5

Genetic Resources

CROPS

Germplasm conservation

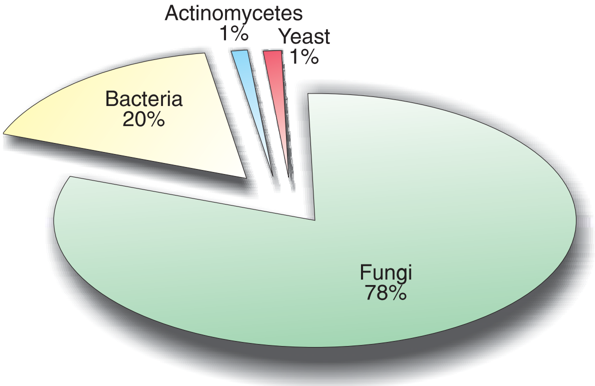
About 2,892 accessions were collected, including 869 of wild species. A total of 294 herbarium specimens were added to the National Herbarium of Cultivated Plants, taking the total holdings to 20,012 specimens. Germplasm added to the National Genebank for long-term storage included 9,526 accessions of orthodox seed species; 247 accessions of non-orthodox species were cryostored and 42 accessions were added to the *In vitro* Genebank.

Germplasm under exchange comprised 32,260 accessions imported from 45 countries, which included international trial material (5,373) and transgenics (132). Promising introductions were—wheat with disease resistance (EC638278-315) from USA; barley with higher yield, lodging-resistant, good malting quality, resistant to covered and false loose smut, spot form of net blotch and spot blotch (EC657888-89) from Canada; paddy with high yield (EC637732-35) from USA, blast-resistant and *Rice tungro* virus-tolerant (EC638157-178) from Philippines and drought-tolerant (EC639584-757) from Kenya; soybean resistant to soybean mosaic virus, stem canker, bacterial pustule, frogeye leaf spot and peanut root-knot nematode (EC638228-642) from Taiwan; and tomato resistant to bacterial wilt, fusarium wilt and tomato mosaic virus (EC654678-686) and heat tolerant lines (EC654694-699) from Taiwan.

A total of 8,280 accessions were characterized and evaluated. Germplasm field days (5) were organized for maize, *kharif* and *rabi* pulses, oilseed brassicas and wheat, barley and triticale. Besides, 5,093 accessions were supplied for research and crop improvement within the country. Phytochemical evaluation of germplasm was undertaken in rapeseed-mustard (721), sunflower (36), safflower (352), linseed (85), walnut (17), almond (39) and *Salvadora oleoides* (20) for oil content and medicinal and aromatic plants (485) for active principles.

Promising genotypes were identified for high yield in advanced varietal trials (based on three-year data) in chenopods (IC415477 with 10.34 q/ha), *Momordica dioica* (SKNK-679 with 8.13 q/ha) and *Jatropha curcas* (JH-1 with 16.59 q/ha). Phytochemical characterization of promising genotypes identified in advanced varietal trials was done for lysine content in grain amaranth (RMA-30 with 4.9%) and protein content in rice bean (PRR-2 with 21.5%), adzuki bean (EC340267 with 21.3%) and buckwheat (IC 125938 with 13.4%).

The National Bureau of Agriculturally Important Microorganisms has more than 2,000, 500, 30 and 20 culture collections of fungi, bacteria, actinomycetes and yeasts respectively.



Germplasm registration

Registration of germplasm of potential importance is an important activity and 720 germplasm lines have so far been registered. About 117 proposals including cereals (46), millets (10), legumes (13), oilseeds (7), fibre and forage crops

(15), vegetables (5), fruit/nut crops (1), ornamentals (6), medicinal and aromatic plants (8), commercial crops (3) and tuber crops (3) were approved for germplasm registration.

DNA fingerprinting

Germplasm accessions of various medicinal plants (190) such as vetiver (*Vetiveria zizanioides*), palmarosa (*Cymbopogon* sp.), giloe (*Tinospora cordifolia*), brahmi (*Bacopa monnieri*) and kalmegh (*Andrographis paniculatus*) were fingerprinted with RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeat) markers. The SSR (Simple Sequence Repeat) markers were developed in finger millet, jute (EST-SSR/ Expressed Sequence Tag-SSRs) and *Vigna* (genomic SSRs). Analysis of SSR polymorphism data in natural populations of *Vigna* (30) indicated substantial gene flow between the cultivated and wild forms/types of greengram (*V. radiata* and *V. radiata* var. *sublobata*) and blackgram (*V. mungo* and *V. mungo* var. *silvestris*).

Phytosanitation

A total of 73,217 imported samples including transgenics and trial material were processed for quarantine clearance. Of the 3,364 samples found infested/infected with different pests 3,293 samples were salvaged. Sixteen Phytosanitary Certificates were issued for export of 1,120 samples. Important interceptions included insects (*Bruchus emarginatus, Carpophilus hemipterus, Rhizopertha dominica, Sitophilus oryzae, S. zeamais, Systole coriandri, Tribolium castaneum, Typhaea stercorea*; nematodes (*Aphelenchoides besseyi, Pratylenchus penetrans, Rotylenchulus reniformis, Tylenchorhynchus* sp.); fungi (*Acremonium strictum, Alternaria raphani, A. sesami, A. ricini, A. solani, Colletotrichum dematium, C. graminicola, C. capsici, Drechslera maydis, D. oryzae, D. sorghicola, D. sorokiniana, Fusarium longipes, F. moniliforme, F. oxysporum, F. poae, Macrophomina phaseolina, Peronospora manshurica, Puccinia carthami, P. helianthi, Rhizoctonia solani* and *Sporisorium* sp.); and viruses (Alfalfa mosaic virus, Bean yellow mosaic virus, Broad bean stain virus, Broad bean wilt virus, Cowpea mild mottle virus, Pea enation mosaic virus, Peanut mottle virus and Pea seed-borne mosaic virus).

