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Original Research Article

Effect of TLR6-5 Gene on Different Economic Traits Along With Its Polymorphism, Sequencing and Phylogenetic Analysis in Swine

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A B S T R A C T

The current study was proposed to see the effect of TLR6 gene on different selective economic traits (Growth +Reproductive traits) along with polymorphism, sequence and phylogenetic study in Sus scrofa. Nine primers were used in this study. The data pertaining to parameters for the 5th primers (TLR6-5) has been documented here. DNA was extracted from 48 animals at R.V.C. Pig farm belonging to three genetic groups namely Tamworth, Desi and T&D and were subjected to SSCP. A total of six growth traits (body weight at 0-day, 7-day, 14-day, 28-day, 42-day and 56-day) and reproductive traits (litter size at birth and weaning and litter weight at birth and weaning) were taken. The results after analysis with SPAB were sent for sequencing at Xcleris, Hyderabad. Sequencing result was studied with DNASTAR and further used for phylogenetic studies using BLAST and NCBI. The effect was not significant. But Variations were witnessed in different haplotypes. High responders performed better and there were positive correlations in majority of cases. These studies could be used for MAS for disease resistance. The research coupled with further work on the same topic could be crucial for breeding, genetics as well as evolutionary studies.

Keywords

SSCP, DNA, MAS, Phylogeny, Sequencing and Haplotypes

Introduction

Aeons ago when man started domesticating livestock species, he knowingly or unknowingly started selecting and breeding them for one or the other required characters. Centuries later, with the advancement of science and technology, genetic improvement was carried out with more developed techniques of selection and breeding with an emphasis on improvement of economic traits. But unfourtunately, little importance has been given to the selection based on disease resistance capability of animals specially in Swine. Among the different genes involved in immune response of different livestock particularly pig, TLR (TOLL-LIKE RECEPTOR) gene is of prime importance in innate immunity response.

So the objectives of the current study are to find the association between TLR6-5 and the economic traits, to study the polymorphism, sequencing and the sequencing of TLR6-5 in Swine.

Materials and Methods

The total number of experimental animals used for the current study were 48 and they were from the three genetic groups (Tamworth, Desi and T&D) maintained at pig farm of Ranchi Veterinary College. Under controlled managerial conditions, observations of different growth traits and reproductive traits under study were recorded for the experimental animals. Isolation of blood (5ml) along with anticoagulant from each of the 48 animals was followed by extraction and purification of DNA from white blood cells standard protocol as described by Sambrook *et al.*, (1989). A total of nine primers were taken out of which the result of 5th primer (TLR6-5) with the forward and reverse sequences as 5' GTCCTCAGGTACCAAGCACA 3' and 3' TGGAAAGGCTGCTAAAGGAA5' respectively of size of 453 Kb has been discussed here. After standardization, PCR was run using the taken set of primers (Fig. 1.and 2.). Purified PCR products were subjected to SSCP through polyacrylamide gel electrophoresis. After silver staining method as described by Bassam *et al.*, (1991), the data was statistically analyzed with available software like SPAB and least square Analysis Harvey's model (1990). Confirmatory sequencing was done on samples from Xcleris Lab, Ahmedabad) and the result was analyzed with Meg Align software included in DNASTAR (Lasergene 10). NCBI BLAST was used for phylogenetic studies based on sequencing data of the samples.

Results and Discussion

Haplotype Frequency with Primer-5 of TLR 6 Gene

In case of Desi population, haplotype A had maximum genotype frequency of 57.14% while haplotype D had the lowest genotype frequency of 0%. In Tamworth population, out of the four haplotypes A, B, C, and D obtained, haplotypes A and C had the maximum genotype frequency of 35.71% followed by haplotype B (28.57%). T&D was found to have the maximum value of genotype frequency for haplotype C (60.00%) and 25.00% for haplotype D and a lowest of 00.00% was found for A (Table.1.).

The main principle behind the calculation of haplotype frequency in this case was Hardy-Weinberg Law. According the Hardy Weinberg law in a large random mating population with no selection, migration and mutation, the gene frequencies and genotype frequencies are constant fron generation to generation and furthermore, there is a simple relationship between the gene frequencies and genotype frequencies. Hence the frequencies of all alleles at any one locus must add up to unity, or 100 percent (Falconer and Mackay, 1998). From the data of Mukherjee *et al.*, (2014) on DNA sequences of cell-surface TLR genes, excess of rare variants and a large number of low frequency haplotypes in each gene were observed as in the present study.

