

Genetic Diversity of *Rhizopora mucronata* in the Timor Island as Learning Sources on Genetic Course Based on Local Natural Resources

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ABSTRACT

This research aimed at discovering genetic diversity of *Rhizopora mucronata* in the Timor Island, in which it could be implemented as learning sources on the genetic course based on local natural resources. There were two stages of this research, namely quantitative-qualitative descriptive research, and development research used 4D model. 16 mangroves vegetation existed in the Timor Island were taken as the sample of research. Samples were analyzed at the Cell and Molecular Biology Laboratory and Plant Physiology, Tissue Culture and Microtechnical Laboratory, Brawijaya University. Field test was carried out in 5th semester of Departement of Biology Education, Universitas Muhammadiyah Kupang. The results of this study indicated that the genetic diversity values of (*He*) *R. mucronata* were 0.666. The highest value of genetic diversity (*He*) was in Sumlili, Abudenok, and Tanjung Bastian (0.667), while the lowest value was in Sulamu and Tanjung Tuamese (0.664). Based on the results of dendrogram, generally the *R. mucronata* grouping was not related to the geographical position. Distant populations tended to form one subgroup. The analysis results of genetic diversity were further developed into leaflets, in which it had been validated by the experts. The mean scores of quality assessment of teaching materials were 3.755, while the mean results of material assessment were 3.755. The results of field test or wider scale test show that leaflets used as teaching materials are included in the effective category or can be declared as effective learning resources.

Keywords: Genetic Diversity, *Rhizopora mucronata*, Timor Island, learning sources, leaflet, genetic, local natural resources

1. INTRODUCTION

Timor island is one of areas in East Nusa Tenggara (NTT), Indonesia, in which it is surrounded by the sea. Administratively, the Timor island is divided into four regencies/cities, i.e. Kupang City, Kupang Regency, South Central Timor Regency, North Central Timor Regency and Belu Regency (1). This island is also a land border between Indonesia and República Democrática de Timor-Leste (RDTL) (2). In the coast of Timor Island, mangrove vegetation is often found, one of which is the *Rhizopora mucronata* species [3,4].

Mangrove ecosystem has an important role ecologically, economically, and socially for the coastal communities (5–7). The mangrove ecosystem can grow up well in the tidal zone along the coastline of tropical regions i.e. lagoons, swamps, deltas, and river mouths [8,9]. This ecosystem is complex and dynamic but unstable (10). Factors affecting the zoning distribution are related to the response of plant species to soil conditions, wave exposure, tides and salinity as well as a combination of local chemical and physical conditions. Such a land condition impacts on

the zoning construction of plants and animals distribution i.e. the difference of crab species on the different land condition (11).

Genetic diversity is a gen variation on one species determined by the numbers of gen which have more that alleles (polymorphic genes), and the numbers of alleles for each gen (12). The existence of polymorphic genes also indicated that several individuals in a population have heterozygote gen, in which genetic diversity contributes on the population abilities to adapt on the environmental changes (12). Furthermore, the genetic diversity plays an important role in the adaptabilities of certain species, because when a specie's environment changes, small variations of genes are needed so that the species are able to and adapt (13–15). Various concepts and development on the genetic reviews are necessary to be communicated to the communities, especially to the students/ prospective biology teachers (16–20).

Consequently, during this time the learning process on genetic course carried out by Departement of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Kupang, was still deemed not utilizing the surrounding environment as a source of

learning, and still relied on the use of textbooks that were not in accordance with the learning competencies to be achieved (21). In fact, a learning using textbook seems to still be conventional, and tends not to provide motivation for students in understanding the concepts related to the lecture material provided [22.23]. According to the observation results, discussion with the lecturers and analysis of learning outcomes can be stated that the *genetic diversity* is not yet fully understood by the students. Of course, this will impact on the weak competence of these biology teacher candidates. Of course, this will impact on the weak competence of these biology teacher candidates.

Truthfully, science learning must be designed to educate the students to develop scientific thinking skills or known as scientific learning and participate actively in the learning process (24–27); thus, a pattern on the students' self can be constructed to develop their thinking ability, learning, and caring attitude towards the surrounding natural environment (28). Hence, an appropriate teaching material is needed to be designed so that the *genetic diversity* material is easy to be understood by the students and the learning process becomes more meaningful. The learning is expected to be more meaningful and will be more effective if the learning uses appropriate learning sources (29). One of main components that determines the quality of learning sources is the involvement of environmental elements (30–33). Learning material integration using surrounding environment issues and varied learning method can ease the students to solve the environmental problems and construct the caring attitude towards the surrounding environment. The integration of information, material, and local resources are expected to be able to motivate the students in learning [29.34.35].

The students' creativity can be developed by means of active mindset by utilizing the environment and even local potency as the learning sources (36). Learning sources based on local potential can be packaged in the teaching materials designed to achieve the learning objectives (37). The accuracy in utilizing the learning sources in the teaching and learning processes has a profound effect on the achievement of learning objectives. One of the choices of teaching materials that is thought to be interesting, effective and has an impact is the media of *leaflets*. Some previous studies conclude that *leaflets* are easy to be used as a medium for the delivery of interesting learning materials and reduce boredom. (38). *Leaflet* are usually designed carefully and completed by an illustration using simple languages, short and easy to understand. *Leaflets* should contain materials that can lead the students to master one or more basic competencies (39).

Besides, leaflets are attractive to look at, easy to understand, stimulate the imagination in understanding the content, and are more concise in delivering the information content (40). Another advantage of using leaflets are the target can adapt and learn independently. Leaflets are also practical because they reduce the need for taking notes, the target can see the contents while relaxing. In addition, leaflets are very economical, the various information can be given or read by the members of the target group, so that it can be discussed. Leaflets can provide the detail information which not given

orally, easy to make, to reproduce, to improve and easily adapted to the target group (41), (42). In this research, the researchers integrate the research results about genetic diversity of *Rhizophora mucronata* in the Timor Island.

Scopus and Google Scholar search results show that in a limited amount, the integration of mangroves or the results of mangrove research have been carried out by previous researchers. Some topics that can be raised as examples are "mangrove wetland ecology exploration project" (43), "diversity of mangrove forest gastropods" (5), "mangrove forest genetic resources - a training manual" (44), "mangrove ecosystem introduction on elementary students" (45), "integrating nature conservation and biodiversity education in teaching in general schools" (46), and "booklet development based on research identification of fiddler crab (*Uca* spp.) diversity in mangrove ecosystem". According to previous research data, it can be concluded that there are only five researches/reviews related to "topic mangrove" as a learning source, in which it exists in the books or modules. Those reviews are still general, not specific on the *genetic diversity*, species of *R. mucronata*, and leaflets. Hence, based on the given explanation and it is in line with the needs of genetic learning in the Department of Biology education, this research is necessary to be carried out. Therefore, this research is a development research on the results of previous studies by utilizing the results of research to be used as a learning resource in the form of leaflets, so that this article is different from other existing publication articles. This research contributes in providing an alternative source of genetic learning in college (especially for biology education students – who are future biology teacher candidates). This learning source has an element of novelty because it is developed based on the results of the research of species *R. mucronata* that has been done previously on the Timor Island (47) (based on local resources)..

