

MOLECULAR CHARACTERIZATION OF Mx GENE POLYMORPHISM IN GERZE CHICKEN BREED AND PURE LINE CHICKEN BREED

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ABSTRACT

Mx proteins, found in many organisms including birds and mammals, are reported to show antiviral activity by inhibiting replication of various viruses. In the present study, 43 samples of conserved Gerze chicken from Gerze District Directorate of Agriculture and 50 samples of pure lines from Ankara Poultry Research Institute were examined for determining of allelic frequencies of reported as a marker loci related to resistance "NE-F2 and R2 / R" and "NE-F2 and R2 / S" using RsaI and SspI restriction endonucleases by Polymerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP) method. Allele frequencies of resistant (Mx⁺) and sensitive allele (Mx⁻) were found to be 98 and 2 % in Gerze chicken breed population respectively. Virus resistant allele (Mx⁺) was 52 % and sensitive allele (Mx⁻) frequency was 48 % in the pure line population. As a result of the study, Gerze chicken breed population was concluded as a valuable resource that could be used for disease resistance in chicken breeding.

Keywords: Gerze chicken, Mx proteins, Mx gene, PCR-RFLP, Resistant allele

INTRODUCTION

Avian influenza is a viral infection that affects respiratory, digestive and nervous systems in poultry and birds. It is influenza A virus from the family Orthomyxoviridae (Fadhil, 2014). The Mx gene found in various organisms, including human, vertebrates, fish and yeast (Ko *et al.*, 2002; Wantanabe, 2007) have been reported to promote antiviral activity by inhibiting replication of various viruses. The Mx protein is interferon-induced, dynamin-like, large GTPases and has association with influenza virus resistance in laboratory mice (Fulton *et al.*, 2014). In 1995 the researchers cloned from a white Leghorn strain of chicken and found to be devoid of detectable antiviral activity. Subsequently, Ko *et al.* (2002) reported that the chicken Mx gene was highly polymorphic, and that the Mx alleles of some breeds of chicken had activity against

influenza virus and vesicular stomatitis virus (VSV). These authors showed that the amino acid at position 631 of the chicken Mx protein is a crucial determinant of anti-VSV activity (Asn631 is active and Ser631 is inactive against VSV) (Benfield *et al.*, 2010; Sironi *et al.*, 2010). The Mx gene was determination in chickens during the spread AI virus in domestic fowl indicated that the Mx gene is located on chromosome 1 in a 20.767 bp fragment. It consists of 13 exons, with as many as 2.115 bp coding regions and 705 amino acids. Resistance against the AI virus was determined at exon 13, nucleotide number 1.892 where it undergoes alkaline transition mutation (single mutation) (Solihin *et al.*, 2013). The aim of the research was to examine the proportion of allele frequency of Turkey native chicken and pure lines.

MATERIALS AND METHODS

Ninety three chickens (43 natives, 50 pure lines) were used in this study. Blood samples were taken from the winged veins of the chickens (brachial vein). Genomic DNA was extracted from whole blood using the BILATEST kit methods. The PCR-RFLP techniques were used to determine the polymorphism between two chicken breeds. Sartika *et al.* (2011) primers were used in this research. The specific primers sequences were: Forward primer NE-F2 (5'CCTT CAGCCTGTTTTCTCCTTTTAGGAA3') and Reverse primer NE-R2/R (5'CAGAGGAATCTGATTGCTCAGG CGTGTA3'). Amplified fragment 100 bp was separated on 2 % agarose gel and stained with ethidium bromide. 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. The PCR conditions of reaction was used with an initial pre-denaturation at 94°C for 5 minutes, followed by 35 cycles of 60 seconds at 94°C, annealing temperature for 60 seconds at 60°C, and 72°C for 60 seconds, and final extension at 72°C for 5 minutes. Amplicons were digested with the Rsa1 (1U/μg) for 3 hours at 37°C following the manufacture's instruction. The digested fragments were checked by 4 % agarose gel.

