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GENETIC VARIATION AMONG LOCAL SHEEP IN INDONESIA USING MICROSATELLITE DNA

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ABSTRACT

The objectives of this study were to evaluate the genetic variation and examine the genetic distance of Indonesian local sheep among east part and west part using CSSM018; ILST054 and IDVGA30 microsatellite loci. Data were collected from eight sheep populations, four populations from East part (Rote, Sumbawa and Donggala) and five populations from west part (Ciomas, Jonggol Margawati Madura and Indramayu). Genetic variation for each sheep population were calculated in the form of allelic (total number alleles, mean number of alleles per locus, frequency of every allele in each population) according to Nei (1987): The phylogenetic trees were constructed using UPGMA (unweighted pair-group method with arithmetic mean) based on the genetic distance (D). The result showed that CSSM018; ILST054 and IDVGA30 microsatellite loci. Exhibited a total of 13 alleles from eight populations. Allele d in locus CSSM018 has only found in Madura sheep. Allele b in locus loci ILST 054 was found only in Margawati (0.0833) and allele d was found only in Jonggol (0.0833) and in Indramayu (0.0455). The heterozygosity value ($\hat{h}$) of loci CSSM 018 was high (0.5680) followed by IDVGA-30 (0.5190) and ILST054 (0.3486) and statistically different $t = 6.75$, $p<0.05$. The Indramayu population has the highest average heterozygosity ($\hat{H}$) value (0.5426) followed by Margawati (0.5372), Madura (0.4887), Jonggol (0.4758), Sumbawa (0.3309), Ciomas (0.3105), and Donggala (0.2935) and Rote (0.1163). The population which genetic distance was nearest to Margawati was Indramayu (0.0028) followed by Sumbawa (0.0528), Madura (0.0747), Rote (0.0848), Ciomas (0.0854), Jonggol (0.1166) and Donggala (0.1401). Population was furthest to Rote was Jongol (0.2855), and Ciomas (0.2540).

Key words: *Indonesian local sheep, microsatellite DNA and genetic distance*

INTRODUCTION

Local sheep from East area of Indonesia have body size smaller than west area. However, it has many desirable characters such as being adapted to the low quality vegetation and to withstand seasonal shortages of food and water during dry season. Genetic variation should be taken into account to guide genetic conservation programs, while highly polymorphic microsatellite DNA marker provide a good tool to asses the genetic variation (Mougel *et al.,* 1997). So using microsatellite markers to analyze the genetic variation is critical for identification and characterization of Indonesian native sheep.

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MATERIALS AND METHODS

Sampling

Blood samples were collected randomly from 8 populations; which were three populations from East part (Rote, Sumbawa and Donggala) and 5 populations from West part (Garut fighting type from Ciomas commercial farm, Jonggol Teaching and Research Farm of Fact of Anim. Sci, Bogor Agric. Univ. Garut meat type from Margawati Breeding Center, fat tail type originally from east Java but breed for special purposes for almost 10 years in Indramayu, fat tail type from small farm in Madura).

Microsatellite genotyping

The totall number of DNA samples was 96 from 8 populations. The genomic DNA was extracted by a standard phenol-cloroform protocol with some modification of Sambrook *et al.*, (1989). Three microsatelitte loci were chosen (Cockett *et al.* 1994). The primers used for the amplification of these microsatellite loci were listed in Table 1. The PCR reactions were carried out in *thermocycler* (TaKaRa PCR Thermal Cycler MP4) in 12.5 µl final volume consisting of 1 µl (100-150) ng DNA, 0,5 µl (10pM) forward primer and 0,5 µl (10pM) Reverse primer, 1 µl (µM 100) dNTP.. 1 µl (1.5 nM) $MgCl_2$, 10 x PCR Promega *buffer* 1,25 µl and 0,15 µl supertaq. After an initial 5 min denaturation at 94^0C, 30 cycles were performed as follow: 55 s denaturation at 94^0C, 60 annealing at 56^0C, and 70 s extension at 72^0C. A final elongation was carried out for 7 min at 72^0C. PCR products were separated on electrophoresis in 8 % polyacrylamide gels for 2.5 hours and constant electric current 165 mA; bands were visualized by rapid silver staining according to Tegelstrom (1992).

