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SNP of ZFY gene in Yak (*Poephagus grunniens* L) and their F1 hybrid

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ABSTRACT

ZFY is a potential candidate gene known to influence fertility in yak and their hybrids. Genetic polymorphism and diversity study of ZFY gene in Yak (*Poephagus grunniens* L), yak hybrids and related bovines in Arunachal Pradesh has been attempted in this study. PCR based protocols, direct sequencing and bioinformatic tools have been adopted to find out SNPs within the species and variation across the species. With reference to standard template SNPs have been observed in position A76C, G272A & G428A. *In-silico* translation is performed which revealed nonsynonymous mutation which is of missense type substituting metheonine for valine. Restriction digestion study is also performed with *Alu 1*. Gene identity in yak and homology studies were also conducted following standard protocol

Key words : F1 hybrids, *in-silico,* Polymorphism, SNPs, , SSCP, Yak translation

INTRODUCTION

Understanding the molecular basis of fertility is of immense importance not only for the advancement of knowledge but has profound practical implication for genetic improvement of livestock, reproductive health care of human and domestic animals and wild-life conservation. Since ancient days fertility or lack of fertility aroused human curiosity and he tried to overcome it as per his existing knowledge and experience. Many domestic animals often face the problem of sterility which not only affects their propagation and population size but also livelihood of people. One such domestic animal is Yak (*Poephagus gruenneins* L.) figure 2 which is often known as Ship of the plateaus and is one among the bovine family distinct from its wild predecessor i.e wild yak (*Bos mutus*). One possible candidate gene along with the other MSY/NRY gene (Male specific gene of Y Chromosome/ Non Recombining Region of Y Chromosome) associated with male fertility was detected in the short arm of the Y chromosome by Page *et al.,* 1987. This gene was reported to encode a Zinc Finger protein and hence named as Zinc Finger gene on Y chromosome with abbreviation ZFY.

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Initially this was projected as the master gene Testis Determining Factor (TDF) which was contradicted by others. Zinc finger proteins forms a big protein family and they are of great interest to molecular biologist since they regulate the expression of many genes and referred to as transcription factor. Genes for Zinc Finger Protein are a group of trans-acting element which synthesize the so called Zinc Finger Protein. They are also known as transcription factors because they modulate transcription by guiding the RNA polymerase to the promoter (cis-acting element) which is generally in upstream and in close physical proximity to the structural gene. The Zinc finger protein have a common DNA binding motif as finger like protrusions which consist of approximately 30 amino acids folds in two N-terminals β-strands and a C-terminal α-helix, surrounding a central zinc ion. This zinc ion is generally co-ordinated by four cysteine and/ or histidine residues. Such transcription factors (Zinc Finger Protein) generally have three or more finger like protrusions. Lau and Chan (1989) isolated and sequenced a testis specific ZFY c-DNA. The corresponding ZFY transcript encodes a protein that has 801 amino acids and a calculated molecular weight of 90.6 KD. When first detected ZFY was describes as 'Master' switch – a function attributed to TDF (Testis determining factor). The *zfy* gene is highly conserved in many species of mammals, and has been used as a marker for the Y chromosome in population studies of rodents and humans (Tucker and Lundrigan,1993; Mukhopadhyay *et al.,* 2011) and as a sex-specific marker in wildlife studies (Okuyama *et al.,* 2014). Limited studies have been conducted with Indian yak and their hybrids. So the present study is conducted with an aim to study SNPs and variation of the participating individuals of parental species, figure 1 (Yak 2n=60, Tibetian Hill Cattle 2n=60), hybrids (2n=60) and Back Crosses (2n=60) of ZFY gene.

MATERIALS AND METHODS

DNA from 124 individuals were isolated viz. from yak, indigenous hill cattle, F1 male hybrids and members from back crosses following high salt method as described by Montgomery and Sise (1990) with minor modification . The purity and concentration of DNA samples were checked by UV-VIS Spectroscopy. The optical density (OD) was checked at 260 nm and 280 nm in an UV spectrophotometer (Systronics-2202) and was found in between 1.7 to 1.9. The working solution was prepared by diluting the stock to 70 to 100 ng/µL for further PCR related work.

