

5. Genetic Resources

Crops

Plant genetic resources of cultivated, naturalized and wild crop species and genera are collected from the domestic locations as well as from the international sources. Their cataloguing, characterization and conservation are undertaken for crop improvement activities. Similar approach is taken up for agriculturally important microbial and insect species also.

Germplasm augmentation, conservation and use:

In 20 states, 40 explorations were undertaken and 2,676 accessions were collected; of which 371 accessions were of wild species. In the National Herbarium of Cultivated Plants, 350 herbarium specimens were added. In the National Genebank, 6,550 germplasm accessions of the orthodox seed species were added for the long-term storage; 26 of non-orthodox species were cryo-stored and 28 were added in *in-vitro* culture. A total of 48,422 accessions were characterized and evaluated, and 6,271 accessions were supplied for research and crop improvement within the country.

Germplasm exchange comprised 41,210 accessions imported from 50 countries, including international trial material (35,150). Promising introductions were of rice: for thermogenic male sterility (EC 720903-4), tolerance to drought (EC 725224-5), tolerance to salinity and low-input lines (EC 733948-54), tolerance to heat (EC 733828-46), tolerance to salinity and resistance to brown planthopper and bacterial blight (EC 750230-57) from the IRRI, Philippines; high-yielding hybrid (EC 720905) from Indonesia; and drought-tolerant lines (EC 726475-54) from the USA; of wheat: nullisomic/tetrasomic/monosomic Chinese spring lines (EC 731579-636); cultivar Anton with low level of polyphenol oxidase (PPO) (EC 732856) and substitution/deletion/aneuploid lines (EC 736143-62) from the USA; and Vijay (BL 3063) (EC 721736) resistant to Ug 99 and terminal heat tolerant for normal/late sown conditions from Nepal; of mungbean: resistant to Mungbean Yellow Mosaic Virus (EC 718740-5) from the AVRDC, Taiwan; of tomato: high beta-carotene content lines (EC 721238-41) from Taiwan; of cucumber: recombinant lines (EC 738814-9038) from the Netherlands; of colocasia: leaf-blight tolerant lines (EC 719534-48) from Fiji; and of castor: low ricin-content line (EC 736481) from the USA.

One-hundred wild *Arachis* accessions belonging to six different sections—*Arachis* (54), *Caulorhizae* (1), *Erectoides* (7), *Heteranthae* (7), *Procumbentes* (6) and *Rhizomatosae* (40) were maintained in the field gene bank, and 9,024 accessions were conserved in the medium-term cold storage module.

Germplasm registration: Seventy-one novel germplasm of cereals (11), grain-legumes (7), oilseeds (10), fibres and forages (7), vegetables (3), tubers (2),

beverages (26), commercial crops (3) and agroforestry (2) were registered.

Rice germplasm accessions (124) collected from different districts of Odisha and West Bengal included 59 from drought-prone areas, 62 from saline areas, and three of *Oryza coarctata* Tateoka.

Eleven wheat genetic stocks, including one of durum (DDW 12) with novel traits, were registered with the NBPGR, New Delhi. These potential donor lines are included in the National Genetic Stock Nursery (NGSN) from time-to-time for use in wheat improvement and/or for genetic studies at the research centres.

Two maize hybrids registered are DMH1 (Matungha) and NAH 2049, and five composites are Aravali Makka 1, JM 8, Jawahar Makka 216, Jawahar Composite Makka 12 and Pratap Makka 5. Three hybrids and two varieties of pearl millet were registered in the PPV&FRA.

Wheat genetic stocks registered during 2011-12

Name	Developed by	Traits
DDW 12 (durum)	DWR, Karnal	High iron, zinc and manganese content; resistance to rusts and Karnal bunt
DBW 46	DWR, Karnal	Resistance to yellow and brown rusts and leaf blight
DBW 51	DWR, Karnal	High iron content, protein content; good <i>chapati</i> -making and with good biscuit spread factor; resistance to rusts and leaf blight
HTW 6, HTW 11	DWR, Karnal	Terminal heat tolerance
DWRL1	DWR, Karnal	Triple dwarf, resistance to rust (<i>Lr19</i>), high protein content
KBRL 76-3	PAU, Ludhiana	Karnal bunt resistance
WCF 12-7, WCF 12-19, WCF 12-61, WCF 12-208	IARI, New Delhi	Drought tolerance

A total of 300 accessions of six fodder species (cultivated/rangeland) were procured from the international agencies, USDA, ICARDA, ICRISAT, ILRI and national agencies, NBPGR and IIVR. Germplasm catalogue on 367 accessions (219 landraces and 148 exotic accessions) of fodder sorghum was published following DUS guidelines. A rare sexual obligate plant of *Cenchrus ciliaris* (IGFRI-CcSx-08/1) has been

registered as a novel genetic stock (INGR 11062) with the NBPGR.

Four novel genetic stocks of rapeseed-mustard, *moricaudia*-based CMS line of *karan rai* (*Brassica carinata*) and MCA 1 (IC 0589777, IC 059009D3, INGR 11049), and three of Indian mustard (*Brassica juncea*) germplasm, BPR-349-9 for thermo-tolerance at the juvenile stage (IC 0589778; Regd. No. INGR 11048), DRMR WFM 1 (IC 059926; INGR12007) for white petals and RH 0116 (IC 0584669; INGR 11033) for tolerance to salinity (10ds/m) at juvenile stage were registered with the NBPGR, New Delhi. A yellow stem mutant of castor (INGR 11059) and a breeding line 96-508-2-90 (INGR 11060) with resistance to wilt, tolerance to aphid and moisture stress were also registered.



