Characteristics of the Genetic Variation of a Swamp Buffalo (Bubalusbubalis) of South Sumatra Based on Polymerase Chain Reaction- Random Amplified Polymorphic DNA (PCR- RAPD)

By Arum Setiawan

WORD COUNT 3124 TIME SUBMITTED 18-NOV-2019 10:58AM
PAPER ID 52159861

Characteristics of the Genetic Variation of a Swamp Buffalo (*Bubalusbubalis*) of South Sumatra Based on Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD)

Yuanita Windusari[1,*], *Laila* Hanum[1], *Arum* Setiawan[1], and *Veronika* Larasati[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, South Sumatera

Abstract. Swamp buffalo (*Bubalusbubalis*) is one of the endemic species that become a wealth of genetic resources of South Sumatra. This study aims to the genetic variation and relationships of kinship 6 variants of swamp buffalo South Sumatera. The methods used by the molecular approach using RAPD-PCR primer 5 i.e. ILO 1204, ILO 1212, ILO 525, OPW 03 and OPY 13. Data was analyzed using SPSS ver 16.0 and presented in dendrogram. The results of the amplification, all primary produce band with a total of 63 band of DNA (14.92%) with an average of every primary produce 12.6 band of DNA. The most primary produce DNA polymorphic bands namely OPW 03 (23.81%) and ILO 1204 (20.63%), while the primary ILO 525 (0.00%) do not generate polymorphic bands. Genetic variation of swamp buffalo has a low genetic variation with 14.92% percentage it generated polymorphic bands. The results of the dendogram obtained two clusters namely cluster 1 included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 of them Kerbau Bule and Kerbau Rebah Belakang. Swamp buffalo variants that have the closest genetic distance. Kerbau Tanduk Langit and Kerbau Tanduk Bulat with 856 coefficient similarity, while the farthest Kerbau Tanduk Langit and Kerbau Bule with the coefficient similarity -972. Swamp buffalo (*Bubalusbubalis*) of South Sumatera, which consists of 6 variants of buffalo have low genetic variation and inbreeding of closekinship.

1 Introduction

The population of swamp buffalo Pampangan South Sumatera adapts to monotonous swamp areas that are not cultivated. Swamp buffalo Pampangan is a source of germplasm was endemic to South Sumatera with low productivity and limited distribution which is one of the genetic wealth of South Sumatera with OganIlir, Ogan Komering Ilir and Banyuasin district [1]. The important role of buffaloes are very strategic in certain regions in

Indonesia. Statistical data population in 2000 until 2008 shows buffalo livestock populations did not increase and even tended to decline by 8.85% with an average rate of decline of 1.03% per year. The influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value. The development of biotechnology at this point give a positive impact against buffalo breeding and preservation in Indonesia are decrease each year. In line with the development of the technology of DNA levels, then weakness identification with morphological markers can be supported by a molecular approach. The use of molecular markers in the form of DNA is used along with the development of the science of molecular biology at this time[2].

Polymerase Chain Reaction (PCR) is a technique in molecular biology to multiply the amount of DNA in-vitro fertilization using the enzyme DNA polymerase and temperature [3]. The use of RAPD (Random Amplified Polymorphic DNA) is DNA analysis techniques are becoming very popular todays. It is based on at the level of DNA [4]. The technique were generally faster, cheaper than any other method for detecting DNA sequence variation and does not require prior sequence information. The fact that RAPD's survey multiple loci in the genome makes the method attractive for analysis of genetic distance and phylogeny reconstruction [5]. Diversity or genetic variation can be used as a starting point for improving the quality and quantity of swamp buffalo. Thus, information about the genetic crossbreeding and kinship in cattle including swamp buffalo is crucial in the preservation of germplasm [6]. Genetic diversity became the important information in evaluating the genetic potential for the development, utilization, and conservation of species[7].

2 Materials and Methods

Blood sampling place the swamp Buffalo (*Bubalusbubalis*) in the area of TanjungSenai, Ogan Ilir Distrik, South Sumatera. Separation of serum is performed in the integrated laboratory Postgraduate Sriwijaya University. The research of DNA isolation, quantification of DNA and PCR-RAPD performed in the laboratory of Biochemistry at the Faculty of biology of the Gajah Mada University.

Fig. 1. Sampling location

A total of 300 l blood from 6 variants buffalo (Kerbau Bule, Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang, Kerbau Tanduk Dungkul, Kerbau Rebah Belakang) from 1 location were used in this study, with the number of samples for each variant were 2individuals.