FP5'-GAAACCCAATTAAAATATAGAAGCA-3 & RF5' AGACCTGATTCCAGACAGTACCA-3' were used after Cathey *et al.,* 1998. The PCR (hot start PCR) reactions were carried with the following programme, initial denaturation at 95ºC for 5 minutes, denaturation at 94ºC for 30 sec, annealing 59ºC for 45 seconds and extension at 72ºC for 1 minute followed by a final extension of 10 minutes at 72ºC. Number of cycle for the programme was 30. The PCR product is then electrophoresed in 1.5% agarose in constant 100V and variable mA for 45 to 60 mins with 1X TAE buffer followed by staining the gel with Ethedium Bromide. SSCP patterns were obtained by PCR amplicons were resolved in PAGE gel 12% non-denaturing Gel. 5 µl of denaturing solution (95% formamide, 10mM NaOH, 20mM EDTA) and 5 µl tracking dye was added to the samples (10 ul PCR product). All samples were denatured in a thermo cycler at 95°C for 13 minutes.

Denatured PCR product was subjected to snap cooling by immediate transfer to ice to prevent renaturation of the denatured PCR product. The gel was run initially for 1 hour at 200 Volt and then at constant voltage of 375 volt for 10 hours. Thereafter the glass plates were removed and the gel was washed in double distilled water. Subsequently the gel was kept immersed in fixing solution (10% ethanol) for 9-10 hours to over night. The SSCP gels were stained after Sambrook and Russel (2001). Following which electrophoretic mobility and scoring the gel for their characteristic pattern was done.

Following scoring of SSCP gels, representative sample products giving unique SSCP patterns were custom sequenced to confirm the mobility shift in each pattern. Sequence data were analysed using Bio Edit software, Clustal W multiple alignments for detecting single nucleotide polymorphisms (SNPs) by comparing the observed sequence with AY079137. *In-silico* translation was done with the help of NCBI ORF finder and the best frame is selected with high precision . The selected ORF frame of each representative population (hybrids, back cross and parental species) were aligned with the help of Bio Edit (Sequence of NCBI ORF selected frames » translate or reverse translate permanent» ClustalW). The restriction digestion is done as per the manufacturer's instruction (Fermentas) and the reaction is inactivated at 64 ºC for 20 mins after its recommended incubation at 37º C with optimum buffer and enzyme unit.

RESULTS & DISCUSSION

The observed ratio of OD 260 to OD 280 was found to be in between 1.7 to 1.9 which is regarded as good in quality for the isolated DNA. The size of the PCR amplicons was 589 bp. Five partial sequence were deposited with NCBI whose accession number are from GU075408.1- GU075416.1. Male yak, hill cattle and hybrids showed amplification in these ZFY regions whereas their female counterpart had partial or no amplification. BLAST analysis of ZFY (589 bp) also confirmed gene identity (figure 8) & the percent homology which is 99% (*Bos grunniens*), 98% (*Bos gaurus*), 97% (*Bos taurus*), 98% (*Bos indicus*), 98% (*Bos frontalis*), 98% (*Bos javanicus*), 96% (*Bubalus bubalis*), 99% (*Bison bison*). PCR-SSCP studies (figure 5) revealed five characteristic band pattern for ZFY fragment (589 bp) figure 3 a &b. SNP studies revealed that ZFY amplicon (589 bp), one transversion was observed at A76C and two transition was observed at position G272A & G428A both for hill cattle and hybrid (figure 6). The restriction digestion product of ZFY by *Alu 1* (figure 4 a & b)reveals fragments of 589 bp, 500 bp, 400 bp, 380 bp, 350 bp, 220 bp, 180 bp, 150 bp & 75 bp. Yak, F1 hybrids and others showed districted band patterns. The *in-silico* translation (figure 7) result showed that for a change in the selected ORF (with NCBI ORF finder) in position G51A & A64G for hill cattle and F1 hybrid, there is a change in amino acid i.e. nonsynonymous change (missense mutation) where methionine (M, uncharged hydrophilic with side chain containing S atom) at position 22 is substituted by valine (V, uncharged hydrophobic with aliphatic side chain) for hill cattle and F1 hybrid. Since the substituted amino acid are of different nature, there is a like chance of change in the protein structure and therefore its function. The partial expression of ZFY in some yak females and F1 hybrids is found as par with the work of Lau and Chan (1989). Moreover expression analysis revealed that ZFY and ZFX

is differentially expressed in both adult and foetal human tissue and even in XX tissue there were ZFY expression which should not have occurred if ZFX is TDF (Lau and Chan (1989).

