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SHORT COMMUNICATION

Association of Two ISSR Markers with the Growth Traits of Saburai Does *(Capra hircus)*

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Abstract

Saburai goat *(Capra hircus)* developed by the farmers at Tanggamus Regency, Indonesia and originated from the crossbreeding between Boer buck and Ettawa grade doe for meat production purpose. This study aimed to evaluate Saburai goat based on two ISSR markers of $(AG)_9C$ and $(GA)_9C$. This study uses 28 adult Saburai does. Results showed that a total of 13 DNA fragments were observed according to two ISSR markers with a total of 11 polymorphic loci. However, the PIC value in $(AG)_9C$ ISSR marker was higher than $(GA)_9C$ ISSR marker. According to both ISSR markers, Saburai goat can be characterized into two clusters. In conclusion, the genetic diversity in Saburai goats is high and potential to improve with molecular selection.

Keywords: Genetic diversity, Indonesia, ISSR marker, Polymorphic, Saburai goat

Introduction

Saburai *(Capra hircus)* is an Indonesian crossbred goat developed through a grading up program by farmers in Tanggamus Regency, Lampung (one of Indonesia›s provinces). The breed is a mix of 75% Boer buck and 25% Ettawa-grade doe. Since 2015, Saburai has been recognized as an official Indonesian native goat breed by the Indonesian Ministry of Agriculture through decision No. 359/Kpts/PK.040/6/2015. Presently, Saburai goats were bred for meat production with 35.93±7.16 kg (bucks) and 31.77±7.58 kg (does) of yearling weight and 1.64±0.56 of litter size. As the native Indonesian crossbred goat, assessing the genetic diversity of the Saburai goat is important for genetic conservation and evaluation of population structure. Inter simple sequence repeat (ISSR) is a molecular marker technique that uses polymerase chain reaction (PCR) to amplify DNA with a single primer made up of a microsatellite sequence. The ISSR method is known for being quick, easy to use, highly reproducible, and polymorphic. Despite the need for genetic characterization, ISSR markers can select sheep with a high breeding value for body weight.

Recently, the two ISSR markers of $(AG)_9C$ and $(GA)_9C$ have been used to study the genetic diversity in ruminant livestock animals such as cattle [1], goats and sheep [2]. Unfortunately, there are limited studies for assessing both ISSR markers in goats. Previously, researching with other

ISSR markers to observe the genetic diversity in the goat population without an association study [3]. Therefore, the current study aimed to determine the association of $(AG)_9C$ and $(GA)_9C$ ISSR markers with the body weight of Saburai does. Furthermore, this study was conducted to observe the genetic diversity in Saburai does based on both ISSR markers. These findings are important for evaluating the genetic potential of Saburai does concerning growth traits.

Material and Methods

Ethic Approval Statement

This study was performed under the animal ethics protocols of the Animal Science Faculty, Universitas Lampung, Indonesia (Certificate No: 6410/UN26.14/2023)

Animals and Research Site

Twenty-eight (28) unrelated Saburai does were selected for the experiment. The does were collected from Tanggamus Regency, Lampung Province of Indonesia. This research site located at latitude 5°05'-5°56'S and longitude 104°18'-105°12'E and placed at 2.115 m asl with 27.3 – 29.6°C of air temperature, 72.1-81.8% of relative humidity and 2.066.5 mm of rainfall per year.

Management of Animal and Data Records

The management of Saburai goat husbandry implemented by the livestock farming group in Gisting Atas Subdistrict aims to maintain animal health, increase production, and ensure business sustainability. The primary feed for Saburai goats in this group consists of green fodder like gamal, kaliandra, and odot grass, which are known for their high nutritional content. Additionally, farmers use plant residues as supplemental feed. The feeding schedule is three times a day: morning, noon, and afternoon. The Gemar Menanam Hijauan (GMH) program encourages members to plant their own animal feed, which helps ensure a year-round supply of high-quality feed.

The goats' housing is managed using a well-structured system, typically elevated pens to maintain cleanliness and animal health. These pens provide ample space for movement and are equipped with efficient waste disposal systems, essential to prevent disease transmission.