2.1 Procedures of Quantity and Quality Test Results DNA Isolation

Before being used in the PCR reaction, DNA quantification is done to determine the purity and concentration of DNA. Results of a DNA sample isolation, diluted as much as10 xin 0.2 ml tube with TE buffer, inserted into the as much as 1 l sample DNA and added 9 l of TE buffer. Then at homogeneous with spin down and spectrophotometer set at wavelength of 260 nm, and washing kuvet with aquadest sterile then conducted measurements of blanko solution first. According to [8], quantitative DNA test with spectrophotometry pure DNA can absorb ultraviolet light because of the existence of bases purin and pyrimidine. So the purity of DNA can be quantified by calculating the values of absorbance λ 260 nm absorbance value divided by 280 λ (Å260/Å280) and the value of DNA purity ranges between 1.8-2.0.

2.2 Electrophoresis

The results of the DNA isolation obtained then in gel electrophoresis by using agarose 1.5%, by dissolving 1.5 gram agarose with 100 ml of TBE 1x, and put in the microvawe until dissolved. After a rather cold in the add 2 l EtBr. Then prepared parafilm, as much as 2 l DNA loading buffer (loading dye), then inserted 8 l sample and mixed with a loading buffer. Then, put in marker, DNA ladder 100 bp into wells. Electrode is then connected to a power supply with a voltage of 100 Volt, ampere 400 speed for 30 minutes. Then the agarose gel soaked in dye gel. Visualization is performed using the UV-transimulator to see the results of electrophoresis. Then photographed as documentation [8].

2.3 PCR-RAPD

Observation 6 variants molecular done with phase DNA templates preparation, DNA amplification, gel electrophoresis, gel electrophoresis quantification results, and data analysis. Before being used in the process of PCR-RAPD primer beforehand suspended using sterile aquabidest. Added with each primer 2x Master Mix PCR with 12,5 L, Primer RAPD (ILO- 1204, ILO-1212, ILO-525, OPW-03 and 2 L OPY-13, 10,5 L dH_2O, totalvolume of 25 L to each microtube). The sample is then placed into the machine performed as many as 30 cycles of PCR with pre-denaturation at 95°C for 5 minute, denaturation at 95°C for 1 min, annealing at 55°C for 45 minutes, extention or elongation at 72°C for 1 minute, and final extention at 72°C for 10 minutes.

2.4 Visualization of Results Amplification

Visualization results of gel electrophoresis was conducted by using UV-Transilluminator and the results photographed as documentation.

2.5 Data Analysis

Data obtained from RAPD method in the form of a band or bands of amplification product was binary data represented by 'No (1) or no (0)'. DNA bands that have been subsequently measured distance migration. Determining the size of the amplification of DNA bands was done by measuring the migration distance of DNA standard (1 kb ladder)

ranging from pitting to place the DNA migration. DNA fragment size standards then created the algorithm, and the log is used as the Y axis while the migration distance of the DNA used as the X axis measurable standards After the X and Y values are known then made linear line equation Y = ax + b through linear regression line equation. The size of the DNA bands are amplified searched by entering the measured values in the equation of the line. Furthermore, the value of Y obtained and will be used as antilog long known bases are amplified by a primer. After lengthy bases are known and obtained binary data is expressed with 'No (1) or no (0)' [9], the data is then analyzed its genetic variation with the program SPSS (Statistical Package for the Social Sciences) ver 16.0 and presented with a form of cluster analysis, a dendogram using the coefficientsimilaritas.

3 Results and Discussions

Based on the results amplification of the swamp Buffalo (*Bubalusbubalis*) South Sumatera based on PCR with RADP primary 5 (ILO 1204, ILO 1212, ILO 525, OPW 03and OPY 13) using 6 variant of the Buffalo swamp. The resulting DNA bands is analyzed, then conducted by calculating the distance of migration of DNA. After that,if there is a DNA bands in the give a score of (1) and there is no DNA bands in the give the score (0). According with [10] that the DNA fragment pattern profiles of each primary based on give the score or not the fragment using the binary code. Based on the scores are then analyzed by using SPSS ver 16.0.

The results of the analysis kinship swamp buffalo South Sumatera by using SPSS 16.0 versi can be displayed in the form of a diagram called a dendogram. According [11], that cluster formation process describes the dendogram expressed in form of pictures. Horizontal lines above dendogram indicates scale that describes the level of similarity, the smaller the scale value indicates the increasingly similar to thatindividual.

