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Short Communication

Allelic Diversity of the Endangered Taro White Cattle Population from Bali using BoLA Microsatellite Loci

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Abstract | Taro white cattle is an indigenous and endangered cattle population in Taro village, Bali and only inhabiting restricted areas in forests of Taro village. Current population are 57 head. To provide basic information concerning allele and locus in BoLA gene, the BoLA microsatellite loci (DRB3 and BM1815) were used to evaluate allel diversity using 18 samples of Taro white cattle. Genetic variability was low, with observed heterozygosity and expected heterozygosity for locus BM1815 was 0.778 and 0.51, and for DRB3 was 0.556 and 0.478, respectively. Moreover, the polymorphic information content (PIC) for these two microsatellite was 0.397 for BM1815 and 0.413 for DRB3. The Taro white cattle showed low level of genetic diversity. This result would be used as an initial guide for conservation decisions in future for survival of this valuable genetic resource. The alleles identified from the present study should be further confirmed by sequencing.

Keywords | Taro white cattle, Microsatellite, BoLA, Allel, Heterozygosity

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INTRODUCTION

In Bali, apart from popular Bali cattle, there are small population of cattle inhabiting restricted areas in forests of Taro village. Taro white cattle is one of local cattle breed in Bali which tropically well adapted to local environment and raised by farmers in limited area of Taro village, district of Tegalalang, Gianyar Regency (Oka et al., 2020). The local people named these cattle as Taro white cattle due to their white skin and coat color. Because of its appearance as albino, this animal can be easily distinguished from Bali cattle. While it is known that Bali cattle is derived from *Bos sondaicus* (*banteng*), the origin of Taro white cows is not clearly understood as yet.

The Taro white cattle, sanctified by the surrounding communities and Balinese society utilized them for rituals and ceremonial purposes only. Its habitat is only in the forest of Taro village. The population of this breed is 57 heads. Now the breed status is at critical level and close to extinction according to FAO (2007).

Current management guidelines for populations at risk frequently emphasize genetic uniqueness over genetic diversity (Funk et al., 2012). Genomic tools can contribute to genetic resource management through accurate estimation of genetic uniqueness (Pertoldi et al., 2014). Genetic characteristics of breed and the uniqueness of genetic characteristics in a given population can be determined by

performing molecular analysis, particularly microsatellite (Wang et al., 2021). Microsatellite is quite useful for breed identification as it can not be influenced by environmental factors. Moreover, microstellite has been known to be highly polymorphic for population due to variation in the number of repeated nucleotides (Wang et al., 2021). Currently, microstellite is a genetic marker mostly used for molecular characterization. Some of molecular studies applying microsatellite involves analysis in plant genetics studies (Vieira et al., 2016), population structure (Moges et al., 2016), prediction of genetic variation and inbreeding (Radko et al., 2021), pedigree recording (Ivankovic et al., 2021), and individual identity (Miller et al., 2016).

Microsatellite is a simple and short sequence (particularly a di-, tri-, and tetranucleotide) reappearing in sequence in eukaryote genome (Sathishkumar et al., 2011). It is also known as simple sequence repeats (SSRs) or simple tandem repeats (STRs). Polymorphism of microsatellite allele occurs due to difference in the number of motive copies (unit of sequence). Such polymorphism is often regarded as simple sequence length polymorphisms (SSLPs) or short tandem repeat polymorphisms (STRPs). Change in one nucleotide sequence results in movement of pair of bases and improper pair of bases. Improper pair of bases can be corrected by reducing or adding the number of motives or units of sequence. This in turn leads to polymorphism of microsatellite allele (Vieira et al., 2016).

Microsatellite-based mapping of major histocompatibility complex (MHC) is very useful in selection and can provide important information about variations and kin relationship in a given population (Bastos-Silviera et al., 2008). The high microsatellite polymorphism is a good molecular marker in study of population genetics. Such a high polymorphism results in different expression variation for each individual. Thus, microsatellite is a genetic marker that provides information about variation of allele at gene locus (Abdul-Muneer, 2014).

