

3. RESEARCH AND DEVELOPMENT

Though the recommendations of BRPC and 16 Task Forces, projects were recommended and reviewed during the year. Some achievements are briefly given in this section.

3.1. BASIC RESEARCH AND EMERGING AREAS

The Task force on Basic Research on Modern Biology which aims at generation of new knowledge for providing base for application oriented research approved 20 such projects during the year.

Studies were carried out at IISc, Bangalore to elucidate the factors involved in the Sex specific heterochromatization of paternal genome- a phenomenon related to genomic imprinting in mealybugs. During the year two middle repetitive sequences that are differentially organised between the two sexes and also within the same diploid nucleus were identified. These two sequences form a part of the male-specific nuclease-resistant chromatin (NRC) fraction of a mealybug *Planococcus lilacinus*. Components of NRC were analysed by cloning the DNA sequences present. The studies revealed that the middle repetitive sequences within NRC are functionally significant and may be involved in male-specific chromatin organisation.

In a study of hydrogel nanoparticles (10-100 nm) on the targeting of mammalian cell type markers compounds like FITC-dextran or ¹²⁵I tagged tyraminylinulin were entrapped. In the aqueous core of reverse micellar droplets within a hydrogel polymer of vinylpyrrolidone produced by polymerising the monomer in the in aqueous core presence of marker compound/s and cross-linking agent. The nanoparticles evoked in reticuloendothelial system (RES) in animal studies. This is the first reported hydrophilic nanoparticles, which completely evade RES and remains in blood for a long time.

Northern hybridisation studies carried out under a project on Identification, Expression and Modulation of Tissue specific Genes That Cause Ageing at BHU, Varanasi showed that expression of the CPK gene declines as a function of age in the heart, but its expression does not change in the skeletal muscle. The role of trans-acting factors in the regulation of the CPK gene was analysed by sub cloning the promoter of the gene and electrophoretic mobility Shift Assay (EMSA) was carried out.

Attempts were made to immobilise lipase in gelatine containing microemulsion based organogels (MBGs) under a project Investigation into the formulation of micellar phases for enzymatic reactions and protein recovery at Sardar Patel University, Vallabh Vidya Nagar. The behaviours of this novel, predominantly hydrophobic matrix as an esterification catalyst has been examined for the synthesis of ethyl-butyrate and ethyloctanoate as food flavours under different water-surfactant ratios. The system was compared with silica based immobilisation system. Both systems have been examined for their repeated use providing a better approach for catalysing reactions in non-conventional media and exhibiting higher potentiality.

The FtsZ protein homologue of *M. tuberculosis* H37Rv (FtsZMt) was identified and the gene (ftsZMt) has been cloned and expressed in *E. coli* at IISc, Bangalore. The sequence of the protein is quite similar to its *E. coli* homologue, with several conserved domains, but with some specific differences. Experiments are in progress in order to characterise the structural differences and to understand the implications of the structural features on the biological function of the protein in the tubercle bacillus.

Studies carried out under the project “Expanded bed chromatography for protein purifications” being implemented at IIT Delhi and UDCT, Mumbai, revealed that in expanded beds the axial mixing, unlike assumed universally, is not a function of system, physical property alone. It was found out that the bead properties were better than those of imported matrices like those based on agarose or other polymers.

Significant achievements of the project on expression of wild type and mutant human eukaryotic initiation factor2 (α eIF2) sub-unit in *Spodoptera frugiperda* insect cells to characterise the importance of α eIF2 phosphorylation in the regulation of protein synthesis at University of Hyderabad are i) impressive expression (15-20% of the total protein) of factor α eIF2, ii) the specific recognition of the recombinant viruses by southern hybridisation and the trans-proteins by monoclonal human eIF2 α antibody. The expressed sub-units of human eIF2 α are found to be phosphorylated to the same extent in-vitro by purified raticulocyte heme - regulated eIF2 α kinase.

A relationship between the sequences and structures of several legume lectins vis-à-vis their ligand recognition propensities was successfully defined with the studies on knowledge based redesign of tumour associated T-antigenic specificity of peanut agglutinin by protein engineering at IISc, Bangalore. It was observed that an explicit correlation between the lengths of their D loop with their monosaccharide specificities exist. These results provided a framework for understanding the molecular basis of carbohydrate recognition by legume lectins and also a rationale for redesign of their ligand binding properties.

Significant changes in the distribution of *Arg*, *Ile*, *Lys* isoaccepting tRNA species were observed after phytohormone and/or light treatments to dark-grown ragi seedlings under a project studies on structure and developmental regulation of expression of plastid and nuclear tRNA genes in higher plants at IISc, Bangalore. These data show that increases in the level of aminoacylation of plastid t RNAs and specific alterations of plasmid tRNA population to occur during phytohormone and light-induced plastid development in ragi coleoptiles.

Studies on "Biochemical studies on basidiospore formation in oyster mushroom (*Pleurotus spp.*)", revealed that high increase of hexokinase and succinic dehydrogenase activities are associated with initiation of basidiospore formation in gill tissue of fruit-body. It was also observed that pentose phosphate (PP) pathway plays a minor role in fruit body development of oyster mushroom.

Localisation of the transcriptional activator protein Pdc2p, and the protein Pdc1p encoding the enzyme structure was demonstrated under a project on “Molecular analysis of PDC2: A novel transcriptional activator and its role in pyruvate decarboxylase synthesis in yeast” at TIFR, Mumbai. It was further found that one of the suppressors SXP9 restores pyruvate decarboxylase activity. It also expressed enhanced level of glycolytic enzymes and showed increased level of alcohol production compared to the wild-type strain.

Studies were carried out on cysteine proteases which have importance in medicine and industry under a project on the structural basis of differences in catalytic mechanism of different cysteine proteases by X-ray crystallography and molecular modelling at Bose Institute, Calcutta. One protein was studied and the results are encouraging.

An ordered cloned DNA map of chromosome of the hypertoxigenic strain 569B of *Vibrio cholerae* has been constructed at IICB, Calcutta and a cosmid library of the size of about 4000 clones containing more than 120 Mb of *V. Cholerae* genomic DNA (40-genome equivalent) was generated. Interesting results are anticipated in these studies.

3.2. AGRICULTURE BIOTECHNOLOGY

Genetic improvement of major Indian crop species has been achieved mainly through classical genetics and plant breeding methods. Recent advances in cellular and molecular genetics have opened new avenues to apply biotechnology tools for improving the conventional approaches. Several projects are being pursued using such recent tools.

Rice (*Oryza sativa*): The Indian isolates of rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) are being characterised and diagnostic probes for these viruses are being developed in a multi-centric project involving three institutions at Bidhan Chandra Krishi Vidyalaya (BCKV), Kalyani, Delhi University South Campus, New Delhi and IARI, New Delhi. Studies revealed certain important differences in the gene organisation in the Indian isolates as compared to the sequences available from the database.

The Cry 1A (c) gene incorporated rice was developed at ICAR research complex for NEH region, Barapani; the transformation was confirmed through PCR carried out on transformed tissues.

Brassica sps: On transfer of male sterility from different sources to vegetable Brassica's through protoplast fusion and incorporation of cry 1A (b) into combiner lines implemented at IARI, interesting results have been achieved. The donor-recipient protoplast fusion technique was employed to transfer CMS-inducing cytoplasm into cabbage, cauliflower and broccoli. Three different CMS - inducing cytoplasm were transferred to obtain 5 CMS-lines, which are to be assessed for male sterility phenotype. However, Southern hybridisation pattern of mt-DNA of these lines shows similarities to respective CMS inducing cytoplasm. In cabbage transgenic plants were shown to carry Bt gene as evidenced by southern hybridisation of genomic DNA with Bt gene probe. Insect feeding bioassay with Diamond black moth larvae confirmed that transgenic plants were tolerant to insect pests.

Brassica juncea transgenic plants resistant to fungal pathogen were developed by introducing the suitable constructs of chitinase and glucanase. Since chitinase and glucanase work synergistically, two chimeric constructs for the purpose of gene pyramiding have been made. These constructs have been transferred to *Agrobacterium spp.* and will also be used for Brassica transformation in near future.

On hybrid seed production in mustard using barnase and barstar gene at UDSC, New Delhi, six constructs were successfully used to generate male sterile lines; the marker gene was either *bar* or *npt II*. The plants were allowed to set seeds under contained conditions. Single copy barstar lines both with wild type gene as well as with codon-usage modified gene driven by A 9 or TA 29 promoter were obtained. They were selfed to obtain homozygous lines. Two single copy plants were also crossed with male sterile barnase containing plants to assess their restoration capabilities. Antibodies against barstar protein have been developed and will be used to assess the expression levels of barstar protein in different plants.

Pigeonpea (*Cajanus cajan*): At IARI, New Delhi, the vector pCambAc carrying Cry 1 A(c) gene was mobilised into *Agrobacterium tumefaciens* and was used for pigeonpea transformation. The Cry 1 A(c) gene was also modified by site directed mutagenesis with a view to enhance insecticidal efficacy.

On genetic transformation of pigeonpea at UDSC, New Delhi, the system was found to be most responsive to transformation via particle bombardment. In order to enhance the growth of transformed tissues, bombarding shoot apices tested a cytokinin biosynthetic gene from *Agrobacterium*. In addition, new constructs were developed which carry a proper *nptIII* gene and

isopentenyl transferase (*ipt*) gene. The new constructs will be tested on shoot apices, to study the effects of *ipt* gene as positive selection marker in further studies.

Other crops: At Bose Institute, Calcutta an antisense RNA to delay fruit ripening had been subcloned in pBSks. A transformation method using *Agrobacterium tumefaciens* was developed for tomato. Transformation of tomato using partial and full-length antisense constructs had also been initiated. Also suspension cell culture of banana was established. An efficient protocol for production of embryogenic calli from leaf bases of tissue culture grown plants had also been developed.

In another project on rapid clonal propagation and *in vitro* regeneration of Tikka disease resistant varieties of groundnut at Visva-Bharati, Santiniketan, clonal propagation of selected accessions was achieved using different explants. Apart from callus lines, attempts were made to induce variability through somatic embryos. In the second phase of experiment, mutagen-treated zygotic embryos would be used for the production of genetically variable plantlets through somatic embryogenesis.

In a project on RFLP and RAPD analysis of powdery mildew resistant gene in pea at IARI, New Delhi, inheritance of *er* gene and its molecular mapping studies were undertaken. The inheritance of *er* gene was confirmed to be monogenic recessive. Regarding the location of *er* gene on chromosome VI, 15 crosses were made for raising F1 generation. The F2 population will be raised for detailed mapping on chromosome VI to determine precise location of *er* gene in relation to other major genes. Further, the SCAR (Sequence characterised amplified region) marker reported to be linked with *er* gene was included in the mapping. The joint segregation of RAPD and (sequence characterised amplified region) SCAR markers resulted in a map.

Project mode support: The department is supporting focused programmes at 6 institutions/universities namely; University of Delhi South Campus, NBRI, Lucknow, MKU, Madurai, TNAU, Coimbatore, Bose Institute, Calcutta and Osmania University, Hyderabad. The salient achievements made in these programmes are given as below: -

University of Delhi South Campus: Organ-specific regulatory elements isolated from rice revealed the presence of 2 inflorescence specific cDNA; one of which was novel. A root-specific new cDNA codes for tetra-functional protein and genomic clones have been isolated to obtain organ-specific promoters in rice employing a genomic library in Lambda DASH II. Transgenic tobacco was produced by employing binary vectors containing GUS gene under the control of deletion constructs of leaf-specific gene promoters. Initial studies showed that these promoters might provide tissues specific and light- regulated expression.

Constitutively photomorphogenic mutants of Arabidopsis, the HLSI/PHOI gene, encoding N-acetyltransferase, was amplified by PCR from hlsI/phol I mutants and its wild-type parent and sequenced. The HLS I/PHO I ortholog has also been PCR-amplified from *B.napus* and its sequence analysis shows 84% homology to that of *Arabidopsis*. Several phytochrome gene clones from wheat was also isolated.

National Botanical Research Institute, Lucknow: The group at NBRI has cloned various ripening related genes from banana. The expression of these genes over the entire period of ripening shows a specific and differential pattern on Northern analysis. The antisense construct has been expressed in *Agrobacterium* as identified by probing Northern blot.

In another project related to nuclear organelle genome interaction some interesting results were obtained. Studies on identifying the 5 ends of different chloroplastic mRNAs from *Populus deltoides* revealed for the first time that there may be more than two 5 ends of rbcL mRNA as

compared to what was reported earlier in other plants. The studies related to plastid gene expression suggest that accumulation of transcript is more prominent in light as compared to dark. Interestingly, psbD/C operon accumulated more polycistronic transcript under light conditions.

Tobacco transformation using coat protein gene of TLCV-pBI 121 construct was done through *Agrobacterium tumefaciens*. PCR amplification and Southern hybridisation screened these plants. The transgenic plants of R0 generation were inoculated by viruliferous white flies and checked for symptoms. Further PCR amplification was carried out using specific primers for TLCV replicase. In addition, the mobilisation of CP gene in tomato, co-cultivation of *Agrobacterium tumefaciens* with tomato explants was done. Also constructs of coat protein and replicase gene of TLCV in a high copy number expression vector are being generated.

Transformed lines of chickpea and pigeonpea expressing Bt-endotoxin gene were generated; a genotype independent rapid *in vitro* regeneration system in *Cajanus cajan* was established. The regeneration system of pigeonpea was used for co-cultivation with *Agrobacterium tumefaciens* and particle bombardment for introduction of desired gene constructs. The extents of transformational events were monitored through transient expression of reporter gene GUS. In chickpea, the vexation problem of acclimatisation and hardening of *in vitro* grown primary transgenic plants were accomplished to about 55-65% success for F1 seed setting. A comprehensive package for genetic transformation and development of transgenic plants in chickpea using direct organogenesis protocol was established.

Madurai Kamraj University, Madurai: One group is cloning and characterising the *Vigna mungo* yellow mosaic virus (VMYMV) in order to introduce the replicase and coat protein genes of the virus for generating resistance to yellow mosaic disease in blackgram and mungbean. *Agrobacterium* strain harbouring binary vectors will be used for transforming *V.mungo*.

Protocols for purification of chloroplast DNA of cotton and tobacco has been standardised. Also trn A and trn 1 genes from cotton chloroplast were PCR amplified and these genes were individually cloned into pGEM vector and were introduced into *E. coli* JM 109 cells. Sequencing of these two genes has also been done. The 16 S rDNA promoter fragment of cotton was also PCR amplified, cloned into pGEM and sequenced. This shows 90% homology to that of tobacco.

Attempts were made to transform two indica rice cultivars (IR-50 and ADT-36 with (pyroline-5-carboxylate synthase) p5cs gene. A protocol for high frequency plant regeneration from embryogenic calli derived from matured seed embryo explants has been developed. In the meantime, the presence of p5cs gene in hygromycin resistant calli by doing PCR analysis was confirmed. Oligo primers specific to p5c s gene were obtained which will amplify 274-bp region between 152 to 425 region of the gene. Further studies are in progress. On development of transgenic cardamom expressing coat protein gene during current year, 1.8 kb RT-PCR product containing partial Nib (RNA polymerase) and complete coat protein codons was cloned in pBS 11 KS and sequenced. Further, regeneration of cardamom from calli derived from rhizome has been achieved.

Tamil Nadu Agricultural University, Coimbatore: In the present study, map based cloning strategy was adopted to tag gene(s) of resistance to leaf folder. The two hybrids between susceptible and resistant lines were forwarded to succeeding generations. Two sets of such mapping populations were made. The first is at F7 stage and the second at F4 stage. Phenotyping revealed the polygenic nature of leaf folder resistance and by bulk segregate

analysis first set of mapping population with 325 random 10 – mers, three closely linked RAPD markers were identified.

In another project on tagging genes for brown plant hopper (BPH) resistance in rice, recombinant inbred lines were developed by crossing IR- 50 and a resistant line and this was used for tagging and positioning gene resistant to BPH. RAPD and SSRs were used for gene tagging. Four RAPD and two microsatellites were found to be tightly linked with BPH resistance. These markers can be used for genotype selection in breeding programme. On DNA fingerprinting of rice varieties released by TNAU, 43 varieties were available for the analysis. Usual primers amplified a total of 1588 loci. Among the varieties, the number of loci amplified ranged from 68 to 96. Out of 116 common loci amplified among all the varieties, 76 were polymorphic and 40 monomorphic. PCR was also performed with the complete set of 43 varieties using four operon primers. In a project on molecular mapping for yellow stem borer (YSB) resistance in rice, a moderate resistant variety to YSB was crossed with a susceptible variety and F1 was fixed morphologically as well as by molecular markers.

