5. Genetic Resources

Crops

Germplasm augmentation, conservation and use: The ICAR institutions are involved in the augmentation and conservation of genetic resources, and their utilization for improvement of crop varieties, beneficial insects and microorganisms of agricultural importance.

In 21 states, 32 explorations were undertaken, and 1,785 accessions were collected; of which 976 accessions were of the wild species. In the National Herbarium of Cultivated Plants, 316 herbarium specimens were added, totalling to 20,300 specimens. Germplasm accessions added to the National Genebank for the long-term storage were 7,441 of the orthodox seed species; 278 were of the non-orthodox species for the cryo-storage and 25 accessions were for *in-vitro* culture in the Genebank. A total of 13,470 germplasm accessions were characterized and evaluated, and 11,742 were supplied for research and crop improvement within the country.

Germplasm exchange comprised 32,617 accessions imported from 37 countries, including international trial material (6,127) and transgenics (132).Promising introductions were—rice (EC 6599 13-14) resistant to sheath and bacterial panicle blight and narrow brown leaf spot from the USA, rice (EC 670423-45, EC 674492-871, EC 678586-634) resistant to brown plant-hopper and heat/salt tolerant from the Philippines; maize (EC 659915-17) with low aflatoxin and resistant to rusts and ear-rot from Nigeria; wheat (EC 671601-02) resistant to stripe rust and tolerant to high temperature, wheat (EC 673058) resistant to hessian-fly, conferred by the gene *H 25* from the USA; sunflower (EC 668894-925) with high oleic acid content from Belgium; lentil (EC 666214-952) with early maturity from Syria and recombinant inbred lines (EC 676027-132) from the USA; Frenchbean (EC 677210) with multiple disease resistance from the USA; tomato (EC 664585-99) with early maturity and cold resistance from Canada, tomato (EC 671594-95)with resistance to tomato mosaic virus from Taiwan, and tomato (EC 676413) with insect resistant gene *ARG* from the USA; and potato (EC 670754-64) with medium to late maturity and good processing quality from the USA.

Phytosanitation: A total of 87,069 imported samples including transgenics and trial material were processed for quarantine clearance. Important interceptions included **insects:** *Acanthoscelides obtectus*, *Bruchus ervi* and *Trogoderma variabile*; **fungi:** *Dendryphion penicillatum* and *Peronospora manshurica*; **nematodes:** *Tylenchorhynchus* sp., *Helicotylenchus* sp. and *Tylenchulus semipenetrans*; and **weeds:** *Anthemis cotula, Carrichtera annua, Galium boreale* and *G. trifidum, Ostrya virginiana, Phalaris arundinacea, Ranunculus bulbosus* and *Taraxacum officinale, Avena sterilis, Bifora testiculata* and *Silene noctiflora.*

Registration of improved genetic stocks: Three genetic stocks of **wheat**—HS 491 for high spread factor and soft-grain texture, UP 2698 for high protein (>13%) and AKAW 3717 for drought and heat tolerance have been registered. Wheat genetic stock VL 876 (INGR No. 09056) developed for high-bread loaf volume and quality spread has also been registered.

Kagazi Madira (B 29), an easy de-hulling-type **barnyard millet**, has been registered vide INGR No. 09023.

In Himachal Pradesh, nine districts were surveyed out of 12, and 28 collections of **sugarcane** germplasm, *Saccharum spontaneum*, *Erianthus fulvus* and *Miscanthus* species were made; 11 out of 13 districts of Uttarakhand

Maize registered germplasm

Inbred line	Characteristics
HKI 47	Late, bright orange, flint, good combiner
HKI 287L	Late, yellow, flint, long cob, productive
HKI 327T	Tall, late, yellow, flint
HKI 326	Late, yellow, flint, productive
HKI 1040-5	Late, yellow, flint, good combiner, highly productive
HKI 1341	Late, white, flint, productive, rust resistant
HKI 1342	Late, white, flint, long cob, rust resistant, Maydis leaf blight(MLB) resistant
HKI 288-2	Late maturity, yellow, flint, MLB resistant
HKI 1126	Late maturity, yellow, flint, resistant to MLB
HKI 1040-4	Medium maturity, orange, flint, resistant to MLB
HKI 1015WG-8	Medium maturity, orange, flint, MLB resistant
HKI 1347-4LT	Late maturity, white, flint , MLB resistant
HKI 1 70 (1 +2)	Late, yellow, flint, high tryptophan (0.84%)
HKI 164D-4(O)	Late, quality maize protein (QPM), yellow, semi-dent, MLB resistant
HKI 164-7-6	Late, QPM, orange ,semi-dent, MLB resistant
VQL 3	Early, orange, flint, high tryptophan (0.83%)
VQL 8	Medium, orange, flint, high tryptophan (0.94%)
VQL 12	Early, orange, flint, high tryptophan (0.75%)
VQL 16	Early, yellow, flint, high tryptophan (0.73%)
VQL 30	Early, orange, flint, high tryptophan

