GENETIC CHARACTERIZATION IN FOUR INDONESIAN CATTLE BREEDS INFERRED FROM ILLUMINA PARENTAGE SNP MARKERS

W.P.B. Putra[1,2]* Hartati[3] Mariyono[3] R.R. Noor[4] C. Sumantri[4,] E.T. Margawati[2]
Researcher Researcher Researcher Researcher Researcher Researcher
[1]Study Program of Animal Production and Technology Science, Postgraduate School of Faculty Animal Science, Bogor Agricultural University, Bogor, West Java 16680, Indonesia
[2]Research Center for Applied Zoology - National Research and Innovation Agency (BRIN), Cibinong, Bogor, West Java 16911, Indonesia
[3]Research Center for Animal Husbandry - National Research and Innovation Agency (BRIN), Cibinong, Bogor, West Java 16911, Indonesia
[4]Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor West Java 16680, Indonesia
***E-mail: widya.putra.lipi@gmail.com**

ABSTRACT

Genetic characterization in native livestock is essential to conserve the genetics standard of livestock for pure-breeding programs. This research aimed to characterize four Indonesian cattle breeds using 156 sites of Parentage SNP Markers, includingARS-USMARC-Parent (119 sites) and ARS-USMARC (37 sites). Total of 113 mixed-sex animals were used for the evaluation, including 16 Bali (*Bos javanicus*), 16 Madura (*Bos indicus*), 33 Ongole grade (*Bos indicus*), and 48 Sumba Ongole (*Bos indicus*) cattle breeds. Results showed Bali, Madura, and Ongole lineage (Ongole grade and Sumba Ongole) cattle can be discriminated against with the Parentage SNP Markers. The pairwise genetic distance analysis revealed that Bali cattle had a closer genetic relationship with Madura cattle (0.043 - 0.151) than Ongole lineage cattle (0.073 - 0.325). However, the phylogenetic tree explained that the Ongole lineage cattle are grouped into two different clades. At the same time, Bali, Madura and Ongole lineage cattle are grouped into their original cluster. The study suggests that the Parentage SNP Markers can be used to characterize Bali, Madura, and Ongole lineage cattle accurately.

Key words: ***Phylogenetic tree, genetic characterization, Indonesian cattle, Illumina Parentage SNP Markers***

مجلة العلوم الزراعية العراقية 2024-:55(عدد خاص):284-292 بوترا وآخرون

التوصيف في أربع سلالات من الماشية الإند نوسية مستمدة من علامات ILLUMINA PARENTAGE SNP

بوترا هارتاتي ماريونو نور سومانتري ماركاواتي
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المستخلص

يعد التوصيف الوراثي للماشية المحلية أمرًا ضروريًا للحفاظ على المعيار الوراثي للماشية من أجل برامج التربية النقية. يهدف هذا البحث إلى توصيف أربع سلالات من الماشية الإندونيسية باستخدام 156 موقعًا من علامات Parentage SNP، بما في ذلك (ARS–USMARC–Parent 119 موقعًا) و (ARS–USMARC 37 موقعًا). تم استعمال إجمالي 113 حيوانًا مختلطًا للتقييم، بما في ذلك 16 سلالة من الماشية بالي (Bos javanicus)، و16 مادورا (Bos indicus)، و33 سلالة من أبقار Bos indicus) Ongole)، و48 سلالة من الماشية Bos indicus) Sumba Ongole). أظهرت النتائج أن أبقار بالي ومادورا وأونجول (درجة أونجول وسومبا أونجول) يمكن التمييز ضدها باستعمال علامات Parentage SNP. كشف تحليل المسافة الوراثية الزوجية أن أبقار بالي كانت لها علاقة وراثية أوثق مع مادورا (0.043 – 0.151) من أبقار نسب أونجول (0.073 – 0.325). ومع ذلك، أوضحت شجرة النشوء والتطور أن أبقار سلالة الأونجول تم تجميعها في فرعين مختلفين. وفي الوقت نفسه، تم تجميع أبقار بالي ومادورا وأونجول في مجموعتها الأصلية. تشير الدراسة إلى أنه يمكن استخدام علامات Parentage SNP لتوصيف أبقار بالي ومادورا وأونجول بدقة.