2. MATERIAL AND METHOD

2.1. Time and Place

Sampling was carried out in 2017 to 2018. There were 16 mangrove vegetation taken as the mangrove leaf samples, in which those were from the Timor Island, NTT including; Kupang Regency (Pulau Pasir, Sumlili, Salupu, Tesabela, Oematnunu, Nunkurus, Bipolo, Pariti, and Sulamu), Kupang City (Paradiso), Malaka Regency (Kletek and Abudenok), and Timor Tengah Utara Regency (Humusu C., Tanjung Bastian, Oemanu, Tanjung Tuamese). Subsequently, the samples were analyzed the Cell and Molecular Biology Laboratory and Plant Physiology, Tissue Culture and Microtechnical Laboratory, Brawijaya University.

2.2. Research Procedures

The research method referred to the stages of Research and Development (39). The research began by analyzing the material requirements. This research was conducted based on observing facts as a systematic sign of plants (48). The data that analyzed were the emergence of DNA bands analyzed to obtain the value of genetic diversity.

The results of *polymerase chain reaction (PCR)* that had been electrophoresis were taken a picture and analyzed using band pattern *scoring*, in which it used the *software* PyElph version 1.4., the bands pattern that emerged (positive) were given the score of 1 and the bands pattern that did not emerge (negative) were given a score of 0. Furthermore, from the calculation results, it was carried out a grouping analysis (*cluster analysis*) using the UPGMA method based on the coefficient similarity of Jaccard's by means of *software* MVSP 3.2 program. The data obtained from the research results were about the genetic diversity; then, those were used as learning sources in which packaged in a learning material in the form of local resource-based *leaflet*.

The development procedures of *leaflets* learning material referred to the development model of Thiagarajan, namely 4-D model (49).

2.3. Teaching Materials Development Model

The development research was carried out in Departement of Biology Education for the students of VA and VB semesters to find out the effectiveness of learning (lectures) in Genetics courses using the teaching materials in the form of *leaflets* after the 4-D development stages. The 4-D development model included of the *Define* stage, the *Design* stage, the *Develop* stage, and the *Disseminate* stage. Data collection techniques used were questionnaire, test, and observation sheets. While the instruments used were expert validation questionnaires, student response questionnaire to learning using *leaflets* that were developed as well as a test on the genetic diversity material. Overall data obtained in this research were analyzed using qualitative descriptive analysis. Overall, the 4 D development stages were described in the following explanation.

2.3.1. Define Stage.

In this stage, the researcher collected the information relating to the product to be developed. The *define* stage included five main stages, namely: a) front-end analysis; b) student analysis; c) task analysis; d) concept analysis; and e) formulation of objectives. The front-end analysis began with the knowledge, skills and initial attitude of students in learning the genetic diversity material. Then, need *assessment* was carried out in the form of material developed in the development results of leaflets. The next

stage was to determine the content of the material to be designed in the learning process or lecture.

2.3.2. Design Stage.

In the design stage, several stages were carried out, namely (1) determining the objectives of developing leaflet teaching materials, (2) preparing benchmark reference tests, namely the initial steps connecting between the define and design stages using a questionnaire, (3) preparation of leaflets teaching materials based on local resources (4) the initial design was the initial result of the product design in the form of a leaflet that would be developed (5) preparation of instruments to measure the validity and effectiveness of the leaflet.

2.3.3. Develop Stage.

The develop stage covered up: Expert validation, products produced from the second stage were then validated by Biology material experts and leaflet teaching material experts so that it could be known whether the products produced were appropriate and suitable for use and the measuring instruments used had met the condition or not yet eligible. In addition to knowing the validity of leaflet teaching material products developed, the validation also aimed at determining the appropriateness of the instruments used to measure the validity and effectiveness of the product. In addition, the validation also aimed at obtaining the recommendations, and suggestions for improvement of products that had been developed.

Data analysis techniques included the feasibility analysis of teaching materials on genetic material leaflets based on validation stages I and stage II by the validators, student responses, and analysis of student test results during the learning process. The data obtained in this research were in the form of qualitative data obtained during a small group test (consisting of 8 students) that were analyzed descriptively qualitatively and converted to a scale table. The scores obtained were then converted to four scale qualitative data adopted from Millah (50), in which those were elucidated on the Table 1.

Table 1. The Qualitative Data of Scale Four

Values	Criteria	Scores	
		Number	Calculation
A	Very Good	4	3.51 – 4.0
B	Good	3	2.51 – 3.50
C	Quite Good	2	1.51 – 2.50
D	Not Good	1	1.00 – 1.50

After the validation by experts, then an analysis of the results of the validation was carried out. If the results of the analysis of draft product data 1 were valid, the product was stated to be suitable for use. Meanwhile, to determine the effectiveness or improvement of student learning outcomes in local resource-based genetic courses was by using the formula 1:

$$g = \frac{\text{posttest scores} - \text{pretest scores}}{\text{max scores} - \text{pretest score}} \quad (1)$$

Then, the results of Gain calculation were categorized into 3 groups (51), in which it was elucidated in the Table 2.

Table 2. Conversion of Gain Scores to Qualitative

Gain Score	Category
$g > 0.7$	High
$0.7 > g > 0.3$	Moderate
$g > 0.3$	Low

2.3.4. Disseminate Stage.

Disseminate stage was the stage of using tools and teaching materials that had been developed on a broader scale. After conducting a field test, and the product leaflets for teaching genetic diversity material were declared to have been valid

and effectively used in the learning process, the next step was to disseminate or spread to other classes.

3. RESULT AND DISCUSSION

3.1. Genetic Diversity of *R. mucronata*

Amplification of DNA bands from 48 *R. mucronata* samples using six RAPD primers, OPA-05. OPA-07. OPA-08. OPA-10. OPA-11 and OPA-14 showed the results of DNA band amplification from each of the various primers with different resolutions ranging from bleak and thin to clear and thick. The PCR product was then screened using PyElph software version 1.4. Then, the results could be analyzed. The DNA bands that were successfully amplified totaled 820 bands with sizes ranging from 200 to 1500 bp as shown in the Figure 1.