Sorghum registered genetic stocks

Characteristics	Entry
Shoot-fly resistant and multiple resistance to leaf diseases	NRCSFR 06-1
High biomass, high grain yield and drought-tolerant	PEC 17 (IC 392140)
Grain-mold resistant	PDSR-GM 25,PDSR-GM 83, PDSR-GM 92, PDSR-GM 98, PDSR-GM 124, PDSR-GM 169, PDSR-GM 170, PDSR-GM 203
Superior stover quality	MS 3042A, B; MS 3151A, B; MS 3062A, B; MS 3009A, B; MS 3182A, B
Multiple foliar diseases resistance	EC 1 (IC 345703), EC 31 (IC 345733), EC 32 (IC 345734), SEVS 6 (IC 347572)

were surveyed, and 25 clones of *Saccharum spontaneum* and *Miscanthus* were collected. In general, variability in height was limited to short, medium and medium-tall. From Kesardeshi (Uttarakhand) (1,950 above mean sea level), *S. spontaneum* was collected, and near Almora, shortest clone (IND09-1542) — 30 cm of this species — was collected. *Erianthus fulvus* and *Miscanthus* sp. were found only above 2,000-metre elevation.

Three genetic stocks of **cotton** *Gossypium arboreum* race *cernuum* — 30814 (INGR No. 09117), 30826 (INGR No. 09118) and 30856 (INGR No. 09119)— with immunity to grey mildew disease; and ABGMS, a male sterile of *G. hirsutum* with curved stigma, developed by induced mutation, have been registered. **Soybean** genotypes IC 210 and NRC 106 developed for high oleic acid for improving oxidative stability of soybean oil have been registered with identity No.INGR10052 and INGR10053. In addition, NRC 101 and NRC 102, which are free from *kunitz* trypsin inhibitor polypeptide, have been registered with identity No.INGR10054 and INGR10055. A vegetable-type soybean NRC 105 has been registered with No.INGR10056.

Agriculturally important microorganisms

Twenty-three national explorations and extensive surveys were undertaken in Uttarakhand, Uttar Pradesh, Himachal Pradesh, Rajasthan, Bihar, Odisha, West Bengal, Arunachal Pradesh, Asom, Sikkim, Meghalaya and Kerala.

Aerobic thermophilic bacteria were isolated and characterized from water and sediment samples collected from hot springs. Out of 53 morphotypes, 11 grew at 60°C. In soil samples from Gurudongar lake (North Sikkim), 50 bacteria were isolated that could grow at 4°C.

A total of 192 morphotypes of actinomycetes were isolated using different media. Cultures were identified by using classical and 16S-rDNA sequencing approaches. Cultures identified using 16S-rDNA sequences were submitted to the NBAIM culture collection—*Streptomyces viridodiastaticus*, *S. heliomycini*, *S. albogriseolus*, *S. griseorubens* and *S.macrosporeus* along with NCBI accession numbers GU817410, GU817411, GU817412, GU817413 and GU817414.

Abiotic stresses such as extremes in temperature, drought, salinity, heavy metals and radiation have detrimental effects on plant growth and yield. Sambhar salt lake isolates possessed NaCl tolerance up to 20% along with plant growth promoting (PGP) traits (IAA, Ammonia, ACC deaminase, siderophore and P-solubilization) and extracellular enzyme activity. Evaluated potent isolates (S-121 *Bacillus pumilus,* AS-40 *Pseudomonas mendocina,* AS-18 *Arthrobacter* sp., SL-11 *Nitronicola lacisaponensis* and SL-9 *Halomonas* sp.) in wheat, and observed better crop growth and stress tolerance crop responses at different salt concentrations under the greenhouse conditions. Rhizosphere extracts from plants bacterized with isolates SL-11 and S-121 showed maximum seed germination in comparison to control and other treatments. *Nitrinicola lacisaponensis* (SL-11 isolate) showed better amplification of osmoprotectant choline dehydrogenase gene.

Bradyrhizobium japonicum-ISR-33 and *Bacillus megaterium*-ISP-3 increased soybean grain yield with integrated nutrient management by 18% over balanced fertilization and 54% over farmers' practices.

Cu and Zn efficient *Bacillus* and *Pseudomonas* strains have been identified as potential organisms for sorghum and pigeonpea. *Bacillus* strain B66 promoted Cu and Zn uptake in pigeonpea seedlings while seed treatment with *Bacillus* strain B101 resulted in increased uptake of Na and Ca. *Pseudomonas* (P33) and *Bacillus* (B61) efficiently solubilized zinc in zinc deficient soils to make it available to maize seedlings.

