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Repeated G-nucleotides from DNA Sequences from RAPD Results in Indonesian Local Etawah Goats Derived from Natural Service and Artificial Insemination

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Abstract. Indonesian local Etawah goats (ILEG) are a livestock that has adapted to the environment and management of community farms in Indonesia, with systems of natural service (NS) and artificial insemination (AI). The livestock potential needs to be studied molecularly as a source of information on genetic diversity. The purpose of this study was to determine the repeated of guanine nucleotides (GG, GGG, GGGG, GGGGG and GGGGGG) from DNA sequenced with RAPD results in two ILEG populations of NS and AI. The method used was an experiment with sequencing analysis based on RAPD results with the marker OPA-19 with five replications. The sequencing results were descriptive analysis using SPSS V. 23 with unpaired t-test on the two ILEG populations. The results showed that the ILEG from NS had a very significantly higher number of repeated G-nucleotides ($P < 0.01$) than ILEG from AI. While the data of repeated GGG, GGGG, GGGGG and GGGGGG were not used for unpaired t-tested because it did not pass normality test. Descriptively repeated GGG, GGGG, GGGGG and GGGGG on ILEG from NS results were higher than AI. Particularly in repeated G-nucleotides (GGGG, GGGGG and GGGGGG), in ILEG from NS results were still found to be 100% in all sequencing samples, but in ILEG from AI results showed decreased percentages of 60%, 40% and 0%. The conclusion of this study was that repeated G-nucleotides in ILEG from NS are more common than in ILEG from AI.

Keywords: ILEG, RAPD, sequencing.

INTRODUCTION

There are many local goat breeds in Indonesia, one of which is the Indonesian local Etawah goat (ILEG) with a population of 18,410,379 in 2017.[1] ILEG is one of Indonesia's local goats, a cross between the Etawah goat from India and Kacang goat of Java Island, which has developed to have different forms of black/brown and white coloration. There are two types of mating systems for the ILEG population, those being natural service (NS) and artificial insemination (AI) with different genetic resources than bucks.[2] It is necessary to research the genetics of the ILEG through molecular studies. It is interesting to do research on genetic differences of ILEG through molecular studies.

Molecular studies of local livestock have been and continue to be used for breeding, conservation and determining specific traits that could be passed to their offspring as well as mapping the genetic superiority of the local livestock. Randomly amplified polymorphic DNA (RAPD) is a sequencing method that could be used to determine genetic diversity. [3, 4, 5, 6, 7]

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The sequencing results based on RAPD with OPA-19 found novel repeated-G in two ILEG populations. Therefore, the aim of this research was to determine the genetic diversity through the differences in repeats of guanine nucleotides (GG, GGG, GGGG, GGGGG and GGGGGG) from sequenced DNA of RAPD results between ILEG populations from NS and AI.

METHOD

The method of research was an experiment with purposive sampling for selecting does. Doe samples were taken from a population of ILEG derived from NS and AI mating with a known pedigree estimate, healthy and no reproductive disorders. ILEG blood collection was done with the following steps: the livestock was in a calm and comfortable position, the part of the body from which blood was to be taken was rubbed slowly with cotton soaked in 70% alcohol, taking blood slowly through the jugular vein to a volume of 3-5 mL by syringe with needle, after which the jugular vein area was gently pressed with cotton that had been soaked in 70% alcohol. DNA extraction included isolation of white blood cells and DNA isolation from white blood cells, RAPD amplification and visualization of fragments with electrophoresis. Selected RAPD results were analyzed by sequencing.

The method of this study was an experiment by performing RAPD analysis using the OPA-19 marker (CAAACGTCGG) with five replications for each ILEG population followed by sequence analysis with Korean Macrogen.[7] The sequencing results were descriptive analyses and SPSS V. 23 software was used for unpaired t-test.

RAPD analysis (21 samples of NS population and 19 samples of AI population) was performed by the stages of isolation and amplification of DNA. DNA isolation was done by extracting DNA including white blood cell isolation and DNA isolation from white blood cells. A master cycler gradient was used for amplification that included: denaturation at 95 °C for 5 minutes; followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 36 °C for 45 seconds and extension at 72 °C for 1 minutes; and post extension at 72 °C for 10 minutes.[4]

RESULTS AND DISCUSSION

The sequencing results based on RAPD with OPA-19 marker (Figure 1) showed a novelty with the discovery of repeated G-nucleotides, which are potential microsatellite markers on the ILEG. In accordance with the opinion of Bagshaw, microsatellites are repeated nucleotides of 1-6 bp with various combinations.[8] Various studies on repeated-G4 (quadruplex-G DNA) were performed by various methods to obtain them, among others, with monoclonal antibody 1H6 as well as the effect of CpG methylation on pure oligodeoxyribonucleotides.[9] The repeated G study was conducted because it was thought to be related to many of the biological processes in the body.[10,11]

This research found that the data of repeated GG was normally distributed so the data were analyzed by unpaired t-test, while the data of repeated GGG, GGGG, GGGGG and GGGGGG were not analyzed by t-tested because it did not have the normality test data. The average data and statistical test results can be seen in Table 1.

