Genetic variability of pepper mutants (*Piper nigrum* L.) based on morphological and RAPD markers

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Abstract. The area expansion and volume of Indonesian palm oil production is not in line with its productivity. The productivity of Indonesian oil palm is inseparable from the plants age, most of which have exceeded their productive age. There are also significant indications of the use of illegitimate seeds, causing a gap between yield and potential productivity. Efforts to increase the productivity and sustainability of oil palm plantations have been carried out by forming a particular agency that collects and manages oil palm plantation funds. One of the programs to increase productivity and sustainability is the "Replanting of Smallholder Palm Oil (PSR)" with a grant scheme for participating farmers. The progress of replanting realization is plodding. The target of 180,000 hectares per year cannot be realized. Accelerated breakthroughs without neglecting the primary mission of increasing productivity and sustainability are very urgent. This paper is a review on the importance of supporting the PSR seed institutional innovation in accelerating the productivity and sustainability of Indonesian oil palm.

1. Introduction

Pepper (*Piper nigrum* L.) is one of the leading spice commodities in the plantation sub-sector in Indonesia [1]. Pepper is one of the essential species in the family Piperaceae because of its benefits [2]. Pepper is used by the community as a spice, herbal medicine, cosmetics [3] as well as as an antibacterial agent [4].

The total area of pepper cultivation in Indonesia has decreased by 1.46% year⁻¹ in 2011 to 2015. Cultivation techniques and the use of a minimalist technology result in low pepper production so that farmers shift their plantation business to other commodities which are considered more profitable than pepper plants [1]. Since 2015 there has been a decline in black and white pepper production from 690 kg/ha to 641kg / ha [5]. Based on data from the [6], in 2010-2011 there was a fluctuation most significant decline in pepper exports which reached 50% of the total production of the previous year. From 2015 to 2016, Indonesian pepper export output decreased by 131,614,000 US $ [5]. Some farmers used Petaling1 and Natar 1, but mostly used local varieties.

Black pepper has been propagated vegetatively, so it might experience genetic stagnation. The new genetic combination can not be easily obtained. Vegetatively propagated plants generally genetically similar or identical so that they may be vulnerable to biotic stress such as disease attacks caused by fungal pathogens. Black pepper is susceptible to foot-rot disease caused by *Phytophthora capsici*. Generating variability may be done through hybridization or sexually crossing plants within the same
species, however, it requires adequate variability within the species populations. Introduction of varieties from abroad for such an important spices is not that simple. Another way to increase genetic variability and generate new genetic variants is by mutagenesis [7].

The existence of mutations has an impact on the amount of genetic variability available in an individual plant [8]. The mutation method was chosen because it can reduce the risk of losing mutant characters due to segregation in vegetative plants [9]. One of the mutation treatments given to plants is irradiation using electromagnetic rays, namely gamma rays [10]. The use of radiation with gamma rays is considered more effective and economical and has high penetration strength [11].

To find out the genetic variability of a population with the same species can be done through phenotypic and genotypic analysis. The phenotypic analysis is carried out as a first step in estimating plant diversity through observing several quantitative and qualitative characters. The difference obtained indicates that there is a possibility of genotype variations between plants that were found so that further genotypic analysis is needed. Besides, phenotypic analysis is still influenced by external factors of plants, for example, environmental conditions so that the validation of the data obtained needs to be analyzed using molecular analysis [12].

Genetic markers can be used to distinguish components of the genetic makeup between individuals of an organism or population [13]. The use of DNA markers has been widely used in the analysis of genetic variation and diversity between species or populations in plant conservation management [14]. The use of RAPD markers for genetic diversity analysis in addition to morphological characters that it is not influenced by the environment, also provides faster results ([15]. The used of RAPD markers have a high degree of polymorphism in the analysis of the diversity of several pepper species [16]. Combination of morphological characters observation and RAPD markers, expected will give more accurate information that can be obtained in finding the genetic diversity of pepper mutants observed. If this research result can get wide variability of pepper mutant, then easy to do next selection in genetical trial for pepper yield.

