

**PROFORMA FOR SUBMISSION OF ANNUAL PROGRESS REPORT
OF RESEARCH PROJECTS**

Part - I : General information

600 Project Code

6001 Institute Project Code No : *XI/66B-2.1*

6002 ICAR Project Code No.

601 Name of the Institute and Division :

6011 Name and Address of Institute : Central Institute for Research on Goats,
Makhdoom, Farah, Mathura

6012 Name of Division/Section : Genetics and Breeding Div.

602 Project Title

Molecular analysis of major genes and quantitative trait loci influencing growth, reproduction and disease resistance traits in Indian goats.

603 Priority Area

6031 Research Approach: **Applied Res./Basic Res./** Process / Transfer of Tech.
or Tech. Dev 01

02 03 04

604 Specific Area : Genetic improvement and genomic tool

605 Duration of Project : 5 years

6051 Date of start of Project : September, 2007

6052 Likely date of completion of Project : 2012

6053 Period for which report submitted : September 2007---Mar, 2008

606 Total cost of Project

6061 Expenditure of date

607 Summary Achievements

Genotyping of Myf-5 gene was carried out by analyzing samples from 3 different goat breeds for the presence of different variants by allele specific polymerase chain reaction. PCR amplified product was observed as 980 bp. The population analysis revealed three different genotypes and BB genotypes was showing association with high growth rate at 3 months of age in Barbari goats.

Part-II : Investigator Profile

(Please identify clearly changes, if any in Project personnel)

610 Principal Investigator :
6101 Name : Dr. P. K. Rout
6102 Designation : Sr. Scientist
6103 Division/Section : G.G. & B. Division
6104 Location : C.I.R.G., Makhdoom
6105 Institute Address : C.I.R.G., Makhdoom
611 Co-investigator :
6111 Name : Dr. Ajoy Mandal
6112 Designation : Scientist (SS)
6113 Division/Section : G.G. & B. Division
6114 Location : C.I.R.G., Makhdoom
6115 Institute Address : C.I.R.G., Makhdoom
612 Co-investigator :
6121 Name : Dr. A.K.Das
6122 Designation : Scientist
6123 Division/Section : G.G. & B. Division
6124 Location : C.I.R.G., Makhdoom
6125 Institute Address : C.I.R.G., Makhdoom
613 Co-investigator :
6131 Name : Dr. S.K.Singh
6132 Designation : Principal Scientist
6133 Division/Section : G.G. & B. Division
6134 Location : C.I.R.G., Makhdoom
6135 Institute Address : C.I.R.G., Makhdoom
613 Co-investigator :
6131 Name : Dr. M.K.Singh
6132 Designation : Sr. Scientist
6133 Division/Section : G.G. & B. Division
6134 Location : C.I.R.G., Makhdoom
6135 Institute Address : C.I.R.G., Makhdoom
613 Co-investigator :
6131 Name : Dr. R. Roy
6132 Designation : Principal Scientist
6133 Division/Section : G.G. & B. Division
6134 Location : C.I.R.G., Makhdoom
6135 Institute Address : C.I.R.G., Makhdoom

Part-III : Technical Details

620 Introduction and objectives

The major focus of this project is to identify and characterize genetic variation underlying economically important traits in Indian goats. During the domestication, the goat has undergone intense natural selection pressure for various phenotypes. Selection in this species

has led to distinct phenotypes associated with meat, milk, fibre production thriving in tropical environments in some part and tolerating specific pathogens. These selective pressures have differentiated sub-populations and produced phenotypes according to the need of the region. Therefore, it is necessary to utilize molecular markers to select high performance individuals for suitable environment for enhancing productivity and sustainability in goat production. It is necessary to combine molecular markers and production traits in an efficient manner for attaining higher productivity. DNA marker information, which identifies important allelic variation within the genome, could be incorporated into genetic evaluations to provide producers with selection tools that increase the rate of genetic improvement for lowly heritable traits.

Genetic diversity in Indian goats has been established using microsatellite and mtDNA markers. The geographical structuring exists within Indian goats and the genetic distances between breeds are significant indicating that they are unique population. The association of some markers with different traits has also been studied. Therefore, we propose to analyse the major genes/QTL influencing production and other traits in Indian goats. We will select the established loci in cattle, goat and human for growth, milk production and analyse the region with PCR-RFLP, microsatellite and SNP based approach. This will be again validated in population and will give an idea to use marker studies for attaining sustainability in goat production through this approach. As bovine genome sequence draft has been declared, therefore the information yielded from the bovine genome will have direct application to goat, sheep and other farm animal research. The bovine genome physical map and genome sequence will help to select desirable production traits, identify genes involved in disease resistance and enable to produce better matching products for market specifications.

