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PHENOTYPIC AND GENETIC DENDOGRAM BETWEENTWO POPULATIONS OF INDONESIAN LOCAL ETTAWAH GOATS

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Abstract – There are two populations of Indonesian Local Ettawah Goats (ILEG) in Ampelgading District, East Java, Indonesia, derived from different selected bucks by natural service (NS) and artificial insemination (AI). The objectives of this study were to provide genetic and phenotypic information of Indonesian Local Ettawah Goats that has been able to adapt in rural areas. The informations explained the phenotypic and genetic variation based on dendogram analysis. The phenotypic dendogram based on taxonomic relationships with morphological characteristics, the genotypic dendogram based on the RAPD analysis, and phenotypic and genotypic dendogram resulted by the combined data between morphological characteristics and molecular of the both local goat populations. This study used RAPD with 11 primers from OPP and RAPD primers. The samples were 23 ILEG does derived from natural service population and 20 ILEG does derived from artificial insemination population. Analysis of dendogram were calculated using numerical taxonomy and multivariate analysis system (NTSYS) software version 2.0. The results showed that similarity phenotypic, genetic, phenotype and genetic combination of natural service and artificial insemination population were 0.45, 0.56, 0.57 and 0.68, 0.60, 0.65, respectively. The conclusion of this study was genetic and phenotypic variation was higher in the population derived from natural service than artificial service. The phenotypic and genetic variation between population of natural service and artificial insemination was low.

INTRODUCTION

Indonesian Local Ettawah Goats (ILEG) was a cross between the Ettawah goat from India brought by Dutch colonists to Indonesia with the local goat (Kacang goat) in Indonesia (Shodiq and Setianto, 2011). ILEG that one of the local livestock was developed to support food security based domestic livestock resource in Indonesia (Department of Animal Husbandry and Animal Health, 2010)

Lately, many groups of ILEG breeders have grown with ownership of more than 50 heads, one of them is in the village of Ampel Gading Malang. That village is divided into two of population derived from mating systems are used. The first population used natural service which the semen came from bucks were selected from only outside performance without recording (NS group).

The second population used artificial service that the semen came from selected bucks based on recording of productivity and semen quality, namely AI group. It could be presumed that the genetic and phenotypic variation between NS population and AI population was different.

The diversity of phenotypics could be measured through dendogram by looking at the phenotypic character of each individual and then compared with other individuals in the group as well as comparing between populations. While the genetic diversity was analyzed by using RAPD (random amplified polymorphic DNA). RAPD advantages compared with other DNA method were fast, cheap, could be used for a limited sample of DNA, suitable for many genomes and detection in various sequences (Hardys *et al.*, 1992). But there were some limitations on the sensitivity of RAPD fragments in

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a small change of conditions or there could be no amplification of DNA. To avoid these limitations, preliminary research needs to be done to ensure that the reaction occurs and selecting primer could work well (Paramanik and Chikkaswamy, 2014).

RAPD analysis could be widely used for detecting DNA polymorphism, to generate molecular markers, species identification and population genetic structure that were used in a variety of different applications (Arif and Khan, 2009; Huang *et al.*, 2003) and to analyze genetic variation or molecular characterization within and between populations of plants and animals (Paramanik and Chikkaswamy, 2014; Maciuszonek *et al.*, 2005; Atil *et al.*, 2011; Maiti *et al.*, 2009; Ozbey *et al.*, 2004). RAPD analysis was used to detect genetic similarities among individuals (Stepniak *et al.*, 2002), to uncover the polymorphism of cattle and sheep (Guneren *et al.*, 2010; Devrimand Kaya, 2006; Kumar *et al.*, 2008; Ali, 2003), to reveal goat polimorfism (Kumari *et al.*, 2013; Oliveira *et al.*, 2005)

The objective of this study was to detect genetic diversity of ILEG of NS and AI population with RAPD or and taxonomy analysis in the form of dendogram. In the future, the result of this study could serve as a source of information for policy-making fondation for a breeding program of ILEG goat as a superior local Indonesian goat in the short, medium and long program.