HORTICULTURE

Mango: One of the largest field gene banks of mango germplasm has been maintained at CISH, Lucknow, having 735 accessions collected from diverse agro-ecological zones of the country. Under AICRP on STF, 78 accessions and 16 superior clones were collected at different centres. At IIHR, Bangalore, morphological characterization was carried out for 20 mango accessions as per Bioversity International (IPGRI) Descriptor. Fourteen STMS primers, which showed high PIC values, were employed for the gene-scan analysis of 80 mango cultivars using automated DNA sequencer. The genetic analysis for 14 STMS primers data was generated for a total of 269 mango cultivars from the germplasm collection, using Cervus 3.0 software.

Guava: At CISH, Lucknow, 122 accessions of guava on its National Active Germplasm Site were added.

Litchi: Four new accessions and two clones of Bombai were added to field gene bank at GBPUA&T, Pantnagar, and BCKV, Mohanpur, of AICRP on STF centres.

Banana: At NRC on Banana, Trichirapalli; 24 accessions of banana which included wild species, land races and cultivated varieties were added to the field gene bank.

Citrus: A total number of citrus accessions collected and maintained at NRC on Citrus, Nagpur, are 602. Among them, Exotic-50, Rootstock-19 and Scion-31 (Mandarin, Sweet orange, Pummelo and grapefruit) were collected mostly from USA, Japan, Australia and Niger. In AICRP on Tropical Fruits, nine new accessions were collected at Tinsukia by survey in Asom, Mizoram and Arunachal Pradesh.

Grape: At NRC on Grapes, Pune; the national grape germplasm was further strengthened by adding 12 wine and 2 rootstock genotypes, thus bringing the total number of accessions to 458.

Pomegranate: More than 25 variants from 'Bhagawa' population were identified from Maharashtra, Karnataka and Andhra Pradesh.

Different variants from cv. 'Bhagawa'

Arid fruits: In ber, 318 *Ziziphus* genotypes/ strains are being maintained in the National Germplasm Repository of CIAH, Bikaner. A total of 70 accessions of *Z. nummularia* were collected and eight promising accessions are being evaluated for suitability as rootstock and tolerance to abiotic stress. The date palm offshoots of cultivar Siwi and Amhat were introduced from Arab Republic of Egypt in 2009 and planted in the field for evaluation. A total of 60 date palm varieties/ genotypes are being maintained in field repository and evaluated for morphological, yield and quality attributes. In pomegranate, a total of 154 germplasm are maintained at CIAH, Bikaner, and 45 at CHES, Vejalpur. Seventeen germplasm of bael at CIAH, Bikaner, and 15 at CHES, Vejalpur, are being maintained and evaluated. Recently, 5 genotypes were collected from Sikar, Chomu and Jaipur districts of Rajasthan.

Plantation crops: Five distinct coconut accessions were collected from Andhra Pradesh, bringing the total coconut germplasm collections to 370. The coconut collections include aromatic coconut, Jonnalarasi tall and spicata types with orange and yellow nuts. In arecanut, five ecotypes from North Bengal have been added to the germplasm bringing the total to 153. In cocoa, one hybrid and 19 clonal accessions were obtained from Malaysia and UK, respectively, bringing the total to 222.

Oil palm: The genetic diversity of the 23 *E. oleifera* palms were assessed using 54 random primers. About 238 alleles were scored, of which (72.69%) were polymorphic. Maximum similarity was found to be 0.895. Cluster analysis revealed 5 clusters and 4 palms were standing apart**.**

Cashew: Fourteen germplasm accessions collected from NEH region (4) and Andaman (10) were added to National Cashew Field Gene Bank (NCFGB), raising the total germplasm conserved so far to 527. Two wild cashew types (Jungly cashew) were collected from Andaman and Nicobar Islands for conservation in NCFGB. Forty-four germplasm accessions from nine morphological clusters were fingerprinted with nine primer pairs of SSR markers of cashew. The accessions, NRC 97 and NRC 12, were highly divergent and pair of accessions like NRC 12 and NRC 67, NRC 71 and NRC 121, NRC 9 and NRC 126, and NRC 111 and NRC 112 showed highest similarity supported by 83–100% bootstrap values.

Fruit of wild cashew (*jungly caju*)

Under AICRP on Cashew, 47 accessions had bold nut character with a nut weight ranging from 7.0 to 15.0 g, 81 accessions had 28.0–38.5% shelling at Bhubaneshwar, while accession NRC 131 had a high shelling percentage of 32.7% at Jagdalpur. At Bapatla, T.No. 10/19 had maximum mean annual nut yield (14.32 kg/tree) and maximum cumulative nut yield for 12 harvesting (76.13 kg). H 303 gave a cumulative yield of 80.3 kg for 12 harvesting at Bapatla and 77.64 kg for 13 harvesting at Bhubaneshwar.

Potato: JX 90, a new parental line, has been registered with NBPGR with INGR No. 09069. It is a late blight and early blight resistant and high-yielding potato parental line with high yield under early and medium crop duration. JX 90 is a selection from the progeny of cross CP 1346 (Krirrinee) × MS/78-62. It possesses high general combining ability for horizontal resistance to late blight and resistance to early blight. Generally, early bulking varieties are susceptible to late blight. However, JX 90 combines high horizontal resistance to late blight with early bulking. This line performs well for yield under early (75 days) and medium (90 days) maturing in Indian plains and plateau region. Under early (75 days) maturing, it gives yield of 25–30 tonnes/ha, which is at par with early-maturing variety Kufri Ashoka. Under medium (90 days) maturing, it gives 33–38 tonnes/ ha yield, which is at par with cultivar Kufri Pukhraj.

