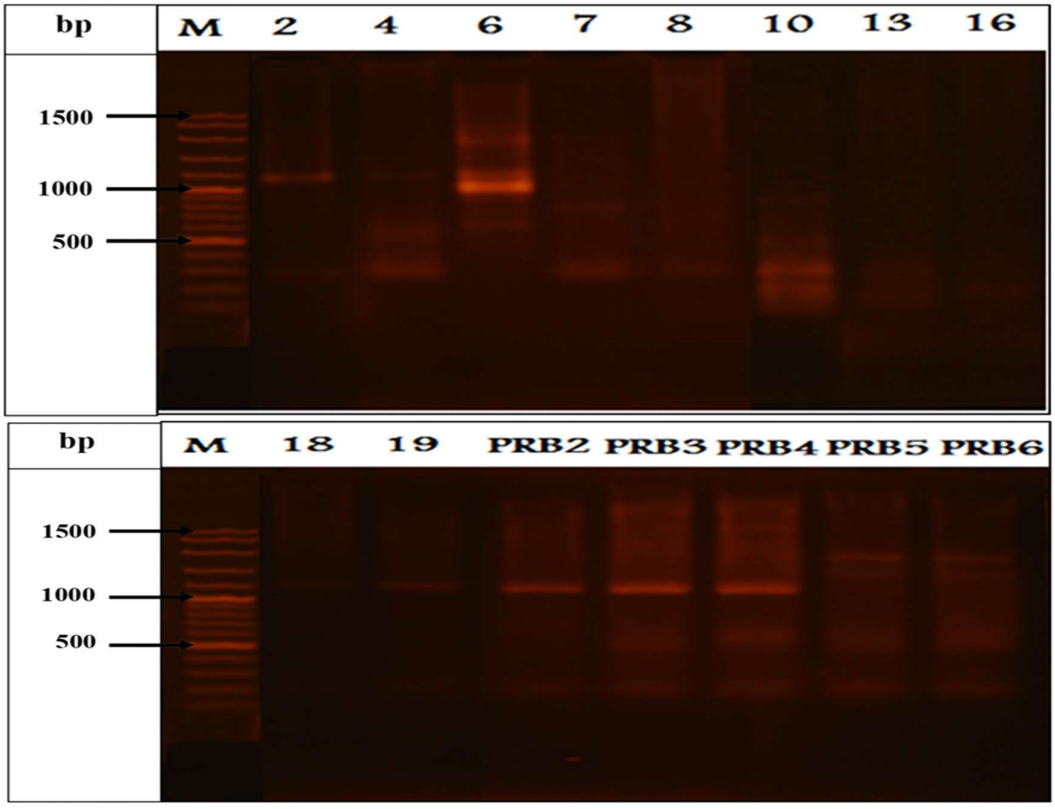
(e)


bp
M 2 4 6 7 8 10 12 13 14 16
1500
1000
500

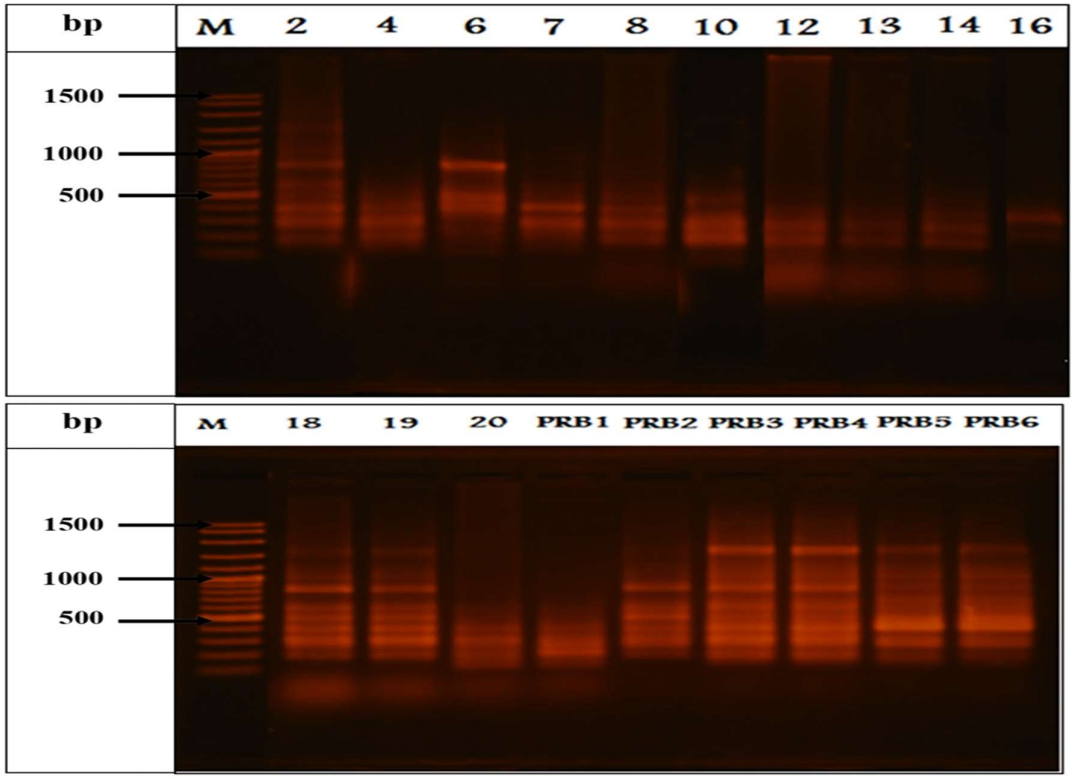
(f)


bp
M 3 4 6 7 8 10 13 14
1500
1000
500
bp
M 18 19 PRB1 PRB3 PRB4 PRB5 PRB6
1500
1000
500

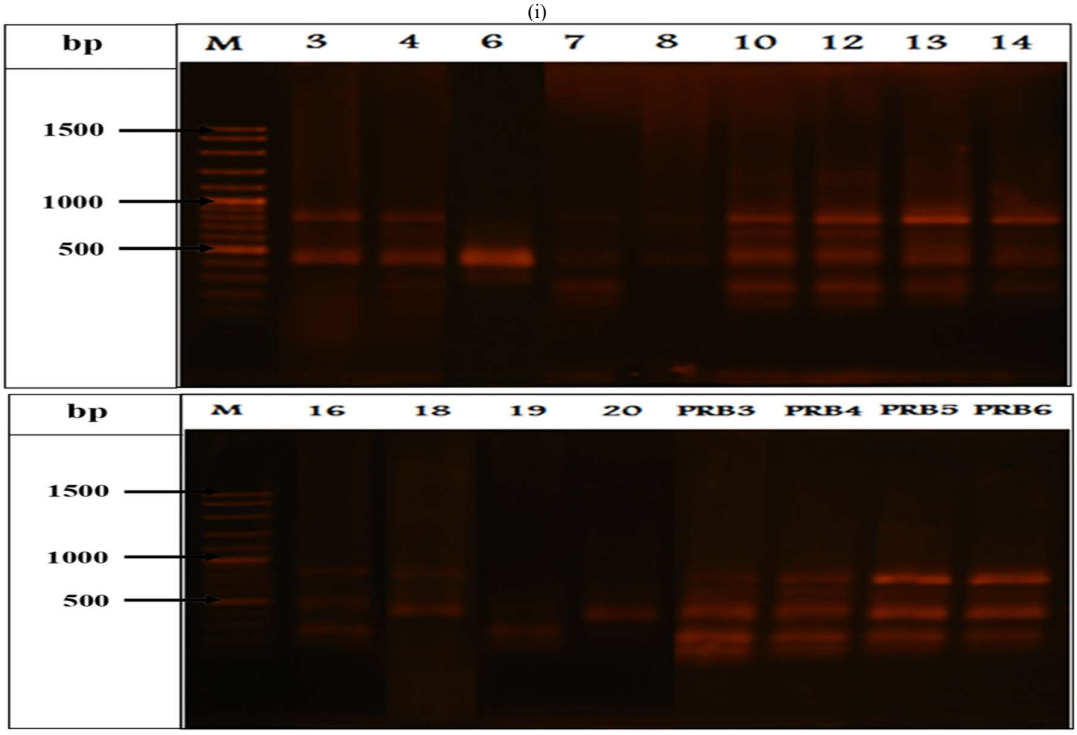
(g)


bp
M 2 4 6 7 8 10 13 16
1500
1000
500
bp
M 18 19 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(h)



(i)



(j)

Figure 1. Gel electrophoresis profiles for PCR–RAPD results using (a) OPA6, (b) OPA8, (c) OPA10, (d) OPA20, (e) OPA19, (f) OPD8, (g) OPD12, (h) OPE8, (i) OPE15, (j) OPE16. M is the DNA size ladder

Table 3. The record of the number of locus found at 15 genotypes of *P. lunatus* using 10 random primary

Codes	Locus (bp)												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description: (1): DNA band was existent, (0): DNA band was non-existent.