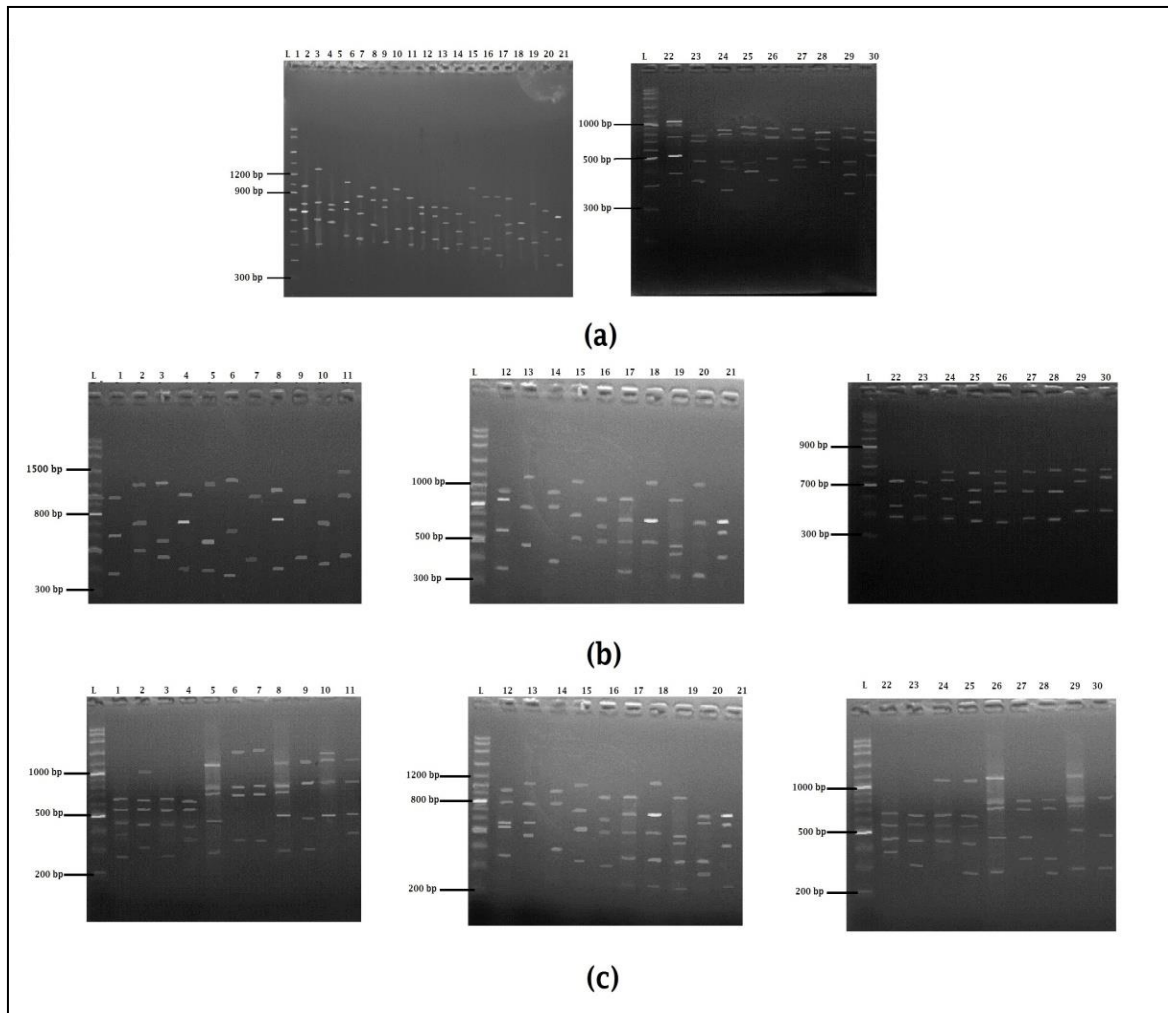


Figure 1. DNA Profile of the Amplification Results of PCR-RAPD Using Primers (a) OPA-05. (b) OPA-07 and (c) OPA-08

Based on the Figure 1, it could be seen that the OPA-05 Primer showed a pattern of DNA bands totaling 23 bands with sizes ranging from 280 to 1345 bp and the percentage of polymorphic bands at 73.91%, OPA-07 primers totaling 23 bands with sizes ranging from 450-1110 bp and polymorphic percentages of 86.96%, and OPA-08 primers amounted to 27 bands with sizes ranging from 270-1315 bp

and the percentage of polymorphic bands was 81.48%. While the level of primary informativeness was 0.93. The DNA band size from PCR-RAPD results, number of DNA bands, percentage of polymorphism and *Polymorphic Information Content* (PIC) of the six primers used in the PCR-RAPD reaction in 48 samples of *R. mucronata* were presented in the Table 3.

Table 3. The Bands Size of DNA *R. mucronata* and the Informativeness of Each Primer

No	Name of Primers	Size Range of bp	Number of Bands	Σ Polymorphic DNA Bands	% Polymorphic	PIC
1	OPA-05	280-1350	124	128	86.96%	0.953
2	OPA-07	300-1480	116	113	93.48%	0.953
3	OPA-08	210-1500	155	150	90.74%	0.953
4	OPA-10	200-1325	139	135	91.305%	0.953
5	OPA-11	200-1120	155	151	90.91%	0.953
6	OPA-14	200-1210	131	127	90.91%	0.953
Total			820	804		

The results of the research in Table 3 above showed that in the 6 (six) primers used, a range of bp size of 200-1500 was obtained with the number of bands ranging from 116-155. While the percentage of polymorphic bands ranged from 86.96-93.48% with *Polymorphic Information Content*

(PIC) of 0.953. The data were then analyzed to determine the level of genetic diversity of *Rhizopora mucronata* growing on Timor Island, East Nusa Tenggara. The Dendrogram results from the analysis of the level of genetic diversity of *R. Mucronata* were presented in Figure 2.