Onion and garlic: About 212 germplasm of onion and garlic were collected, which included 109 red onion, 57 white onion and 46 garlic germplasm from Karimnagar, Nizamabad and Adilabad districts of Andhra Pradesh; Garchiroli, Chandrapur, Yavatmal and Nanded districts of Maharashtra; and Narainpur, Bastar and Dantewada districts of Chhattisgarh.

Tuber crops: The germplasm was enriched with the collection of 30 indigenous and 29 exotic collections of sweet potato. In addition, 48 new accessions of yams collected from Meghalaya, Tripura, Asom and Kerala were added to the existing (873) collections. Besides, wild yams were also collected from Khasi hills of Meghalaya, Barmurah forest of Tripura and Western Ghats. In Aroids group, 42 new accessions comprising taro (37), *Xanthosoma* (3) and *Amorphophallus* (2) were collected from North-Eastern Hill Region.

Mushroom: About 217 specimens of different wild mushrooms were collected. Out of them, 192 were identified up to genus level. Studies on germplasm characterization revealed wide interspecific variation among 41 strains of *Pleurotus* species.

Black pepper: The characterization of 734 germplasm resulted in identification of high-yielding accessions, namely KM III, Angamali, KM II and Valiyaramundi, with more than 2 kg/ vine of green berry yield.

Cardamom: Five new unique cardamom collections, namely Pacchaikai, Pink Stem, Parrot Green, Nattu Vazhukka, and Koadi Mysore, were collected from Megamalai area of Tamil Nadu and added in collection. Among 313 cardamom germplasm evaluated at Mudigere centre, accession Pothamedu recorded highest green capsule yield (208 kg/ha), followed by D 141 and Cl 730. Under CVT, entries CL 722, PS 27, MCC 309 and MCC 246 were found promising for dry capsule yield (347.96 kg/ha). At Myladumpara centre, accessions MHC 26 (1317 kg/ha), MCC 73 (1172 kg/ha), MCC 246 (1155 kg/ha) and MCC 309 (1033 kg/ ha) were identified as high-yielders.

Turmeric: A total of 140 microsatellites containing genomic DNA fragments were isolated from turmeric, adopting selective hybridization method with di and trinucleotide biotinylated probes. Eight polymorphic primers were identified for amplifying SSR containing ESTs. The biologically active peptide turmerin isolated from all the 13 species of *Curcuma* showed highest concentration in C*urcuma sylvatica* (320 mg/100 g).

Ginger: An accession, Acc. No. 195, was identified with high pollen fertility of 67.73% and is being utilized in crop improvement.

Paprika: Twenty-seven new paprika accessions were purified by single plant selection and selfing. The capsaicin content among germplasm accessions varied from 0.0081 to 0.513%. The lines, ICBD-10, Kt-pl-19 and EC-18, were found promising with high colour value and low pungency.

Tree spices: A total of 38 clove, 122 nutmeg, 42 cinnamon and 10 cassia germplasm have been collected. Characterization of cinnamon germplasm at Pechiparai led to the identification of Sel-65 with a bark yield of 420 g of dried bark/tree and leaf yield of 6.3 kg/tree. A local collection from Pechiparai was also identified for high leaf yield of 6.0 kg/tree and bark yield of 314.75 g/tree.

Floriculture: Under AICRP on Floriculture at Bhubaneswar, five new HT and 3 miniature varieties of rose were added to the existing germplasm of 201 HT, 73 Floribunda, 45 Miniature and 8 climbing roses. The Delhi and Pantnagar centres added 20 and 54 new collections, whereas Ludhana centre added 20 new collections and are maintaining a germplasm collection of 117 HT, 55 floribunda, 5 miniature and 3 polyanthas.

Orchid: The characterization of 89 endangered and rare orchid species were done. About 162 orchid species are maintained evaluated for various useful horticultural trials. In *Coelogyne nitida*, three new variants were identified. Diversity analysis and DNA profiling in 10 *Cymbidium* species were done using RAPD markers. Out of 20 decamer primers, 10 arbitary primers produced a total of 180 distinct major bands. Of which 98% were polymorphic. RAPD analysis of 15 vandaceous orchids indicated 98% polymorphism. In eight *Dendrobium* orchids, morphological trials were analysed using RAPD analysis. The genetic distance measured based on Squared Euclidean Distance ranged from 5–23%.

Ashoka: Forty-two accessions are maintained and evaluated at KAU, Trichur. The accessions collected from Thrissur and Trivandrum districts showed vigorous growth represented by its increased height, number of leaves and higher girth of stem.

Ashwagandha: Out of 131 evaluated, accessions MWS-312, MWS-315, MWS-108, RAS-53 and RAS-39 had significantly higher seed yield as well as root yield per plant than the best check, JA-20. The new collections, 31 at CCSHAU, Hisar; 67 at RVSKVV, Mandsaur and 137 at MPUAT, Udaipur, have been made for further evaluation and maintenance.

Giloi: Characterisation of 43 germplasm was carried out. On the basis of starch granules size, five classes, namely very small, small, medium, big and very big, have been made. Starch shape also varied among different accessions. Out of 43 accessions, 34 were characterized using RAPD markers and maximum polymorphism was found in OPC followed by OPA and OPD primers.

ANIMALS

Registration of animal germplasm: Registration of Animal Germplasm was started at the NBAGR, Karnal, provide protection to the valuable animal genetic diversity and facilitate its access for genetic improvement. Accession numbers were given to each of the 129 well defined breeds of species of livestock and poultry, and these were published as a special feature in the *Indian Journal of Animal Sciences*. Guidelines, application form for registration were prepared and are being distributed to potential stakeholders for registration of new populations as breeds. The information is also available on the NBAGR website (www.nbagr.ernet.in/GUIDELINES.pdf).

