CHARACTERISTIC OF LOCAL SWAMP BUFFALO (*BUBALIS BUBALIS* LINN.) GENETIC VARIATIONS IN RAMBUTAN SUB-DISTRICT, SOUTH SUMATRA BASED ON POLYMERASE CHAIN REACTION-RANDOM AMPLIFIED POLYMORPHIC DNA (PCR RAPD)

Rahmat Pratama[1], Yuanita Windusari[2,*], Laila Hanum[2], Rizki Palupi[3], Afnur Imsya[3], Erwin Nofyan[2] and Melda Oktapia[2]

ABSTRACT

Swamp buffalo (*Bubalus bubalis*) is a germ plasm specific of Pampangan and endemic in South Sumatra with low productivity and limited distribution so it was important to analyze characteristics of Genetic Variation Buffalo Swamp in Pampangan. The method used is Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD). data were analyzed using genetic variations of the NTSys program ver. 2.1 and presented in the form of dendrogram. The dendogram shows that the South Sumatra swamp buffalo consists of 2 clusters, namely cluster 1 consisting of black buffalo, and cluster 2 consisting of Lampung buffalo, striped buffalo and red buffalo. The closest genetic distance value from the swamp buffalo variant was 0.46 found in Lampung, striped and red buffalo variants, while the furthest genetic distance was found in black buffalo.

Keywords: *Bubalus bubalis*, buffaloes, Pampangan swamp buffalo, bovinae, Artiodactyla, PCR-RAPD, genetic variation

INTRODUCTION

Swamp buffalo (*Bubalus bubalis* Linn.) Pampangan is a native buffalo variety and one of the germplasm riches in South Sumatra with distribution covering the Pampangan Sub-district (Ogan Ilir and Ogan Komering Ilir Regency) and Banyuasin regency. According to Windusari *et al*. (2016); Pratama *et al*. (2019), based on morphological characteristics, there are four variants of swamp buffalo found in the Sub-district of Pampangan, South Sumatra, namely black buffalo, red buffalo, striped buffalo, and Lampung buffalo.

Some researchers have conducted morphological analysis and genetic variation approaches to estimate the relationship of kinship and genetic variation in local buffalo swamps in South Sumatra. Among them, Windusari *et al*. (2015), reported on the analysis of the habitat of the swamp buffalo (*Bubalus bubalis*) Pampangan, Rambutan Sub-district, Banyuasin regency, South Sumatra. Windusari *et al*. (2016), reported on the diversity and kinship of swamp buffalo (*Bubalus bubalis*) in Pampangan, South Sumatra based on morphological characteristics. Windusari *et al*. (2017); Windusari *et al*. (2019) reported on genetic

[1]Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya, South Sumatra, Indonesia
[2]Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University, South Sumatra, Indonesia, *E-mail: ywindusari@gmail.com
[3]Department of Technology and Livestock Industry, Faculty of Agriculture, Sriwijaya University, South Sumatra, Indonesia

variation in local swamp buffalo (*Bubalus bubalis*) Pampangan based on the profile of blood plasma proteins.

Some researchers have not much information in molecular DNA, especially the local swamp buffalo Pampangan, Banyuasin Regency. Therefore it is necessary to conduct research on "Characteristics of genetic variations in swamp buffalo (*Bubalus bubalis* Linn.) in Pampangan, Rambutan Sub-district, Banyuasin regency based on Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD)". The RAPD method is a DNA analysis technique that many researchers use because it is faster, cheaper than other methods to detect variations of DNA sequences without requiring prior information. The advantage of this technique is that it can analyze genetic distances, reconstruct phylogeny and require small amounts of DNA (Paraguison *et al*., 2012; Sangwan, 2012).

MATERIALS AND METHODS

Time and place of research (study area)

This research was conducted in October 2018 until July 2019. The process of DNA isolation, and PCR-RAPD was carried out in the Biotechnology laboratory, Faculty of Mathematics and Natural Sciences, Sriwijaya University. Swamp buffalo blood samples (*Bubalus bubalis* Linn.) were taken in Pampangan, Rambutan Sub-District, Banyuasin regency and the blood sampling method is Purposive random sampling. Blood is taken as much as 200 µl from 4 variants of the Pampangan swamp buffalo (black buffalo, Lampung buffalo, striped buffalo and red buffalo) with the number of samples for each variant is 2 individuals.

