Identification of genetic diversity of Bali cattle in Bali and Nusa Penida Island with microsatellite DNA

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Abstract. Bali cattle is one of Indonesia's biodiversity with some superiority. Bali cattle scattered in various region, built centered in Bali Island. Polymorphism is very important to keep a population. Microsatellite is one of easiest methods to identify genetic diversity. The aim of this research was to identify genetic polymorphism loci Bali cattle from SPS115, ETH225, and INRA37 in Bali island and Nusa Penida island with microsatellite DNA labeling system. SPS115, ETH225 and INRA37 had been analyzed from total sample of 48 Bali cattle in Bali Island and 47 in Nusa Penida Island. The results of sequent were analyzed by GenAlEx 6.41. The results of this research showed that SPS115, ETH225 and INRA37 are of higher diversity. The highest heterozygosity was found in loci ETH225 in Bali island. The highest PIC was found in loci INRA37 in Nusa Penida Island. The inbreeding rate of Bali cattle was up to 14.1%. Bali cattle in Bali island and Nusa Penida island were different.

1. Introduction

Bali cattle (Bos javanicus) as native Indonesian cattle were originated from domesticated banteng [1] and is a livestock genetic resource which have been recognized by FAO as one of the world's cattle breeds [2]. The distribution of Bali cattle in Indonesia, apart from Bali Island, is also spread over several main areas of the Bali cattle population, namely South Sulawesi, East Nusa Tenggara, and West Nusa Tenggara [3]. Bali cattle have several advantages, namely being able to adapt to marginal environments and having high reproductive power, especially in poor feed conditions [4], has a very good fertility and conception rate [5], a high carcass percentage of 52 to 57.7% [1] and is resistant to internal and external parasites [6].

Bali cattle conservation and development program are located on the island of Bali. There is the possible relocation of the Bali cattle breeding program to other areas, especially outside the island of Bali, namely Nusa Penida Island. Nusa Penida Island has a large population of Bali cattle. The Directorate General of Livestock and Animal Health has launched the Bali Cattle Breeding Program on Nusa Penida Island with Decree No.18020/kpts/PD.420/F2.3/02/2013. One of the efforts to improve the quality of Bali cattle genetic resources is selection and crossbreeding. Genetic diversity in a population is an important information in the process of conserving genetic resources of livestock in a sustainable manner. Genetic diversity can be detected through many
loci, including ETH225, INRA037, and SPS115. The three loci according to FAO [7] have a fairly high number of repetitions. Weber [8], the higher the polymorphism if the repetition unit is multiplied more than 10 times. Increasing the number of alleles at different loci will increase the average genetic diversity in the population. The development of biotechnology in the field of molecular genetics allows the use of molecular markers to measure the status of genetic diversity through the use of marker assisted selection or MAS. One technique that is easy to do to identify genetic diversity is the DNA Microsatellite method with a labeling system.

2. Materials and methods

2.1. Sample collection and extraction DNA
The blood samples came from 48 samples of Bali cattle on the island of Bali and 47 samples on the island of Nusa Penida. Sampling of bovine blood was carried out on the external jugular vein using a needle and collected in a venoject tube containing EDTA; and the DNA extraction was conducted based on protocol [9]. DNA fragments were amplified with PCR technique.

2.2. Primer, amplification, fragment analysis
The extracted DNA sample of 2 μL was put into a PCR tube, then 28 μL of premix solution was added. The premix is composed of 0.3 μL Primary, 12.4 μL DW, 6 μL Green Master Mix. Initial denaturation was carried out at 95 °C for 15 minutes, then denaturation lasted for 20 seconds at the same temperature. PCR conditions included pre denaturation at 95 °C for 5 minutes, followed by a denaturation step at 95 °C for 10 seconds, 20 seconds annealing at 58 °C, the elongation step at 72 °C for 30 seconds and the final elongation at 72 °C for 5 minutes. This DNA amplification process was carried out for up to 35 cycles. Multiplex fragment analysis was performed on fragment analysis services on 1st base (http://www.base-asia.com/fragment_analysis/).

Table 1. The description of microsatellite markers used

| Locus  | Chr. | Motif | Primer Sequence (5’-3’) Forward-Reverse | Label | Size (bp) |
|--------|------|-------|-----------------------------------------|-------|-----------|
| ETH225 | 09   | (CA)14| GATCACCTTGCCAACATTTTCCT                | HEX   | 131-185   |
|        |      |       | ACATGACAGCCGCTGCTACT                   |       |           |
| SPS115 | 15   | (CA)18| AAAAGTGACACAACAGCTTCTTCA               | HEX   | 234-258   |
|        |      |       | AACGAGTGTCCTAGTTTGACGCTG              |       |           |
| INRA037| 10   | (TG)12| GATCTTGCT ATATTTAACAC                 | TAMN  | 112-148   |
|        |      |       | AAAATCCATGAGAGAGAAC                  |       |           |

2.3. Data analysis
Each microsatellite locus was determined for calculating the alleles, number of alleles (NA) from various loci, observed values of heterozygosity (H0) and expected heterozygosity (He), number of effective alleles (NE) at each locus, genetic distance and analysis of molecular variation using the GenAlEx 6.501 program [10].