RESULTS

PCR-RFLP method used for identification of resistant and sensitive chicken Mx gene. The PCR product was digested with the of the Rsa1 restriction enzyme 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). To determine the allelic variation, the Gerze chicken samples of NE-F2 and NE-R2/R loci were cutting by RsaI. The cut-out obtained gel images are given in Figure 1.

NE-F2 and NE-R2/R locus of Gerze chicken frequency of alleles related to resistance and sensitivity was calculated according to the cutting enzyme scores. The evaluation of the results was based on the criteria reported by Seyama *et al.* (2006). Accordingly, individuals having a single fragment of 100 bp: AA genotype (homozygous, resistant Mx gene allele (Mx⁺), individuals which have two fragments of 100 and 73 bp: AG genotype (heterozygous, sensitive and resistant Mx gene allele (Mx⁺), individuals who had only band 73 bp single fragments: GG genotype (homozygous sensitive Mx gene allele (Mx⁻). Mx gene allele frequencies are given in Table 1.

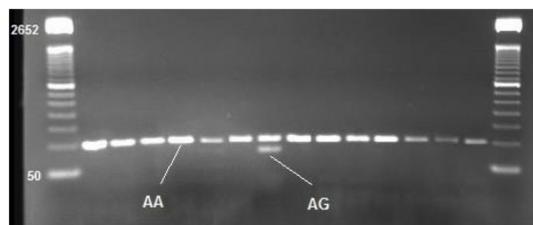


Figure 1: The gel image of the Gerze chicken population with NE-F2 and NE-R2/R locus cut-off with the RsaI enzyme

Table 1: Mx gene allele frequencies in Gerze chicken

Samples	Genotype		Allele frequency
	AA/Mx ⁺	AG/Mx ⁺	
29	27	2	f(A) 98 %
14	14	0	f(G) 2 %

The samples belonging to the pure chicken line was different from Gerze chickens. Three alleles (AA, AG and GG) were identified in pure chicken line. The cut-out obtained gel images are given in Figure 2. Mx gene allele frequencies of pure chicken line are given in Table 2.

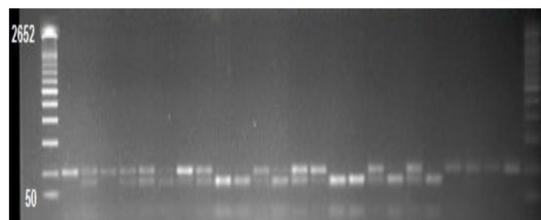


Figure 2: The gel image of the pure chicken line population with NE-F2 and NE-R2/R locus cut-off with the RsaI enzyme

Table 2: Mx gene allele frequencies in pure chicken line

Samples	Genotype			Allele frequency
	AA/Mx ⁺	AG/Mx ⁺	GG/Mx ⁻	
40	16	10	14	f(A) 52 %
10	3	4	3	f(G) 48 %

DISCUSSION

In chickens, it has been reported that the substitution of asparagine with serine amino acid at amino acid position 361 of the Mx protein resulted either in a positive and negative antiviral response (Ko *et al.*, 2002; Seyama *et al.*, 2006; Sartika *et al.*, 2011). In another study, Sartika *et al.* (2011) reported 60 – 65 % Mx⁺ resistant Mx gene allele and 35 – 40 % Mx⁻ sensitive Mx gene allele in indigenous chicken breeds in Indonesia. In this study, the resistant Mx gene allele (Mx⁺) frequency was 98 % and the sensitive Mx allele frequency (Mx⁻) was 2 % in Gerze chicken. Seyama *et al.* (2006) reported 40.8 % Mx⁺ resistant Mx gene alleles and 59.2 % Mx⁻ sensitive Mx gene alleles in different chicken breeds and red forest hen. In addition, Quan *et al.* (2010) found 35 % resistant Mx gene alleles (Mx⁺) and 65 % sensitive Mx gene alleles (Mx⁻) in China native chicken breeds. In this study, the Mx gene allele frequency in the pure line chicken population was 48 % and the resistant allele frequency was 52 %. Allele frequencies in the pure line population appear to be in agreement with research conducted by various researchers. In the Gerze chicken population the Mx gene contains polymorphic alleles and thus considered as a valuable population. This population showed high frequencies of alleles which can be used in genetic improvement programs. This study thus provides base line data for future genetic assessments of this population.