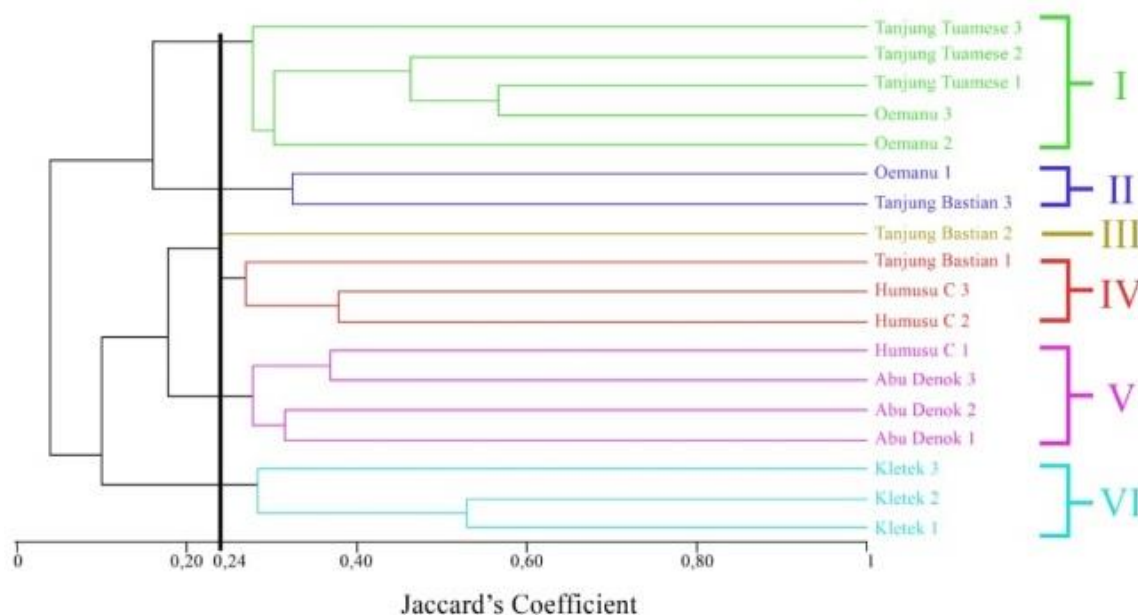


Figure 2. The Dendrogram 18 Mangrove Samples of *R. Mucronata* in the Timor Island Area – NTT. The Numbers to the Right of the Dendrogram Indicated Grouping

Based on Figure 2, it could be seen that 18 samples of *R. mucronata* in the eastern part of Timor Island formed 6 clusters with similarity coefficient values of 0.24 or 24%. Samples that clustered in the first cluster were Oemenu 2, Oemenu 3, and Tanjung Tuamese (1, 2, 3). Samples that clustered in the second cluster were Tanjung Bastian 3 and

Oemenu 1. Samples that clustered in the third cluster were only Tanjung Bastian 2. Samples that clustered in the fourth cluster were Humusu C 2, Humusu C 3, and Tanjung Bastian 1. Samples that clustered in the fifth cluster were Humusu C 1 and Abudenok (1,2,3). While the sixth cluster consisted of Kletek (1, 2, 3).

Meanwhile, 30 mangrove samples of *R. mucronata* in the western part of the Timor Island (unpublished data) formed 3 main groups and formed 12 subgroups with a similarity coefficient value of 0.42 or 42%. The first group formed 2 sub-groups, namely the first sub-group consisting of Pariti 2, Pariti 3, Sulamu 1, Sulamu 2, and Sulamu 3 and the second sub group consisting of Bipolo 1, Bipolo 2, Bipolo 3, and Pariti 1. While the second group consisted of 6 sub-groups, namely the first sub-group that consisted of Paradiso 1 and Oematnunu 3, the second sub-group consisted of Oematnunu 2 and Tesabela 3, the third sub-group consisted of Tesabela 1, Tesabela 2, and Oematnunu 1, the fourth sub-group consisted of Salupu 2 and Salupu 3,

the fifth sub-group consisted of Salupu 1 and Sumlili 3, and the sixth sub-group consisted only of Sumlili 2. The third group consisted of 4 sub-groups namely the first sub-group consisting of Nunkurus 1, Nunkurus 2, Paradiso 2, and Paradiso 3, the second sub-group consisting only of Sumlili 1. The third subgroup consisted of Nunkurus 3 and Pulau Pasir 2, and the fourth sub-group consisted of Pulau Pasir 1 and Pulau Pasir 3. Furthermore, to find out the genetic diversity of the 6 *R. mucronata* populations in northern Timor Island, calculations were made based on the size of the PCR-RAPD band and the number of bands according to the genetic diversity parameters as presented in the Table 4.

Table 4. The Measurement Results of Genetic Diversity on the 16 population of *R. mucronata* in the Timor Island, East Nusa Tenggara.

No	Population	Number of Samples	Na	Ne	He
1	Kletek	3	2.993	25.333	0.666
2	Abudenok	3	3.000	25.000	0.667
3	Humusu C	3	2.997	26.000	0.666
4	Tanjung Bastian	3	2.999	24.667	0.667
5	Oemanu	3	2.997	25.000	0.666
6	Tanjung Tuamese	3	2.974	25.333	0.664
7	Pulau Pasir	3	2.996	12.667	0.666
8	Sumlili	3	2.999	11.167	0.667
9	Salupu	3	2.990	10.667	0.666
10	Tesabela	3	2.998	10.333	0.666
11	Oematnunu	3	2.982	10.833	0.665
12	Paradiso	3	2.990	10.833	0.666
13	Nunkurus	3	2.995	10.500	0.666
14	Bipolo	3	2.976	12.000	0.664
15	Pariti	3	2.987	12.500	0.665
16	Sulamu	3	2.967	11.833	0.663
Total		48	47.84	264.666	10.65
Mean			2.99	16.542	0.666

Notes: Na = Number of alleles observed; Ne = Number of effective alleles; He = Genetic diversity

The parameters used to indicate the genetic diversity in the population were the number of observed alleles (Na), the number of effective alleles (Ne), and genetic variation (He) (52). Based on the Table 4, it could be seen that the diversity value (He) of *R. mucronata* on the Timor Island, East Nusa Tenggara ranged from 0.663-0.667. This indicated that the genetic diversity of *R. mucronata* on the Timor Island was classified as moderate.

The grouping of *R. mucronata* individuals from the same population into one subgroup showed that in a subgroup was influenced by geographical location. This was presumably because the distribution of *R. mucronata* on the Timor Island originated from the same elders which caused a close kinship between the individual plants. This tendency was caused by genetic recombination (53). Genetic proximity between the populations was often associated with geographical proximity (54), although this was not always the case in population genetic studies (55).

The genetic diversity of a population could also occur due to the interaction of several factors, namely mutation, migration, recombination, selection, and drift. Mutation, migration and recombination of genes would enrich diversity in natural populations, whereas genetic selection and drift tended to reduce variation (56).

The existence of difference was also expected due to the distribution of *R. mucronata* assisted by the flow of sea water at low tide. This sea water flow allowed the mangroves of *R. mucronata* to be carried out by currents so that they could be washed away to other locations. In addition, the *R. mucronata* could also do a cross-breed with the help of insects, although it was very unlikely. According to Uslan (57), random marriages allowed the process of gene transfer or migration through pollen transfer and could cover large areas of spread.

High diversity values in the mangrove population of *R. mucronata* could be caused due to environmental conditions

in which to grow. As it was known that the climatic conditions in East Nusa Tenggara, especially on the Timor Island in general was a dry climate. These environmental stress conditions became a limiting factor that had selected the *R. mucronata* for a long time to produce *R. mucronata* that had genetic resistance to environmental conditions. Pratiwi (58) asserted that geographical location could isolate a plant which caused a gene flow to be obstructed. Haryjanto et al., (59) added that variations in a trait in a type of plant could occur between geographical regions. Growing environmental differences could be a major driver in the process of differences in genetic makeup due to local adaptation. The difference in genetic order would affect the appearance of a certain character. In addition, high diversity could also be caused by cross-marriages (*outcrossing*) between the individuals who had distant kinship relationships.