الكلمات المفتاحية: شجرة النشوء والتطور، التوصيف الوراثي، الأبقار الإندونيسية، علامات

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INTRODUCTION

Genetic and morphological characterization in the native livestock are essential in the pure-breeding program and valuable to develop the breed standard and the conservation program. Bali (*Bos javanicus*), Madura (*Bos indicus*), Ongole grade (*Bos indicus*), and Sumba Ongole (*Bos indicus*) cattle are four Indonesian cattle that have the standard of qualitative and quantitative traits (15). Recently, the crossbreeding program with exotic bulls was used to increase meat production (44). Consequently, many farmers applied crossbreeding with the frozen sperm of exotic cattle to increase meat production. As a result, the crossbred cows produced from the crossbreeding between exotic bulls (*Bos taurus*) and native cows have low reproductive performance (17,23,34). Despite reducing the reproductive traits, the crossbreeding between native cows and exotic cattle can influence the phenotypic and physical characteristics (41,3). Consequently, the origin of genetic structure in native cattle will be changed when crossbreeding is not controlled. Hence, genetic characterization helps select purebred cattle for the breeding program. Currently, there are many studies to characterize Indonesian cattle breeds with many regions of mtDNA genes such as Cytb (35,25,42,31). Despite those previous studies, many studies were conducted for genetic characterization in Indonesian cattle breeds with microsatellite markers (1) and SRY gene (17). Currently, the genetic characterization of cattle can be evaluated based on their genomic diversity through Illumina Bovine Beadchip (49,32,26). Many previous studies used the Bovine Beadchip toolkit to characterize native cattle breeds in South Africa (30), China (47), semi-arid areas of Africa (6), Bangladesh (5), Ethiopia (46), Peru (8) and Turkiye (9). Moreover, the Bovine Beadchip toolkit was used to evaluate the genomic diversity in the population of Colombian Chino Santandereano (14), Korean Jeju Black (2), German Black Pied (33) and Indonesian Bali cattle (39) cattle. Interestingly, many SNP markers in the Illumina 50K Bovine Beadchipwere used for genetic characterization in many cattle breeds (26). Commonly, three SNP markers ID of ARS-USMARC-Parent, ARS-BFGL-NGS, and BOVINEHD were recommended for the genetic characterization in cattle (22). Therefore, many SNP markers can discriminate between *Bos indicus* and *Bos taurus* cattle breeds (27). Furthermore, those SNP markers can be used for parentage checking in inbred cattle (13). Unfortunately, studies on Indonesian cattle breeds with SNP markers were not reported. Hence, this study aimed to characterize four Indonesian cattle breeds with Illumina Parentage SNP Markers. The results of this study are significant as the additional findings explain the kindship of Indonesian cattle breeds.

MATERIALS AND METHODS

Ethics approval: This study was approved by the Animal Ethics Committee of the Indonesian Ministry of Agriculture (Protocol No. Balitbangtan/Lolitsapi/Rm/11/2018).

Sample and DNA extraction

A total of one hundred and thirteen (113) mixed-sex cattle comprising Bali (16 heads), Madura (16 heads), Ongole grade (33 heads), and Sumba Ongole (48 heads) breeds were used for the experimental animals. The blood samples were taken from each animal from the jugular vein using a venoject vacutainer tube containing EDTA. The DNA extraction was performed using Genomic DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's instruction.

Selecting SNP markers

SNP Markers were obtained by using Illumina Bovine 50 K SNP BeadChip with the extracted DNA through a commercial laboratory service (Macrogen, South Korea). Therefore, two PLINK output files (pad and map) were obtained from the initial analysis with Genome Studio 2.0. software (48). A TASSEL 5.0. software (7) was also used to obtain the SNP markers from pad and map files. Subsequently, all the SNP markers belonging to SNP ID: ARS-USMARC-Parent (119 sites) and SNP ID: ARS-USMARC (37 sites) were selected for genetic characterization. In the present study, 156 sites of SNP markers were obtained from each cattle breed according to the Illumina Bovine 50 K SNP BeadChip.