MATERIALS AND METHODS

The tools used in this study are stationery, label paper, 3 ml syringe, pain 10 ml vacutainer, centrifuge, parafil paper, electrophoresis (electrophoresis), hot plate, UV device, digital camera, water bath, microtube, micropipette, microtip, microtip, freezer, vortex and PCR. The materials needed in this study are swamp buffalo blood from the Sub-district of Rambutan, Banyuasin regency as much as 200 µl, alcohol, cotton, distilled water, agarose 1%, TNIamp blood DNA Kit consists of (Buffer CL, Buffer GS, Buffer GB, Buffer GD, Buffer PW, Buffer TB and proteinase K), RAPD primer (ILO 1204, ILO 525, IL0 1212, 0PW 13, and OPY 13), 2X Taq plus master mix with dye consisting of (0.1 ul Taq plus polymerase, 500 uM dNTP each, 20 mM Tris-HCL (pH 8.3), 100 mM KCL, 3 mM MgCl2, stabilizer and enchancer).

Isolation of blood DNA

The process of DNA isolation is carried out in accordance with TNIamp Blood DNA Kit work procedures.

Eletrophoresis

DNA isolation results in electrophoresis using 1% agarose gel by dissolving 0.5 g of agarose with 50 ml TAE 1x and heated on a hot plate until all the powder dissolves. After a little cold, add 2 µl of red gel, shake until completely mixed and pour into the mold. 5 ml sample was taken and mixed with 2 ml loading buffer by inverting until homogeneous. Then put into the first and last wells with a voltage of 80 volts, 400 mA for 45 minutes. After running, a visualization using UV-transmulator is performed to see the electrophoresis results and to be photographed as

the result of documentation (Othman *et al*., 2012).

PCR product loading and running and RAPD analysis

The PCR composition was made with a volume of 25 µl consisting of (2x) taq plus master mix (12.5 µl), 2 µl of primary RAPD (ILO-1204, ILO-1212, ILO-525, OPW-03, OPY-13), DNA Tamplate 6 µl and ddh_2O 4,5 µl. The DNA amplification reaction was carried out under the following PCR conditions: Pre-denaturation of 95°C for 7 minutes, followed by denaturation of 95°C for 1 minute, annealing 34 to 36°C for 30 seconds, extension 72°C for 1 minute repeated 30 times.

Electrophoresis of PCR-RAPD DNA amplification results

The results of the amplification were then electrophoresed at 1% agarose concentration with a voltage of 60 volts for 60 minutes to get the DNA band or band from the PCR-RAPD results (Koh *et al*., 1998).

Data analysis

Determination of the size of DNA bands amplification is done by measuring the distance of standard DNA migration (1kp ladder) starting from the pit to the place of DNA migration, so that binary data is obtained either stated (either) or absent (0). Then the data were analyzed using genetic variations of the NTSys program ver. 2.1 and presented in the form of dendrogram. Dendrogram created using Unweighted Pair-Group With Arithmetic Average or UPGMA with similarity coefficient using Jaccard Coefficient of Similarity (Hanum *et al*., 2017).

Polymorphic percentage analysis is calculated to see what percentage of polymorphism is formed in each primer used. According to (Zarringhabaie *et al*., 2012; Simbolon *et al*. 2017) calculate the percentage of polymorphic bands, the formula is used:

$$\text{Polymorphic percentage} = \frac{\text{NPB} + \text{NMB}}{\text{NPB}} \times 100\%$$

NPB = Number of Polymorphic Bands

NMB = Number of Monomorphic Bands

RESULTS AND DISCUSSION

The results of RAPD PCR amplification using 5 primers for blood samples of 4 variants of the Pampangan swamp buffalo showed that each primer was able to produce DNA bands with unequal band intensity (Figure 2). Sunandar and Imron (2007), stated that the intensity of the amplified band was influenced by $MgCl_2$ concentration, Taq DNA polymerase enzyme, and annealing temperature. Purwanta (2009), explains the success of amplification is determined by the primary ability to (1) amplify printed DNA with the help of the enzyme DNA polymerase and the appropriate temperature; (2) primary adhesion at the printed DNA site; and (3) DNA polymerization. In addition, the success of a primer in amplifying printed DNA is determined by the presence or absence of homology between the primary nucleotide sequence and the printed DNA nucleotide sequence.