MCA 1—Flower with rudimentary anthers



White-flowered mustard

Four unique germplasm of urdbean, a mutant with protruded stigma IPU 99-167m (IC 0594172; INGR 12005), with sympodial bearing SPS 5 (IC 0594173 Regd. No.; INGR 12006) and two MYMV resistant, VBG09-012 and VBG04-008, and two of mungbean, extra early germplasm IPM 409-4 (IC0589310) and IPM 205-7 are registered at the NBPGR.



(A) Sympodial bearing urdbean; (B) Urdbean mutant with protruded stigma; (C) Extra early genotype of mungbean

Drought-tolerant culture of cotton CNH 30I (Regd No. INGR 11061; IC 0587405) is registered at the NBPGR. Two of the canes, suitable as feed stock in cogeneration and alcohol distillation industries, were identified and are registered with the NBPGR.

Agriculturally important micro-organisms

From salt-affected areas of eastern Uttar Pradesh, 55

New sugarcane germplasm from north-east India

An exploration was conducted in Nagaland and in Manipur for sugarcane germplasm. Tall, medium-tall, short- and medium-short forms of *Saccharum spontaneum*, *Erianthus bengalensis*, *E. longisetosus*, *E. procerus*, *Sclerostachya* and *Phragmites* were found in Nagaland.

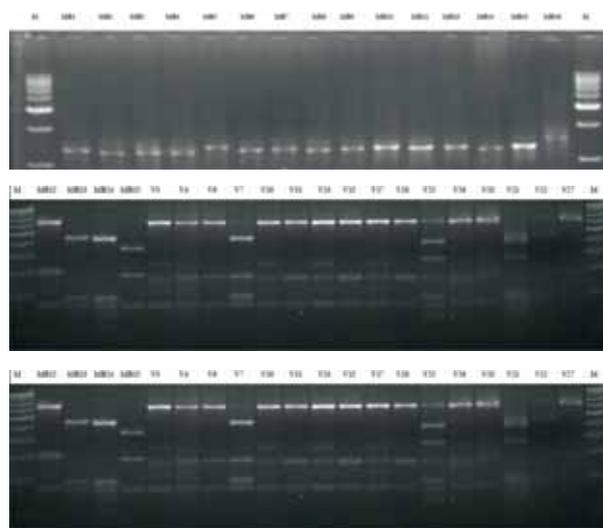
From Manipur, 67 germplasm were collected, including different forms of *S. spontaneum*, *S. officinarum* and *E. rufipilus*.



Saccharum spontaneum on a river bank in Nagaland

isolates of *Bacillus* and 72 of predominant genera, which grew on more than 4 % NaCl concentration, were selected for diversity analysis. The molecular characterization on the basis of 16S and 16-23S rDNA PCR-RFLP analysis with three restriction endonucleases *Alu I*, *Hae III* and *Taq I* revealed greater diversity among the isolates.

Based on the 16S rDNA sequencing, *Bacillus* isolates identified were: *B. megaterium*, *B. subtilis*, *B. licheniformis*, *B. horikoshi*, *B. pumilus*, *Bacillus* sp., *B. cereus*, *B. simplex*, *B. flexus*, *B. arsenicus*, *B. thuriangiensis*, *Bacillus firmus*, *Lysinibacillus*, *B. marisflavi* and of predominant genera were: *B. licheniformis*, *B. niabensis*, *b. aryabhatai*, *B. subtilis*, *B. thioparans*, *B. flexusm*, *B. marisflavi*, *B. endophyticus*,



Amplified 16-23S rDNA restriction analysis of 55 *Bacillus* isolates of eastern Uttar Pradesh with *AluI*, *Hae III*, *Taq I*

B. cereus, *B. pumilus*, *Lysinibacillus xylanilyticus*, *Pseudomonas stutzeri*, *Staphylococcus*, *Enterobacter cloacae*, *Micrococcus* sp., *Cellulosimicrobium funki*, *Ochrobacterium* sp., *Acinetobacter* sp. etc; 16S rRNA gene sequences of *Bacillus* were submitted to the NCBI and accession numbers assigned to them were from JN215486 to JN215522.

In Nimar region of Madhya Pradesh, occurrence of zinc-solubilizing *Bacillus cereus* isolates in soybean rhizosphere is a first report; four isolates solubilized three zinc compounds.

Agriculturally important insects and mites

New accessions: *Sticholotis magnostriata* Poorani (Coccinellidae) from Asom and *Microterys chaetococci* Hayat & Poorani (Encyrtidae) from Karnataka have been described. A distinctive species, akin to the Palaearctic region of the world, *Trichogramma bistratae*, was found to occur in Odisha.

Two new species of Platygasteridae, *Odontocolus markadicus* Veenakumari and *Odontoscelio vikata* Veenakumari and Rajmohana, have been described from southern India. A checklist of Indian Microgastrinae was prepared with 21 genera and 231 species with information on distribution and biology. A total of 42 species of pseudococcids were identified, of which *Phenacoccus Cockerell* was predominant. Ten anthocorids including a new species of *Xylocoris* (*Proxylocoris*) sp. from maize and a new species of *Orius* sp. have been recorded. *Physopleurella armata* collected from coconut is a new record from India. Twenty-nine species of phytophagous mites and 19 species of predatory mites were collected from vegetable, fruit, cereal and ornamental crops from different locations in Punjab. Twenty new mites were

recorded from fruit and vegetable crops of Himachal Pradesh. The new phytophagous mites are being studied for their possibility of becoming pests. The predatory mite fauna are characterized for their utility in biological control.

