

Nucleotide sequence analysis of Mx1 gene in Japanese quail

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ABSTRACT

During the process of increasing production potential of poultry, other economically important traits like disease resistance are neglected and ultimately leading to decrease in quality food production. The poultry health management is important due to emergence of highly pathogenic diseases like Avian Influenza. Mx1 gene has been reported to provide resistance against avian influenza virus in chicken. Therefore, present research work was focused to identify the genetic variation of Mx1 gene in Japanese quail. Total three fragments *viz.* Frag-I of 185 bp (Exon 3 region), Frag-II of 161 bp (Exon 7 region) and Frag-III of 176 bp (Exon 13 region) of Mx1 gene in 170 Japanese quail birds were amplified and screened for polymorphism by PCR-SSCP technique. All the three fragments were found to be monomorphic which was confirmed by dideoxy nucleotide sequencing. Comparative analysis of Mx1 sequence of Japanese quail by MegAlign programme of DNASTAR showed 100% homology with common quail and more than 80% homology with Rhode Island Red and Silki chicken breeds.

Key words: Allele, Genotype, Mx1, PCR-SSCP, Quail, Virus.

INTRODUCTION

The poultry production is a profitable business due to its low input and high output. The increasing demand for eggs and poultry meat to meet the recommended nutritional requirement paves the way for rearing of alternate poultry species like ducks and quail which are known for their ability to produce more eggs and better meat in comparison to chicken. Japanese quail (*Coturnix coturnix japonica*) is a migratory bird of Japan, now domesticated throughout the world as a good source of egg and meat. In India also it has gained popularity among poultry breeders. For economic and sustainable production, good health and survival of birds along with optimum productive performance are fundamental factors. But, the new emerging diseases and some important zoonotic diseases are posing serious threats to the poultry industry, out of them avian influenza is the most important one being its widespread occurrence (de Jong and Hien, 2006). Current methods to control diseases like antibiotic treatment and vaccination are not much effective due to rapid changes in the pathogenicity, vaccination failure and problem of drug residues in poultry products. So the future research activities shall mostly focus on utilization of diversified approaches such as development of disease resistant lines, well suited to prevailing climatic conditions and having better adaptability and production capacity. The genetic markers can provide useful information to identify genetic basis of disease resistance (Orita *et al.*, 1989). Myxovirus resistance (Mx1) gene is an interferon induced gene that inhibits single

stranded negative sense RNA viruses' proliferation including avian influenza virus (Ko *et al.*, 2002; Sasaki *et al.*, 2013; Wang *et al.*, 2014). The chicken Mx1 gene consists of 21289 bp linear DNA, 14 exons and 2118 bp long coding sequence. The Mx protein of chicken (*Gallus gallus*) is predominantly cytoplasmic consisting of 705 amino acids (Bernasconi *et al.*, 1995). However, study on quail Mx1 gene was known very little globally. Therefore, the present investigation was undertaken to find out the DNA polymorphism in Mx1 gene of Japanese quail using PCR-SSCP and nucleotide sequencing techniques.

MATERIALS AND METHODS

Isolation of DNA: The blood samples were collected from 170 quail birds maintained at Central Avian Research Institute (CARI), Izatnagar, Bareilly. The genomic DNA was isolated (Sambrook and Russell, 2001). The quality and purity were assessed by agarose gel electrophoresis and spectrophotometer.

Design of PCR Primers: Three different regions (Fragment I of 185bp, Fragment II of 161 bp and fragment III of 176bp) of quail Mx1 gene were selected for amplification (Figure 1). The PCR primers were designed on the basis of available sequences of chicken and common quail (wild) using Primer3 and OligoAnalyzer softwares (Table 1).