Marriage between the individuals with large genetic distance or extended kinship had the effect of increasing heterozygosity (60). *Outcrossing* could cause random recombination in samples which were very common in types of flowering plants cross pollinating, causing diversity between the individuals (61). While the low value of diversity could be estimated because the marriage that occurred only involved the individuals who were genetically close together. Marriage between the individuals who were genetically close or the same kinship had the effect of increasing the homozygosity (60). Plants that had a close genetic distance illustrated the high genetic similarity that when crossed would produce individual plants with low genetic diversity. Low genetic diversity would have implications for individual survival which was

also quite low due to the lack of diversity of genes derived from the bloodstocks (62).

3.2. Leaflets Design of the Genetic Diversity of *R. mucronata*

To obtain a teaching material in the form of leaflets that met the valid and effective criteria, it had been carried out in accordance with the development stages using the 4-D model. The define stage was carried out in the form of a preliminary analysis which was a needs analysis related to the learning process or lectures in the Genetics course. Based on observations, it was found that the more lectures were provided a lecture with lecture methods and textbooks, in which it had not been able to explain the concept of material that was easily understood by the students. The genetic diversity material was chosen in research development because this material was a material that required more understanding of the concept compared to other material. The initial stage taken was to choose one of the appropriate Basic Competencies.

Development of teaching materials began with analyzing the material needed in accordance with the competencies to be achieved. The leaflet teaching material compiled was the result of research on the genetic diversity of *Rhizopora mucronata* on the Timor Island. The leaflets developed were then used as a learning resource for genetics courses based on the local natural resources. Whereas the Recapitulation of Draft Validation Results I by material experts and teaching material experts was presented in the Table 5.

Table 5. The Results of Leaflets Revision Based on the Validation Results of the Media and Material Experts

Revision Aspects	Before Revision	After Revision
Cover	<ul style="list-style-type: none"> • University logo was laid above the title • The writing of Genetic word should be complete with the course • In order to be interesting and simple, the author's name did not need to use a title • There was a cropped part of the image 	The cover part was updated based on the recommendations
Completeness of Material	<ul style="list-style-type: none"> • Mentioned the population and place to grow the mangrove plants of <i>R. Mucronata</i> • The material was still too long and not very interesting and the objectives had not been conveyed in accordance with the achievements of the Genetic Course learning indicators 	<ul style="list-style-type: none"> • The population of growing place were listed on the material completeness part • The material had been summarized and added interesting sentences according to the indicators of genetic learning achievement

Content Design	<ul style="list-style-type: none"> • There was an error on the writing of mangrove plant name • An error on the similarity placement of dendrogram • The image placement of DNA profile of the PCR results should be ordered based on the primer profile of RAPD listed in the leaflets 	The content design was updated based on the recommendations
Images	Supporting images such as dendrogram, DNA image of PCR results using Transilluminator photo needed to be displayed to clarify the materials; thus, the students learned more about the concept of genetic diversity based on dendrogram and DNA images displayed so that the material about the level of genes as inheritance was not only abstract but also real	The suggested images had been displayed with attractive color gradations so that the students were happy in learning the Genetics Course

The next stage was the *design* and development of leaflets, followed by data validation stage obtained in the development of learning devices and teaching materials consisting of product feasibility testing data, limited trial data, and field trial data. In general, the characteristics of local resource-based leaflets developed were the data and information on local natural resources based on the genetic

information (genetic diversity). Based on the material experts and the teaching material development experts, the leaflets were in the Very Good category and ready to be implemented in the learning process. The results of the *R. mucronata*'s genetic diversity leaflet design was presented in the Figure 3.