KSB2 *Bacillus subtilis* isolate showed highest K mobilization in mica and feldspar. One potent P solubilizer, *Klebsiella* sp. and two efficient N fixers *Bacillus subtilis* and *Bacillus cereus* have been identified.

Several mushroom specimens of the following genera were recorded for the first time—*Phellodon tomentosus, Laetiporus sulphureus, Thelephora* and *Dictyophora.*

A study revealed the presence of fluorescent pseudomonads having multiple plant growth-promoting determinants in the rhizosphere of groundnut grown in high salinity conditions of up to 10% in Kutchch eco-region of Gujarat. The identification of the archaea on the basis of complete 16S rDNA sequencing revealed five predominant genera of archaea in the salt pan — *Halorubrum*, *Haloarcula*, *Halobacterium*, *Haloterrigena* and *Halogeomatricum*. The bacilli and archaea, which were successfully cultivated at the saturated NaCl condition, can be the source for prospecting of novel genes for osmo-tolerance.

Forty-six (46) cyanobacterial strains belonging to 9 genera have been isolated from different ecological habitats of Manipur, Arunachal Pradesh, Asom, and Mizoram states of the north-eastern region of India, and have been added to the pool of existing four hundred five (405) cyanobacterial strains.

Metagenomic nifH library highlighted the prevalence of *nifH* genes in Uttarakhand Himalayan region. Shotgun clone sequences reveal diversity among nitrogen-fixing community in Pithoragarh soil including some rare and unique bacterial species like *Dechloromonas* and *Sideroxydans*.

Thermotolerant strains P6, P7, B30 are found to be superior under heat stress and *GroES* (hsp 10) gene amplified in *Pseudomonas* sp. strains. Induction of heat shock proteins (hsp60) under heat stress in *Pseudomonas aeruginosa* P6 was confirmed by western blotting and strain P6 improved yield of wheat with late sowing date.

The most potent β-HCH degrading bacterium (PCRBHCH) has been identified as *Methylobacterium* (sequence accession no. G340522) by 16S rDNA sequencing.

Saccharomyces cerevisiae strain has been developed through regular recycling on a galactose medium. It produced about 30% more ethanol from kinnow waste through SSF, compared to the conventional *S. cerevisiae* strain.

Adaptation of *Candida tropicalis* to rice-straw hydrolysate obtained with dilute acid pre-treatment helped in enhanced ethanol production from rice straw. The adapted strain produced 20.32 g/litre ethanol using SSF process. Ethanol concentration of 26 g/litre was obtained in 36 hr from fermentation of hydrolysate obtained with the use of cellulolytic enzymes from alkali pre-treated rice straw.

Novel endophytic bacteria from watermelon were isolated and identified. Total 28 endophytic bacterial cultures were deposited with the NBAIM along with partial 16S rRNA sequence information. PGPs studies with papaya and capsicum seed inoculation led to identification of PGP effect of *Brevibacillus, Bacillus pumilus, B. megaterium, B. fusiformis, Sphingomonas* sp. in capsicum and watermelon seedlings.

All twenty-one bacterial strains have been isolated having phytate mineralizing activity from fish-gut and sediment.

Fruit crops

Genetic resource of **mango** (732) was maintained, 92 new accessions were added, which included unique collection for late and early maturity. Database and information system were developed for researchers, which contain characterization information on 360 mango accessions and a collection of molecular data on curetted ESTs (26), proteins (30) and nucleotides (285) based on NCBI.

Seedling population of **litchi** cultivars, viz. Shahi and China, exhibited a high diversity of phenotypic traits. Twenty elite clones of litchi identified for extended harvesting season and improved quality were multiplied for further evaluation. In **guava**, 120 accessions were maintained and interspecies variability was assessed using mPgCIP primer. In existing collection of **banana**, 88 exotic accessions were added, of which 34 were established. One of the most important accessions, *Musa textilis*, known for banana fibre has been introduced for the first time in India. A total of 17 accessions were added, from eastern region of the country to indigenous collection; 121 accessions were characterized for morphological traits.

In **grape**, four new accessions were added, totaling 425 accessions consisting of 112 indigenous and 313 exotic ones. Genotyping of 134 accessions was done using 25 microsatellite primers. In **pomegranate**, 178 accessions were maintained and screening of germplasm showed tolerance to bacterial blight in ACC 15, ACC 50 and ACC 51 accessions. Among 240 accessions/hybrids screened under net house, only 4 plants (one each of Nana, Nana × Ruby and 2 of Kalpitya × Ruby) were found free from bacterial blight. In **arid fruits**, 318 genotypes of *Ziziphus*, 60 of date palm, 154 of pomegranate and 17 of bael are being maintained. The germplasm of karonda, phalsa, chironji, mahua, jamun, tamarind and wood-apple are being maintained and evaluated.