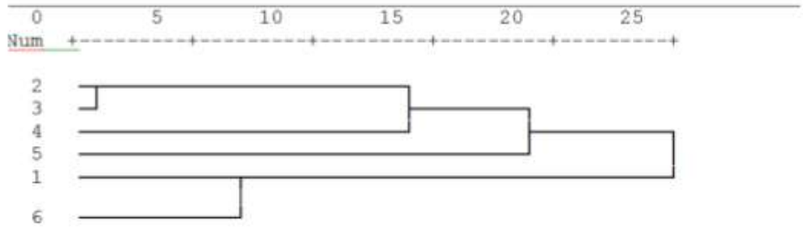

Fig. 2. Dendogram of swamp buffalo (*Bubalusbubalis*) South Sumatra based on PCR- RAPD

Description : (1) Kerbau Bule, (2) Kerbau Tanduk Bulat, (3) Kerbau Tanduk Langit, (4) Kerbau Tanduk Melintang, (5) Kerbau Tanduk Dungkul, (6) Kerbau Rebah Belakang

The results of the analysis similarity matrix coefficients PCR-RAPD similarity between 6 variant of the swamp buffalo South Sumatera based on polymorphic DNA bands that 47 amplified similarity coefficients obtained with ranges of values -972 to 856. The value of the lowest similarity coefficients -972 are indicated with the variant number 3 (Kerbau Tanduk langit) with number 1 (Kerbau Bule) this indicates that due to the presence of the genetic distance is far enough, while the highest coefficients value i.e. 856 indicated by variant number 3 (Kerbau Tanduk Langit) and number 2 (Kerbau Tanduk Bulat), that is because the genetic distance is close enough.

The value of the coefficient of 1.000 (100%) indicates that the absence of genetic distance among the variants. It is in accordance with statement [12], that the value of the

coefficient similarity 100% indicate that it is not just the distance between genetic variants in one with the other variants. The smaller the distance its genetic then the higher its genetic similarities instead its genetic distance is so large it will lower its genetic difference salso.

Based on the results of the analysis of the dendo gram can be seen that there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Swamp Buffalo variants that have the closest genetic distance is kerbau tanduk langit and kerbau tanduk bulat, while the farthest variants kerbau tanduk langit and kerbau bule. According [10] to the genetic distance is the degree of difference between the two populations and genes can be used in constructing phylogenetic.

It is supported by research [13], about the relationship of kinship based on morphological characteristics. Cluster 1 had the same character on the shape of the tip of the horn, the color of the nose, eye, eyebrows eyelashes, color necklace neck and color hair tail, while the cluster 2 has the same character on the color of the nose, ear, eye color, the color of the lashes, the color of the body and tail of the hair color.

According to [14], that the population had a close kinship This indicates the occurrence of inbreeding that can degrade the quality of genetic offspring, because it can increase homozygosity populations. The level of genetic similarity of a population can be described by the genetic distance of the individuals members of the population. The greater the genetic distance of individuals in a population, then the population has members who are increasingly diverse. Instead the smaller the genetic distance between individuals in a population, the more uniform the population.

Based on those results show that swamp buffalos South Sumatera have low genetic variation and genetic distance near or close relationship is suspected of inbreeding. It agreed with [7], which stated the inbreeding suspected of causing a decrease in the nature of phenotypes. Inbreeding among a variety of swamp Buffalo is certainly not only lowers quality but also genotip and phenotypes. According to [6], that inbreeding affects the productivity of the cattle. The low productivity of the herds of Buffalo are associated with genetic reduction of occurrence prediction.

This is coherent with the research conducted by kinship of swamp Buffalo had previously done by [15], that measures the of kinship based on morphology. The correlation coefficient is 0.57 shows more kinship between swamp Buffalo post enumerates the variance relatively close. Suspected cross between variants tend to be high. Advanced research by [7], about genetic characteristics based on protein profile showed an average value of results of all loci (H) 0.1286. Based on the average heterozygosity, swamp Buffalo (*Bubalusbubalis*) Pampangan have low genetic variation and genetic relationship of the nearest. Triwulaningsih (2005) *in* [16] states that traditional maintenance systems cause the quality of buffalo seedlings to decline and result in the development of a slow population. Indicators of the occurrence of inbreeding in buffalo livestock populations are characterized by symptoms of genetic disorders / defects such as downward curved horns, and the high incidence of albino.

[16] added that the influence of inbreeding on a large livestock on livestock productivity. Inbreeding depression or inbreeding pressure on buffalo can usually cause a decrease in the performance of livestock (growth), high mortality and low reproducibility. One of the economic impacts of the high level of inbreeding is inbreeding depression where there is a decrease in phenotypic averages, especially in traits that have high economic value.