Phenotypic characterization

Marathwadi buffaloes: The Marathwadi buffaloes are found in the Marathwada region of Central India, especially in Parbhani, Nanded, Bid, Hingoli and Latur districts of Maharashtra state. The average body length, height at withers, heart girth, horn length and face length were 134.21±1.28

Morphometric parameters of mithun

			Adlt wt (kg)	Ht at with (cm)	Body length (cm)	Heart girth (cm)	Face length (cm)	Tall length (cm)	Neck circle (cm)	Neck length (cm)	Ear length (cm)	Horn length (cm)	Horn circ. (cm)
Nagaland	Female	n	7	11	11	11	11	11	11	11	11	11	11
		Mean	370.29	128.25	190.29	182.95	41.29	88.79	76.22	45.34	21.96	28.21	29.38
		STDV	36.75	4.95	9.07	7.72	4.68	3.76	8.17	4.83	1.96	3.85	5.02
		Error	13.89	1.49	2.73	2.33	1.41	1.13	2.46	1.46	0.59	1.16	1.51
	Male	n	4	11	11	11	11	11	11	10	11	11	11
		Mean	411.50	128.23	185.29	188.54	45.17	91.73	94.85	44.28	20.95	31.35	40.34
		STDV	38.86	8.30	13.51	12.89	2.16	6.27	7.72	5.17	1.68	5.81	5.65
		Error	19.43	2.50	4.07	3.89	0.65	1.89	2.33	1.63	0.51	1.75	1.70
Arunachal Pradesh	Female	n	5	7	7	7	7	7	7	7	7	7	7
		Mean	339.40	123.96	185.21	177.41	40.64	86.33	74.79	43.71	21.79	23.64	27.36
		STDV	31.37	3.60	9.08	7.28	2.06	5.93	7.84	3.90	2.23	6.25	3.54
		Error	14.03	1.36	3.43	2.75	0.78	2.24	2.96	1.48	0.84	2.36	1.34
	Male		335	123	200	179	44	83	76	49	24	29	40
Mizoram	Female	n	8	8	8	8	8	8	8	8	8	8	8
		Mean	363.94	125.90	186.60	176.79	40.76	83.29	73.00	45.76	19.88	23.81	28.54
		STDV	32.68	2.87	7.31	5.60	2.21	11.24	3.63	5.17	1.81	4.24	4.19
		Error	11.55	1.01	2.58	1.98	0.78	3.97	1.28	1.83	0.64	1.50	1.48
	Male		398.5	132	195	178	43	81	81	40	21	31	37
Manipur	Female	n	4	6	6	6	6	6	6	6	6	6	6
		Mean	334.00	124.67	186.42	182.68	41.47	81.92	73.75	49.33	19.08	22.50	25.00
		STDV	14.38	4.37	5.52	10.32	2.43	3.32	6.93	15.76	1.02	3.13	4.86
		Error	7.19	1.78	2.25	4.21	0.99	1.36	2.83	6.43	0.42	1.28	1.98
	Male	n		2	2	2	2	2	2	2	2	2	2
		Mean		127.50	197.50	186.00	43.50	85.00	95.00	47.50	21.00	33.25	45.50
		STDV		7.78	9.19	1.41	2.12	5.66	8.49	7.78	1.41	0.35	7.78
		Error		5.50	6.50	1.00	1.50	4.00	6.00	5.50	1.00	0.25	5.50

Adlt wt: Adult weight, Ht at with: Height at wither, Horn circ.: circumference

cm, 125.60±0.627 cm, 182.55±1.08 cm, 54.47±1.11 cm and 45.95 ±0.30 cm respectively. Marathwadi buffaloes are reared under low input system and have moderate milk production varying from 4 to 8 litres/day.

South Kanara buffaloes: South Kanara buffaloes are medium built animals distributed in Mangalore, Udupi and Shimoga districts of Karnataka. The average body length, height at withers, heart girth, horn length and face length were 112.8±3.3 cm, 113.1±2.9 cm, 154.2±2.9 cm, 42.1±2.2 cm, 42.5±0.7 cm respectively. The average daily milk yield of South Kanara buffaloes is about 3.9 ±0.3 litre. The length of lactation varied from 210 to more than 360 days with an average of 313.6±10.2 days. The lactation milk yield varied from 420 to 2,520 litre with a mean of 1,206.8 ±110.1 litre. The average age at first calving was 41.4±1.9 months and the mean calving interval 543.4 ±51.3 days.

Sangamneri goat: The Sangamneri goat, a dual-purpose breed of India, has made its place in the rural economy of Nasik, Ahmednagar and Pune districts of Maharashtra. About 40% of the goats have long hair at the thigh. In adult animals the average height at wither, body length, chest girth, paunch girth, face length, horn length, ear length, tail length (cm) and body weight (kg) are 68.78 ± 0.38, 68.63 ± 0.38, 72.66 ± 0.43, 76.93 ± 0.46, 16.73 ± 0.09, 13.23 ±0.10, 17.21 ± 0.08, 15.13 ± 0.08 and 32.21 ± 0.22, respectively. The average daily milk yield recorded under farm conditions was 0.860 litre and lactation yield of 77.40 litre in about 160 days of lactation.

Morphometric characterization of mithun: The recording of morphometric parameters of four strains of mithun (Nagaland, Arunachal, Manipur and Mizoram) kept in the institute farm are given here.