CONCLUSION

The present study revealed distinct SSCP patterns of individuals of parental species, hybrids and Back crosses. Three SNPs was observed in Hill cattle and F1 hybrid and *in -silico* translation was performed and significant change was observed at amino acid substitution level. Restriction digestion of the amplicon of ZFY is also done and distinct type was observed with hill cattle and F1 hybrids.

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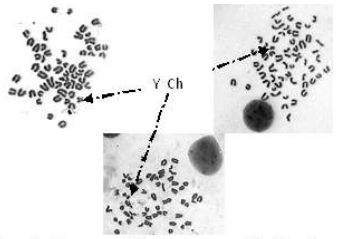
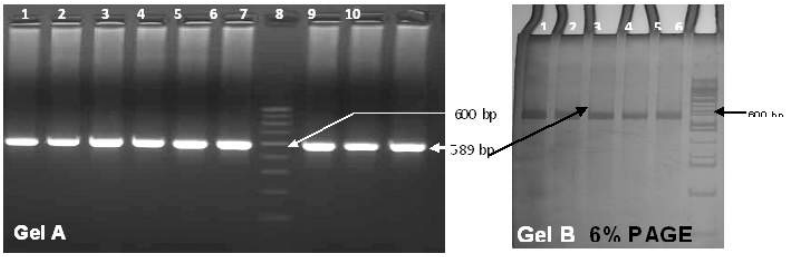
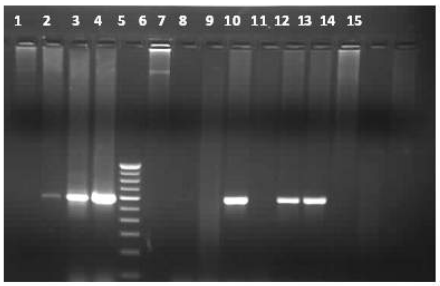


Figure 1: Presence of Y Chromosome. Starting from left Metaphase plate of Yak, Hill Cattle and F1 hybrid

Figure 2: Different types of Indian Yak, Starting from left, Common yak, Hairy forehead, Bare Back, Bisonian type and White Yak





Gel A	Gel B	Gel C
Lane	Lane	Lane
1 Yak male	1 Yak male	1 Female yak
2 Yak male	2 Yak male	2 F3 hybrid female
3 Hill cattle male	3 F1 male hybrid	3 Yak male
4 F1 male hybrid	4 Hill cattle male	4 Yak male
5 F2 male hybrid	5 F3 male	5 100 bp ladder
6 F1 male hybrid	6 100 bp ladder	6 F1 hybrid female
7 100bp ladder		7 F1 hybrid female
8 F1 male hybrid		8 F2 hybrid female
9 F2 male hybrid		9 F3 hybrid male
10 F1 male hybrid		10 F3 female hybrid
		11 Yak male
		12 Yak male
		13 Yak female
		14 F1 female hybrid
		15 F2 hybrid female

Figure 3 Agarose gel electrophoresis and PAGE of ZFY, 589 bp. Gel A represent agarose gel electrophoresis (1.5%) of male yak and male hybrid. Gel B is simple PAGE (6%) to confirm for the absence of any artifact or non specific band. Gel C (1.5 %) agarose represents the absence or partial presence of amplification in female yak and related female hybrids.