Association of TLR6-5 haplotypes with different traits

A Total of four haplotypes (A, B, C and D) were observed (Figure 2). As observed from Tables No 2 and 3, the population means were 09.378 ± 00.7323, 10.945 ± 00.8840 kg, 09.030 ± 00.5850and 81.275 ± 6.7156, 01.178 ± 00.086, 01.689 ± 00.3480, 02.950 ± 00.5387, 04.057 ± 00.5821, 05.976 ± 00.5881 & 06.935, ± 00.9902 kg, for litter size at birth, litter weight at birth, litter size at weaning, litter weight at weaning, body weight at birth, body weight at 7-day, body weight at 14-day, body weight at 42-day and body weight at 56-day respectively. Haplotypes for primer -5

(TLR6-5) had non-significant effect on all the traits (Table no.2 &3).

Non-significant association of different haplotypes of TLR6-5 with different economic traits might be due to small sample size and the result could be compared after being pooled over for all the primers.

Relative superiority of T&D over both the parental breeds i.e. Tamworth and Desi would be due to heterosis/hybrid Vigour in T&D.

Kumar (2013) found that the statistical analysis of TLR1-2 indicated SNP genotypes were non-significantly associated with piglet titre, dam titre, litter size at birth, birth body weight, litter weight at birth, litter size at weaning, 28-day weight and 42-day weight and 56-day body weight. He further revealed that TLR1-2 was significantly associated with Litter weight at Weaning, 7day body Weight and 14dayWeight. The allelic variants in the sequence of nucleotide base pairs were analysed.

Results of Paul *et al.,* (2015) demonstrated that TLR and HSP genes could possibly play a significant role to combat the deleterious effect of thermal stress so as to maintain homeostasis in Black Bengal goats and as HSPs are the endogenous ligands of TLR2 and TLR4 thus, they play a essential modulatory role in activation of immune system in Black Bengal goat during heat stress. This supports our finding where effect of TLR6 haplotypes on growth traits was found to exist.

TLR6 Gene Sequence Analysis

The PCR products representing different SSCP patterns in swine resource population of present study were directly got sequenced using DNA sequencing service (Xcelris Hyderabad).

The nucleotide sequence alignments were carried out using alignment tools, viz. Clustal W (DNA star Inc. USA) and BLAST to reveal single base variations. These allelic variants in the sequence of nucleotide were analysed.

The DNA sequences showing polymorphism were used to identify SNPs. The fifth primer of the TLR6 exhibited the principal SNPs at positions 61, 64, 65, 70, 225, 235, 275, 285, 289, 302 and 303 of the nucleotide bases (Fig.3.).

One reason for these SNPs might be point mutations attributed to various reasons.

Similar to the present findings, synonymous and non-synonymous SNPs were identified using Sanger sequencing which gave 3 non-synonymous SNPs in TLR2, 4 in TLR3, 10 in TLR4, 5 in TLR5, 2 in TLR7, 3 in TLR8 and 1 in TLR9.

Maximum number of 10 non-synonymous SNPs was found in TLR4. The Signal P results reveal that TLR1, 6 and 10 are non-secretory proteins whereas rest of the TLR genes in buffaloes are secretory and carry signal peptides (Banerjee *et al.,* (2012).

The DNA sequence showing polymorphism observed were used to identify SNPs.

Principal SNPs were found at various locus of gene sequence with different primers (Kumar 2013).

According to Hayashi, (2014) one advantage of PCR-SSCP over other methods of PCR based techniques in mutation detection is its simplicity.