The breeding management in this group has focused on Saburai goat reproduction since 2014. They use natural mating methods while keeping several bucks to avoid inbreeding, ensuring good genetic diversity in their herd.

DNA Extraction

The blood samples were taken from each animal using venoject and vacutainer tubes containing EDTA, then stored at -20°C for subsequent DNA extraction. The DNA extraction was conducted using the Genomic DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's instructions. Nonetheless, individual body weight data at birth, weaning (4 months old), and yearling (12 months old) were collected from each animal for the association study.

DNA Amplification

The DNA amplification was performed in a total volume of 10 µL containing 3 µL of DNA template, 5 µL of PCR Master Mix (DreamTag™, USA), 0.5 µL of $(AG)_9C$/$(GA)_9C$ ISSR primer and 1.5 µL of free-nuclease water. The amplification of each primer consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min and final extension at 72°C for 5 min. The electrophoresis for DNA visualisation was performed at 100 V for 35 min with 2% agarose gel stained with safe DNA gel stain (SYBR™, USA). The DNA fragments'visualisation was captured by G-Box Documentation System (Syngene, UK).

Data Analysis

Five genetic diversity parameters were calculated in this study belonging to the total of DNA fragments, number of polymorphic loci, number of effective allelles (n_e), Shannon's diversity index (I) and polymorphic informative content (PIC). While, the n_e, I and PIC values were calculated referring as follows [4]:

$$n_e = 1/\sum P_{ij}^2 \quad (1)$$

$$I = -\sum P_i \ln(P_i) \quad (2)$$

$$PIC = 1-\sum P_i^2 \quad (3)$$

where: n_e is the number of effective alleles; I is the Shannon's diversity index, PIC is the polymorphic informative content and P_i is the frequency of the j^{th} pattern in the i^{th} band. The dendrogram of animals was computed with a Heatmapper computer program. The association study of ISSR markers was performed by comparing the growth traits of animals at different clusters using General Linear Model (GLM) methods and analyzed with the SPSS 16.0 computer program.

Results

Sixteen (16) DNA loci were obtained in Saburai does based on two ISSR markers belonging to eleven (11) DNA patterns for $(AG)_9C$ and five (5) DNA patterns for $(GA)_9C$ *(Table 1)*. Three DNA loci of S1.2 (5 animals), S1.4 (5 animals) and S1.5 (4 animals) were shown as the three common DNA loci in $(AG)_9C$ ISSR marker for experimental animals. While S2.3 (12 animals) and S2.4 (10 animals) were shown as the two common DNA loci

Table 1. *Polymorphisms of two ISSR markers in Saburai does*

ISSR Marker	Locus Name	Number of Fragment	Length (bp)	N	Frequency
$(AG)_9C$	S1.1	6	350, 500, 650, 780, 1500, >3000	1	0.04
	S1.2	5	500, 650, 780, 1500, >3000	5	0.19
	S1.3	5	350, 500, 650, 780, >3000	2	0.07
	S1.4	5	500, 550, 650, 780, >3000	5	0.19
	S1.5	4	500, 650, 780, >3000	4	0.15
	S1.6	4	500, 550, 650, 780	1	0.04
	S1.7	3	500, 650, >3000	3	0.11
	S1.8	2	500, 650	3	0.11
	S1.9	2	500, 550	1	0.04
	S1.10	1	550	1	0.04
	S1.11	1	500	1	0.04
$(GA)_9C$	S2.1	4	500, 600, 650, >3000	2	0.07
	S2.2	4	300, 490, 500, >3000	1	0.03
	S2.3	3	300, 500, >3000	12	0.43
	S2.4	2	300, 500	10	0.36
	S2.5	1	500	3	0.11

