

## 5. Genetic Resources

Access to a range of genetic diversity is critical for success of the breeding programme. The efficient and complimentary use of all resources and technological tools will be required to meet the challenges posed by the food demand.

### Crops

#### Germplasm augmentation, conservation and use:

A total of 36 explorations were undertaken in 15 states and 2,713 accessions, including 570 of wild species, were collected. In the National Herbarium of Cultivated Plants, 321 herbarium specimens were added, making specimens' holdings total to 20,560. Germplasm for long-term storage to the National Genebank comprised 4,302 accessions of the orthodox seed-species, 24 cryostored non-orthodox species, and 29 accessions were added to *in-vitro* Genebank. A total of 10,334 accessions were characterized and evaluated.

Germplasm under exchange comprised 42,947 accessions, imported from 42 countries, including international trial material (12,488) and transgenics (536). Promising introductions are: rice blast monogenic lines (EC 694979-5010), rice lines tolerant to heat (EC 695984-7020), tolerant to salinity (EC 699185-257; EC 706195-223), resistant/tolerant to rice blast

and water stress (EC 703152-236), and tolerant to drought (EC 704343-46) from the Philippines; wheat lines tolerant to pre-harvest sprouting (EC 675838-41; EC 692009) from Australia, resistant to rust (EC 693252-324) from the CIMMYT, Mexico, resistant to hessian-fly, stripe rust and *Fusarium* head blight (EC 675845) from the USA; two-rowed barley resistant to Russian wheat aphid (EC 698889-95) from the USA; maize tolerant to downy mildew (EC 707957-21) from Indonesia; safflower cytoplasmic male-sterile lines (EC 675847-8) from the USA; chilli lines resistant to anthracnose disease (EC 695166-75); and tomato lines resistant to tomato yellow leaf curl virus, tomato mosaic virus, bacterial wilt, grey leaf-spot and heat-tolerant (EC 687094-108; EC 692268-77) from the AVRDC, Taiwan, and resistant to bacterial wilt (EC 698844-75) from the USA.

**Germplasm registration:** One hundred-and-sixty proposals, including cereals and pseudo-cereals (48), millets (18), grain-legumes (18), oilseeds (36), fibres and forages (11), vegetables (6), medicinal and aromatic plants (9), ornamentals (10), tubers (3) and agroforestry species (1) have been approved for germplasm registration.

Maize inbred lines (24), including eight of normal

#### High-protein rice from Asom

Two high-protein rice cultivars (crude grain protein content, 15-16.41%), ARC 10063 and ARC 10075, identified from the stock of the Asom Rice Collections of the CRRI Rice Gene Bank have been found to have an additional slow-moving globulin band. Three glutelin bands are highly expressed in the high-protein cultivars. They showed higher activity of nitrate reductase (NR) and glutamic dehydrogenase (GDH) at seedling stage (one-week-old) and maximum tillering stage (three-week-old).



#### Wild sugarcane from West Bengal

Forty-one clones of *Saccharum spontaneum*, *Erianthus rufipilus*, *E. elephantinus* and *E. arundinaceus* were collected from West Bengal. *S. spontaneum* clones were assembled from different habitats and also from many morphotypes, excepting from very tall types. All collections were established in the pots, and were quarantined in the glasshouse. *S. spontaneum* accession IND 101568 collected from the hill slope at 1,270 msl near Kurseong is with thick cane and broad leaves.



*Erianthus rufipilus* near Kurseong

and 16 of speciality corns (quality maize protein – 2, sweet-corn – 8, popcorn – 3, high oil – 3) have been registered. During the year, 11 applications of new hybrids — PMH 4, PMH 5, DHM 119, Rajendra Hybrid Makka 3, HQPM 4, HSC 1, Vivek Maize Hybrid 39, Vivek Maize Hybrid 43, DHM 117, DHM 113 and DHM 111— were filed under the Plant Protection of Variety & Farmers' Right Act.