Bose Institute, Calcutta: In a project on metabolic engineering of inositol pathway in rice, sequencing of *Oryza* and *Porteresia* inositol synthase cDNAs (1.5 kb long) have been done. Also *in vitro* bacterial expression of the cloned inositol synthase(s) (RINO/PINO) was done. Standardisation of protocols for generation of *Oryza* and *Porteresia* in culture has been attempted before rice transformation work. In another project on cloning of molecular markers involved in salt tolerance and their over expression in salt sensitive and high yielding rice, amplification of cDNA for samdecarboxylase done. After the amplification, rice cDNA for OSBZ 8 (the ABRE binding transcription factor) and verification by DNA sequencing has been taken up. Two oligonucleotides containing tandom of ABRE and basal 35 S promoter sequence was synthesised. Tissue culture of rice seeds scutellum has been initiated.

The third project is on genetic approaches for the improvement of aromatic rice suitable for agroclimatic conditions in West Bengal and mapping of aroma gene(s). In this project agromorphological characterisation of more than 2000 F3 plants has been completed. Data analysis and quality characterisation is underway. For generation advance of the segregating population, a total of 1608 F4 families were raised in microplots during 1999 and more than 3000 F4 individuals have been selected and harvested for advancing. Also mapping of aroma gene using linked RAPD primers and chromosome 8 specific markers is being taken up.

Osmania University, Hyderabad: In order to introduce insecticidal genes of *Bacillus thuringiensis* and snowdrop lectin of *Galanthus nivalis*, transformation studies were undertaken with 18 indica rice varieties. The transformation efficiency was determined based on transient expression of GUS A and GFP reporter genes using the agro vectors. The infection of rice explants was carried out using different *Agrobacterium tumefaciens* strains. It was found that all cultivars tested, exhibited GUS and GFP activities after co-cultivation. Among the cultivars tested, it was found that the shoot apices of *Tellahamsa*, IR 74 and Pusa Basmati showed higher frequency of transformation events with the *Agrobacterium* strains. The hygromycin and ppt resistant calli obtained after infection with pSB 11 bar GNA pSB 11 bar Ubi. Cry 1 A (b) plasmid constructs were transferred to different regeneration media. Plants regenerated from ppt – resistant calli were analysed with herbicide basta test to see the tolerance. The putative transformants when treated with herbicide basta showed no damage with the herbicide.

Multi-institutional projects:

(i) **Development of transgenics in cotton, rice, mungbean and tomato:** A multi-institutional project with 12 implementing institutions was sanctioned in July 1999. The project

envisages collaboration between different labs. to develop transgenics of cotton, rice, mungbean and tomato resistant to biotic stresses. For cotton the main goals are insect resistance and resistance to cotton leaf curl virus (CLCV); for *Vigna*; resistance to Yellow mosaic virus (YMV); for rice, resistance to tungro virus and for tomato, resistance to tomato leaf curl virus (TLCV) and cucumber mosaic virus.

As an ongoing activity with most of the investigators, a set of three *Agrobacterium* based vectors with lox sites is being developed at the Dept. of Genetics, University of Delhi South Campus which would be made available to all those given the responsibility of cotton transformation. The modified Cry 1A(c) gene is likely to be made available in the next six months. The NCL, Pune would identify one proteinase inhibitor, which is strong antifeedent to *H. armigera* feeding on cotton. As the useful clones of tomato leaf curl virus and transformation system are available, transgenics would be developed within the next one year and subsequently could be tested. It was noticed that transformation frequencies in *Vigna* sps are low, therefore, attempts are being made to further optimise transformation conditions. M.D.University, Rohtak and MKU, Madurai would collaborate for the transformation work. Also IISc, Bangalore, would identify one or two sequences for generating transgenic cotton plants resistant to leaf curl.

(ii) Characterization and using the quality traits in wheat aided by molecular markers:

The main emphasis of the project had been to characterise and use the quality traits in wheat using molecular approaches so as to develop better end product making varieties. During the last one-year studies have been undertaken towards development of the mapping population and data generation for identification of molecular markers. As several genes govern most of the quality characters, studies for identification of molecular markers linked to these require a fixed mapping population. In view of above, recombinant inbred lines (RILs) have been developed for both bread and durum wheats. Success has also been achieved in identifying STMS and STS markers linked with the grain protein content and with pre harvest sprouting tolerance. The QTL analysis using associated primer (wmc 41) with the protein content, in the RILs was done through linear regression. Many putative markers have also been identified for the rest of characters, which are being confirmed using the respective mapping population.

Stable transgenic wheat plants with *bar* and GUS genes have been produced in high yielding cultivars. The transformation protocol has been modified for maximum efficiency. Expression of *bar* gene under maize ubiquitin promoter had been strong enough to offer protection against glufosinate concentration that killed most of the weeds.

3.3. BIOLOGICAL CONTROL OF PLANT PESTS, DISEASES AND WEEDS

Research and Technology Development: Projects under the National Biocontrol Network Programme have made steady progress. Achievements of these R&D projects in specific areas are as under:

Microbial Pesticides, Parasites and Predators: At **KAU, Kerala**, large-scale demonstration of the IPM for two important pests of coconut palm viz. *Opisina arenosella* (leaf eating caterpillar) *Oryctes rhinoceros* (Rhinoceros beetle) has been taken up in two centres in South Kerala and North Kerala besides in Coastal Karnataka. Stage specific parasitoids viz., *Goniozus nephantidis*, *Bracon hebator*, *Elasmus nephantidis* and *Brachymeria* sp. proved to be effective biocontrol agents against leaf eating caterpillars in large areas (2000 ha). Two effective bio-control agents namely *Baculorhynchus oryctes* and *Metarhizium anisoplae* are employed for the bio-suppression of rhinoceros beetle on coconut in a total areas of 1500 ha. At **University of Mysore**, formulations of *Beauveria bassiana* for the control of coffee berry borer using purified talcum powder, diatomaceous earth and corn starch as carrier materials and sodium alginate for

pelletization. At **KAU, Kerala**, the entomopathogenic fungus *F. pallidroseum* was used to control cowpea aphids. Trial was conducted covering an area of 41.09 acres. The predator *Chrysoperla carnea* was also multiplied and distributed to 71 farmers in Thiruvananthapuram, Kollam and Kottayam districts. The field efficacy of the combined use of fungal pathogen *F. pallidroseum* and the predator *C. carnea* were demonstrated for the management of pests of cowpea. At **NCL, Pune**, *Myrothecium verrucaria*, has been used for controlling the root infecting fungi like *Sclerotium rolfsii*. A patent is being filed for screening antifungal agents. A method for the preparation of novel enzyme inhibitor useful against agriculture pest has been standardised; a process patent is being filed. At **HPKV, Palampur**, mass production and utilization of biotic agents of important pests of cole crops and peas was done. Significant reduction in the population of *P. xylostella*, *P. orichalcea*, *H. armigera* and aphids was observed after the releases of *Trichogramma chilonis*, *Trichogrammatoidea*, *Diaeretiella rapae* and *Apanteles glomeratus* in cole crops and pea. Extension personnel/officers from Department of Agriculture, H.P. were imparted training on mass production and utilisation of biotic agents. At **MPKV, Rahuri**, a new method was developed for releasing the parasitoid in the field against potato tuber moth. Four Farmers' Melas and two Mahila Melas on "Production of biocontrol agents" were organised which were attended by a large number of participants. At **NARDI, Secundrabad**, *Trichoderma viride* a biofungicide was distributed to 2501 cotton and chilli growers in Andhra Pradesh. The areas covered in 2908 ha which was spread over 193 villages in nine districts. The fields treated with *Trichoderma* showed reduction of wilt caused by root pathogens. In Nellore, soil application of *T. viride* was done in 75 sugarcane growers field spread over an area of 131 ha, that were identified as hot spots for the red rot disease. In citrus growing areas of AP, 300 Kg of *T. viride* was distributed for soil application to control the root pathogens. At Nellore, *Trichogramma chilonis* cards (1300) were given to 46 sugarcane growers in 62 ha area to control early shoot borer. At **GBPAUT, Pantnagar**, a potential native egg parasitoid of *Pygaera* could be reared successfully on the eggs of *Corcyra cephalonica* that gave 9% parasitisation on the eggs of *Chilo* spp. *Trissolcus* spp.

Pheromones/Kairomones/Othersemiochemical: At **IICT Hyderabad**, Kairomones were used for the Integrated Management of Brown Plant Hoppers (BPH) and Yellow Stem Borer (YSB) in Rice Crop. In a project on "Insect sex pheromones process & dispenser development and isolation & identification as tools for Integrated Pest Management (IPM)", "Day and Night Regime facility" was established by installing "Reverse photoperiod climatic Chamber /Room" in the pheromone laboratory as a National Facility for IPM research in India. Under the development of Insect sex pheromones as a tool for IPM, bench scale preparation of two pheromone components at a batch size of 10g each, was successfully completed. Olfactory response was obtained towards several of the general plant volatiles that were tested through electroantennography on the male *Nephantis serinopa* (black headed coconut caterpillar). At **PDBC, Bangalore**, Semiochemicals were used to increase biocontrol potential of some important predators and parasitoids. Dodecane was found to enhance the parasitisation by *T. chilonis*. Higher EAG response was noticed from extracts of frass derived from *C. partellus* feeding on the host plant maize than frass from artificial diet. At **DRR, Hyderabad**, management of yellow stem borer, (*Scirpophaga incertulas*) was done through pheromone mediated mating disruption and mass trapping. Mass trapping was very effective over large areas. A field day was organised at Mandapaka and Suryaraopalem villages to popularise mass trapping technology among the farmers. Over 500 farmers, representatives from state government, and Scientists from state Agriculture University participated.

Botanical Pesticides (Plant Products): At **SPIC, Guindy, Chennai**, the photostability study of pure azadirachtin, an isolated from *Azadirachta indica* A. Juss under different conditions has been completed. Studies on bio-efficacy of the photodecomposition products suggest that photoproducts in some cases were as active as the parent compound. At **CIMAP, Lucknow**, for the development of Insect Growth Regulators (IGR) from natural products, bioassays of different plant part extractives of *Catharanthus roseus*, led to effective mean insecticidal IGR values against *Myzus persicae* Sulz, *Spodoptera litter F.* and *Helocoverpa amigera* Hub.

Transgenic Plants: For breeding tobacco varieties against biotic stresses at **CTRI, Rajahmundry, Hyderabad**, the two transgenic tobacco cultivars namely Hema and Jayasri were evaluated in contained “Open field” trial during 1998-99 season as per the approval given by the government. Through somaclonal variation, resistant lines were developed for tobacco leaf curl virus (TLCV) from the variety ‘Candel’. At **SPIC Science Foundation, Chennai**, studies were carried out on inducible promoters for the regulated expression of insecticidal toxins in plants. A proteinase, aprotinin was produced as a fusion protein with thioredoxin in *E. coli* and was purified. The purified product was used in in-vitro assays for testing its efficacy against insect gut proteinases in yellow stem borer (*Scirpophaga incertuals*), rice leaf folder (*Cnaphalocrocus medinalis*), rice grasshopper (*Oya nitidual*) and cotton garsshopper (*Cyrtacanthacris tartaricus*). Rice and tobacco plants were transformed with aprotinin constructs. Screening of the putative plants with PCR analysis indicated the presence of the gene in the plant.

IPM - as a component of INM: This programme has a major component on Biological control under Integrated Pest Management. This particular component of the programme has been developed based on the success achieved in the biocontrol R&D network programme and subsequently in the mission mode programme of the department, which was launched by DBT covering 14 states of the country. In this programme 14 centres are involved in various states viz., U.P, Kerala, Tamil Nadu, J&K, Assam, Gujarat, Karnataka, Andhra Pradesh, Maharashtra and Andaman & Nicobar. The achievements observed are that at **RARS, Lam, Guntur**, *Trichogramma chilonis* and Ha NPV were multiplied for use in the *Heliothis* management programme on test crops. For monitoring the adult population of *Helicoverpa*, pheromone traps 10/ha were arranged. Similarly bird purchase at the rate of 50/ha were arranged to attract bird predation. The *Trichogramma chilonis* was released at weekly intervals. Farmers were trained for IPM with emphasis on biopesticide technology. At **RRL, Jammu** - mass production of PGPR, biofertilizer and biocontrol bacteria was undertaken by fermentation using the SOPs. In total 120 demonstration plots were sown covering an area of 265 kanals in District of Jammu, Kathua and Udhampur. Crops grown were Bhindi, Beans, Brinjal, Cucumber, Chillies and tomato. Seed treatment was given to Bhindi, Beans and Cucurbits whereas in case of tomato chillies and brinjal seedling dip was applied. The results are very promising. At **PDVR, Varanasi**, NPV culture of *Helicoverpa armigera* and *Spodoptera litura* have been collected from different parts of the country and the adjoining areas. All the cultures have multiplied separately in the laboratory reared population of the host insects. Studies on the relative virulence of these viruses are going on in the laboratory by determining the LD₅₀ through bioassay on the test insects. At **AAU, Jorhat, Assam**, in a laboratory trial, *Beauveria bassiana* was found to produce more than 90% mortality of the tested population of the tea mosquito-bug, one of the menacing pests of tea. A few botanicals are combined with *Verticillium lecanii* for the management of the red spider mite, one of the persistent pests of tea in North-East region of India.

Development of Biopesticides / Biocontrol Technologies and Technology Transfer: Under the demonstration programme, 14 states were covered which include - Kerala, Karnataka, Madras, Maharashtra, Punjab, Haryana, Rajasthan, Uttar Pradesh, Andhra Pradesh, Gujarat, Jammu & Kashmir, Assam, Orissa and Andaman Nicobar. Farmers have adopted this biopesticides technology under IPM and have expressed their satisfaction about the performance of biopesticides technology in IPM. Successful demonstration trials in this programme have culminated as a recommendation by the State Dept. of Agriculture in various states. At **MPKV, Rahuri**, two villages adopted for field trials were declared as "Biocontrol village" by the University. At **Tocklai Experimental Station (TES), Jorhat** 24 Tea states were covered throughout Assam for biological control of stem and root diseases of tea. Success has been achieved in reducing the rehabilitation period of Tea from 2 yr. to 3-6 months for replanting purposes. At **CTRI, Rajahmundry**, an IPM kit was developed for the management of *S. litura* in tobacco nurseries and kits were distributed to farmers as free incentives for adoption of this technology. At **RRL, Jammu**, fermentation based mass production technology of *Trichoderma viridae* was perfected, standardised and has been transferred as an integrated package for biofertilizers and biopesticides. Mass production technologies of some of the other biocontrol agents like – *Myrothecium verrucaria*, *Trichogramma*, *Trichoderma viride*, *T. virens* and *Beaveria bassiana* and *Verticillium lecanium*, *Bacillus spp.* also being patented. The biocontrol products developed were distributed among the farmers free of cost for use in various crops viz., cotton, rice, groundnut, sunflower, pulses, sugarcane, tobacco, Chilli, and vegetables.

Under this programme, RRL, Jammu has transferred the technologies as an integrated package for biofertilisers and biopesticides to two industries viz., M/s Pratishta Industries Ltd., Secundrabad, A.P. and M/s Javeri Agro-industries & Investment Co. Ltd., Javeri Nursery Premises, Amrawati, M.S. Besides, 4 new potential biopesticide technologies are being revalidated for commercialisation. These include bioformulations of (1.) *Aspergillus niger* (fungal antagonist), (2.) *Debaromyces hansenii* (yeast antagonist), (3.) *Steinernema carpocapsea* (nematode) and (4.) internode borers lure. (Sex pheromones An IPM package/module has been developed for green/organic cotton. A fungus (*B. bassiana*) based technology is also under development for the management of coffee berry borer pest. The results obtained are promising.

The completed mission mode programme was reviewed to consolidate the achievements in the individual projects as well as to assess/analyse the overall impact of this goal-oriented, time-bound programme. Important issues related to the constraints in adoption of biopesticide technology in IPM and technology transfer, limitation in commercialisation; involvement of industry as joint partnership alongwith steps to be taken up by Govt. and Non-govt. organisations for the promotion of biopesticides were addressed and deliberated at great length and some major recommendations have emerged out. Issues related to guidelines/data requirements for registration of biopesticides was also taken up in the context of the latest notification by the Central Insecticide Board wherein all the biocontrol agents including antagonistic fungi and bacteria have been put in the schedule to the Insecticide Act, 1968.