Horticulture

Fruit crops: Of the total 538 mango accessions, 55 were morphologically characterized as per the International Descriptors. Evaluation of these accessions indicated that mango variety, Safed Mulgoa, had maximum fruit weight (1,813 g), while Ramphalya and Ruswani recorded maximum TSS of 24.40° and 24.38° Brix respectively. Maximum pulp content of 88.95% was observed in Nymath. The chloroplast DNA (cpDNA) isolation protocol was standardized (100-150 ng/ml from 100 g of leaf). Eight cultivars were characterized for *trnL* and *trnF* genes (transfer RNA) and variations were ascribed to indels and SNPs. Sequence of mango hybrid, Arunika, revealed maximum similarity with the pollen parent, Vanraj, for *trnF* gene. Further, phylogenetic analysis of five chloroplast gene sequences revealed evolutionary pattern and varietal divergence.

Attempts were made to map the physical positions of certain chloroplast genes, viz. *trnL*, *trnF*, *petB-petD* and *atp-rbcL*, using partial chloroplast genome in mango for sequence alignment and then positioning of genes. The partial chloroplast genome consisted of a partial Large Single Copy (LSC) region, complete Inverted Repeats (IR) regions and Small Single Copy (SSC) regions. This mapping study is an effort towards understanding the molecular phylogeny using cpDNA markers and would be a valuable tool for developing sequence-based markers for cultivar identification.



New species of natural enemies (Left to right: top left - *Sticholotis magnostriata*, middle - *Microterys chaetococci*, right - *Xylocoris* (*Proxylocoris*) sp.; bottom left - *Odontocolus markadicus*, middle - *Odontoscelio vikata*, right - *Orius* sp.)

Citrus germplasm consisting of 614 accessions, including 23 rootstocks from exotic sources, 552 from indigenous sources and 39 scion cultivars was maintained. Twenty exotic accessions received from Brazil are being screened/tested for presence of pathogen. Banana accessions, Aittakola, Mizo Cavendish, Sabri, Behula (cooking type) and *M. acuminata* collected from Tripura, promising Cavendish clone with short duration from the secondary source in Odisha and *M. acuminata* ssp. *burmannica* from Anamalai Hills, Tamil Nadu and 88 exotic accessions from ITC, Belgium, were added to the field genebank. DNA fingerprinting profile was developed for 14 major commercial varieties. Further, expression profiling of Resistant Gene Analogus (RGAs) in nematode (*Pratylenchus coffeae*) infected samples indicated that C1, C5 and C6 transcripts might be related to root lesion nematode resistance in Karthobiumtham. Isolation and sequencing of full length genes of ATP synthase showed 98% homology to *M. acuminata* ATP synthase beta sub-unit and 97% homology to Maize and Rice ATP synthase. Seven new accessions were added to grape collection, totalling to 464. Microsatellite data of 317 accessions were analysed and several misnomers and duplicates were identified. Rootstock, B2/56, was identified as a clone of 110R. Cheema Sahebi was grouped to be a selection of Spin Sahebi and not Pandhari Sahebi as reported earlier.



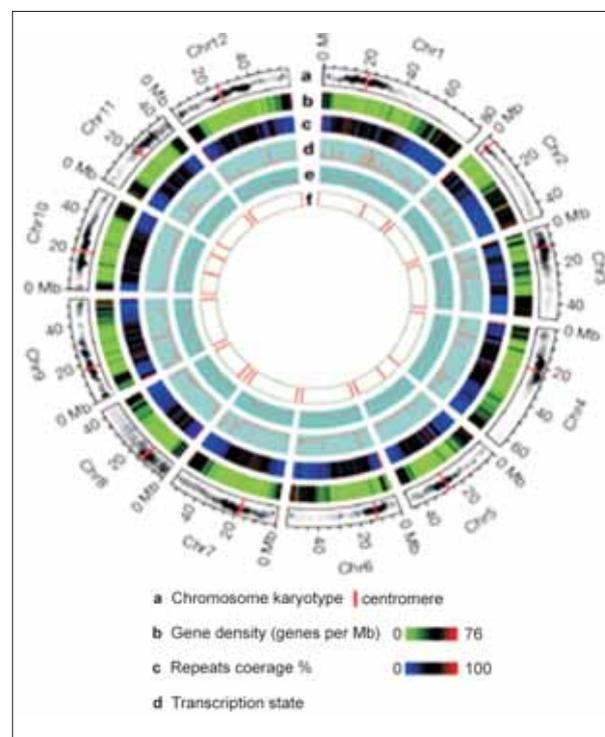
Strawberry accessions collected from Sub-Himalayan region are promising

Fifteen pomegranate accessions were collected from Maharashtra and Odisha. The exotic accessions introduced from University of California, USDA, showed 86.0% survivability after one-and-a-half year of planting at Bhowali, where they are being maintained. Similarly, five litchi cultivars, collected from Spain have been established. Fifty-four clones of litchi from Vaishali and other districts were characterized and five were identified as promising. Among 11 longan germplasm evaluated, accession, Lgc-6, excelled in fruit yield (10.5 kg/plant). Two accessions of strawberry collected from Sub-Himalayan region (Rohtang Pass-Manali: altitude 3,978 m) were multiplied for further utilization.