2. Materials and Methods
This research was conducted from May 2018 to August 2018, at the Indonesian Spice and Medicinal Research Institute, Bogor, West Java. The plant materials used were seedlings of 25 mutant plants derived from gamma-irradiated of black pepper seeds or germinated seeds of Cinten variety at 25-50 Gy (table 1). Each survived seed from gamma irradiation dose, from each replication is selected individually and propagated vegetatively until the fourth generation (M1V4). Gamma irradiated seeds could survive from both irradiation dose 25 and 50 Gy, but those sustained for the germinated seeds (seed with radicle) were from lower irradiation dose (25 Gy) only [17].

2.1. Identification of mutant variability based on morphological characters
Observation of morphological characteristics in pepper mutants was carried out on quantitative and qualitative traits followed IPGRI descriptor [18] on five months old mutant plants grown in polybags in the shading house. Quantitative morphological characters include the number of leaves, plant height, leaf length, leaf width, leaf thickness, and stem diameter. While qualitative morphological characteristics include leaf colour, stem colour, leaf shape, leaf base shape, and leaf margin. The observation on plant colour was measured based on the RHS colour chart [19].
Table 1. Code of 25 mutants and its mutation treatments

| Mutant Number | Treatment | Irradiation dose (Gy) | Material treated | Seed number |
|---------------|-----------|-----------------------|------------------|-------------|
| 1             | I.25.14S  | 25                    | Seed             | 14          |
| 2             | I.25.16S  | 25                    | Seed             | 16          |
| 3             | III.25.6S | 25                    | Seed             | 6           |
| 4             | III.25.9S | 25                    | Seed             | 9           |
| 5             | III.25.10S| 25                    | Seed             | 10          |
| 6             | III.25.17S| 25                    | Seed             | 17          |
| 7             | III.25.28S| 25                    | Seed             | 28          |
| 8             | I.50.1S   | 50                    | Seed             | 1           |
| 9             | I.50.2S   | 50                    | Seed             | 2           |
| 10            | I.50.7S   | 50                    | Seed             | 7           |
| 11            | I.50.8S   | 50                    | Seed             | 8           |
| 12            | I.50.9S   | 50                    | Seed             | 9           |
| 13            | I.50.13S  | 50                    | Seed             | 13          |
| 14            | I.50.16S  | 50                    | Seed             | 16          |
| 15            | I.50.17S  | 50                    | Seed             | 17          |
| 16            | I.50.18S  | 50                    | Seed             | 18          |
| 17            | I.25.3R   | 25                    | Germinated seed  | 3           |
| 18            | I.25.4R   | 25                    | Germinated seed  | 4           |
| 19            | I.25.5R   | 25                    | Germinated seed  | 5           |
| 20            | I.25.13R  | 25                    | Germinated seed  | 13          |
| 21            | II.25.3R  | 25                    | Germinated seed  | 3           |
| 22            | II.25.5R  | 25                    | Germinated seed  | 5           |
| 23            | III.25.11R| 25                    | Germinated seed  | 11          |
| 24            | III.25.8R | 25                    | Germinated seed  | 8           |
| 25            | III.25.12R| 25                    | Germinated seed  | 12          |

2.2 Data Analysis of morphological characters

Morphological data were tested using analysis of variance (F test) at a 5% significance level. If the results are significantly different then further tested with Duncan's Multiple Range Test (DMRT). Estimation of quantitative character genetic parameters was analyzed using R-Studio software. The linear model used in the analysis of quantitative character data with complete random designs according to [20], namely:

\[ Y_{ij} = \mu + t_i + \epsilon_{ij}, \]