TABLE 1. The Average number of repeated-G resulted from sequencing based on RAPD between ILEG of NS group and AI group

Reapeated-G	N	NS	AI
GG (G-2)	5	$25.4 \pm 4{,}16^{a}$	10.4 ± 1.95^{b}
GGG (G-3)	5	$13 \pm 5{,}74$	3.6 ± 1.95
GGGG (G-4)	5	7 ± 3.67	1 ± 1
GGGGG (G-5)	5	3.4 ± 3.71	0.4 ± 0.54
GGGGGG (G-6)	5	2.2 ± 2.78	0 ± 0

[a, b] different notations were a very significant difference ($P < 0.01$)

Based on Table 1, repeats of GG in NS were very significantly higher ($P < 0.01$) than in AI, the NS group had GG repeats 144% higher than the AI group. Repeated GGG, GGGG, GGGGG and GGGGGG descriptive analysis showed that ILEG from NS tended to be higher than ILEG from AI, and ILEG from AI had no G-6 repeats. The visualization of RAPD results and profile of repeated G-nucleotides in each replication of the NS and AI populations can be seen in Figures 1 and 2.

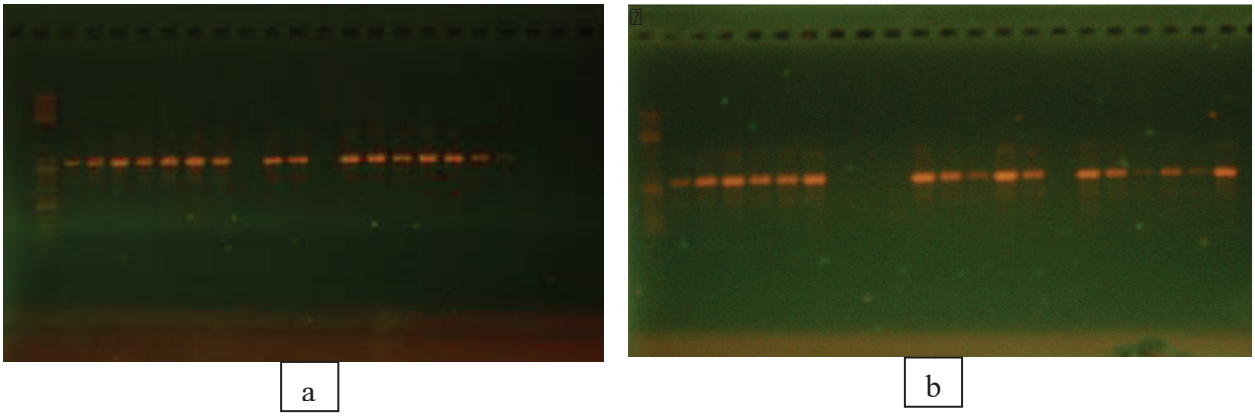

FIGURE 1. Visualisation of RAPD amplification in ILEG derived from the AI (a) and NS (b) population.

Based on the visualization results of RAPD amplification (Figure 1), ILEG derived from the NS population resulted in DNA fragments that tended to be more variable than ILEG derived from the AI population. The DNA from the NS population showed variable fragments, three fragments (64.71% of total amplified replications), two fragments (29.41% of total amplified replications) or one fragment (5.88% of total amplified replications), with an average band number of 2.59 ± 0.62. In contrast, the number of DNA fragments in the AI population resulting from RAPD amplification were all three fragments (100% of total amplified replications) with an average value of 3.00 ± 0.00. The average of band number in RAPD amplification from the ILEG from both NS and AI populations was lower than that in lambs (8.04 ± 0.18).[12]