**Cotton:** Five genetic stocks of cotton with novel and unique traits were identified and registered.

Name	Species and race	Registration no.	Traits
SLL 33	<i>G.hirsutum</i> , race – <i>latifolium</i>	INGR 10060	Single leaf-lobe morphological marker
YPLL 29	<i>G.hirsutum</i> , race – <i>latifolium</i>	INGR 10061	Yellow pigmented leaf-lobe morphological marker
CSLL 59	<i>G.hirsutum</i> , race – <i>latifolium</i>	INGR 10062	Cup-shaped leaf-lobe morphological marker
CISA 2	<i>G.arboreum</i> , race – <i>bengalense</i>	INGR 10057	Sterile mutant morphological marker
CINA 333	<i>G.arboreum</i> , race – <i>bengalense</i>	INGR 10059	Morphological marker for shoot claw of petal

**Castor:** One castor line ICS106 (INGR 10137) was registered as a *Fusarium* wilt and leaf-hopper resistant male line.

**Patents granted:** Two patents, an improved process for the enrichment of *babchi* drug from seeds of *Psoralea corylifolia*, and the other process enabling simultaneous detection of transgene 5-enol pyruvylshikimate-3-phosphate synthase (*cp4epsps*) gene and CaMV 35S promoter in maize utilizing novel primers in multiplex PCR, were granted.

### Fruit crops

Thirty-five accessions of **mango** and 14 of **guava** were collected from various locations of Uttar Pradesh, Bihar and Goa. Three more **litchi** varieties were added to the field gene bank and nine litchi cultivars were obtained from Spain under FAO sponsored project. **Jamun** accessions, CISH-J 42 and CISH-J 37, were registered with the NBPGR, New Delhi. In **banana**, a black stem accession, Karuvazhai (AAA), was collected from Kolli Hills of Tamil Nadu and 41 superior clones of cv. Grand Naine were collected from Theni district based on high yield, earliness and short stature in tissue culture banana plantations.

Field gene bank of **grape** germplasm was added with 32 more accessions. One hundred and twelve accessions were analyzed with 25 microsatellite primers. A total of 418 alleles were detected and matrix among these accessions was estimated and genetic relationship was established. In **pomegranate**, 109 exotic germplasm from the USA and 31 wild germplasm from Uttarakhand

were collected. The DNA fingerprinting of 64 pomegranate germplasm was done in collaboration with the NBPGR. In **temperate fruits**, 1,994 exotic and indigenous germplasm have been collected, conserved and evaluated for growth, yield and quality attributes.

Five genotypes of **walnut** superior in yield and kernel quality, namely CITH-Walnut 6, CITH-Walnut 7, CITH-Walnut 8, CITH-Walnut 9 and CITH-Walnut 10, were identified for release.

### Plantation crops

The CPCRI, Kasaragod, has the largest germplasm accessions: 398 of **coconut**, 164 of **arecanut** and 291 of **cocoa**. A large-fruited coconut accession was collected from Maharashtra. Five new local ecotypes of coconut were collected (Jappanam from Kerala; Devermute, Mavinkuruva, Kalache and Yana from Karnataka) for further studies. The cocoa collections were enriched with 11 exotic clones (resistant to mirids, thrips and canker) acquired from Reading University, UK and six local collections from Kanyakumari district.

In Andhra Pradesh, 36 **palms** have been identified for higher yield. Accessions collected from Little Andaman had high specific leaf weight, a trait indicator of drought tolerance.

In **oil palm**, 14 dura, 6 pisifera and 9 tenera palms were identified in Kerala and two palms (one each of tenera and dura) were identified in Karnataka from oil palm plantation (mutants of BARC) in Bhadra Reserve forest with comparatively less canopy spread. In cashew, out of 527 accessions maintained, 433 were characterized as per IPGRI descriptors for their growth, yield and nut characters.

### Potato and tuber crops

In tuber crops, 4,738 accessions are being maintained in active field gene bank after careful elimination of duplicates. In addition, 34 new accessions were collected from West Bengal, Odisha and Lakshadweep, and added to the existing collection, besides 84 accessions of **elephant-foot yam**, 50 cultivated and 34 wild species received from the NBPGR, Thrissur centre. Of these total accessions, 1,042 were brought under *in-vitro* active gene bank. About 1, 222 accessions mainly of **yams** and **aroids** are maintained in shade net house. About 789 germplasm accessions are maintained in field.

### Spices

About 103 new accessions of **black pepper** from Alapuzha district in Kerala and 73 and 30 new accessions of **cardamom** from Pampadumpara (Kerala) and Mudigere (Karnataka) were added, thus the total collection of **spices** maintained in germplasm repository includes 2,695 of black pepper, 550 of cardamom, 1,026 of turmeric and 590 of ginger.