MATERIALS AND METHOD

The research method consisted of two stages: the first stage of the survey research for blood sampling and the second stage was experimental laboratory for DNA analysis by RAPD.

Materials

Samples taken must fulfill the following criteria: 1) they have pedigree estimation; 2) they were healthy and not experiencing reproductive disorder. Samples were used 19 AI goats (code number A23-A41) and 19NS goats (code number IB 20-IB 38).

Phenotypic Characteristics

Each doe sample was recorded characteristic phenotypic. Observed phenotypic variation were back line, face profile, face form, forehead profile, horn shape, foldears, udder, and number of nipples, the colour of body hair, tail, face and legs. Data tabulated by converted into binary data. Only a distinct phenotypic was scored for these estimation of various parameters. Only distinct phenotypic was scored for estimation of various parameters.

The presence and absence of phenotypic variation was recorded as "1" and "0", respectively. The binary coded characters (1, 0) were used for phenotypic variation analysis. The phenotypic similarities between individuals of same or different groups were calculated by NTsys version 2.0 software. Dendogram illustrated through phenotypic similarity of the qualitative traits of the two populations in two rural areas of Indonesian Local Ettawah goat breeding.

Extraction DNA

Blood samples (3-5mL) were taken from vena jugularis of ILEG using syringe with needle (terumo) with ethical clearance with number 845-KEP-UB from research ethics commission of Brawijaya University and then inject to vaculab EDTA (Onemed). As soon as possible blood samples were isolated or stored at -20^{0}C. DNA isolation used salting out method. DNA extraction technique was through several phases with the addition of RBC lysis, celllysis buffer, PCI, CI and several treatments incubated using water bath (Yamato BT 100), vortexed (Genie), centrifuged (Hettick) and microcentrifuge (refrigerated centrifuge Micro22R). DNA isolation was harvested by the addition of absolute EtOH and 3 M Na-acetate, followed by addition of 70% EtOH. Storage of extracted DNA pellet with the addition of TE buffer and temperature of3-6° C

RAPD Analysis

This study used 12 primers and only 11 primers that could amplify DNA of more than 3 bands of DNA (Table 1). DNA amplification used a *master cycler gradient*. RAPD amplification consisted of following stages: pre denaturation at a temperature of 95 ° C for 5 minutes, denaturation at a temperature of 95 °C for 1 min, annealing at a temperature of 36 °C for 1 min, extension at a temperature of 72 °C for 2 minutes, and post extension at 72 °C for 10 minutes. Samples were amplified with 35 cycles (Mudawamah *et al.*, 2014)

Visualization of DNA amplification was done by electrophoresis (Lee *et al.*, 2012). Running RAPD products performed in 1.5% agarose. Only distinct and clear bands were scored for the estimation of genetic variation. The presence and absence of the band were given a score of "1" and "0". Genetic

variation between individuals of the same or different groups were calculated using NTSYS software version 2.0 through observation of similarity and dendogram.

Table 1. Primer sequens and amplification DNA characteristic

Name of Primers	Sequence	Polymorphic (Yes /No)
OPP 8	5′ ACATCGCCCA 3′	Yes
OPP 12	5′ AAGGGCGAGT 3′	Yes
RAPD 2	5′ CCGCGCCGGT 3′	Yes
RAPD 4	5′ CAGCCTCGGC 3′	Yes
RAPD 7	5′ ACGTCGAGCA 3′	Yes
RAPD 11	5′ GCACTGAGTA 3′	Yes
RAPD 15	5′ GCTAGCTACG 3′	Yes
RAPD 19	5′ TCGCGAGCTG 3′	Yes
RAPD 21	5′ GTGACGTAGG 3′	Yes
RAPD 23	5′ GTCCACACGG 3′	Yes
RAPD 25	5′ TTAGCGCCCC 3′	Yes

RESULTS AND DISCUSSION

Phenotypic Variations based on Dendogram

Phenotypic dendogram based on 15 phenotypic characteristics in NS group which resulted phenotypic similarity (Figure 1) which resulted phenotypic similarity was varied between 0.45-1.00 or phenotypic variation was ranged between 0-0.55**.**

Phenotypic similarity of does derived from NS (Figure 1) included specification of triangle face, folded ears and dropped ears. The phenotypic variations of does derived from NS included line back and symmetric udders, the colour of tail, legs, foot, and face.