3. Result and discussion
The three microsatellite loci namely SPS115, ETH225 and INRA037 (table 1) were successfully amplified with annealing temperature of 50°C for 20 seconds (Figure 1).
3.1. Microsatellite locus diversity

The results of the analysis of the SPS115, ETH225, and INRA037 loci based on the results of genotyping were grouped by population, namely the island of Bali and the island of Nusa Penida. The allele frequencies of each locus and each population of Bali cattle are presented in Table 2.

![Figure 1. PCR product of microsatellite loci on Bali Cattle](image)

**Table 2. Allele frequencies of Bali cattle**

| Locus     | Bali (n=47) | Nusa Penida(n=46) |
|-----------|-------------|-------------------|
| SPS115    |             |                   |
| Number of alleles | 6           | 6                 |
| Number of allele effective | 3.920       | 3.605             |
| Allele type |             |                   |
| 242       | 0.043       | 0.011             |
| 244       | 0.276       | 0.413             |
| 246       | 0.213       | 0.250             |
| 248       | 0.351       | 0.141             |
| 250       | 0.085       | 0.152             |
| 252       | 0.032       | 0.033             |
| ETH225    |             |                   |
| Number of alleles | 7           | 6                 |
| Number of allele effective | 3.201       | 3.938             |
| Allele type |             |                   |
| 149       | 0.011       | 0.011             |
| 153       | 0.011       | 0.800             |
| 155       | 0.011       | 0.117             |
| 157       | 0.304       | 0.202             |
| 159       | 0.022       | 0.043             |
| 163       | 0.234       | 0.329             |
| 165       | 0.402       | 0.298             |
| INRA037   |             |                   |
| Number of alleles | 10          | 11                |
| Number of allele effective | 6.162       | 6.686             |
| Allele type |             |                   |
| 114       | 0.000       | 0.022             |
| 118       | 0.064       | 0.217             |
| 120       | 0.160       | 0.076             |
| 122       | 0.075       | 0.054             |
| 124       | 0.011       | 0.174             |
| 126       | 0.234       | 0.196             |
| 128       | 0.234       | 0.022             |
| 130       | 0.032       | 0.141             |
| 132       | 0.117       | 0.054             |
| 134       | 0.043       | 0.032             |
| 136       | 0.032       | 0.011             |

The number of Bali cattle alleles at the SPS115 locus on Bali Island and Nusa Penida Island was the same, namely 6. The results of the study by Soldatovic et al. [11] and Vucinic [12] in Yugoslavian cattle in Serbia showed that the SPS115 locus had the same number of alleles. However, in the research of
Radko et al. [13] the number of alleles in Polish Red-White cattle at the SPS115 locus was 5 and the SPS115 locus was a low polymorphic locus. The effective number of alleles in the population of Bali Island was higher (3.920) than in Nusa Penida (3.605). The ETH225 foci had a different number of alleles in the two regions, namely on the island of Bali 7 alleles while on the island of Nusa Penida. Abdullah's study [14] found that the ETH225 locus in Bali cattle had four alleles, while in the study of Yugoslovian cattle in Serbia the ETH225 locus had 6 alleles [11]. The ETH225 locus had the highest allele frequency in the 165 allele types, which was 0.402. The allele type 153 at the ETH225 locus was only owned by Bali cattle originating from the island of Bali. The presence of specific alleles in Bali cattle found on the island of Bali can be used as a special marker for Bali cattle on the island of Bali. The highest allele effectiveness number at the ETH225 locus was 3,938 in the population of Nusa Penida Island. The INRA037 locus had 10 alleles on the island of Bali and 11 on the island of Nusa Penida. The INRA037 locus had the highest number of alleles compared to other loci. Armstrong et al. [15] in his research on Uruguayan Creolo cattle stated that the INRA037 locus was the most polymorphic locus in microsatellite with ten alleles. The INRA037 locus had the highest allele frequency in allele types 126 and 128, namely 0.234. The allele type 114 was not found on the island of Bali but was found on the island of Nusa Penida. The presence of this specific allele in Bali cattle on the island of Bali can be a defining allele for Bali cattle on the island of Bali. The highest allele effectiveness number at the INRA037 locus was found in the population of the island of Nusa Penida.