### Floriculture

As many as 3,130 accessions of 360 species in 124

### Microbial Genome Resource Repository

Presently, Microbial Genomic Resource Repository possesses a total of 1,231 genomic DNA isolates from bacterial, fungal, cyanobacterial and actinomycetes cultures; 64 different cloning, gene-silencing, expression vectors and 92 gene sequences. In addition to this, 188 environmental samples, 6,720 clones from genomic library of *Mesorhizobium ciceri* Ca 181 and different strains of *E. coli* competent cells (DH5a, XL1 Blue, JM107, JM109) and *Agrobacterium* spp. have been preserved.

genera of **orchids** were collected and conserved in repository. NRCO-Coll 77 of red vanda (INGR 10113) and NRCO 42 (No. 09131/IC 574581) have been registered. Molecular variation and fingerprinting of native *Cymbidium* and *Vanda* species were done using ISSR and microsatellite markers for identification and determination of relationships among the species.

### Agriculturally important microorganisms

National explorations and extensive surveys were undertaken in different agro-ecological regions and extreme habitats.

**Culturable halo-alkali-tolerant bacteria from Sambhar lake:** The lake abounds in halo-alkali tolerant bacteria. Based on the 16S rRNA gene sequence analyses, 93 isolates could be categorized into 32 groups; each representing different taxa (*Bacilli*, *Actinobacteria* and *Proteobacteria*) of 3 phyla. Majority of them are found related to phylum Firmicutes bacteria. These are mainly from genus *Bacillus* such as *Halobacillus*, *Thalassobacillus*, *Virgibacillus*, *Sediminibacillus*, *Oceanobacillus*, *Amphibacillus*, *Exiguobacterium* and *Alkalibacterium*. The Sambhar lake samples have a relatively high abundance of Firmicutes. Only two isolates (6.25%) have high G+C, gram-positive bacteria associated with phylum *Actinobacteria* and gram-negative bacteria group contains 13 strains (40.63%) from *Halomonas*, *Marinobacter hydrocarbonoclasticus*, *Nitrincola* sp. and *Alcaligenes* sp.

**Fungi from Kachchh region:** Seventy-seven fungal isolates were separated from the region by different enrichment techniques, and count of colony-forming units (Cfu) ranged from 2 to  $4 \times 10^5$ /g of soil. Of

### Microbial consortium for PCP degradation

Pentachlorophenol (PCP) degraders were isolated from pulp and paper mill effluents contaminating soil. Isolates were grouped into four major lineages of bacterial domain- $\alpha$ ,  $\beta$  and  $\gamma$ -Proteobacteria, Firmicutes. Out of these, 7 isolates degraded PCP at concentrations varying from 100 to 300 mg/litre. Isolates PCP 1 and PCP 42 were found to tolerate PCP at 300 mg/litre, with percentage degradation of 72.91 and 72.07 in 144 hours. PCP 13, PCP 15, PCP 16 and PCP 20 tolerated PCP at 100 mg/litre with percentage degradation of 72.98, 72.90, 69.76 and 72.75.

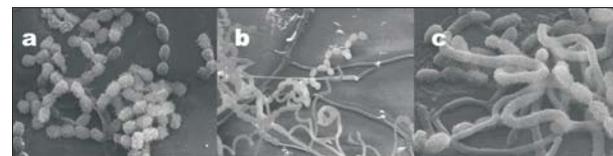
these, 7 isolates were found tolerating 25% NaCl and 22 isolates, 20% NaCl.

**Culturable and unculturable bacteria in brackishwater:** Methylophilic bacteria are ubiquitous, and play an important role in recycling of hazardous compound. Five composite samples of sediments from Chilika lake were subjected to metagenomic DNA extraction. The *mxhF* gene-coding alpha subunit of methanol dehydrogenase was amplified with primer sets *mxhF* f100/r. The microbes belonged to uncultured methylophilic bacteria, *Methylobacterium organophilum*, *Ancyclobacter aquaticus*.

### Release of first whole genome sequence of a bacterium, *Mesorhizobium ciceri*

The estimated genome size of *M. ciceri* Ca181 is 6.47 Mbp, and it has 6,742 predicted genes in 4,116 transcription units. Several of the identified genes are involved in the biological nitrogen fixation (34 genes) and stress tolerance (184 genes). The information can be utilized for the preparation of the genetically modified improved rhizobia strains that may increase productivity and yield in pulses.