Of the 28 ber varieties assessed, highest polyphenols were recorded in cv. Mehrun (140.93 mg/100 g), followed by ZG-3 (122.27 mg/100 g), and highest content of flavonoids and flavonols were observed in cv. ZG 3 (173 mg/100 g) and Aliganj (70.59 mg/100 g), respectively. The total antioxidant activity was the highest in cv. ZG 3

(6.3 MTE/100 g), followed by Thar Sevika (5.3 MTE/100 g), and Gola and Illaichi (5.2 MTE/100 g). The kachri lines were screened for incidence of diseases and fruit fly infestation. Based on maturity, fruit size, shape, yield potential and overall performance, kachri lines, AHK 119, AHK 200, AHK 411, AHK 564 and AHK 572, were promising.

Vegetable crops: Six potato hybrids, J/2-14, MS/6-819 and MS/6-1947, PS/5-73, PS/5-75 and PS/6-88, for table purpose and 2 (MP/04-578, French fry and MP/04-816, chips) for processing were introduced in AICRP for multilocation testing. The accessions, J 93-98, MS/82-638 and MS/82-398, were identified for efficient nitrogen uptake than Kufri Gaurav based on nitrogen requirement for getting equivalent yield. Complex genome of potato (*Solanum phureja*) was deciphered by a consortium of 26 international institutes including CPRI, Shimla. A hybrid approach including Sanger and next generation sequencing were adapted for sequencing of 727 Mb constituting 86% of the complete potato genome.



Potato genome for future

Palms and nuts: The field gene bank at Kidu in Karnataka has the largest number of accessions of coconut (269 indigenous and 132 exotic), arecanut (141 indigenous and 23 exotic) and cocoa (36 indigenous and 255 exotic). Four coconut ecotypes, viz. Puvar, Edawa Oblong, Edawa Round and Kappil, with good quality fibre, were identified from Thiruvananthapuram district of Kerala. Horned coconuts were collected for conservation from Andamans to study the segregation pattern and evolution in coconut. Cluster analysis based on palm and fruit characters showed Devermute and Mavinkuruva coconut ecotypes (from Honavar, Karnataka) as distinct populations and quite different from West Coast Tall (WCT) populations. Coconut



Horned coconuts in National Gene Bank

accessions, viz. IND085S, IND001S, IND013S, IND086S, IND026S, IND027S, IND038S, IND054S, IND01S, IND015S and IND071S, were identified for high copra yield. Among arecanut accessions, VTL 28I, VTL 29I and VTL 12, exhibited high yield potential of 3.86, 3.98 and 3.70 kg dry kernel/palm/year, respectively. Evaluation of cocoa collections showed high yield potential in VTLC 65 (128.8 pods/tree/year) and VTLC 85 (102.7 pods/tree/year).

Forty-five new accessions of cashew germplasm characterized using RAPD, ISSR and SSR markers revealed less diversity. Further, 19 germplasm accessions were characterized as per the IPGRI (Bioversity International) Descriptors, bringing the total number of accessions evaluated to 452, out of 527 maintained in field. The evaluation indicated highest cumulative nut yield in BLA 39/4 (66.48 kg/tree) at Bapatla (Andhra Pradesh). More than 60 out of 94 accessions yielded bold nuts (7.0–16 g) with high shelling capacity (28–36%) at Bhubaneswar (Odisha), while HC 6 showed dwarfing feature at Vriddhachalam (Tamil Nadu).

Ornamental crops: One hundred and seventy-two orchid accessions were collected, especially from north-eastern Himalayan regions. The characterization of collected orchid germplasm revealed two novel variants of *Pleione humilis*, and alba varieties of *Coelogyne nitida*, *Coelogyne punctulata* and *Dendrobium amoeneum* which were not reported so far. The explorations resulted in addition of four new records, *Calanthe manni* Hook f., *Calanthe yucksomnensis* Luckson, *Calanthe pachystylis* and *Cymbidium cyperifolium* Lindl. The two significant observations, are *ex situ* germination in *Calanthe yucksomnensis* and vivipary in *Coelogyne nitida*. The efforts are to isolate mycorrhizal fungi and use them for improvement in seed germination and hardening of tissue cultured plants. True vivipary, an alternative reproductive strategy, was also noticed which had so far not been observed in members of family Orchidaceae.

Medicinal crops: Explorations undertaken in different parts of the country revealed that of the total 121 accessions, 43 were of *Desmodium gangeticum*, 44 of *Asparagus adscendens*, and 34 of *Asparagus recemosus* and *Gymnema sylvestre*. In *Gymnema sylvestre*, two high leaf-yielding lines, DGS 22 and DGS 6, were identified. Another line, DGS 2, has been identified to possess high

(>3%) gymnemic acid content as well a high root-yielding *Asparagus adscendens*, DAA 2. Two morphotypes, pure white (DAA 1) and purple white flower (DAA 38), were identified in *Asparagus adscendens*. In *D. gangeticum*, different morphotypes, viz. prostrate dwarf plant (DDG 6) and tall erect plants (DDG 15), narrow long leaves (DDG 29) and broad large leaves (DDG 8), white flowers (DDG 18) and pink flowers (DDG 8) types of plants, were identified. A total of 140 *ashwagandha* germplasm accessions were maintained and characterized. For dry root yield, DWS 132 and DWS 135 performed significantly better than the control, JA 20 and JA 134.

Spices: A total of 236 accessions of black pepper were collected from Idukki, Sabari hills and Goodrickal ranges in Kerala. Endangered species such as *Piper barberi* and *P. hapnium* were collected from Sabari Hills. Ninety-five core collections of cardamom germplasm were profiled using 25 ISSR and 3 microsatellite markers.