Setting up of PCR reactions: The PCR master mix, containing all the reaction components except genomic DNA, was prepared (Table 2). Care was taken to add *Taq DNA*

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Table 1: Primer sequences used to amplify Mx1 gene in Japanese quail

Fragments	Primer	Primer Sequence	Length (Base)	Amplification length
I	Forward	5'- GCAGCAGAACACAGCTTTCA -3'	20	185
	Reverse	5'- CTAGGAAGAGCAACACCAGAC -3'	21	
II	Forward	5'- TCCTCACTAAACCAGATCTGGTG - 3'	23	161
	Reverse	5'-TTGCTGGATTACAGAGGCCAAGGA- 3'	24	
III	Forward	5'- GCAAGCAACAGCTGCGAAAA - 3'	20	176
	Reverse	5'-AAACCATTTCAGGGCAAAGCTGG -3'	24	

polymerase at the end. After all the components were added, the master mix was mixed gently, followed by spinning and dispensed (24 µl) to each labeled PCR tube kept in ice. Finally, 1 µl (80-100 ng) of good quality diluted genomic DNA was added to each tube. The PCR tubes were kept in a preprogrammed thermocycler (Eppendorf gradient, Germany). The PCR programme were as, initial denaturation (94°C for 4min), denaturation (94°C for 1 min), the annealing (61°C, 59.2°C and 61.2°C for 1 min for fragment I, II and III, respectively), extension (72°C for 1 min) and the final extension (72°C for 10 min)

Checking of the amplified product: Horizontal submarine agarose gel electrophoresis was carried out to check the amplified products. A 1.5% w/v agarose (Low EEO) gel was prepared in 0.5 X TBE and subsequently 5 µl PCR product mixed with 1 µl of 6 X gel loading dye (Bromophenol blue and xylene cyanol) was loaded along with 100 bp ladder (Gene Ruler™ Plus) as a marker in a separate lane. The electrophoresis was done at 60 volt for 2 hr. The amplified product in the gel was observed under UV transilluminator and documented by photography.

PCR-SSCP analysis: The PCR products were resolved on 15% polyacrylamide gel. About 6 µl of PCR product and 12 µl of denaturing formamide dye (Formamide, 95%; Xylene cyanol, 0.025%; Bromophenol blue, 0.025%; 0.5 MEDTA, 4%) were taken in a 0.2 ml PCR tube and mixed properly. The mixture of PCR product and formamide dye were denatured at 95°C for 10 min (by keeping on 95°C hot

Table 2: PCR reaction components for fragment- I, II and III

Reaction components	Amount 1X (25 µl)	Final concentration
dNTPs mix (10 mM)	1 µl	100 µM
Primers		
Forward Primer (E3 QF)	1µl	10 pmole
Reverse primer (E3 QR)	1µl	10 pmole
MgCl ₂ (25 mM)	2µl	2mM
10 x PCR assay buffer	2.5 µl	1 X
Taq DNA polymerase (1U/ml)	1 µl	1 unit
Genomic DNA	1 µl	80-100 ng
Nuclease free water	15.5 µl	
Total	25 µl	

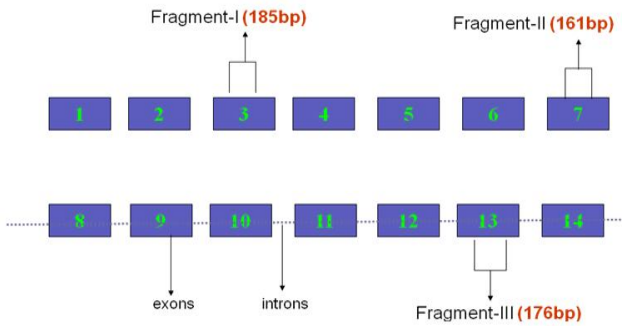


Fig 1: Amplified regions of Mx 1 gene in quail

water) and snap chilled on ice for 15 min. The product was loaded in gel carefully. The electrophoresis was performed at 4°C for 13-16 hr at 130 constant volts with subsequent silver staining for visualization of bands under gel documentation system (Bassam *et al.*, 1991). The genotypes were identified by visualizing SSCP patterns of each sample in the gel. The gene and genotype frequencies were estimated by applying standard procedure (Falconer and Mackey, 1996).