Plantation crops

A total of 398 accessions of **coconut** were maintained and evaluated including 266 indigenous and 132 exotic accessions. A total of 28 new accessions were added from the exploration of eastern India and Andaman and Nicobar islands. A total of 164 accessions consisting of 141 indigenous and 23 exotics were maintained and evaluated in **arecanut**.

Potato and tuber crops

Potato germplasm repository was strengthened by adding 91 accessions from exotic sources, totaling 3,900 accessions of cultivated and wild species. A total of 1,800 accessions were evaluated for biotic and abiotic stresses and also for adaptability and biochemical characters. Three accessions with unique characters were registered with NBPGR as elite germplasm lines.

Germplasm consisting of 5,774 accessions of **tuber crops** were maintained in field gene bank and evaluated. A total of 144 new accessions of cassava, sweet potato, *Alocasia*, *Xanthosoma*, *Colocasia*, yams and minor tuber crops were collected from Jharkhand, Lakshadweep islands and Wayanad in Kerala.

Spices

In black pepper, 2,595 accessions are being conserved. In cardamom, 5 accessions were collected from Sabarimala area of Kerala, making the total accessions to 447. In turmeric, 1,173 accessions are being maintained in field germplasm conservatory.In ginger, 600 accessions are being maintained at the field germplasm conservatory.

Mushroom

A total of 154 specimen of mushroom were collected and 123 have been identified up to genus level.

Medicinal and aromatic plants

A total of 14 endangered species of medicinal plants were rescued by intensive exploration across the country and collection have been planted and are characterized for phenotypic and genotypic traits.

LIVESTOCK

Database on genetic characterization of animal genetic resources: The integrated database provides information on relevant published literature, microsatellite loci, statistical methods and software for data analysis, locus-wise, within-breed and inter-breed genetic diversity. Literature on genetic diversity of breeds can be searched by keywords, author and year.

Scoring-based phenotypic identification methodology for AnGR: The scoring based technique was refined to make a decision on breed of an animal in case of a single breed and if the test animals do not belong to any of the breed under comparison. Experiments were conducted on actual field data on cows and bullocks of Dangi and Khillar cattle breeds to view location of scores of animals among the scores of 1,000 animals simulated from breed descriptors. The procedure helps in decision support on breed of an animal.

The system has facilities for adding new breeds, breed descriptors, weights assigned to traits of breeds. The analysis report includes the identified breed on the basis of scores for selected breeds.

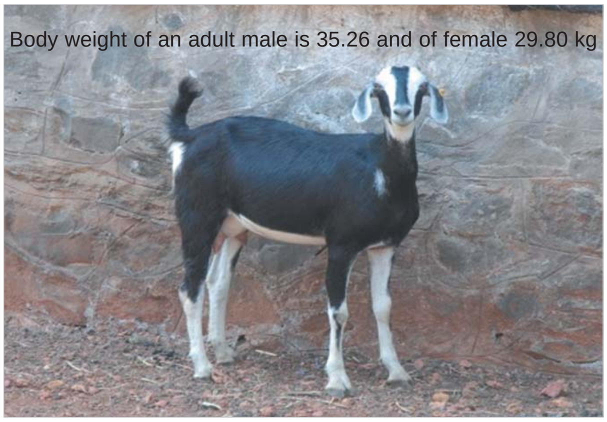
Characterization

Manipuri buffalo: Manipuri buffaloes, the Hill type animals, are stouter than their valley counterparts. Two white stripes, one in the neck and another slightly above the brisket, are the distinct features. The buffaloes are draught type and used in agricultural operations predominantly on hills.

Manipuri buffaloes are hill type animals which are stouter than their valley counterparts

They possess a diploid count of 2n=48 chromosomes (23 pairs of autosomes and a pair of sex chromosomes) typical of swamp a buffalo. Comparison of mitochondrial D-loop sequence of these buffaloes with representative riverine and swamp type buffaloes further corroborated this conclusion. The mtDNA based phylogenetic tree positioned the river and swamp haplotypes into specific and distinct grouping, which confirmed the unequivocal classification of Manipuri buffaloes as true swamp type.

Konkan Kanyal goats: Kanyal goats, found in villages of Sindhudurg district of Maharashtra state, are reared mostly by Dhangar and Maratha communities for meat purpose. These goats are mainly black with white marking in a specific pattern–ventral surface of the body is white and legs have white stockings. Konkan Kanyal goats have bilateral white strips from nostrils to ears; flat and broad forehead; flat, long drooping ear; backward, straight, pointed, cylindrical horn; white muzzle and long legs, laterally black, medially white for knee to fetlock joint. The body weights of adult male and females were 35.26 and 29.80 kg respectively.