Vembur sheep: Vembur sheep also known as Pulli Adu, is found in Virudhunagar, Tirunulveli and Thuthukudi districts of Tamil Nadu. Vembur

Rams of Vembur sheep

Camels as a draught animal

Camel keepers were studied in 7 *tehsils* of Bikaner district. The transformation of camel use is investigated on pre-tested survey performa by participatory approach from villages of Bikaner district, viz. Bachchhasar, Gadwala, Jasrasar, Udasar, 8 KYD Chak, Khajuwala, Lunkaransar, Mahajan, Pugal, Nada, Seruna, Lakhmisar, Salasar, and Kolayat. Most of the camel keepers (77.52%) are putting their camel to work at an age of 4 years whereas few (22.48%) put them to work at 3 years of age. Mostly (76.82%) male camels are being used for carting, farming and other agriculture operations whereas only23.18% female are used for this purpose. An average income of Rs 350–450/day is generated from camel carting at the village level but fetches more in the city areas.

sheep are medium to tall, well-built, hardy and strong with a straight topline. The sheep can trek long distances in search of grazing material. The average body weight, body length, height at wither and chest girth are 38.5±1.24 kg, 69.3±1.07 cm, 78.2±0.83 cm and 84.3±0.95 cm in rams and 28.5±0.27 kg, 64.1±0.27 cm, 73.9±0.24 cm and 75.1±0.28 cm in ewes, respectively. Age at first lambing is about 18 months and lambing interval is 11.6 months. Lambs are sold @ Rs 700–800 at marketing age of about 3 months after which the sale price is about Rs100/kg live weight. Mutton fetches a price of about Rs 150/kg.

Munjal sheep: Data on body biometry of Munjal sheep was obtained from Karnal (Haryana), Bhatinda (Punjab) and Hanumangarh and Ganganagar (Rajasthan). Very few animals of Munjal type sheep are now available in these areas. Average body length, height at withers, chest girth, paunch girth, ear length, tail length are 83.64, 80.38, 91.76, 94.33, 18.81 and 47.90 cm, respectively, in male, and 75.59, 73.24, 83.77, 86.90, 18.47 and 42.01 cm, respectively, in female. Adult weight averaged 60.04 and 43.94 kg in male and female, respectively.

Evaluation and characterization of indigenous pigs: Phenotypic characterization of 3 indigenous pigs (Nagaland local, Khasi local and Ghungroo) of Eastern and North-eastern India was completed. The weight at weaning was higher in female (in comparison to male) in Khasi local pig, whereas, it was reverse in Ghungroo pigs.

Zanskari ponies: Biometric indices of true-to-Zanskari breed of horses were recorded in their home tract in and around Leh, Laddakh (Jammu and Kashmir). The average height at wither of Zanskari breed was 126 cm, lesser than the standard height criteria, i.e. 150 cm, for differentiating horses from ponies and as such these equines come under the category of ponies. Average height was slightly higher in stallions (127.21 ± 7.57 cm) than mares (125.45 ± 4.74 cm). Beside this, average body length (123.07 vs 129.5 cm), heart girth (144.4 vs 148.9 cm), hind leg length (80.11 vs 79.95 cm), canon length (16.18 vs 15.80 cm), height at knee (37.57 vs 36.95 cm), face length (53.79 vs 53.75 cm) and face width (15.68 vs 15.25 cm) did not vary significantly among the stallions and mares. Hair coat was thick and quite similar to that of Spiti ponies. Grey was the most prominent coat colour followed by bay and black.

Molecular characterization

Molecular characterization of Motu, Ghumsuri, Binjharpuri, Kumaoni Hill cattle; Marathwari, South Kanara buffalo; Changthangi, Deccani, Muzaffarnagri, Ganjam, Jalauni, Marwari, Sonadi sheep and Arunachali mithun was completed using microsatellite based genotyping.

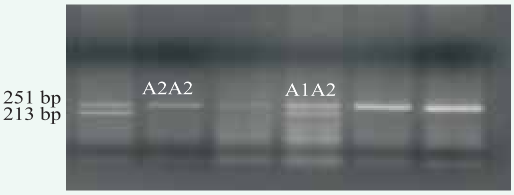
Diversity analysis of livestock breeds: The diversity analysis of different breeds of cattle, buffalo and goats was carried out to establish the relationship among different breeds. The global F-statistics revealed F_{ST} value of 4.5% across South Kanara and Marathwada buffalo populations. The Nei's genetic distance between individual animals revealed 2 distinct clusters of South Kanara and Marathwada buffaloes with some overlapping. In phylogenetic tree using average square distance with UPGMA, the 3 studied cattle populations of Orissa clustered together and then with Kumauni cattle population. Within Orissa, Binjharpuri and Ghumsuri cattle populations joined first and then with Motu cattle.

Immune response genes in indigenous cattle: Analysis of the sequences of tlr-4, tlr-9, mcp-1 gene and nos2a genes in indigenous cattle breeds revealed that tlr-4 gene contained 91 snps spread over the length of 10.8 Kb of gene. Of these approximately 22% of snps were present in the coding regions of the gene. Only one snp present in the exon-2 of the gene resulted in an amino acid change from Ile to Asn. Over the length of approximately 5.1 Kb, only 19 snps could be observed in Sahiwal cattle. All the snps were substitution mutations. The mcp-1 gene showed the occurrence of 23 snps across the gene length of 3156 bp of the gene. There was approximately one snp for every 137 bp length of the mcp-1 gene. The fezl gene was studied across the *Bos indicus*, exotic and crossbred cattle populations and the Mehsana, Murrah and Banni buffalo populations. All the animals studied contained a glycine insertion in them. Accordingly all of them got grouped as being potentially susceptible to mastitis.

Expression analysis of Bcl2, Hsp72 and TLR4 genes: The expression analysis of Bcl2, Hsp72

ß-casein A1/A2 variant in indigenous cattle breeds

Animals (618) of 15 Indian zebu cattle breeds from different agro-climatic regions of India along with 231 buffaloes of 8 river buffalo breeds were genotyped in beta-casein (CSN2) locus by PCR-RFLP method. The ß-casein A1/A2 frequency data indicated predominance of A2 variant (0.987) in zebu cattle breeds while buffaloes indicated only A2 milk type.