DNA sequencing: Different genotypes of amplified fragment of Mx1 gene were selected for sequencing and subjected to BLAST (www.ncbi.nlm.nih.gov/BLAST) analysis to ascertain that sequences were of Mx1 gene. Nucleotide sequences were then aligned with the corresponding common quail (NCBI Acc. No. EF575605) and different breeds of chicken *viz.* RIR (NCBI Acc. No. DQ788613), SILKIE (NCBI Acc. No. DQ788614) and WLH (NCBI Acc. No. DQ788615) Mx1 gene sequences using the ClustalW method of MegAlign Programme of Lasergene Software (DNASTAR). The amino acid sequences were deduced from different alleles of Mx1 gene in quail and compared with the corresponding deduced chicken amino acid sequences. The nucleotide and amino acid substitutions and diversity analyses (by DNASTAR) were reported. Percentage similarity and phylogenetic analysis were also performed to determine the evolutionary relationship.

RESULTS AND DISCUSSION

DNA Polymorphism of Mx1 gene: The 185 bp fragment in 3rd exon, 161 bp fragment of 7th exon and 176 bp fragment of 13th exon of Mx1 gene of quail were amplified (Figure 2a, 2b and 2c). The SSCP analysis revealed

monomorphic pattern in all the three fragments (Figure 3a, 3b and 3c). The PCR-SSCP of all the three fragments of Mx1 gene has not been studied so far in quail or any other breeds of chicken. Therefore, present findings could not be compared.

Nucleotide sequence analysis: Different amplified fragments of Mx1 gene of Japanese quail were sequenced by Sanger's dideoxy chain termination sequencing method