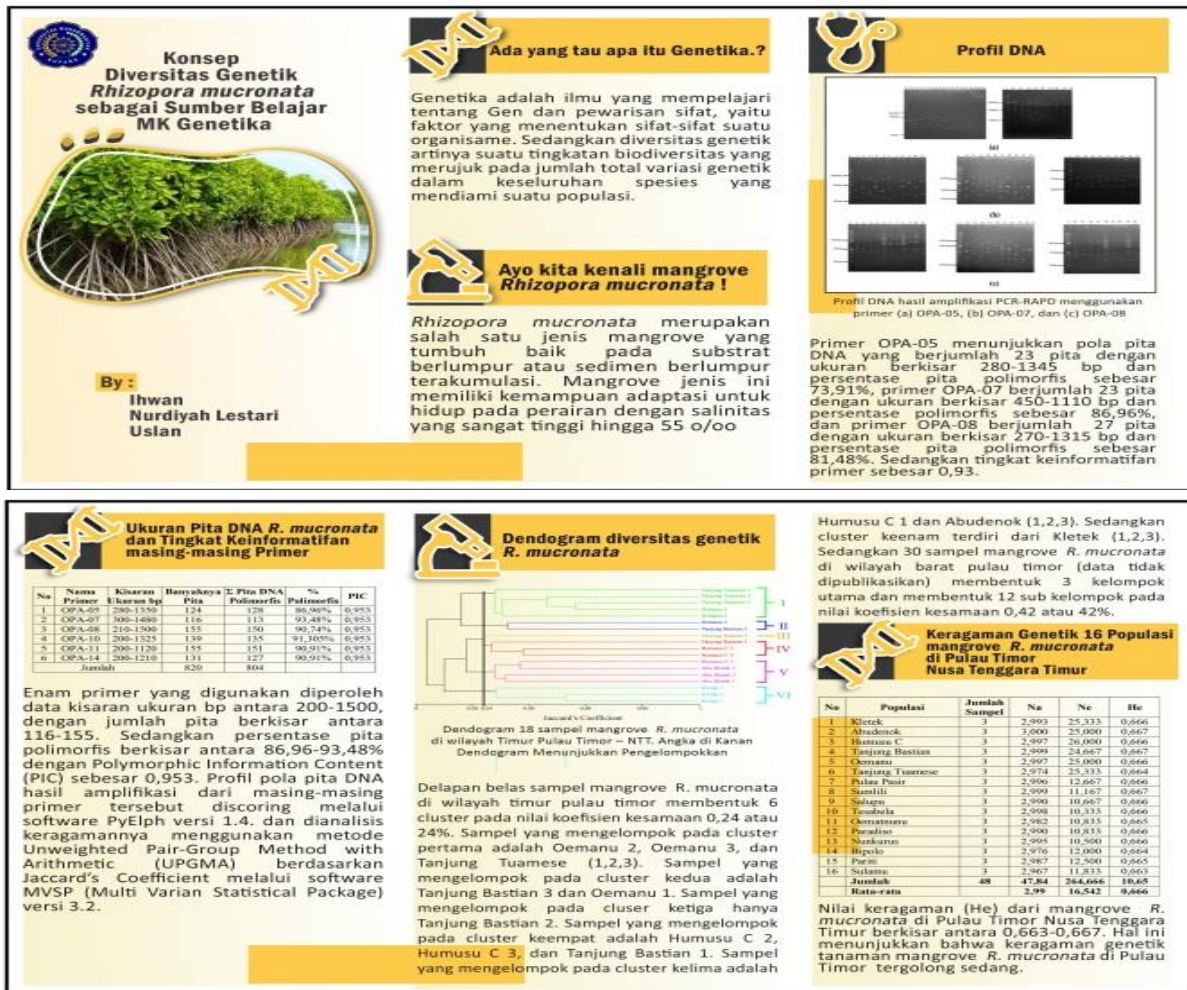


Figure 3. The Genetic Diversity Leaflets of *R. mucronata*

The *develop* stage was a stage that aimed at producing the final product after validation, revision, and small group trials or limited trials. At this stage, all the material and all components supporting the leaflet including the learning tools used were validated by the material experts and the

teaching material experts. The development tests included of individual trials, small group trials, and actual field or class trials. The results of the leaflet assessment by media experts (validator 1) and material experts (validator 2) were presented in Table 6.

Table 6. The Revision Results of Leaflets Based on the Validation Results by the Media and Material Experts

Assessment Aspects	Assessment Results			Category
	Validator 1	Validator 2	Mean	
Cover	3.61	3.60	3.60	Very Good
Contents Design	3.61	3.60	3.60	Very Good
Material Completeness	3.91	3.90	3.90	Very Good
Material Contents / Substances	3.89	3.92	3.90	Very Good
Total	15.02	15.02	15	
Mean	3.755	3.755	3.75	

Based on the data in Table 6 above, it showed the average rating of the developed teaching material was 3.755 while for the average results of the evaluation of the

material developed was 3.755. This assessment was included in the category of very valid so that the *leaflets*

could be used as additional learning resources in Genetics courses based on the local natural resources.

Based on the results of small-scale test, it was carried out revisions in accordance with the recommendation from the material experts and teaching materials experts. The next stage was a field test or a broader scale test conducted on the students in the actual class. In this large-scale test, the research data was collected after the learning was given using leaflets based on local resources, in which it was obtained from the pre-test and post-test scores. The average pre-test and post-test scores and normalized Gain values were presented in Table 7.

Table 7. The Average of *pre-test* and *post-test* Scores, as well as N-Gain

Components	Scores
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Average Scores of <i>pre-test</i>	58.36
Average Scores post-test	74.21
Gain	15.85
Normalized Gain t (N-Gain)	0.95

Based on the Table 7, the average pre-test score was 58.36 and the average post-test score for learning outcomes was 74.21. While the gain score was 15.85 and the N-gain was 0.95. This indicated that the improvement of pedagogic abilities in the Genetics course was in the high category, and shows that the selection of leaflets as teaching materials is very effective. The advantage of using leaflets in learning in Genetics courses was that it could improve the students' ability to understand the concept of genetic diversity more easily. The histogram of the normalized gain value of the study results was presented in Figure 4.

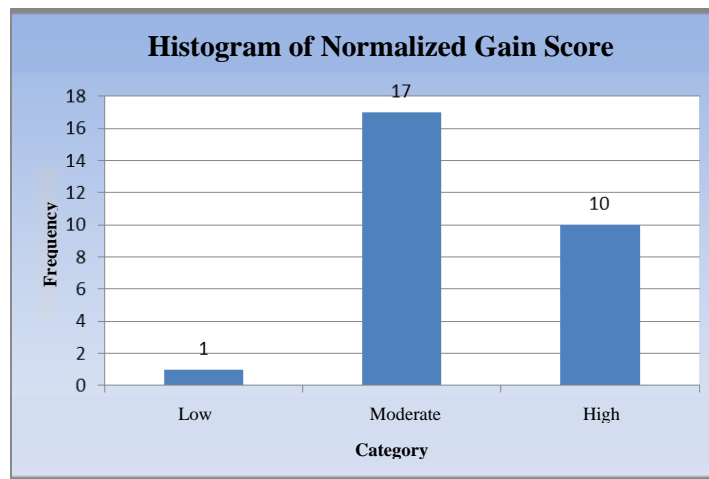


Figure 4. The Histogram of Normalized Gain Scores

The figure 4 showed that the number of students whose normalized gain scores were included in the low category was 1 person, the normalized Gain value was 17 people, and 10 students were included in the high normalized Gain score category. This indicated that the learning using genetic diversity material leaflets eased the students to learn the concept of material when compared to the learning using the books since the material contained in leaflets were usually arranged systematically and sequentially

4. CONCLUSION

Departing from the explanation above, it can be concluded that the genetic diversity values (*He*) of *R. mucronata* population that grow in the Timor Island are 0.666, in which the highest genetic diversity value (*He*) is 0.667 located at Sumlili, Abudenok and Tanjung Bastian; while the population that show the lowest genetic diversity is 0.664 at Sulamu and Tanjung Tuamese. Population grouping of *R. mucronata* in the Timor Island is not related with the

geographical position. Distant populations have a tendency to form one sub group. The assessment results of teaching material are 3.755 whereas the material assessment results are 3.755, as well as the leaflets developed is categorized in a very good category. The broader results of field test and scale test show that the leaflets used as the teaching material are categorized as very effective or be able to stated as very effective learning sources. Then, the implementation of leaflets can be reviewed from the effectiveness on semester level, background, students' initial ability, and lecturers' competencies. Those are recommended to be carried out by further researchers.

ACKNOWLEDGMENT

We would like to thank Ahmad Yani, Abdul Hakim, Vinsensius K. Sabon, Bayu Albarkah, Abka Abdullah, Dian Lestari, Ahmanat Mustafa, Ibnu Rusid, and Maria Fatima Hoar for their assistance during this study. We also express our gratitude to the Ministry of Research, Technology, and Higher Education for funding this study under the PEKERTI scheme 2017 and 2018.

