

# Diversity\_of\_Phaseolus\_lunatus

*by* Elly Purwanti

---

**Submission date:** 08-Jul-2022 10:14AM (UTC+0700)

**Submission ID:** 1867937325

**File name:** ah\_Maftuchah\_Hidayati\_Fauzi\_-\_Diversity\_of\_Phaseolus\_lunatus.pdf (469.25K)

**Word count:** 4407

**Character count:** 22602

5

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

Elly Purwanti<sup>1</sup>, Mohamad Amin<sup>2</sup>, Siti Zubaidah<sup>2</sup>, Maftuchah Maftuchah<sup>3</sup>,  
Siti Nur Hidayati<sup>4</sup>, and Ahmad Fauzi<sup>1,\*</sup>

6

<sup>1</sup>Department of Biology Education, Faculty of Teacher Training and Education, University of Muhammadiyah Malang, Jl. Raya Tlogmoas No. 246, Malang 65144, Indonesia; <sup>2</sup>Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Semarang No. 5, Malang 65145, Indonesia; <sup>3</sup>Department of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Indonesia; <sup>4</sup>Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, USA

Received: April 1, 2021; Revised: May 9, 2021; Accepted May 10, 2021

### Abstract

14

*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.

20

**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

\* Corresponding author e-mail: [ahmad\\_fauzi@umm.ac.id](mailto:ahmad_fauzi@umm.ac.id).

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 °C.

**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-Cl, pH 8.3; 500 mM KCl; 15 mM MgCl<sub>2</sub>; 0.01% gelatin); *dNTPS mix* (dGTP, dATP, dTTP and dCTP) (Roche); dH<sub>2</sub>O; 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.

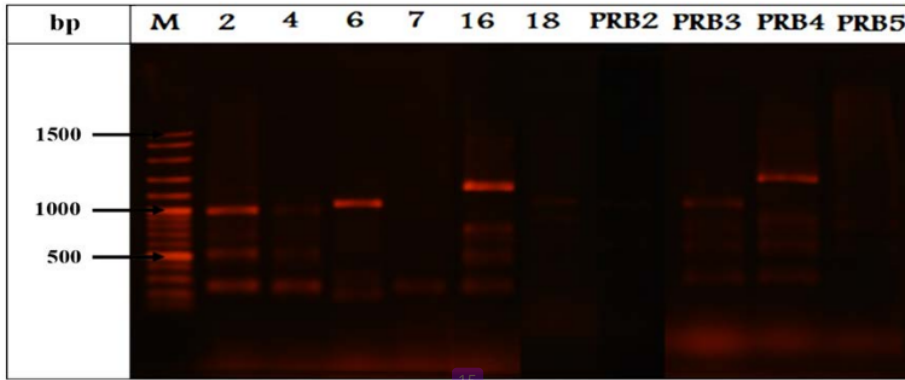
### 2.5. Analysis on the DNA Bands Yielded from RAPD

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).