Where:
- \( Y_{ij} \) = Observation value of a character in the i-genotype and j-replication
- \( \mu \) = Common midpoint
- \( t_i \) = Effect of additives from the i-genotype
- \( \epsilon_{ij} \) = Effect of trial error from the i-genotype on j-replication

Based on the linear model, the variance can be arranged as follows:

Table 2. Analysis of Variance for quantitative morphological characters

| Source of variation (SV) | Degree of freedom (DF) | Mean Square (MS) |
|-------------------------|------------------------|------------------|
| Treatment               | t-1                    | MS_1             |
| Error                   | t(n-1)                 | MS_2             |

Note: \( t = \) treatment (25); \( n = \) number of replications (3)
Variance components include genetic variance, environmental variance, phenotypic variance, which are calculated based on Singh and Chaudary (1979):

\[
\text{Error variance } (\sigma^2_e) = MS_2 \\
\text{Genetic variance } (\sigma^2_g) = \frac{MS_1 - MS_2}{n} \\
\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \frac{\sigma^2_e}{n}
\]

Genetic variance deviation (\(\sigma_{\sigma^2_g}^2\)) was calculated based on [21] as follows:

\[
\sqrt{\frac{2}{n^2} \left( \frac{KT_1}{db_1 + 2} + \frac{KT_2}{db_2 + 2} \right)}
\]

The value of genetic variability of a character is determined based on genetic variance (\(\sigma^2_g\)) and the standard deviation of genetic variance. If \(\sigma^2_g > 2 \sigma_{\sigma^2_g}^2\), then the genetic variation is broad, whereas if \(\sigma^2_g \leq 2 \sigma_{\sigma^2_g}^2\) then the genetic variation is narrow [22]. Estimation of broad-sense heritability values (\(h_{bs^2}\)) followed [23]:

\[
\text{Broad sense heritability } \left( h_{bs^2} \right) = \frac{\sigma^2_g}{\sigma^2_p} \times 100\%
\]

With the criteria of \(h_{bs^2}\) (Stanfield, 1991):

- High : \(h_{bs^2} > 50\)
- Medium : \(20 \leq h_{bs^2} \leq 50\)
- Low : \(h_{bs^2} < 20\)

Data qualitative and quantitative morphological characters were entries into MS Excel with the customized format PBSTAT-CL 1.7 software. All data in PBSTAT-CL 1.7 software, were analyzed using the method UPGMA (Unweighted Paired-group Method Arithmetic Average) and a dendrogram was generated.

2.3. Identification of mutant variability based on RAPD markers

This activity includes DNA isolation, purification, determination of the quality and quantity of DNA, as well as amplification reactions.

2.3.1. DNA isolation

DNA isolation using the young leaf and undertaken based on modified method from [25]. DNA concentrations were calculated using [26]. DNA concentration can be calculated by multiplying the absorbance value of 260 nm, the dilution factor (1: 100).

Amplification reaction and electrophoresis

The RAPD primers used were 38 primers (Table 3). The amplification reaction was carried out using a 0.5 ml microtube volume containing 12.5 \(\mu\)l of a mixture of solutions consisting of 6.25 \(\mu\)l kappa taq, 3.25 \(\mu\)l ion free water, 1 \(\mu\)l primer, and 2 \(\mu\)l DNA. The final volume of the amplification reaction mixture is 12.5 \(\mu\)l. PCR condition for DNA amplification was pre denaturation at 92\(^\circ\)C for 2 min, followed by denaturation at 92\(^\circ\)C for 3 seconds, annealing 33\(^\circ\)C for 1 second, extention 92 \(\circ\)C for 2 min and extension at 72 \(\circ\)C for 7 min. Thirty-eight RAPD primers were screened for genetic variability analyses (table 3). The amplified product is given a loading dye and then electrophorized at 1% agarose in a 1x TBE solution with 2 \(\mu\)l (1: 9) red gel.
2.4. Data Analysis

DNA band patterns obtained from molecular activities are converted into binary data by scoring. A value of one (1) if the band/character exist and zero (0) if the band / character does not exist. The binary data were entry using DARwin 6.0.17 software. All data in DARwin 6.0.17 software were analyzed using the UPGMA (Unweighted Paired-group Method Arithmetic Average), and a dendrogram of genetic variability among 25 mutants was generated.