**Alkali halophilic actinomycetes:** A total of 465 isolates of actinomycetes were procured from Chilika lagoon; 18 were moderately alkali-halophiles exhibiting growth at 9.0 pH and 1.71 M NaCl (w/v) were identified by scanning electron microscopy and 16SrDNA sequencing. Identification based on the percentage similarity are with accession numbers from JN400094 to JN400111 as *Micromonosporae chinospora*, *Streptomyces albogriseolus*, *S. acrimycini*, *S. albus*, *S. mutabilis*, *S. thermocarboxydus*, *S. bacillaris*, *S. geysiriensis*, *S. achromogenes*, *S. vinaceusdrappus*, *S. fradiae*, *S. macrosporeus*, *S. griseorubens*, *S. labedae*, *S. aureofaciens*, *S. spiralis*, *S. erythrogriseus*, *S. fumigatiscleroticus* at NCBI Genbank.



Scanning Electron Microscopy of actinomycetes isolated from Chilika Lake, (a) S3RS5 (*S. mutabilis*), (b) S6SS2 (*S. erythrogriseus*), (c) S5MS3 (*S. griseorubens*)

**Cytokinin-producing methylobacteria from leaf phyllosphere:** Methylophilic bacteria were isolated from sugarcane, pigeonpea, mustard, potato and radish. Amplification of *mxhF* gene resulted in identification of *Methylobacterium radiotolerans*, *M. mesophilicum*, *M. hispanicum*, *M. organophilum*, *M. suomiense*, *M. oryzae*, *M. salsuginis*, *M. phyllosphaerae* and its several other species. Extract of cell-free culture filtrate of these strains enhanced seed germination of wheat (*Triticum aestivum*); up to a maximum of 98.3% with *M. mesophilicum* (NC4) in comparison to control (85.0%).

## Mushroom

A total of 111 specimens of mushroom were collected and 108 were identified up to genus level. New genera recorded for the first time are: *Humidicutis*, *Leucoagaricus*, *Leucopaxillus*, *Micromphalea*, *Otidea*, *Schizostoma*, *Tulostoma* and *Vascellum*. A Pink Oyster Mushroom Arka OM 1 with short cropping period (20-23 days), synchronus cropping, better shelf-life and rich in antioxidants was identified.

Pure culture of *Lentinus* collected from Andaman and Nicobar was prepared. On the basis of other morphological characteristics and DNA fingerprinting the species was identified as *Lentinus sajor-caju*. This mushroom was successfully cultivated on sawdust at 28-30°C.

## Agriculturally important insects

Scanning electron microscopy studies were focused on chorionic micro-sculpturing in fruit-flies, and external thoracic-scent efferent systems of bugs to explore their utility in species diagnostics and insect classification. Ultrastructural studies were undertaken on the sensilla of whiteflies for deploying these in diagnostics. Development of DNA barcodes in terms of COI sequences was achieved in more than 178 insect species and their biotypes/intraspecific populations, and 278 sequences were submitted to the NCBI GenBank. Stage-specific molecular studies published on four species of *Bactrocera* fruit-flies are of significance in plant quarantine/import and export of fruits and vegetables.

For development of field-diagnostic aids, 919 visuals were documented on the field crop pests, and five inventories were prepared. Two new species of weevils, *Rhamphus* and *Synorchestes*, were discovered.

**Biodiversity of predatory anthocorid insects:** Twenty-five surveys were conducted in Karnataka, and *Orius* spp., *Cardiastethus exiguus*, *Blaptostethus pallescens*, *Anthocoris muraleedharani*, *Carayanocoris indicus* and five unidentified species of anthocorids were collected. It was observed that rearing of *C. exiguus* at 25 and 30°C was most suitable for survival and reproduction. Eggs of *C. exiguus* can be stored for five days at 10°C (with 64% hatching and 64% adult emergence) and for 10 days at 15°C (with 68% hatching and 68% adult emergence).

## Livestock

### Animal genetic resources, characterization and evaluation

**Hill cattle:** The hill cattle of Kumaun and Garhwal region of Uttarakhand are small with compact body and strong legs. Body colours are white, grey, black, red, brown or combinations of any of these colours. The discriminate step-wise cluster analysis based on various morphometric traits revealed that cattle population of Rudraprayag and Chamoli districts are the closest, while cattle from Rudraprayag and Pithoragrah districts are distant apart. The daily milk

yield was significantly higher in Garhwal than that in Kumaun region.