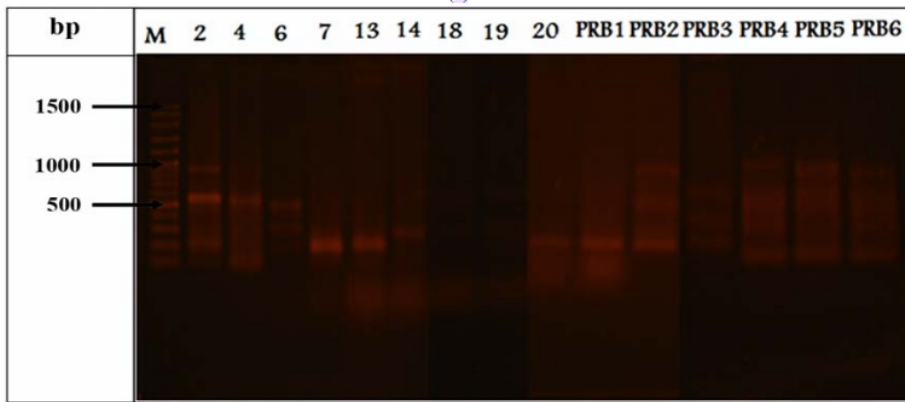
**3. Results and Discussion**

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

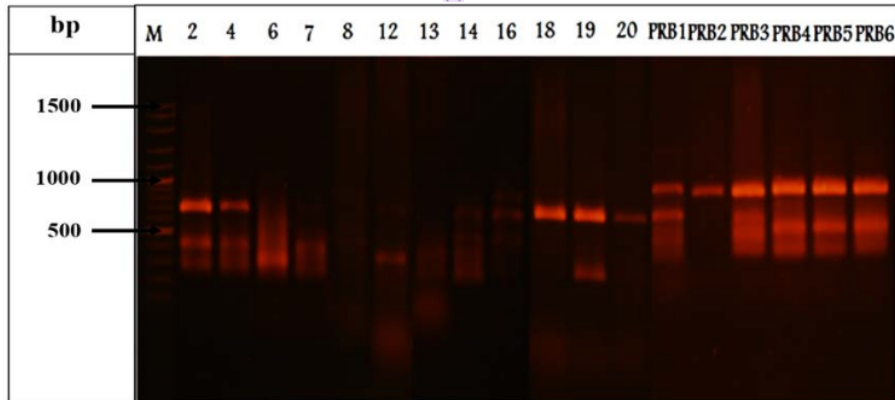
genotypes (Figure 1), the bands were assessed based on the binary data, with the description of 1 for the amplified band and 0 for unamplified. The following Table 3 showed the record of the number of loci found.



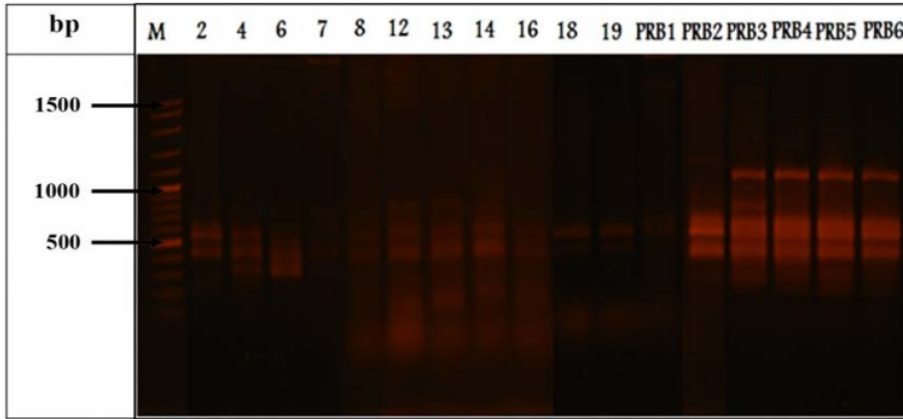
(a)



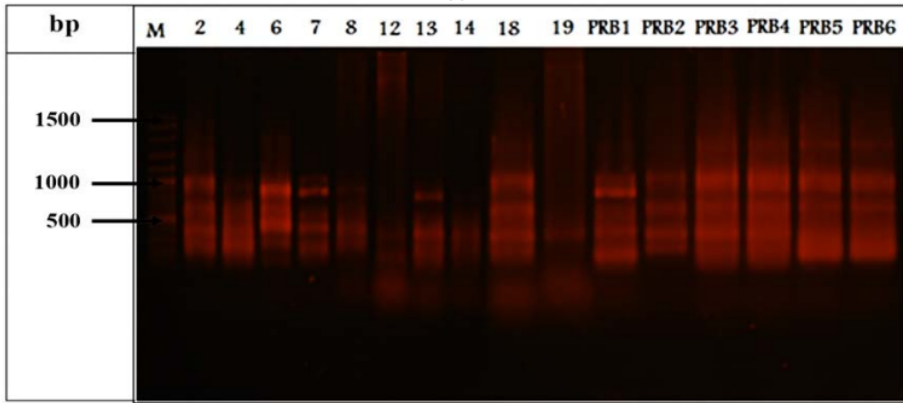
(b)