PCR-RFLP of ß-Casein (CASB) Taq I showing A2A2 and A1A2 genotypes

and TLR4 genes carried out in the thermal challenged (48°C/56%RH for 2 h) Murrah buffalo along with Tharparkar and Karan-Fries cattle revealed no change in expression profile of these genes. Hsp72 expression increased in buffalo, 2h after heat shock and returned back to 0h level after 4h, whereas, Karan-Fries animal showed decreased expression of Hsp72 during 0h, 2h and 4h after heat shock treatment.

Diversity analysis of buffalo breeds: Nei's genetic distance between individual animals of Marathwadi and South Kanara buffaloes revealed two monophyletic clusters of respective source populations. F_{ST} value of 0.071 indicated considerable genetic differentiation among these two breeds.

Leptin and mammary derived growth inhibitor genes: A non-synonymous change resulting in amino acid variation from alanine to valine in leptin gene was detected and *HaeIII* genotyping protocol was developed for screening of SNP (single nucleotide polymorphism). A polymorphic nucleotide T/C was detected at 53Int2 position in MDGI gene, and Bah RFLP genotyping protocol was developed for the screening of SNP. Screening of 200 Mehsana buffaloes along with association studies in 67 samples having milk production records showed no significant association of SNP with any of the performance records.

Diversity analysis of sheep breeds: Genetic profiling of unrelated individuals belonging to Deccani, Muzaffarnagri, Ganjam, Jalauni, Marwari and Sonadi sheep, revealed high level of genetic diversity in them. The level between breed differentiation was moderate (<15%). Global F_{ST} values indicated that 11.2% of the total genetic variation could be explained by breed differences and the remaining 88.8% by differences among individuals. Marwari and Sonadi were the closest, while Deccani and Sonadi showed highest degree of genetic differentiation.

Genotyping of Kathiawari and Marwari breeds: Genotyping of Marwari and Kathiawari horses showed high heterozygosity, indicating adequate genetic diversity among both the populations.

Microsatellite analysis in Indian dromedary breeds: Forty microsatellite primers known to be polymorphic in camelids were successfully amplified in the Indian dromedary breeds. Number of alleles at these loci ranged from 2 to 7. Maximum number of alleles were in Bikaneri (76) followed by Jaisalmeri (74) and Kachchhi (69) breeds. The consensus tree was also constructed. A high degree of homozygosity was observed among the Indian dromedary breeds.

Marker for higher adult body weight in yaks: An AFLP (amplified fragment length polymorphism) allele was found with higher frequency in yaks with higher adult body weight. Adult male and female yaks with body weight greater than or equal to mean $+1.5\sigma$ were grouped as elite animals. Frequency of AFLP marker allele in the elite group was 78% in male and 66% in female group. The overall frequency in the male and female groups was as 0.28 and 0.12, respectively, suggesting a stronger selection pressure on sires than on dams. The marker information could be incorporated in marker assisted selection for higher adult body weights in yak, as the body weight determines draught and pack ability and quantity of meat production.

Cloning, sequencing and homology studies on *SRY* gene: The *SRY* gene typing in yak, cattle and their hybrids was done based on *SRY* nucleotide sequence data for cattle (AB039748) and yak (AY079143). Numbering of restriction sites refer to the Genbank entry AB039748 for bovine *SRY*. The individuals showing polymorphisms were identified and further probed by direct DNA sequencing.

The study indicated high degree of sequence homology in *SRY* gene among yak and cattle. Mutations in *SRY* gene were used to develop simple assays to identify male mediated introgression in yak × cattle hybrids. Delineation of the sequence homology in *SRY* gene among yak, cattle and their hybrids may help analyze the cause of male infertility at gene level.

Milk fat associated genes polymorphism: DNA sequencing of milk fat percentage regulating genes FASN, MOGAT1 and MOGAT2 in yaks revealed polymorphism in these genes at various positions resulting in amino acid changes. Homology studies showed 97, 99 and 98 similarity in the FASN,

Population genetic diversity in yaks

An RAPD analysis showed that yak population reared in an organized herd is highly homogenous (similarity coefficient=1). A comparison with few other individuals belonging to different yak tracts of India also produced strikingly similar genotypic profiles. This remarkable homogeneity could be attributable to either high level of inbreeding or to the limited diversification among the yak population in India.

MOGAT1 and MOGAT2 genes, respectively, between *Bos grunniens* and *Bos taurus*. No transcription-start-sites and zinc-finger-residues were found in the sequenced region of the 3 genes.

Genetic studies on mithun: Karyotypes for male and female mithuns were prepared and cytogenetic analysis was carried out, which included estimation of relative length and centromeric index. Idiograms were also prepared for both male and female mithuns. The protocol of C- and R-banding (RB-FPG method) was standardized for the mithun chromosomes.

Genetic characterization of kappa casein gene of mithun: Kappa casein genetic variation among mithun strains were studied using PCR-RFLP technique. PCR products between exon 4 and intron 4 region of CSN3 gene was 270 bp. HindIII produced definite restriction sites in mithun yielding approximately 230 bp, 180 bp and 90 bp fragments. EcoRI, MboII and Sau3AI were having no cutting site in the region of exon 4 and intron 4 of mithun CSN3.

Genetic characterization of microbes available in mithun rumen fluid: The PCR amplification of 16S rDNA was done for *Prevotella ruminicola* (ferments protein, hemicellulose and starch), *Prevotella bryantii* (ferments protein, hemicellulose and starch), *Ruminobacter amylophilus* (ferments protein and starch), *Selenomonas ruminantium* (ferments sugar/dextrins) and *Treponema bryantii* (ferments cellobiose, xylose, arabinose and glucose) using 20–50 ng total DNA in 25 µl reaction volume. Some of the PCR products of above mentioned bacteria were cloned into *Escherichia coli* DH5α using pGMET vector.