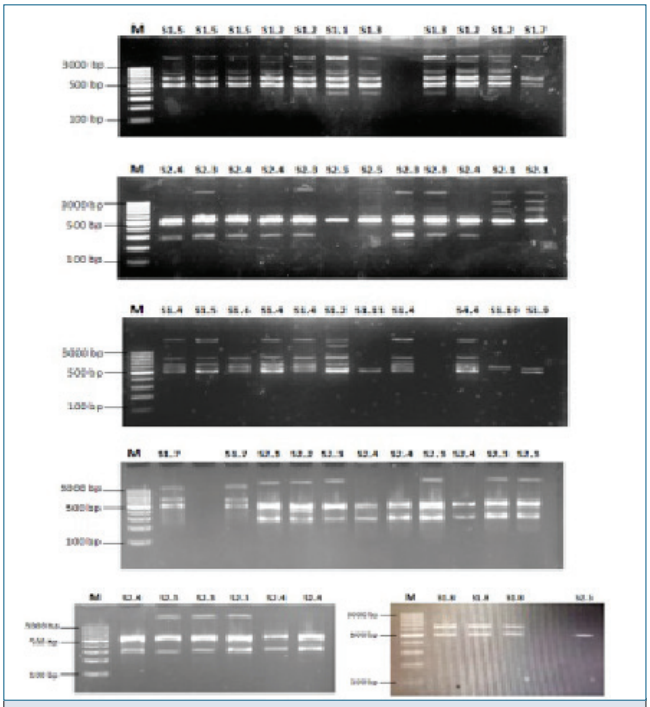

Fig 1. Pattern of DNA fragments in Saburai does based on PCR-ISSR analysis with $(AG)_9C$ (S1.- code) and $(GA)_9C$ (S2.- code) ISSR Markers on 2% agarose gel. M: DNA ladder 100 bp

Table 2 . *Genetic diversity in two ISSR markers of Saburai does*

ISSR Marker	Total Number of Fragment	Number of Polymorphic Locus	Frequency of Polymorphic Locus	Number of Effective Allele (n_e)	Shannon's Diversity Index (I)	PIC
$(AG)_9C$	7	6	0.86	7.59	2.50	0.87
$(GA)_9C$	6	5	0.83	3.01	1.26	0.67

PIC = polymorphic informative content

in the $(GA)_9C$ ISSR marker for experimental animals. In addition, the number of DNA fragments in both ISSR markers ranged from 1 to 6 bands for $(AG)_9C$ and 1 to 4 bands for $(GA)_9C$ *(Fig. 1)*. Therefore, the PIC and I values in the $(AG)_9C$ were higher than $(GA)_9C$ *(Table 2)*. In general, two clusters of Saburai does were determined according to two ISSR markers such as cluster 1 with 15 animals) and cluster 2 with 13 animals *(Fig. 2)*. Therefore,

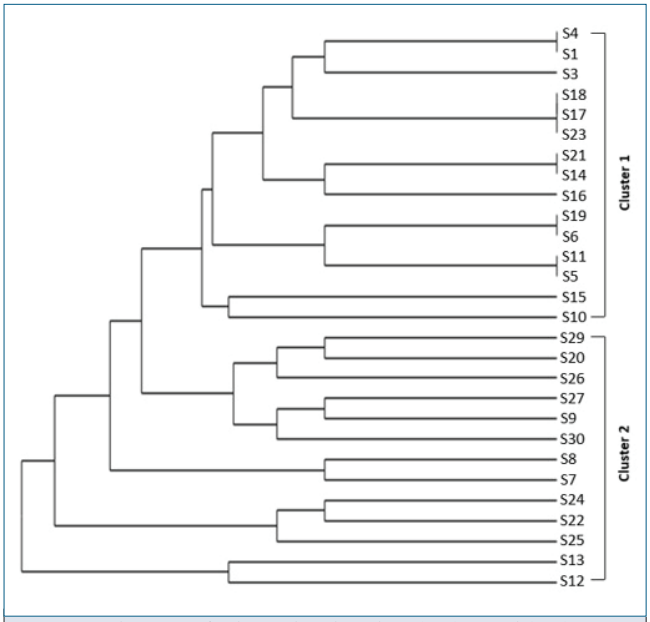

Fig 2. Dendrogram of Saburai does based on $(AG)_9C$ and $(GA)_9C$ ISSR markers. S- code is the sample ID