*Piper hapnium* (male), an endangered wild species collected from Sabari Hills

Tuber crops: One hundred fifty-three accessions of cassava, 15 of sweet potato and 57 of aroids were collected. Further, a land race, *Aniyoor*, has been identified as potassium uptake-efficient cassava genotype suitable for table consumption.

Livestock

Breed registration

Breed Registration Committee of the ICAR approved nine new populations of indigenous farm animals as breeds. Now there are 144 registered indigenous livestock breeds—37 of cattle, 13 of buffalo, 23 of goat, 39 of sheep, 6 of horse and pony, 8 of camel, 2 of pig, 1 of donkey, and 15 of chicken. Details of new breeds are as follows:

Pulikulam cattle (INDIA_CATTLE_1800_PULIKULAM_03035)—are maintained in large migratory herds in Madurai and adjoining districts of Tamil Nadu, and are used in Jallikattu (bull riding) games.





Kosali cattle (INDIA_CATTLE_2600_KOSALI_03036)—is a small size, draught cattle breed of Chhattisgarh. The bullocks are employed for removal of weeds from paddy field.



Malnad Gidda (INDIA_CATTLE_0800_MALNADGIDDA_03037)—are small cattle from Western Ghat region of Karnataka. Breed is reared for milk and light draught work.



Kalahandi buffalo (INDIA_BUFFALO_1500_KALAHANDI_01013)—is a dual purpose breed, used for milk and draught purpose in Kalahandi and Rayagada districts of

Odisha. Animals are of medium size with long and half circled horns.



Konkan Kanyal goat (INDIA_GOAT_1100_KONKAN_KANYAL_06022)—is a meat type breed adapted to high rainfall and hot and humid climate of Konkan region. Animals have typical white

bands on black face and black ear with white margin.



Berari goat (INDIA_GOAT_1100_BERARI_06023)—is reared mainly for meat purpose in Vidarbha region of Maharashtra. As a unique feature, animals have light to dark strips on lateral

sides from horn base to nostrils.



Ghoongroo pig (INDIA_PIG_2100_GHOONGROO_09001)—is found in Darjeeling and Doar area of West Bengal. The animals are black and

have upwardly curved snout and large heart shaped ears.



Niang Megha pig (INDIA_PIG_1300_NIANGMEGHA_09002)—is found in Meghalaya. The animals have small erect ears extended vertically.

Spiti donkey (INDIA_DONKEY_0600_SPITI_05001)—is found in Lahaul and Spiti regions of Himachal Pradesh. The animals are utilized for transportation at high altitude (around 3,500 m above

msl) with low level of environmental oxygen. These animals can survive well in scarcity of feed and fodder during harsh winter when the area is completely snow bound.



Phenotypic characterization and conservation of farm animal genetic resources

Nari cattle: Nari cattle are found in Sirohi district and Bali tehsil of Pali district in Rajasthan. The breed is reared by Raika/ Rebari communities. Nari cattle are very hardy and sustain well in the hilly terrain. The herd size varies from 20 to 100 animals. They travel 150–200 km during migration even to neighboring Gujarat state. The coat colour is mainly white. The horns are long and spirally curved outwards or forward. The age at first calving is 3.5 to 5 years. The per day milk yield varies from 3 to 8 kg.



Nari cattle on an average gives 3 to 8 kg milk/day

Tripura cattle: These cattle are found in West and South districts of the Tripura. Animals are compact and of small size with brown, reddish, black and grey, and white coat; small hump, dewlap and horns; small ears which are horizontal in orientation. The average age at first calving, daily milk yield, lactation length, dry period, service period, herd life and number of calving during life time are 44 months, 1.36±0.06 kg, 145 days, 272 days, 231 days, 427 days, 12–15 years and 8–10 calving, respectively.



Tripura cattle are well built and compact

Purnea cattle: These cattle are distributed mainly in the Purnea, Araria, Katihar and Kishanganj districts of Bihar. The coat is light to deep red and greyish white; head profile is straight in females and slightly bulged in males; and horns are short, crescent shaped and pointed forward. The age at sexual maturity in males is 2.5 to 3 years. Age at first calving ranges between 3 and 4 years. Average daily milk yield of cows varies between 0.5 and 2 kg.



Ghumsuri goats: These are medium in size and reared for meat in Ghumsuri of Khandamal district of Odisha. The coat is black or light brown to black brown. The adult body weights of females and males are 19.88 and 20.86 kg respectively. The flock size varies from 5 to 25. Female animals attain sexual maturity at 12–18 months of age.



Ghumsuri goats are reared mainly for meat in Ghumsuri of Khandamal district of Odisha

Local non-descript donkeys: Comparative biometric analysis revealed that donkeys of Poitu breed were significantly taller than the indigenous donkeys of Himachal Pradesh and Gujarat. Donkeys from Spiti area of Himachal Pradesh were the smallest. However canon, face length and width as well as hoof length and width were similar in both the indigenous donkey populations. Coat colour and biometric indices generated will serve as baseline information for defining and identifying donkey populations as different breeds.

Mithun: Cytogenetic analysis was carried out on all mithuns available in the Institute farm, and data were compiled and presented in the form of a digital album.

Molecular genetic characterization

FecB mutation in Edka sheep: In Edka sheep 37% of the Edka animals were *FecB*⁺ heterozygous carriers, whereas remaining 63% animals were *FecB* non-carriers. Edka is the third prolific sheep in India after Garole and Kuzi/Kendrapada. Genotyping of *FecG* locus in five sheep breeds Garole, Kuzi, Shahabadi, Balangir and Bonpala showed presence of only one genotype i.e. *FecG*^H, suggesting fixing of *FecG* gene in these breeds.

