SELECTION OF MX GENE GENOTYPE AS GENETIC MARKER FOR AVIAN INFLUENZA RESISTANCE IN INDONESIAN NATIVE CHICKEN

T. SARTIKA[1)], S. SULANDARI[2)] AND MSA. ZEIN[2)]

1). Indonesian Research Institute for Animal Production (IRIAP), PO BOX 221 Bogor-16002, Indonesia. Email: tikesartika@hotmail.com.
2). Research Centre for Biology, The Indonesian Institute of Sciences (LIPI),Cibinong- 16911, Indonesia.

ABSTRACT

This research was aimed to select one of the native chicken breeds which found out resistance to avian influenza using the molecular technique. The selected breed will then be used as the base population to improve native chicken breed in Indonesia. Marker Assisted Selection (MAS) method was used in this research to accelerate the selection process, since the disease resistance had low heritability value. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique used to select the genotype of Mx^{++}, Mx^{+-} and Mx^{--} that corresponded to the positive antiviral activity (Mx^{++}), or those which have could positive or negative activity (Mx^{+-}) and negatively antiviral activity (Mx^{--}). There were 200 native hens and 40 cocks used in this experiment. Allele frequency of Mx Gene was calculated. The productivity indicators such as age at first laying, egg weight and hen weight at first laying and egg production were also measured. The chicken that has Mx^{++} and Mx^{+-} genotypes, were selected to produce offspring. Result showed that the resistant allele (Mx^{+}) for laying hen had frequency of 65% and for cocks had frequency of 60%, while the susceptible allele (Mx^{-}) for hen had frequency of 35% and for cocks had frequency of 40%. Age, egg weight and hen weight at first laying and egg production for susceptible genotype was slightly better than those the resistant genotype which were 172,41 VS 178,81 days; 33,94 VS 32,84 g; 1450 VS 1439 g and 54,32 VS 48,30 % respectively.

Keywords: *Indonesian native chicken, Mx gene, seletion*.

INTRODUCTION

Indonesia has many varieties of native chicken Based on phenotypic performances there are about more than 32 distinctive breeds that are being raised under extensive and/or intensive systems (Sartika and Iskandar, 2007). Estimated population was about 230 millions. In Indonesia, native chicken meat is more expensive than the commercial hybrid chicken is. The consumers like to pay more as it is tastier and low fat content. The native chicken eggs also more expensive than commercial chicken eggs,

because it can be used as part of traditional herbal drink call “Jamu” which is very popular in Indonesia.

Since 2003, Indonesia has outbreaks of Avian Influenza (AI). Naturally, native chicken has ability to resist the virus controlled by antiviral gene. The Mx proteins are key components and its coding protein had been shown to be induced by interferon (IFN) and to inhibit the replication of RNA virus (Ko *et al.*2002). Their resistant or sensitive genetic character was shown to result from the difference in genomic structure of Mx gene. Watanabe (2003) examined many more chicken Mx cDNAs from other breeds whether these chickens carry resistant or sensitive character of Mx gene to the VSV/*vesicular stomatities virus* infection, compared with the dfferential antiviral activity with amino acid substitutions at 15 positions. Only an amino acid substitution at position 631 was emerged to determine the difference between the antiviral activity of chicken Mx protein; that is asparagine (Asn) corresponded to the positively antiviral activity and serine (ser) corresponded to the negatively antiviral activity (Ko *et al.*, 2002; Watanabe, 2003; Li *et al.*, 2007). The chicken Mx protein spans about 2,118 bp, with 13 exons on chromosome 1 of the chicken genome. A total of 237 single nucleotide polymorphisms were found in the chicken Mx gene by comparison among 4 directly sequenced Mx genomic DNA sequences. In this study identification of Mx gene by mismatching PCR-RFLP method could analyses whether the chicken carry positive or negative virus activity. Sulandari *et al* (2009) reported the examination of 485 samples from 15 breeds of Indonesian native chicken by a specific PCR-RFLP technique showed that the averaged frequency of resistant allele (A/Mx^+ allele) was 62.73% and that of sensitive allele (G/Mx^- allele) was 37.27%. Investigation of distribution of the allele A (Mx^+) and G (Mx^-) on chickens has also been reported by other researcher (Ko *et al.*, 2002; Watanabe 2003; Li *et al.*, 2006; Seyama *et al.*, 2006; Balkisson *et al.*, 2007 and Watanabe *et al.*, 2007).