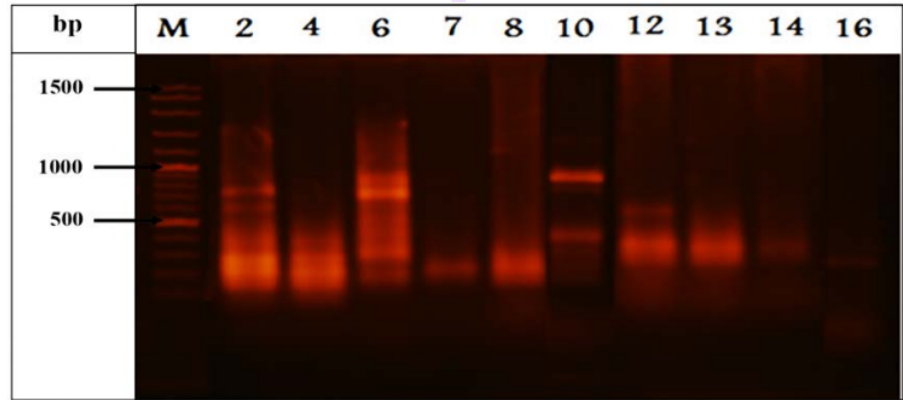
(c)



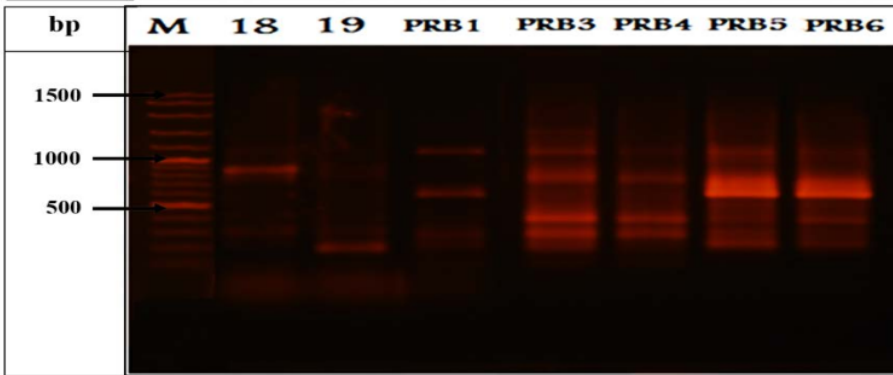
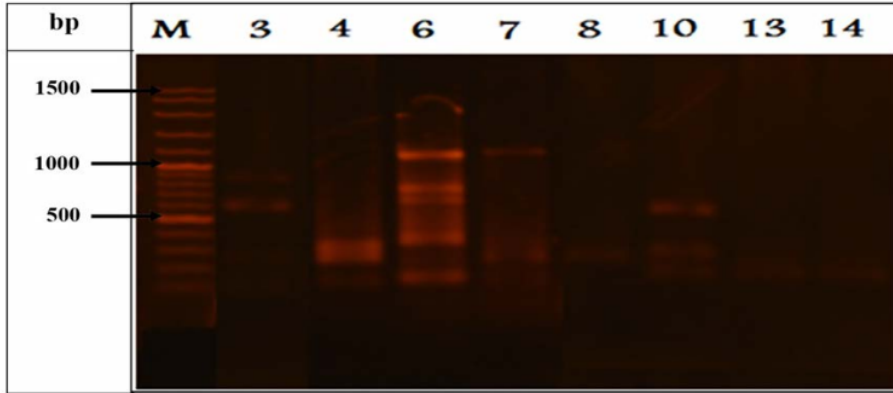
(d)