Indigenous pigs: Indigenous pig breeds, viz. Ghoongroo and Niang Megha were characterized using 22 ISAG/FAO recommended microsatellite markers, all of which were found to be polymorphic. The Nei's genetic identity and other estimates indicated varied genetic distance among different populations. The study on genetic relationship among the other breeds found in the adjoining areas, revealed lower genetic distance between Niang Megha and Naga Local (Suho) pigs than Ghoongroo pigs.

Polymorphism in toll-like receptor (TLR) genes: Buffalo *TLR4* gene was analysed by sequencing of 24 different riverine and swamp animals. Total 28 polymorphic nucleotide sites were identified in buffalo *TLR4*. Polymorphic nucleotide sites were also identified in 3'UTR of *TLR2* and buffalo *TLR7* promoter region. Genotyping protocols, developed for the SNPs in *TLR4*-3463C > T, 3'UTR of buffalo *TLR2* 79C > T and -322G > T SNP in *TLR7* promoter within Oct1 binding site, revealed differences in the allele frequencies among riverine and swamp buffaloes. These SNPs could be the potential loci for association analysis with disease resistance traits. Characterization of goat *TLR2* gene showed presence of seven polymorphic nucleotides. Of the five SNPs in the coding region one SNP 1732A > G was non-synonymous changing amino acid 578Ala > Thr within LRRCT domain. Overall, goat *TLR2* gene was less polymorphic than cattle and buffalo.

KIT gene in Nukra horses: *KIT* gene is responsible for development of the specific coat colour in horses. Till now, 17 variations leading to altered or truncated function of *KIT* gene, viz. W1-W17 have been identified and found associated with the dominant white coat colour across various horse breeds. Exon 7 region was amplified and sequenced in Nukra horse, in which GAAC deletion with respect to wild type was observed. The mutation was reported earlier as W10 variation in other horse breeds.

Leptin gene and its association with performance of mithun: The gene frequency for better meat quality was more in farm animals than that in animals from field. The result found after PCR-RFLP was quite encouraging, as some unique bands were noticed in animals belonging to a specific region. Initial analysis of data related to growth, nutritional performance and carcass quality traits for various strains of mithun, showed that the marbling and average fat thickness in mithun meat is moderately abundant, whereas the rib eye area is comparatively larger indicating the better meat quality of mithun.

Identification of Internal control genes (ICG) in cows: The study involved 4 periparturient Sahiwal cows (–21 days to 0 day to +21 days relative to parturition) and 10 known internal control genes (*ICG*) from different functional classes (*GAPDH*, *ACTB*, *UXT*, *RPS15A*, *B2M*, *RPS23*, *HPRT1*, *EEF1A1*, *GTP*, *HMBS*). All the genes showed acceptable expression stability as per recommended threshold values. Most of the 10 genes evaluated, showed consistent expression level in PMN at different time points of peripartum Sahiwal cows.

ACTB and B2M were the most abundant while HMBS was the least abundant transcripts in PMN of Sahiwal cows. The expression stability measure (M) of the 10 candidate ICG ranged from 0.236 (RPS9, RPS15A) to 0.636 (HPRT1). All candidate genes performed well displaying M values below the default limit of 1.5. The genes were ranked from the most stable (lowest M value) to the least stable genes (highest M value): RPS9, RPS15A>RPS23> ACTB> B2M > UXT> GAPDH> GTP> HMBS> HPRT1.

Transcriptomic response of buffalo mammary explants during heat stress: The changes in expression of inducible heat shock protein throughout the time course after heat shock indicated the responsiveness of mammary explant to heat stress in buffalo. In addition to HSP70, expression profiles of IL-6, TNF- α , BCL2, NF-kappaB, PRLR, BAX and c-Jun mRNA were also evaluated in heat stressed mammary explants to understand their responsiveness to heat challenge *in vitro*. Global expression analysis of 2 hr heat stressed mammary explant revealed 138 differentially expressed genes with ≥ 2 -fold change (75 were up-regulated and 63 were down regulated). Gene ontology and pathway analysis revealed several pathways, including Interleukin signaling, chemokine and cytokine signaling, complement and coagulation cascades, antigen processing and presentation, MAPK signalling and JAK-STAT signaling pathway. The transcriptome data would help to understand the modulation of buffalo mammary expression signature in response to environmental heat load.

Transcriptome analysis of mammary epithelial cells (MEC): Agilent 44K bovine microarray data based on two- or more-fold change criteria revealed 89 differentially expressed genes in MEC of two species during early lactation stage. Majority genes were found upregulated in buffalo MEC, which was also revealed by gene set enrichment analysis. Number of biological pathways like ribosomal pathway, glycolysis, pentose phosphate synthesis, oxidative phosphorylation, fructose and mannose metabolism, proteasome; and signalling pathways like NOTCH, mTOR, insulin, MAPK, chemokine and JAK-STAT were identified through GSEA analysis. Ribosomal pathway was identified as the most significant pathway having maximum enrichment score. Microarray analysis suggested increased expression of genes associated with translation and metabolism activities in buffalo MEC during early lactation stages.

Molecular sex typing in pigs: A PCR based method for identification of sex from porcine DNA samples was designed for determination of sex in domestic pigs. This assay utilizes two sets of primers, one control and the other male specific multiplexed for sex typing. Repeatability of the assay was confirmed by testing different samples.