**Berari goat:** Berari goat is found in Vidarbha region. The main breeding tract is located in Nagpur, Akola, and Wardha districts of Maharashtra and Nimar district of Madhya Pradesh. Animals are tall, light to dark tan coat colour. The face is convex with Roman nose. The ears are leafy. Black hair-line along vertebral column extending up to tail is a prominent feature. The average body weight is 40.00 kg and 28.25 kg in male and female, respectively.



A Berari buck weighs around 40 kg and is found in Vidarbha region

**Bundelkhandi goat:** Bundelkhandi goat is found in and around Jhansi district of Uttar Pradesh and Datia taluka of Madhya Pradesh. These goats are large in size, with narrow face and Roman nose. The coat colour is black. The ears are long and pendulous. The average body weight is 39.33 kg and 33.01 kg of male and female respectively.

**Uttarakhand goat:** The goat population was studied from 15 villages of Rudraprayag and Okhimath blocks of Rudraprayag district of Uttarakhand. The breed tract is at altitude ranging from 800 to 5,000 m above sea-level. Animals are having medium sized body with white, black, brown and mottled coat colour. The average body weight is 42.14 and 40.13 kg of male and female respectively.

**Patanwadi sheep:** Patanwadi sheep is reared for mutton, milk and wool. It is found in Kachchh,



Patanwadi sheep is reared for mutton, milk and wool



Ghumsuri cattle



Khariar cattle



Motu cattle

Surendernagar, Rajkot and Amreli districts of Gujarat. The animals are large in size having compact body. The face and neck is dark brown while rest of the body is white. The face is prominent with Roman nose. Ears are medium to large and tubular with a hairy tuft. Both sexes are polled. Tail is thin and short. The ewes have well developed udder. Average body weight of rams and ewes is  $46.1 \pm 1.28$  and  $34.7 \pm 0.26$  kg respectively.

**Spiti donkey:** The main breeding tract in Spiti taluka of Lahaul and Spiti district and Puh taluka of Kinnaur district is at an average altitude of 3,700 m above sea level. The total donkey population in Lahaul and Spiti district was 2,007 and in Kinnaur district was 2,361 (2007 livestock census). The animals are small in size, with compact body. The back and legs have thick coat of long hair. The prominent coat colour is black, brown, tan and white. The face is covered by long hair. The tail extends up to the hocks.

### Registration of new breeds

Four new breeds of cattle, namely Binjharपुरी, Ghumsuri, Khariar, Motu from Odisha; and two buffalo breeds, namely Banni from Gujarat and Chilika from Odisha, were registered.

### Candidate gene analysis

**Buffaloes:** Complete open reading frames (ORFs) of ten buffalo toll-like receptor (TLR) genes were amplified into overlapping fragments and sequenced using cattle primers. Buffaloes have same ORF in all the TLRs like cattle except the TLR6. Buffalo TLR6 showed insertion of 3 nt (TTA) at 545 nucleotide position adding amino acid tyrosine at position 182. The buffalo TLR2, TLR5, TLR6 and TLR10 showed maximum identity with cattle, followed by that of goat, sheep and pig. But buffalo TLR3 showed the maximum identity with sheep, followed by cattle and pig.

**Sheep:** Studies on the expression of *BMP4*, *BMP7* and *BMPRIA* genes in *FecB* carrier and non-carrier ewes revealed similar expression of *BMP7* across different genotypes. However, the expression levels of *BMP4* and *BMPRIA* were significantly higher in ewes having high fecundity than that of low-fecundity ewes. *BMP4* mRNA abundance in ovaries of high-fecundity ewes suggested their role in regulation of ovulation rate.

**Cattle:** Amplified fragments of *CXCR1* (311 bp),

*CRBR<sub>1</sub>* (316 bp) and *CRBR<sub>2</sub>* (382 bp) genes were polymorphic in both mastitis affected as well as tolerant groups of crossbred cows. However, no significant association was found between different genotypes and mastitis, PCR-RFLP studies of 482 bp amplicon encompassing exon 3 and 4 of *CatSper1* gene in Vrindavani and Tharparkar cattle revealed monomorphism. Sequence analysis revealed high nucleotide homology, indicating the conserved nature of this gene.