Data analysis

The nucleotide data of 156 SNP markers were computed as the sequence data using BioEdit software (19). The sequence data changes to

the numerical data are then used for discriminant analysis using SPSS 16.0 software with criteria of breeds as the factor and SNP markers as the variable. The discriminant analysis determined the individual discriminant plot based on the SNP markers and detected the discriminating SNP markers (38). At the same time, the UPGMA tree was performed using TASSEL 5.0 software for determining the kinship among Indonesian cattle breeds based on the Parentage SNP Markers. A MEGA software (20) was also used to calculate the pairwise genetic distance (F_{st}) in the animals under study. A structure software (10) was then used to observe the genetic admixture of animals under study with the numerical data.

RESULTS AND DISCUSSION

Genetic diversity: The genetic diversity in four Indonesian cattle breeds based on 156 sites of Parentage SNP markers was presented in Table 1. The MAF value in Bali was lowest than other breeds. While Madura, Ongole grade, and Sumba Ongole cattle had a similar range of MAF value (0.14 - 0.16). According to Illumina Bovine 50 K SNP Beadchip, the R (22.58%) was detected as the most frequent heterozygous nucleotide in cattle breeds of Indonesia (Ongole grade and Friesian Holstein) and followed by Y (19.04%) and M (10%). Thus, S (1%) was the lowest frequency in the heterozygous nucleotides (29). Similar findings were reported in six Chinese cattle breeds that R (34.89/47.10%) and Y (46.55/34.73%) are two standard heterozygous nucleotides based on Whole Genome Sequencing (WGS) method (45). Interestingly, R and Y were also observed as the two highest frequencies of heterozygous nucleotide in animals under study. The Illumina Bovine 50K SNP BeadChip revealed that many cattle breeds in the world have a close MAF value to the animals under study, such as Podolian (0.19), Yakutian (0.19), Kankraj (0.18), Tharparkar (0.18), Gir (0.16) and Sahiwal (0.16) cattle (32, 24,26). In general, many studies reported that the MAF value was higher than animals under study in Pakistani Zebu cattle (0.21) and Turkish cattle (0.358) breeds (32,9). The difference of the MAF value can be influenced by the breed (genetic) and the number of evaluated SNP markers (11,43).

Table 1. Genetic diversity in four Indonesian cattle breeds based on 156 sites of Parentage SNP Markers

Parameter	Breed			
	Bali	Madura	Ongole grade	Sumba Ongole
Number of animals (taxa)	16	16	33	48
Taxa × Site	2,496	2,496	5,148	7,488
Number of not missing site	2,448	2,489	5,146	7,472
Number of missing sites	48	7	2	16
Number of heterozygous	66	504	1,163	1,700
Minor allele frequency (MAF)	0.014	0.143	0.155	0.165
Number of nucleotides				
A	508	426	855	1,248
C	762	642	1,241	1,753
G	672	555	1,199	1,733
T	442	368	689	1,050
R (A/G)	36	203	443	657
Y (C/T)	14	148	328	467
S (C/G)	1	17	41	72
W (A/T)	3	52	117	162
K (G/T)	10	38	102	146
M (A/C)	0	47	133	200

Otherwise,the changes in population structure, such as selection, migration, inbreeding, and crossbreeding, can influence the MAF value (28). The MAF value can be classified into three different categories of Rare (<0.05), Intermediate (0.05 - 0.25), and >0.25 for Highly polymorphic (36,40). In the present study, the Parentage SNP Markers revealed that Madura, Ongole grade, and Sumba Ongole had an intermediate MAF value. Hence, the Parentage SNP Markers used in this study are potential for detecting the candidate genes for economic traits in those cattle breeds. Moreover, a high MAF value

associated with greater genetic diversity has more significant effects and better genomic predictive ability for quantitative traits in cattle (4). Contrastly, Bali cattle have rare MAF values and indicating highly proportion of fixed alleles. Consequently, detecting candidate genes in Bali cattle must involve large SNP marker numbers. Interestingly, most Bangladeshi indigenous cattle also have the rare MAF value based on Illumina Bovine 50K BeadChip (5).