Table 3. RAPD primers used for genetic variability analyses of 25 black pepper mutants

| No | Primers   | Base sequence (5'-3') | No | Primers   | Base sequence (5'-3') |
|----|-----------|-----------------------|----|-----------|-----------------------|
| 1  | OPA-05    | AGGGGTCTTG            | 20 | OPM-18    | CACCATCCGT            |
| 2  | OPA-09    | GGGTAAACGCC           | 21 | OPN-01    | CTCA GTTGG            |
| 3  | OPB-18    | CCACACGAGT            | 22 | OPN-03    | GTACTCCCC             |
| 4  | OPC-01    | TTCGAGGCCAG           | 23 | OPN-05    | ACTGAACGCC            |
| 5  | OPC-02    | GTGAGGCGTGC           | 24 | OPN-06    | GAGGGCCACA            |
| 6  | OPC-04    | CCGCATCTAC           | 25 | OPN-07    | CAGC CCCAGAG          |
| 7  | OPC-05    | GATGACCGGCC           | 26 | OPN-08    | ACCTCAGCT            |
| 8  | OPC-08    | TGGACCGGTG           | 27 | OPN-10    | ACAACTGGGG            |
| 9  | OPC-09    | CTCACCGTGCC          | 28 | OPN-13    | AGGGTCACCT            |
| 10 | OPC-02    | GAGACCAACC           | 29 | OPN-15    | CAGCGACTG            |
| 11 | OPD-11    | AGCGCCATTG           | 30 | OPN-20    | GTGCTCCGT            |
| 12 | OPD-15    | CATCCGGTCT           | 31 | OPO-01    | GGCGTAAG             |
| 13 | OPF-09    | CCAAGCTTTC           | 32 | OP2-02    | AGCTAGCCT            |
| 14 | OPG-13    | CTTCTGCGCCA          | 33 | OP3-03    | GAGGGCTCC            |
| 15 | OJP-01    | CCCGGCATAA           | 34 | OP5-03    | ACTTTGGCGG           |
| 16 | OPJ-09    | TGGACCTCAC           | 35 | OPT-06    | CAAGGGCCAGA          |
| 17 | OPK-12    | TGGCCCTCAC           | 36 | OPW-11    | CTGATGGCT            |
| 18 | OPM-14    | AGGGTCGTTTC          | 37 | OPY-04    | GGTGCTAAAT            |
| 19 | OPM-15    | GACCTACCAC           | 38 | OPZ-20    | ACTTTGGCGG           |

3. Results and Discussion

3.1. Identification of mutant variability based on morphological characters

There was variation on morphological characters among the 25 mutants. On leaf shape, four kinds of shape were observed namely ovate (figure 1-1), elliptical (ovate-elliptic) (figure 1-2), ovate lanceolate (figure 1-3), and cordate (figure 1-5). Similar results were also found in the first vegetative generation (M1V1) [17].

![Figure 1. Variation in the shape of leaf blade on mutants](image-url)

The shape of leaf base from pepper mutant clones varies from cordate (figure 2-A), and round (figure 2-B). Twenty-five pepper mutants, all have the entire leaf margin. The figure shows that the shape of leaf blade and the shape of leaf base vary, while the leaf margin of all pepper mutant clones is uniform. The color of the leaves and stem color in the twenty-five pepper mutant showed variation. The leaf color of pepper mutant plants ranged between 137A and 143A, which belongs to the green group [19]. The
color of the stem in general in the twenty-five pepper mutant clones are categorized as yellow green group (YGG), ranged from YGG 146A, YGG 147A, YGG 147B, YGG 148A, and YGG 148B [19].