Konkan Kanyal doe

Ramnad White sheep: Ramnad White sheep breed is distributed mainly in Ramnathapuram and Sivaganga districts of southern Tamil Nadu. The animals are medium to large in size, having 43.9±1.07 and

Ramnad White-Ewe

31.5±0.26 kg weights in rams and ewes respectively. The coat is white with black belly and extremities. The legs below the hock/knee joints are black. Some of the animals are complete white.

Chevaad sheep: Chevaad, a medium size sheep is distributed in Tirunelveli and Thoouthukudi districts of Tamil Nadu. Average body weight is 26.5±0.75 and 22.6±0.21 kg in rams and ewes respectively. They have deep brown body coat with small and shiny hair, straight noseline and long and semi drooping ears. Age at first breeding is 16-18 months with a breeding life of 3-4 years.

Chevaad sheep

Bhutia pony: Bhutia equids also known as Bhutia or Bhotia ponies have their origin in the Himalayan region of India, and are now found both in Bhutan and India (Sikkim, Darjeeling, Arunachal Pradesh). In Sikkim, population of Bhutia ponies is only 546 (18th Livestock census). Mean height at withers is 126.5 cm. Bay is the most common colour (69%), followed by chestnut (23%), grey and other colours (8%). Average body length and heart girth are 129 and 148.5 cm, respectively.

Genetic characterization

SNP profiling of toll like receptor-1 (TLR-1) gene in Indian native cattle: The comparative sequence analysis of TLR1 region of 11 Indian cattle breeds (*Bos indicus*), viz. Amritmahal, Deoni, Gir, Haryana, Khillar, Kankrej, Kangyam, Rathi, Red Sindhi, Sahiwal and Tharparkar with *Bos taurus* revealed 17 different SNPs. These SNPs were distributed across different regions of TLR1 gene. Seven of the SNPs are non-synonymous (d_N) and 10 were synonymous (d_S). At protein level, change in 17 amino acids was detected, out of which 12 were found novel when compared with the reported SNPs in *Bos taurus* from database.

Mitochondrial D-loop analysis of Indian native and *Bos taurus* cattle: The mitochondrial D-loop based evolutionary relationship among the native cattle (*Bos indicus*) studied in 20 breeds from different agro-climatic regions of India revealed 134 haplotypes with a high fraction of singletons (92, 68.8%). Diversity indices indicated that grey/white, draught breeds from Southern region are with higher mtDNA diversity. The overall pair-wise mismatch distribution with unimodal peak revealed population expansion, except for Red Sindhi and Sahiwal breeds, which showed different demographic history with bimodal peaks and high raggedness value. The distant grouping of *Bos taurus* haplotypes from that of Indian cattle haplotypes in the network analysis, supported earlier concept of distinct dichotomy and independent domestication events for the two cattle species.

Temporal expression of Hsp70 mRNA: The transcriptional responsiveness of buffalo mammary gland tissue to heat stress challenge was studied *in-vitro*. In comparison to control samples, the expression of heat inducible heat shock protein 70 (HSP70) mRNA by real time q-PCR increased after 2h of heat shock and maintained till 8h after heat shock. For normalization of q-PCR expression data, α-actin and GAPDH were used as house keeping genes.

Variation in regulatory and coding region of inducible heat shock protein 70-1 gene: The comparative sequence analysis revealed that coding region of heat shock protein (HSP70-1) gene in Indian native cattle, *Bos taurus* and buffalo was similar in length. A total of 9, 19 and 4 SNPs in 5′UTR, CDS and 3′UTR, respectively, were identified across all the native cattle samples. Comparison of the Indian cattle and *Bos taurus* amino acid sequences revealed three non-synonymous changes at position 5 (Met/Thr), 590 (Asp/Glu) and 626 (Gly/Ala). Buffaloes showed a longer 5′ and 3′UTRs of 204 and 293 nucleotides, respectively, in comparison to Indian native cattle and *Bos taurus*. The variation highlighted in the present study gave the first evidence of unique changes in HSP70-1 gene in Indian cattle and buffaloes that might have some role in regulating the gene expression or protein function in response to thermal stress in different agro-ecological zones in the country.