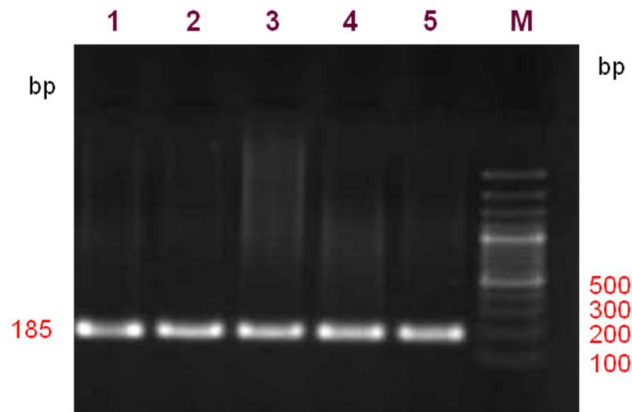


Fig 2a: Specific 185 bp amplified PCR product of Mx1 gene in Japanese quail

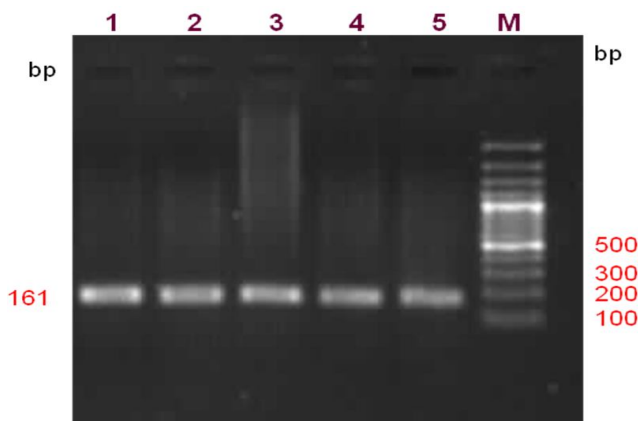


Fig 2b: Specific 161 bp amplified PCR product of Mx1 gene in quail

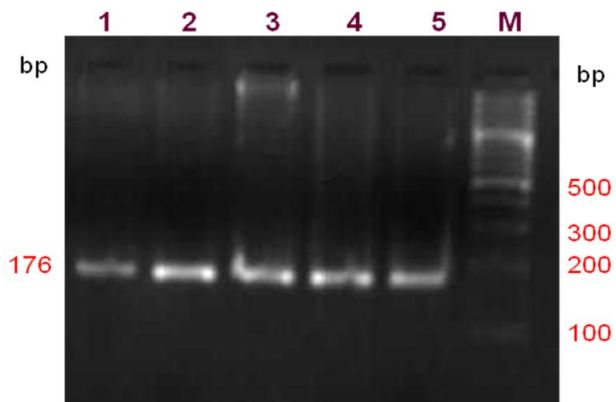


Fig 2c: Specific 176 bp amplified PCR product of Mx1 gene in Japanese quail



Fig 3a: PCR-SSCP genotypes of 185 bp fragment of Mx1 gene



Fig 3b: PCR-SSCP genotypes of 161 bp fragment of Mx1 gene



Fig 3c: PCR-SSCP genotypes of 176 bp fragment of Mx1 gene

Diversity analysis among alleles: Percentage similarity/dissimilarity was done for three fragments of Japanese quail Mx1 gene and common quail along with other breeds of chicken (RIR, Silki and WLH). The 100% similarity was observed between Japanese quail and common quail for fragment I and fragment II of Mx1 gene (Figure 7a and 7b). However, 99.4% similarity was observed for fragment III between Japanese quail and common quail (Figure 7c). In contrast to this Japanese quail was more distinct from RIR, Silki and WLH breed of chicken (Range 83.2% to 89.2%).

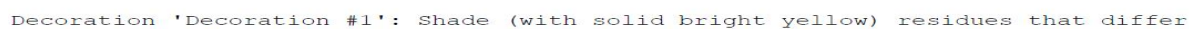


Fig 4: Nucleotide sequence alignment of 185 bp fragment of Mx 1 gene of Japanese quail with Common quail and different breeds of chicken