3.4. BIOFERTILIZERS

To understand the basic mechanism of Nitrogen Fixation, which will help in technology development, a number of projects, were supported on crop specific biofertilisers. High Nitrogen fixing strains of Blue green algae, Rhizobium, Acetobacter, Azospirillum and Azotobacter and high phosphate solubilising Mycorrhiza have been isolated and demonstrated in field conditions. These strains are suitable for different agroclimatic regions. Technologies developed for mass

production of Rhizobium and Mycorrhiza have been transferred to small entrepreneurs and corporate body for commercial production. A network programme on integrated use of biofertilisers in nutrient management for region specific cropping systems has been started.

3.4.1. Integrated Nutrient Management Programme: INM project implemented at G.B. Pant University of Agriculture and Technology for sugarcane based cropping system indicated that microbial population increased. Populations of Azotobacter and fluorescent pseudomonads in sugarcane have shown good stability and competent behaviour of introduced bio-inoculants. Soil dehydrogenase levels confirm this information further highlighting increased microbial activity in the rhizosphere zone as a measure of good soil and plant health. In situ soil DNA and total lipids are to be used to decipher change in the microbial community structure.

The project at MS Swaminathan Research Foundation is being implemented in six villages of Pondichery with the main objective of testing and demonstrating the role of various proven and locally available biofertilisers for rice-rice-rice and rice-rice-pulse cropping systems for three consecutive years in the same field with four treatments using three kinds of biofertilisers.

Analysis of surface soil samples collected during survey of Udaipur district by Rajasthan Agricultural University, Udaipur showed that in general chemical soil properties were favourable for establishment and multiplication of strains. Native population of Azotobacter and Azospirillum were low and hence, responded to inoculation. Field trials of Sorghum and Soybean showed the beneficial effect of biofertilisers when used in combination with organic manure and chemical fertilisers.

Seven Pseudomonas isolates, 16 *Azospirillum* isolates and 8 phosphate solubilizing bacterial isolates were obtained from rice rhizosphere by Assam Agricultural University, Jorhat. Growth of a HYV rice was improved due to application of either Azospirillum + PSB + Compost or NPK fertiliser compared to the control. Organic carbon content and microbial biomass in soil improved due to application of compost + biofertilizer.

Indian Agricultural Research Institute, New Delhi is concentrating on integrated use of biofertilisers for Rice-wheat-legume based cropping system. The results indicated that presence of BGA and / or Azolla lead to increase in grain yield at different levels of inorganic nitrogen. The inoculation of BGA alone increased the grain yield at lower levels of nitrogen (N₃₀ and N₆₀). There was significant increase in grain yield with inoculation of Azolla. Due to the application of biofertilisers a significant increase in grain weight of rice variety PNR 381 was observed. A combined application of two or more biofertilizers was always superior.

Panjabrao Deshmukh Krishi Vidyapeeth Akola implemented INM project for wheat-legume -millet-cropping system. The trial with bajra in Kharif has been conducted.

Banaras Hindu University, Varanasi studied colonisation of rice roots with more than 25 strains of cyanobacteria. Only two species of symbiotic cyanobacterium, *Anabaena cycadeae* showed active colonisation. Nitrogenase activity of colonised filaments was always higher than the free-living counterpart. Rice plants colonised by N₂ - fixing *A. cycadeae* showed significant increase (upto 20%) in fresh wt. 14-C labelling experiments revealed transfer of metabolites from *A.cycadeae* to rice plants. Endophytes isolated from stem and root parts of Saryu-52 have been studied in greater detail. Maximum number of putative endophytes was present in stem part just above the root. Few of them were common in root and stem.

U.P. Council of Science & Technology, Lucknow is working on rice based cropping system. The three cropping system proposed are Rice-Wheat-mung/Daincha, Rice-Pea-

Mung/Dhaincha and Rice-Gram-Mung/Dhaincha. Studies on this cropping system have already started in 8 districts of Uttar Pradesh, which represent 8 different agro-climatic zones.

3.4.2. Other Programmes on Biofertilizers: In addition to INM programme Department also supported projects on various biofertilisers specific to crops.

Rhizobium: National Research Centre for Groundnut, Junagarh has implemented project on isolation of efficient nitrogen fixing strain of Bradyrhizobium for groundnut growing regions of Gujarat. A survey of groundnut growing areas was made in five districts of Saurashtra and collected soil and plant samples of groundnut in order to isolate efficient strains of Bradyrhizobium. A total of 263 isolates were obtained from root nodules and soil samples. Antibodies, bacteriocinogeny and siderophore production was the basis of determining the competitiveness traits. The competitive isolates are being tested for nodulation and efficiency of nitrogen-fixation efficiency in pot and growth chamber.

Six Nod strains of Rhizobium sp. (*Cajanus*) were isolated from root nodules of pigeonpea plants growing in CCS HAU Farm. Work is in progress for the isolation of heat shock resistant mutant strains of Rhizobium sp. (*Cajanus*) through high temperature treatment and chemical mutagenesis.

Acetobacter and Azobacter: Tamil Nadu Co-operative Sugar Federation Ltd, Chennai isolated strains of nitrogen fixing *Acetobacter diazotrophicus* and *Herbaspirillum* spp. isolated from sugarcane varieties. These were used in growing micropropagated sugarcane commercial varieties. These platelets after a growth period of 15 days were analysed for their whole plant nitrogenase activity and enumeration of inoculated microbes.

Jawaharlal Nehru University, New Delhi has developed a transgenic Azotobacter, which can fix more nitrogen than the normal strains. This has been achieved by making the *nifL* gene inactivated by insertion of a constitutive tetracycline promoter. This has resulted in three-fold increase in the synthesis of NH_4^+ .

Mycorrhiza: A survey of various crop-fields of minor millets- viz. *Paspalum scrobiculatum*, *Eleusine coracena*, *Panicum*, *Setaria pumila*, *Setaria italica* and *Echinolca frumentacea* of Santhal pargana and Bhagalpur divisions has been conducted by T.M. Bhagalpur University for the AM-fungi associated with these crops. Various species belonging to different genera of AM-fungi were identified and isolated. Total phosphorus, organic carbon, potassium content, and pH of soil of crop-fields surveyed and the experimental plots were determined and findings were correlated with the density of AM-spores / frequency of AM association. Three crops, *Sorghum*, *biocolor*, *Zea mays* and *Panicum miliaceum* grown in the experimental plots were found to have dense association of VAM fungi. On the basis of their positive responses, these crops were used as the host plants for inoculum production/multiplication of the AM fungi.

Blue Green Algae: Forty two species of Cyanobacteria belonging to the genera *Microcystis*, *Chroococcus*, *Gloeocapsa*, *Aphanothecea*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Microcoleus*, *Cylindrospermum*, *Wollea*, *Nostoc*, *Anabaena*, *Aulosira*, *Scytonema*, *Calothrix*, *Glboetrichia* and *Westiellopsis* were identified at Rajendra Agricultural University, Pusa, Bihar. Wide variation was recorded in cyanobacterial growth and nitrogenase activity in incubated soil samples collected from different soil types. Field trails revealed that application of different cyanobacterial inoculants alone or in combination with fertiliser nitrogen brought significant increase in grain and straw yield over the respective controls.

Allahabad University surveyed forty localities in ten districts viz., Allahabad, Pratapgarh, Raibareli, Fatehpur, Jaunpur, Hardoi, Bareilly, Varanasi, Basti and Moradabad for the growth of Blue-green Algae in rice field. Ten localities of Allahabad district were kept under close

observation for gradual change in floristic pattern during the paddy season and also for their recurrence in subsequent years and strains were isolated. At present non-heterocystous and filamentous forms (*Oscillatoriales*) 58 strains, and *Nostoc*, *Anabaena* complex (Nostocales) 80 strains are being maintained. These strains were characterised on the basis of morphological and physiological parameters. C.S. Azad University of Agriculture and Technology, Kanpur also conducted similar studies and a number of promising strains of BGA were isolated and characterised.

3.4.3. Transfer of Technologies of Mass Production of Biofertilisers

Mycorrhiza: Mass production technology of AM fungi which is suitable for almost all crops including plantation crops and forest trees developed with DBT's support at TERI, New Delhi transferred to M/s Cadila Pharmaceuticals, Ahmedabad.

Rhizobium: Fermenter based production technology of Rhizobium suitable for soybean, rajmash, mungbean and french bean to M/s Pritistha Agro Industries, Secunderabad and Javeri industries on Rs. 8.00 lakhs and 2.5% royalty from each.

Patents: a) Role of flavanoids in root nodulations of legumes, b) Large-scale production of AM fungi, c) Large-scale production of Rhizobium biofertilizer in fermenter.

3.5. TREE AND WOODY SPECIES AND APPLICATION OF TISSUE CULTURE

1. Plant Tissue Culture: Studies were continued on standardizing/developing complete protocols for regeneration of economically important plant species - Forestry, Horticulture and Plantation Crops. Regional hardening facilities and Green Houses continued plant production in order to demonstrate the feasibility of large-scale production of important plant species through tissue culture. Specific programmes were supported for field demonstration of those plants for which large-scale production protocols have been developed and evaluation is necessary before commercialization.

(i) Forest Trees: In the arid zone region, large scale production of *Wightii arborea* and *Boswellia serrata* continued. 20,000 plants of *W. arborea* have been produced and hardened. There were 80 – 90% successes during hardening and 99% survival on transfer to field. In the case of *B. serrata*, 10,000 plants have been produced, however hardening percentage is very low. Multilocation field trials are now being initiated and field data evaluated. The region is effectively using the hardening facility at Jodhpur. In addition, the Centre itself has perfected protocols for a number of Arid Zone tree species and nearly 20,000 plants transferred to the field at different locations in collaboration with the State Forest Department and other agencies.

The technology for mass multiplication of *Commiphora wightii* through somatic cell culture and embryogenesis is being perfected. Presently cultures - embryogenic and non-embryogenic are growing and attempts are being made to reinstate embryogenic cell suspension and develop methods of obtaining embryogenic cultures from non-embryogenic cultures. Somatic embryos from mature leaf serve as a better system for regeneration and propagation of plant species.

Studies continued on Micropropagation of high yielding azardiachitin clones of Neem. 2 lakh plants have been produced and planted in approximately 15 multilocation sites. In the Temperate and Himalayan region tree species, protocol standardisation for *Sapium* and *Bauhinia* continued. The growth performance was evaluated and *in vitro* raised high yielding superior plant material of *Sapium* and *Bauhinia* was established in experimental plots in arboretum. The growth performance has been evaluated and results suggest that the *in vitro* raised plants

gradually adapted to the natural environment and thereafter performed better in terms of plant height and leaf number. Plantlets are now ready for plantation in the marginal lands.

Plant regeneration has also been standardised in *Pinus* sp, *Cedrus* sp and *Querus* sp. Studies are being conducted on field establishment of the plants and the effect of various strains of plants and the effect of various strains of bacteria, microorganisms and mycorrhizae on the germination, growth etc. A multi-institutional programme has also been initiated on Biohardening of tissue culture raised plants with different strains of Mycorrhizae. Studies have been initiated on different multipurpose and medicinal tree species.

A project has also been initiated for multiplication and field establishment of Seabuck thorn. Studies were also undertaken for regeneration of species of the Bay Island. Protocol for 3 Timber species – *Pterocarpus dalbergioides*, *Terminata bilata* and *Diospyros marneorata*. The studies on Mangrove species also continued successfully. A large number of plants were transferred to the Genetic Resource Centre at Pichavaram and also along the Coastal region. RAPD and RFLP studies are also being continued for studying the genetic variability.

A Regional Hardening Unit has been set up at Jammu for large-scale production of Medicinal and Aromatic Plants and Horticulture Crops.

(ii) Horticulture Crops: Studies on regeneration of Mango continued in the commercially important varieties of Dashera, Chausa, Langra, Alphonso, Amrapali, Totapuri and Neelam. The regeneration protocol from nucellar and zygotic embryo has been perfected. Axillary bud shoot proliferation has also been achieved. Studies are continuing for standardising the synchronised development of nuclear embryo and establishment of plants in the soil.

In the case of citrus, mother grove of disease free Darjeeling orange plants has been established in insect-proof green houses in experimental fields. Double grafted plants have been transferred to the greenhouse and some of them have begun fruiting. A comparison of the double grafted plants vis-à-vis the single grafted reveals that the heights, branching and flushing were higher in the case of double grafted.

Tissue culture raised plants of Rangpur Lime and Rough Lemon have been produced and 600 plants field planted. Protocol for mass multiplication of Khasi mandarin has also been perfected. Shoot tip grafting has also been perfected and micrografts are being produced.

The Bunchy Top Virus in Banana is being characterised and the host response studied. Banana plantlets derived from meristem culture were transferred to glass house. A mutant clone showing resistance has been multiplied and the resistant plants are being screened. Protein studies are being conducted for characterisation of BBTV and PCR based system has been developed for detection of BBTV.

A project has also been initiated for large-scale production and extension of Tissue Culture raised Banana. Different cultivars performing well in Maharashtra were identified and plants raised in vitro. Large-scale plantlet production was achieved and evaluation trials are being conducted in some districts and selected sites. Studies have also been initiated for developing virus resistant papaya plants by introduction and expression of the PRV Coat Protein (Cp) gene in both sense and antisense orientation. Regeneration system from somatic embryos was perfected. Isolation and purification of Poly A⁺ RNA from infected leaves of Papaya is in progress. A study has been initiated for mass propagation and field evaluation of Tissue Culture raised rootstock of Apple. The protocol for regeneration of Walnut (*Jugalans regia*) is also being developed. Indirect somatic embryogenesis has been reported from callus derived from embryonal axis and cotyledonary explant.

Programmes have also been initiated for improvement of certain floricultural crops. A protocol for genetic transformation of *Dianthus* through *Agrobacterium tumefaciens* mediated transfer is being worked out in order to transfer gene related to control of flower colour development. The regeneration protocol via organogenesis and somatic embryogenesis is also being perfected for large-scale multiplication.

Rare and endangered flowering plants of the Western Ghats are also being regenerated through tissue culture. The protocol has been standardised and the plants established in the Green House.

Study continued for Breeding of interspecific and intergeneric hybrids of Orchids. A number of successful intravarietal and interspecific and intergeneric crosses involving sympodial and monopodial varieties have been made. 37 crosses have already produced pods / capsules.

(iii) Plantation Crops: A major initiative of the Department has been the Network Multi-institutional Programme on Coffee improvement using biotechnology tools. The programme aims at developing improved coffee varieties with low caffeine and resistant to diseases. The present “Network Programme” envisages several molecular approaches for Coffee, such as characterisation, mapping and inventorisation of coffee gene pool and hybrids in India, developing linkage map of coffee for use in marker assisted breeding, molecular basis of abiotic stress tolerance, *Agrobacterium* based transformation technologies for disease tolerance, microbial degradation of caffeine and ‘Bt’ based transformation technologies for pest resistance etc. The biotechnology programme in coffee, which is being implemented as an inter-institutional and multi-centric collaborative programme, is expected to generate a wealth of information on molecular aspects of coffee and to establish the potential application of biotechnology in coffee genetic improvement.

In addition the field evaluation of the tissue culture raised plants is also being conducted. Approx. 2000 plants have been hardened and field planted in the field. Data collected on these plants raised via somatic embryogenesis reveal that the growth parameters are at par with the seedling-raised plants.

Field evaluation of tissue culture raised Tea plants of 3 UPASI clones – UPASI – 3, 26 & 27 is being conducted under different agro-climatic conditions at multilocations. Data collected indicate a better growth of TC raised plants. R&D efforts are also being continued for establishing an Anther Culture Protocol for Tea. Androgenic calli establishment and plantlet regeneration is being attempted. High frequency embryogenesis and embryo conversion has been difficult. 78% explants could be successfully induced to follow the normal route of embryo maturation and development giving rise to healthy plants.

A complete protocol for regeneration of Black Pepper has been standardised. More than 400 plants have been produced. 250 in vitro raised plants have been field planted at different locations in three districts and are being evaluated vis-à-vis the commercially raised propagules. No significant variation was reported in the morphological characterisation, however better establishment was observed for TC regeneration, mainly due to a better root system establishment.

Protocol regeneration studies are also continuing for Nutmeg, Clove and Cinnamon. Plantlet regeneration has been achieved in the case of Clove, however the protocol is still being perfected for large-scale multiplication.

The protocol for Saffron regeneration has also been developed. The technology is now being refined for scale-up. The protocol is based on in vitro cornlet production via multiple shoot induction. Large-scale multiplication of the cornlets in Bioreactors is being attempted.

Studies have also been supported on Tissue Culture regeneration of other priority crops – Ginger and Cashew.