Statistical Analysis

Genetic variations for each sheep population were calculated in the form of allelic (total number alleles, mean number of alleles per locus, frequency of every allele in each population) according to Nei (1987):

The gene frequency of A1 is given by:

$$X_i = (2N_{11} + N_{12}) / (2N)$$
$$= X_{11} + X_{12}/2$$

Obviously, the gene frequency of A_2 is $X_2 = 1-X_1$

Heterozygosity ($\hat{h}$) estimated) as a follow:

$$\hat{h} = 2n\,(1-\Sigma x_i^2) / (2n-1)$$

Where, x_i = frequency of the i_{th} allele at a locus

n = the number of individual sampled

$\hat{h}$ = heterozygote at locus

In a random mating population, the variance ($V_{sl}(\hat{h})$) can be determined by formula

$$V_{sl}(\hat{h}) = \frac{2}{2n(2n-1)} \{2(2n-2)\,\{\Sigma xi^3 - (\Sigma xi^2)^2\} + \Sigma xi^2 - (\Sigma xi^2)^2\}$$

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And standard error (SE) obtained from square root of $(V_{sl}(\hat{h}))$

The average heterozygosity ($\hat{H}$) is estimated by sampling r loci from the genome namely:

$\hat{H} = \Sigma \hat{h}_j / r$

Where : $\hat{h}_j$ = is the value of $\hat{h}$ for the jth locus
r = number locus tested
$\hat{H}$ = average heterozygosity

The phylogenetic trees were constructed using UPGMA (unweighted pair-group method with arithmetic mean) based on the genetic distance (D) followed formula (Nei, 1987), namely

$D = \ln [J_{XY} / (J_X J_Y)^{1/2}]$

RESULTS AND DISCUSSION

Genetic variation within populations

The band pattern of each locus were shown for CSSM018 in (Fig, 1) ILSTS054 in (Fig. 2) and for IDVGA-30 in (Fig. 3). Three microsatellite loci exhibited a total 13 alleles from the 8 populations (Table 1). The number alleles were 5, 4 and 4 for CSSM018, ILSTS054 and IDVGA-30, respectively. And the estimation of PCR product were126-144 bp, 106-112 bp, and 120-128 for CSSM018, ILSTS054 and IDVGA-30, respectively. The result differed with Freking *et al.* (1997) who reported that the PCR product for CSSM018, IDVGA-30 and ILSTS054 were 120 bp,120 bp dan 140bp, respectively. Due to the differences of breed of sheep used. And also with Hidayat (2004) who reported that the PCR product for CSSM018 and IDVGA-30 were 126-144 bp and 126-130 bp, respectively.

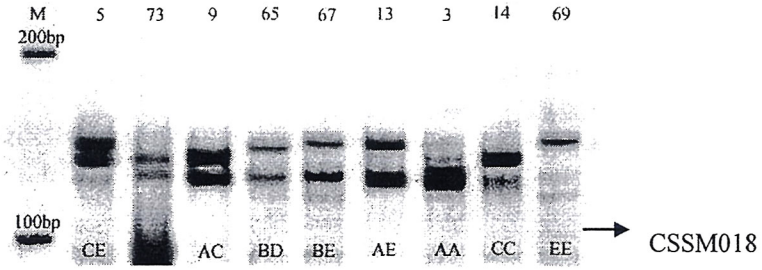

Fig. 1. Band pattern of CSSM018

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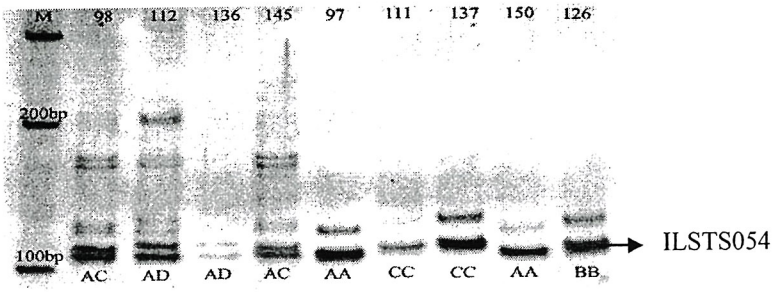