Genetic characterization

The pairwise genetic distance (F_{st}) among four Indonesian cattle breeds is presented in Table 2. According to Table 2, Bali and Madura's cattle had a close distance (0.043/0.151) and similar to Ongole grade and Sumba Ongole (0.000/0.188). Generally, Madura has a lower genetic distance with Ongole lineage (0.032/0.251) than Bali (0.073/0.325). Lin *et al.* (27) reported a close distance among Myanmar, Laotian and Cambodian cattle (0.009 to 0.010) based on 58 SNP markers. These values are similar to the present study in Ongole grade - Sumba Ongole (0.000 to 0.010). In the African cattle breeds, the F_{st} value with Illumina Bovine 50K BeadChip among Nguni, Drakensberg, and Bonsmarawas about 0.043 (30) and similar to Madura - Bali (0.043) in the present study. As a result, the discriminant plots of Ongole grade and Sumba Ongole cattle were located at a similar cluster, as shown in Figure 1. While, Bali and Madura cattle were located at the different cluster. Otherwise, the discriminant analysis revealed that twenty-three (23) SNP markers were detected as the discriminating SNP markers to characterize four Indonesian cattle breeds, as shown in Table 3. Additionally, the discriminant analysis with 156 SNP markers able to characterize four Indonesian native cattle with the canonical correlation of 95.20% (function 1) and 82.70% (function 2). Interestingly, the number of discriminating SNP markers with discriminant analysis was higher than that obtained by Zwane *et al.* (49) with Lewontin and Krakauer's (FLK) statistics test (23 SNP markers vs 11 SNP markers). Furthermore, the genetic admixture study revealed that Bali, Madura, and Ongole lineages had different genetic composition (Figure 2). According to Figure 2, Madura cattle had the genetic composition from Bali (*Bos javanicus*) and Ongole (*Bos indicus*) lineages (k=4). A similar finding was reported by Firdhausi *et al.* (12) that detecting two maternal lineages in Madura cattle, *i.e.*, *Bos javanicus* and *Bos indicus*, based on mitochondrial Cyt-b and D-loop genes. Therefore, Ongole grade and Sumba Ongole cattle have two main genetic lineages based on the same parameters. Hartati *et al.* (16) reported that the Ongole grade cattle have two main genetic introgressions from Indian Zebu (Gir and Nellore) and Brahman cattle based on Illumina Bovine 50K BeadChip. Hence, two main genetic lineages in Ongole grade and Sumba Ongole may be originated from Indian Zebu and Brahman cattle. Agung *et al.* (1) supported it, which constructed the phylogenetic tree among Indonesian cattle breeds close to the present study based on 12 microsatellite loci. Additionally, the UPGMA tree revealed that Madura, Ongole lineage (Ongole grade and Sumba Ongole), Madura, and Bali cattle were characterized into their origin clusters, as shown in Figure 3. At the same time, the Ongole lineage cattle could be characterized into clade A (63 animals) and clade B (18 animals). Otherwise, the Ongole lineage cattle at clade B were close to the Madura cattle. Hence, the SNP markers under study can not discriminate between two different Ongole lineages clearly. However, Ongole grade and Sumba Ongole cattle can be discriminated against with their body measurements. Body length (BL), withers height (WH), hip height (HH), and chest depth (CD) in Ongole grade bulls were 123.97±11.58 cm; 124.74±6.70 cm; 130.81±6.39 cm and 58.63±5.94 cm, respectively (21). These values were higher than BL, WH, HH, and CD in Sumba Ongole cattle, *i.e.*, 112.50±10.12 cm, 113.75±9.81 cm; 120.50±6.35 cm and 57.25±30.27 cm, respectively (37). In the future, a depth study involving other Indonesian cattle breeds and many SNP markers will be essential to evaluate the potential genetics of Indonesian cattle breeds.