6201 Immediate objectives:

To identify genetic variation in major genes that influence growth, fecundity, and disease resistance traits

To characterize quantitative loci that control production, reproduction, disease resistance and meat quality traits in Indian goats.

6202 Long term objectives:

To identify chromosomal regions containing QTL/QTN for production, reproduction and disease resistance traits.

To design breeding programme to improve overall productivity in low input environment for sustainable production.

6203 Specific objectives for the year as detailed in RPF-I

Organization of facilities, procurement of chemicals, Blood collection and DNA isolation, Allele specific characterization of Myf-5 gene .

6211 Technical Programme :

(Indicate briefly plan of procedure, techniques, instrument and special materials, organisms, special environment etc.)

1. Procurement of chemicals, equipments; organization of facilities.

2. Resource population selection and phenotypic data collection: Genetic variation will be analysed in 10 different Indian goat breeds and association study will be carried out in Barbari and Jamunapari goats.

3. Blood collection and DNA isolation.

4. Selection and synthesis of primer and PCR standardisation.

5. Genotyping by PCR-RFLP and SSCP analysis

TECHNICAL PROGRAMME :

DNA isolation: DNA from all the goat samples will be isolated using a standard protocol.

Selection of marker by comparing goat, cattle and human map

Analysis of genetic variation in genomic regions influencing growth, meat quality and milk quality (MYF, Myostatin, etc), fecundity (Boorola and inverdale gene), Disease resistance (MHC region variability and specific resistance to parasitic disease) by PCR-RFLP, Microsatellite and SNP analysis.

Genotyping will be carried out in population and half-sib offspring, and additional intercross can be developed as per experiment requirement

Phenotypic measurement for Growth trait, Reproduction (fecundity), Milk yield trait and Carcass trait:

Linkage analysis

Statistical analysis: Various statistical analyses will be carried out to establish the association of marker with the traits such as interval mapping and regression analysis.

6212 Man months involvement of component Project workers for the specified year.
As Per specification in original project document

622 Progress of work

6221 Achievements in terms of targets fixed for each Activity

Achieved all the target as per objectives.

6222 Questions - Answered :

Genetic variation in Myf-5 gene was carried out in 3 different breed and association between Myf-5 gene and growth traits was observed.

6223 Process/Product/Technology/Developed during the year.

6224 Utility of results obtained so far

The data obtained can be used for the improvement of Indian goat breeds.

623 Publications and Material Development :

(One copy each to be supplied with this proforma)

6231 Research papers: Nil

6232 Popular articles: Nil

6233 Reports : Annual Report

6234 Seminars and workshops (relevant to the project) in which the scientists have participated.

Yes

6235 Infrastructural facilities developed:

**Part-IV : Project Expenditure
(Summary)**

Recurring Expenditure

630 6301 Salaries (Designation with pay scale) : Nil

i) Scientific

ii) Technical

iii) Supporting

iv) Wages

Sub total

6302 Consumables:

i) Chemicals

ii) Glasswares

iii) Others

Sub total

6303 Travel

6304 Miscellaneous
(other costs)

6305 Sub Total
(Recurring)

631 Non-recurring Expenditure : (Equipments)

i)

ii)

iii)

632 Total
(630 and 631)

Pr
Signature of the Project Investigator:

Co-investigators:

- 1.
- 2.
- 3.

Signature and comments of the Head of the Division/Section

Pr
6/5/08

Signature and comments of the Joint Director (Research)

Signature and comments of the Director

The major focus of this project is to identify and characterize genetic variation underlying economically important traits in Indian goats. During the domestication, the goat has undergone intense natural selection pressure for various phenotypes. Selection in this species has led to distinct phenotypes associated with meat, milk, fibre production thriving in tropical environments in some part and tolerating specific pathogens. These selective pressures have differentiated sub-populations and produced phenotypes according to the need of the region. Therefore, it is necessary to utilize molecular markers to select high performance individuals for suitable environment for enhancing productivity and sustainability in goat production. It is necessary to combine molecular markers and production traits in an efficient manner for attaining higher productivity. DNA marker information, which identifies important allelic variation within the genome, could be incorporated into genetic evaluations to provide producers with selection tools that increase the rate of genetic improvement for lowly heritable traits.