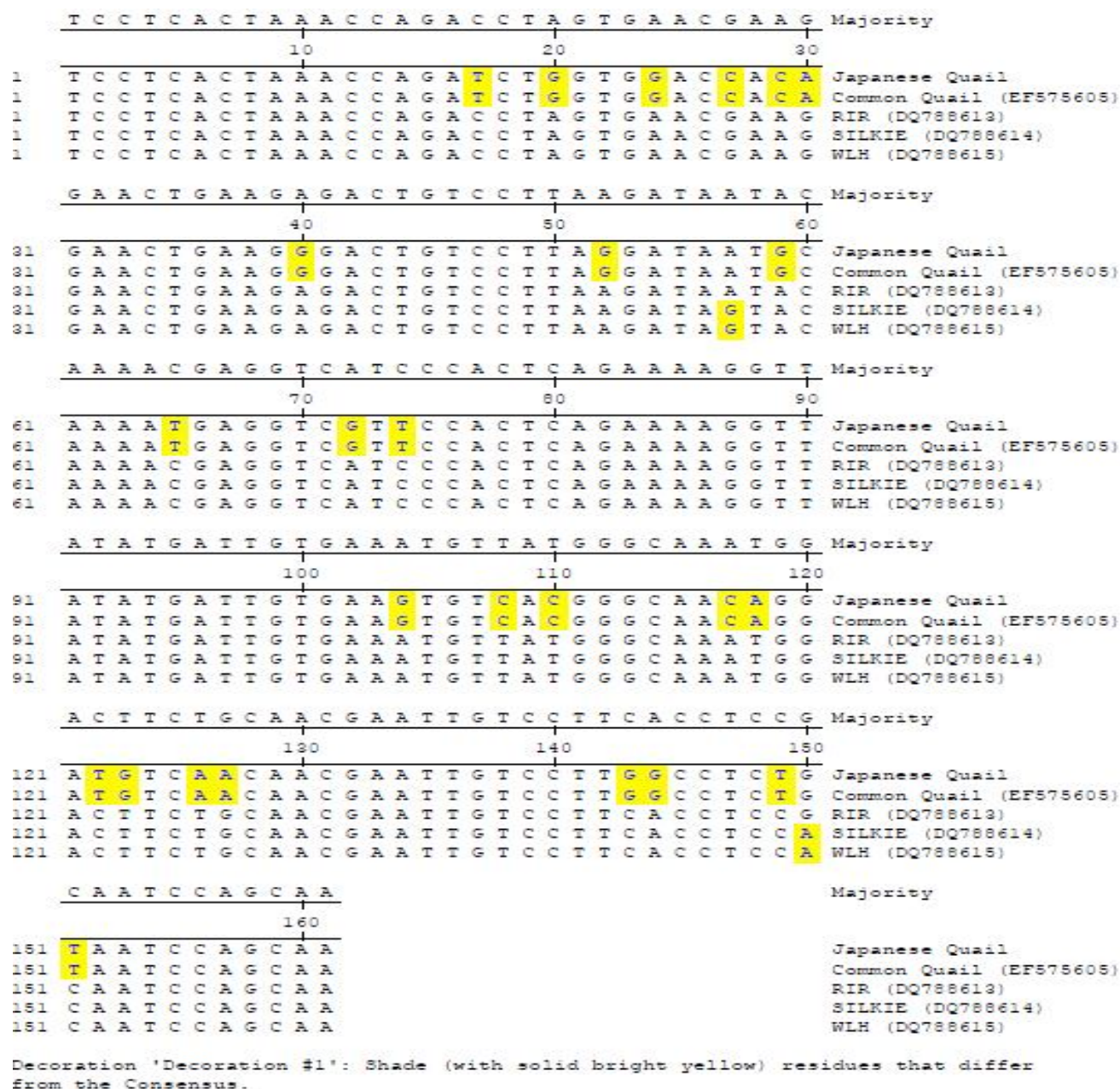


Fig 5: Nucleotide sequence alignment of 161 bp fragment of Mx 1 gene of Japanese quail with Common quail and different breeds of chicken

Phylogenetic analysis : The phylogenetic analysis showed that Japanese quail and common quail formed single cluster while all chicken breeds remain in another cluster for all the three fragments (Figure 8a, 8b and 8c).

Comparison of amino acid sequences: The deduced amino acid sequences of 185, 161 and 176 bp fragments of Mx1 gene quail was compared with those of the published sequences of chicken breeds viz. RIR, Silki and WLH (Figure 9a, 9b and 9c). Total forty six (Twelve in fragment I, fourteen in fragment II and twenty in fragment III) amino acid substitutions were observed in quail Mx1

gene as compared to chicken (Table 3). The highest similarity of amino acid composition was observed between Japanese quail and common quail and more dissimilarity was observed between quail and different breeds of chicken. However, when amino acid sequences of Japanese quail and common quail were compared, amino acid variation was observed at 60th position (Alanine was present in Japanese quail and Valine was present in common quail) in fragment III. The fragment I and II showed 100% identity in Japanese quail and common quail.