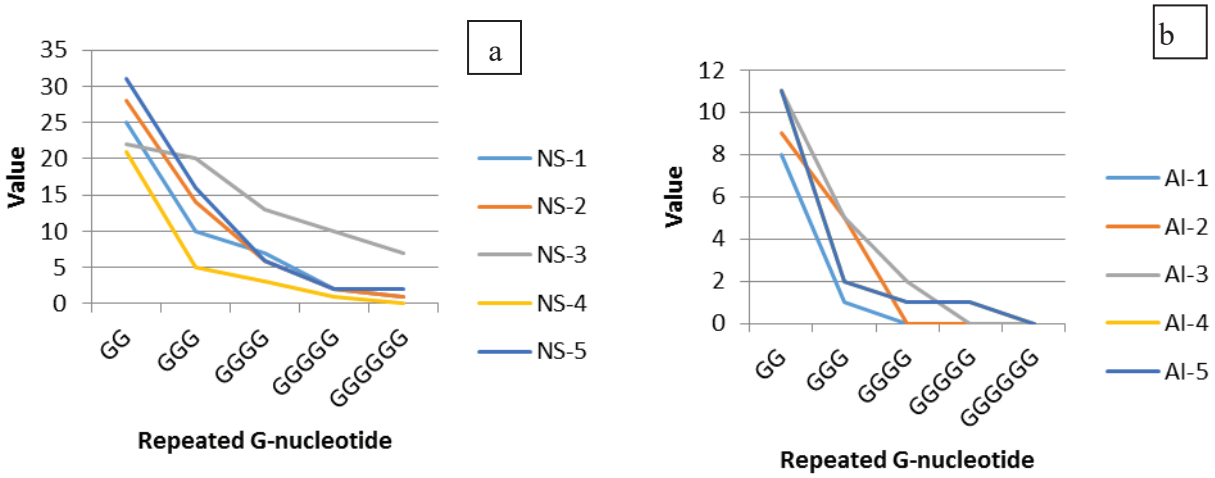

FIGURE 2. The profile of repeated-G in each replication of the AI (a) and NS (b) population.

Descriptive analysis (Figure 2) showed that repeated G-nucleotides (GG, GGG, GGGG and GGGGG) in ILEG derived from NS was still found 100% in all sequencing samples, only GGGGGG was found 80% (4 of 5 sequencing samples). Repeated GG and GGG of ILEG derived from AI was found at 100% in all sequencing samples, whereas repeated GGGG, GGGGG and GGGGGG were not present in all sequencing samples, 60% (3 of 5 sequencing samples), 40% (2 of 5 sequencing samples) and 0% (0 of 5 sequencing samples), respectively. This means that repeated G is more commonly found in the NS population than AI population, it could be said that the sequencing of the NS population was more uniform than the AI population. This is supported by other research showing that the genetic similarity of ILEG derived from NS based on RAPD with OPA 4, OPA 6-10, OPA 15-17 and OPA 19 was 3% higher than ILEG derived from AI.[2]

CONCLUSIONS

Repeated G-nucleotides (GG, GGG, GGGG, GGGGG, GGGGGG) in Indonesian local Etawah goats derived from natural service were more common than in Indonesian local Etawah goats derived from artificial insemination. Repeated G-nucleotides in ILEG has the potential to be developed as a microsatellite marker.

SUMMARY

Goat is the livestock with the highest population in Indonesia, it shows goats are able to grow well within the environment (feed, management and breeding) of Indonesia. One of the local breeds of Indonesian goats that already has national breed guidance through the Indonesian National Standard (SNI) is the Indonesian local Etawah goat (ILEG). ILEG is the result of crossing the Etawah goat and Kacang goat for decades. As concerns germplasm, there is still little information about molecular studies on ILEG. The molecular research on repeated G-nucleotides in ILEG does not yet exist and needs to be developed in order to support the availability of genetic information as the basis of breeding. In this study, RAPD analysis was performed using the OPA-19 marker followed by sequencing in two ILEG populations with different mating systems, namely natural service (NS) and artificial insemination (AI). Sequencing results observed differences in repeated G-nucleotides of GG (G-2), GGG (G-3), GGGG (G-4), GGGGG (G-5) and GGGGGG (G-6) between the NS population and AI population. The results showed that repeated G-nucleotides (GG, GGG, GGGG, GGGGG and GGGGGG) were present in the ILEG in both NS and AI populations, but the GG repeat in the NS population was significantly higher ($P < 0.01$) than in the AI population (25.4 ± 4.16 vs. 10.4 ± 1.95), while other G repeats (GGG, GGGG, GGGGG and GGGGGG) in the NS population also tended to be higher than in ILEG derived from AI (based on repeated G average). The results of this study showed that repeated G-nucleotides have potential for development of a novel satellite marker in ILEG livestock.

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