REFERENCES

1. Kause WL, Helfiarne MR, Komba YT, Salim A, Djesse ST, Pendidikan B, et al. Study of NTT province's status as an island province: Overview from law perspective. *J Borneo Adm.* 2013;9(2):137–61.
2. Mangku DGS. Implementasi Joint Border Committee (JBC) untuk penyelesaian sengketa perbatasan darat antara Indonesia-Timor Leste. *J Yuridis.* 2017;5(1):43–66.
3. Kurniadi R, Koeslulat EE. Short Communication: The tolerance level of local sorghum genotypes from Sabu-Raijua and Belu Districts, Indonesia to saline soil. *Trop Drylands.* 2018;4(1):1–4.
4. Alongi DM. In the coast of Timor Island, mangrove vegetation is often found. In: Faridah-Hanum I, Latiff A, Hakeem KR, Ozturk M, editors. *Mangrove Ecosystems of Asia: Status, Challenges and Management Strategies.* 2014. p. 1–471.
5. Romdhani AM, Sukarsono S, Susetyarini RE. Keanekaragaman Gastropoda Hutan Mangrove Desa Baban Kecamatan Gapura Kabupaten Sumenep sebagai Sumber Belajar Biologi. *JPBI (Jurnal Pendidik Biol Indones.* 2016;2(2):161–7.
6. Setyoko S, Rohman F, Suwono H. Development of Animals Ecology Module for Macrozoobentos Community at Higher Institution. *JPBI (Jurnal Pendidik Biol Indones.* 2017;3(1):80–7.
7. Ajizatunnisa A, Wahyuni S, Waluyo L, Miharja FJ. Booklet development based on research identification of fiddler crab (*Uca* spp.) diversity in mangrove ecosystem. *JPBI (Jurnal Pendidik Biol Indones.* 2018;4(1):61–6.
8. Kathiresan K, Bingham BL. *Biology of Mangroves and Mangrove Ecosystems.* Vol. 40, *Advances in Marine Biology.* 2001. 81–251 p.
9. Sukardjo S. Fisheries associated with mangrove ecosystem in Indonesia: A view from a mangrove ecologist. *Biotropia (Bogor).* 2004;2004(23):13–39.
10. Samad A, Burhanuddin B, Iskandar AM. Association of Arbuscular Mycorrhizal Fungi (FMA) on the *Rhizophora* spp In The Terusan Village Mempawah Hilir Districts West Kalimantan. *J Tengkwang.* 2017;7(1):11–8.
11. Rusydi R, Ihwan I, Suaedin S. Struktur Dan Kepadatan Vegetasi Mangrove Di Teluk Kupang. *J Segara.* 2015;11(2):147.
12. Indrawan M, Primack RB, Supriatna J. *Biologi konservasi.* Jakarta: Yayasan Obor Indonesia; 2007. 625 p.
13. Razgour O, Forester B, Taggart JB, Bekaert M, Juste J, Ibáñez C, et al. Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proc Natl Acad Sci U S A.* 2019;116(21):10418–23.
14. Mable BK. Conservation of adaptive potential and functional diversity: integrating old and new approaches. *Conserv Genet.* 2019;20(1):89–100.
15. Jill T. Anderson, Willis JH, Mitchell-Olds T. Evolutionary genetics of plant adaptation Jill. *Trends Genet.* 2011;27(7):258–266.
16. Donovan BM, Semmens R, Keck P, Brimhall E, Busch KC, Weindling M, et al. Toward a more humane genetics education: Learning about the social and quantitative complexities of human genetic variation research could reduce racial bias in adolescent and adult populations. *Sci Educ.* 2019;103(3):529–60.
17. Illingworth S, Prokop A. Science communication in the field of fundamental biomedical research (editorial). *Semin Cell Dev Biol.* 2017;70:1–9.
18. Abrori FM, Adhani A. Developing an atlas based on frond venation patterns analysis of polypodiales in Tarakan. *JPBI (Jurnal Pendidik Biol Indones.* 2017;3(3):222–37.
19. Fleischner TL, Espinoza RE, Gerrish GA, Greene HW, Kimmerer RW, Lacey EA, et al. Teaching Biology in the Field: Importance, Challenges, and Solutions. *Bioscience.* 2017;67(6):558–67.
20. Slamet A, Taharu FI, Hudha AM. Developing genetic learning module based on blue eyes phenomenon in Buton Island, Southeast Sulawesi. *JPBI (Jurnal Pendidik Biol Indones.* 2019;5(1):69–76.
21. Lestari N, Basri KI, Yusuf SM, Suciati S, Masykuri M. Life Skill Integrated Science-PBL Module to Improve Critical Thinking Skills of Secondary School Students. *Univers J Educ Res.* 2020;8(7):3085–96.
22. Milrad M. Designing an Interactive Learning Environment to Support Children ' s Understanding in Complex Domains Designing an Interactive Learning Environment to Support Children ' s Understanding of Complexity. *Thinking.* 1998;(September 1999):1–178.
23. Dunlosky J, Rawson KA, Marsh EJ, Nathan MJ, Willingham DT. Improving students' learning with effective learning techniques: Promising directions from cognitive and educational psychology. *Psychol Sci Public Interes Suppl.* 2013;14(1):4–58.
24. Hayati N, Berlianti NA. Critical thinking skills of natural science undergraduate students on biology subject: Gender perspective. *JPBI (Jurnal Pendidik Biol Indones J Biol Educ.* 2020;6(1):83–90.
25. Ilma S, Al-Muhdhar MHI, Rohman F, Saptasari M. The correlation between science process skills and biology cognitive learning outcome of senior high school students. *JPBI (Jurnal Pendidik Biol Indones.* 2020;6(1):55–64.
26. Nursa'ban E, Masykuri M, Yamtinah S. Improving student learning outcomes in science subjects