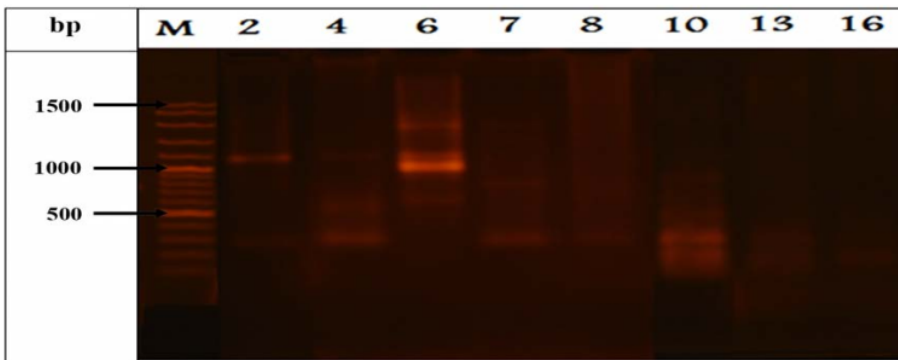
(e)



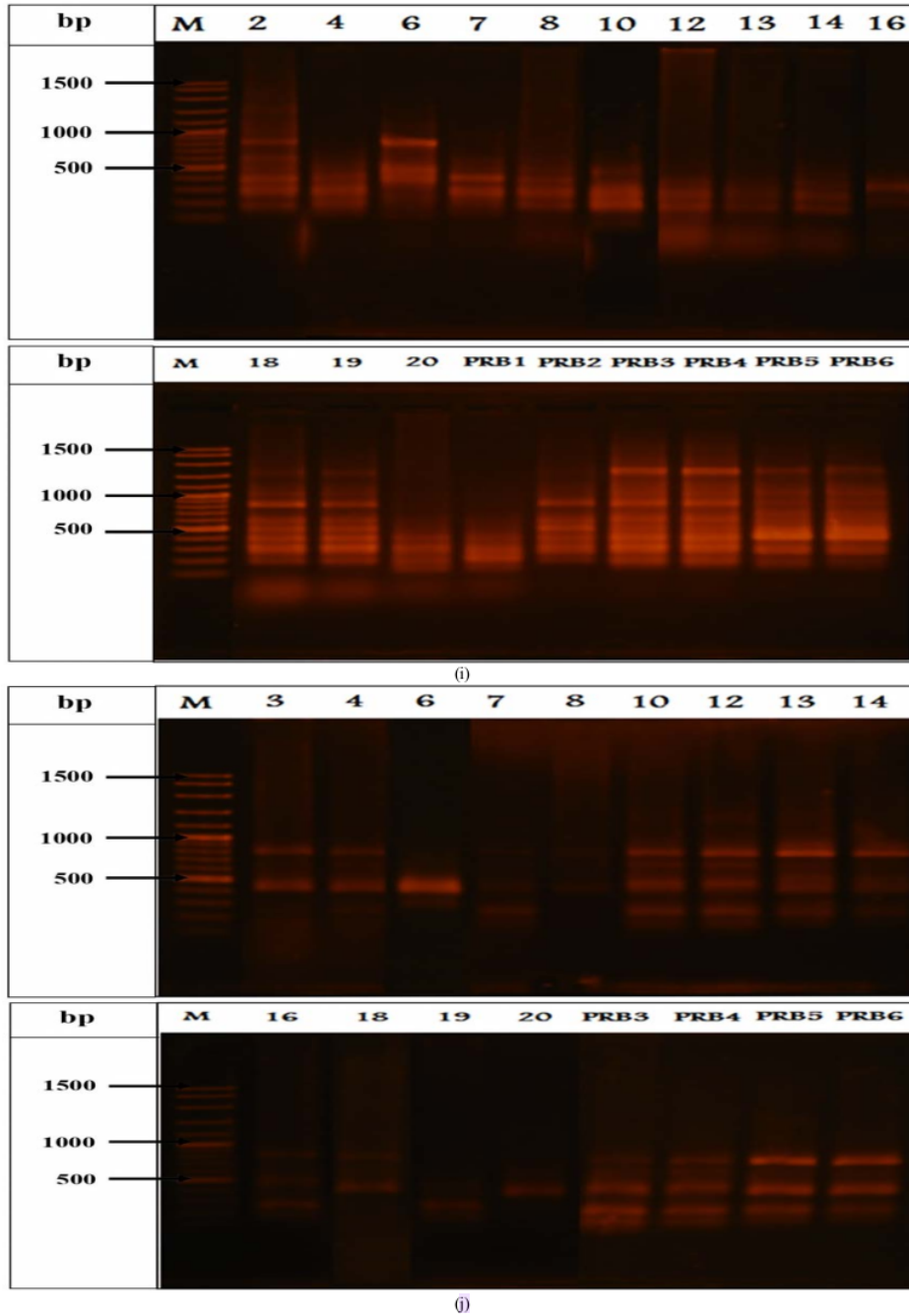
(f)