After PCR was conducted, DNA fragments of diverse sizes (polymorphic) were produced (Table 4). A total of 68 amplified bands were obtained, out of 67 were polymorphic. The percent polymorphism was 96% to 100%. The total number of amplified bands varied between 5 (OPA10 and OPA20) to 9 (OPA12), with an average of 6.8 bands per primer. Ten unique bands were detected in several accessions. The size of the unique band ranged from 200 (Mdr6) to 1 300 bp (Prb5).

Table 4. RAPD primers used for diversity analysis of *P. lunatus*.

No.	Markers (100 bp–2000 bp)	∑ Band	Polymorphic	% Polymorphic	Unique band		
					Total	Locus	Accession
1	OPA 6	8	8	100%	2	700	Mdr16
						200	Mdr6
2	OPA 8	7	7	100%	0	-	-
3	OPA 10	5	5	100%	0	-	-
4	OPA 20	5	5	100%	1	300	Mdr4
5	OPC 19	6	5	96%	0	-	-
6	OPD 8	8	8	100%	1	500	Prb1
7	OPD 12	9	9	100%	2	1 200	Prb3
						900	Mdr19
8	OPE 8	7	7	100%	2	1 300	Prb5
						500	Mdr4
9	OPE 15	7	7	100%	0	-	-
10	OPE 16	6	6	100%	2	700	Mdr12
						500	Mdr16
	Total	68	67		10		

The cluster analysis upon the 68 RAPD bands was administered. The phylogenetic tree using Neighbor-Joining method was produced, equipped with similarity coefficient that ranged from 64% to 100%, or there was genetic variation with the range of 0% to 36% (Figure 2). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close.

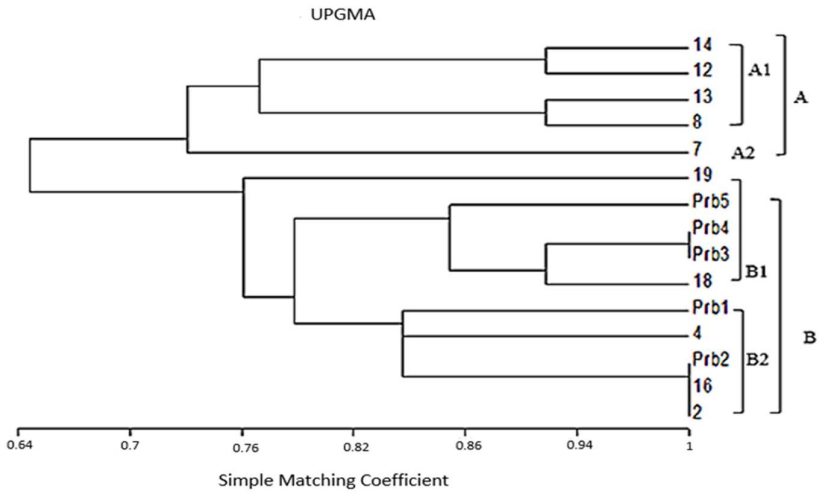


Figure 2. The dendrogram showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96% to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez *et al*., 2018; Chacón-Sánchez and Martínez-Castillo, 2017). The existing high polymorphism had indicated the vast range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes a series of parameters to define genetic diversity on particular species (Singh and Kulathinal, 2013). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of the gene pool. According to the previous study, the 15 accessions of *P. lunatus* included in the current research were originated from five different gene pools, name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti and Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The ecological condition was closely interconnected with the agro-climatic zone in which it was grown. The origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity considered high had brought about an urgent implication in terms of crop improvement attempt, including the breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not effective as the main food resource (Doria *et al*., 2012). Consequently, the process of maturation in *P. lunatus* seeds needed proper and acceptable process; thus it could reduce the anti-nutritional substances (Sukatiningsih *et al*., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factors. In addition, the breeding procedure could be designed that way to yield the generation of *P. lunatus* with highly pest-resistant performance and a shorter period of maturation phase.

Next, concerning the phylogenetic tree formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width, and pod width compared to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti and Fauzi, 2019).

Despite this fact, cluster analysis also indicated the existence of accessions of which pod lengths were quite different but still grouped in the same cluster. In contrast, when the pod length was not significantly different, cluster analysis classified those accessions in different clusters. This was probably due to DNA related to the RAPD marker used in this current research being unrelated to the character set. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, the use of RAPD was not only affordable but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. However, there were some studies of genetic variation on germplasm of beans that were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al*., 2015), South Africa (Adesoye and Ojobo, 2012), Turkey (Ince and Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al*., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al*., 2018) and SNP (Chacón-Sánchez and Martínez-Castillo, 2017). In

short, this current research has advocated the credibility of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

8. Conclusion

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analysed. The result of RAPD using ten primers resulted in 68 bands in which nine primers possessed 100% level of polymorphism, and one primary was equipped with 96% level of polymorphism. In addition, ten unique bands were detected in eight accessions, i.e. Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6. Based on cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13, and 14, while the latter comprised the 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

References

Adesoye 0A and Ojobo OA. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv*., **4** (13): 453–460.

AlRawashdeh IM and AlRawashdeh NQ. 2015. Evaluating the genetic relatedness within *Lupinus pilosus* L. species based on RAPD analysis. *Jordan J. Biol. Sci.* 8 (1): 61–64.

Basheer-Salimia R, Shtaya M, Awad M, Abdallah J and Hamdam Y. 2013. Genetic diversity of Palestine landraces of Faba bean (*Vicia faba*) based on RAPD markers. *Genet. Mol. Res*, **12** (3): 3314–3323.

Ben-Ari G and Lavi U. 2012. Marker-assisted selection in plant breeding. In: Chapter 11, **Plant Biotechnology and Agriculture**. Academic Press. Elsevier. USA.pp 163–184

Bhanu AN. 2017. Assessment of genetic diversity in crop plants - An overview. *Adv. Plants Agric. Res*. **7** (3): 279–286.

Bukhari A, Bhat MA, Ahmad M and Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol.,* **14** (6):451–458.

Carvalho YGS, Vitorino LC, de Souza UJB and Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*, **11** (4): 1–21.

Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, Nugraha GI, Gibson RS and Houghton L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLo S One,* **12** (4): 1–17.

Diniyah N, Windarti WS and Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagia bahan pangan alternatif pensubtitusi kedelai [Development of food technology based on koro-koroan as an alternative food material to substitute soybeans] Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi. UPN Veteran, Surabaya, Indonesia. [in Bahasa Indonesia]

Diniyah N, Windrati WS and Riady S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (*Phaseolus lunatus* L.) dengan perlakuan lama perendaman [The functional properties of black, red and white koro kratok flour (*Phaseolus lunatus* L.) with long soaking treatment. *Jurnal Hasil Penelitian Industri*, **28** (2):70–77. [in Bahasa Indonesia].

Doria E, Campion B, Sparvoli F, Tava A and Nielsen E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (*Phaseolus vulgaris* L. and *Phaseolus lunatus* L.) landraces cultivated in southern Italy. *J Food Compost Anal.* **26** (1–2): 72–80.

Doyle J J. and Doyle JL. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.***19**: 11–15.

Fu YB. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet*. **128** (11): 2131–2142.

Govindaraj M, Vetriventhan M. and Srinivasan M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. Article ID 431487..

Herry B, Windarti WS and Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein [The potential of koro-koroan as a source of local ingredients for the manufacture of various processed protein products] Prosiding Seminar Nasional. Universitas Muhammadiyah Jember, Jember, Indonesia. [in Bahasa Indonesia]

Huang W, Zhao X, Zhao X, Li Y and Lian J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. Ecol. Evol. **6** (22): 8256–8266.

Ickowitz A, Rowland D, Powell B and Salim MA. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLo S One* **11** (5): 1–15.

Ince AG and Karaca M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics* **4** (4): 220–227.

Kalaminasih D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (*Canavalia ensiformis* L.) terhadap mutu organoleptik tempe koro [Effect of the proportion of *Phaseolus lunatus* and *Canavalia ensiformis* L.) on the organoleptic quality of tempe koro]. *E-Journal Boga,* **2** (3): 104–113. [in Bahasa Indonesia].

Kumar NS and Gurusubramanian G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis.* **11** (3): 116–124.

Kumari N and Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. *Am J Anim Vet Sci.* **9** (1): 6–13.

Lim TK. 2012. Lablab purpureus. In: **Edible Medicinal And Non-Medicinal Plants, Vol. 2, Fruits.** Springer Netherlands. pp. 730–741.

Madanijah S, Briawan D, Rimbawan R, Zulaikhah Z, Andarwulan N, Nuraida L, Sundjaya T, Murti L, Shah Priyali, Bindels J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: A cross-sectional dietary and nutrient intake study. Br J Nutr **116**: 1–10.

Maftuchah and Zainuddin. 2006. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (*Jathropa curcas* L.) [Development of genomic DNA isolation methods in *Jathropa curcas* L.]. *Humanity* **2** (1): 63–69. [in Bahasa Indonesia].