One of the factors that determine individual resistance to diseases is MHC known as bovine leucocyte antigen (BoLA) in cattle (Bohórquez et al., 2019). MHC can be classified into 3 classes: Class I, II, and III (Bastos-Silveira et al., 2008). MHC is characterized by large number of alleles at each locus and big difference in the number of amino acids in each allele. Bastos-Silviera et al. (2008) reported that there are significant differences in allele variation in microsatellite locus region of BoLA gene in eight species of cattle in Portugal, and these differences could be related to their physical position in the chromosome. Moreover, it was reported that BM1815 locus is situated in the side of BoLA-DRB gene and located at 5'. This locus has a low variation. Meanwhile, DRB3 and DRBP1 loci are located in the intron of BoLA gene and DRB3 gene can be considered as the most polymorphic gene amongst genes of BoLA (Takeshima et al., 2018; Mandefro et al., 2021).

The present study was aimed to characterize the genetic profile of Taro white cattle to get information that can be useful for designing breeding strategies to preserve genetic variation of the Taro white cattle.

MATERIALS AND METHODS

Animal

This experiment was conducted at Taro village and was the part of Taro white cattle conservation project. Cattle used in this study were 3-4 years old. The age was determined by examination of the teeth. Age was estimated by the stage of eruption of permanent pair of incisors. All animals were clinically examined prior to use. The study was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine Udayana University.

DNA Exatraction

Eighteen cattle of Taro white breed were genotyped for BoLA microsatellite loci. Approximately 10 mL **b**lood samples were collected from each animal. The DNA from blood samples were then extracted using QIAamp DNA Mini Kit (Qiagen).

BoLA microsatellite loci Amplification

Two microsatellites that were previously mapped along the BoLA genome segment on chromosome 23 (DRB3 and BM1815) were amplified (Table 1). Amplification reaction in PCR was carried out in PCT 100 (MJ Research, Inc., Watertown, Mass, USA) as much as 30 cycles with procedure as follow: denaturation at 94°C (35 seconds), annealing at 54°C for 35 seconds, and extension at 72°C for 35 second. Result of amplification was separated with gel bis-acrylamide 6% (electrophoresis) and visualization was done using silver staining. DNA typing was performed by measuring migration length of each DNA tape in gel compared to DNA tape standard 100 bp ladder.

Data analysis

Frequency of allele, the number of allele, H_O (observed heterozygosity, and H_e (expected heterozygosity) was calculated using microsatellite toolkit v.3.1 program (Park, 2001).

RESULTS AND DISCUSSION

During the data collection, the total population of Taro white cattle was 57 head. From the total population, 18 cattle were used as sample. The cattle used in the study

Table 1: Primers used for the amplification of microsatellite markers in the Taro white cattle.

No.	Primer	Chromosome	Sequence	Reference
1	DRB3	23	F: 5'GAGAGTTTCACTGTGCAG 3: R: 5'CGCGAATTCCCAGAGTGAGTGAAGTATCT 3'	Bastos-Silveira et al. (2008)
2	BM1815	23	F: 5'AGAGGATGATGGCCTCCTG 3' R: 5'CAAGGAGACAAGTCAAGTTCCC 3'	

Table 2: Analysis of heterozygosity and polymorphism information content (PIC) in Taro white cattle using BoLA microsatellite markers.

Microsatellite marker	No.of Alleles	Allele size range (bp)	PIC	Heterozygosity	
				Observed	Expected
DRB3	4	166-172	0.413	0.556	0.478
BM1815	3	152-176	0.397	0.778	0.51

showed the same exterior characteristics with white coat color (Figure 1). The cattle were reared intensively in colony houses and fed with grasses twice daily.

Figure 1: Taro white cattle.

A total of 7 alleles were detected in BoLA microsatellite of Taro white cattle. The BoLA microsatellite loci was polymorphic, with 4 alleles in BoLA DRB3 microsatellite locus and 3 alleles in BoLA BM1815 microsatellite locus. The mean number of allele, the size of allele, PIC, the H_O and H_e were presented in Table 2.