Fig.1 Standardization of primer-4 and 5 of TLR6 gene (TLR6-4 AND TLR6-5)

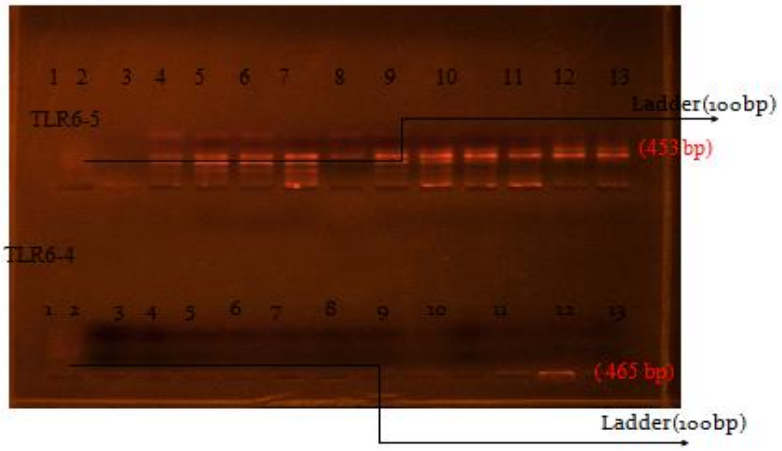


Fig.2 Haplotyping pattern under TLR6-5 (Gel-2)

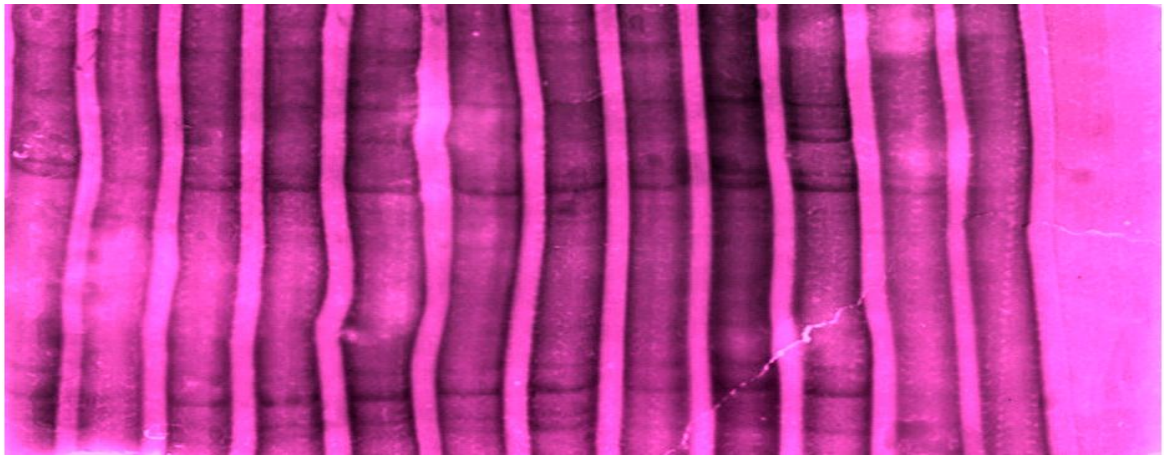
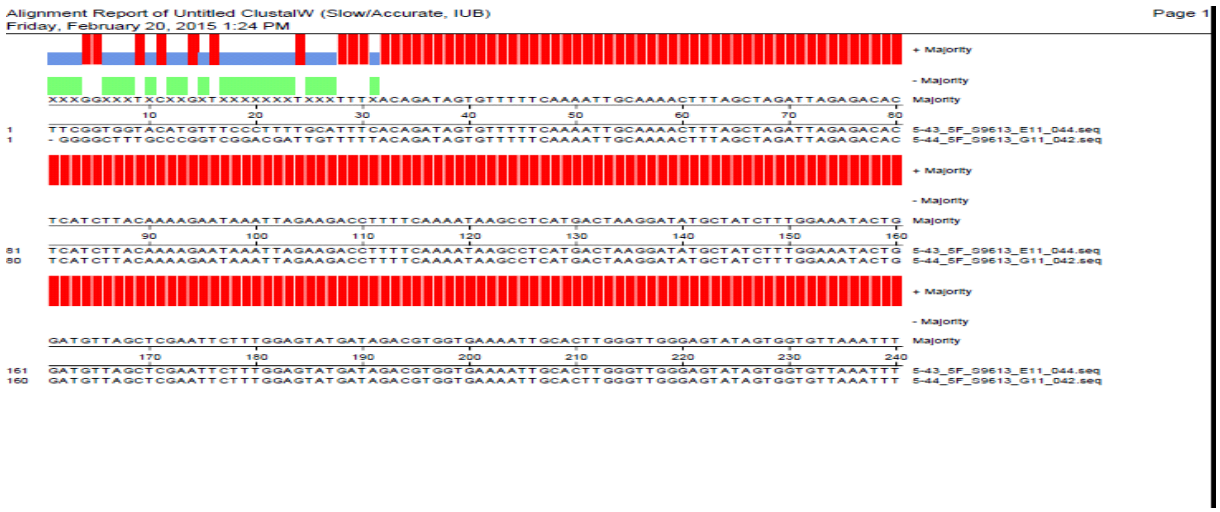