Nafi A, Diniyah N. and Hastuti, FT. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (*Phaseolus lunatus* L.) termodifikasi yang diproduksi secara fermentasi spontan [Physicochemical and technical functional characteristics of modified Phaseolus lunatus L. flour produced by spontaneous fermentation]. *Agrointek*, 9 (1): 24–32. [in Bahasa Indonesia].

Praseptiangga D, Tryas A A, Affandi DR, Atmaka W, Ariyantoro AR and Minardi S. 2018. Physical and chemical characterization of composite flour from Canna flour (*Canna edulis*) and Lima bean flour (*Phaseolus lunatus*). *AIP Conf Proc*. 1927 (030020): 1–6.

Purwanti E and Fauzi A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. *IOP Conf. Ser. Earth Environ. Sci*. **276** (012017):1–10

Serrano-serrano ML, Hernández-torres J, Castillo-villamizar G, Debouck DG and Chacón, MI. 2010. Molecular phylogenetics and evolution gene pools in wild Lima bean (*Phaseolus lunatus* L.) from the Americas: Evidences for an Andean origin and past migrations. Mol. Phylogenet. Evol. **54** (1): 76–87.

Silva RNO, Lopes ACA, Gomes RLF, Pádua JG and Burle ML. 2019. High diversity of cultivated Lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. Genet. Mol. Res. **18** (4), gmr18441: 1–15.

Singh RS and Kulathinal RJ. 2013. Polymorphism. In: Maloy S and Hughes K (Eds.). **Brenner's Encyclopedia of Genetics 2nd Edition.** Academic Press - Elsevier. Cambridge, Massachusetts, USA, pp. 398–399.

Sukatiningsih, Yustian AM and Windarti SW. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet] [Addition of soy protein isolate and toxic sucrose to the sprouts of Phaseolus lunatus (L) sweet. *Agritrop Jurnal Ilmu-Ilmu Pertanian,* **11** (1): 1–7.

Syuaib MF. 2016. Sustainable agriculture in Indonesia: Facts and challenges to keep growing in harmony with environment. *Agricultural Engineering International: CIGR Journal*, **18** (2):170–184.

Yeh, F.C., and T.J.B. Boyle. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian J. Bot,* **129**:157–163

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Diversity of *Phaseolus lunatus* L. in East Java, Indonesia based on PCR-RAPD technique

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Abstract

Phaseolus lunatus L. is one of the legume plants found in some parts of Indonesia and has potential as alternative food rich in protein. This current research aimed at analysing genetic accessions of *P. lunatus* distributed in some areas in East Java, Indonesia, based on the RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) marker. 15 accessions originated from four locations were analysed. Ten primers were used and produced 68 bands out of 67 were polymorphic. The percent polymorphism was 96% to 100%. Ten unique bands were detected in eight accessions (Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6). Using the Neighbor-Joining method, a phylogenetic tree was yielded by a similarity coefficient of 64% to 100%. On the genetic similarity coefficient (GSC) of 0.6, there were two clusters: the first and second major clusters (Cluster A and B). The former contained the accessions 7, 8, 13, and 14, while the latter comprised 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5. In conclusion, based on phylogenetic trees formed, *P. lunatus* from the same region cluster in the same cluster.

Keywords: Genetic diversity; Lima bean, Phylogeny, Polymerase Chain Reaction, Random Amplified Polymorphic Deoxyribonucleic acid

1. Introduction

Phaseolus lunatus L. is categorized as a legume plant with great potential to become nutritious food. In Indonesia, *P. lunatus* can be found in some islands, such as Java and Madura (Purwanti and Fauzi, 2019). The distribution of *P. lunatus* in this country implies the probability of various accessions existing in Indonesia. The diversity of *P. lunatus* accessions and relative ease offered to cultivate the plant bring about probability for Indonesian societies as well as government to make use of it as an alternatively functional food source (Diniyah *et al*., 2013; Diniyah *et al*., 2015; Herry *et al*., 2013; Nafi *et al*., 2015). Nowadays, Indonesia is dealing with the severe issue of protein shortage in some areas (Diana *et al*., 2017; Ickowitz *et al*., 2016; Madanijah *et al.,* 2016), so maximizing consumer consumption *P. lunatus* will be an effective solution.

With respect to elevating *P. lunatus* as one of food sources, identification on genetic diversity typifying the accessions with high protein content needs actualization. The obtainability of information regarding genetic diversity of intra- and inter-species is the most essential foundation to run all the programs of food source enhancement (Bhanu, 2017). Also, information that pinpoints natural variability and difference that lies on the plant itself is used as the primary capital to design a betterment scheme for the species since the beginning of systematic plant breeding (Bhanu, 2017). Furthermore, such information can be used to reach a phase of sustainable crop production (Fu, 2015). Moreover, the research underpinning genetic diversity in a particular plant also leads to conservation (Carvalho *et al*., 2019). For that reason, to support the attempt, a series of assessments on genetic diversity have been routinely administered using numerous techniques such as morphological identification, biochemical characterization, and analysis of molecular markers (Govindaraj *et al*., 2015). Related to those techniques, the selection of molecular markers is considered more appropriate and effective to avoid any bias due to environmental influence and provide eclectic information about genetic diversity in a more acceptable way (Fu, 2015). Some molecular markers are included and considered particularly promising in helping analyse genetic diversity, such as RAPD, RFLP, and SCAR.

Among those markers, RAPD (Random Amplified Polymorphic Deoxyribonucleic acid) is the most popular marker in many research projects (AlRawashdeh and AlRawashdeh, 2015; Ben-Ari and Lavi, 2012). RAPD constitutes a PCR-based (Polymerase Chain Reaction) technique that involves a primary set with a relatively short size and can be a PCR-based technique that involves a relatively short size and can randomly amplify many DNA segments (Kumari and Thakur, 2014). This

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technique is equipped with outstanding excellence compared to others, which occupies a universal primary set without undergoing the DNA sequencing phase in its actual implementation. In addition, the RAPD marker is effective to demonstrate reasonable speed and is deemed more efficient (Kumar and Gurusubramanian, 2011), as it can be used for limited DNA samples, is not costly (Kumari and Thakur, 2014), and is applicable for various laboratory situations (Kumar and Gurusubramanian, 2011). Therefore, RAPD has often been used as a genetic marker in much research on the genetic variation of various legumes.

However, analyses of the genetic diversity of *P. lunatus* in Indonesia are still rare. In fact, in some parts of the world, such kinds of analyses are intensively published, such as in North America (Serrano-serrano *et al.*, 2010), Central America (Camacho-Pérez *et al.*, 2018), and South America (Silva *et al.*, 2019). On the one hand, in Indonesia, research about *P. lunatus* was still limited on its potency as an alternative food source (Diniyah *et al*., 2013; Herry *et al*., 2014) alongside its essential substances (Diniyah *et al*., 2015; Praseptiangga *et al*., 2018; Sukatiningsih *et al*., 2013; Tejasari, 2016). In addition, researches that study the diversity of *P. lunatus* are still restricted to its morphological characteristics (Purwanti and Fauzi, 2019). Therefore, this research is focused on the genetic diversity of *P. lunatus* based on the RAPD marker.

2. Material and Methods

2.1. Collection of Samples

P. lunatus used in this present study was originated from seeds collected from some areas in East Java, Indonesia, such as Madura, Tulungagung, Malang, and Probolinggo. Based on the result of identification in previous research (Purwanti and Fauzi, 2019), the collection of *P. lunatus* consisted of 15 accessions. All those fifteen are listed in the following Table 1. Further, each of the accessions was planted in a polybag in which one polybag was distanced one meter long from the next one. In addition, the plantation was done without any extraneous additions of neither fertilizer nor other kinds of growth agents.

Table 1. List of accessions to analyze

Accession Codes	Origins
2	Madura
4	Madura
7	Madura
8	Madura
12	Madura
13	Madura
14	Madura, Tulungagung
16	Madura
18	Madura
19	Madura
Prb1	Probolinggo
Prb2	Probolinggo
Prb3	Probolinggo
Prb4	Probolinggo, Malang
Prb5	Probolinggo, Madura

2.2. DNA Isolation

DNA isolation was administered based on the CTAB method of Doyle and Doyle (1984), which was modified by Maftuchah and Zainuddin (2010). The used tissue stemmed from the leaf organ of 3 mo (month-old) plants. First, leaves were cut out and were crushed using liquid nitrogen. Then, Natrium bisulfited was weighed for each of 12 samples and dissolved into the buffer. The results of isolated DNA were stored under a temperature of -20 ºC.