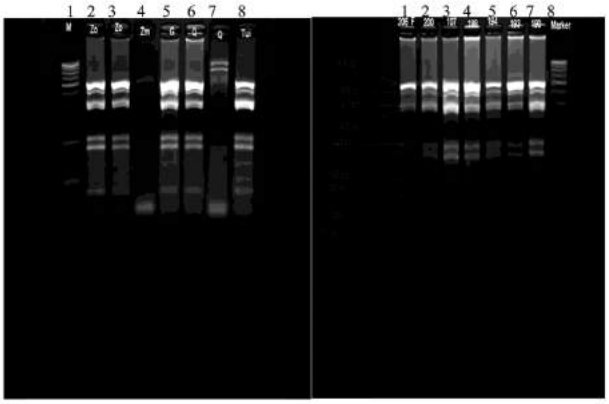


Figure 4a &b: Restriction Enzyme digestion (*Alu I*) of 589 bp ZFY fragment showed distinct features in some of the hybrids

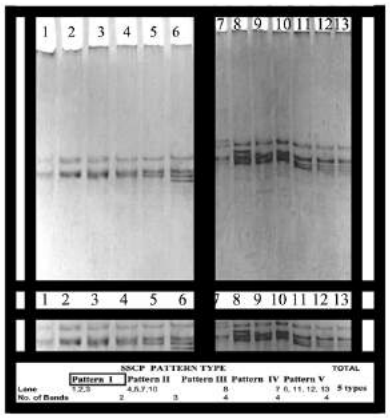


Figure 5: SSCP pattern of ZFY-1 589 bp.

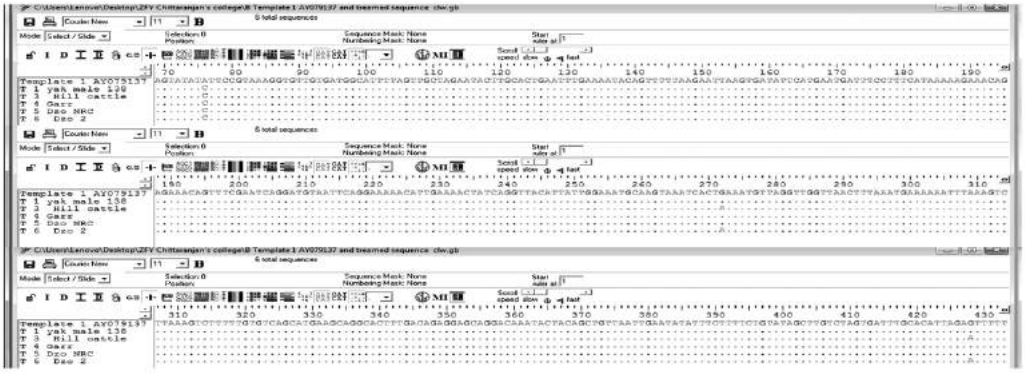


Figure 6: Clustal W results of the ZFY (589bp) fragments of yak, Fi hybrid, hill cattle showing mutation at position A76C (transversion) , G272A (transition), G428A (transition).

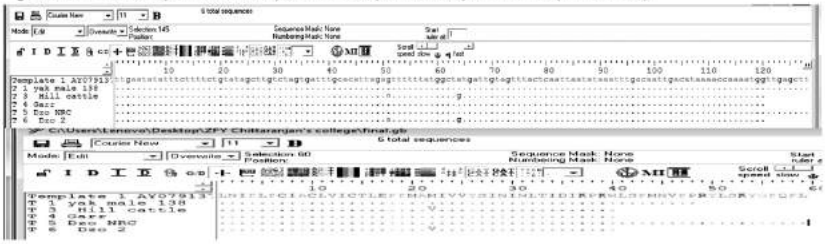


Figure 7: *In-silico* translation of 589bp ZFY amplicons of yak, hill cattle and their hybrids with the help of NCBI ORF finder.

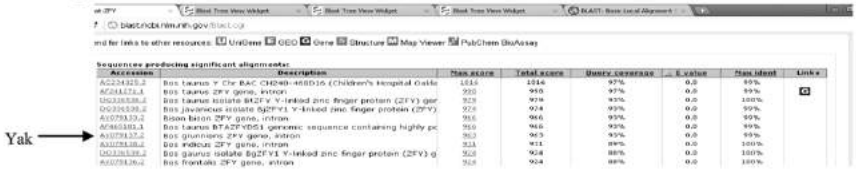


Figure 8: Gene Identity by BLAST analysis

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