2. Genetic Variability of Tissue Culture Raised Plants: In order to scale-up and commercialise the technology developed for tissue culture regeneration, it is essential to establish the genetic fidelity of the TC raised plants. Detailed studies have been conducted on the use of molecular markers for the study of genetic variation of tissue culture raised plants. The micrografted plants have been assessed at gene expression level. Using differential display approach, studies have been conducted on *Eucalyptus*, *Populus*, Teak, Pepper, Tea & Coffee. A suitable method for extraction of quality DNA in Coffee, Pepper, Teak and Tea has been developed. In the case of *Eucalyptus* sp 12 arbitrary, 10-mer primers and six synthetic oligonucleotide sequences successfully used to amplify genomic DNA from in vivo and in vitro materials produced 133 fragments that were monomorphic. This report indicated that enhanced axillary branching culture of mature trees could be utilised commercially for mass clonal propagation of *Eucalyptus* sps, which have been recalcitrant to vegetative propagation. Studies also indicate only 2.8% polymorphism in tissue culture derived plants of Coffee.

DNA fingerprinting studies are also being conducted for documentation of Tea clones and developing RFLP / RAPD markers for phenotypic characters of economic importance.

3.6. BIOPROSPECTING AND MOLECULAR TAXONOMY

A multi-institutional collaborative programme on “Bioprospecting of Biological Wealth using Biotechnological Tools”, was initiated in Oct’97 and the main objectives of the programme are (a) landscape & biodiversity characterisation, biome level ecological zone map; (b) collection and documentation, molecular characterisation, and (c) gene / biomolecule prospecting. There are 15 institutes involved in the study, which is concentrating mainly in the two hot-spot regions – North-Eastern Himalayas and South-Western Ghats and also the Western Himalayas. The Institutes involved and species being studied are detailed in the table below:

INSTITUTES INVOLVED	SPECIES BEING STUDIED
Indian Institute of Remote Sensing, Dehradun	<ul style="list-style-type: none"> • <i>Taxus baccata</i> • <i>Rhododendron spp.</i> • <i>Hippophae spp.</i>, One or two species in Western Ghats
National Chemical Laboratory, Pune Botanical Survey of India, Pune Tropical Botanic Garden & Research Instt., Thiruvananthapuram.	<ul style="list-style-type: none"> • <i>Symplocos laurina</i> • <i>Symplocos racemosa</i> • <i>Ternstroemia japonica</i> • <i>Gaultheria fragrantissima</i> • <i>Eurya nitida</i> • <i>Rhododendron arboreum</i> • <i>Mahonia leschnaultii</i>
Delhi University, Delhi G.B.Pant Instt. of Himalayan Environment & Development, Almora Y.S.Parmar Univ., Solan	<ul style="list-style-type: none"> • <i>Valeriana jatamanasi</i> • <i>Gentiana kurroo</i> • <i>Aconitum heterophyllum</i> • <i>Acpnitum balfourii</i> • <i>Podophyllum hexandrum</i>

	<ul style="list-style-type: none"> • <i>Piccorrhiza kuroa</i> • <i>Dactylorrhiza hatageri</i>
Instit. of Himalayan Bioresource Technology, Palampur	<ul style="list-style-type: none"> • <i>Hippophae rhamnoides</i> • <i>Hippophae tibetana</i> • <i>Potentilla spp.</i> • <i>Dactylorrhiza hatagirea</i> • <i>Arnebia euchroma</i> • <i>Aquelgia spp.</i> • <i>Artemissia spp.</i> • <i>Gentiana spp.</i> • <i>Genum elatum</i> • <i>Hordeum spp.</i> • <i>Picorrhiza kurrooa</i>
Guwahati University, Guwahati	<ul style="list-style-type: none"> • <i>Nepenthes khasiana</i> • <i>Plumbago rosea</i> • <i>Coptis teeta</i>
G.B.Pant University of Agriculture & Technology, Pantnagar	<ul style="list-style-type: none"> • <i>Berberis aristata</i> • <i>Boenninghauseni albiflora</i> • <i>Cleome viscosa</i> • <i>Erigeron karvinskianus</i> • <i>Polygonum spp</i>
Central Instit. for Medicinal and Aromatic Plants, Lucknow	49 Species of Medicinal Plants
Tata Energy Research Institute, New Delhi	<ul style="list-style-type: none"> • <i>Eucalyptus camaldulensis</i> • <i>Callistemon lanceolatus</i>
M.S.Swaminathan Research Foundation, Chennai	<p>Ten species of the family Rhizophoraceae</p> <ul style="list-style-type: none"> • <i>Avicennia marina</i> • <i>Lichens</i>

The and achievements are as follows:

a. Landscape and biodiversity characterisation: The Department of Space is involved in this study and is working towards developing complete Biome maps of the identified area. A prototype study has been carried out in the state of Meghalaya, which has provided a basic framework for further studies. The digital analysis is in progress for the vegetation and land use mapping from different state. Under the phase-I of the programme, studies have mainly been concentrated on North-Eastern region and maps have been prepared at a scale of 1: 2,50,000. Deptt. Of Space has been able to Map Taxus and its associated species in the Tale Valley of Arunachal Pradesh. This is significant as this species occurs gregariously and is well protected.

Indian Institute of Science, Bangalore involving 13 colleges in 5 States, has evolved a Biomonitoring system. Monitoring of 15 organismic groups and the number of study sites from where information on these organismic groups have been collected has been recorded. Nine training courses have been conducted, one prospectus and two field guides prepared and a

publication of five focal organismic groups has been carried out as part of the attempt to develop monitoring methodologies and human resource development for biodiversity monitoring.

b. Documentation and molecular characterisation: Complete documentation and genetic characterisation is being done for 10 identified priority species which are endangered and of medicinal and economic importance. The genetic DNA fingerprinting of 26 accessions of *Symplocos laurina* and 34 accessions of *S. racemosa* which are common to South-Western and North-Eastern regions has been carried out. DNA finger-printing of 12 accessions of *Gaultheria fragrantissima* and 9 accessions of *Eurya nitida* have also been carried out.

DNA and cytological analysis of different genotypes of four identified priority species has been carried out for all samples of leaf, root tips and somatic cells. The analysis results have reported for the first time, the allopolyploid nature of *Aconitum balfourii*. These results would be very beneficial in the efficient use of the wild species of the germplasm. Analysis of species of *Aconitum* and *Podophyllum* for Podophyllotoxin content is being carried out. 59 accessions of *Andrographis paniculata* from various phyto-geographical regions of the country have been collected and maintained. Primary analysis of the germplasm comprising of 16 accessions indicates moderate genetic diversity and results of the chemical prospecting indicate the uniform amount of andrographolide is present in all accessions. ISSR polymorphism has been studied in 16 different accessions.

Studies on the microbial diversity along the coastal agri-ecosystem and forest system in the Western Ghats is being conducted. For the Lichen component, 39 transacts have been laid and various parameters recorded. Study of the diversity with reference to the functional aspects and stress-tolerance is underway.

15 plant species from coastal systems have been collected and screened for potential anti-microbial and anti-pesticidal properties against 10 microbes.

c. Gene/Biomolecule Prospecting: Isolation, characterisation and cloning of potential genes for stress tolerance is being carried out. A salt tolerant gene has been identified and successfully expressed. The complete c-DNA library from mangrove species has been constructed and this has been screened for stress related genes. A gene resistant to cold temperature has also been identified and is now being isolated and characterised.

Five plant species having a broad spectrum of biocidal activities have been screened from 123 plant species belonging to 6 families. 5 Plant extracts have been found to exhibit strong anti-bacterial activity against human pathogenic bacteria. 4 plant extracts have exhibited strong anti-fungal activity against dermatomycotic fungi. Plant extracts from 2 species have been fractionated with strong insecticidal properties.

3.7. PROGRAMME ON MOLECULAR TAXONOMY

Closely linked to the Bioprospecting Programme, the Department has initiated a major Programme on Molecular Taxonomy with the main objective of inventorisation and documentation and molecular characterisation of identified economically important and / or endangered species from different regions of India for their sustainable utilisation. A collaborative effort between the molecular biologists and taxonomists have been evolved. Each group comprising of a Molecular Biologist and a Taxonomist would work in a specific region targeting identified species. All the available accessions of the plant species – wild, cultivated and landraces would be identified and catalogued according to threat-perception, economic importance and DNA fingerprinting. A specie-specific database has already been initiated,

would finally be evolved. Details of the above programme have been summarised in the table below:

<u>INSTITUTES INVOLVED</u>	<u>REGION</u>	<u>SPECIES BEING STUDIED</u>
National Chemical Laboratory, Pune Sri Krishnadevaraya Univ., Anantpur & M.S.Swaminathan Research Institute, Chennai	Western Ghats Eastern Ghats	a) Legumes : Cajanus & Trigonella b) Legumes & Millets : <i>Vigna</i> , <i>Dolichos</i> and <i>Phaseolus</i> complex; <i>Cajanus</i> , <i>Atylosia</i> , <i>Rhynchosia</i> , <i>Paracalyx</i> and <i>Dunbaria</i> complex; <i>Crotalaria</i>
Regional Plant Resource Centre, Bhubaneswar & National Chemical Laboratory, Pune Univ. of Delhi, South Campus, New Delhi & Univ. of Delhi, Delhi Rajasthan Univ, Jaipur & Tata Energy Research Instt., New Delhi	<u>Western Ghats, Orissa</u> Desert (Rajasthan)	a) Tree Legumes : <i>Acacia</i> , <i>Albizia</i> , <i>Sesbainia</i> b) Non-Timber : <i>Acacia catechu</i> ; <i>Madhuca latifolia</i> ; <i>Stericulia urens</i> c) Desert Tree : <i>Prosopis</i> ; <i>Tecomella</i> ; <i>Lasiurius</i>
Central Instt. for Medicinal and Aromatic Plants, Lucknow		a) <i>Phyllanthus</i> b) <i>Asparagus</i> c) <i>Vitievaria</i>
Tropical Botanic Garden and Research Instt., Thiruvananthapuram.	Western Ghats Western Peninsular India	a) <i>Piper</i> b) <i>Curcuma</i>
Himachal Pradesh Krishi Vishwavidyalaya, Palampur	Himalayan region	a) <i>Delphinium</i>

3.7.1. Jute Biotechnology: Under the Phase-1 of the UNDP-GOI supported project being implemented at 3 centres – IIT Kharagpur, M.S.Univ Baroda and Calcutta University a laboratory scale protocol for enzyme retting for jute fiber was developed. The process involves retting of the fiber using an enzyme obtained from different micro-organisms. A number of fungal and bacterial strains have been identified which secrete large quantities of retting enzymes. These organisms have been identified from the soil and water available in the natural retting environment and also from other sources. Both solid and liquid state fermentation protocols were standardized for optimal growth of these organisms in culture conditions. A laboratory level protocol was found to be suitable for both green and dried jute ribbons and the lab level results indicated that the fiber obtained after enzymatic retting was superior in quality without deterioration of the physical characteristics.

A field demonstration was conducted to test the efficacy of the protocol. The results of the demonstration trial indicated that further process optimisation was required in order to tackle the environmental conditions. The field trials also gave an insight into the type of infrastructure required both for large scale enzyme production and the retting tanks. From the results obtained there was a clear understanding of the temperature conditions required for storage of both green and dried ribbons.

A second demonstration has also been conducted in 1999 alongwith National Centre for Jute Diversification, Calcutta. The results of this demonstration indicate that the enzyme could now be produced on a large scale at the farmers field. However there have been clear results which now indicate that the enzyme consortia to be prepared should take into account other related parameters such as maturity of the raw-material, temperature for storage, ambient temperature for retting and other environmental factors which effect the retting process.

Based on the results achieved during the two demonstration trials the scientists from the three institutes are now working on fine tuning of the technology. A complete survey and collection has been done of the micro-organisms from both soil and water available in the natural retting environment. Superior strains have been identified which yield higher quantities of enzymes. There is also an attempt to genetically manipulate these micro-organisms so that not only is there higher production of enzyme, but it is also possible for the enzyme to tackle different types of raw-material. There is an concerted effort to develop a user and environmental friendly enzyme which can be conveniently and cost effectively produced by the farmers themselves. This process has enormous potential not only for reducing the duration of the retting process but also in reducing the cost.

3.8. MEDICINAL AND AROMATIC PLANTS

Application of biotechnology for conservation, micropropagation, production of secondary metabolites, biotransformation of intermediates into pharmaceutically important products and genetic improvement of medicinal and aromatic plants have been taken up. The salient achievements of recently completed/on-going projects are as follows: -

National Gene Banks for Medicinal & Aromatic Plants:

Tropical Botanic Garden and Research Institute, Thiruvananthapuram: A total of 2360 accessions of 89 targeted and 485 other species of medicinal & aromatic plants (MAP) were selected and introduced into the gene bank. Over 1360 herbarium specimens were also collected using which 574 taxa were identified taxonomically. The collected accessions were propagated, multiplied and over 10,000 plants were under cultivation over 20 Field Gene Bank (FGB) plots spread in about 50 acres of protected forest land. Over 100 rare/endangered/threatened and endemic species of MAP were

propagated, cultivated and maintained in the FGB. Morphological, cytological and molecular characterisation with passport data was done in about 80 variants of 26 MAPs species. Reproductive biological studies were carried out in 7 species. Quality seeds of 48 MAP were collected, dried and stored in the seed bank. In the *in vitro* bank, 29 accessions belonging to 26 targeted rare and important MAP are maintained. Micropropagation protocols were standardised for 24 MAP species. Protocols on cryopreservation of three targeted species were also standardised. Over 50,000 medicinal plants raised at the nursery were distributed to the farmers, local physicians and rural women for cultivation.

National Bureau of Plant Genetic Resources, New Delhi: Seventeen exploration trips were conducted and a total of 700 accessions were collected in form of seeds, roots, rhizomes and bulbs etc. A total of 125 herbarium specimens were also collected and deposited at National Herbarium of Cultivated Plants and Wild Relatives at NBPGR. Germplasm collections of *Acorus*, *Elsholtzia*, *Skimmia*, *Mucuna*, *Psoralea* from wild habitats were analysed for their chemical constituents. One hundred collected accessions are maintained in the field gene bank at Delhi and its regional stations at Bhowali, Shimla, Jodhpur and Shillong depending on the agro climatic requirement of the species. A total of 569 seed samples belonging to 62 genera and 97 species have been conserved for long term at -20° C in the seed bank. Twenty eight seed samples are maintained in medium term repository at 4° C and 15% RH. Seed samples belonging to 85 genera and 100 species comprising 220 accessions are maintained in cryobank at the temperature of -150° C (Vapour phase). In the *In vitro* bank, shoot cultures of *Picrorhiza kurroa*, *Gentiana kurroa*, *Saussurea lappa* and *Valeriana wallichii* could be conserved for 12-16 months at 4° C.

Central Institute of Medicinal & Aromatic Plants, Lucknow: A total of 30 expeditions have been organised for survey and collection of the target species covering 120 locations in 20 different states of the country. Accessions have also been procured from the other countries. A total of about 1500 accessions belonging to 185 genera of 57 families have been collected and are being maintained in different forms in the gene bank. About 185 species of MAP are being maintained in the field gene bank. A total of 41 species in seed gene bank and 24 species in DNA bank are being conserved. In addition to these, 17 plant species are also being maintained in the form of callus, multiple shoots, microplantlets and hairy root cultures in the *in vitro* bank. Experiments are underway for long term conservation of tissues in cryobank. Morphological and chemical characterisation of different accessions of *Papaver somniferum*, *Mentha* spp., *Centella asiatica*, *Withania somnifera*, *Ocimum* spp, and *Catharanthus roseus* have been accomplished. Molecular characterization of *Bacopa monnieri* and *Catharanthus roseus* have been carried out to establish distinct homogenous population for marker assisted selection programme. Based on evaluation of their genetic variability, comprehensive descriptors of *Cymbopogon flexuosus*, *C.martini*, *C.winterianus*, *Matricaria chamomila*, *Mentha* species, *Papaver somniferum* and *Pelargonium graveolens* as per international standards are under preparation. A total of 259 farmers and entrepreneurs were provided training on improved agronomic practices and processing technologies for the cultivation and production of medicinal and aromatic plants and plant products.

Regional Research Laboratory, Jammu Tawi: Studies have been initiated on the present status of resource availability of 10 target species (critically endangered high altitude medicinal plants) in North-West Himalayas. Several accessions of 66 species of medicinal plants were procured from Himalayan region and planted at field germplasm banks at Jammu, Palampur and Srinagar. Seeds and propagating material of 20 temperate medicinal plants were procured and stored under low temperature conditions. Investigations have been initiated for developing

micropropagation protocols for 15 Himalayan medicinal plants. The Cryobank facility is in the making.