Table 2. List of primers

Primers	Sequence 5'-3'	GC Content (%)
OPA6	GGTCCCTGAC	70
OPA8	GTGACGTAGG	60
OPA10	GTGATCGCAG	60
OPA20	GTTGCGATCC	60
OPC19	GTTGCCAGCC	70
OPD8	GTGTGCCCCA	70
OPD12	CACCGTATCC	60
OPE8	TCACCACGGT	60
OPE15	ACGCACAACC	60
OPE16	GGTGACTGTG	60

2.3. PCR-RAPD

Ten primers having 60 to 70 GC content were used in this present study (Table 2). The total volume of PCR reaction used signified 22.5 µL, containing the mixture of liquid DNA of *taq* polymerase and 10-fold buffer of *taq* polymerase (100 mM Tris-CI, pH 8.3; 500 mM KCl; 15 mM $MgCI_2$; 0.01% gelatin); *dNTP'S mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH_20; and 30 ng DNA template. The condition for PCR reaction was designed under the pre-denaturation temperature of 94 °C (in 5 min), denaturation temperature of 94 °C (in 1 min), primary attachment temperature of 36 °C (in 1 min), extension temperature of 72 °C (in 2 min), the post-extension temperature of 72 °C (in 5 min), and post PCR reaction temperature of 4 °C (in 2 min). For multiplication, the cycle of the PCR reaction was repeated 36 times.

2.4. Agarose Gel Electrophoresis

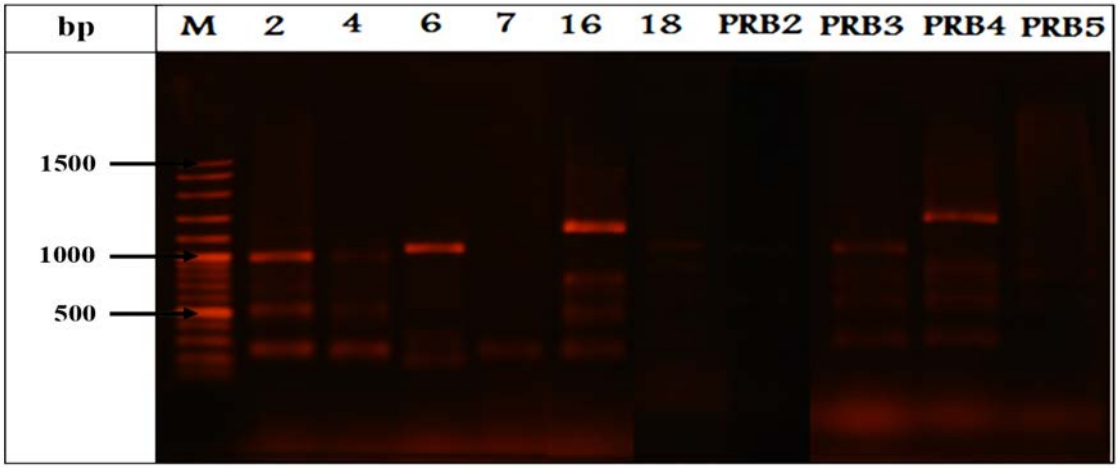
There were three stages of procedure to confirm the result of isolation process and PCR reaction after implementation. The first stage was creating agarose gel with a concentration of 0.8% (for isolation result) and 1% (for PCR result) as a medium of running DNA. Next, the stage was labelled electrophoresis with the electrophoresis buffer of TBE (1×), loading dye (6×) under the condition of 60 V, 400 mA within 45 min. At last, the stage was the coloration using 10%-concentrated ethidium-bromide and the documentation of the DNA using UV-Trans illuminator.

2.5. Analysis on the DNA Bands Yielded from RAPD

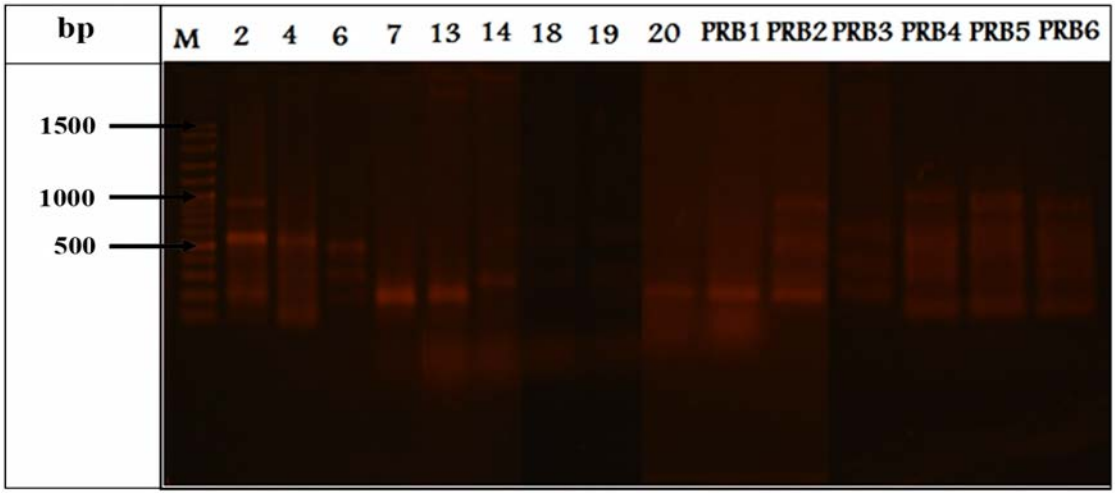
Data analysis was performed by observing the pattern of visible bands from the electrophoresis process in each primary locus. In addition, DNA bands were converted into binary data (0 and 1), indicating the existence and inexistence of bands typifying specific sizes. Afterward, the existing bands were observed to identify the percentage of polymorphic and monomorphic bands and create a phylogenetic tree by creating a phylogenetic tree with Popgen software version 3.1 (Yeh and Boyle, 1997).

3. Results and Discussion

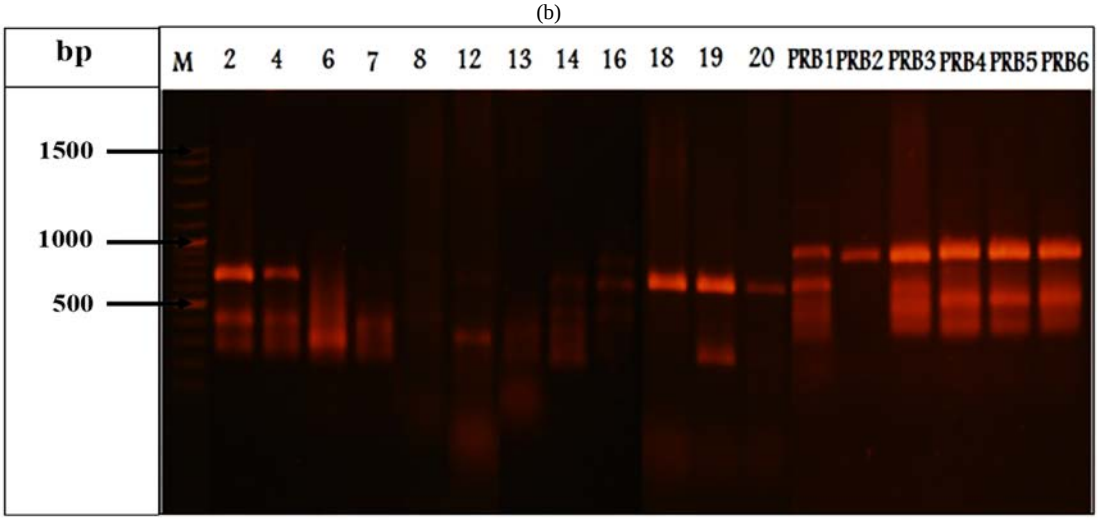
In this current research, ten primers were occupied for DNA amplification at 15 genotypes of *P. lunatus* L. Further, as a result of PCR–RAPD amplification at those genotypes (Figure 1), the bands were assessed based on the binary data, with the description of 1 for the amplified band and 0 for unamplified. The following Table 3 showed the record of the number of loci found.