Table 2. The lower (below diagonal) and higher (above diagonal) pairwise genetic distance among four Indonesian cattle breeds based on Parentage SNP Markers

Breed	Bali	Madura	Ongole grade	Sumba Ongole
Bali	-	0.230	0.256	0.325
Madura	0.043	-	0.151	0.230
Ongole grade	0.073	0.032	-	0.188
Sumba Ongole	0.119	0.033	0.000	-

Table 3. Twenty-three (23) discriminating SNP markers to characterize four Indonesian cattle breeds

SNP marker	BTA	Position	Allele	MAF	SNP marker	BTA	Position	Allele	MAF
AUP-DQ451555	1	29,524,658	R	0.07	AU-186	10	8,394,690	R	0.01
AUP-DQ404152	2	5,306,838	R	0.29	AUP-DQ837646	11	1,703,612	M	0.11
AUP-AY776154	2	26,997,623	R	0.12	AUP-AY851162	11	46,411,100	R	0.05
AUP-AY841151	2	45,832,887	K	0.06	AUP-DQ786764	12	25,668,974	K	0.24
AUP-DQ786757	2	111,155,237	R	0.48	AUP-DQ846691	14	48,380,429	Y	0.02
AUP-DQ435443	3	58,040,470	M	0.03	AUP-DQ846694	16	33,521,338	M	0.06
AU-666	5	25,643,874	M	0.02	AUP-DQ381152	17	17,616,950	W	0.15
AU-646	5	25,800,581	G	0.00	AUP-EF093511	21	26,620,013	K	0.08
AU-674	5	44,187,145	K	0.01	AUP-EF026085	21	65,198,296	M	0.23
AU-636	5	45,774,499	R	0.43	AUP-AY937242	23	27,306,795	R	0.36
AU-670	7	98,534,197	R	0.33	AUP-EF034085	28	5,913,226	R	0.26
AUP-DQ786766	10	3,530,271	R	0.46					

BTA: *Bos taurus* autosome; AUP=ARS-USMARC-Parent; AU=ARS-USMARC; MAF=minimum allele frequency; R=A/G; M=A/C; K=G/T; Y=T/C; W=A/T

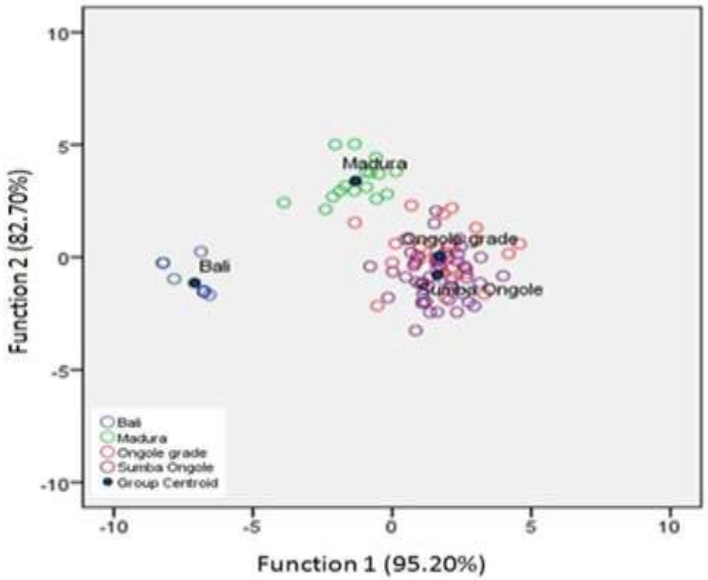

Figure 1. Clustering of four Indonesian cattle breeds inferred from Parentage SNP Markers