Table 3 . *Comparation between the growth traits of Saburai does from different clusters*

Parameter	Cluster 1 (N=15)	Cluster 2 (N=13)	P-Value
Birth weight (kg)	3.27±0.81	2.97±0.72	0.634
Weaning weight (kg)	17.08±3.24	15.90±2.90	0.634
Yearling weight (kg)	38.25±9.71	34.67±8.69	0.634
Preweaned daily gain (kg/day)	0.11±0.02	0.10±0.01	0.631
Postweaned daily gain (kg/day)	0.09±0.03	0.08±0.02	0.544

N = number of animal

the association analysis revealed that the growth traits of Saburai does at cluster 1 were higher than in cluster 2 but it's not significantly different *(Table 3)*.

Discussion

In Saburai does, the PIC value of ISSR markers in $(AG)_9C$ was higher than $(GA)_9C$. A high PIC value also indicated high genetic diversity in the observed animals. Thus, the PIC value is classified in low (<0.30), moderate (0.31-0.50) and high (>0.50) categories. In Tuvinian short-tailed sheep, the PIC value of $(AG)_9C$ ISSR markers was about 0.20-0.0.40 [5] and lower than in animals under study. Contrastly, there are 21 DNA loci of $(AG)_9C$ ISSR markers were observed in Tuvinian short-tailed sheep and higher than in Saburai does. In Kermani sheep, a total of 28 DNA loci of $(AG)_9C$ and 36 DNA loci of $(GA)_9C$ were observed and higher than in Saburai does [6]. In Mehraban sheep, total 28 and 36 of DNA loci were observed with $(AG)_9C$ and $(GA)_9C$ ISSR markers, respectively [7]. In goats, the PIC value ranged from 0.09-0.19 for $(AG)_9C$ and 0.14-0.21 for $(GA)_9C$ [2].

The variation of DNA loci can be affected by the type of species and breed. As the crossbred goat, Saburai had higher genetic diversity and its supporting these findings. According to the quantitative genetic aspect, the heritability (h^2) value of growth traits in Saburai goat from low (<0.10) to moderate (0.11-0.30) categories such as 0.24±0.08 for body weight, 0.29±0.17 for weaning weight and 0.10±0.08 for yearling weight [8]. Hence, the quantitative genetic aspects explained that Saburai goat had high genetic diversity and possible to improve their productivity traits with conventional and molecular selection methods. Hence, the ISSR analysis revealed that Saburai does at cluster 1 had higher growth traits in comparison to cluster 2 but it is not different significantly.

In the present study, the genetic diversity of Saburai does was able to be observed based on two ISSR markers. A study of microsatellite markers was reported able to identify the genetic diversity in Indonesian native goat breeds [9]. Therefore, a molecular selection involving functional genes may be used to obtain the genetic markers for growth traits. The growth hormone (GH) gene is one of candidate genes that had a potency for

molecular selection of growth traits in Saburai goats [10] and Merino cross sheep [11] in Indonesia. Subsequently, Myostatin (MSTN) gene was polymorphic in Saburai goat and can be used for molecular selection of growth traits [12]. Furthermore, assessing the genome-wide association study (GWAS) can detect many potential candidate genes across the autosomal chromosome regions of goats accurately [13,14]. Despite this, a whole genome sequencing (WGS) technique can obtain the genetic markers in the livestock accurately [15].

In conclusion, two ISSR markers of $(AG)_9C$ and $(GA)_9C$ were polymorphic in Saburai does. However, the ISSR marker of $(AG)_9C$ had a higher PIC value rather than $(GA)_9C$. In this study, Saburai does can be characterized into two clusters based on both ISSR markers. Commonly, the average growth traits in the does on cluster 1 were higher than those in cluster 2. In the future, further research involving large samples and ISSR markers is important to select Saburai goats based on specific genetic markers accurately.

Declarations

Availability of Data and Materials: The authors declare that data supporting the study findings are also available to the corresponding author (W.P.B. Putra).

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