The PCR-RAPD technique can detect polymorphic DNA caused by the absence of amplification at a locus caused by differences in the nucleotide base sequence at the primary attachment point. The polymorphism produced by PCR-RAPD technique is caused by changes in nucleotide bases, deletions, and insertions

(William *et al*. 1990; Semagn *et al*. 2006).

The presence of polymorphic bands in the amplification results indicates the presence of nucleotide components. Different nucleotides can be caused by deletions or insertions in DNA. This factor causes the RAPD band to show polymorphic results (Naurala and Srivastava, 2005). Insertion and deletion are the addition or loss of nucleotide pairs in genes (Suprabha *et al*., 2005).

The success of genomic DNA amplification using the RAPD technique is determined by the primary base sequence and the suitability of PCR conditions such as annealing temperature and extension. According to Salem *et al.* (2010), annealing temperature is the temperature to attach the primer to the DNA template. The high temperature is calculated based on the Melting temperature (Tm) value for each primer. Melting temperature (Tm) is the temperature when 50% of the DNA double chains are separated. Handoyo and Ari (2010) also emphasized that choosing the primary Melting temperature (Tm) is very important. Melting temperature (Tm) is related to the primary composition and length of the primer, and is calculated according to the formula (2 (A + T) + 4 (C + G)). In this study, the anneling temperature for primary ILO 1204, ILO 1212, OPY 13 and OPW 03 is 34^{o}C, while for primary ILO 525 is 36^{o}C.

The results of this study indicate that the banding pattern produced from the 5 primers used shows different banding patterns. The resulting band size varies between 200 bp to 1900 bp. Total band from 5 to as much as 20 primary emerging band with the average 4 tape polymorphic for each primer, and none were found tape monomorphic or polymorphic percentage of 100%. These results support the study of Windusari *et al*. (2018), which states that the use of primary OPW 03 in swamp buffalo blood samples in South Sumatra produces 15 polymorphic bands (23.81%) which are more than polymorphic bands than other primers (ILO 1204 as many as 13 DNA bands (20, 63%); ILO 1212 11 DNA bands (17, 46%), OPY 13 8 DNA bands (12, 70%), and ILO 525 does not produce polymorphic bands.

The number of polymorphic bands formed shows the genetic variation of Pampangan swamp buffaloes is low except for black buffalo variants which have high genetic variation. This is indicated by the more polymorphic bands formed in the ILO 525 primer (Figure 1). Windusari *et al*. (2017), stated that the presence of transferrin locus in black buffalo blood protein shows high genetic variation because the presence of this locus is an indication of polymorphic characteristics. Locus transferrin in blood protein buffalo there are two kinds of alleles Tf^{A} to 0.7500 genotype and allele frequencies Tf^{B} with genotype frequency of 1.000. Black buffalo only has Tf^{B} allele which is an indication of polymorphic characteristics, while other buffalo variants have both types of alleles.

The value of heterozygosity at the locus can be caused by high population dynamics (Sumantri *et al*., 2007). Some of the factors that affect it is the presence of a mutation, migration, and cross (Bourbon, 2000). The diversity of protein types at each locus caused by the existence of a mating system. Marriage in one nation tends to increase homozygosity genes and marriage with other nations will increase heterozygosity genes which also shows genetic variation (Rao *et al*., 1996).

Kinship of Pampangan local swamp buffalo

The kinship between the variants of the *Bubalus bubalis* Linn. swamp in South Sumatra is shown in Figure 3.

The dendogram shows that the South

Table 1. The percentage of DNA bands swamp buffalo (*Bubalus bubalis* Linn.) Pampangan of RAPD PCR results.

No	Primary type	Band length	Band		Total bandwidth specified	Polymorphic%
			Polymorphic	Monomorfik		
1.	ILO 1204	GCAGGCGCAA	4	0	4	100
2.	ILO 1212	GCGGCCGTAA	4	0	4	100
3.	ILO 525	CGGACGTCGC	3	0	3	100
4.	OPY 03	GTCCGGAGTG	3	0	3	100
5.	OPW 13	GCGTCTCGGT	6	0	6	100
Average			4	0	4	100

Figure 1. Four variants of the Pampangan swamp buffalo. (a: red buffalo, b: Lampung buffalo, c: black buffalo, d: striped buffalo).