Candidate genes expression in muscle of broiler and layer chicken: Expression of troponin C and troponin slow type protein were upregulated in broiler day-old chicken, while only troponin slow type protein

was upregulated in layer. At 6 weeks of age, expression of troponin C, troponin slow type protein and myoglobin gene were upregulated in both broiler and layer lines. In broiler line, calmodulin revealed about 200-fold greater expression than that in layer line. Myostatin, IGF-1 and GHR had higher expression in layer than broiler at day 1. In heart, calmodulin, troponin C, troponin slow type protein, myoglobin and cardiac related protein revealed upregulation of expression at day-old as well as 6 weeks of age in broilers while in layers, only myoglobin and cardiac related protein genes were upregulated both at day 1 and 6 weeks. In liver, activin, calmodulin, calreticulin, collagen, troponin C, troponin slow type protein, sarcolipin, myoglobin, cardiac related protein, myelin, myostatin, IGF1 and GHR genes were down regulated. At day 1, all genes showed higher expression in broiler than layer birds. At 6 weeks of age, troponin slow type protein, myoglobin and myelin had higher expression in layer than broiler chicken.

Fish

First record of extended distribution of *Tor tor*: Mahseer, *Tor tor*, a well known game and food fish that inhabits streams and fast flowing rivers along mountains was first time reported from Penganga river. River Penganga (N 19° 45.987, E 078° 43.058, Altitude 639 ft.) is a major tributary of River Godavari marking the boundary between the states of Maharashtra and Andhra Pradesh. The identification was supported by morphometric and molecular investigations. The occurrence of various life stages of this species in river suggested that the species has adapted in wide range of distribution and established self-recruiting population in this river of Peninsular India.

New records of species of fish and prawn reported: A fish, *Pinniwallago bhagirathiensis* sp. nov. and a prawn *Macrobrachium hooghliense* sp. nov. were described and reported as new to science by the CIFRI. The holotypes were deposited in the National Zoological Collections of the Zoological Survey of India, Kolkata with holotype IDs ZSI FF4488 and ZSI C5914/2 respectively.



Prawn *Macrobrachium hooghliense* sp. nov. was described and reported as new to science

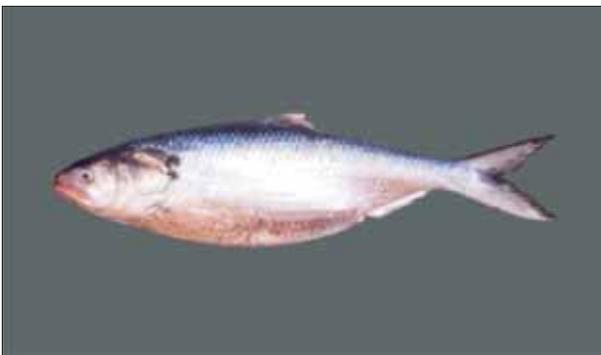
The sea horse species, *Hippocampus kuda*, an endangered fish species, listed in the Schedule-I of the Indian Wildlife (Protection) Act, 1972, was collected



The first record of sea horse species, *Hippocampus kuda*, an endangered fish, from the riverine section of Hooghly estuary

from Kakdwip area. This is the first record of sea horse species from the riverine section of Hooghly estuary.

The CIFRI recorded a female hilsa (*Tenualosa ilisha*) of 614 mm in length and 4,250 g from commercial catch of Tapti estuary at Surat. As on record, the specimen was the biggest from the Indian waters.



Giant hilsa (*Tenualosa ilisha*) from Tapti estuary — the biggest specimen from the Indian waters

Genetic characterization

Cell line as *in vitro* model: A cell line developed from eye tissue of *Puntis chelynoides* was used for genotoxicity and cytotoxicity assessment of heavy metal salts (cadmium, zinc) and nanoparticles. Toxicity potential of titanium oxide, zinc oxide, silver and gold nanoparticles was also evaluated. Heavy metal salts were cytotoxic as well as genotoxic to the studied cell line at various concentrations, cadmium salts being more toxic than zinc. Tested nanoparticles were found

genotoxic to cell line but did not exhibit any significant cytotoxicity. Results strongly validated the efficacy of this cell line as an *in vitro* model for toxicity studies.

Macrophage cell line developed from *Catla catla*:

A cell line designated as *Catla catla* macrophage (CCM) was developed. The cultured cells are avidly phagocytic as revealed by ingestion of yeast cells and fluorescent latex beads. The CCM cells showed increased production of reactive oxygen and nitrogen intermediates following stimulation with lipopolysaccharide and phorbol esters. The culture supernatant from CCM cells revealed lysozyme-like activity. The CCM cells had Fc receptors on their surface in fluorescent antibody test. Moreover, the cells were positive for alpha-naphthyl acetate esterase enzyme and took up acridine orange, indicating that CCM cells are of macrophage lineage. This cell line was successfully used for cytotoxicity assay, as revealed by uptake of neutral red. It can be a useful tool to study the role of macrophages in teleost immune response and development of markers for macrophages.

