Y Chromosome Microsatellites Variation in Bali Cattle *(Bos sondaicus)* Population

(Variasi Mikrosatelit Kromosom Y pada Populasi Sapi Bali)

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Abstract. Seven Y chromosome specific microsatellites were typed in a sample of 36 unrelated males from Bali cattle breed. Analysis variation of microsatellites marker in Bali cattle were determinate from PCR products by using 7 primer pairs that flanking microsatellites (INRA008, INRA057, INRA062, INRA124, INRA126, DYS 199, and INRA 189). PCR products were separated by 10% polyacrylamide gel electrophoresis (PAGE), and silver staining method was used to detect allele polymorphism at each locus. From two different geographical breed origin showed that Bali cattle from Bali island has higher heterozygous (h=0.33) level than Lombok island (h=0.30), but the number of allele was few (only two alleles) in all of locus. It is indicated that Bali cattle from two geographical origins were not significant in genetic variation. We know that Y chromosomal microsatellite in general has tend to specific allele in breed comparing to autosomal chromosome, because allele come from only male or Y sex chromosome and it contrary to autosomal chromosome where allele is contributed from male and female. FAO has specified that minimum four distinct alleles per locus for proficient judgment of genetic differences between breeds and this study we only used one breed, so it may not significantly to discriminate in Bali cattle population. For next study we need more Y chromosomal microsatellite marker to discriminate more Indonesian breeds related to tracing the genetic potential and because males animal has roles in genetic spreading which can have an enormous impact on highly selected domestic animal populations.

Key Words : Bali cattle , Y chromosome, microsatellite, allele

Introduction

Asia continent has more than three hundred millions cattle and less than two millions located in India sub continent. Asia and Africa cattle breed in general could be differed by has hump and humpless, and more than 170 breeds has been identified including Bali cattle (domesticated from Banteng, *Bos [Bibos] banteng)* which found in South East Asia, especially in Indonesia and Philippine. So, Asian farmers were has important role in domestication processed or agricultural ecology in this continent.

Bali cattle is very popular native cattle in Indonesia related to its status in development of farmer community, especially as a drought and meat animal served. Beside Bali cattle, Indonesia has others breed where it existence has common ancestry from Banteng (like Madura cattle) or from *Bos taurus* and *Bos indicus*, that is in past years caused of economics and politic policy (colonialism or after independence) were imported to Indonesia and could adapted in local climate and be a part of Indonesian native cattle. So, it is interested to study Indonesian native cattle because of large genetic variation and to maintain the genetic characters and the importance of repairing breeding management related to conserve genetic traits or preventing extinction of Indonesian native cattle.

This study is effort to find the breed specific alleles or DNA marker in Bali cattle by using microsatellites marker in term of Y chromosome

location. Different area of Bali cattle origin that is Bali and Lombok islands were used. From the study before that based on 16 microsatellites locus in autosomal chromosome but not in sex chromosome were found that markers could giving the genetic relation between Bali, Madura, Ongole Offspring and Brangus cattle (Winaya *et al.*, 2000) and also Verkaar *et al.* (2002 & 2003), Nijman *et al.* (2003) and Ugla (2008) could be differentiated many breeds of Indonesian cattle using autosome microsatellite, but this result could not illustrated yet the relationship between microsatellite marker and economic traits of native cattle, like reproduction ability of Indonesian native cattle male.

The important of assumption in using molecular marker for genetic study because polymorphism in molecular level is neutral and although using less number of microsatellite locus, the allele segregation is independently, so it could be using as a good tool to predict the entire genomic variation of population. In the other word, the allele frequencies between populations could be giving the illustration of genetic variation distribution between or inter population.

Research Methods

Sample collections

Blood samples were collected from 36 male unrelated Bali cattle that randomly selected at Bali and Lombok islands. Cattle were selected from different villages after interviewing with the owners in details to ensure the family is unrelated cattle in pedigree. Samples for DNA analysis was extracted from whole blood cells (5–10 mL) that were collected from jugular vein of cattle and preserved in vaccutainer tubes containing EDTA as anticoagulant.

The DNA isolation procedure was based on phenol-chloroform standart protocol according to Sambrook *et al.* (1989). After genomic DNA from isolation purified and measured the concentration, then prepared for a template in PCR reaction.

Molecular techniques

In the reaction of DNA amplification or PCR reaction, the genomic DNA cattle was used as a template and the pairing primers (forward and reverse) which flanking microsatellite sequence in Y chromosomal locus were used as complement sequence. Seven primers was used in the PCR reaction to generate microsatellite genotyping data from panel of 36 males Bali cattle. Because microsatellite markers are codominant, 36 samples are correspond to 54 alelles for each locus.