**Chicken:** A total of 16 haplotypes for *GnRH1* gene were found in layer chicken populations; H1H13 haplotype had the highest egg production (191.1); while H1H5 haplotype showed the lowest egg production (176.2) up to 64 weeks age. In *Pit-1* gene, 10 haplotypes and 11 haplotype combinations were found. The pit 1 expression at mRNA level was similar in PB-1 and control broiler populations with differences from IWI population during juvenile age up to seventh week.

### Molecular genotyping

**Buffaloes:** Buffaloes from Dibrugarh of upper Asom region, Mizoram and Nagaland were genotyped using microsatellite markers and compared with swamp and other river buffaloes. The Nagaland buffaloes were placed in a distinct cluster. Assamese buffaloes were clustered in between riverine and true swamp types. Multi-dimensional scaling showed swamp buffaloes from different parts of north-eastern region together, while Asomese and Riverine buffaloes were clustered separately.

**Camels:** The Mewari breed showed polymorphism only at 21 loci out of 41 microsatellite loci screened. The number of alleles ranged from two to five. The observed and expected heterozygosity ranged from 0.14 to 0.83 and 0.264 to 0.720 respectively. The polymorphic information content (PIC) ranged from 0.244 to 0.649. Bikaneri, Jaisalmeri, Kachchhi and Mewari breeds showed heterozygote deficiency at several loci.

**Horses and ponies:** Genetic characterization studies of six indigenous equine breeds revealed more genetic diversity within breeds. The thoroughbred horses clustered separately in phylogenetic tree. The indigenous breeds clustered into two distinctive classes. One cluster grouped Kathiawari and Marwari horses, while the other cluster had Manipuri, Spiti, Zanskari and Bhutia ponies.

**Chicken:** The genetic diversity analysis of various

chicken populations was carried out using 16 microsatellite markers. All the microsatellites except MCW048 were polymorphic. The average number of alleles varied from 3 to 3.8. The genetic distance within broiler and layer lines was relatively low, while between broiler and layer was high. The native populations were found to be closer to broiler than layers.

**Buffalo genome sequence:** The first version of draft assembly of buffalo genome sequence was constructed using the cattle genome (Btau 4.0 assembly) as a reference. The buffalo assembly represents 91–95% coverage in comparison to the cattle. The assembly has 185,150 contigs with the median contig length of 2.3 Kb and the largest contig length of 663 Kb. The mitochondrial genome is fully covered by a single contig. Buffalo assembly and of cattle genome revealed 52 million mismatches/indels. The present analysis also unveils about 300 structural variants in the buffalo genome. The buffalo assembly has been integrated into a publicly available genome browser (<http://210.212.93.84/cgi-bin/gb2/gbrowse/bovine/>).

**Conservation of animal genetic resources**

**National Animal Gene Bank:** The National Gene Bank at NBAGR now stores about 84,200 frozen semen doses from 26 breeds of cattle, buffalo, goat, sheep, camel, horse and yak.

**Killakarsal sheep breed:** The Killakarsal sheep breed has been conserved in Tirunelveli district of Tamil Nadu, by establishing a nucleus flock under TANUVAS, Chennai. The elite rams are produced and distributed to farmers. The numbers of progenies of Killakarsal have been produced in farmers flocks.

**Krishnavelley cattle breed:** The Krishnavelley cattle has been conserved in Maharashtra and parts of

Karnataka. Frozen semen doses were produced at BAIF, Pune and inseminations are being done in farmers' herd and calves have been produced.