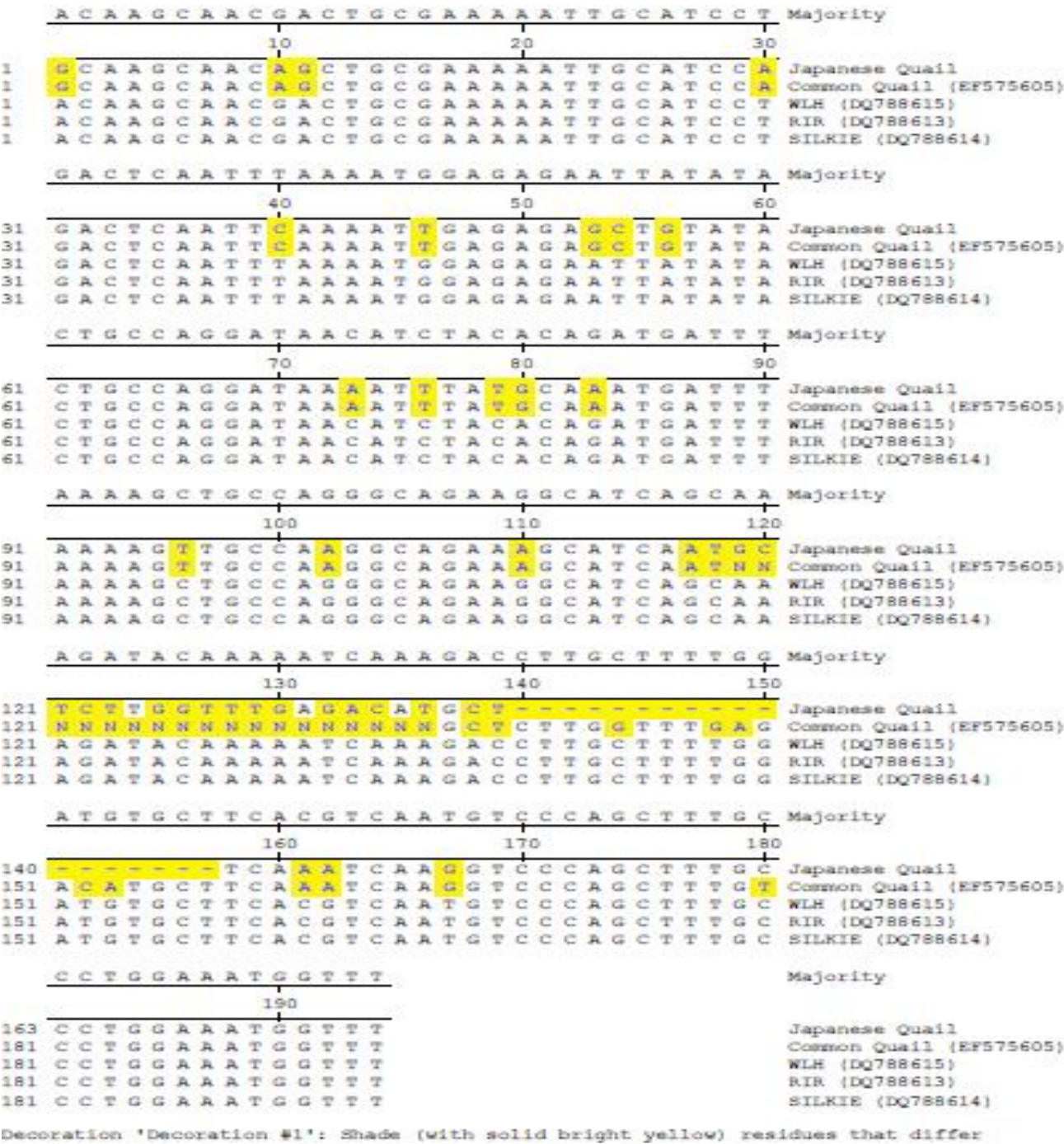


Fig 6: Nucleotide sequence alignment of 176 bp fragment of Mx 1 gene of Japanese quail with Common quail and different breeds of chicken

Table 3: Amino acid substitutions at different positions of three fragments of Japanese quail Mx1 gene

Fragment	Positions																		
I	3	4	6	7	9	12	22	26	32	37	39	44							
II	8	9	10	13	17	19	24	36	39	41	42	47	48	50					
III	4	10	15	18	19	24	27	28	32	34	37	39	46	48	49	50	51	54	56

		Percent Identity					Divergence	
		1	2	3	4	5		
1	■	100.0	88.6	89.2	89.2	1		Japanese Quail
2	0.0	■	88.6	89.2	89.2	2		Common Quail (EF575605)
3	12.5	12.5	■	99.5	99.5	3		RIR (DQ788613)
4	11.8	11.8	0.5	■	100.0	4		SILKIE (DQ788614)
5	11.8	11.8	0.5	0.0	■	5		WLH (DQ788615)
	1	2	3	4	5			