![Figure 2. Variation in leaf base on mutants](image)

Quantitative characters observed included leaf number, leaf length, leaf width, plant height, leaf thickness, and stem diameter. Then the data is used to estimate the variation of all observed mutant numbers. Genetic variability, phenotype, and heritability in the broadest sense of quantitative morphological characters are presented in table 4.

Genetic variability of six morphological characters of pepper mutants (table 4), shows that all characters fit into broad criteria according to [22] except leaves thickness. The value of diversity criteria is obtained from a comparison between the diversity of genotypes with twice the standard deviation of genetic diversity. The value of variation criteria on the character of the number of leaves, leaf length, leaf width, plant height, and stem diameter shows the area criteria, which means that the influence of genotype on character formation is greater than the effect of phenotype. In contrast, leaf thickness character has narrow variability criteria which shows that the effect of phenotype is greater than genotype on the appearance of the phenotype formed.

| Characters                  | $\sigma^2_g$ | $\sigma^2_p$ | $2\sigma^2_g$ | Variation criteria | $\sigma^2_p$ | $h^2_{bs}$ | Criteria $h^2_{bs}$ |
|-----------------------------|--------------|--------------|----------------|-------------------|--------------|------------|-------------------|
| Number of leaf              | 8792.3       | 16.01        | 32.02          | H                 | 9056.2       | 97.08      | H                 |
| Leaf length                 | 10107.7      | 16.1         | 32.2           | H                 | 10107.7      | 99.98      | H                 |
| Leaf width                  | 8835.8       | 15.05        | 30.1           | H                 | 8835.9       | 99.99      | H                 |
| Plant height                | 137.63       | 2.05         | 4.1            | H                 | 143.5        | 95.91      | H                 |
| Leaf thickness              | 0.0011       | 0.007        | 0.014          | N                 | 0.0012       | 87.07      | H                 |
| Stem diameter               | 0.157        | 0.077        | 0.154          | H                 | 0.173        | 90.58      | H                 |

Note: Variation criteria: H = high, N= Narrow  h2bs criteria: H= High

Estimation of heritability can be used to determine genetic or environmental factors that most influence the phenotypic appearance of plants [26]. Heritability illustrates how far the phenotype that appears is a reflection of the genotype possessed. Based on [27], values of 87% to 100% indicate that all characters observed have high criteria heritability. This value is obtained from the comparison of genotype variants with phenotype variants. The higher the estimated heritability, indicates that the greater the influence of genotypes on the phenotypes seen in plants [28]. The high estimated heritability also indicates that genetic factors have a greater influence than environmental factors on plant phenotypes [8]. The higher the heritability value, the simpler the selection process and the higher the response to the selection [29].

Dendrogram of the twenty-five pepper mutants was formed into three main groups (figure 3). Group II is based on the same leaf base shape. Group I based on the same leaf color 137A (green group). Group III based on leaf thickness and stem diameter that did not have significant differences.
The dissimilarity coefficient for twenty-five pepper mutant numbers shows a range of values ranging from 0.09-0.62 with similarities formed between 0.38-0.91. The coefficient value of dissimilarity with a value greater than 0.5, among others, 6-1 (0.60), 24-1 (0.52), 25-1 (0.50), 10-5 (0.5), 19-6 (0.62), 19-7 (0.53), 10-8 (0.54), 19-13 (0.50), and 24-13 (0.53). The mutant with the highest dissimilarity value is shown by the relationship number 19-6, which means they have the most distant relationship compared to the relationship between the other mutants. The two mutants have a significant difference in the number of leaves, plant height, leaf blade shape, leaf base shape, leaf color, and stem color. The lowest dissimilarity coefficient value is found in the relationship between mutant number 21-18 (0.09), both of them only have differences in the number of leaves and plant height that are not significant. The next researches are selection against important characters such as foot rot disease, stem borer, abiotic stress, yield and quality.