Fig.3 Sequencing report of the samples with Primer -5 (Page1, 2)



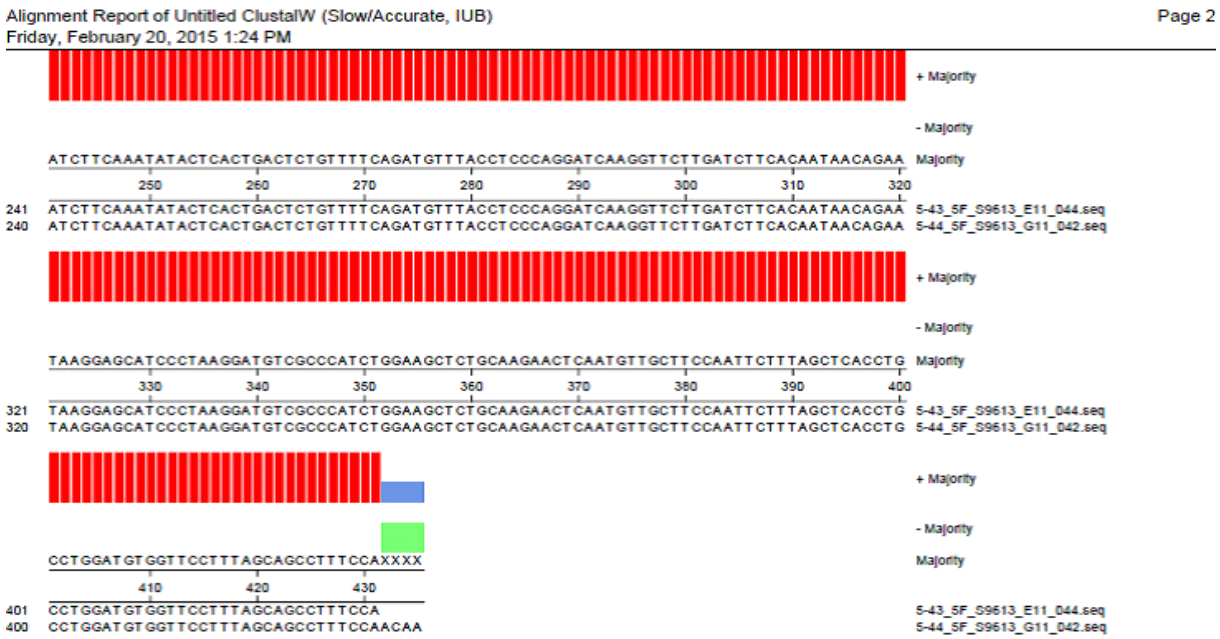


Fig.4 Phylogenetic trees under TLR6-5

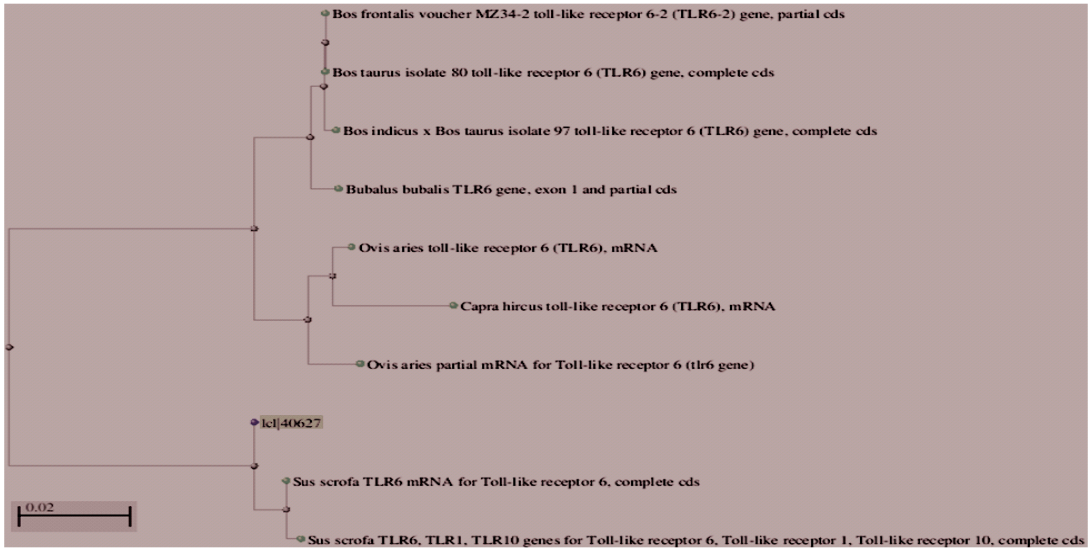


Table.1 Haplotype frequency with primer-5 of TLR 6 gene

Haplotypes	Desi	Tamworth	T&D
A	57.14	35.71	0.00
B	28.57	28.57	15.00
C	14.29	35.71	60.00
D	00.00	00.00	25.00

Note- The sum of all haplotypes' frequency in a column for each primer equals to unity.

Table.2 Association of TLR6-5 PCR-SSCP Variants/SNPs with growth Traits

Traits	Body weight (Kg) at different ages					
	Birth	**7-day**	**14-day**	**28-day**	**42-day**	**56-day**
µ Haplotypes	1.177 ±0.086	1.689 ±0.348	2.950 ±0.539	4.097± 0.582	5.976 ±0.582	6.935 ±0.990
A	1.083 ±0.120	1.792 ±0.483	**3.377 ±0.748**	**4.550 ±0.808**	6.083 ± 0.816	6.933 ±1.374
B	1.120 ± 0.121	1.414 ±0.489	2.705 ±0.757	3.688 ±0.818	5.826± 0.826	6.961 ±1.391
C	1.207 ±0.109	1.432 ±0.442	2.873± 0.683	3.635 ±0.738	4.893 ±0.746	5.913 ±1.256
D	**1.298 ±0.135**	**2.118 ±0.545**	2.843 ± 0.840	4.514 ±0.911	**7.102 ±0.920**	**7.935 ±1.549**

Table.3 Association of TLR6-5 PCR-SSCP Variants/SNPs with reproductive traits

Factors	Litter Size (No.) at		Litter weight (Kg) at	
	Birth	**Weaning**	**Birth**	**Weaning**
µ Haplotypes	09.378±0.723	09.031±00.5850	10.975±00.8840	81.275±06.7156
A	09.774 ±01.0165	**09.526 ±00.8121**	**11.645± 01.2271**	**84.876± 09.3217**