***De novo* whole transcriptome analysis of freshwater fish louse:** The transcriptome sequencing of *Argulus siamensis*, a major pest capable of causing huge economic losses in freshwater aquaculture sector,

Development of Fish Genomic Resource Databases

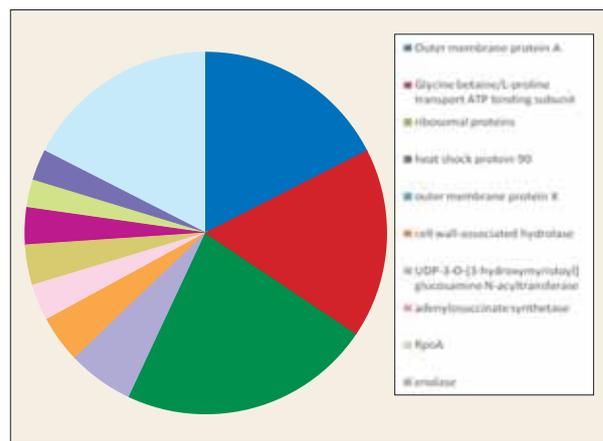
Five fish genomic resource databases, namely Fish Barcode Information System (FBIS), Fish Karyome, Microsatellites Database of Indian Fishes (MIDIF), Ribosomal DNA/RNA, and Fish ESTs, were developed under the NAIP funded project entitled 'Establishment of National Agricultural Bioinformatics Grid in ICAR (NABG)' and are being updated periodically. Databases FBIS and Fish Karyome, are available online. FBIS contains 2,658 barcode records of 518 species. The web interface of FBIS was integrated with tools and applications for retrieving and viewing information about database statistics and taxonomy, including species identification as well as inter- and intra-sequence divergence estimation. The database can be accessed at URL: <http://mail.nbgr.res.in/fbis/>. Fish Karyome database contains karyological information of 154 Indian fish species belonging to 42 families under 10 orders. It enables quick sharing of cytogenetic information on chromosome number, morphology, sex chromosomes and markers, like nucleolar organiser regions, constitutive heterochromatic regions etc. of the fish species, in addition to physical and phenotypic information. The database is accessible at URL: <http://www.nbgr.res.in/fishkaryome/>. MIDIF presently contains 993 non-coding microsatellite sequences for 34 Indian fish species belonging to 11 families, including 531 records of *Labeo rohita*. The database is accessible at URL: <http://mail.nbgr.res.in/microsatellite.indices.php>. Ribosomal DNA/RNA database presently contains 1,080 GenBank records for 219 Indian fish species. Fish EST database contains 2,662 GenBank records for *L. rohita* (1631 ESTs) and *Schizothorax richardsonii* (1,031 ESTs). It is anticipated that these databases would be useful as an appropriate source for characterization, cataloguing, conservation and management of Indian fish diversity.

was performed on Illumina HiSeq 2000. Of the 75,126,957 high quality reads generated, 46,352 transcript contigs were assembled with average length of 1,211 bp and N50 length of 2,302 bp. A total of 19,290 CDS including 184 novel CDS and 59,019 ORF were identified from the assembled contigs. Further, 28,035 transcripts were identified without CDS which include 2,931 novel contigs. Maximum percentage of *A. siamensis* CDS, showed significant similarity with *Lepeophtheirus salmonis*, the salmon louse. From the assembled contigs, 1,171 SSRs were identified. The transcriptome data generated will facilitate future functional studies on the genome of the parasite which will subsequently be applied for vaccine development and other control strategies.

Ceruloplasmin, a potential indirect marker:

Ceruloplasmin, an acute phase protein, was found to be activated by the host immune system during stress conditions. The expression of ceruloplasmin gene was detected at 9 h post-fertilization onwards in rohu carp, *Labeo rohita* during ontogeny study. A strong level of expression was detected at 24 h (38-fold) and 15 days (34-fold) post-fertilization in real time PCR. The ceruloplasmin transcripts were evident in liver, spleen, stomach, and heart but undetectable in gill, brain, eye, skin, muscle, intestine, anterior and posterior kidney tissues of healthy juvenile rohu. The level of ceruloplasmin was also compared in rohu selectively bred for higher growth and disease resistance to *A. hydrophila*. The estimated heritability was also quite high for this parameter. Thus, ceruloplasmin could be a useful marker trait for selection against *A. hydrophila* resistance in fish.

Salt responsive genes of *Mangrovibacter* spp: The genes responsible for salt tolerance in *Mangrovibacter* spp., a facultative anaerobic, nitrogen-fixing salt-tolerant bacterium usually associated with mangrove plants, were identified by using suppression subtractive hybridization (SSH). Clones (160) obtained from forward subtraction resulted in 37 uni-ESTs that comprised 25 contigs and 12 singletons. The outer



Salt responsive genes in *Mangrovibacter* spp. identified by suppressive subtractive hybridization and RT-qPCR

membrane protein A (ompA) (17%) and glycine betaine/L-proline transport ATP binding subunit (16%) genes constituted the maximum number of ESTs in the library. During salt-stressed condition, the glycine betaine/L-proline transport gene showed the highest up-regulation in RT-qPCR analysis indicating that this transport system has a major role in *Mangrovibacter* spp. at salt-stressed conditions. Other important up-regulated genes identified include, outer membrane protein A, mechanosensitive channel, outer membrane protein X, UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase, enolase and translation elongation factor G (EFG).

Detection of enterotoxin producing *Bacillus cereus* in fish: A PCR-based method for rapid detection of enterotoxin producing *B. cereus* was developed based on *hbla* gene, which yielded 834 bp amplified product from both the naturally positive and artificially spiked samples. The conventional method for detection of enterotoxin producing *B. cereus* requires imported expensive kit. This *hbla* gene specific PCR method is cost-effective and takes less time as compared to conventional RPLA-based method, which takes 3 days for detection of enterotoxin producing *B. cereus* in food.