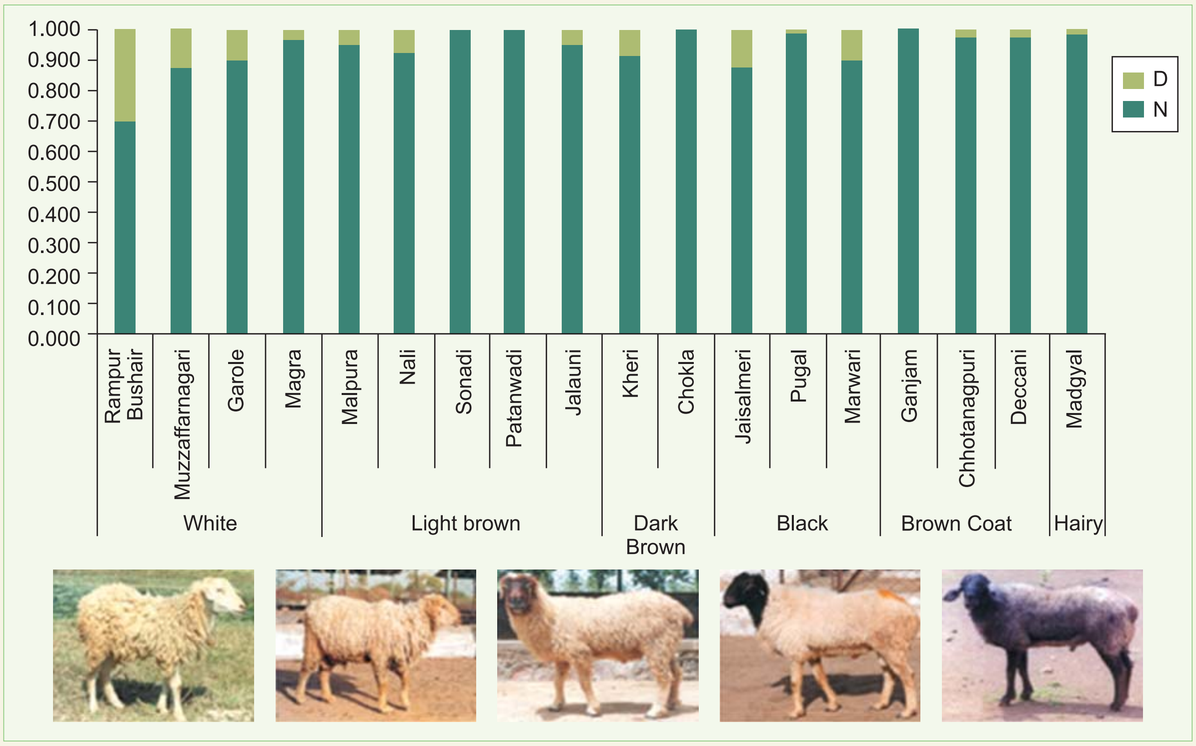
Network Project on Animal Genetic Resources

Genome sequencing: Whole genome sequencing of one female Murrah buffalo (Murrah 5620, NDRI, Karnal) was undertaken using the next generation sequencing platform; 65 GB DNA sequence data were generated providing 21X genome coverage. Short read genome sequence data were submitted to NCBI in SRA database (Accession Numbers SRR032564.1; SRR034148.7; SRR034232.2 and SRR035526.1). A draft assembly of buffalo genome was done with cattle as reference and all the 24 autosomes and X chromosome sequence were assembled based on buffalo radiation hybrid map.

The available commercial bovine SNP chip from Illumina (54k) was evaluated in 8 samples from 6 diverse buffalo breeds (Murrah, Nili-Ravi, Jaffrabadi, Pandharpuri, Toda and Surti). The SNP data analysis revealed 88% positive amplification of bovine SNPs in buffalo while only about 1800 SNPs were informative (polymorphic) in buffalo.

Genetic profiling of indigenous sheep

Genetic polymorphism at a 142 bp segement of Agouti (ASIP) gene was studied using PCR-SSCP in 18 sheep breeds, namely, Rampur Bushair, Muzzaffarnagari, Garole, Magra (white); Malpura, Nali, Sonadi, Patanwari, Jalauni (Light Brown); Kheri, Chokla (dark brown); Jaisalmeri, Pugal, Marwari (black); Ganjam, Chota Nagpuri, Deccani (brown) and Madgyal (hairy) representing colour types. N and D alleles were observed in most of the breeds. All the sheep breeds investigated revealed a predominance of N allele. No common pattern of N and D alleles was observed across the individual breeds and breed groups based on their distribution and face/fleece colour patterns at this locus. These indigenous sheep breeds were also evaluated using microsatellite markers. The analysed data revealed poor resolution of individual breeds in the Northwestern arid and semiarid region but clear separation of the breeds of Eastern and Southern Peninsular region.



Distribution of N and B alleles at ASIP gene locus across indigenous sheep breeds

Polymorphic male fertility: Murrah bulls categorized under sub-groups of high and low field conception rates recorded at farm, were genotyped for seminal plasma protein gene, using orthologous gene sequence based primers. Study revealed that said gene is polymorphic in Murrah bulls and indicated relation with semen quality attributes such as sperm count motility, head size and viability estimated by computer assisted semen analyzer (CASA) and field conception rate of bulls. Direct correlation was elucidated between rapid and progressive motility of sperm (CASA estimates) and per cent conception rate.