The aim of the research was to examine the proportion of allele frequency of Indonesian Native chicken, especially Kampung chicken at breeding population in IRIAP (*Indonesian Research Institute for Animal Production*). Selection using resistant Mx gene is effective for breeding program to increase selected breed as resistant to RNA virus.

MATERIALS AND METHODS

DNA Collection

A total of 240 samples from one of native chickens in Indonesia (selected Kampung chicken for egg production for 6 generations) were used in this study, which were 200 samples from female chicken (hens) and 40 samples from male chicken (Cocks).

DNA Extraction

The fresh blood from chickens was collected and preserved in 96% absolute alcohol. Genomic DNA was extracted from whole blood using the phenol-chloroform method (Sambrook and Russell, 2001).

Genotyping of Mx gene

PCR-RFLP method was employed to genotype the G/A SNP at nucleotide position 1,892 in the 13th exon of coding sequence of the Mx gene using PCR-RFLP mismatched primers. The mismatch primer sequences (Seyama *et al.*, 2006) which amplify approximate 100 bp long fragment were as follows: Forward primer NE-F2 (5'CCTTCAGCCTGTTTTTCTCCTTTTAGGAA3') and Reverse primer NE-R2/R (5'CAGAGGAATCTGATTGCTCAGGCGTGTA3') or Reverse primer NE-R2/S (5'CAGAGGAATCTGATTGCTCAGGCGAATA3'). The Rsa1 restriction enzyme was used with a recognition sequence of 5'GT↓AC3' to cut the fragment at the position of interest when there is an allele G using primer NE-F2 and NE-R2/R, while the Ssp1 restriction enzyme was used with a recognition sequence of 5'AAT↓ATT3' to cut the fragment at the position of an allele A using primer NE-F2 and NE-R2/S. The mismatch RFLPs using Rsa1 yielded one visible fragment of either 100 bp for allele A without a recognition site or 73 bp for allele G, and/or in the contrary for using Ssp1 (Figure 1).

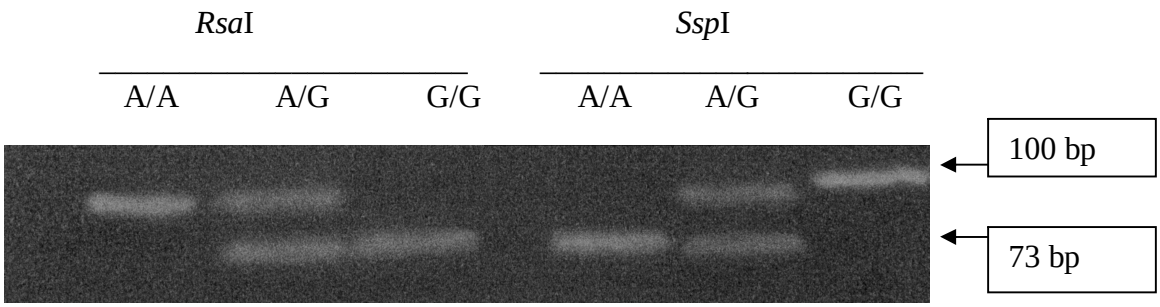

Figure 1. Elektroforesis agarose gel 3%, Seyama *et. al* (2006).

The PCR condition was used: an initial denature at 94°C for 5 min, followed by 35 cycles of 60 s at 94°C, annealing temperature for 60 s at 60°C, and 72°C for 60 s, and final extension at 72°C for 5 min. PCR product were analyzed by electrophoresis through 2% agarose gel in 1x TAE buffer, and stained with ethidum bromide. Amplicons were cleaved with the restriction enzyme the Rsa1 and/or Ssp 1 (1U/µg) for 6-8 hours at 37°C following the manufacture's instruction. The digested fragments were visualized by 12% polyacrylamide gel in constant voltage 160 volt for 4 hours. The gel was stained with silver nitrate (Sulandari and Zein, 2003) and scanned for an image using Adobe Photoshop.