A follow-up study on the relationship of kinship according to [15], about diversity and kinship swamp buffalo *(Bubalusbubalis*) Pampangan of South Sumatera based on

morphological characteristics. The result of the analysis of kinship based on morphological characteristics shows the correlation coefficient value of 0.85. Inbreeding and adaptation factor cause the difference in phenotypes and morphology. A high level of inbreeding can lead to inbreeding pressure which is characterized by a decrease in livestock production and reproduction performance [17] which results in a decrease in the profits of livestock businesses [18]. Some reports suspect the pressure of inbreeding in a population group that results in a decrease in productivity and a slow increase in the livestock population. According to [16] close relatives or inbreeding led to the emergence of recessive traits such as albino. The factors that led to the occurrence of inbreeding in this group of livestock due to closed populations, the inbreeding system was not directed, lack of knowledge about breeders and male limitations. Based on those results can be that they variants swamp buffalo Pampangan tend to be low due to inbreeding and adaptation to the environment that result in differences of phenotype.

4 Conclusion

Genetic variation of swamp buffalo (*Bubalusbubalis*) has a low variation with the percentage of polymorphic band 14.92 %. Swamp buffalo (*Bubalusbubalis*) South Sumatera there are two clusters namely cluster 1 that included Kerbau Tanduk Bulat, Kerbau Tanduk Langit, Kerbau Tanduk Melintang and Kerbau Tanduk Dungkul, while the cluster 2 included Kerbau Bule and Kerbau Rebah Belakang. Kerbau tanduk langit and kerbau tanduk bulat has a close kinship on coefficient of 856 while kerbau tanduk langit and kerbau bule have a kinship with the farthest distance coefficient-972. To study the characteristics of genetic diversity of swamp buffalo, it is necessary to use a different approach

References

1. Windusari, Y., E. Nofyan., M. Kamal., L. Hanum., dan R. Pratama. J. of Biological Researches **19** (2014[a])
2. Langga, I. F., M. Restu dan T. Kuswinanti. J. Sains & Teknologi **12**, 3 (2012)
3. Herman., Y. P. Ardila, F. Nugraha dan D. I. Roslim. Jurnal Dinamika Pertanian **29**, 1 (2014)
4. Anggereini, E. J. *Biospecies.* **1**, 2 (2008)
5. Sangwan, M. L. *Journal of Buffalo Science* **1** (2012)
6. Windusari, Y., L. Hanum., dan R. Pratama. Sriwijaya Journal of Environment **1**, 3 (2016[c])
7. Windusari, Y., L. Hanum., dan R. Wahyudi. Genetic Characteristic of Swamp Buffalo (*Bubalusbubalis*) from Pampangan, South Sumatra Based on Blood Protein Profile. *Proceedings of the 3rd International Conference on Construction and Building Engineering (ICONBUILD)2017* (2017[e])
8. Fatchiyah., E. L. Arumingtyas., S. Widyarti., dan S. Rahayu. *Biologi molecular* (Prinsip Dasar Analisis, Jakarta, 2011)
9. Hanum, L., R. S. Kasiamdari., Santosa., dan Rugayah. Indonesian Journal of Biotechnology **17**, 2 (2012)
10. Maesaroh, A., A. Amurwanto dan A. Yuniaty. Scripta Biologica **1**, 1 (2014)

11. Rachmatin, D. Infinity Jurnal Ilmiah Program Studi Matematika STKIP Siliwangi Bandung **3**, 2 (2014)
12. Hadipoentyanti, E., D.Ratnadewi dan L. Solihat. Zuriat **12,** 1 (2001)
13. Windusari, Y., *et al*. *Report of Competention Grant Research by Sriwijaya University. Not Publication* (2016[d])
14. Arifin, O. Z., E. Nugroho dan R. Gustiano. Berita Biologi **8**, 6 (2007)
15. Windusari, Y., L. Hanum., E. Nofyan., M. Kamal dan A. Amsar. Animal Review **3**, 1 (2016[b])
16. Praharani, L., E. Juarini dan I. G. M. Budiarsana. Parameter Indikator Inbreeding Rate pada Populasi Ternak Kerbau di Kabupaten Lebak, Provinsi Banten. *Seminar dan Lokakarya Nasional Kerbau 2009 (*2009)
17. Mrode, R., G. J. T. Swanson, and M. F. Paget. J. **32** (2004)

Characteristics of the Genetic Variation of a Swamp Buffalo (Bubalusbubalis) of South Sumatra Based on Polymerase Chain Reaction- Random Amplified Polymorphic DNA (PCR-RAPD)

ORIGINALITY REPORT

7%

SIMILARITY INDEX

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

★biodiversitas.mipa.uns.ac.id
Internet
3%

EXCLUDE QUOTES	OFF	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	OFF		