Fig.2. Band pattern of ILSTS054

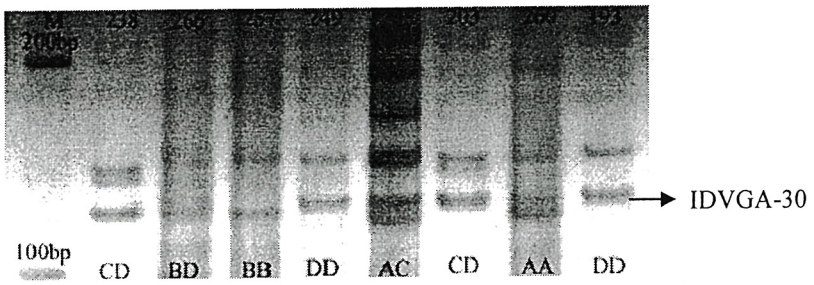

Fig.2. Band pattern of IDVGA-30

Genetic variation among 8 sheep populations

Table 1, showed that the frequency of allele an in locus CSSM018 generally found in 8 populations. The highest (0.5833) was in Margawati followed in Ciomas and Indramayu (0.5417) and the lowest in Rote (0.083). Allele d has only found in Madura sheep. The frequency of allele an in locus ILST 054 almost high in 8 populations, the highest (1.00) was in Sumbawa and Rote and followed in Ciomas (0.8750), Madura (0.8333), Indramayu (0.7273), Margawati (0.7083), Donggala (0.6250) and Jonggol (0.5417). The Allele b was found only in Morganatic (0.0833) and allele d were found only in Mongol 0.0833 and in Intraday 0.0455. The frequency of allele an in locus IDVGA-30 was very low and only found in Madura (0.0417) and in Sumbawa (0.0833). However, allele d almost high found in Ciomas (0.9545) followed by Jonggol (0.9167) and Donggala (0.91667) and the lowest was in Rote (0.100). Hidayat (2004) who reported that the loci IDVGA-30 was very polymorphic with 12 genotypic variation but loci CSSM018. was monomorfic.

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Heterozygosity

Table 2, showed the heterozygosity value ($\hat{h}$) of loci CSSM 018 was high (0.5680) followed by IDVGA-30 (0.5190) and ILST054 (0.3486) and statistically different $t = 6.75$, $p<0.05$. The Indramayu population has the highest average heterozygosity ($\hat{H}$) value (0.5426) followed by Margawati (0.5372), Madura (0.4887), Jonggol (0.4758), Sumbawa (0.3309), Ciomas (0.3105), Donggala (0.2935) and Rote (0.1163). The average heterozygosity ($\hat{H}$) in this research was 0.4785 lower than 0.6899 (Hidayat 2004).

The result suggested that the genetic variation was greater in Indramayu than in the Donggala and Rote population. The possible reason might be due to the sheep from Donggala and Rote has a long–time breeding history and stationary genetic property at isolated area, while the Indramayu has only ten years of breeding history.

Genetic distance

The genetic distance among 8 sheep populations was listed in Table 3, from which the phylogenic trees were conducted as Figure 4. The result showed that the population which genetic distance was nearest to Margawati was Indramayu (0.0028) followed by Sumbawa (0.0528), Madura (0.0747), Rote (0.0848), Ciomas (0.0854), Jonggol (0.1166). Donggala (0.1401). Population was furthest to Jonggol was Rote (0.2855), and population was furthest to Ciomas was Rote (0.2540).

Table 2. Heterozygosity ($\hat{h}$) and average heterozygosity ($\hat{H}$) in 8 sheep populations

Population	Heterozygosity ($\hat{h}$)			Average Heterozygosity ($\hat{H}$)
	CSSM 018	ILST 054	IDVGA-30	
Ciomas	0.6123±0.0676	0.2283±0.1006	0.0909±0.0799	0.3105±0.0827
Jonggol	0.6848±0.0568	0.5833±0.0573	0.1594±0.0932	0.4758±0.0691
Margawati	0.5073±0.0391	0.4674±0.1002	0.6368±0.0593	0.5372±0.0662
Indramayu	0.5544±0.0480	0.4372±0.1020	0.6364±0.0489	0.5426±0.0663
Donggala	0.2283±0.1006	0.4891±0.0528	0.1630±0.0978	0.2935±0.0837
Madura	0.7428±0.0520	0.3030±0.1422	0.4203±0.1080	0.4887±0.1007
Rote	0.1594±0.0932	0.0000±0.0000	0.1895±0.1063	0.1163±0.0665
Sumbawa	0.4746±0.1038	0.0000±0.0000	0.5181±0.0270	0.3309±0.0436
Average Heterozygosity ($\hat{H}$)	0.5680±0.0248	0.3486±0.0385	0.5190±0.0281	0.4785±0.0305