Micropropagation: A protocol for obtaining somatic embryos with high frequency in liquid suspension cultures was developed for sandalwood at IISc, Bangalore. The biopolar/torpedo – shaped embryos recovered were found to be fairly uniform in size and morphology, most of which germinated into plantlets. A method of embryo multiplication known as repetitive embryogenesis or ‘Embryo cloning’ was standardised in order to enhance the production of embryos.

In vitro studies have been initiated on *Swertia chirata* at RRL, Jammu. Live plant material has been collected from different locations in Darjeeling region and Himachal Pradesh to be used as source for initiation of tissue cultures. Cultures initiated on different media compositions to find out optimum composition for ‘enmasse’ shoot proliferation. At Birla Institute of Scientific Research, Jaipur, axillary buds of *Crataeva magna* gave multiple shoots when cultured on modified MS medium. These shoots when cultured on half the strength of MS medium incorporated with 0.1 μ M NAA rooted after 30-40 days. The lupeol content in 10 plants of *C. magna* has been estimated and the explants for regeneration have been selected from the plants with highest lupeol content. Field trips were conducted to collect uprooted plants as well as fruits of two medicinally useful Asclepiadaceous members – *Decalepis hamiltonii* and *Hemidesmus indicus* at Sri Krishnadevaraya University, Anantapur. Different seedling explants such as root, cotyledon, internode and leaves of 60 days old aseptic seedlings were cultured on MS medium supplemented with various growth hormones. The explants from axenic cultures were tested for their morphogenic as well as callogenic vigour by employing different concentrations and combinations of hormones. At RRL, Bhubaneswar, sodium chloride tolerant lines of palmarosa grass (*Cymbopogon martinii*) were selected by exposing the wild type callus to increasing concentrations of NaCl in MS medium. The selected callus lines retained their salinity tolerance following 3-4 subcultures on NaCl free medium. Plantlets regenerated from NaCl tolerant callus were transferred to soil and grown under green house conditions with about 70% survival. Hardened and acclimatized plantlets obtained from the salinity tolerant callus lines are showing normal growth in salinized soil medium (100 mM NaCl – 0.6% NaCl) in the green house experiments.

The protocol for micropropagation of *Gymnema sylvestre* (an antidiabetic plant) from nodal segment has been developed at Kerala Agricultural University, Trissur. One hundred fifty tissue culture-derived plantlets were transferred to the field and are showing comparable growth rate with the conventional plants. At G.B. Pant Institute of Himalayan Environment & Development, Almora, studies have been initiated on evaluation and propagation of selected endemic medicinal plants of the Himalayas. Over forty percent of potential high altitude sites in Kumaun and Garhwal have been covered to identify and locate the populations of target taxa (*Arnebia benthamii*, *Angelica glauca* and *Saussurea obvallata*). Four populations of *A. benthamii* and *A. glauca* each and five populations of *S. obvallata* have been identified. Preliminary attempts have been made to standardize the protocols for protein and isozyme patterns in seed and rhizomes of different species collected from all possible sites.

Production of secondary metabolites/Biotransformation of intermediates: Cell suspension cultures were established from *Azadirachta indica* and *Withania somnifera* for production of therapeutics at Kakatiya University, Warangal. The addition of fungal elicitors and adsorbents (charcoal and kaolin) has not affected the production of azadirachtin and

withaferin–A. However, squalene has significantly enhanced the production of azadirachtin and withaferin–A over the controls in cell cultures of *A. Indica* and *W. somnifera*, respectively. At Kakatiya University, Warangal, the production kinetics of baccatin-III and deacetyl baccatin-III (DAB-III) in cell cultures of *Taxus wallichiana* have been studied. The elicitation of *T.wallichiana* with methyl jasmonate enhanced the baccatin-III and DAB-III over the control cultures. Arachidonic acid also improved the production of baccatin-III and DAB-III.

At JNU, New Delhi, hypoglycemic activity of *Momordica charantia* seed extract was established by testing its blood glucose lowering ability in diabetic animals and its lipogenic activity in cultured adipocytes. It was identified that the blood glucose lowering ability of *M. charantia* is due to a proteinaceous molecule whereas the lipogenic activity is due to a non-proteinaceous molecule. Two patent applications are being filed one for hypoglycemic peptide and the second one for lipogenic compound. Future studies are planned to be undertaken on complete characterization of peptide and lipogenic compound. Calli obtained from *Mappia foetida* Syn. *Nothapodytes foetida* – a source of anticancer compounds were screened by TLC for presence of camptothecin or 9 – methoxy camptothecin at NCL, Pune, HPLC was done to quantify the active principle in the callus. Promising calli lines have been identified for production of quinoline alkaloids.

Investigations have been carried out at AIIMS, New Delhi on cardioprotective effects of the bark of *Terminalia arjuna* in terms of oxidant stress that occurs during myocardial stunning (a type of cardiac ischemic – reperfusion injury) and in causing induction of endogenous defense mechanism e.g. heat shock protein. *In vitro* and *in vivo* models of myocardial stunning of heart have been used for screening these activities. Neem bark extract contains bioactive compounds which have been found to be highly effective in preventing gastric hyperacidity and ulcer in animal models. The isolated bioactive compound has been characterized to be a high molecular weight complex organic compound (phenolic glycoside). The isolated compound has potential for the development of a new class of novel anti-ulcer drug for therapeutic control of gastric hyperacidity and ulcer.

Callus culture of *Podophyllum hexandrum* (procured from H.P.K.V., Palampur) is being maintained at IIT, New Delhi. Cell suspension culture was initiated from the callus in MS and B5 media using pectinase and polyvinyl pyrrolidene (PVP). Further planned studies are to be undertaken for the production of podophyllotoxin in bioreactor using these cell cultures.

Studies on biopesticidal evaluation of plant species used traditionally in N.E. India to control mosquitoes and termites have been initiated at Gauhati University, Guwahati. Twenty plant species collected from Arunachal Pradesh, Nagaland and Assam have been screened with aqueous extract against mosquito larvae of *Aedes aegyptii* and *Culex quinquefasciatus*. At Manipur University, Imphal investigations have been carried out on isolation and identification of lectins present in plants belonging to the local flora of Manipur. Lectins present in *Vigna umbellata*, *Vigna cylindrica* and *Gynura angulosa* have been assayed and partially characterized with respect to their sugar specificity. Lectin from *Vigna umbellata* has been found to be specific for binding of D- glucosamine whereas those from other two have been found to be specific for D- galactonolactone. The work on screening of plant extracts for the manipulation of cytokine network has been taken up at ITRC, Lucknow. Preliminary studies suggest the possible use of water soluble ethanol extract of two plant species (ITRC bnp 101 and ITRC bnp 102) for prevention of the onset of xenobiotic induced pulmonary fibrosis. The studies on plant based immunomodulators as therapeutic strategy for acquired immuno deficiency syndrome (AIDS) have been carried out at NII, New Delhi. An immunomodulatory compound, NII–16, that

protects immunocompromised animals against opportunistic infection by *Candida albicans* has been identified. Since one of the major complications in AIDS cases is due to opportunistic infection, NII-16 could be used as an adjuvant treatment for prevention and treatment of opportunistic infections.

Screening of biological properties of plants having anti-cancerous and anti-diabetic properties has been taken up jointly at NII, New Delhi and Anna University, Chennai. *In vitro* bioscreens were developed to understand how some of the plant extracts used in the traditional medicine modulate cellular and molecular signals in the host cells. Popular antidiabetic plants such as *Trigonella foenum graceum*, *Szygium cumini*, *Gymnema sylvestre*, *Azadirachta indica* and *Aegle marmelos* were tested. It was observed that extracts from *Gymnema sylvestre* and *Aegle marmelos* were modulating glucose uptake in the cells or activating glucose metabolism. *In vitro* screens to examine the antiproliferative effect of several of the plant products that are used in traditional system for the treatment of cancer has been established. Five plants – *Phyllanthus urinaria*, *Withania somnifera*, *Aegle marmelos*, *Polygala arvensis* and *Hybanthus enneaspermus* were shown to have antiproliferative activity by monitoring the thymidine incorporation. Active fraction has been purified from *Phyllanthus urinaria* and was tested in the above screen and found to have the antiproliferative action. Studies on exploring medicinal plants of Bay Islands for bioactive compounds of therapeutic use have been recently initiated jointly at Centraal Agricultural Research Institute, Port-Blair and ICMR Virus Unit, Calcutta. Survey on phytogeographical distribution and possible occurrence of different ecotypes of *Alstonia macrophylla*, *Mallotus peltatus* and *Raulvolfia sumatrana* was made. Crude aqueous and methanol extracts of the leaves and bark of *Alstonia macrophylla* and *Mallotus peltatus* were evaluated in bacterial and protozoal systems for their antimicrobial activities. Inhibition of *Escherichia coli* and *Proteus* spp. at 64 ug/ml of *A. macrophylla* leaf extracts was achieved. Preliminary studies indicate the antileishmanial activity of *A. macrophylla* bark at a concentration of 15 ug/ml in ³H thymidine uptake under *in vitro* study against *Leishmania donovani*.

Genetic Improvement: The project on developing *Mucuna pruriens* lines with high L-Dopa content, improved yield characters and reduced intensity/lacking of itching trichomes on pods continued at Zandu Foundation for Health Care, Ambach, Gujarat. Five F-1, one BC-1 and two BC-2 lines have been selected as deserving lines from the previous year breeding programme for further backcrossing and stabilizing their promising characteristics. Seeds of these lines were sown in the field during June, 1999 for the assessment of their promising characters. Protoplasts were isolated from actively growing freshly harvested suspension cells of *Rosa damascena* and *R. bourboniana* for somatic hybridization studies at Institute of Himalayan Bioresource Technology, Palampur. Isolation of protoplasts from microspores of different species of *Rosa* was also standardized. Attempts are being made to induce regeneration in protoplast derived callus.

Demonstration of improved agrotechnologies of some of the medicinal and aromatic plants has been continued at CIMAP, Lucknow. The studies have shown that improved menthol mint varieties – *Himalaya* and *Kosi* yielded about 40 to 50% more oil than the existing varieties. Planting material of newly developed lemongrass var. *Krishna*, Senna var. *Sona*, periwinkle vars. *Nirmal* and *Dhawan* and Citronella var Bio-13 were made available to the growers for further multiplication and large scale cultivation. An improved variety of citronella - *Jalpallavi* has been found to be ideal for cultivation in waterlogged conditions. Ninety five growers and entrepreneurs were trained in cultivation and processing of improved varieties of mint, aromatic grasses, senna, ashwagandha and periwinkle. Studies have been undertaken to develop stable

autopolyploids in the elite clones of *Mentha arvensis* to elucidate effect of polyploidy on yield contributing component – the essential oil secretory cells. Data suggest that there is 16% enhancement in the surface area occupied by the secretory cells in the autopolyploids as compared to its source diploids. Genetically stable artificial autopolyploids are developed in three grasses namely – *Cymbopogon winterianus*, *C. khasianus x pendulus* and *C.jwarcushe* with increase in the essential oil concentration at the level of 15%, 20% and 20% respectively.

Integrated Programme on Taxol: Various callus lines raised from *Taxus baccata* were screened by HPLC/TLC for taxol/10-DAB (a taxol precursor) production at NCL, Pune. Three promising cell lines with 10 – DAB and taxol were identified. Cell suspensions are being initiated from these cell lines for scale-up. Enhanced axillary budding with production of two or three axillary sprouts per mature explant derived from a century-old tree was successfully achieved. These axillary sprouts elongated and grew when detached from the mother plant. These results are useful for development of a possible protocol for clonal multiplication. At RRL, Jammu, biotransformation studies on taxol congeners with a bacterial strain RRL – 189 have been continued. The concentration of 10-DAB-III could be enhanced to two folds. Biotransformation with two fungal strains (mucorales) shows enhancement of 10-DAB-III approximately two folds in crude taxoids.

At CIMAP, Lucknow, various cell lines have been generated from the needling and stem explants of *Taxus* collected from different Himalayan regions of India. Out of these, a promising cell line has been identified. It produces four taxoids and a phenolic compound (catechin) in suspension cultures. Conditions for release/down processing of the compound in the culture medium are being worked out for its commercial exploitation. RAPD analysis has been done to determine the molecular relatedness for estimating the genetic diversity among *T. wallichiana* plants growing in the Northern – Himalayan regions in India. This will help into tagging high yielding genotypes using DNA markers for micropropagation and mass multiplication.

About 27,000 stem cuttings of *Taxus* have been raised using the technologies developed by IHBT, Palampur at three different forest divisions – Banjar, Kullu (10,000), Dalhausie, Chamba (7000) and Palampur (10,000). Practical demonstrations were imparted by the scientists of IHBT to the forest officials of the above forest divisions for raising cuttings. About 1500 plants have already been established in its natural habitat within the periphery of the Great Himalayan National Park and Banjar Forest Areas in Kullu district. Seventy percent survival rate has been observed in these plantations. On the basis of chemical evaluation for taxol, three plants of *Taxus* from each location of Chamba and Dharmashala areas have been identified as elites for further multiplication.

At RRL, Jorhat, mapping of the precise localities of *Taxus baccata* growing areas in Arunachal Pradesh has been carried out on the basis of case studies in field and in consultation with State Forest Department. Induction of callus culture has been achieved from the nodes, needles and basal portion of apical shoots of the tree. Nature of callus development and their growths are observed to be varied according to the variations of hormonal supplements.

Technology transferred: The work carried out under a completed project at the NII, New Delhi has led to the identification and isolation of an isoquinoline alkaloid, berberin from *Berberis aristata* that down regulates the TNF - induced expression of inter cellular adhesion molecule-I (ICAM-I) on the endothelial cells. Experimental studies confirmed that treatment with berberin inhibits attachment of neutrophils to glomerular capillary endothelium in mice challenged with endotoxin and also prevents lethal effects of endotoxin or *E.coli* induced septic shock. NII has signed an agreement with Gufic Health Care Ltd, Mumbai for development of a

berberin-based drug formulation for prevention of septic shock in burn patients. Under this agreement, the industry will develop the formulation, compile toxicological data and will carry out the clinical trials after obtaining permission from Drug Controller General of India.

3.8.1. Biotechnological approaches for herbal product development: National Jai Vigyan Science and Technology Mission: The project has been recently initiated under the umbrella of “National Jai Vigyan Science & Technology Mission” for converting the available leads into potential herbal products using various biotechnological approaches. Four institutions – CIMAP, Lucknow; RRL, Jammu; BHU, Varanasi and NII, New Delhi are involved in this programme. The project aims at (a) developing improved ergot production technology (b) developing agrotechnologies for high yielding *Artemisia annua* (anti-malarial plant) (c) therapeutic application of a compound isolated from *Tinospora cordifolia* (d) developing herbal product for hyperlipidemia, and (e) developing immunomodulators and anti – arthritic agents of herbal origin.

At CIMAP, Lucknow, experiments have been initiated by isolating *Claviceps purpurea* from the sclerotia obtained from rye crop. Standardisation of protocol for the generation of variants/mutants from the existing isolate of *C. purpurea* is under process. Further improvements are being made over an agrotechnology already developed for new variety – Jeevan Raksha of *Artemisia annua* whose leaves and flowers contain 0.5 to 1.3% artemisinin at different stages of post rosette stage plant life. Preliminary results from a recently completed field trial have shown that the strain of *A.annua* can produce up to 25 quintals of dry leaves and flower material and 12.5 kg of artemisinin per hectare land area.

Studies carried out at NII, New Delhi has led to identification of an immunomodulatory compound, NII-70 which induces hematopoiesis. Experimental studies demonstrate that bone marrow cell incubated *in vitro* with different concentrations of NII-70 without addition of any cytokines or growth factors significantly increases total colony counts. Estimation of cytokines in the culture supernatant showed that NII-70 induces significant amounts of IL-6 which is one of the key cytokine involved in haematopoiesis. Based on these results, a method for *ex-vivo* propagation of bone marrow cells has been developed for transplantation therapies. A patent application has been filed and collaboration with a pharmaceutical industry is being finalised for clinical trials.

At BHU, Varanasi, all the five constituents (*Commiphora mukul*, *Boswellia serrata*, *Strichnos nux-vomica*, *Semecarpus anacardium* and Shankha bhasma) of an Ayurvedic preparation – BHU-X have been collected and characterised with respect to pharmacognostical parameters. Their extractive value (alcoholic and water) have been determined and the fingerprints are being developed on thin layer chromatography and HPLC to have consistency in the supply of raw drugs all along the study.