3.2. Identification of mutant variability based on RAPD markers
The number of primers used to analyse genetic variability using RAPD markers were 38 primers, but only 11 primers showing polymorphism. Amplification of 11 primers for 25 pepper mutants produced 89 bands with 72 polymorphic bands (80.9%). OPC 2, OPC 9, OPN 6, OPN 13 primers showed the highest percentage of polymorphic bands (100%) (table 5).

According to Darojah (2017), the high number of polymorphisms shows the great diversity in DNA samples at the amplified loci. This shows that the increasing number of DNA samples that have sequences that complement with sequences owned by a primer so that the use of the same primer can amplify different loci even in the same species. OPC 5 primers showed a polymorphism band percentage of 86%. Band polymorphisms formed with primers are found at molecular sizes 600bp, 700bp, 800bp, 900bp, 1000bp, 1100bp, 1200bp (figure 4).
Table 5. Percentage of polymorphisms of 11 primers of RAPD markers

| No | Primer  | Number of bands | Number of polymorphic bands | Percentage of polymorphism (%) |
|----|---------|-----------------|-----------------------------|--------------------------------|
| 1  | OPK 12  | 8               | 6                           | 75                             |
| 2  | OPD 2   | 10              | 7                           | 70                             |
| 3  | OPB 18  | 10              | 8                           | 80                             |
| 4  | OPG 18  | 11              | 9                           | 82                             |
| 5  | OPC 2   | 8               | 8                           | 100                            |
| 6  | OPC 9   | 7               | 7                           | 100                            |
| 7  | OPC 5   | 7               | 6                           | 86                             |
| 8  | OPN 6   | 5               | 5                           | 100                            |
| 9  | OPN 20  | 10              | 5                           | 50                             |
| 10 | OPN 13  | 3               | 3                           | 100                            |
| 11 | OPM 15  | 10              | 8                           | 80                             |
|    | Average | 89              | 72                          | 80.9                           |

Figure 4. DNA pattern of 25 black pepper mutants with OPC 5 primer

Figure 5. Dendrogram 25 pepper mutants based on RAPD markers

A dendrogram generated from RAPD markers showed that the dissimilarity coefficient of twenty-five pepper mutant based on 72 polymorphic DNA bands shows values ranging from 0.31-0.98 with a similarity of 0.02-0.69. The coefficient value of dissimilarity with values above 90% is shown by the
relationship between 6 and 1 (0.98), 6 and 3 (0.93), 6 and 4 (0.96), 7 and 6 (0.93), 8 and 6 (0.96), 10 and 6 (0.94), 11 and 6 (0.91), 12 and 6 (0.97), 11 and 6 (0.93), 17 and 6 (0.94), 19 and 6 (0.92), 24 and 6 (0.95), and 25 and 6 (0.91). From these results it can be seen that the mutant number 6 has the farthest relationship compared to other mutants. Whereas the mutants with the lowest dissimilarity coefficient is between number 22 and 15 (0.31) (figure 5).

This result is slightly lower than diversity reported on 24 accessions of local Indian P. nigrum L. obtained a coefficient of diversity between 0.34-0.76 [31]. While from three local varieties of P. nigrum L. in China, diversity was obtained from 0.05-0.11 [32]. The dissimilarity coefficient value with RAPD markers among pepper mutants is higher than the dissimilarity coefficient with morphological markers. The grouping of twenty-five pepper mutants using the RAPD divides the mutant into three groups. Group I has 2 sub-groups, group II has 2 sub-groups, and group III. When directed at the primer used, each primer has a band specification that appears for a specific mutant.