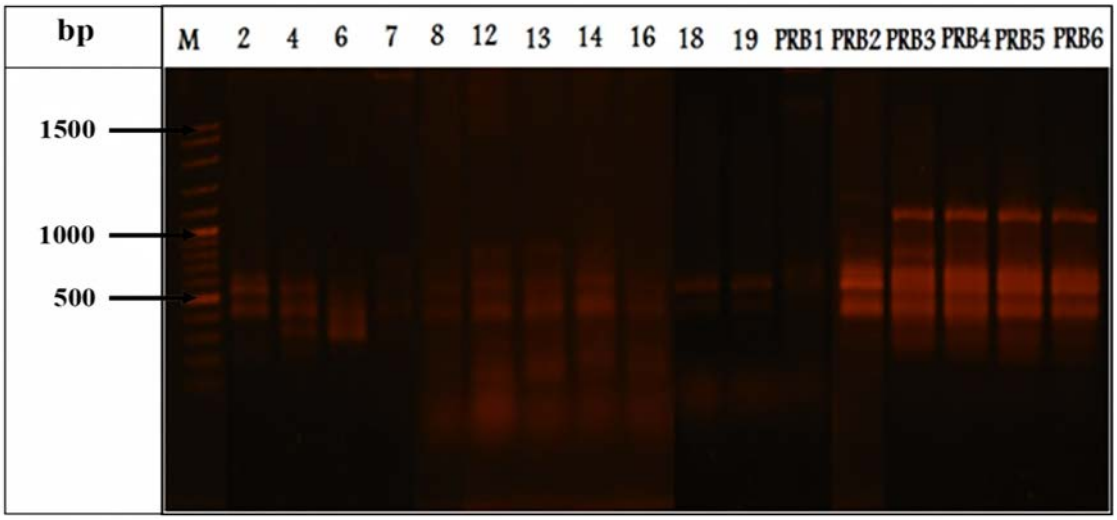
(a)



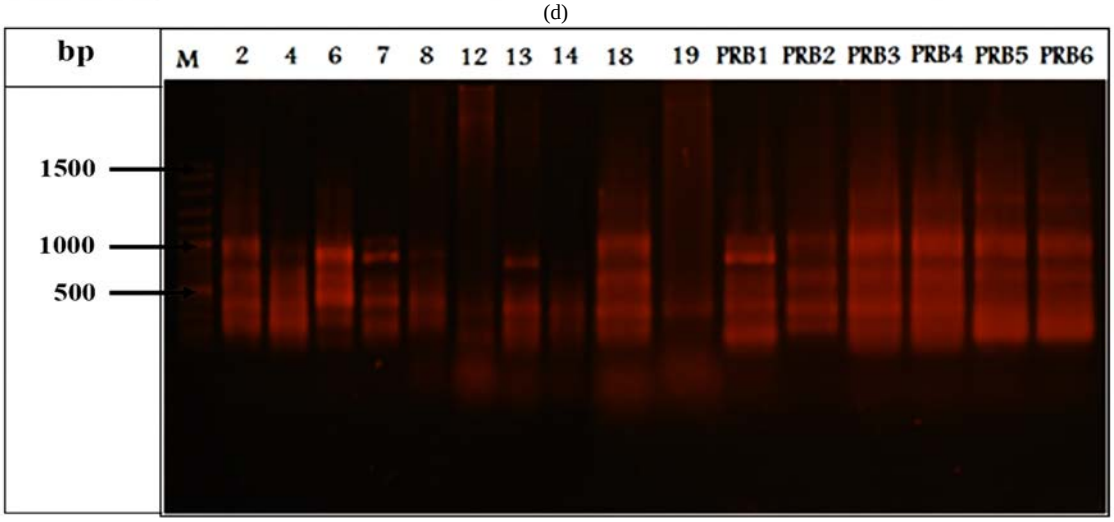
(b)



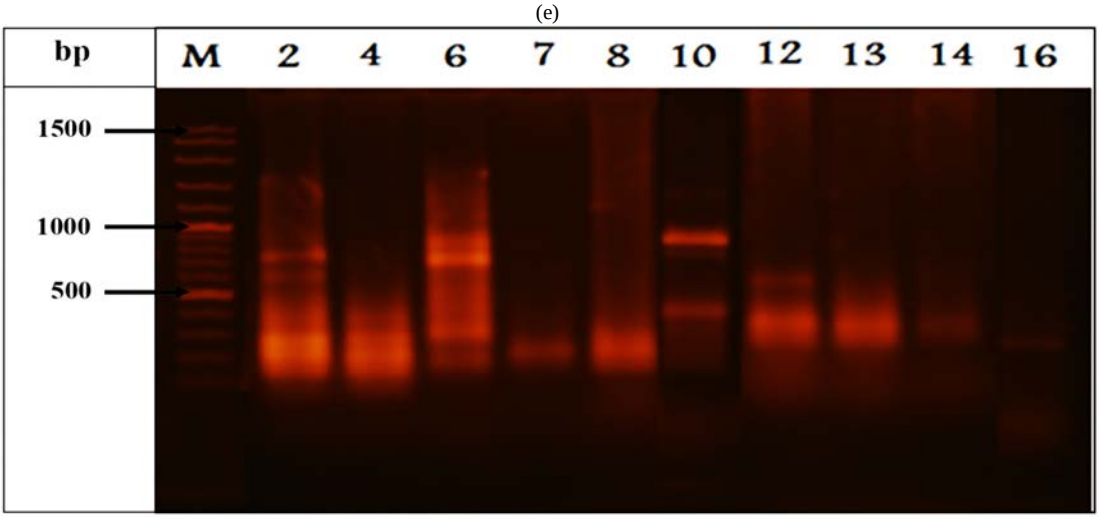
(c)



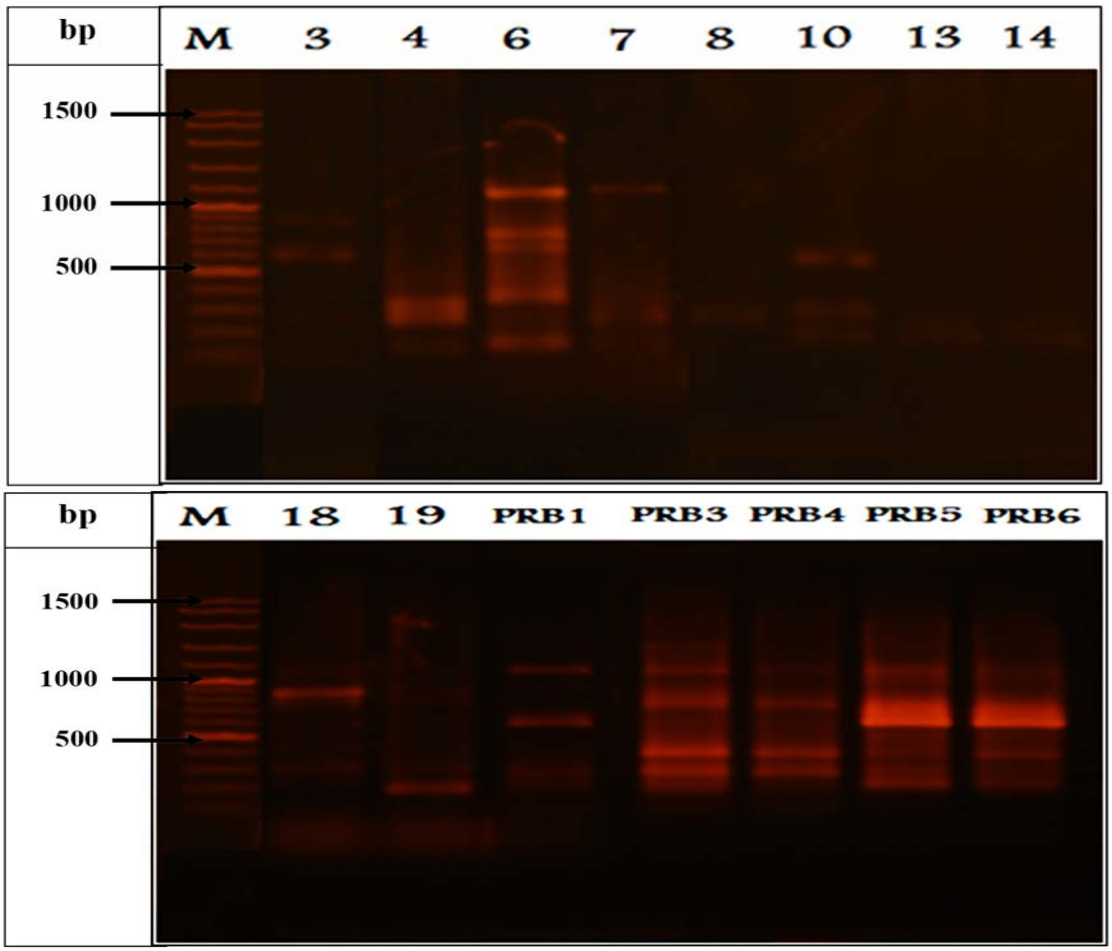
(d)