Statistical analysis

Allele frequencies were calculated for hens and cocks. Genotype frequency were obtained by counts, and productivity such as age, egg weight and hen weight at first laying and egg production based on genotype Mx gene were analyse using Anova, Minitab V.14.

RESULT AND DISCUSSION

Mx Gene Genotyping

The genomic DNA of 200 hens and 40 cocks were successfully amplified. Identification of resistant and sensitive chicken Mx gene was examined by mismatch PCR-RFLP. The PCR product was cleaved with the restriction enzyme of the Rsa 1 and the digested showing polymorphism bands, one band with 100 bp in length (A/A, homozygous resistant Mx allelic genes); two bands with 100 bp and 73 bp in length (A/G, heterozygous Mx allelic genes); and one band with 73 bp in length (G/G,

homozygous sensitive Mx allelic gene). An example of genotyping results is presented in Figure 2, and calculated of allele frequency is presented in Table 1.

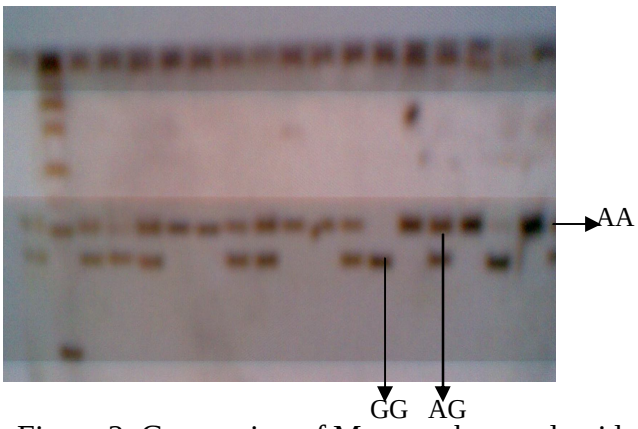

Figure 2. Genotyping of Mx gene by acrylamide gel

Table 1. Frequency of allelic hen and cock native chicken

Samples	**GENOTYPE**			Frequency of allele
	AA/Mx^{++}	AG/Mx^{+-}	GG/Mx^{--}	
Hens native chicken (200)	80	102	18	f(A) =0,65 f(G) =0,35
Cocks native chicken (40)	15	18	7	f(A) = 0,60 f(G) = 0,40

It is shown in Table 1, as resulted from 240 samples by specific PCR-RFLP indicating polymorphisms of the Mx gene (which putatively associated with AI resistance/susceptibility in chickens). The frequency of resistance allele (A allele) for the hen native chicken was 65%, while for cock native chicken was 60%, and that for sensitive allele (G allele) was 35% for hen and 40% for cock native chickens. Investigation of distribution of the allele A and G on chickens has also been reported by other researchers (Ko *et al.*, 2002; Watanabe, 2003; Maeda, 2005; Li *et al.*, 2006; Seyama *et al.*, 2006; Balkisson *et al.*, 2007; and Sulandari *et al.*, 2009). As reported by Sulandari *et al.*, (2009), Indonesian native chicken had averaged frequency of resistant allele (A allele) of 62.73% and sensitive allele (G allele) of 37.27%. A representative various breed of Indonesian native chicken such as White Kedu, Golden Arab, Sentul, Dwarf, Black Kedu, Pelung, Gaok, Kalosi, Tolaki, Merawang, and Cemani chickens, tent to have a higher frequency of the resistant allele. Frequency of A allele in each

breed was 0.58, 0.62, 0.63, 0.66, 0.68, 0.69, 0.70, 0.70, 0.74, 0.81, and 0.87 respectively, while Kapas, Wareng, Nunukan and Silver Arab chickens, as founder local chicken, had a higher frequency of the sensitive allele, frequency of A allele was 0.32, 0.44, 0.45, and 0.47 respectively.