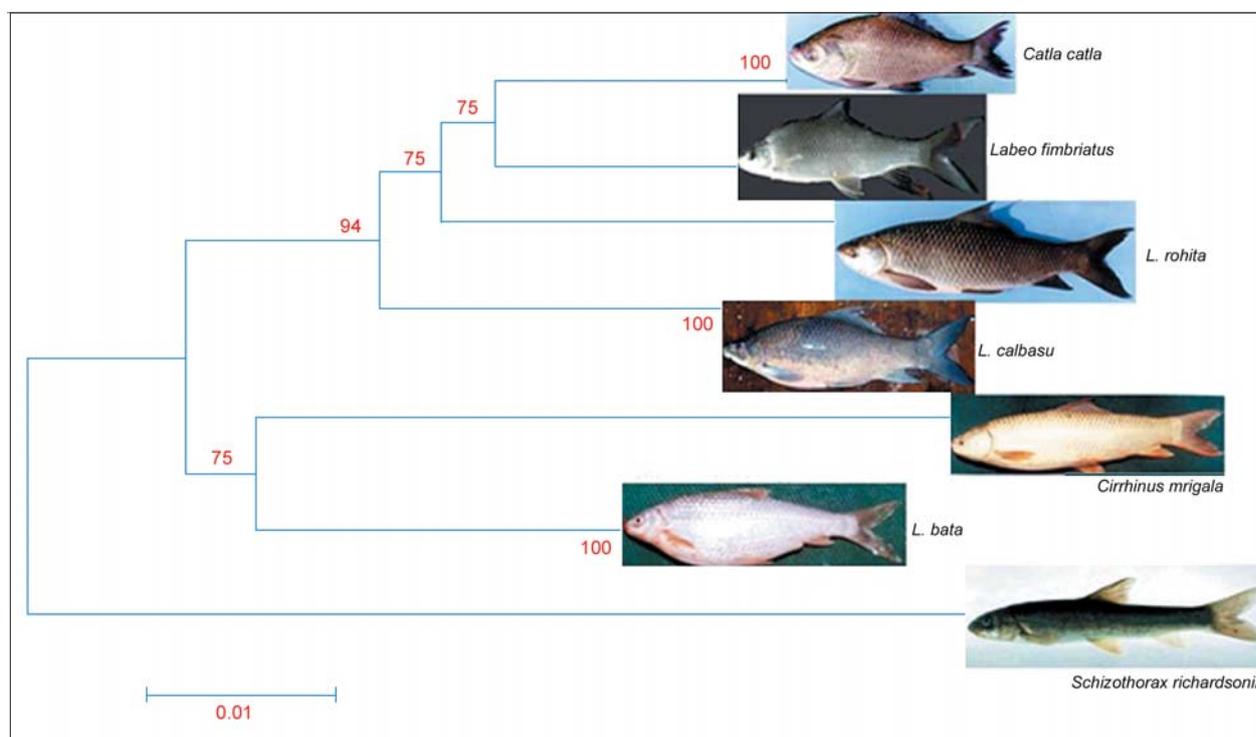
**Rumen microbial diversity:** A total of 120 clones of archaea 16sRDNA clone library were sequenced and data were subjected to phylogenetic analysis to assess diversity of archaea in the rumen of buffaloes. *Methanobacterium* genus was predominant in the rumen. Copy number of fibre degrading bacteria like *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* were significantly higher in buffaloes fed high fibre diet (75% straw + 20% concentrate + 5% green) vs. high concentrate diet (60% concentrate + 40% green). *Ruminococcus flavefaciens* were not detectable in the group fed high concentrate diet.

**Buffalo fetal fibroblasts:** Primary culture of buffalo fetal fibroblasts showed expression of alkaline phosphatase and pluripotency genes, viz. *OCT-4*, *NANOG* and *SOX-2*. Further, changes in relative expression of transcriptional factors were determined and found up-regulation of all the three genes up to P15, followed by up-regulation of *SOX-2* up to P45 but down-regulation of *NANOG*. Upon induced-differentiation, these cells showed properties of adipogenic and osteogenic cells.

**Fish**

**Phylogenetic relationships of cultured Indian carps:** Partial sequence of *Cytochrome C Oxidase I (COI)* gene was used to elucidate taxonomy and phylogenetic relationships among *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *L. calbasu*, *L. fimbriatus* and *L. bata* sampled from culture conditions.

One of the potential applications of this study will



Neighbour Joining (NJ) consensus trees of Indian carps inferred from mtDNA COI sequencing. *S. richardsonii* was used as out group

be for accurate identification of seeds of carp species using DNA barcoding. Further, the data would help to identify and differentiate taxonomic units and deduce genetic relationships between the carp species, thereby providing valuable information for breeding, conservation, systematic, ecological and evolutionary studies.

**Genetic variability in *Penaeus (Fenneropenaeus) indicus*:** Polymorphic microsatellites (81) in Indian white shrimp, *Penaeus (Fenneropenaeus) indicus*, were developed through cross-amplification from other penaeids. The putative microsatellites developed were cloned, sequenced and confirmed to contain repeats. The suitability of the identified polymorphic loci for stock identification was evaluated on a heterogeneous collection of 23 *F. indicus* samples from India and Oman. The number of alleles per locus ranged from 5 to 14, and the observed heterozygosities were from 0.237 to 0.889. The probability test did not detect any significant deviation in allele frequencies from that expected under Hardy–Weinberg equilibrium. None of the loci showed significant linkage disequilibrium and occurrence of null alleles. The polymorphic microsatellite DNA markers developed in this study will be useful for commercial shrimp breeding and selection programmes and genetic studies of both wild and cultured stocks of *F. indicus*.