The phenomenon of high allele numbers in Bali cattle is due to the genetic variation of Bali cattle at these microsatellite loci. The diversity of microsatellites is due to variations in the number of repeats of the base sequence. The differences that arise are considered to be different alleles. The resulting allele differences are caused by differences in the number of base repetitions [16]. Locus variation is important to provide an overview of genetic diversity in a population. According to Lan et al. [17], microsatellite loci that have more than 4 alleles were very effective to be used as a mean of evaluation of genetic diversity.

3.2. Heterozygosity value
The loci SPS 115, ETH225, and INRA037 had high diversity based on their heterozygosity values. Table 3 presents the results of heterozygosity values.

| Population          | Locus | N   | Ho    | He    | PIC  |
|---------------------|-------|-----|-------|-------|------|
| Bali Island         | SPS115| 47  | 0.745 | 0.753 | 0.703|
|                     | ETH225| 46  | 0.717 | 0.695 | 0.628|
|                     | INRA037| 47  | 0.532 | 0.847 | 0.819|
| Nusa Penida Island  | SPS115| 46  | 0.652 | 0.713 | 0.688|
|                     | ETH225| 47  | 0.872 | 0.754 | 0.663|
|                     | INRA037| 46  | 0.413 | 0.860 | 0.462|

Marson et al. [18] stated that the genetic diversity of a population can be measured using the heterozygosity value which aims to assist the selection program. Table 3 shows that the observed heterozygosity value (Ho) of Bali cattle on the island of Bali is quite high, namely 0.745, 0.753 and 0.553. Bali cattle on the island of Nusa Penida have a Ho value that is not too different when compared to Ho on the island of Bali. However, both regions have a fairly high heterozygosity value, according to Javanmard et al. [19] that the heterozygosity value below 0.5 (50%) indicates the low variation of a gene in the population. Tombasco et al. [20] stated that if the value of Ho (observation heterozygosity) is lower than He (expected heterozygosity) then it can indicate an intensive selection process. This can be seen from Table 3 Bali cattle found on the islands of Bali and Nusa Penida at the SPS115 and INRA037 loci the value of Ho is lower than He. According to Tambasco et al. [20] the difference between the observed heterozygosity value (Ho) and the expected heterozygosity value (He) can be used as an indicator of an imbalance of genotypes in the observed cattle population, which indicates that selection activities have been carried out and there is no random mating. Allendorf et al. [21] stated that a
population is said to be in equilibrium if the genotype and allele frequencies are constant from generation to generation due to the incorporation of gametes that occur randomly in a large population. The balance of genes in a population occurs in the absence of mutation, selection, migration, and genetic drift.

The ETH225 locus on the island of Bali is a locus that has a high Ho value, but a high He value is shown at the INRA037 locus in Nusa Penida. High heterozygosity in a population indicates that these cows contain alleles of other cows or mutation alleles with low frequency [14]. The highest PIC value was found at the INRA037 locus on the island of Bali. A high PIC value describes the level of information on the marker or locus that is used as a very informative marker.

3.3. Genetic distance and performance of Bali Cattle
The results of the calculation of the genetic distance value in Bali cattle on the island of Bali and Nusa Penida are in Table 4.

| Population            | Genetic Distance | Genetic similarity | Fit  |
|-----------------------|------------------|--------------------|------|
| Bali – Nusa Penida    | 0.111            | 0.895              | 0.141|

Fit: total inbreeding rate

Pairwise genetic distance matrix, showed that Bali cattle on the island of Bali have a difference rate of 11.1%. This means that the Bali cattle found on the island of Bali with Nusa Penida Island have similarities. The similarity between Bali cattle on the island of Bali and Nusa Penida is 89.5%. This study illustrates that Bali cattle found on the islands of Bali and Nusa Penida contain almost the same genetic material.

The AMOVA results showed that the performances at the 3 loci used for Bali cattle found on the islands of Bali and Nusa Penida were significantly different with a difference of 3% level. This shows that the Bali cattle found on the island of Bali and the island of Nusa Penida are different. The results of the analysis of three microsatellite loci used showed the value of the total inbreeding rate (Fit) (0.141) in both populations of Bali cattle. High inbreeding rates will tend to eliminate genetic variation. This is not expected, if the missing genetic variation is a favorable genetic variation.

Bali cattle are still being developed and increasing their productivity, both on the island of Bali and outside areas known as areas where Bali cattle are developed. Bali cattle found on the mainland of Nusa Penida are designated as breeding and refining areas for Bali cattle. The low value of diversity in the population can also be caused by the use of a limited number of loci. The number of loci used in this study were 3 loci. According to FAO [7] the number of loci that should be used to identify genetic diversity in cattle is 30 microsatellite loci.

4. Conclusion
Based on microsatellite DNA, Bali cattle have a high degree of heterozygosity. The loci INRA037, ETH225, and SPS115 had high diversity. Based on the results of the analysis of molecular variation, it can be concluded that the Bali cattle found on the island of Bali and the island of Nusa Penida are different.

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