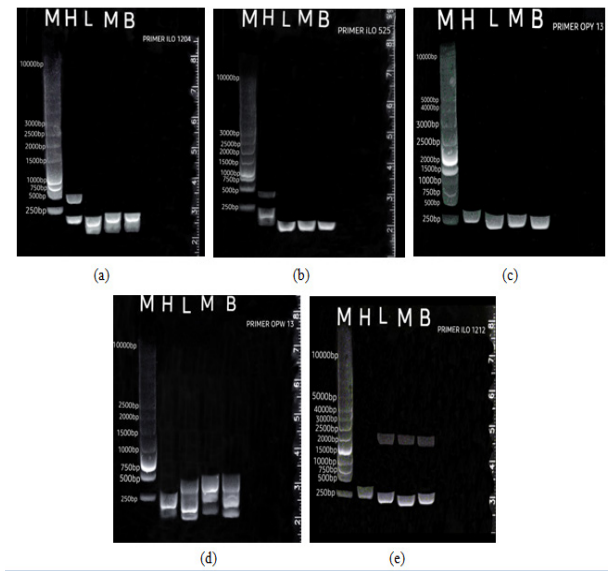

Figure 2. Electropherogram of DNA bands; (a) ILO 1204, (b) ILO 525, (c) OPY 13, (d) OPW 03, (e) ILO 1212.

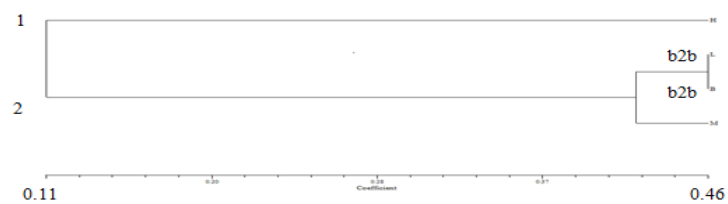

Figure 3. Dendogram kinship of buffalo variant (*Bubalus bubalis* Linn.) Pampangan based on PCR-RAPD, (H): black; (L): Lampung, (B): striped, (M): red.

Sumatra swamp buffalo consists of 2 clusters, namely Cluster 1 consisting of black buffalo, and Cluster 2 consisting of Lampung buffalo, striped buffalo and red buffalo. The closest genetic distance value from the swamp buffalo variant is 0.46 found in Lampung, striped and red buffalo variants, while the furthest genetic distance is found in black buffalo. According to Nei and Kumar (2000), stated that genetic distance is the level of gene differences between two populations and can be used in building phylogenetic trees. Othman *et al*. (2012); Barwar *et al*. (2008) confirmed that the level of genetic similarity within a population is illustrated by the genetic distance of the individual members of the population. Benson (2002) states that similarity values range from 0 to 1. Kinship is said to be close if the similarity value approaches or equals 1. According to Windusari *et al*. (2015) the kinship level of the Pampangan swamp buffalo can also be done through morphological analysis. Pampangan swamp buffalo similarity coefficient based on morphology is 0.57.

The results of the study showed that the similarity coefficients of the four variants of the *Bubalus bubalis* Linn. swamp buffalo based on PCR-RAPD had a range of values between 0.06 to 0.46. The coefficient values show variants swamp buffalo *Bubalus bubalis* Linn. Pampangan included in the low category. Windusari *et al*. (2016) research explains that morphologically, diversity and kinship of swamp buffaloes (*Bubalus bubalis*) Pampangan, South Sumatra tend to be low with a correlation coefficient of 0, 57. Windusari *et al*. (2017) also explained that based on blood plasma protein the relative relation between the variants was classified as high with a similarity coefficient of 0.89. The similarity coefficient value of 100% showed no genetic distance between variants in the population. The smaller the genetic distance between variants, the higher the genetic similarity or otherwise (Hadipoentyanti *et al*., 2001; Witherspoon *et al*., 2007)

CONCLUSION

Based on research that has been done can be concluded as follows;

Genetic variations of the Pampangan swamp buffalo (*Bubalus bubalis*) for the striped, red and Lampung variants are in the close category except genetic variations for the black swamp buffalo marked by more polymorphic bands formed in DNA amplication

Kinship among the variants of the Pampangan swamp buffalo (*Bubalus bubalis* Linn.) is relatively low with a similarity coefficient value of 0.46 (less than 1).

The kinship dendogram between the Pampangan swamp buffalo variants (*Bubalus bubalis* Linn.) Divides 2 variant clusters namely Cluster 1 consisting of black buffalo, and Cluster 2 consisting of Lampung, red and striped buffaloes.

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