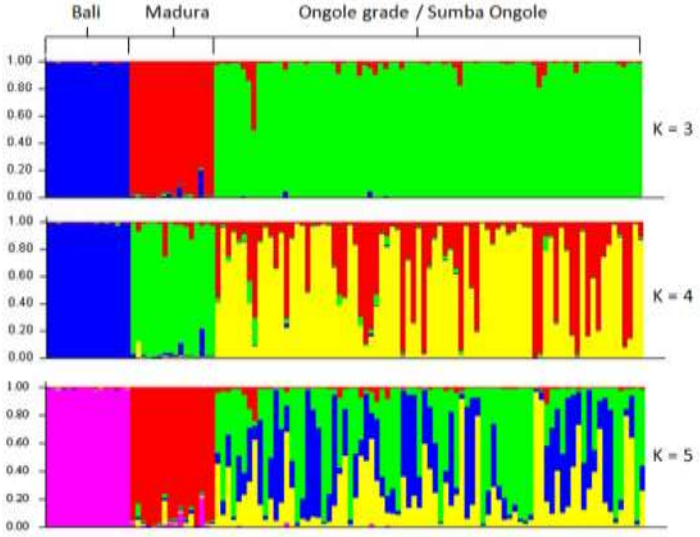

Figure 2. Genetic structure analysis using 156 sites of Parentage SNP Markers (k=3 to k=5) in four Indonesian cattle breeds of 113 individuals

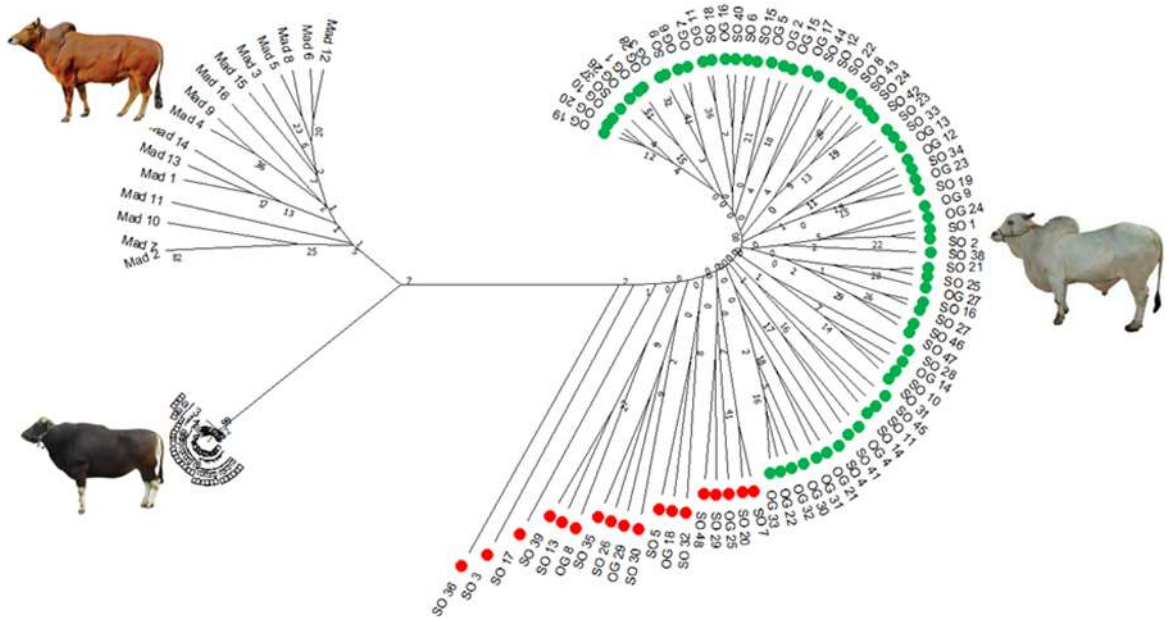

Figure 3. Dendogram among Bali (Bal), Madura (Mad), Ongole grade (OG) and Sumba Ongole (SO) using 156 sites of Parentage SNP Markers. Two genetic clusters of clade A (green circle) and clade B (red circle) were determined in the Ongole lineage (*Bos indicus*)

CONCLUSION

The Parentage SNP Markers can be used to discriminate Bali (*Bos javanicus*), Madura (*Bos indicus*), and Ongole lineage (Ongole grade and Sumba Ongole) cattle. Thus, two different clades were observed in the Ongole lineage cattle. In this study, the MAF value in the *Bos indicus* lineage belongs to the intermediate category (0.05 - 0.25). Hence, the Parentage SNP Markers under study could be used for a genomic selection program to reveal the candidate genes for economic traits in Indonesian *Bos indicus* cattle.

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