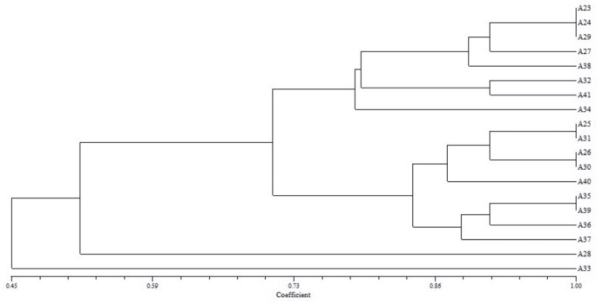

Fig. 1. Dendogram phenotypic of does derived from NS (sample code A23-A41)

Phenotypic dendogram based on 15 phenotypic characteristics in AI group which resulted phenotypic similarity was varied between 0.56-1 (Figure 2) or the phenotypic variation was ranged between 0 –0.44

Phenotypic similarity of does derived from AI (Figure 2) included symmetric udders, and folded ears. The phenotypic variations of does derived from AI included line back, specification of triangle face, dropped ears, the colour of tail, legs, foot, and face.

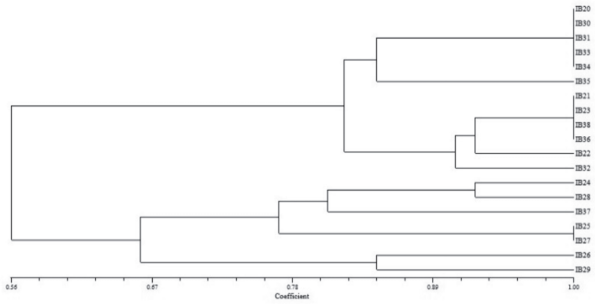

Fig. 2. Dendogram phenotypic of does derived from AI (sample code IB20-IB37)

Phenotypic dendogram based on 15 phenotypic characteristics in NS and AI group (Figure 3) which resulted phenotypic similarity was varied between 0.58-1.00 orthe phenotypic variation was ranged between 0 – 0.32.

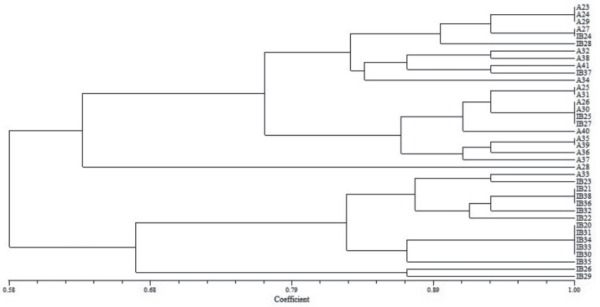

Fig. 3. Dendogram phenotypic of does derived from NS (sample code A23-A24) and AI (sample code IB20-IB37)

Genetic Variations based on Dendogram

RAPD Dendogram derived from NS group which used OPP 8, OPP 12, RAPD 2, RAPD 4, RAPD 7, RAPD 11, RAPD 15, RAPD 19, RAPD 21, RAPD 23, and RAPD 25 primers, generated the lowest of genetic similarity was 0.58 or the highest of genetic variations was 0.42 (Figure 4).