Candidate marker approach for selected economic traits: Comparative evaluation of reproductive traits, viz. estrogen receptor (ESR), prolactin receptor (PRLR), retinal binding protein 4 (RBP4) and growth hormone (GH) gene in Ghungroo, Meghalaya Local, Naga Local and crossbred pigs revealed the presence of heterozygous genotype (AB genotype) of ESR gene in Ghungroo and Hampshire × Assam local animals.

Molecular characterization of chicken germplasm: Five chicken pure lines of PDP (PD1, PD2, PD3, PD4 and PD5) being used for development of varieties for rural poultry, were characterized using 15 microsatellite markers, of which 14 markers were polymorphic. The number of alleles for different microsatellites varied between 2 and 6. The Nei's genetic identity and other estimates indicated varied genetic distance among different lines. Five alleles were found for each of the five microsatellites (located on chromosome E48, 10, 5, 2 and 1) in Naked neck population.

Genetic marker and trait association in chicken: In prolactin promoter of White Leghorn chicken 28 haplotypes were identified. The h1/h22 haplotype combination produced significantly highest number of eggs up to 52 and 64 weeks of age and significant effect of haplotype combinations was recorded on several egg quality traits. The prolactin gene expression was the lowest in h1/h22 and highest in h1/h5 combination. The birds with higher prolactin expression showed lower egg production.

A 390 bp fragment of melanocortin receptor-4 was analyzed in 2 layer (IWH and IWK), 2 native (Aseel and Kadaknath) and Naked neck populations. Three alleles (*A*, *B* and *C*) were observed in IWH, IWK and Kadaknath populations, while only 2 alleles (*A* and *B*) were observed in Naked neck and Aseel breeds. The amino acid sequence of this fragment indicated that *C*-allele was different from *A*- and *B*- alleles by having lysine amino acid at 28th position as against glutamic acid in *A*- and *B*- alleles.

Effect of Naked neck gene on economic parameters: The body weight varied significantly among the genotypes carrying Naked neck gene in homozygous and heterozygous condition. The *NaNa* genotype recorded heavier weights at 4 and 6 weeks of age. The *NaNa* birds had significantly higher dressing percentage, lower feather percentage and lower abdominal fat. The cell-mediated (PHAP) and humoral (SRBC) immune responses were higher in *NaNa*/*Nana* genotypes. The serum cholesterol content was lower in *NaNa* and *Nana* genotypes, compared to normal birds.

Native fowl genomics for disease resistance and molecular breeding for high yielding chickens: Profiling the genetic basis of disease-resistance against avian leukosis and sarcoma virus (AL/SV) [against sub types: B, D and E] was taken up in native breeds Kadaknath (KN) and Aseel [including the varieties: Aseel Peela (AP) and Aseel Kagar (AK)] vis-à-vis White Rock, a commercial broiler breed. The KN, AP and AK segregated for both the susceptible and resistant alleles, i.e. TvbS1 and TvbR. The TvbR was represented through heterozygotes only in the native populations, and no carriers could be detected either in AP or White Rock. Frequency of TvbR in KN and AK was 0.10 and 0.0625 respectively. The PCR-RFLP concluded that our native flocks also segregated for the same resistant allele, as found in their custom-bred stocks. Incidentally, the TvbS1 was the type of susceptible allele found in these test-samples, while the international reports state records an additional type of susceptible allele TvbS3 besides TvbS1.

The frequency of TvbR could be enhanced by identifying all the carriers from a population of Kadaknath chickens and by their subsequent *inter-se* matings. It was concluded that genetically-resistant populations (for ALVB resistance) could be developed from within KN and Aseel, by selective breeding and its introgression into other high-yielding chicken populations, particularly broilers and layers.

Allelic profiles of Aseel and Kadaknath: Microsatellite allelic profiles of Aseel and Kadaknath breeds of native chicken were developed with 24 markers and out of these, 2 loci, MCW266 and LE174 demonstrated monomorphic and 14 demonstrated bi-allelic patterns. Five loci revealed 3 alleles and 2 loci demonstrated 4 alleles with varied frequencies in Aseel and Kadaknath breeds.

MHC haplotypes resistant to Marek's disease (MD): IWI and IWK pure line layer populations were screened for MHC class II alleles. The predominant MHC type was B2 followed by B21. In IWK, B13, B15 and B19 were not found. In IWI, all B types tested were found, except B13. B13, B15, B19 were considered susceptible to MD while B19 and B2 were considered resistant to MD. MHC screening of MD tumors revealed that majority were of B19 type, which is considered susceptible to MD.

Collection, preservation and molecular characterization of veterinary pathogens: Standard operating procedures (SOPs) for animal cell culture, bacterial culture, storage of bacterial/viral pathogens, and working guidelines for sample acquisition, characterization, and reposition were developed. Bacterial isolates (90) and viral isolates (6) were preserved. PCR amplification and partial sequencing of 16S rRNA genes has led to the confirmation of *Aeromonas* spp., *Bordetella bronchiseptica*, *Streptococcus* spp., *Corynebacterium* spp., *Pseudomonas* spp., *Proteus* spp. and ß-hemolysine positive *Staphylococcus* species. A significant finding was isolation and identification of *Pantoea agglomerans* strains from cases of equine abortions.