Studies have been initiated for developing immunomodulators and anti-arthritic agents from plants at RRL, Jammu. Two plants – *Boswellia serrata* and *Allium sativum* were procured and authenticated. A bipolymeric fraction (Polysal) has been isolated from *B.serrata* and pharmacological investigations carried out with respect to acute toxicity and general behaviour, anti-inflammatory and anti-arthritic activities. Lyophilised aqueous alcoholic extract of *A.sativum* was prepared and studied for acute toxicity and anti-inflammatory activity.

3.8.2. National Bioresource Development Board:

The Hon'ble Finance Minister had, in this budget speech, announced the setting up of the National Bioresource Development Board under the Chairmanship of Hon'ble Minister of Science and Technology. In pursuance of this the Department of Biotechnology had sought the approval of the government for the establishment of the same. Approval has now been received for the constitution of the Board with several ex-officio and expert members.

The terms of reference of the Board will be as given below:

- i) To decide the broad policy framework for effective application of biotechnological and related scientific approaches for research and development and sustainable utilisation of bioresources specially for development of new products and processes.
- ii) To develop a scientific plan of action for contributing to the economic prosperity of the nation through accelerated research and development using the modern tools of biosciences. The scientific programmes to be undertaken under the guidance of the Board would be interdisciplinary, inter-institutional and time bound with clear cut milestones. Some of the illustrative areas include:
 - To evolve effective ex-situ conservation strategies for bioresources of potential scientific and economic value.
 - To develop predictive groupings of biological resources through well established molecular lineages.
 - To construct gene maps of bioresources that can be used for locating useful genes.
 - To promote the use of biological software in the management of agricultural pests and pathogens
 - To promote value addition to bioresources
 - To train human resource for the achievement of above mentioned objectives
 - To strengthen bioinformatics vis-à-vis bioresources

The Board will be supported by the Steering Committee under the Chairmanship of the distinguished Biologist Prof. A.K.Sharma, University of Calcutta, Calcutta. The Steering Committee also consists of a number of ex-officio members from all the concerned Ministries/Departments/Agencies of the Government of India as well as experts representing various aspects of bioresources.

The first meeting of the Board was held on 12.1.2000. The Board will work very closely with other agencies in expediting the sustainable utilisation of the country's rich bioresources through the application of modern biology.

3.9. R&D IN OIL PALM AND COCONUT

The detailed studies on pollination biology of oil palm (*Elaeis guineensis*) were carried out in plantations near Bhadrawati (Karnataka) jointly by University of Delhi, Delhi and Bangalore University, Bangalore. Natural pollination takes place by weevils and air. Pollen grains constitute the major food of weevils. Freshly opened male flowers emit fennel – like fragrance and attract a large number of weevils. More than 2000 weevils are observed around the freshly opened male flowers of the spikelets during morning hour. Freshly opened female

flowers also emit similar but less intense fragrance as of male flowers. Weevils visit the female flowers by deceit and probe for the reward thereby affecting pollination. Studies revealed that during some part of the year, the number of plants in the male phase gets reduced and pollination becomes a constraint. Assisted pollination needs to be carried out under such conditions to sustain yield. Protocols were developed to store pollen grains and to use them for assisted pollination. Assisted pollination result in to a normal fruit set.

The studies on developing molecular markers to fingerprint coconut accessions continued at Central Plantation Crops Research Institute (CPCRI), Kasaragod. A partial genomic library of coconut was constructed in pUC18 plasmid vector. One hundred thirty five positive clones were identified. Hundred primers were screened for their ability to amplify coconut DNA and to reveal polymorphism between West Coast Tall (COD) DNA. Only 47 primers amplified both WCT and COD DNA, 4 primers only COD DNA. Thirty-four primers detected polymorphism between WCT and COD DNA. The polymorphic primers were used to detect intervarietal polymorphism in 14 different coconut accessions of significant importance in breeding programme.

At Indian Institute of Science (IISc), Bangalore no evidence of endogenous lipase activity in oil palm mesocarp that is responsible for release of free fatty acids was found. However, a bacterium (*Citrobacter freundii*) isolated from the mesocarp is found to be capable of hydrolysing palm oil efficiently through the excretion of the lipase. The results suggest that exogenous lipase could be responsible for the release of free fatty acids in oil palm mesocarp. Future studies are planned to be undertaken on using a known probe for isolating lipase from oil palm and *Citrobacter freundii*, preparation of genomic library from mature oil palm fruit and purification of the enzyme (lipase) from fruits and other organs of oil palm with the improved protocol.

3.10. SERIBIOTECHNOLOGY

Several projects have been launched on application of biotechnology for improving the productivity and quality of silk alongwith improvement of host – plants in both mulberry and non-mulberry sericulture.

Development of better races of silkworm for increased productivity: At Indian Institute of Science, Bangalore, a method has been developed to dechorionate and devitellinize the silkworm (*Bombyx mori*) eggs without damage. Confocal microscopic examination technique has been applied to study the limb formation during silkworm development by using the heterologous antibodies. Attempts have been made to study the silk gland formation in early embryonic stages of development using *Wingless* from *B. mori* as probe.

Studies on development of mariner element vector for the transformation of *Bombyx mori* continued at Madurai Kamraj University, Madurai. All the screened *B.mori* races are positive for all types of mariner elements such as Mellifera, Cecropia and Mori. Millefera element is highly conserved among different strains whereas cecropia elements are highly diverged even within a strain.

RFLP studies were continued at Central Sericultural Research & Training Institute, Mysore for identification of DNA markers closely linked to cocoon – shell character with the aim of supporting the directional breeding in silkworm (*Bombyx mori*). Bulked segregant analysis was carried out using the silkworm inbred lines showing contrasting shell characters. One hundred twenty low copy probes were screened. Eight probes revealed distinct polymorphic banding pattern in the parents and the F-2 bulks. The study indicated the possibility of close

association of at least eight RFLP markers with cocoon shell characters in silkworm. The results would help in establishing tight linkages of these markers with QTLs and ultimately leading to their use as molecular tags in silkworm breeding.

At University of Calcutta, High Mobility Group of proteins of *Bombyx mori* (HMG-B) have been found to be associated with active chromatin. The HMG-B mRNA has already been prepared and partially purified.

A major protein of tasar silk, fibroin has been purified from the posterior silk gland of tasar silkworm (*Antheraea mylitta*) larvae at Indian Institute of Technology, Kharagpur. Fibroin of *A. mylitta* is composed of two polypeptides of the same molecular weight of 175 kDa and has sequence homology to *A. pernyi* fibroin. The sequence comparison of amplified DNA shows significant homology with *A. pernyi* (92%) as well as *A. yamamai* (95%) fibroin genes.

At the Institute of Advanced Study in S&T, Guwahati, esterase isozyme comparison of different ecoraces of *Philosamia ricini* were undertaken using PAGE technique. The gut and haemolymph of 5th instar larvae fed on castor leaves were analysed. Three esterases were detected in seven ecoraces. The pattern of esterase isozyme activity of different organs in all ecoraces were consistent but showed different intensities.

Studies on molecular biology of silk protein genes of eri silkworm (*Philosamia ricini*) continued at NII, New Delhi. Antibodies have been raised against the silk proteins (fibroin and sericin). These are being used for isolating the corresponding gene by screening the eri cDNA library. Attempts have been made at understanding the molecular basis of tissue and development-specific gene expression in eri silkworm. Fibroin gene expression could be seen at 4th and 5th instar larvae but not at the 3rd instar stage.

In the fifth instar larvae, fibroin expression, however, could also be detected in the middle silk gland as well as posterior silk gland. Studies on molecular genetics of tropical tasar silkworm (*Antheraea mylitta*) have been recently initiated at IIT, Kharagpur.

Development of control measures for major diseases/pests of silkworm: Studies on molecular characterisation of a virus causing grasserie disease in tasar silkworm (*Antheraea mylitta*) continued at Indian Institute of Technology, Kharagpur. A new and novel type of cytoplasmic polyhedrosis virus (Am CPV) infecting *A. mylitta* has been identified. It is a double stranded segmented RNA virus. Cloning and sequencing of the ninth segment of RNA genome revealed no sequence homology of Am CPV with other known CPVs. AmCPV grows very slowly in heterologous cell lines which indicates its host specificity.

At Tamil Nadu Agricultural University, Coimbatore, field trials of integrated management of uzifly (*Exorista bombycis*) – a serious pest of silkworm have been completed at Tharmapuri village of Erode district. Cocoon yields were higher in plots treated with uzicide and *Nesolynx thymus*.

Studies have been continued on immune response of multivoltine and bivoltine races of silkworm (*Bombyx mori*) at KSSR&DI, Bangalore. Inoculation of non-pathogenic bacteria, *Escherichia coli* into the haemolymph and the bacterial clearance tests conducted showed that more than 80% of bacteria were eliminated within 0.5 to 12 hrs of post infection by cell mediated defence reactions such as phagocytosis, nodulation and encapsulation process. High degree of tolerance against invading bacteria due to cell mediated and humoral response was observed in multivoltine races as compared to bivoltine races.

At Karnatak University, Dharwad, studies on red fluorescent protein (RFP) synthesis in silkworm (*Bombyx mori*) continued. RFP found in the gut juice of the silkworm, *Bombyx mori* was isolated by ammonium sulphate precipitation, acetone fractionation and gel filtration

chromatography. This protein was found to contain pigment-chlorophyllide-b, chlorophyll-b and traces of protoporphyrin. Preliminary studies have shown the antiviral activity of RFP.

Silkworm Genome Programme: At CDFD, Hyderabad, two parental strains of *Bombyx mori* (diapausing and non-diapausing) identified on their high degree of homozygosity as revealed by multilocus probe were crossed to obtain F-1 hybrids which were crossed among themselves to obtain F-2 mapping population. Twenty-eight microsatellite loci previously identified, cloned and characterised from silkworm genome were used to analyse their allelic status in the parental strains and F-1 off springs. These genetically well-behaved loci are being used to map the F-2 population. Besides, a search of *B.mori* sequences in the EMBL and Gene Bank database yielded 85 microsatellite motifs of different types. The primers for the flanking sequences of some of the longer microsatellite motifs were designed and PCR amplification conditions were standardised. These loci alongwith 28 previously characterised microsatellite loci are being mapped using F-2 populations. Eight species of non-mulberry silkworms were also genetically analysed using 23 *Bombyx* microsatellite loci. Out of 23 loci 16 were found to be conserved in muga silkworm (*Antheraea assama*), 11 in the eri silkworm (*Philosamia ricini*), 5 in the tropical tasarworm (*A. mylitta*) and temperate tasarworm (*A.proylei*). Cloning and sequencing of the PCR products and the allelic status of the conserved microsatellite loci are being carried out. The results showed that microsatellite DNA-based markers obtained in the silkworm (*B.mori*) greatly facilitate the genetic analysis of non-mulberry silkworms.

Silkworm as a bioreactors: Studies on expression of recombinant viral antigens through *Bombyx mori* Nuclear Polyhedrosis Virus (Bm NPV) system continued at IISc., Bangalore. The expression of recombinant protein (Hepatitis-E-ORF 3) in the haemolymph and fat bodies of the infected silkworm larvae has been demonstrated. However, the protein did not show antibody reactivity in Western Blots since the overall yield was not very high. Efforts are being made to increase the synthesis of desired proteins. So far, the attempts to generate Bm ORF₂ have not been successful. The *luc* and *gfp* tagged viruses have been utilised by CSR&TI, Mysore to monitor and study the progress of BmNPV infections in different silkworm races.

Processing of silk: Studies on the development of a better oak tasar cocoon cooking method using pineapple extract continued at Manipur University, Imphal . An assay procedure for the proteolytic fruit has been standardised. The proteolytic activity in the reaction medium has been found.

Improvement of host plants: At University of Delhi, Delhi, gynogenic haploids have been produced in cvs. S-1 and K-2 of mulberry. Stomatal analysis and cytological studies revealed that out of 20 plants, 12 were haploid and the remaining were aneuploids. Triploids have been developed in cvS-36 of mulberry through endosperm culture. Triploid plants have been sent to KSSR&DI, Bangalore for field evaluation. Friable pale yellow callus was obtained from leaf explants of *Morus alba* var. Chinese White at University of Jammu, Jammu. The callus showed the formation of embryo-like structures with prominent cotyledonary leaves and root upon transfer to medium lacking 2,4 – D but containing kinetin. The observation suggests the embryogenic potential of the mulberry callus raised from a mature explant.

Studies on development of methodologies for sustainable utilisation of castor for seed production and eri silkworm rearing continued at JNV University, Jodhpur. The growth and yield of castor varieties revealed that the increase in the fertiliser application enhanced all growth parameters including seed yield. Maximum yield was obtained in variety Aruna followed by GCH-4 and RHC-1. The Borduar strain of eri silkworm (*Philosamia ricini*) produced more silk than Titabar strain.

At Kakatiya University, Warangal, direct regeneration of *Terminalia tomentosa* -a host tree of tasar silkworm was observed from nodal explants on MS medium supplemented with 2 mg/IBAP. Protocol for the isolation of leaf mesophyll protoplast has been standardised in mulberry at Kakatiya University, Warangal. Out of three elite mulberry varieties (K-2, S-13 and S-36) tried, K-2 showed good protoplast yield followed by S-13 and S-36. Multiple shoot formation from nodal explant of K-2 cultivar has been achieved. Root formation in generated shoot of K-2 cultivar on MS basal medium was also observed.

At University of Calcutta, 28 varieties of castor, a host plant of eri silkworm, collected from different regions are being screened with DNA probes. Castor accessions and some of its F1 progenies (studied so far) showed polymorphism in amplified DNA bands. Ten oligonucleotides were designed for PCR – mediated DNA amplification. These are being used to fingerprint castor varieties.

Insect Host - Plant Interaction: Phytochemical screening of live collections of *Terminalia arjuna* and *T. tomentosa* (maintained at CTR&TI, Ranchi) has resulted into selection of a chemotype with desired characteristics of tropical tasar host plants such as high protein and low tannin contents at University of Delhi, Delhi. This chemotype can enhance quality and quantity of tasar silk. Studies have demonstrated the existence of chemical polymorphism and this polymorphism can be used to select the elite host-trees. The high mortality rate of early instars is associated with proteinase inhibitors and there is an extensive developmental polymorphism in the of proteinase inhibitors. Extensive polymorphism in the distribution pattern of different phenolics (gallic acid, ellagic acid) and some triterpenoids (β -amyrine, oleic acid and others) have been recorded which have bearing on the growth and development of instars as well as on the quality and quantity of silk.

Studies on the effect of botanicals, iodine and inorganic compounds on eri egg production and rearing performance of eri silkworm (*Philosamia ricini*) on different castor genotypes continued at University of Agricultural Sciences, Dharwad. Among the 64 castor genotypes evaluated for eri silkworm rearing, the genotypes RG-323 and RG-902 recorded significantly higher gain in larval weight, economic traits of cocoon and fecundity over local cultivar Aruna. Spraying of potassium iodide (30 ppm) on mated female eri moths has resulted in considerable increase in fecundity. Fortification of castor leaves with zinc sulphate during fifth instar significantly increased the larval weight, cocoon weight, shell ratio and fecundity.

At University of Agricultural Sciences, Dharwad, studies have been initiated on the comparative developmental biology of the tropical tasar silkworm (*Antheraea mylitta*) on *Terminalia tomentosa* and *Ricinus communis* (castor). Investigations revealed that the tasar silkworm, *A. mylitta* could be reared indoor successfully on the cut branches of *T. tomentosa*. Tasar larvae fed on the leaves of castor completed the life cycle successfully and took 64 to 85 days. The growth performance and the silk cocoon of the silkworm reared on castor were normal. The study indicated that castor may be a new potential host plant for indoor rearing of tasar silkworm.

3.11. BIODIVERSITY CONSERVATION AND ENVIRONMENT

To facilitate adoption and utilisation of technology, major emphasis has been given on involvement of user industry from the beginning and demonstration of technologies developed at the site of industry. In over a dozen projects, a number of industries viz. Mathura & Barauni Refinery, Shyam Dyeing, Sanganer & Sanganer Dye Manufacturer Association, Gharda Chemicals Ltd., Thane, M/s NATCO Pharma Ltd., Hyderabad, ETOSHA & CPCB, New Delhi, Karnataka Oswal Oil Mills, Hindustan Zinc Ltd., Tannery in Unnao, Kanpur Century paper

mills, M/s Akar Impax Pvt. Ltd., A.P. Co-operative Oil Seeds Growers Federation Limited, SAIL, Ranchi are involved in the process development, process optimisation, validation and sharing of cost. The task force met twice and considered new R&D projects and also reviewed progress of ongoing projects. Reports of completed projects are being evaluated with the help of experts and task force members to make an assessment of potential leads which can be pursued to logical conclusions. Significant achievements are as follows:

Bioremediation:

Ecorestoration of mine spoil dumps: In a project at Delhi University, ecological restoration technology was developed and it was successfully demonstrated in revegetating limestone belt of Bhatta and Lambadar region near Mussorie, U.P. and was replicated in Bhatti and Asola Mines in Delhi. Within 5 years, the entire rocky terrain was transformed into lush green forests with grasses and lagoons.