Genetic diversity in Indian goats has been established using microsatellite and mtDNA markers. The geographical structuring exists within Indian goats and the genetic distances between breeds are significant indicating that they are unique population. The association of some markers with different traits has also been studied. Therefore, it was proposed to analyse the major genes/QTL influencing production and other traits in Indian goats. The established loci in cattle, goat and human for milk production and growth will be selected and will be analysed by PCR-RFLP, microsatellite and SNP based approach. This will be again validated in population and will give an idea to use markers for enhancing productivity in the population. As bovine genome sequence draft has been declared, therefore the information yielded from the bovine genome will have direct application to goat, sheep and other farm animal research. The bovine genome physical map and genome sequence will help to select desirable production traits, identify genes controlling economic traits and disease resistance traits and enable to produce better matching products for market specifications. As a part of objective, we have analysed genetic variations in MyoD family genes which are responsible for growth and meat quality.

Molecular Analysis of Myf-5 gene

Myofibrillar formation in mammals occurs only during embryonic development and is controlled by the MyoD family genes. MyoD gene family consists of four genes namely myogenin, MyoD1, myf5 and myf6. The myf5 and myoD genes are expressed in proliferating single nucleotide precursor cells called myoblasts. The Myf6 gene is mainly expressed postnatally. Myogenin has a crucial role during myogenesis and expressed in all myoblasts during early differentiation (i.e. Fusion of myoblasts into multinucleated myofiber), and its expression continues during cell fusion. The myf-5 gene has been considered to play an important role in growth and development of mammals. Myogenin expression also marks the end of the proliferation of myoblasts. Therefore different myogenin function or timing of expression could have a major influence on the number of muscle fibers that develop during myogenesis. Therefore polymorphism in Myf-5 coding sequence in different breeds will give an idea regarding body growth and meat quality in Indian goat breeds.

Myf-5 gene polymorphism was carried out in 35 samples of Barbari, 25 samples of Jamunapari and 25 samples of Black Bengal. DNA was isolated from the samples using standard protocols and were checked for its quality, purity and concentration. Subsequently DNA samples were purified by Gene Elute PCR Clean up Kit of Sigma for further use. PCR-RFLP was carried out to analysis myf5 gene using following set of primer F₁=5'-CCT ATC TGG TCC AGA AAG AGC AG-3' R₂=5'-TAT ATA AGT TAA GCA TTG CAA CAA-3' The PCR reaction was performed in a 50 µl final volume containing 0.5 unit of *Taq* DNA polymerase, 1×PCR buffer, 1.5 mM MgCl₂, 200 µM each dNTP, 10 pmole of each primer and approximately 100 ng of goat genomic DNA. The PCR conditions were: 94°C for 4 min, followed by 38 cycles of 94°C (30 sec), 58°C (1 min), 72°C (1 min), and final extension at 72°C (4 min). About 30 microliters of the PCR product were digested with 5 units of the restriction endonucleases *Taq*I (New Biolab) overnight at 37°C. The resultant fragments were separated by electrophoresis in a 4% agarose gel stained with ethidium bromide.

Genotyping of Myf-5 gene was carried out by analyzing DNA samples of 3 different breeds of goats for the presence of different alleles by allele specific polymerase chain reaction (AS-PCR). PCR amplified product was observed to be 980 bp. The PCR product of 980 bp long was digested with *Taq*-I restriction enzyme. The PCR-RFLP pattern revealed three genotypes, BB (580 bp + 400 bp), AB (980 bp + 580 bp + 400 bp) and AA (980 bp). The

Notes. The frequency of AA genotype was very low in all the analysed samples. The genotypic frequencies of BB and AB genotypes were 0.62 & 0.32 in Barbari , 0.54 & 0.42 in Jamunapari goats and 0.50 & 0.45 in Black Bengal goats. The frequency of B allele was higher than A allele in all the three breeds. The myf-5 gene has been considered to play an important role in growth and development of mammals. Myf-5 knock out mouse showed no muscle growth, which indicates that the gene myf-5 had an effect on muscle development . The significant association between the myf-5 gene and these growth traits suggest that the gene may be one of the causative genes that control growth traits in beef cattle or that the gene is very close to the causative genes. Naturally occurring genetic variation in myf-5 gene could affect muscle fibre number and thus lean meat production .