B	08.922 ±01.0288	08.746 ±00.8219	10.433± 01.2420	83.588 ±09.4346
C	08.990± 00.7421	08.847 ±00.9289	10.957± 01.1214	77.247 ±08.5186
D	**09.969 ±01.1459**	08.861± 00.9154	10.864 ± 01.3833	79.387 ±0.5083

Phylogenetic Studies

In the current study, phylogenetic studies based on sequence data using the NCBI BLAST was done. With respect to TLR 6-5 gene fragment, phylogenetic studies were done. The Fig. 4. Showed the genetic distance among the different species of animals with reference sequence ICI / 40627 of TLR6-5 gene fragment from which TLR6 mRNA and TLR 6, TLR 1and TLR10 seems to have evolved. Further based on this sequence sus scrofa is equidistant from other domestic animals with respect to phylogeny (Figure 3.)

Similarly, Banerjee *et al.*, (2012), found that the buffalo (*Bubalus bubalis*) TLR family repertoire consists of ten genes. In their study, they sequenced buffalo TLR genes and placed it in context of vertebrate genomic evolution. Phylogenetic analysis shows that the TLR genes of buffalo are more close to *Bos indicus* and *Bos taurus* species.

The phylogenetic sequence analysis based on complete TLR1 gene revealed that reference sequence were genetically closer to *Sus scrofa* TLR1 and TLR6 (Kumar 2013). This supports the current finding.

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