Industrial effluent treatment: Number of projects have been supported in the area of heavy metal removal, treatment of effluent from paper and pulp mills, dye industry, automobile industry etc.

Palm oil mill effluent treatment: In a collaborative project between CBT, New Delhi and NCL, Pune, a laboratory scale process for treatment of palm oil mill effluent from M/s Karnataka Oswal Oil Palm Limited, Bangalore has been standardised. The process generates biogas from effluents at the rate of about 300 litres per kilo dry matter, which can be captively used by the factory for the energy needs. Efforts are on to undertake a pilot scale trial. The quantity of waste required for the trial will be 1000 litres per day. M/s Akar Impax Private Limited will supply the engineering design and mechanical equipment also to undertake the erection of the plant.

Microbial Treatment of Cassava Starch Factory Waste: Scientists at CTCRI, Trivandrum have isolated potential microorganisms from the starch factory effluents for production of enzymes such as cellulase, amylase, invertase, polyphenol oxidase. Laboratory scale studies were conducted using a conventional bioreactor of 75-litre capacity. Batch digester of 500-litre capacity is being designed. The wastewater treatment technology developed has been tested in Cassava Starch Factory at Salem for its efficacy.

Bioremediation of soil contaminated with chlorinated pesticides: A working group was constituted to formulate collaborative projects on bioremediation of chlorinated pesticides. The working group met on 26-27th July 1999 and made recommendations for further work. At RRL, Trivandrum, a 6.2 litre reactor with recycling system has been fabricated to fluidise the activated carbon to study the aerobic biodegradation of 2,4 dichlorophenol. In a project at TNVASU, Chennai, DDT assay has been carried out on ELISA plates coated with indigenously developed antibody. The antibody was tested for matrix studies against dairy samples like milk, buttermilk, cheese, kalakhand and khoa. No matrix effect interference was noticed. The ELISA method developed by PI showed comparable results to gas chromatography methods.

Dye industry effluent treatment: At Jaipur University, process for dye wastewater treatment has been standardised using wetland technology. The treated waste water had no adverse effect even on the growth of the most susceptible aquatic plant species such as Azolla, Hydrilla, and Spirodela including cultivated ones of Cymopsis. The process has been validated by Sanganer Dye Manufacturers Association. It has been proposed to demonstrate the process at 4-5 locations afflicted with dyestuff. At University of Madras, decolorisation of azo dyes has been achieved after 8 hours of incubation with a potential organism DLK1.

At RRL, Trivandrum, screening of consortia grown in textile effluent of Tirupur area resulted in the isolation of four fungi and four bacterial colonies. The degradation of 15 textile

dyes mainly sulphonated azo dyes were achieved with two bacteria and two fungi. In a project at University of Madras, microbial consortia having a broad degradative potential and a carrier system comprising extra cellular polysaccharide have been developed. Isolation of EPS producing organisms from polluted soil environment (dye, tannery and fertiliser industries) has been undertaken. The organisms were also screened for the degradation of azo dyes. One isolate was found to be very efficient in the degradation of a wide variety of azo dyes (Acid black 48, Sudan 3, and methyl red). Studies on the characterisation of the organisms Vc1 and Vc33 and their antibiotic resistance / sensitivity spectrum were undertaken. Efforts are being made to develop a network programme on dye effluent treatment.

Tannery waste treatment: At ITRC, Lucknow, chromium resistant bacteria were isolated by plating raw tannery effluent on chromium added agar plate. One hundred sixty seven bacterial isolates thus obtained were screened for chromium resistance. Forty-one chromium resistant bacteria were also screened for tolerance for other heavy metals. They were 100% tolerant to cupric copper.

Distillery waste treatment: At ITRC, Lucknow, anaerobically treated distillery effluent was analysed for its physico-chemical properties. Potential aerobic bacterial strains were isolated from the degrading sludge of anaerobically treated distillery effluent. At Pune University, distillery, dairy and palm oil mill effluents have been characterised and a two-stage anaerobic waste treatment process has been developed. Acclimatisation of microbial consortia to distillery waste has been achieved.

Treatment of BOF slag samples from steel industry: SAIL, Ranchi using the microbial collection available with TERI, NEERI, IARI and GBPNAU, undertook preliminary work on treatment of BOF slag sample provided. A collaborative multiinstitutional project is being formulated.

Petroleum sludge degradation: In a project at TERI, New Delhi, highly efficient crude oil and oily sludge degrading bacterial consortium and pure bacterial strains have been selected. Eight different carrier materials have been tested for immobilisation of the selected crude oil and oily sludge degrading bacterial strains. Application of carrier based materials at the selected contaminated sites of two oil refineries (Mathura & Barauni) has led to complete mineralisation of hydrocarbons and restoration of the sites. Similar experiments are on at Digboi refinery and BPCL Refinery, Bombay. Efforts are being made to transfer this technology to industry. In a project at Goa University, studies are being conducted for bioremediation of oil spills and tarballs using the bacterial consortia.

Diagnostic for pathogen detection in drinking water: A New initiative has been taken to convert the multiplex PCR test for detection of pathogenic organisms in drinking water into a colour strip method so that water samples can be checked for potability.

Germplasm characterisation: In a project at TERI, New Delhi, genetic diversity has been determined through AFLP markers in 37 neem accessions from different eco-geographic regions of India and 4 exotic lines from Thailand. The cluster analysis indicated that neem germplasm within India constituted a broad genetic base with the values of genetic similarity coefficient ranging from 0.74 to 0.93. Also, the Indian genotypes were more dispersed on the principal correspondence plot indicating a wide genetic base. The 4 lines from Thailand, on the other hand, formed a narrow genetic base. At IHBT, Palampur, DNA fingerprinting of *Artemisia* species has been carried out using 10 more primers of OPA series.

At IHBT, Palampur, 27 important diverse clones of tea have been characterised using RAPD and RFLP analysis. A collaborative proposal on "Conservation of Endangered Animals",

between CCMB, Hyderabad; Nehru Zoological Park, Hyderabad and Andhra Pradesh State Forest Department has been supported with the major objective of establishing gene and semen banks for lion and tiger.

3.12. MEDICAL BIOTECHNOLOGY

The major emphasis is on the developments of diagnostics, vaccines for prophylactic and therapeutic use, drug delivery systems, production of important biological/biomolecules and creation of infrastructures in hitech biomedical areas in order to undertake further research. Two meetings of the Task Force were conducted; 42 new projects were considered and 55 ongoing projects monitored. Major new R&D projects implemented were in the areas of genetic variations of *V.cholerae*, multiplex PCR based diagnostics development for chlamydial infections, identification of the chromosomes where the gene(s) encoding for antigens for *L.donovani* having protective potentials, technology development for xenotransplant of hepatic cells and advanced programme on histocompatibility and transplantation.

The National Jai Vigyan Science and Technology Mission on Development of New Generation technologies for vaccines was initiated. An interactive meeting with the industries was organised to discuss modalities of transfer of the diagnostic technologies developed from the programmes funded by the department in this area. Guidelines for generating preclinical and clinical data for r-DNA based vaccines, diagnostics and biologicals were finalised for the first time in the country which will be useful for the researchers and the industries.

HIV/AIDS: The multicentric HIV/AIDS programmes on the genotypes of HIV-1 in circulation in India revealed that of the 237 isolates of HIV-1 studied by HMA, 80% were of subtype C. Seventy five percent of the HIV subtype C belonged to quasi species C3. Other subtypes identified were B, E & A and naturally occurring recombinant subtype A/C. The nucleotide sequence analysis of V3-C3 (loop region) of HIV-1 *env* gene revealed Indian isolates to be closer to isolates from sub-Saharan African region. The delivery of HIV-I peptide through oral/nasal routes led to the development of HIV-I specific immune response. Such a delivery system is ideal since local immune response have significant role to interfere with the entry of the HIV. A viral repository at NARI is now fully functional. It has 39 HIV-I isolates from symptomatic HIV infected individuals and another 22 from asymptomatic individuals. In addition, 5 HIV-II isolates have also been stored. In another study, two anti HIV factors with molecular weights of 100 KDa(Epap I) and 60 KDa(Epap 1 alpha) have been isolated and purified from first trimester human placenta tissue. These molecules have been found to neutralise HIV-III B infection in HUT-78 and PBMC interaction through inhibition of gp120 CD4 interaction.

Japanese Encephalitis: The department has funded a multi-institutional, multi-investigator project on JEV covering various aspects specially ,diagnostics for early detection and cost effective vaccines. IgM-MAC ELISA and immuno-fluorescence test systems have been developed and are presently being validated at CMC, Vellore. An Indian strain of JE obtained from NIV, Pune has been grown in Vero cell on micro carriers and an optimal growth has been achieved to make the vaccine from this method which would be cost effective. Presently, immunological studies are being conducted to assess the efficacy of this tissue culture derived vaccine. Work has also been initiated to compare the efficacy of this vaccine with the commercially available suckling mouse brain derived vaccine.. A complete genomic map of one of the Indian strains of JEV has been completed.

Efforts have also been made to use some structural proteins namely, NS1& NS3 for their immunogenicity. Partial protection in immunised mice has been achieved. Work has also been initiated to immunise mice using plasmid DNA. Synthetic chimeric B-cell T helper cell peptide has been employed successfully as an immunogen to develop neutralising antibodies to JEV in animals. Efforts are presently being made to assess the effect of neutralising antibodies on the course of JEV infection in mice. immunogenicity of chimeric peptide in human has revealed the ' peptide' to be recognised by human host.

Hepatitis: Studies on Molecular Epidemiology of Hepatitis B Virus Infection at SGPGIMS, Lucknow indicated that multi-transfused children with thalassemia have a very high incidence of HBV-DNA. However, only 12.5% had 'S' gene vaccine escape mutant. The antibody response by HBV vaccination in this group is very low and frequency of infection in non-responders is more than in responders. The prevalence of HbsAg positivity in the general population is lower than generally reported (1.86%) with an overall exposure rate of 10.9%. SSCP and ARMS-PCR for vaccine escape mutation have been standardised.

Tuberculosis: The department has taken major initiatives which include collection of well-characterised samples and isolates of Mtb; development of appropriate test systems for pulmonary and extra - pulmonary tuberculosis as well as MDR varieties. Efforts have also been made for the development of a recombinant BCG vaccine using suitable vectors. Work has been initiated for the development of candidate DNA vaccines for protection against TB. There has been good progress in maintenance of well characterised strains of about 1200 at JALMA, Agra.

This repository is networked with about 30 laboratories in the country. Four DNA-probes have been prepared for PCR identification of Mtb out of which two have given good results.

In order to develop composite Amplified rDNA Restriction Analysis (ADRDA) techniques for pathogenic mycobacteria, several primer combinations targeting 16S rRNA, 23S rRNA, 5S rRNA and spacer sequences of pathogenic mycobacteria were designed at JALMA, Agra. A primer combination targeting 16S rRNA gene and amplifying 1.2 Kb fragment from various strains of mycobacteria showed only limited variations within the region amplified. This region showed specificity at species level and thus is suitable for detection and rapid identification of pathogenic species of *Mycobacterium*. Primer combinations targeting 16S, 23S and entire spacer region and amplifying 1.8 Kb fragment yielded variations among strains of *M.tuberculosis* and *M.leprae*. Such a system is suitable to develop DNA fingerprinting for molecular epidemiology. The assays targeting the 16 S as well as spacer regions of ribosomal RNA have already been converted into non-radioactive DIG based systems.

Studies on immune responses and host genetics in tuberculosis being carried out at Madurai Kamraj University, Madurai revealed that DRBI*1501 and DQBI*0601 are high alleles for pulmonary tuberculosis while DPBI*04 protects. Susceptibility to *M.tuberculosis* was also linked to non DRB1*02 allele, BCG scar negative and IL-10 positive status. The immune responses to Esat-6 peptide varied in individuals carrying different combination of susceptible/preventive alleles. Two castes, IT5SU and IT2PK, the first, a recent migrant from Gujarat (500 years ago) and the second, a paleo Mediterranean stock and an ancient settlers to this land (15000 years ago) have been identified to possess higher frequency of high risk allele DRBI*15 and high prevalence of pulmonary tuberculosis.

Mycobacterium habana as candidate vaccine alternative to BCG for tuberculosis is being investigated at CDRI, Lucknow. Immunisation of immuno-competent mice with varying doses of live *M.habana* provided protection to 70% animals following challenge with virulent strains of *M.tuberculosis*. Immunised and protected animals had mean survival time of 32 days as compared to 20 days of mean survival time of control mice. Almost identical results were obtained in immuno- compromised animals.

Studies at AIIMS, New Delhi have been carried out to specifically identify *M.tuberculosis* genes that are expressed within the macrophages. The dev R- dev S genes have earlier been studied as co transcribed and their transcripts are lower in avirulent H37 Ra *M.tuberculosis* strain. The expression of Dev R has been observed in the human monocytes infected with *M. tuberculosis* H37 Rv. The Dev R protein is synthesised in phagosome environment of human monocytes, suggesting thereby its possible role in adaptive response of *M.tuberculosis* in host milieu.

Cholera: The department has supported the development of an oral recombinant vaccine for Cholera based on VA1.3 strain. Phase I clinical trials for the vaccine have been conducted at two centres in India and the data generated have shown that the vaccine is safe. However, the immunological responses from the doses used is being analysed. Simultaneously, work has been initiated for studying stability (physiological and genetics) of this vaccine on scale-up. Preparation for Phase II clinical trials has also been initiated under National Jai Vigyan Mission Programme.

Shigella: Studies on the construction of a physical map of the whole genome of *Shigella dysenteriae* type-I at IICB, Calcutta revealed intra species genome conservation through CeuI restriction analysis of several genomes. It was found that genome structure of epidemic and

pandemic causing *S.dysenteriae* type 1 is unique as compared to the other species as well as other serotypes (type 3 & 7) of *S.dysenteriae* examined so far.

Rotavirus: The studies at NICED, Calcutta on the comparative genome analysis of Rotaviral strains prevalent in North Eastern part of India revealed that G4 to be most prevalent serotype followed by G3. These strains of rotavirus are unique in producing clinical disease in adult humans. These strains have nucleotide sequence homology to the strains from China causing Chinese Adult Diarrhoea. The nucleotide sequence homology of strains from North Eastern part of India and that from south India appears to be different. The electrophoretic patterns of these viruses, however, were, often similar to the conventional rotaviruses (Group-A), but antigenically they were sometimes different with respect to their Group specific inner capsid antigen (VP6) and the serotype specific outer capsid antigen (VP7). These studies indicate the need for developing regional specific Rotavirus vaccine.

Helicobacter pylori: A good collection of clinical isolates of *H.pylori* strains have been maintained in two laboratories. A modified broth medium for rapid culturing of *H.pylori* has been standardised. PCR using *H. pylori* targeting *CagA* and *vacA* genes have been standardised using published primers. A PCR system for deletion of *H. pylori* directly in gastriobiosy specimens has been developed & standardised. The *H.pylori* strains from southern India are unique as they carry plasmid which may have role in drug resistance.

Leishmaniasis: A multi investigator programme to identify targets for newer drug delivery systems and genome mapping of some of the chromosomes of *Leishmania donovani* is being implemented at IICB, Calcutta. DNA topoisomerase I&II have been studied as target for plant derived flavonoids, luteolin and quercetin for anti leishmanial activity. For the first time, it has been shown that luteolin inhibits growth in vitro and inhibit DNA synthesis of *L. donovani* promastigotes. The compound induce cell cycle arrest leading to apoptotic cell death in *L.donovani*. Oral administration of these flavonoids four days after *L.donovani* infection in hamster resulted in significant reduction of splenic parasite burden. The compound(luteolin)is ready for pre-clinical investigations. In addition, two novel indigenous compounds i.e. amarogentin and bacopasaponin isolated from medicinal plants *Swertia chirata* and *Bacopa moniera* respectively were found to have anti leishmanial activity *in vivo*. Niosomal preparation of amarogentin was found to be more efficacious than the liposomal form in clearing leishmania bodies in experimental hosts. Another study on evaluation of indolylquinoline derivatives as antileishmanial drugs at IICB, Calcutta revealed that a novel synthetic anti-leishmanial compound (S-4) is non-toxic and has high degree of antileishmanial activity. Application has been filed to patent the process and the product.