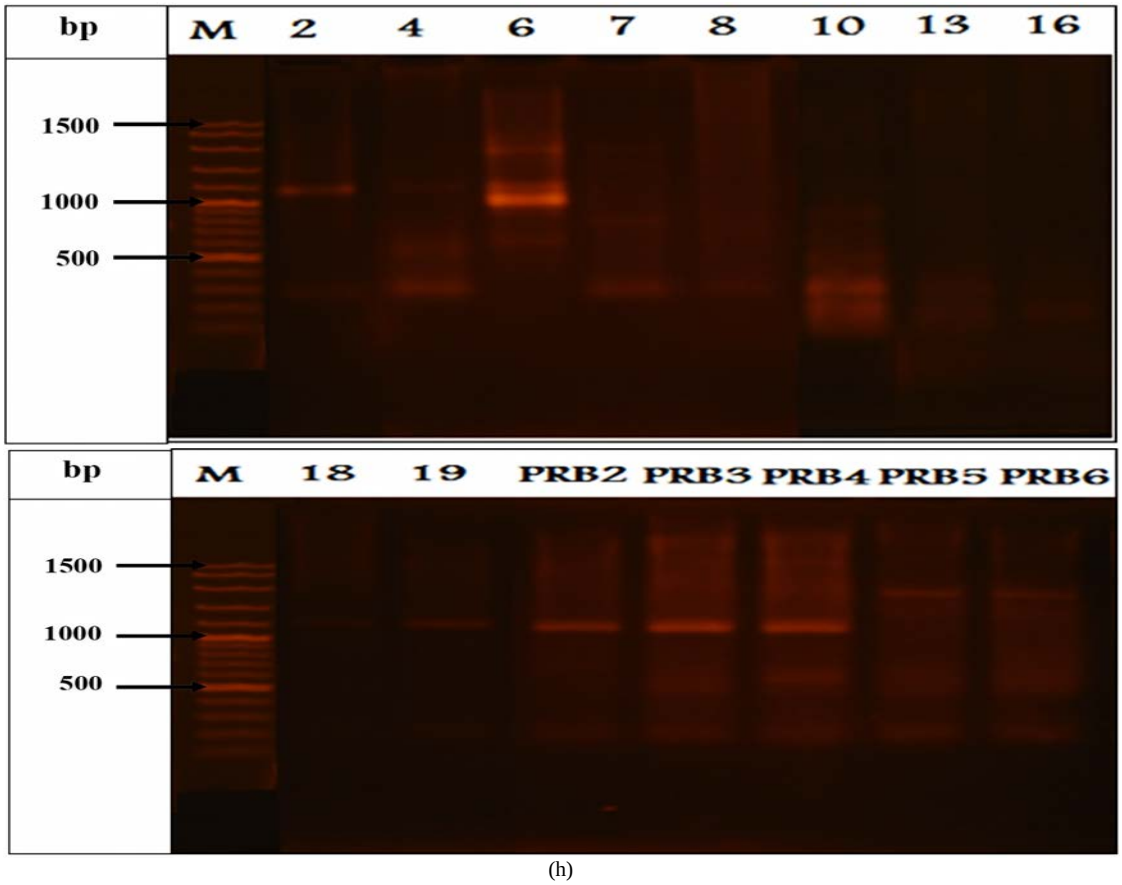
(e)



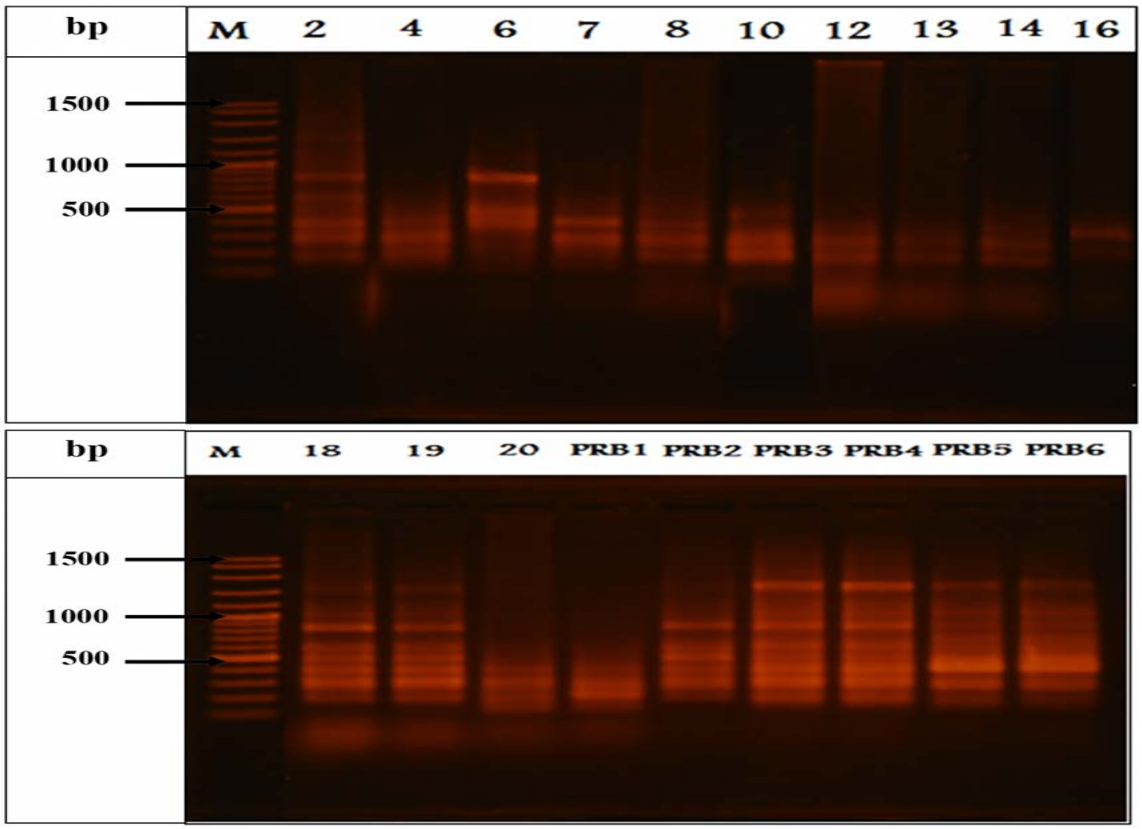
(f)


bp
M 3 4 6 7 8 10 13 14
1500
1000
500
bp
M 18 19 PRB1 PRB3 PRB4 PRB5 PRB6
1500
1000
500

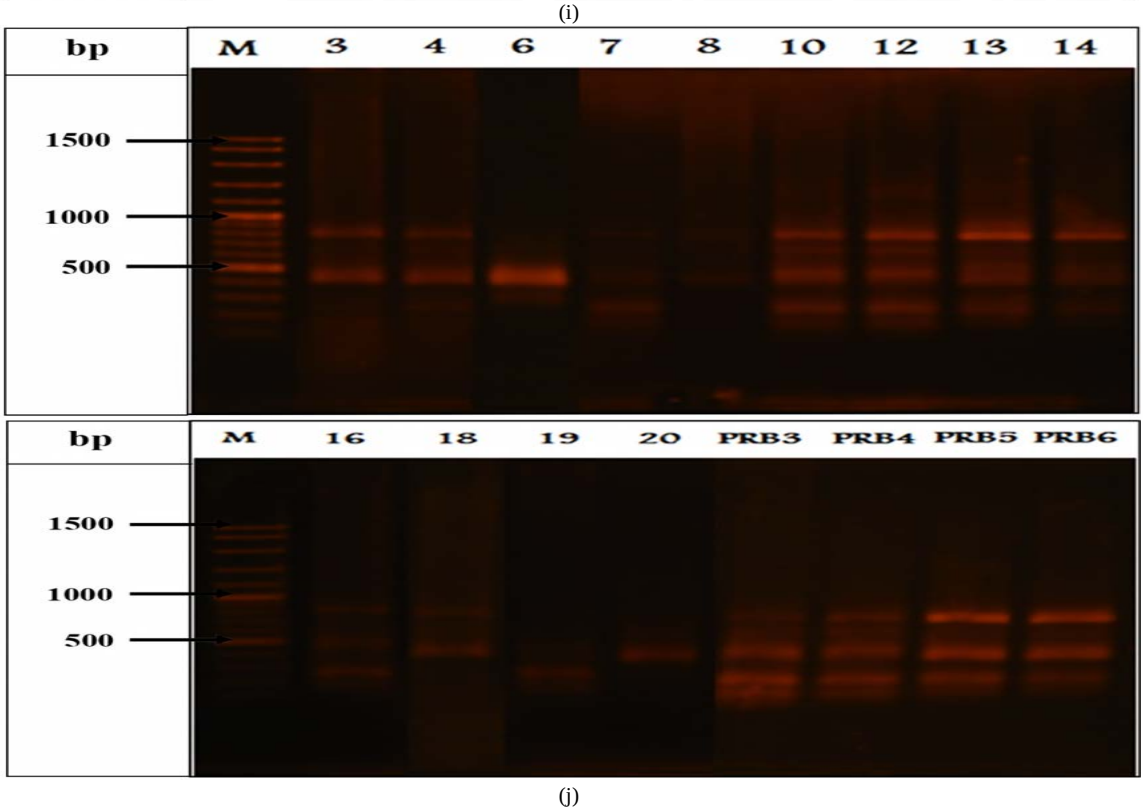
(g)


bp
M 2 4 6 7 8 10 13 16
1500
1000
500
bp
M 18 19 PRB2 PRB3 PRB4 PRB5 PRB6
1500
1000
500

(h)



(i)



(j)

Figure 1. Gel electrophoresis profiles for PCR–RAPD results using (a) OPA6, (b) OPA8, (c) OPA10, (d) OPA20, (e) OPA19, (f) OPD8, (g) OPD12, (h) OPE8, (i) OPE15, (j) OPE16. M is the DNA size ladder

Table 3. The record of the number of locus found at 15 genotypes of *P. lunatus* using 10 random primary

Codes	Locus (bp)												
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
2	0	1	1	1	1	1	1	1	1	1	1	0	0
4	1	1	1	1	1	1	1	1	0	1	1	0	0
7	1	1	1	1	0	0	1	1	0	1	1	0	0
8	0	1	1	1	1	0	0	1	0	1	0	0	0
12	1	1	1	1	1	1	1	1	0	0	0	0	0
13	1	1	1	1	1	0	0	1	0	1	0	0	0
14	1	1	1	1	1	0	1	1	0	0	0	0	0
16	0	1	1	1	1	1	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	0	1	1	0
19	0	1	1	1	1	1	1	1	0	0	1	1	0
Prb1	1	1	1	1	1	1	1	0	1	1	1	0	0
Prb2	0	1	1	1	1	1	1	1	1	1	1	0	0
Prb3	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb4	1	1	1	1	1	1	1	1	1	1	1	1	0
Prb5	1	1	1	1	1	1	1	1	1	0	1	1	1

Description: (1): DNA band was existent, (0): DNA band was non-existent.