(g)



(h)



**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	0	1	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 1300 bp (Prb5).

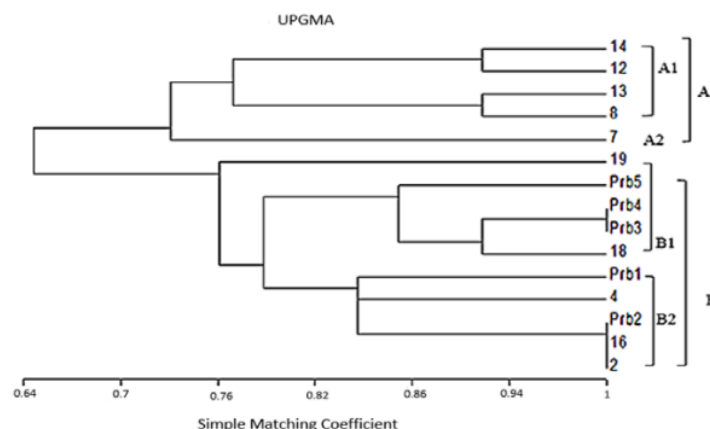
**Table 4.** RAPD primers used for diversity analysis of *P. lunatus*.

No.	Markers (100 bp–2000 bp)	Σ 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
					2	1200	Prb3
7	OPD 12	9	9	100%	900		Mdr19
					2	1300	Prb5
8	OPE 8	7	7	100%		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 (Sukatningsih *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 OA and Ojubo 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 substitusi 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, Windarti 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 Compos 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, ML 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 2<sup>nd</sup> 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.

# Diversity\_of\_Phaseolus\_lunatus

---

## ORIGINALITY REPORT

---

11%

SIMILARITY INDEX

7%

INTERNET SOURCES

8%

PUBLICATIONS

3%

STUDENT PAPERS

---

## PRIMARY SOURCES

---

- |   |   |    |
|---|---|----|
| 1 | <a href="http://eprints.umm.ac.id">eprints.umm.ac.id</a><br>Internet Source   | 1% |
| 2 | E Purwanti, A Fauzi. "The Morphological Characteristics of Phaseolus lunatus L. in Different Areas of East Java, Indonesia", IOP Conference Series: Earth and Environmental Science, 2019<br>Publication                                      | 1% |
| 3 | Submitted to Universitas Brawijaya<br>Student Paper   | 1% |
| 4 | David Goldman. "Failure to detect DUP25 in lymphoblastoid cells derived from patients with panic disorder and control individuals representing European and American populations", European Journal of Human Genetics, 06/2004<br>Publication | 1% |
| 5 | <a href="http://opac.wsb.torun.pl">opac.wsb.torun.pl</a><br>Internet Source   | 1% |
-