- through the implementation of PBL-based module. JPBI (Jurnal Pendidik Biol Indones. 2019;5(2):269–76.
27. Hernawati D, Amin M, Al Muhdhar MHI, Indriwati SE. Science literacy skills through the experience of project activities with assisted local potential based learning materials. JPBI (Jurnal Pendidik Biol Indones. 2019;5(1):159–68.
 28. Kurniawati MW, Anitah S, Suharno S. Developing Learning Science Teaching Materials Based on Scientific to Improve Student Learning Outcome in Elementary School. Eur J Educ Stud. 2017;34(20):319–30.
 29. Supriadi S. Pemanfaatan Sumber Belajar Dalam Proses Pembelajaran. Lantanida J. 2017;3(2):127–39.
 30. Graetz KA. The Psychology of Learning Environments. In: Oblinger DG, editor. Learning Spaces. Winona State University; 2006. p. 6.1–6.14.
 31. Jalloh MG, Schmalz K. Acquiring and Utilizing Resources To Enhance and Sustain a Safe Learning Environment. Northwest Regional Educational Laboratory; 2002. 1–50 p.
 32. Akareem HS, Hossain SS. Determinants of education quality: what makes students' perception different? Open Rev Educ Res. 2016;3(1):52–67.
 33. Milrad M. Designing an Interactive Learning Environment to Support Children's Understanding in Complex Domains. EdMedia + Innovate Learning. Waynesville, NC: Association for the Advancement of Computing in Education (AACE); 1999. 1–178 p.
 34. Mumpuni KE, Susilo H, Rohman F. Potensi tumbuhan lokal sebagai sumber belajar biologi. In: Seminar Nasional XI Pendidikan Biologi FKIP UNS. Surakarta, Central Java, Indonesia: Pendidikan Biologi FKIP UNS; 2013. p. 825–9.
 35. Hafid HA. Sumber dan Media Pembelajaran. J Sulesana. 2011;6(2):69–78.
 36. Susilo MJ, Munajah. Potensi Sumber Belajar Biologi SMA Kelas X Materi Keanekaragaman Tumbuhan Tingkat Tinggi di Kebun Binatang Gembira Loka. Jupemasi-Pbio. 2015;1(2):184–7.
 37. Situmorang R. Analisis Potensi Lokal Untuk Mengembangkan Bahan Ajar Biologi di SMA Negeri 2 Wonosari. J Pendidik Sains Univ Muhammadiyah Semarang. 2016;4(1):51–7.
 38. Majid A. Perencanaan pembelajaran: Mengembangkan standar kompetensi guru /. Bandung: Remaja Rosdakarya; 2006. 291 p.
 39. Karyadi B, Ruyani A, Susanta A, Dasir S. Pembelajaran Sains Berbasis Kearifan Lokal pada Sekolah Menengah Pertama di Wilayah Bengkulu Selatan (Pemanfaatan Ikan Mungkus (*Sicyopterus cynocephalus*) Sebagai Sumber Belajar dalam Pembelajaran Sains di SMPN 20 Bengkulu Selatan). In: Seminar Nasional Pendidika Sains. Surakarta, Central Java, Indonesia: Universitas Sebelas Maret; 2016. p. 231–8.
 40. Falasifah. Pengembangan Bahan Ajar Berbentuk Leaflet Berbasis Sejarah Lokal Dengan Materi Pertempuran Lima Hari Di Semarang Pada Siswa Kelas XI IPS Di SMA Negeri 2 Pemalang Tahun Ajaran 2013-2014 [Internet]. Skripsi. Universitas Negeri Semarang; 2014. Available from: <http://lib.unnes.ac.id/20172/1/3101410001.pdf>
 41. Suiroaka IP, Supariasa IDN. Media Pendidikan Kesehatan. Pertama. Yogyakarta: Graha Ilmu; 2012. 116 p.
 42. Lucie S. Teknik Penyuluhan & Pemberdayaan Masyarakat. Jakarta: Ghalia Indonesia; 2005. 83 p.
 43. Chang CS, Wong WT, Chang CY. Integration of project-based learning strategy with mobile learning: Case study of mangrove wetland ecology exploration project. Tamkang J Sci Eng. 2011;14(3):265–73.
 44. Deshmukh S V, Balaji V. Conservation of Mangrove Forest Genetic Resources - a Training Manual. Madras: M. S. Swaminathan Reserach Foundation; 1994. 1–455 p.
 45. Purwanty R, Sunarni, Monika NS, Tambaru R, Fakhriyyah S. Mangrove ecosystem introduction on elementary students through nature school in coastal area of Samkai urban village Merauke regency. IOP Conf Ser Earth Environ Sci. 2019;235(1).
 46. Oai LT, Thanh Hoi PT. Integrating Nature Conservation and Biodiversity Education in Teaching in General schools in Vietnam. J Phys Conf Ser. 2019;1340(1).
 47. Ihwan, Uslan, Widodo, Hakim L. Genetic diversity of *Rhizophora mucronata* in eastern region of Timor Island, Indonesia as revealed by RAPD. Biodiversitas. 2019;20(11):3364–71.
 48. Hadi A, Irawati M, Suhadi S. Karakteristik Morfo-Anatomi Struktur Vegetatif Spesies *Rhizopora Apiculata* (Rhizoporaceae). J Pendidik - Teor Penelitian, dan Pengemb. 2016;1(9):1688–92.
 49. Trianto T. Mendesain model pembelajaran inovatif-progresif. Jakarta: Kencana Prenada Media Group; 2009. 314 p.
 50. Millah E. Pengembangan buku ajar materi bioteknologi di kelas XII SMA IPIEMS Surabaya berorientasi sains, teknologi, lingkungan, dan masyarakat (SETS). BioEdu. 2012;1(1):19–24.
 51. Hake RR. Analyzing change/gain scores. 1999;
 52. Finkeldey R, Hattermer HH. Tropical Forest Genetics. Springer-Verlag Berlin Heidelberg; 2007. 316 p.
 53. Poerba YS, Martanti D. Genetic variability of *Amorphophallus muelleri* Blume in Java based on Random Amplified Polymorphic DNA. Biodiversitas, J Biol Divers. 2008;9(4):245–9.

54. Sreekanth PM, Balasundaran M, Nazeem PA, Suma TB. Genetic diversity of nine natural *Tectona grandis* L.f. populations of the Western Ghats in Southern India. *Conserv Genet.* 2012;13(5):1409–19.
55. Tsuda Y, Ide Y. Wide-range analysis of genetic structure of *Betula maximowicziana*, a long-lived pioneer tree species and noble hardwood in the cool temperate zone of Japan. *Mol Ecol.* 2005;14(13):3929–41.
56. Bagindo M. Keragaman Genetik Aksesori Pinang (*Areca catechu* L.) Asal Papua, Sulawesi Utara dan Sumatera Utara (Random Amplified Polymorphic DNA). Institut Pertanian Bogor; 2011.
57. Uslan U. Analisis Keragaman Genetik Tumbuhan Faloak (*Sterculia quadrifida* R.Br) Yang Tumbuh Di Kota Dan Kabupaten Kupang Nusa Tenggara Timur Berdasarkan Penanda RAPD (Random Amplified Polymorphic DNA). Program Pascasarjana Universitas Udayana; 2015.
58. Pratiwi P. Analisis variasi genetik beberapa populasi *Globba leucantha* Miq. di Sumatera Barat dengan Random Amplified Polymorphic DNA (RAPD). Program Pascasarjana Biologi Universitas Andalas; 2012.
59. Haryjanto L, Prastyono. Variasi pertumbuhan Nyawai (*Ficus variegata* Blume) pada umur 2 tahun. *Wasian.* 2015;2(1):47–54.
60. JULISANIAH NI. Cucumber (*Cucumis sativus* L.) relationship analysis using RAPD-PCR and isozyme methods. *Biodiversitas, J Biol Divers.* 2008;9(2):99–102.
61. Poerba YS, Wawo AH. Keragaman Fenotipe RAPD *Santalum album* L. di Pulau Timor bagian timur [RAPD phenotypic variation of *Santalum album* L. in Eastern Part of Timor]. *Berita Biol.* 2007;8(6):537–46.
62. Yulita KS, Naiola BP. Keragaman Genetik Beberapa Aksesori Jagung dari Nusa Tenggara Timur Berdasarkan Profil Inter Short Sequence Repeat (ISSR) (Genetic Diversity of Several Accessions of Maize from East Nusa Tenggara Based on Inter Short Sequence Repeat (ISSR) profiles). 2013;9(2):255–63.