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Table 1. Frequency Allele of microsatellite locus CSSM018, ILSTS054 and IDVGA-30 in each eight sheep populations

Locus	Allele, Length (pb)	Populations							
		Ciomas	Jonggol	Margawati	Indramayu	Donggala	Madura	Sumbawa	Rote
CSSM018	a(126)	0.5417	0.3333	0.5833	0.5417	0.1250	0.2500	0.1667	0.0833
	b(128)	0.0417	0.0833	0	0.0417	0	0.0833	0.1250	0
	c(136)	0.3333	0.4583	0.4167	0.4167	0.8750	0.4167	0.7033	0.9167
	d(138)	0	0	0	0	0	0.0417	0	0
	e(144)	0.0833	0.1250	0	0	0	0.2083	0	0
ILSTS054	a(106)	0.8750	0.5417	0.7083	0.7273	0.6250	0.8333	1	1
	b(108)	0	0	0.0833	0	0	0	0	0
	c(110)	0.1250	0.375	0.2083	0.2273	0.3750	0.1667	0	0
	d(112)	0	0.0833	0	0.0455	0	0	0	0
IDVGA-30	a(120)	0	0	0		0	0.0417	0.0833	0
	b(122)	0	0	0.5000	0.4545	0	0.1667	0.5417	0.9000
	c(124)	0.0455	0.0833	0.1500	0.1364	0.04167	0	0	0
	d(128)	0.9545	0.9167	0.3500	0.4091	0.91667	0.7500 0.1000	0.4583	0.1000

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Table 4. Genetic distance index between 8 sheep populations

Subpopulation	Subpopulation						
	Jonggol	Margawati	Indramayu	Donggala		Madura	
	Sumbawa		Rote				
Ciomas	0.0260	0.0854	0.0669	0.0663	0.0192	0.1017	0.2540
Jonggol		0.1166	0.0912	0.0263	0.0308	0.1310	0.2855
Margawati			0.0028	0.1401	0.0747	0.0528	0.0848
Indramayu				0.1154	0.0561	0.0447	0.0875
Donggala					0.0472	0.0958	0.1954
Madura						0.0508	0.1514
Sumbawa							0.0270

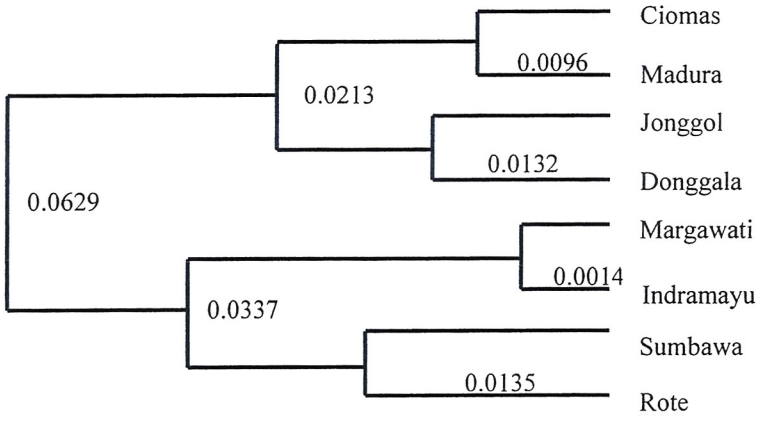

Fig 4. The UPGMA genetic cluster among 8 sheep populations

CONCLUSION

The result showed that CSSM018; ILST054 and IDVGA30 microsatellite loci. Exhibited a total of 13 alleles from 8 populations. Allele d in locus CSSM018 has only found in Madura sheep. Allele b in locus loci ILST 054 was found only in Margawati (0.0833) and allele d were found only in Jonggol (0.0833) and in Indramayu (0.0455). The heterozygosity value ($\hat{h}$) of loci CSSM 018 was high (0.5680) followed by IDVGA-30 (0.5190) and ILST054 (0.3486) and statistically different $t = 6.75$, $p<0.05$.

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