Network Project on Animal Genetic Resources

***In-situ* conservation:** Under *in-situ* conservation of different breeds of goats, 50 Beetal male kids are being reared at the flocks of farmers. At Navsari, the number of selected elite Surti goats is now 170. The physical traits and body measurements were recorded. Male kids were selected on the basis of dam's milk yield, which ranged from 180 to 188 litres. The birth weight of selected male kids ranged from 2.4 to 3.3 kg and their weaning weight ranged from 9.0 to 11.0 kg.

Characterization of the ectoine biosynthesis genes halodurans

Ectoine, a cyclic tetrahydropyrimidine and a compatible solute, plays a major role in preventing plasmolysis of bacteria in saline environments. Ectoine has recently gained importance as it is used in dermopharmacy in anti-ageing creams, skin creams, as a component of shampoo and as an adjuvant for vaccines. The putative ectABC gene cluster from *Bacillus halodurans* was heterologously expressed in *Escherichia coli* and the production of ectoine was confirmed by HPLC analysis. The activity of the ectA protein was confirmed by an acylation assay. The transgenic *E. coli* accumulated up to 4.6 mg ectoine/litre culture. This is the first report of an engineered *E. coli* strain carrying the ectoine genes of the alkaliphilic bacterium, *B. halodurans*.

***Ex-situ* conservation programme:** Four Ponwar cattle bulls, were used for semen donation. Only two semen samples, having post-thaw motility of 40% were found fit for cryopreservation. Out of the 11 Kherigarh cattle bulls put under training for semen donation, 6 bulls are being used for semen production. Post-thaw motility of two bulls was 55–60%.

On an average 1,100 semen doses/bull of cattle from the 8 procured bulls of Krishna Valley cattle were stored. Semen doses were supplied to cattle development centers in Bagalkot and Belgam districts. Artificial inseminations (AI) resulted in average conception rate of 55.95%.

Semen doses were also frozen from prized bulls owned by farmers for conservation of superior germplasm. About 3.5 lakh doses of frozen semen from superior test and progeny tested proven bulls are being maintained.

National gene bank on animal genetic resources: In addition to already existing semen doses from 9 breeds of cattle, 6 breeds of buffalo, 1 breed of sheep, 2 breeds of goat and 1 breed of camel, 15,000 semen doses of Jaffarabadi buffalo, 8,000 of Kherigarh cattle, 230 of Ponwar cattle and 360 semen doses of Arunachali yak were added to the Gene Bank.

FISH

Microorganisms for aquatic bioremediation: Efficient heterophilic nitrifying, aerobic denitrifying, agarolytic and sulphur oxidizing

Forensic identification of whale shark meat

Whale shark (*Rhyncodon typus*) is an endangered species protected under schedule 1 of the Indian Wild life Protection Act 1972 and was designated as endangered species under "Convention on International Trade" in April 2001. Forensic investigations using DNA barcoding resulted in the identification of whale shark meat and hence, revealing the illegal trading of this species. Thus, DNA barcoding provides scientific evidence which can be used effectively in curtailing illegal trade of endangered organisms and the technique will go a long way in preserving biodiversity.

Endangered whale shark (*Rhincodon typus*)

bacteria which can be used for bioremediation of shrimp farm discharge were identified. The diversity of chemolithoautotrophic bacteria isolated from coastal waters was examined by the creation of metagenomic clone libraries for functional genes such as ammonia monooxygenase gene (amoA), nitrite oxido-reductase (norB), nitrous oxide reductase gene (nosZ), nitrite reductase (nirS) and nifH gene. Sequences obtained were deposited in the GenBank. The amoA gene sequences exhibited 81–82% identity to *Nitrosomonas europaea*, *Nitrosococcus mobilis* and *Nitrosomonas eutropha*, which were also similar to particulate methane monooxygenase (pmoA) gene sequences. The norB genes are closely affiliated with *Nitrobacter* sp. especially *Nitrobacter winogradskyi* and other uncultured beta-proteobacteria available in the GenBank. The levels of nucleotide similarity with uncultured bacteria bearing *norB*, *nirS* and *soxB* ranged from 78–82%, 79–82% and 77–82%, whereas amino acid similarity was 79–87%, 80–88% and 76–86% respectively. A molecular tool was also developed for the detection of chemolithoautotrophic bacteria, and is being offered to private entrepreneurs for commercialization.

Genetic characterization

DNA barcoding of fishes: DNA barcodes (1,509) of over 400 fish species were prepared for Indian fishes. More than 1,107 barcode sequences were submitted to the National Centre for Biotechnology Information Gene Bank. In addition, 21 DNA barcodes of helminth parasites of fishes were also prepared. The material was procured from both marine and freshwater sources. A total of 3,403 fish samples of 656 marine species from almost all coastal states and 739 samples of 132 finfish species and 57 samples of 12 shellfish species from freshwater sources (Ganga, Brahmputra and Peninsular rivers) were assessed for the study.

Barcoding of five species of tuna was carried out and deposited with the gene bank of NCBI (http://www.ncbi.nlm.nih.gov/taxonomy).

Microsatellite markers in Indian catfish: Seventeen Type-I markers were identified in Indian catfish (*Clarias batrachus*), which will be particularly valuable for genetic mapping and serving as anchor loci for comparative genomic studies. In addition, 12 polymorphic microsatellite loci were identified, which are useful in determining genetic variations in wild populations of *C. batrachus*.

Vitellogenin gene expression: A rapid and sensitive reverse transcriptase-PCR method was standardized to detect change in fish vitellogenin gene expression. This method successfully detects vitellogenin gene expression in male *Labeo bata* exposed to 17-ß estradiol at a concentration of 100 µg/litre. As vitellogenin gene is not expressed in male fishes under normal condition, expression in males provides a sensitive indicator of fish exposure to estrogenic compounds and endocrine disruption. The method can be used in bio assessment of aquatic pollution, which causes endocrine disruption hampering fish breeding and recruitment.

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