Productivity of native chicken

The productivity of native chicken was divided by 3 groups of Mx genotype presented in Table 2. Statistical analyses showed that in all measurement was not significantly difference, however, descriptively the GG genotype/sensitive allele was relatively slightly better than the AG resistant and sensitive allele and AA genotype/resistant allele. Age at first laying in GG genotype was more quickly than the AG and AA genotype. Similar to those, egg weight, hen weight at first laying and egg production was better in GG genotype.

Table 2. The productivity of hen native chicken for 10 weeks at 1st periods lay

	GENOTIPE		
	AA (Mx^{++})	**AG (Mx^{+-})**	**GG (Mx^{--})**
Age at first laying			
Means (days)	178,81	174,28	172,41
Sdv (days)	16,15	13,79	15,20
CV (%)	9,03	7,91	6,97
Max (days)	221	216	202
Min (days)	149	152	155
Egg weight at first laying			
Means (g)	32,84	32,85	33,94
Sdv (g)	3,54	3,88	4,10
CV (%)	10,77	11,82	1,88
Max (g)	46	49	42
Min (g)	26	26	29
Hen weight at first laying			
Means (g)	1439,11	1395,26	1450,06
Sdv (g)	229,31	189,27	231,94
CV (%)	15,93	13,56	106,39
Max (g)	2437	2081	2023
Min (g)	962	1044	1197
Egg Production during 10 weeks			
Means (eggs)	37,20	39,42	41,82

Sdv (eggs)	15,11	13,75	16,36
CV (%)	40,62	34,88	7,51
Max (eggs)	69	69	66
Min (eggs)	9	3	2
Means (%)	48,30	51,19	54,32
Sdv (%)	19,62	17,86	21,25
CV (%)	40,62	34,88	9,75
Max (%)	89,61	89,61	85,71
Min (%)	11,69	3,90	2,60

Hen day production curve was presented in Figure 3. Based on genotype Mx gene, hen day peak production of hen with GG genotype (64%) was slightly better than of hen with AA and AG genotype (60%). Egg production of native chicken in this study was better than in common native chicken (28%) in Indonesia (Sartika, 2005). The result indicated that the sensitive allele/ GG genotype tent to be better in productivity than that of resistant allele/AA genotype.

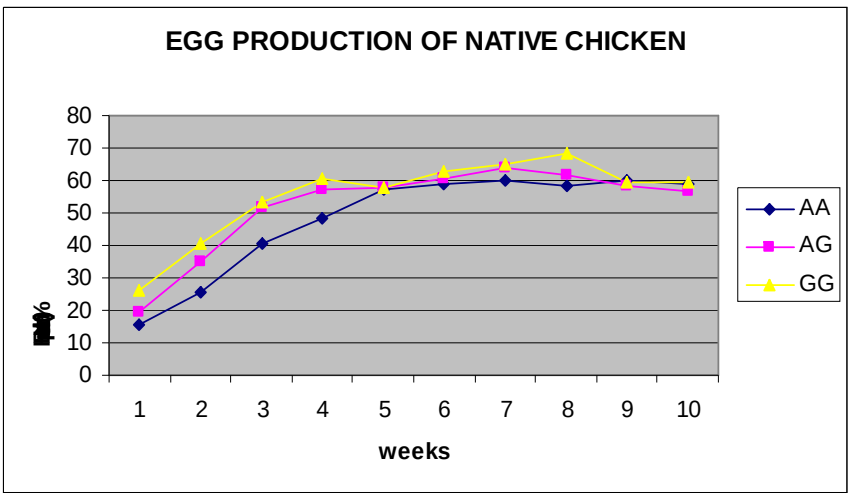

Figure3. Hen day production of native chicken

CONCLUSION

In conclution, the frequency of Mx^{+} gene (A allele) in Indonesian native chicken was relatively high. Based on Mx genotype, the productivity of native chicken measured as age, egg weight, body weight at first laying and 12 weeks egg production statistically was not significantly difference.

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