After PCR was conducted, DNA fragments of diverse sizes (polymorphic) were produced (Table 4). A total of 68 amplified bands were obtained, out of 67 were polymorphic. The percent polymorphism was 96% to 100%. The total number of amplified bands varied between 5 (OPA10 and OPA20) to 9 (OPA12), with an average of 6.8 bands per primer. Ten unique bands were detected in several accessions. The size of the unique band ranged from 200 (Mdr6) to 1 300 bp (Prb5).

Table 4. RAPD primers used for diversity analysis of *P. lunatus*.

No.	**Markers (100 bp–2000 bp)**	**∑ Band**	**Polymorphic**	**% Polymorphic**	**Unique band**		
					Total	**Locus**	**Accession**
1	OPA 6	8	8	100%	2	700	Mdr16
						200	Mdr6
2	OPA 8	7	7	100%	0	-	-
3	OPA 10	5	5	100%	0	-	-
4	OPA 20	5	5	100%	1	300	Mdr4
5	OPC 19	6	5	96%	0	-	-
6	OPD 8	8	8	100%	1	500	Prb1
7	OPD 12	9	9	100%	2	1 200	Prb3
						900	Mdr19
8	OPE 8	7	7	100%	2	1 300	Prb5
						500	Mdr4
9	OPE 15	7	7	100%	0	-	-
10	OPE 16	6	6	100%	2	700	Mdr12
						500	Mdr16
	Total	68	67		10		

The cluster analysis upon the 68 RAPD bands was administered. The phylogenetic tree using Neighbor-Joining method was produced, equipped with similarity coefficient that ranged from 64% to 100%, or there was genetic variation with the range of 0% to 36% (Figure 2). In GSC of 0.6, *P. lunatus* accessions were formed into two main clusters, i.e. Cluster A (comprising 7, 8, 13, 14 genotypes) and Cluster B (comprising 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, Prb5 genotypes). Cluster A contained several sub-clusters, which were A1 with GSC of 0.932 (including 8, 12, 13, 14 genotypes), A2 with GSC of 0.73 (including genotype 7). Meanwhile, Cluster B comprised several sub-clusters, too, with B1 (including 19, Prb5, Prb4, Prb3, 18 genotypes) and B2 (including Prb1, 4, Prb2, 16, 2 genotypes). At GSC of 1.0, there were 2, 4, 16, Prb2, Prb3, Prb4 genotypes. The coefficient was considered higher if it approached the point of 1, indicating that the genetic similarity amongst the genotypes was significantly close.

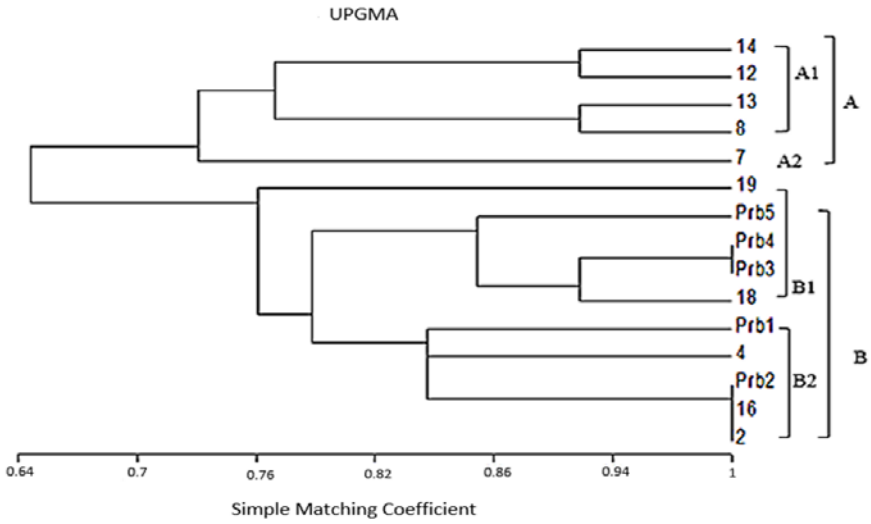


Figure 2. The dendrogram showing the connection amongst 15 accessions of *P. lunatus* referring to RAPD marker

P. lunatus under analysis through this current research was planted using conventional method in some parts of Indonesia of which environmental conditions were of diversity. The result of analysis indicated that the polymorphic level of *P. lunatus* was relatively high (ranging from 96% to 100%). This kind of finding was in line with previous studies that discussed genetic diversity of *P. lunatus* in some areas of Mesoamerica (Camacho-Pérez *et al*., 2018; Chacón-Sánchez and Martínez-Castillo, 2017). The existing high polymorphism had indicated the vast range of genetic diversity from the accessions under analysis.

In nature, polymorphism constitutes a series of parameters to define genetic diversity on particular species (Singh and Kulathinal, 2013). The level of polymorphism in intra-species depended on the level of divergence between the genotypes. Regarding the information, *P. lunatus* used in this research was originated from different types of the gene pool. According to the previous study, the 15 accessions of *P. lunatus* included in the current research were originated from five different gene pools, name Sieva-Big, Potato-Sieva, Big lima, Sieva, and Potato-Sieva (Purwanti and Fauzi, 2019). Genetic diversity, moreover, was also a result of ecological situations that differed (Huang *et al.*, 2016). The ecological condition was closely interconnected with the agro-climatic zone in which it was grown. The origins where the samples were taken had different agro-climatic zone (Syuaib, 2016).

The genetic diversity considered high had brought about an urgent implication in terms of crop improvement attempt, including the breeding procedure for quality improvement (Bhanu, 2017; Fu, 2015). As previously reported, *P. lunatus* contained anti-nutritional components that became a confounding factor why its seeds were not effective as the main food resource (Doria *et al*., 2012). Consequently, the process of maturation in *P. lunatus* seeds needed proper and acceptable process; thus it could reduce the anti-nutritional substances (Sukatiningsih *et al*., 2013). Further, it was quite probable that the generation of *P. lunatus* would remain low anti-nutritional or without anti-nutritional factors. In addition, the breeding procedure could be designed that way to yield the generation of *P. lunatus* with highly pest-resistant performance and a shorter period of maturation phase.

Next, concerning the phylogenetic tree formed, the population tended to be clustered based on the geographical origins of those accessions. In fact, Madura accessions were more dominant within Cluster A, while in Cluster B, Probolinggo clusters were superior. Furthermore, there was also shown a trend of connection between similarity of morphological characteristics amongst accessions grouped in the same cluster. Previous studies have reported that most of Probolinggo accessions possessed better characteristics of seed weight, seed length, leaf length, leaf width, and pod width compared to other accessions. Accession 4 constituted the one of which morphological character was close in characteristics to Probolinggo accessions (Purwanti and Fauzi, 2019).

Despite this fact, cluster analysis also indicated the existence of accessions of which pod lengths were quite different but still grouped in the same cluster. In contrast, when the pod length was not significantly different, cluster analysis classified those accessions in different clusters. This was probably due to DNA related to the RAPD marker used in this current research being unrelated to the character set. For that reason, a further study that highlights types of gen is needed to encode those characters.

In accordance with this research, the use of RAPD was not only affordable but also not complex. Also, it could be used to cluster a collection of accessions that could be connected to their agronomic characteristics. Further, reproducibility and sustainability of RAPD marker in the study of genetic variation were still contested in some previous researches. However, there were some studies of genetic variation on germplasm of beans that were successful by utilizing RAPD, such as some researches about *P. vulgaris* in India (Bukhari *et al*., 2015), South Africa (Adesoye and Ojobo, 2012), Turkey (Ince and Karaca, 2011), and in Vicia Faba, Palestine (Basheer-Salimia *et al*., 2013). In fact, the result of RAPD shown in this current research shared information which was in line with other generic variation researches by the use of other types of marker, such as ISSR (Camacho-Pérez *et al*., 2018) and SNP (Chacón-Sánchez and Martínez-Castillo,

2017). In short, this current research has advocated the credibility of RAPD in the study of genetic variation in beans, such as in *P. lunatus*.