PCR reaction was performed with total volume 25µl from the mix of 50-100 ng genomic DNA template; *Taq* Polymerase DNA with 10 X buffer *Taq* Polymerase (100 mM Tris-Cl, pH 8.3; 500 mM KCl; 15 mM $MgCl_2$; 0.01% gelatin); dNTP'S mix (dGTP, dATP, dTTP dan dCTP) (Roche Applied Science, Germany); and dH_2O sterile. The PCR procedure in thermocycler machine (T-Personal Biometra, Whatman, Germany) was designed by pre-denaturation temperature 95^{o}C for 3 minutes, denaturation 95^{o}C for 45 seconds, annealing 58-60^{o}C for 45 seconds, extension 72^{o}C for 45, finally extension 72^{0}C for 3 minutes and post PCR 4^{o}C. The PCR cycles was repeated by 40 times.

The PCR products were separated on 10% denaturating polyacrylamide gels by homebrew and sized using a 100-bp ladder (Roche Applied Science, Germany) as standard for sizing. Gels were stained by silver staining according to Guillemet & Lewis (Tegelström, 1986), and genotypes score by manually (Leung *et al.,* 1993).

Statistical Analysis

The number and frequency of microsatellite alleles were calculated to get the information of heterozygosity level. Alelle frequency (haplotype) in each microsatellite locus was calculated according to the formula :

$$f(A) = \frac{A}{2n}$$

where :

f (A) = alelle frequency

A = alelle number of the the i^{th} locus

n = number of individu observed

The locus is stated polymorphic if the alelle frequency value is similar or more or less than 0.99. Genetic variation determined by average of

heterozygosity (h) value in all of locus. The heterozygosity value calculated according formula (Nei, 1987) :

$$h = 2n\frac{\left(1-\sum Xi^2\right)}{\left(2n-1\right)}$$

where :
h = locus heterozygosity
X_i = alelle frequency of the i^{th} locus
n = number of individu observed

Allelic frequencies also were utilized for assessing polymorphic information content (PIC), a measure of informativeness of a marker, calculated according to Botstein *et al*. (1980) using the given formula :

$$\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^{\,2} - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2P_i^{\,2}P_j^{\,2}$$

where *k* is the number of alleles and *xi* and *xj* are the frequencies of the i^{th} and j^{th} alleles respectively.

Results and Discussion

Most of study related to microsatellite DNA markers in cattle were applied in autosomal chromosome and less in sex chromosome like Y chromosome. From Hanotte *et al.* (2000) study; by using Y chromosome microsatellite in locus INRA 23 showed that any zebu male introgession into native cattle in Mozambique and Zimbabwe which prediction those allele came from Mozambique gulf. In past years, Mozambique gulf was an international trade area, included cattle trade where it could be an introgession event between breeds, and then was also found a *Bos taurus* breed which has trypanotolerant character.

In the previous study (Winaya *et al.,* 2000) by using sixteen autosomal chromosomal microsatellite has indicated that Bali cattle has higher genetic variation than Madura cattle by heterozygosity (h) level 0.33. In this study we applied seven microsatellites marker (INRA008, INRA057, INRA062, INRA124, INRA126, DYS 199, and INRA 189) at Y chromosome loci were not identified yet in Bali cattle breed. Table 1 showed the number and frequency of all microsatellite alleles at Bali cattle and found that all of the Y chromosomal microsatellite markers were low in frequency (only two alleles).

In general, this allele number is low and according to FAO it was not sufficient to differ or genetic variation between breed, it needed four different alleles per locus microsatellite marker for proficient judgment justification. FAO has specified that minimum four distinct alleles per locus for proficient judgment of genetic differences between breeds (Pandey *et al.,* 2006), however in this study using only one breed with different cattle origin, so markers applied only in Bali cattle populations. From allele frequencies, have been calculated that the average of heterozygosity value less than 50% (33%) or quite variation in Bali cattle population from two origin. In this study assumed that markers may not effectively discrimination within Bali cattle population because the one of segregation allele model of Y chromosome is tend to dominant effect in certain breed, for example INRA 124 locus is presumably an Indicine allele (Hanotte *et al.,* 2000; Edwards *et al.,* 2007). As Kikkawa *et al.* (2003) study which based on mitochondria DNA indicated that one of Bali ancestor came from Zebu allele.

In our past studied which used autosomal chromosomal microsatellites have been found that Bali cattle population more polymorphic than Madura and Indonesian Ongole breeds with four alleles (Winaya *et al.,* 2000). These could be explained that when using Y chromosomal microsatellite, it will be tend to certain male breed specific allele. It is different when using autosomal microsatellite, the allele is originating from two sex parents, male and female, so allele commonly heterozygous compare to Y chromosome which commonly homozygous allele. Other study from Kumar *et al.* (2003) by using 20 autosomal microsatellites showed that the average of expected heterozygosity (h) in seven breeds of Asian cattle was 0,66 higher than this study (0,33) with mean number allele per locus (MNA) 5,45. Similar with our past study in Indonesian Ongole filial has more heterozygous (0,46) than Bali, Madura and Brangus. So, it is indicated that Bali breed has low polymorphic by microsatellite marker.