The average expected hetrozygosity for DRB3 microsatellite locus in Taro white cattle was 0.478, and average observed hetrozygosity was 0.556. The mean polymorphism information content was 0.413. The average expected hetrozygosity for BM1815 microsatellite locus in Taro white cattle was 0.51, and average observed hetrozygosity was 0.778. The mean polymorphism information content was 0.397

In the present study, two locus of BoLA microsatellite were used to evaluate the genetic characteristic of Taro white cattle. Two microsatellites were found polymorphic. The number of alleles in this study were found lower as compared to number of alleles reported in Bangladesh, Ethiopian, and Korean cattle (Mandefro et al., 2021), also in South American Zebu cattle populations (Takeshima et al., 2018). The BoLA locus DRB3 microsatellite exhibited 59 alleles (Mandefro et al., 2021), 46 alleles in South American Zebu cattle populations (Takeshima et al., 2018), and 13 alleles detetcted in the BoLA locus BM1815 microsatellite (Bastos-Silveira et al., 2008).

Average values of H_O and H_e in Taro white cattle were lower than those reported Bastos-Silveira et al. (2008) in Portuguese cattle breeds. Lower values of expected homozygosity in comparison with the observed homozygosity were found. High value of observed homozygosity than the expected homozygosity (van Haeringen et al., 1999) has been reported for DRB3 micrsatellite in cattle. Absence of gene flow, may explain the results observed for this breed

The average PIC value for BM1815 and DRB3 locus was 0.397 and 0.413, respectively. The low value of PIC may indicate the homogeneity of population. The PIC value for Taro white cows noted in the current study was lower compared to PIC value (0.533) in Bali cattle (Puja et al., 2013). Thus, the present findings reveal that population of Taro white cows in their natural habitat at village Taro, Tegallalang, Bali is homogeneous. The low PIC values for markers suggested their no usefulness for genetic polymorphism studies and linkage mapping programes.

A prerequisite for a structured and sustainable animal breeding and conservation programs is the identification of genetic diversity within the population (Unal et al., 2021). The distribution of genetic diversity is an important prerequisite for successful conservation strategies (Ivanova et al., 2021). This research which was carried out by BoLA

microsatellite loci, showed that within population genetic diversity was low. The loss of genetic diversity resulted to a reduction of the effective population size and high levels of inbreeding (Radko and Podbielska, 2021). This situation indicates the difficulty of breeding programs and genetic conservation programs for this population. Therefore, this conservation effort requires scientific production systems to increase production, without losing significant genetic structure of this animal.

CONCLUSIONS

Taro white cattle have low level of genetic diversity and absence of genetic flow. This result, may be useful for conservation decisions in future. The alleles identified from the present study should be further confirmed by sequencing.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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AUTHORS CONTRIBUTION

All of the authors equally contributed and approved the manuscript.

REFERENCES

Abdul_Muneer PM (2014). Application of Microsatellite Markers in Conservation Genetics and Fisheries Management:Recent Advances in Population Structure Analysis and Conservation Strategies. Genetics Research International, Article ID 691759, 11 pages. http://dx.doi.org/10.1155/2014/691759.

Bastos-Silveira C, Luís C, Ginja C, Gama LT, Oom MM (2008). Genetic variation in BoLA microsatellite loci in Portuguese cattle breeds. Animal Genetic., 40: 101-105. https://doi.org/10.1111/j.1365-2052.2008.01791.x

Bohórquez MD, Ordoñez D, Suárez CF, Vicente B, Vieira C, López-Abán J, Muro A, Ordóñez I, Patarroyo MA (2019). Major Histocompatibility Complex class II (DRB3) genetic diversity in Spanish Morucha and Colombian Normande cattle compared to Taurine and Zebu populations. Front. Genet. 10:1293. https://doi.org/10.3389/fgene.2019.01293.

FAO (2007). 'Food and Agriculture Organization of the United Nations, Rome: The State of the World's Animal Genetic Resources for Food and Agriculture' edited by Barbara Rischkowsky and Dafydd Pilling. Rome

Funk CF, McKay JK, Hohenlohe PA, Allendorf FW (2012). Harnessing genomics for delineating conservation units. Trends Ecol. Evol., 27 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Ivanova P, Dzhembekova N, Atanassov I, Rusanov K, Raykov V, Zlateva I, Yankova M, Raev Y, Nikolov G (2021) Genetic diversity and morphological characterisation of three turbot (Scophthalmus maximus L., 1758) populations along the Bulgarian Black Sea coast. Nature Conservation 43: 123–146. https://doi.org/10.3897/ natureconservation.43.64195

Ivankovic A, Bittante G, Konja˘ci´c M, Kelava Ugarkovi´c N, Pe´cina M, Ramljak J (2021). Evaluation of the conservation status of the Croatian Posavina horse breed based on pedigree and microsatellite data. Animals, 11, 2130. https://doi.org/10.3390/ani11072130.

