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IGF1 Gene As A Target For Genetic Diversity Among Indian Poultry Birds

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Abstract: *The genes of Growth Hormone (GH) and Insulin-like Growth Factor 1 (IGF-1) are responsible for growth in animals as they play a key role in the regulation of their growth and development. The native breeds exhibit great Genetic polymorphism and there is a major concern for the necessity of preserving genetic resources. It is very important and significant to characterize and differentiate the genetically indigenous breeds.The present work deals with applying the RFLP Technique on a selected gene (IGF1) to look for the genetic diversity within 40 individuals of chicken belonging to Fast broiler and rainbow rooster. The work is further extended on molecular and bioinformatics level wherein sequencing of randomly selected amplicons was done to check their similarity with the closest sequence(s).*
Keywords: *IGF1, RFLP, genetic diversity, chickens*

I Introduction

During the last two decades India has made remarkable progress in broiler production. High quality chicks, equipment's, vaccines and medicines are available. India has been ranked as the fourth largest producer of eggs and fifth largest producer of poultry broiler in the world with an annual output of 41.06 billion eggs and 1000 million broilers. An annual growth rate of about 15 percent has been recorded in the broiler production resulting in its exponential proliferation in the poultry market. As a result broiler farming and rearing has been given considerable importance and care in the national policy and has a great scope for further development in the upcoming years.

As reported by Tixier-Boichard, Bordas and Rognon (2008), studies using microsatellite markers have revealed large variations in their genetic heterozygosity, that ranges from 28 percent for a fancy breed to 67 percent for a native village population. However the average value (of about 50%) is rather lower than that observed in domestic mammals. The groups wherein the highest levels of within-population diversity were found were the wild ancestor species, the unselected and random local populations, a few standardized breeds kept in large populations, and some commercial broiler lines.[3] A range of values were obtained for European fancy breeds, reflecting the variability of population history within this type of population. Expected values for heterozygosity ranged from 50 to 63% for broilers and 45 to 50% for brown-egg layers, to about 40% for white-egg layers that exhibited the lowest levels of all commercial lines. Their studies suggested that there is a significant reservoir of genetic diversity within local breeds of chickens.[4]

II Materials and Methods

Sample collection

2ML blood was collected in K3 EDTA tubes from 10 male and 10 female individuals each from fast white broiler and Rainbow rooster multicolored dual purpose birds. The supplier of the samples was INDBRO RESEARCH & BREEDING FARMS PVT .LTD. All the samples were stored at –20°C.

Genomic DNA Isolation, purification and qualitative estimation

Genomic DNA was isolated by the Bunce's method in which the anticoagulated blood was treated with a solution of Tris HCl, Sucrose and $MgCl_2$. Further EDTA and NaCl solution was added to the pellet followed by the subsequent treatment with Sodium acetate and ice cold chloroform. To the supernatant obtained an equal volume of ice cold iso propanol was added for proper DNA precipitation. For purification the precipitated DNA was incubated at -20^{o}C overnight and Centrifuged at 6000rpm/10min.The pellet was washed with 1 ml of 70% ethanol and recentrifuged. The air dried DNA pellet was dissolved in100µl of 1xTE buffer.The agarose gel electrophoresis was carried out to check the integrity and quality of the DNA yield. All the samples were run on 0.8% Agarose at 100V for 45 minutes. Furthermore the spectrophotometric readings were taken to obtain the ratio at wavelengths of 260 and 280nm. Samples were then diluted to 25 ng/µl each for using them in PCR.

Primer designing and amplification

Specific primer was designed for the IGF1 gene target region using Primer BLAST and the target region was amplified.The mixture of the PCR reaction had a final volume of 25 µl and contained 50 ng of genomic DNA (2µl), 5 µl of Taq buffer, 1 µl of 1.5mM MgCl2, 2 µl of 0.2 mM dNTP, 2 µl of 20 pmol of each primer,2 µl of 1.00 Unit Taq DNA polymerase ("Fermentas") and 9 µl of nuclease free water. The amplification was performed in 'Bio-Rad Thermo cycler' using the following cycling parameters: pre denaturation (94°C) 5 minutes, 30 cycles of denaturation (95°C) 45 second, annealing (55°C) 30 second, extension (72°C) 1minute,final extension (72°C) 7 minutes.