**Mitochondrial genome:** Complete mitochondrial DNA of *Pangasius pangasius* and *Clarias batrachus* was sequenced. In *P. pangasius* and *C. batrachus*, whole mitochondrial DNA was of 16,476 and 16,571 bp respectively. The base compositions in both the species were as follows: *P. pangasius* T, 25.1%; C, 28.7%; A, 30.5%; G, 15.7%; *C. batrachus* T, 25.0%; C, 27.4%; A, 32.3%; G, 15.4. On the basis of whole mitochondrial sequence, the mean genetic distance, between the two catfish species was recorded at 0.1755.

**Transcriptome sequence assembly of rohu:** Transcriptome sequencing of rohu, *Labeo rohita*, was performed on tissue samples collected from liver, muscle, kidney, spleen, gill, brain and fin tissues of 10 resistant and 10 susceptible lines of fish. Resistant and susceptible families of fish were selected after intra-peritoneal challenge of 87 full sibling families generated during the selective breeding programme to a virulent strain of *Aeromonas hydrophila*. The 15 highest ranked families were selected as the first generation of the resistant line, whereas 10 lower ranked families were selected to form the susceptible line. Over 30 million, 54 base pair sequence reads were produced yielding around 2 billion bases of high quality sequence data. Most homology (>90%) was with genes in the zebra fish (*Danio rerio*) genome. First-pass

filtering of base cell differences identified 57,297 putative SNPs. Contigs 110428 and 111876, showed strong homology to the MH class I antigen and one of the largest allele frequency differences between resistant and susceptible fish. Of the 100 most highly differentially expressed transcripts in the resistant line animals, 6 showed homology to putative heat shock protein genes (*HSP 30, 90* and *70*), 7 to putative zona pellucida glycoprotein, 1 to serum lectin isoform 1 precursor and 3 to putative major histocompatibility (MH) class I antigen or heavy chain genes. Further validation with real time PCR quantification of the contigs showing differential expression confirms higher fold difference of major histocompatibility class I antigen in intestine, gill, brain and in skin. The results may interpret the association of MHC class I molecule in resistance to *Aeromonas hydrophila* in rohu. The present work is the pioneer in providing resources for better understanding of polymorphisms and immune-related genes in resistance to *A. hydrophila* infection in rohu.

**Microsatellite markers in *Schizothorax richardsonii*:** A partial genomic library was constructed using 300–600bp RE digested insert of Indian snow trout (*Schizothorax richardsonii*). RE digested product was cloned in dephosphorylated pUC19 vector. Around 5,000 positive clones were achieved in the form of white colonies, which were screened by colony hybridization using (CA)<sub>n</sub>/(GT)<sub>n</sub>, (GA)<sub>n</sub>/(CT)<sub>n</sub>, (GAA)<sub>n</sub>/(CTT)<sub>n</sub> and (CCA)<sub>n</sub>/(GGT)<sub>n</sub> probes. Out of 5,000 colonies, 450 colonies were detected as having possibly some repeat motifs. Plasmid DNA was isolated from those selected clones and sequenced. Out of 450 sequences, 51 sequences consisted of mono, di, tri and tetranucleotide repeats. GT/CA repeats were most abundant in comparison to tri- and tetra-nucleotide repeats. All the sequences containing microsatellite markers were submitted to the NCBI GenBank having following accession numbers (ACC# HM 591233 to HM 591283). Of the 51 sequences, 57 markers were developed from partial genomic library and, from the 57 markers, 34 markers were validated in two different populations of *S. richardsonii* and found to be highly polymorphic using capillary electrophoresis.

**Bioprospecting of gene and allele mining for cold tolerance:** Partial cDNA of glycerol-3-phosphate dehydrogenase (GPDH) was isolated and characterized in Indian snow trout, *Schizothorax richardsonii*. The nucleotide and deduced amino acids sequences was 92% similar with *Danio* GPDH in GenBank databases. The GPDH gene has shown its possible role in cold acclimation process during winter by accumulation of glycerol in cold aquatic bodies.