4. Conclusions
There are variations in leaf blade shape, leaf base shape, leaf color, and stem color, among all mutants, but uniform in leaf margin. The estimated value of broad sense heritability of the number of leaves, leaf length, leaf width, plant height, leaf thickness, and stem diameter are high, so that the selection process can be made simpler. Amplification of 11 RAPD primers to 25 pepper mutant produced 89 bands with 72 polymorphic bands so that the percentage of polymorphic bands was around 81%. Primers OPC 2, OPC 9, OPN 6, OPN 13 were 100 % polymorphic. The diversity analysis showed the coefficient of diversity based on morphological characters 0.09-0.62 while based on RAPD markers 0.31-0.98. The mutants with the highest diversity coefficient based on morphological characters and RAPD markers is the mutant number 6 (III.25.28).

References
[1] (Kementerian Pertanian) Kementan, Outlook Lada Komoditas Pertanian Sub Sektor Perkebunan. Pusat Data dan Sistem Informasi Pertanian, Jakarta, 2015.
[2] H. Chao-yun et al., “Modeling The Potential Geographic Distribution of Black Pepper (Piper nigrum) in Asia Using GIS Tools,” J. Integr. Agric., vol. 11, no. 4, pp. 593–599, 2012.
[3] I. Sulistiawati, “Pengetahuan, Sikap, dan Tindakan Petani Terhadap Penyakit Kuning pada Tanaman Lada di Kabupaten Bangka dan Bangka Belitung,” Institut Pertanian Bogor. Skripsi, 2011.
[4] E. M. Abdallah and W. E. Abdalla, “Black Pepper Fruit (Piper nigrum L.) as Antibacterial Agent: A Mini-Review,” J. Bacteriol Mycol Open Access, vol. 6, no. 2, pp. 141–145, 2018.
[5] Anonymous, “Country Profile : Indonesia,” 2016. http://www.ipcnet.org/n/map/index.php?path=map&page=id. [Accessed: 08-Dec-2017].
[6] (Direktorat Jenderal Perkebunan) Directorate General of Plantations, Statistik Perkebunan Indonesia : 2015-2017 Lada, Sekretaria. 2016.
[7] J. Jankowicz-Cieslak, O. A. Huynh, M. Brozynska, J. Nakitandwe, and B. J. Till, “Induction, Rapid Fixation and Retention of Mutations in Vegetatively Propagated Banana,” Plant bBotechnology J., vol. 10, no. 9, pp. 1056–1066, 2012.
[8] S. I. Aisyah, “Induction of Physical Mutagens in Carnations (Dianthus caryophyllus Linn.) and Stability Testing of Propagated Vegetatively Mutants,” Graduate School. IPB University. PhD Dissertation (Ind.), 2006.
[9] A. M. Van Harten, Mutation Breeding of Vegetatively Propagated Ornamentals. Kluwer Academic Press, Boston, 2002.
[10] A. M. Van Harten, “Mutation Breeding,” in Theory and Practical Applications, Cambridge University Press, United Kingdom., 1998.
[11] H. R. Moussa, “Role of Gamma Irradiation in Regulation of NO3 Level in Rocket (Eruca vesicaria subsp. sativa) Plants,” Russ. J. Plant Physiol., vol. 53, no. 2, pp. 193–197, 2006.
[12] B. K. Chakravarthi and R. Naravaneni, “SSR Marker Based DNA Fingerprinting and Diversity
Study in Rice (Oryza sativa L.),” *African J. Biotechnol.*, vol. 5, no. 9, pp. 684–688, 2006.

[13] B. C. Y. Collard, M. Z. Z. Jahufer, J. B. Brouwer, and E. C. K. Pang, “An Introduction to Markers, Quantitative Trait Loci (QTL) Mapping and Marker Assisted Selection for Crop Improvement: The Basic Concepts,” *Euphytica*, vol. 142, no. 1–2, pp. 169–196, 2005.

[14] J. M. Rodriguez, T. Berke, L. Engle, and J. Nienhuis, “Variation Among and Within Capsicum Species Revealed by RAPD Markers,” *Theor. Appl. Genet.*, vol. 99, no. 1–2, pp. 147–156, 1999.