III Restriction Digestion

The amplified product was subjected to restriction digestion activity of enzyme Hinf I whose recognition sequence is GANTC. To 7 µl of each of the 20 amplicons 2 µl of Hinf I and 2.5 µl of 10X Assay Buffer were added and the volume was made upto 25 µl by adding distilled water. The enzymatic activity was allowed to run for 1 hour with proper incubation at 37ºC.The digests were run on 1.8% agarose with 5 µl of 6X Loading dye and stained with Ethidium Bromide.

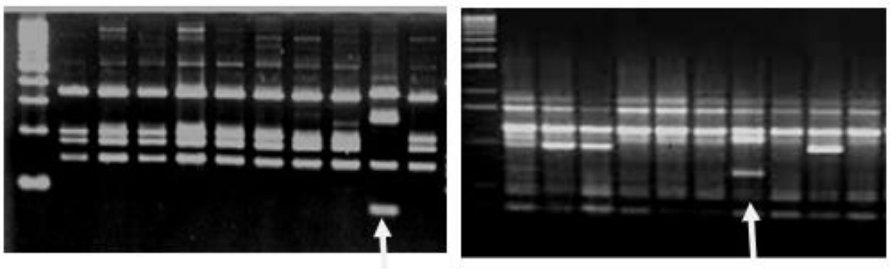
Sequencing and Sequence Analysis

Out of the many amplicons of the digested fragments obtained in gel doc, 4(One from each group) were selected for sequencing by Sanger's method. The sequences obtained were further analyzed by BLAST to look for the sequences most identical to them.

IV Results and discussion

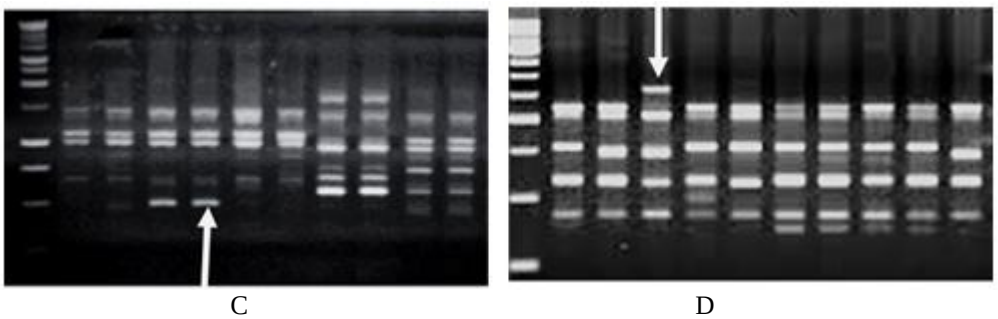
The primer designed and used for amplification of target region of IGF1 was as follows table 1 :

	Sequence (5'->3')	Template strand	Length	Tm	GC%
Forward primer	ATAGAGCCTGCGCAATGGAA	Plus	20	59.82	50.00
Reverse primer	ACATACAGCCATTTTCCAGATCAC	Minus	24	59.36	41.67



A B

Fig 1: *Female (A) and Male (B) Band pattern of IGF1 gene digests of Hinf I in fast white broiler.*



C D

Fig 2: *Female (C) and Male (D) Band pattern of IGF1 gene digests of Hinf I in Rainbow rooster multicolored dual purpose birds (arrows indicate the selected amplicon for sequencing)*

The RFLP results signify that most of the individuals within a group have almost identical band pattern indicating them of belonging to same genera and species. However there exist an exception in group A and group D wherein 9th member of group A and 3rd member of group D are different from the rest of the members of the respective group .Similarly in group B samples 1, 2, 3 and 9 belong to same species 4, 5, 6, 8 and 10

another species and 7 yet another species. Similarly in group C samples 1 to 6 belong to same species 7 & 8 another species and 9 & 10 yet another species.