Fig 7a: Similarity and divergence between Japanese quail, Common quail and different breeds of chicken on the basis of 185 bp Mx1 gene fragment

		Percent Identity					Divergence	
		1	2	3	4	5		
1	■	100.0	84.5	83.2	83.2	1		Japanese Quail
2	0.0	■	84.5	83.2	83.2	2		Common Quail (EF575605)
3	17.8	17.8	■	98.8	98.8	3		RIR (DQ788613)
4	19.5	19.5	1.3	■	100.0	4		SILKIE (DQ788614)
5	19.5	19.5	1.3	0.0	■	5		WLH (DQ788615)
	1	2	3	4	5			

Fig 7b: Similarity and divergence between Japanese quail, Common quail and different breeds of chicken on the basis of 161 bp fragment of Mx 1 gene

		Percent Identity					Divergence	
		1	2	3	4	5		
1	■	99.4	84.1	84.1	84.1	1		Japanese Quail
2	0.6	■	75.3	75.3	75.3	2		Common Quail (EF575605)
3	26.6	19.9	■	100.0	100.0	3		WLH (DQ788615)
4	26.6	19.9	0.0	■	100.0	4		RIR (DQ788613)
5	26.6	19.9	0.0	0.0	■	5		SILKIE (DQ788614)
	1	2	3	4	5			

Fig 7c: Similarity and divergence between Japanese quail, Common quail and different breeds of chicken on the basis of 176 bp fragment of Mx 1 gene

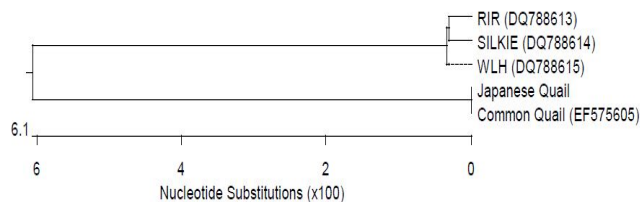


Fig 8a: Phylogenetic tree based on 185 bp fragment of Mx 1 gene in Japanese quail

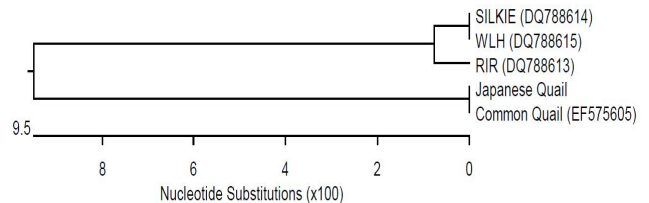


Fig 8b: Phylogenetic tree based on 161 bp fragment of Mx 1 gene in Japanese quail

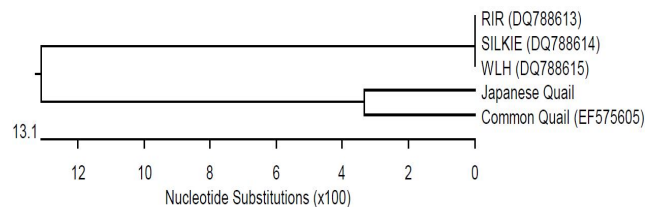


Fig 8c: Phylogenetic tree based on 176 bp fragment of Mx 1 gene in Japanese quail