RAPD Dendogram derived from AI group which used OPP 8, OPP 12, RAPD 2, RAPD 4, RAPD 7, RAPD 11, RAPD 15, RAPD 19, RAPD 21, RAPD 23, and RAPD 25 primers, generated the

lowest of genetic similarity was 0.68 or the highest of genetic similarity was 0.32 (figure 5).

In Figure 4 and 5, the genetic variation between does derived from NS was 42 % lower than genetic variation between does derived from AI (1-68 % = 32 %). The results of this study were higher than among individual variation in a population of Moxoto local goats72,55 % (Oliveira *et al*, 2005), and the genetic variation values were higher compared to Jakhrana goat population with 11% (Harma and Bhusan, 2016).

In Figure 4 and 5, the genetic variation from NS (42 %) was higher than AI group (32 %). It was due to the selection of AI more restrictive compare to the selection of NS. Bucks of artificial insemination had been selected based on production recording and reproduction. While bucks from natural service had been selected based on the estimation of outer appearance only and no recording. Therefore, the selection of AI bucks was more intensity than that of natural mating populations. The intensity of selection increased homozygosity and decrease heterozygosity. In accordance with opinion (Bulut *et al*,2016; Brotherstoneand Goddard,2005) that selection reduced genetic diversity and variation among breeds.

RAPD Dendogram derived from the combination of NS and AI group which used OPP 8, OPP 12, RAPD 2, RAPD 4, RAPD 7, RAPD 11, RAPD 15, RAPD 19, RAPD 21, RAPD 23, and RAPD 25 primers, generated the lowest of genetic similarity was 0.51 or the highest of genetic variation was 0.49 (Figure 6).

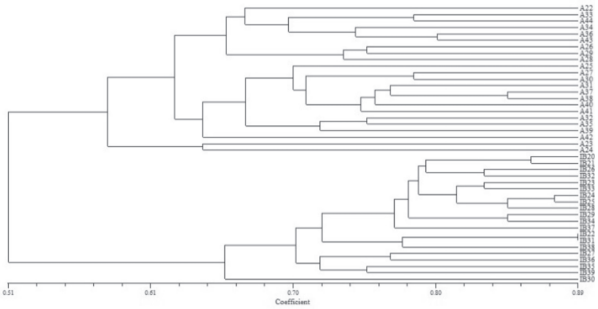

Fig. 6. RAPD Dendogram compared between NS (samples number A22-A44) with AI (samples number IB20-IB39)

Genetic similarity between NS and AI group was 51% (figure 6), and genetic variation between NS and AI grop was 49 %. The genetic similarity was lower than that of 3 local breed goats in Saudi Arabia ranged from 88-93% (El-tarras *et al.,* 2015), but 3% higher than Peranakan Ettawa Goats using primary OPA 4 to OPA 19 (Mudawamah *et al.,* 2014). The genetic similarity between NS and AI groups was a low category. These results reinforced the scientific evidence that the two goat populations were derived from bucks of different pedigrees although both were one breed.

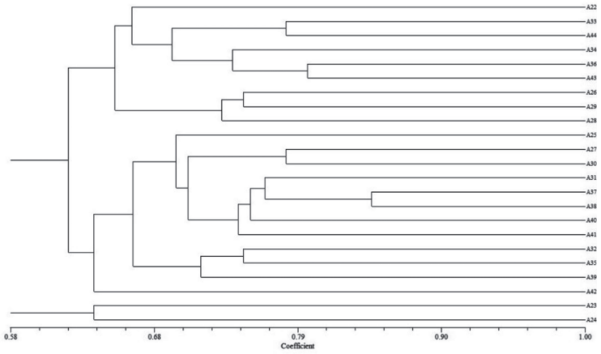

Fig. 4. RAPD Dendogram of does derived from NS (Samples Numbers A1-A21)