Sequence Analysis of selected amplicons by BLAST

Fig 1 (A)

>1
ATTCATGAAAGTTTGCATATGAAGTTTCATGTTATGACCCTAGAATTTCTAGTCATTAATATAAGCT
GTCATTAGGTAAATTTGGCTTCAGTATGTGCACTGACCTCTTTTTCTGTCAATTGGCTCTTGTTCAA
CTTTCCATTATCAACAAATTTATATCCTTCAAGAACTTTTTCTCTTTAGTGGCTTGAAGCACACTTA
TGCAGTTGACAGAATTCTCTTGTTTTAATTTTTAGGGCCTAATTATTTTTTTAGAGAGACTTACACT
TAAGGACACTTAACTCCCTTCCTTACTGTGGTTCAAAAGAGCAAGTCTGTAAATGCAATGGATGAA
ACCATTCTCTTAATGTACAAATGGGCATGCTTTCCAGTCTACTTGAAAGGTGAAGATGTATTTTTTT
TTTTAAGTGTTGAGCAAAGCATTAAGAGACATAAAAGTAATGTGCTAGTTCCTCAGCTGATGCATC
CTGCCAAGCTTCCTTGG

Sequences producing significant alignments, table 2:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anser anser insulin-like growth factor I (IGF-I) gene, intron 1 and partial cds	562	562	99%	2e-156	88%	EU111743.1

Fig 1(B)

>2
ACTCATCCAGCAAGATGCTAAGATCTCTTGCCAAGAAGAAAAGAATACTTACAAATTATTGACTTA
AACAAGAGAAGGCTCAATACCCCACTGGATCAGCTCTCGTATGACCAGACAATAGACAGTTTCAT
CAGTTTTTCAGAAGTGAAAAAATAAGAGGAACAAGTCCCTGAAAGAAACTTCAGGAAATACTAAT
CTTTTTTACTCCAGTTACTAATGTAAGTTTTAAGCCAAGGTTATCTGTACACAAAAGAAAGATTCAT
AACTTCTTACCTGACTGCAATACTAAAATTAACCTTGCAATAGAATGAAATTAAATGCAGATTTAC
ACCCTTTGAGATAGCATTTTTGTCAAATGATCTAAATCAGGTCAGAAAATGCATTAAGCACATTCT
TCACTGTTCTGATACTAAATAGAAATAGATTTTCCTAGGGCTCAAAAAAAATGCTATGTTGAGTAA
GATTTTCTGTTGATATTAACTGATACTAAAGGAAGTAGACAAATACAATTGTGCAAATTTACACCC
ACTGAGGATCAACCTTACTTCAACCATAAGTCTGGAAAATAAATGCTTTGCATCCAAGTTTGAGGA
AACATGCATCACCTCTATTTATACCTCATAGCCAAACCCGAACTAAGTAGACAGAGAAAACATAC
CTGCTTCTGTATTTGTATCAAATACGAAAGATTCCTTCACTATTTCTCCTCTTAATGTCTTGAATCTT
TCCATATGTTATCCTGCTTGTAACAGAAGACTGGAAG

Sequences producing significant alignments, table 3:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anser anser insulin-like growth factor I (IGF-I) gene, intron 1 and partial cds	481	481	100%	1e-131	79%	EU111743.1

Fig 2(C)

>3
ATTCATGAAAGTTTGCATATGAAGTTTCATGTTATGACCCTAGAATTTCTAGTCATTAATATAAGCT
GTCATTAGGTAAATTTGGCTTCAGTATGTGCACTGACCTCTTTTTCTGTCAATTGGCTCTTGTTCAA
CTTTCCATTATCAACAAATTTATATCCTTCAAGAACTTTTTCTCTTTAGTGGCTTGAAGCACACTTA
TGCAGTTGACAGAATTCTCTTGTTTTAATTTTTAGGGCCTAATTATTTTTTTAGAGAGACTTACACT
TAAGGACACTTAACTCCCTTCCTTACTGTGGTTCAAAAGAGCAAGTCTGTAAATGCAATGGATGAA
ACCATTCTCTTAATGTACAAATGGGCATGCTTTCCAGTCTACTTGAAAGGTGAAGATGTATTTTTTT
TTTTAAGTGTTGAGCAAAGCATTAAGAGACATAAAAGTAATGTGCTAGTTCCTCAGCTGATGCATC
CTGCCAAGCTTCCTTGG

Sequences producing significant alignments, table 4:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anser anser insulin-like growth factor I (IGF-I) gene, intron 1 and partial cds	562	562	99%	2e-156	88%	EU111743.1

Fig 2 (D)