[15] S. V. Tingey and J. P. del Tufo, “Genetic Analysis with Random Amplified Polymorphic DNA Markers,” *Plant Physiol.*, vol. 101, no. 2, pp. 349-352., 1993.

[16] S. Sen, R. Skaria, and P. M. A. Muneer, “Genetic Diversity Analysis in Piper Species (Piperaceae) Using RAPD Markers,” *Mol Biotechnol.*, vol. 46, pp. 72–79, 2010.

[17] N. L. W. Meiawati, “Peningkatan Keragaman Genetik Lada (Piper nigrum L.) Varietas Ciinten melalui Iradiasi Sinar Gamma dan Seleksi Terhadap Penyakit Busuk Pangkal Batang (BPB),” Sekolah Pascasarjana. Institut Pertanian Bogor. Tesis, 2016.

[18] (International Plant Genetic Resources Institut) IPGRI, *Descriptor for black pepper* (Piper nigrum L.). International Plant Genetic Resources Institut, Rome, 1995.

[19] (Royal Horticultural Society) RHS, *RHS Colour Chart: Fifth edition*. The Royal Horticultural Society, London, 2007.

[20] R. K. Singh and B. D. Chaudary, *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, 1979.

[21] R. L. Anderson and T. A. Bancroft, *Statistical Theory in Research*. McGraw-Hill, New York, 1952.

[22] S. Pinaria, A. Baihaki, R. Setiamihardja, and A. A. Darajat, “Variabilitas Genetik dan Heritabilitas Karacter-karakter Biomassa 53 Genotipe Kedelai,” *Zurai*, vol. 6, pp. 88–92, 1995.

[23] A. R. Hallauer and J. B. Miranda, *Quantitative Genetics in Maize Breeding 2nd edition*. 1995.

[24] J. J. Doyle and J. L. Doyle, “Isolation of Plant DNA from Fresh Tissue,” *Focus (Madison).*., vol. 12, pp. 13–15, 1990.

[25] K. Weising, H. Nybom, K. Wolff, and G. Kahl., *DNA Fingerprinting in Plants: Principles, Methods, and Applications*. CRC Press, USA, 2005.

[26] E. Wardiana and D. Pranowo, “Pendugaan Parameter Genetik, Korelasi, dan Klasterisasi 20 Genotipe Jarak Pagar (Jatropha curcas L.),” *Bul. Plasma Nutfah*, vol. 21, no. 1, pp. 1–24, 2010.

[27] W. Stanfield, *Teori dan Soal-soal Genetika*. Apandi, M., L.T. Hardy, penerjemah. Terjemahan dari: Theory and Problem of Genetics 3rd Edition. Erlangga, Jakarta, 1995.

[28] J. Welsh, *Fundamentals of Plant Genetics and Breeding*. John Wiley and Sons Inc, United State of America, 1981.

[29] S. Purwiyantri, “Keragaman Genetik Plasma Nutfah Jahe (Zingiber officinale Rosc.) berdasar Karakter Morfologi dan Penanda RAPD,” Sekolah Pascasarjana. Institut Pertanian Bogor, Master Thesis, 2012.

[30] A. U. Darojah, “Genetic Diversity and Segregation of Ornamental Chili (Capsicum annum L.) Crossimg of Royal Black With Peter pepper Based on RAPD Markers,” Gadjah Mada University. Thesis, 2017.

[31] T. Pradeepkumar, J. L. Karihaloo, S. Archak, and A. Baldev, “Analysis of Genetic Diversity in Piper nigrum L. Using RAPD Markers,” *Genet. Resour. Crop Evol.*, vol. 50, no. 5, pp. 469–475, 2003.

[32] Y. Jiang and J. P. Liu, “Evaluation of Genetic Diversity in Piper spp Using RAPD and SRAP Markers,” *Genet. Mol. Res.*, vol. 10, no. 4, pp. 2934–2943, 2011.