Mandefro A, Sisay T, Edea Z, Uzzaman R, Kim KS, Dadi H (2021). Genetic assessment of BoLA-DRB3 polymorphisms by comparing Bangladesh, Ethiopian, and Korean cattle. J Anim Sci Technol, 63(2):248-261. https://doi.org/10.5187/jast.2021.e37

Miller SM, Clarke AB, Bloomer P, Guthrie AJ, Harper CK (2016). Evaluation of microsatellites for common ungulates in the South African wildlife industry. Conservation Genetics Resources, 8(3). https://doi.org/10.1007/s12686-016-0554-7

Moges AD, Admassu B, Belew D, Yesuf M, Njuguna J, Kyalo M, Ghimire SR (2016). Development of microsatellite markers and analysis of genetic diversity and population structure of Colletotrichum gloeosporioides from Ethiopia. PLoS One. 11(3):e0151257. http://dx.doi.org/10.1371/journal.pone.0151257.

Oka AA, Putra IGAA, Suarna IW, Doloksaribu L, Puja IK (2020). Productive potency of the endangered taro white cattle population reared under conservation management system in bali, indonesia. J. Anim. Health Prod. 8(4): 193-198. http://dx.doi.org/10.17582/journal.jahp/2020/8.4.193.198.

Park SDE (2001). Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. PhD thesis, University of Dublin, Dublin.

Puja IK, Wandia IN, Sulabda IN, Suastika P (2013). Asosiasi Polimorfisme Genetika Lokus Deoxyribo Nucleic Acid (DNA) Mikrosatelit gen BoLA dengan Kualitas Semen pada Sapi Bali. Jurnal Kedokteran Hewan.,7(20):163-165

Pertoldi C, Purfield DC, Berg P, Jensen TH, Bach OS, Vingborg R (2014). Genetic characterization of a herd of the endangered Danish Jutland cattle. *J. Anim. Sci.* 92 2372–2376. https://doi.org/10.2527/jas.2013-7206

Radko A, Podbielska A (2021). Microsatellite DNA Analysis of Genetic Diversity and Parentage Testing in the Popular Dog Breeds in Poland. Genes, 12, 485. https://doi.org/10.3390/genes 12040485.

Sathishkumar R, Lakshmi PTV, Annamalai A, Arunachalam V (2011). Mining of simple sequence repeats in the Genome of Gentianaceae. Pharmacognosy Res. 3(1): 19–29. https://doi.org/10.4103/0974-8490.79111.

Takeshima SN, Corbi-Botto C, Giovambattista G, Aida Y (2018). Genetic diversity of BoLA-DRB3 in South American Zebu cattle populations. Genetics,19:33. https://doi.org/10.1186/s12863-018-0618-7

Unal EO, Isık R, Sen A, Kus EG, Soysal MI (2021). Evaluation of Genetic Diversity and Structure of Turkish Water Buffalo Population by Using 20 Microsatellite Markers. Animals, 11, 1067. https://doi.org/10.3390/ani11041067

van Haeringen WA, Gwakisa PS, Mikko S, Eythorsdottir E, Helm LE, Olsaker I, Outteridge P. Andersson L (1999).

Heterozygosity excess at the cattle DRB locus revealed by large scale genotyping of two closely linked microsatellites. Anim. Genet., 30, 169–76.

Vieira MLC, Santini L, Diniz AL, de Freitas Munhoz D (2016). Microsatellite markers: what they mean and why they are so useful. Genet. Mol. Biol. 39(3): 312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Wang H, Yang B, Wang H, Xiao H (2021). Impact of diferent numbers of microsatellite markers on population genetic results using SLAF-seq data for Rhododendron species. Scientifc Reports,11:8597. https://doi.org/10.1038/s41598-021-87945-x