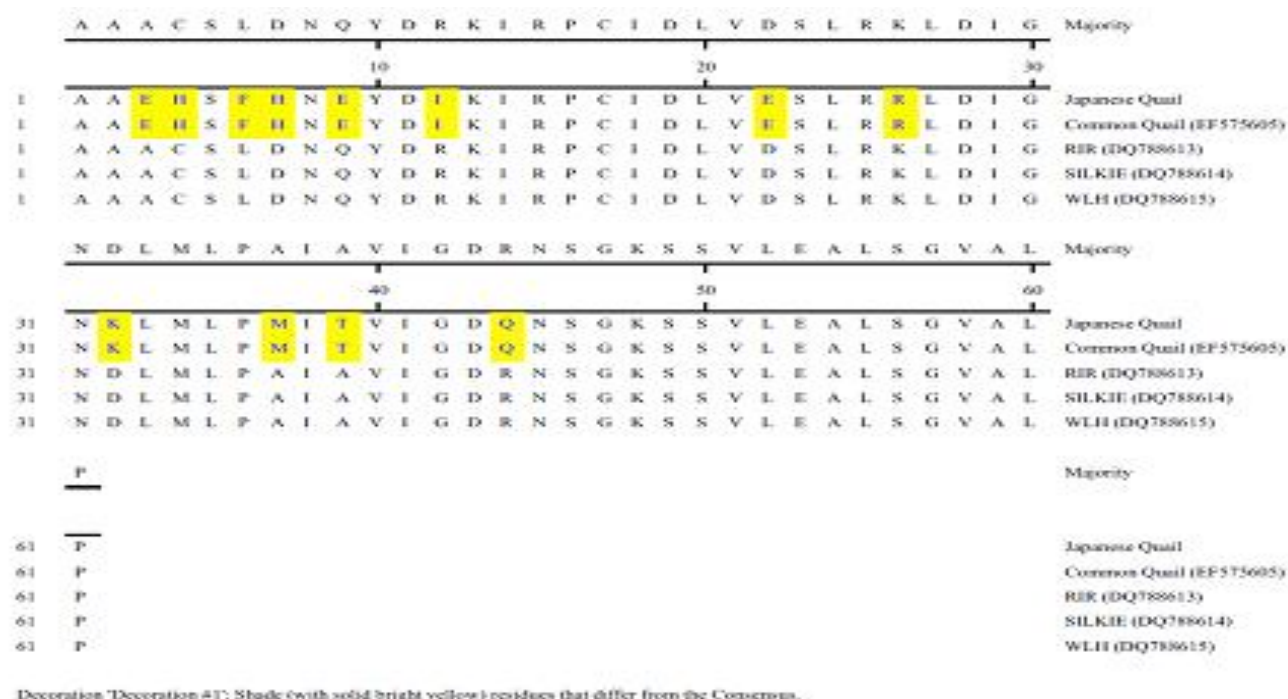


Fig 9a : Amino acid sequence alignment of 185 bp fragment of Mx 1 gene in Japanese quail



Fig 9b: Amino acid sequence alignment of 161 bp fragment of Mx 1 gene in Japanese quail

CONCLUSION

The Mx1 gene was found to be conserved in quail. The other regions of this gene need to be sequenced for the complete characterization of this gene in quail.

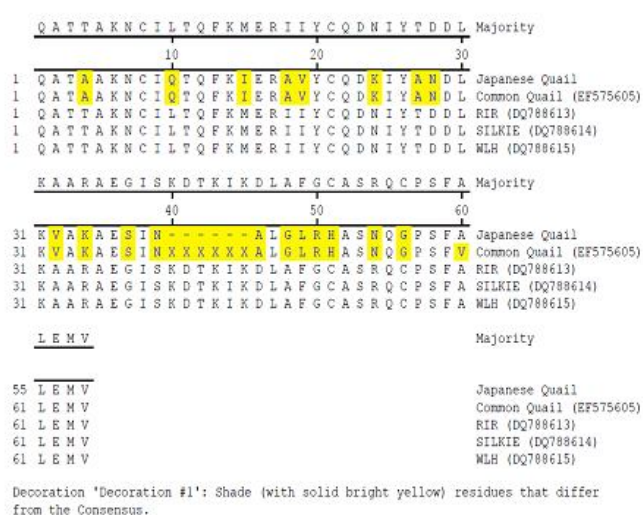


Fig 9c: Amino acid sequence alignment of 176 bp fragment of Mx 1 gene in Japanese quail

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