Conclusion: The native Gerze chicken population used in this study would be considered valuable as it has the polymorphic alleles of the resistance against viral infections. Therefore it can be used for the future improvement programs.

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REFERENCES

- BENFIELD, C. T. O., LYALL, J. W. and TILEY, L. S. (2010). The cytoplasmic location of chicken Mx is not the determining factor for its lack of antiviral activity. *Plos One*, 8: 1 – 8. doi.org/10.1371/journal.pone.0012151.
- FADHIL, M. (2014). *Molecular Characterization of Mx Gene Polymorphism in Gerze Chicken Breed by PCR-RFLP Method*. Graduate Thesis, Ondokuz Mayıs University, Turkey.
- FULTON, J. E., ARANGO, J., ALI, R. A., BOHORQUEZ, E. B., LUND, A. R., ASHWELL, C. M., SETTAR, P., O'SULLIVAN, N. P. and KOCI, M. D. (2014). Genetic variation within the Mx gene of commercially selected chicken lines reveals multiple haplotypes, recombination and a protein under selection pressure. *Plos One*, 9(9): e108054. doi:10.1371/journal.pone.0108054.
- KO, J., JIN, H., ASANO, A., TAKADA, A., NINOMIYA, A., KIDA, H., HOKIYAMA, H., OHARA, M., TSUZUKI, M., NISHIBORI, M., MIZUTANI, M. and WATANABE, T. (2002). Polymorphisms and the differential antiviral activity of the chicken Mx gene. *Genome Research*, 12(4): 595 – 601.
- QUAN, T. Z., XIAO-WEI, W., MIN, S., HAI-YAN, Y., GUO-BING, C. and BI-CHUN, L. (2010). The genetic distribution and polymorphism analysis of antiviral resistant Mx gene locus in fifteen Chinese indigenous chicken breeds. *Journal of Animal and Veterinary Advances*, 9(2): 402 – 405.
- SARTIKA, T., SULANDARI, S. and ZEIN, M. (2011). Selection of Mx gene genotype as genetic marker for avian influenza resistance in Indonesian native chicken.

- BMC Proceedings*, 5(Suppl 4): S37. doi: 10.1186/1753-6561-5-S4-S37.
- SEYAMA, T., KO, J. H., OHE, M., SASAOKA, N., OKADA, A., GOMI, H., YONEDA, A., UEDA, J. NISHIBORI, M., OKAMOTO, S., MAEDA, Y. and WATANABE, T. (2006). Population research of genetic polymorphism at amino acid position 631 in chicken Mx protein with differential antiviral activity. *Biochemical Genetics*, 44(9 – 10): 437 – 448.
- SIRONI, L., RAMELLI, P., WILLIAMS, J. L. and MARIANI, P. (2010). PCR-RFLP genotyping protocol for chicken Mx gene G/A polymorphism associated with the S631N mutation. *Genetics and Molecular Research*, 9(2): 1104 – 1108.
- SOLIHIN, D., ELFIDASARI, D., SOEJOEDONO, R. and MURTINI, S. (2013). Identification of gene resistance to avian influenza virus (Mx gene) among wild water birds. *Makara Journal of Science*, 1: 6 – 10.
- WATANABE, T. (2007). Polymorphisms of the chicken antiviral Mx gene. *Cytogenetic and Genome Research*, 117: 370 – 375.