>4

AATCAATGGAACATATTGATTTAGGTTAAGATGAAGCCCTGTTTTTTTTTTAAACAAAATTAGCAG
TATTTAAACCTTGACCCATCCTGAAGTACATATTCTTTATATACAGTAAATGCTAGGGAATACTGTT
ATTAAATGCTAATTGTAAACTTTTACTATGACTTGGTAATTAGATCCAAAAACTATGTAGCAAAAT
GAATATAACTTTTTTCCTTTAGTATACACTATCCATAAGAATTTTGCTCACATCAAGATATTTATAA
TTATTATGAATGCCATTATGTTATTAAAATGACCAAGCATGTGAAGAATTCCTATGCACAACTTGT
GCCAACTGATAGCTTGTTAGAAGAAGTGGGTTTATATGGAAGATTTAAGATGTAAAAGAGATGTA
GGGTGTTCATATATGACATTCTTCTGTGGTGCCACTCTTCTGGACTATTAAAAGAGGAATGCAGCA
AAAGCTGGTTACTTTACTGAAAAAAAAGAGGAAATTCCAGCTGTGAAAATATGGTCAGTCATTCT
ATGTAACCAAAAGAAGTGTTTAACCAGGCTCATCCTGAACCTGTATTCAATTTCCAAATGTAATTA
GATGCTTCAATAAACCCCACCCACATGCCAATGCATTTTCTTTTAAAGTCTCAACTTTCTAGTGAAA
CTGGGGACATTGTAAGAACTCTGCCTGTTTGCAAAGGACCTGAAGCAATATTGGATTATAACTTTG
TATTTTCTTAAGACTATTCTCCTCAGACATAATGGGAACTGTGATTTAACTATGAACATATTGAATC
AACTAATGTTGTATTTAATTAAATTGATGAAGTAAAGTTAAAGTCTTAAACTATGTTTTGGAATTA
GTTTTGATACAACAACATTTAATTTAAAAAGTAATTGAGTCCCAGAAATATGTTGATCTTTCCTCTC
TTCCAAAGTTTTTACTTGTTTGCAAGTACTTGACTGCTTTTAAATTTGGAGATAAAGTGTAGAAATT
CTCTGTGGTTTATACTGTGCAGCCTTTTTGGCCTCCATTAAAGGAAAAACAGTAGATAATATGTTTT
GTTGTAGTCCAGTGCTGATATTTATCTTAAAATGTAACCCAGCTGAAAAGTCTTGAAAAATAAAAA
ATTGTTAAATGTCTAAGATTTATGTTTATTTCTGGCCCAAATATTATACAAGAACAAACTGTTAAAT
ATGTAACATTCTTGTATTTATAATGTCTTTCAGCCTTCTCAGCCTGTTGTGCTAAACAGCTTTCTGCT
TTACCTCCTCAGAACTAGGAACCATTTTAAACCACAGGTGCATAACATTAAACTTTAAAATTAAAA
AATATGGCAAGGGAAAAAAGAAGTTCGTGTTTTTAAGATATGGAATAATTACAGGAAAGAATGTC
ATGTGTTATCATTCATTTATGATGTGACCTTCCTCCATCTGCCACTGAAGTCATGTCCATGCATTAA
AGCTTGTTGGTTTTTCCATGGCAAAGTTGGCTAAAGACTGTTGTGAATGGGAAAATAATTATTCTTT
CTTTTTATGATATAACCTGGGATTTAGATTTGCTTGTTATTTTCGCAGTGGAAATATTATCAGGAAA
AACAGAGGGGTACATTCTGACTTATTTATAGACTTTTTTCTTACTCAGCTTTGACTTAAGCTGAAAT
AGGGG

Sequences producing significant alignments, table 5:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anser anser insulin-like growth factor I (IGF-I) gene, intron 1 and partial cds	1825	1825	99%	0.0	87%	EU111743.1
Apteryx australis mantelli genome assembly AptMant0, scaffold scaffold27	1528	1528	99%	0.0	83%	LK391419.1
PREDICTED: Corvus cornix cornix insulin-like growth factor 1 (somatomedin C) (IGF1), transcript variant X2, mRNA	91.6	91.6	4%	5e-14	88%	XM_010410452.1

It is evident from the sequence analysis of these 4 amplicons of restricted fragments they mostly share their similarity with Insulin like growth Factor (IGF1) Gene of *Anser anser*.

V Conclusion

It can be concluded that RFLP can be used as a powerful molecular technique for establishing the genetic diversity within a group. The sequences as obtained of the 4 selected amplicons show highest similarity with the fragments of IGF1 of *Anser anser* that is phylogenetically very close to the Indian chicken species.

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