Molecular characterization and candidate gene analysis of chicken germplasm: The genetic diversity analysis of 4 layer populations (IWH, IWI, IWK and layer control), 3 broiler populations (PB-1, PB-2 and control broiler) and 2 gene lines (Naked neck and Dwarf) was carried out. The Nei's genetic identity was the highest between IWH and IWI (0.93) and the lowest between IWI and dwarf (0.48) lines. Cluster analysis revealed that all the layer populations grouped together in a single cluster, while the broiler populations and dwarf formed another cluster.

Candidate gene study revealed 8 haplotypes in IGF-1 gene. Haplotype combination showed significant effect on egg production at 52 and 64 weeks of age in White Leghorn chicken lines. The haplogroups also showed significant effect on egg weights at 40, 52 and 64 weeks; Haugh unit at 52 and 64 weeks; albumen index at 52 and 64 weeks, and yolk weight and shell thickness at 64 weeks of age. In Pit-1 gene, 7 haplotypes were found. The haplotypes showed significant effect on egg production up to 64 weeks. Besides, melanocortin receptor 3 (MC3R) responsible for regulation of energy homeostasis and feeding behaviour showed 3 alleles (A, B and C) in IWI and IWK populations, and 2 alleles (A and B) in Naked neck chicken. For another candidate gene, myostatin (negative regulator of muscular growth) 8 haplotypes were determined in

- Cloned and characterized the complete CDs of BMP15, GDF9 and IGBP3 gene of different goat breeds.
- Identified new SNPs in BMP15 gene of Black Bengal goats.
- Cloned and characterized the promoter and exonic regions of myostatin gene in different goat breeds.

PB-1, control broiler and IWI lines. The haplotype combinations showed significant effect on body weights at 4 and 7 weeks of age and growth rates between 2 to 4 and 6 to 7 weeks of age. The mRNA expression at different ages was analysed where the lowest expression was detected during sixth week in PB-1 and control broiler and IWI layer strains.

Molecular characterization of leptin exon 2 and receptor genes: The leptin receptor was expressed primarily in regions of the brain and regulates feeding behavior and energy balance. Genotyping of leptin receptor exon 4 regions was carried out in Barbari goats by PCR-RFLP. Three genes of Y chromosome amelogenin gene (AMELY), sex determining gene (SRY) and Zinc finger gene (ZFY) were analysed by sequencing in Jamunapari, Barbari, Black Bengal goats.

Molecular genetic studies in Indian camel: The available DNA samples of the unrelated Mewari camels and 21 samples collected from the breeding tract were utilized and 23 microsatellite loci were successfully amplified. Ten samples were found polymorphic and rest were monomorphic.

Evaluation and characterization of indigenous pigs: The morphological breed characteristics were established for Ghungroo and Niang Megha strains of indigenous pig. The performance evaluation (both productive and reproductive) of these pigs was also carried out under standard feeding, breeding and managemental conditions. The registration of the local strain of Meghalaya (Niang Mehga) is being processed.

PSE-pork in indigenous pigs: Molecular screening of indigenous pig for porcine stress syndrome (PSS), which is associated with pale-soft-exudative pork (PSE) causing considerable economic losses, revealed that indigenous pigs are free from the deleterious mutation.

Genetic studies on mithun: The first ever C-banded karyotype for mithun metaphase chromosome was constructed. All the acrocentric autosome chromosomes (i.e. from chromosome number 2 to 28) showed darkly stained centromere at the joint chromatid arms. Whereas, in submetacentric chromosome, i.e. chromosome number 1 and sex chromosomes the centromere were lightly stained.

Stem cell research in buffalo

The cells obtained from amniotic membrane, amniotic fluid, umbilical cord matrix and fetalexplant, could be maintained in culture for different intervals. Cells from all sources could maintain normal morphology for different but limited number of passages except cells from fetal explants, which continued in continuous culture maintaining their morphology at 183 days for 45 passages. The problem of slow growth and partial adherence to flask were overcome by freezing in media containing increased concentration of FBS. Satisfactory results of post-thaw survival were obtained with 20% serum, except in amniotic fluid cells. Cells from all sources were characterized as alkaline phosphatase positive. Characterization of cells from amnion, amniotic fluid and fetal explants established their pluripotency by the expression of marker genes at different passages. These cells tended to differentiate after several passages and change their morphology.

Kappa casein gene: A set of bovine primers was used to successfully amplify 874 bp regions of the exon III of mithun kappa casein gene after success with using bovine primer for amplification of 271 bp amplicon from mithun kappa casein gene (CSN3). In the A allele, this 874 bp fragment contains no site for HindIII. But the B allele is cleaved by HindIII into two fragments of 521 bp and 353 bp as separated in ethidium bromide stained 2% gel electrophoresis from 874 bp PCR product.

Genetic characterization of yak: Genetic variation was quantified by calculating observed and effective number of alleles, observed heterozygosity, expected heterozygosity and within group heterozygotes deficiency (F_{IS}). Out of 30 microsatellite markers tested 27 (90%) successfully amplified yak genomic DNA, of which 23 (85%) were polymorphic with allele number ranging from two to seven, and total 69 alleles were detected. The PIC values were high (> 0.5) in most of the primers studied which proves the utility of genetic diversity analysis.