6

Rr. Eko Susetyarini. "The Level of Glutamic Acid in the Semen of Male White Rat (*Ratus Norwegicus*) after Being Treated with Tannin of *Pluchea Indica*", *Procedia Chemistry*, 2015

Publication

1 %

7

Submitted to Angeles University Foundation

Student Paper

&lt;1 %

8

Tânia da Silveira Agostini-Costa, Ana Flávia Pádua Teodoro, Rosa de Belem das Neves Alves, Leandro Ribeiro Braga et al. "Total phenolics, flavonoids, tannins and antioxidant activity of lima beans conserved in a Brazilian Genebank", *Ciência Rural*, 2014

Publication

&lt;1 %

9

Submitted to Bridgewater-Raritan Regional High School

Student Paper

&lt;1 %

10

Filipe Inácio Matias, Italo Stefanine Correa Granato, Gabriel Dequigiovanni, Roberto Fritsche-Neto. "Be-Breeder - an application for analysis of genomic data in plant breeding", *Crop Breeding and Applied Biotechnology*, 2017

Publication

&lt;1 %

11

Karl J. Indest, Mario T. Philipp. "DNA-Binding Proteins Possibly Involved in Regulation of the Post-Logarithmic-Phase Expression of

&lt;1 %

Lipoprotein P35 in *Borrelia burgdorferi*",  
Journal of Bacteriology, 2000

Publication

12

[krishikosh.egranth.ac.in](http://krishikosh.egranth.ac.in)

Internet Source

<1 %

13

[dev.biologists.org](http://dev.biologists.org)

Internet Source

<1 %

14

[ptti.or.id](http://ptti.or.id)

Internet Source

<1 %

15

[www.biorxiv.org](http://www.biorxiv.org)

Internet Source

<1 %

16

"Origin and Evolution of Biodiversity",  
Springer Science and Business Media LLC,  
2018

Publication

<1 %

17

Andrés J. Cortés, Paola Skeen, Matthew W. Blair, María I. Chacón-Sánchez. "Does the Genomic Landscape of Species Divergence in Phaseolus Beans Coerce Parallel Signatures of Adaptation and Domestication?", *Frontiers in Plant Science*, 2018

Publication

<1 %

18

Dzul-Tejero Félix, Julián Coello-Coello, Jaime Martínez-Castillo. "Wild to crop introgression and genetic diversity in Lima bean (*Phaseolus lunatus* L.) in traditional Mayan milpas from Mexico", *Conservation Genetics*, 2014

<1 %

---

19	<a href="http://bmcbiotechnol.biomedcentral.com">bmcbiotechnol.biomedcentral.com</a> Internet Source	<1 %
20	<a href="http://etd.repository.ugm.ac.id">etd.repository.ugm.ac.id</a> Internet Source	<1 %
21	<a href="http://mafiadoc.com">mafiadoc.com</a> Internet Source	<1 %
22	<a href="http://uni-sz.bg">uni-sz.bg</a> Internet Source	<1 %
23	<a href="http://www.ars-grin.gov">www.ars-grin.gov</a> Internet Source	<1 %
24	<a href="http://www.science.gov">www.science.gov</a> Internet Source	<1 %
25	F Tawati, Y Risjani, M S Djati, A S Leksono, B Yanuwiadi. "Evaluation of water quality using plankton diversity in Sumber Maron River District Gondanglegi Kulon East Java-Malang, Indonesia", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1 %

---

Exclude quotes  On

Exclude matches  Off

Exclude bibliography  On

# Diversity\_of\_Phaseolus\_lunatus

---

GRADEMARK REPORT

---

FINAL GRADE

**/0**

GENERAL COMMENTS

**Instructor**

---

PAGE 1

---

PAGE 2

---

PAGE 3

---

PAGE 4

---

PAGE 5

---

PAGE 6

---

PAGE 7

---

PAGE 8

---

PAGE 9

---

PAGE 10

---