4. Conclusion

In this research, the genetic variations of 15 accessions of *P. lunatus* originated from Malang, Probolinggo, Tulungagung, and Madura were analysed. The result of RAPD using ten primers resulted in 68 bands in which nine primers possessed 100% level of polymorphism, and one primary was equipped with 96% level of polymorphism. In addition, ten unique bands were detected in eight accessions, i.e. Prb1, Prb2, Prb5, Mdr12, Mdr16, Mdr19, Mdr4, and Mdr6. Based on cluster analysis, there were two major clusters, Cluster A and B. The former contained the accessions of 7, 8, 13, and 14, while the latter comprised the 2, 4, 16, 18, Prb1, Prb2, Prb3, Prb4, and Prb5.

References

Adesoye 0A and Ojobo OA. 2012. Genetic diversity assessment of *Phaseolus vulgaris* L. landraces in Nigeria's mid-altitude agroecological zone. *Int. J. Biodivers. Conserv*. **4 (13)**: 453–460.

AlRawashdeh IM and AlRawashdeh NQ. 2015. Evaluating the genetic relatedness within *Lupinus pilosus* L. species based on RAPD analysis. *Jordan J. Biol. Sci*. **8** (1): 61–64**.**

Basheer-Salimia R, Shtaya M, Awad M, Abdallah J and Hamdam Y. 2013. Genetic diversity of Palestine landraces of Faba bean (*Vicia faba*) based on RAPD markers. *Genet. Mol. Res*. **12 (3)**: 3314–3323.

Ben-Ari G and Lavi U. 2012. Marker-assisted selection in plant breeding. In: Chapter 11, **Plant Biotechnology and Agriculture**. Academic Press. Elsevier. USA.pp 163–184

Bhanu AN. 2017. Assessment of genetic diversity in crop plants - An overview. *Adv. Plants Agric. Res*. **7 (3)**: 279–286.

Bukhari A, Bhat MA, Ahmad M and Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *Afr. J. Biotechnol*. **14** (**6)**:451–458.

Carvalho YGS, Vitorino LC, de Souza UJB and Bessa LA. 2019. Recent trends in research on the genetic diversity of plants: Implications for conservation. *Diversity*. **11 (4)**: 1–21.

Diana A, Mallard SR, Haszard JJ, Purnamasari DM, Nurulazmi I, Herliani PD, Nugraha GI, Gibson RS and Houghton L. 2017. Consumption of fortified infant foods reduces dietary diversity but has a positive effect on subsequent growth in infants from Sumedang district, Indonesia. *PLoS One*. **12 (4)**: 1–17.

Diniyah N, Windarti WS and Maryanto. 2013. Pengembangan teknologi pangan berbasis koro-koroan sebagai bahan pangan alternatif pensubtitusi kedelai [Development of food technology based on koro-koroan as an alternative food material to substitute soybeans] Seminar Nasional Pengembangan Sumber Daya Lokal untuk Mendorong Ketahanan Pangan dan Ekonomi. UPN Veteran, Surabaya, Indonesia.

Diniyah N, Windrati WS and Riady S. 2015. Sifat fungsional tepung koro kratok hitam, merah dan putih (*Phaseolus lunatus* L.) dengan perlakuan lama perendaman [The functional properties of black, red and white koro kratok flour (*Phaseolus lunatus* L.) with long soaking treatment. *Jurnal Hasil Penelitian Industri*. **28 (2)**:70–77.

Doria E, Campion B, Sparvoli F, Tava A and Nielsen E. 2012. Anti-nutrient components and metabolites with health implications in seeds of 10 common bean (*Phaseolus vulgaris* L. and *Phaseolus lunatus* L.) landraces cultivated in southern Italy. *J Food Compost Anal*. **26** (1–2): 72–80.

Doyle J J. and Doyle JL. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*. **19**: 11–15.

Fu YB. 2015. Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet*. **128** (11): 2131–2142.

Govindaraj M, Vetriventhan M. and Srinivasan M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. **ID 431487**: 1–14

Herry B, Windarti WS and Nuru. 2014. Potensi koro-koroan sebagai sumber bahan lokal untuk pembuatan aneka produk olahan berprotein [The potential of koro-koroan as a source of local ingredients for the manufacture of various processed protein products] Prosiding Seminar Nasional. Universitas Muhammadiyah Jember, Jember, Indonesia.

Huang W, Zhao X, Zhao X, Li Y and Lian J. 2016. Effects of environmental factors on genetic diversity of *Caragana microphylla* in Horqin Sandy Land, northeast China. Ecol. Evol. **6 (22)**: 8256–8266.

Ickowitz A, Rowland D, Powell B and Salim MA. 2016. Forests, trees, and micronutrient-rich food consumption in Indonesia. *PLoS One* **11 (5)**: 1–15.

Ince AG and Karaca M. 2011. Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers. *Plant Omics*. **4** (4): 220–227.

Kalaminasih D. 2013. Pengaruh proporsi kacang koro sayur (*Phaseolus lunatus*) dan kacang koro pedang (*Canavalia ensiformis* L.) terhadap mutu organoleptik tempe koro [Effect of the proportion of *Phaseolus lunatus* and *Canavalia ensiformis* L.) on the organoleptic quality of tempe koro]. *E-Journal Boga*. **2** (3): 104–113.

Kumar NS and Gurusubramanian G. 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis.* **11** (3): 116–124.

Kumari N and Thakur SK. 2014. Randomly amplified polymorphic DNA-A brief review. *Am J Anim Vet Sci*. **9**(1): 6–13.

Lim TK. 2012. *Lablab purpureus*. In: **Edible Medicinal And Non-Medicinal Plants, Vol. 2, Fruits.** Springer Netherlands. pp. 730–741.

Madanijah S, Briawan D, Rimbawan R, Zulaikhah Z, Andarwulan N, Nuraida L, Sundjaya T, Murti L, Shah Priyali, Bindels J. 2016. Nutritional status of pre-pregnant and pregnant women residing in Bogor district, Indonesia: A cross-sectional dietary and nutrient intake study. *Br J Nutr*. **116**: 1–10.

Maftuchah and Zainuddin. 2006. Pengembangan metode isolasi DNA genom pada tanaman jarak pagar (*Jathropa curcas* L.) [Development of genomic DNA isolation methods in *Jathropa curcas* L.]. *Humanity*. **2 (1)**: 63–69.

Nafi A, Diniyah N. and Hastuti, FT. 2015. Karakteristik fisikokimia dan fungsional teknis tepung koro kratok (*Phaseolus lunatus* L.) termodifikasi yang diproduksi secara fermentasi spontan [Physicochemical and technical functional characteristics of modified *Phaseolus lunatus* L. flour produced by spontaneous fermentation]. *Agrointek*, **9 (1)**: 24–32.

Praseptiangga D, Tryas A A, Affandi DR, Atmaka W, Ariyantoro AR and Minardi S. 2018. Physical and chemical characterization of composite flour from Canna flour (*Canna edulis*) and Lima bean flour (*Phaseolus lunatus*). *AIP Conf Proc*. **1927 (030020)**: 1–6.

Purwanti E and Fauzi A. 2019. The morphological characteristics of *Phaseolus lunatus* L. in different areas of East Java, Indonesia. *IOP Conf. Ser. Earth Environ. Sci*. **276** (012017):1–10

Serrano-serrano ML, Hernández-torres J, Castillo-villamizar G, Debouck DG and Chacón, MI. 2010. Molecular phylogenetics and evolution gene pools in wild Lima bean (*Phaseolus lunatus* L.) from the Americas: Evidences for an Andean origin and past migrations. *Mol. Phylogenet. Evol*. **54** (1): 76–87.

Silva RNO, Lopes ACA, Gomes RLF, Pádua JG and Burle ML. 2019. High diversity of cultivated Lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. *Genet. Mol. Res.* **18** (4): 1–15.

Singh RS and Kulathinal RJ. 2013. Polymorphism. In: Maloy S and Hughes K (Eds.). **Brenner's Encyclopedia of Genetics 2nd Edition.** Academic Press - Elsevier. Cambridge, Massachusetts, USA, pp. 398–399.

Sukatiningsih, Yustian AM and Windarti SW. 2013. Penambahan isolat protein kedelai dan sukrosa racun pada kecambah koro kratok [*Phaseolus lunatus* (L) sweet] [Addition of soy protein isolate and toxic sucrose to the sprouts of Phaseolus lunatus (L) sweet. *Agritrop Jurnal Ilmu-Ilmu Pertanian.* **11 (1)**: 1–7.

Syuaib MF. 2016. Sustainable agriculture in Indonesia: Facts and challenges to keep growing in harmony with environment. *Agri Eng Int: CIGR J.* **18 (2)**:170–184.

Yeh, F.C., and T.J.B. Boyle. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian J. Bot.* **129**:157–163.