Table 1. The animal assayed number, allele number and allele frequency (haplotypes) of Y-chromosome microsatellite in Bali cattle population

LOCUS	BALI			LOMBOK		
	1	2	3	1	2	3
INRA 008	18	2	A = 6 (0.33)	18	2	A = 2 (0.11)
			B = 12 (0.67)			B = 16 (0.89)
INRA 057	18	2	A = 4 (0.22)	18	2	A = 4 (0.22)
			B = 14 (0.78)			B = 14 (0.78)
INRA 062	18	2	A = 3 (0.17)	18	2	A = 2 (0.11)
			B = 15 (0.83)			B = 16 (0.89)
INRA 124	18	2	A = 8 (0.44)	18	2	A = 6 (0.33)
			B = 10 (0.56)			B = 12 (0.67)
INRA 126	18	2	A = 2 (0.11)	18	2	A = 4 (0.22)
			B = 16 (0.89)			B = 14 (0.78)
DYS 199	18	2	A = 3 (0.17)	18	2	A = 4 (0.22)
			B = 15 (0.83)			B = 14 (0.78)
INRA 189	18	2	A = 2 (0.11)	18	2	A = 2 (0.11)
			B = 16 (0.89)			B = 16 (0.89)

Notes : 1) animal assayed number; 2) allele number; 3) allele frequency

Table 2. The polymorphic information content (PIC) score and heterozygousity level of Bali cattle population

LOKUS	BALI		LOMBOK	
	h	*PIC*	*h*	*PIC*
INRA 008	0.46	0.34	0.20	0.18
INRA 057	0.35	0.29	0.35	0.29
INRA 062	0.29	0.24	0.20	0.18
INRA 124	0.51	0.37	0.46	0.34
INRA 126	0.20	0.18	0.35	0.29
DYS 199	0.29	0.24	0.35	0.29
INRA 189	0.20	0.18	0.20	0.18
Mean	0.33 $\pm$ 0.18	0.26 $\pm$ 0.11	0.30 $\pm$ 0.07	0.25 $\pm$ 0.01

In our opinion, Bali cattle or other Indonesian cattle are needs seriously attention regarding to its existence. From polymorphic information content (PIC) values, the Y microsatellite markers showed PIC value between 0.18 to 0.37, and from seven loci only locus INRA 124 has highest value (0.37), so it means this value lower than 0.5 where are normally consider as informative in population genetic analyses (Botstein *et al.,* 1980).

Consequently, locus INRA 124 only was not informative marker yet in this breed. According to Hannote *et al.* (2000) study that locus INRA 124 finding as Y chromosome microsatellite allele specific for Indicine allele, so presumably that Bali breed not only has common ancestor from Indicine allele but also from Banteng allele as suggested by Namikawa *et al*. (1980) study.

The observation of heterozygousity level over seven locus, found score between 0.20 to 0.51 with mean 0.33$\pm$0.18 and 0.30$\pm$0.07 (Table 2), it means that Bali breed in this study showed lower than others study, like in Kherigarh cattle of India (0.495–0.856; Pandey *et al.,* 2006), in Deoni cattle breed of India (0.59; Mukesh *et al.,* 2004), and also 12 west/central of African breeds cattle

(0.506–0,697; Ibeagha-Awemu *et al.,* 2004), also our past study in Madura cattle (0.43). Although in this study the heterozygousity level was lower than others study, it does not mean that Bali breed has trend decreasing genetic variation. In our study before (Winaya *et al.,* 2000) that using 16 markers and 25 Bali cattle in autosome showed that heterozygousity level was 0.33. So, assumed that may the genetic variation of Bali breed still conserved or in other reason the inbreeding level was increasing, although Bali cattle samples were unrelated breed in population or within population. If this phenomenon is an inbreeding effect, presumably that may caused by insufficient of male in breeding region, so this condition could be overcome by introducing male from others territory (especially between region or different geography areas). In other view, suggested that markers may less polymorphic if used in Bali cattle since most of markers which used in this study originated from Taurine allele. So, in future may in the study needed use more markers, especially in Y chromosome to ensure that Bali breed is the one of Indonesian local breeds and indeed the exploration of Banteng as the common ancestor of Indonesian local breed may be supported the genetic status of Bali breed.

Conclusions

Bali cattle breed that was assayed by seven markers of Y chromosome microsatellite showed the low polymorphism level. From the value of PIC and heterozygousity level assumed that Bali cattle may tend to increasing inbreeding level, but in other view Bali cattle may has more Taurine allele as a consequently of cross breed event and markers that used in this study may limited and most of them came from Taurine allele. Bali allele presumably has two common ancestors, Banteng and Indicine (Namikawa, 1980). As a suggestion may use more markers and wide coverage of samples based on region or geographical distribution for the next study.

The recent condition of Bali breed is indicated that these breed need to conserve the genetic diversity, because Bali cattle is a breed that has a high value regarding to its existence as indigenous genetic source. This study suggested that to start conservation of this breed may emphasizes in the importance of genetic regulation and conservation of this indigenous as a fattening cattle beside the traditional needed, also may Bali cattle breed society should be formed, which could be educated and supported for comprehensive safeguarding and upgrading of this breed to make it economically sustainable.

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