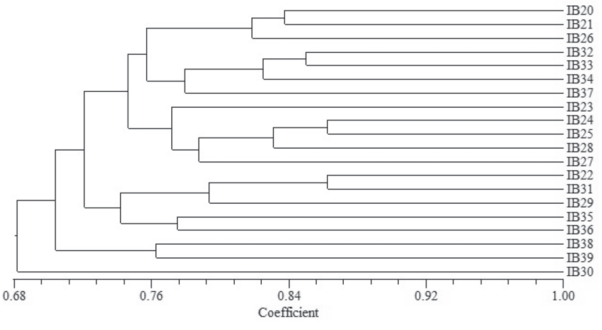

Fig. 5. RAPD Dendogram of PE does derived from AI (sample numbers IB 20-IB39)

Phenotypic and Genotypic Combination based on Dendogram

Dendogram of genetic and phenotypic combination in NS resulted similarity value was varied between 0.60-1.00 (Figure 7).

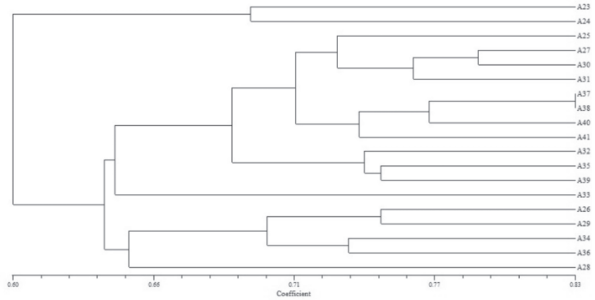

Fig. 7. Dendogram of genetic dan phenotypic combination in NS (sample number A23-A41)

The similarity of genetic and phenotypic combination in NS was 60 % that was lower than AI (69 %), Figure 7 and 8. It meaned that the diversity of genetic and phenotypic combination was higher in NS than in AI. This was caused selection method of NS was only based on observation at the purchase moment and no buck recording. Meanwhile, selection method of AI based on good managemen and individual performance recording.

Dendogram of genetic and phenotypic combination in AI resulted similarity value was varied between 0.53-1.00 (Figure 9).

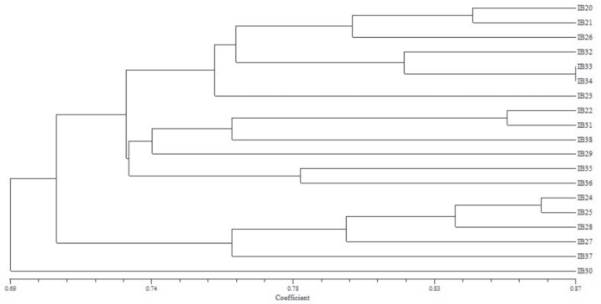

Fig. 8. Dendogram of genetic dan phenotypic combination in AI (sample number IB20-IB38)

Based on Figure 9, dendogram of genetic and phenotypic similarity derived from NS group and AI group was 53 %. It showed that genetic and phenotypic similarity was low categorized but genetic and phenotypic diversity between NS and AI was 47 % (low categorized).

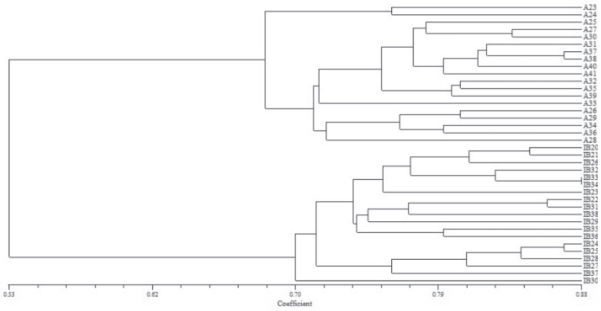

Fig. 9. Phenotypic and Genotypic Dendogram based on the combination of NS (samples number A23-A41) and AI (samples number IB20-IB37)

CONCLUSION

Indonesia Local Does derived from bucks with no recording (natural service program) had genetic variation, phenotypic variation and the combination between phenotypic and genotype variation was higher than does derived from bucks with selection based on recording (artificial insemination program).

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