Phylogenetic analysis using SRY gene: Genetic polymorphism and diversity of SRY gene in yak, yak hybrids and hill cattle of Arunachal Pradesh was investigated. The multiple sequence alignment revealed polymorphism at 61, 122 and 197, positions and genetic variability in yak and other related bovines. Phylogenetic results revealed that yak, yak hybrids and hill cattle have the same origin of evolution.

Genetic variations affecting milk production and growth traits of yak: An amplified fragment length polymorphism or AFLP allele (derived after amplifying the EcoRI and TaqI dieted genomic DNA with EAC/TAGA primer combination) was found with higher frequency in the group of yaks with higher adult body. Adult body weight of individuals harbouring the allele was compared separately for male and female with that of individuals lacking the allele. For male the average adult body was 472±15.37 kg (allele present) and 377±12.45 kg (allele absent) and for female it was 318±13.21 kg (allele present) and 243±9.68 kg (allele absent).

Fish

Genetic characterization: Sixteen polymorphic microsatellite were identified from *Pampus chinensis* to be used in *P. argenteus* and *Parastromateus niger*. For microsatellite analysis in *P. argenteus*, PCR amplification was attempted using 14 loci. All the 14 primer pairs tried (100%) got amplified with 2-6 alleles in each locus. All primer pairs amplified only a single locus. To confirm the occurrence of repeats, the cross-amplified polymorphic microsatellite loci were analyzed by sequencing. After sequencing, all the 11 loci were confirmed to contain microsatellite. The allele size ranged from 104–408 bp. These polymorphic loci will be considered for population genetic analysis of *Pampus argenteus*.

***Puntius denisonii*:** To generate the whole mitogenome of the highly sought after ornamental species from the Western Ghats, the complete ATPase 8 and 6 genes of *P. denisonii* were sequenced. The size of the gene was 842 bp.

Physical mapping of 45S and 5S ribosomal genes in *Tor mosal mahanadicus:* Mahseer are commercially important game fishes and high priced food fishes. There is taxonomic ambiguity in nomenclature/ classification of different mahseer in genus *Tor* in India. Observations on ribosomal genes on chromosomes of *Tor mosal mahanadicus* revealed 45S rDNA sites on three pairs of submetacentric and subtelocentric chromosomes. The presence of 5S rDNA clusters on more than one pair of chromosomes in *T. mosal mahanadicus* is an uncommon feature for the genus *Tor* and can be used as a marker for species identification and germplasm conservation .

Habitat fingerprinting techniques of selected fishes: Habitat fingerprinting technique using otolith as a source material was found useful for determining nursery area affiliation, population structure, and movement of individual fish. Since the elemental composition of fish otoliths reflects some of the environmental conditions under which a fish was reared, otolith chemistry can record differences in ambient water conditions specific to habitats used during the life history of a fish. The samples of *Ompok pabda* and *O. bimaculatus* were collected from different sampling sites in river Gomti and river Betwa. The otoliths from these samples were dissected out and elements were analyzed using ICPMS. Analysis of elements, viz. Ba, Sr, Zn, Pb, Cd, Na, K, and Mg both from water and otolith samples, suggested that otolith elemental finger prints are useful for quantifying the relative contributions of different habitat areas to recruitment in adjacent populations.

Differential gene expression profiling in hepatopancreas of WSSV infected *Fenneropenaeus indicus:* The differentially expressed mRNAs in the hepatopancreas of WSSV-infected shrimp, *Fenneropenaeus indicus*, were identified using suppression subtractive hybridization (SSH) to gain an insight into the shrimp genes involved in immune function and pathogenesis of WSSV infection. Approximately 55% of the genes that were upregulated during the virus infection had no significant match to reported genes. The antimicrobial peptides (AMPs) formed the major portion of known genes during the viral infection. Other genes that were expressed during viral infection belonged to signal transporter molecules, antioxidant enzymes, proteinases, cell adhesion molecules, reverse transcriptase and active transporters all of which belong to the non-specific immune system. This is the first report of differential gene expression profiling of WSSV infection in *F. indicus* and has provided new insight into the shrimp immune system.

Functional shrimp genomics: To identify genes responsible for fighting infection in penaeid shrimps *Penaeus monodon* and *Fenneropenaeus indicus*, 1,500 new EST sequences were identified. For identification of immune-related genes responsible for the virus resistance in WSSV infected shrimp, a suppression subtractive hybridization library of *Penaeus monodon* was created. From this library more than 20 genes were identified, which were considered to be immune related. Of these, toll like receptors, crustin (antimicrobial peptide), caspase 3 and antiviral gene (PmAV) were cloned and expressed.

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