A laminin binding protein of 67kDa on *L.donovani* promastigotes has been identified and has been found to be an “adhesin” molecule responsible for attachment of parasites to host tissue. Such a molecule has potential to develop immunoprophylactic agent. The localisation of genes for DNA topoisomerase II, adenosine kinase, cyclophilin and Gp63 revealed that these are located on chromosomes 5 and 10. These two chromosomes are being mapped.

Studies conducted at AIIMS, New Delhi indicated molecular heterogeneity amongst the different Indian isolates of *L.donovani* by using kDNA and nDNA RFLP analysis. New set of primers has been designed targeting kinesin gene in order to differentiate *L.donovani* strains from clinical cases of visceral leishmaniasis and post kala – azar dermal leishmanoid (PKDL). These primers have been able to differentiate PKDL strains from VL strains of *L.donovani*. This is for the first time that such primers set has been designed. A patent application has been filed.

Malaria: At IISc, Bangalore, studies on the heme biosynthetic pathway of the malarial parasite as a potential drug target have shown that the malarial parasite synthesises heme *de novo* and inhibition of the pathway leads to death of the parasite. The malarial parasite *P.falciparum*, in culture has been found to import the enzyme, δ -aminolevulinic dehydrase (ALAD) from the host red cell to synthesise heme *de novo*. This can also be demonstrated in *P.berghei* in mice in vivo. This phenomenon appears to be receptor-mediated. The import of the host enzyme into the parasite can be inhibited by a deleted fragment of the enzyme and this leads to the death of the parasite. Import of a host enzyme by the parasite for carrying out a vital function is a new facet in parasite biology and is an unique drug target.

Studies on the development of the PCR primers for predicting drug sensitivity in *P.falciparum* malaria were conducted at Delhi University, Delhi. DHPS and DHFR gene domains of 831 bp and 1100 bp, for pyrimethamine and sulfadoxime respectively have been amplified using custom designed conserved oligoprimers. Sequence comparison showed two point mutations in DHFR selected line and additional point mutation in naturally resistant isolate.

In another study on malaria metalloprotease at AIIMS, New Delhi, indicates that *P.falciparum* contains a cross-reacting antigen to that of the *P.vivax* heat-shock metalloprotease. The antigen was expressed by the blood stages of the parasite and was of 55 KDa molecular weight. Further work to purify this antigen is in progress. The ORF of *P.vivax* heat-shock metalloprotease gene was amplified, cloned into pGEMT vector. This *in-vitro* transcribed product was translated by rabbit reticulocyte lysate system. Two bands of 53kDa and 30kDa were obtained which were immunoprecipitated with the Pv9 antibody. Of these two bands the lower band gave the protease activity on zymography. The protease activity was specific to the insert derived protein.

Mw for Immunoprophylaxis of Leprosy: An immunomodular based on killed *Mw* to be used alongwith Multi Drug Therapy, has been commercialised by the M/S Cadila Pharmaceuticals. Trials were conducted in early ninety's for its use as prophylaxis by administering the vaccine to about twenty thousand healthy household contacts in Kanpur Dehat. After the first and second surveys, a third survey by an independent agency is underway to see its potential as an immunoprophylactic agent.

Contraceptive Immunogens: The department has been supporting research in this area for the last 10-12 years. Three candidate vaccines have been under development. Out of these, β hCG, an anti-fertility vaccine for female has undergone Phase II clinical trials. Analysis of the data has shown unpredictable immune response in women. The hCG is a self-protein in humans. Hence, basic work has been initiated to enhance its immunogenicity as well as to have a predictable and sustained response in genetically heterogeneous population. Various strategies are being followed by using different carriers, conjugates, adjuvants, use of T-cell peptides from infectious agents etc. At present, T cell determinants and hCG have been linked chemically and its response in monkeys is being studied. In the quest for greater reproducibility as well as low cost of vaccine production, attempts are also underway to genetically engineer and express β hCG, alongwith selected T helper epitopes, in the form of well defined fusion proteins. This method offers the flexibility to alter the orientation of epitopes, the position of one determinant in relation to the other and also to express β hCG containing variable numbers of T cell epitope(s).

The investigations on other two candidate vaccines, viz., oFSH and anti-RCP are also being carried out. In female rats(outbred) recombinant RCM-RCP not only elicited high specific antibodies but also protected all test animals from pregnancy. This molecule was also found to

reduce fertility following immunisation of male rats/monkey and upon mating with respective females. Recombinant oFSH has been produced.

Markers for infertility: Hyaluronic acid binding protein has been shown to be a good marker for detecting infertility in males. The preliminary data using antibodies against proprotein form of HABPI has shown a defective processing of proprotein to mature form of HABPI in male fertility. Integrins (ITGs) have been reported to be indispensable molecules for various biological processes. ITGs also play a role in sperm-egg interaction. A series of integrin cell adhesion molecules ($\alpha 1$, $\alpha 2$, $\alpha 4$, $\alpha 5$, $\alpha 6$, β^1 , β^3 , $\beta 4$, $\alpha 6, \beta 1$, αv , $\beta 3$) and their ligands (Fibronectin, vitronectin and laminin) on human spermatozoa from fertile and oligoastheno-zoospermic men have been studied. The spermatozoa of the fertile men showed significantly higher expression of $\alpha 6$ and Fibronectin than sub fertile men. Flowcytometric & ELISA studies are in conformity with these observations. It appears $\alpha 6$ & ligand Fn could be used as a clinical markers to assess the qualities and fertilising ability of the spermatozoa.

Oncology: A diphyllin glycoside called cleistanthin A, isolated from the tropical plant *Cleistanthus collinus* is most effective for killing oral carcinoma cell line than cervical carcinoma cell line. In *in vivo* experiments in animals, it drastically reduced tumour development. This treatment led to increase in life span of experimental animals. The compound has low toxicity with no significant effects on lymphocyte counts.

NFI (Nuclear Factor 1) proteins are a heterogeneous family of proteins encoded by multiple genes. NFI is known as ubiquitous transcription factor. Differential binding of NF-1 transcription factor to P53 gene promoter and its depletion in human breast tumours has demonstrated for the first time about a deregulation of NF1 transcription factors during the process of breast tumourogenesis. It is presumed that in normal tissues NFI might have a role in p53 expression, but in tumour samples, p53 gene expression is not dependent on NF1.

Identification of metastatic gene based on expression of CD44 and its varieties in oral squamous cell carcinoma suggested V5 and V6 variants as possible markers of metastasis while down regulation of V3 variant may resist the metastasis of oral cancer. The first generation of transgenic mice carrying purified K14-EF gene construct has been raised. The positive mice are being analysed for phenotypic changes.

Therapeutic Antivenin for Snake Envenomation: Snake bites account for a large number of deaths in all tropical and subtropical countries. Currently, polyvalent antisnake venom (PAV) raised in horse is being used. In recent years, it is increasingly realised that ovine (sheep) and avian (hen) antibodies are better candidates for neutralisation of snake toxins. Specific antibodies from venoms raised in sheep were affinity purified. The immuno-reactivity of affinity purified antivenins was higher than crude preparation. *In vivo* neutralisation in mice suggested affinity purified antivenins to be more effective. Such antivenins appear to have potential in treatment of snakebites subjects.

Drug Delivery: Studies on development of liposome technology for extended ocular drug delivery showed that Liposomal fluconazole was effective in the Candida induced endophthalmitis. The pharmacokinetic profile indicated that the concentration of fluconazole in liposome injected group were significantly higher than that of the group injected with aqueous fluconazole (in ocular compartments like cornea, aqueous humour and vitreous). This indicates that liposomal fluconazole remains for longer time.

Studies on the development of an *in vitro* screening procedure for identifying anti-inflammatory drugs were undertaken at CBT, Delhi. A cell culture system to isolate and grow human umbilical vein endothelial cells has been developed. Adhesion assay of peripheral blood

leukocytes to endothelial cells was developed. The efficacy of the screening system was checked using diclofenac.

Diagnosics: The department has been funding research for the development of indigenous diagnostics for major communicable and non-communicable diseases. The basic purpose has been to develop easy to use, affordable and accurate test systems for Indian Strains which be converted into appropriate kits with the help of industries for large scale use. After reaching to the level of prototype systems, the department is presently making efforts to upscale these technologies with the help of industries. These technologies are IgM Mac ELISA for Dengue, JE and West Nile, haemagglutination of erythrocytes carrying 9-0 – acylated derivatives of sialic acid for Kala Azar, PCR for tuberculosis, LDH based test system for malaria, IFA for rabies, DAT for toxoplasmosis, ELISAs for reproductive hormones, test system for alpha-fetoproteins, acute lymphocytic leukaemia and species specific diagnostic for snake bites. A number of industrial entrepreneurs have come forward and shown interest in joint collaboration for refining and upscaling of these technologies. A profile of diagnostics technologies has been prepared for widely circulation. A market survey for various technologies has been initiated jointly by DBT, DCGI & BCIL.

Miscellaneous: Studies on myositis-specific autoantibodies in patients with idiopathic inflammatory myopathies and their correlation with clinical, epidemiological and immunogenetic characteristics were undertaken at AIIMS, New Delhi. An interesting observation has been made with regard to the time of clinical presentation and time of onset of idiopathic inflammatory myopathy. Temporal clustering was observed with regard to both of these parameters. Thirty four patients out of 71 presented during the period May to August. The onset of muscle weakness was observed in as many as 25 patients during the period November to January. There was truly no evidence of geographic clustering of cases.

3.13. HUMAN GENETICS & GENOME ANALYSIS

The "Human Genetics & Genome Analysis" Programme was initiated during 1994 with the objective to identify, map and characterise genes related to genetic disorders prevalent in India for diagnosis, prevention and management of genetic disorders and to develop new methods of the analysis and interpretations of genomic DNA sequences.

Genetic Diagnosis cum counselling units: Genetic diagnosis cum counselling units established by the department have been providing molecular diagnosis and counselling for the common genetic disorders such as beta-thalassaemia, Duchenne Muscular Dystrophy (DMD), haemoglobinopathies and neurogenetic disorders prevalent in the country, to the affected families.

The five units provided the following genetic disease diagnostic and counselling services to the affected families during the year:

Genetic Disorder (Diagnosis & Counselling)	Genetic Diagnosis cum Counselling Unit				
	IIH, Mumbai	B.J.Wadia Hospital, Mumbai	B.J.Med. College, Pune	GNDU, Amritsar	CMC, Vellore
β-thalassaemia major	245 unrelated Heterozygotes	787 couples	269 couples		546

Sickle cell anaemia, Hb E or Hb S thalassemia	78 couples				
G6PD Deficiency	253				
Haemophilia A&B	10				
Von Willebrand Disease (VWD)	28				
Glanzman's Thrombasthenia	25				49
Down Syndrome		135	25	17	
Cystic Fibrosis			43		
Chromosomal Analysis				172	
Retinitis Pigmentosa				28	
Sex related anomalies				15	
Acute Leukaemia					120

Since the inception of 14 genetic diagnosis cum counselling units, about 11,000 affected families got benefited from prenatal diagnosis and counselling for major genetic disorders and 1a population of 1,700 tribals screened for these disorders.

A novel β thalassemia mutation (codon 10 GCC→GCA) and rare transcriptional mutation (28 A→G) were identified in two families which were referred for second trimester prenatal diagnosis at Institute of Immunohaematology, Mumbai. B.J. Wadia Hospital, Mumbai also identify unknown mutations. A simple and cost effective test (ACQ) was developed by B.J.Medical College, Pune, for screening β - thalassemia trait.

Programme on Functional Genomics: The project implemented at CBT, Delhi, progress has been made in studies related to genomic instability, trinucleotide repeat expansion and neurodegenerative disorder. Fast and efficient methods for isolation of DNA from 10 ml. of blood samples and a PCR based analysis for SCA3 and SCA1 disease detection have been standardised. Trinucleotide repeat sequences have been found to be abundantly interspersed in the human genome. These trinucleotide repeats exhibit extensive length polymorphism in the neurodegenerative disorders. All the probands were analysed for SCA1, SCA2, MJD (SCA3), SCA6, SCA7, DRPLA and SBMA. Seventeen of the 34 families studied have been positively assigned to a locus and appears to have unknown/novel mutations. The 29 β -thalassemia

samples received from VIMS, Calcutta (Eastern region of India), single nucleotide polymorphism were found to be commonest which is unique to eastern region of India.

Gene Therapy: Under the project, "Experimental gene therapy on Oral Cancer" at CRI, Mumbai, cloning of 2 kb cDNA sequence of the Herpes simplex virus thymidine kinase (HSV-tk) gene in a replication deficient viral vector was carried out. An epithelial cell line NT8e was established from an human oral tumour. A model was developed for preclinical studies of gene therapy *in vitro* and *in vivo*. PTK-16 cells were shown to be sensitive to ganciclovir (GCV); using these as effector cells and mixing them with different ratios of target cells the extent of bystander effect was demonstrated.

Human Genome Diversity: The project has been implemented as a collaborative project at ISI, IICB and SINP, Calcutta. Blood samples with appropriate informed consent from unrelated individuals belonging to several population groups of eastern India were collected. Standardisation of protocols for genotyping about 20 DNA marker loci has been completed. Based on genotyping results of 8 human specific insertion/deletion polymorphism in population indicated that the group of modern humans who entered India about 60,000 years ago from Africa underwent a major demographic expansion in prehistoric times within India. Genomic evidence supported the anthropological hypothesis on Austro-Asiatic speaking tribals being the most ancient inhabitants of India. Frequencies of haplotypes based on CTG repeat and three other biallelic markers in and around the myotonic dystrophy (DM) locus were estimated in 13 ethnically, linguistically and geographically diverse populations of India. The range of CTG repeats in caste populations was 5-31, while in tribal populations the range was shorter (5-23). Extensive variation in frequencies of large (CTG) alleles (>18 repeats) was found in Indian populations. One of haplotypes was found to be in particularly had high frequency in Indian populations, and also among Indian DM patients.

Immunogenetics: The studies relating to Immunogenetics on patients with various forms of tuberculosis (PTB, MDR-TB, MTB/DTB, LNTB) and sarcoidosis revealed the increase of HLA-DRB1*15/16, in MDR-TB, MTB and PTB patients. The increased frequency in the above group was not statistically significant as compared to the control population. Significant increase of HLA-DRB1*11 was found in sarcoidosis patients. The studies indicated significantly increased HLA-A2 in tuberculosis patient groups, especially in patients with drug induced hepatotoxicity. These studies indicate the role of host genetics and susceptibility to clinically severe tuberculosis.

National Ethical Committee:

Following adoption of the declaration on "universe declaration of human genome and human rights" by UNESCO, on November 11, 1997, an adhoc working group comprising of representative members status of UNESCO decided to establish Inter-Governmental Committee to keep a liason with Government bodies of different countries of international Bio-ethic Committees. In order to discuss issues specifically related to our country leading to modifications of universal declaration clauses with the approval of Hon'ble Minister of Science and Technology, Government of India, the department has set up a "National Bioethics Committee". The composition of the Committee is provided in Annexure-II. The department earlier had a "Bioethic Committees" which has now been merged with "National Bioethics Committee". This committee will consider any amendment(s) in the universal declaration on human genome and human rights, liason with the International Bioethics Committees of UNESCO, develop guidelines for bioethics and consider/monitor programmes on "gene therapy" research in the country.

3.14. VACCINE RESEARCH AND DEVELOPMENT

National Jai Vigyan Mission on Development of New Generation Vaccines: The department has initiated the programme based on several leads obtained from the R&D projects funded by the department earlier. The objectives of programme includes generating candidate vaccine(s) for diseases like cholera, rabies, Japanese encephalitis, tuberculosis, malaria and HIV. The programme has been grouped under three categories. Category (A) includes those 'candidate vaccines ' where potential research leads are available and which now require clinical trials or generating pre-clinical data. Under this category, vaccines for cholera and rabies are being attempted. The programmes under category (B) are related to available leads on candidate vaccines where efficacy and immunogenicity need to be worked out. The target vaccines are tissue culture based Japanese encephalitis, DNA vaccines, epitope vaccinogenes of Japanese encephalitis and synthetic candidate malaria vaccine. Under category (C), programmes requiring sustained R&D efforts with the possibility for development of candidate vaccine(s). Under this category ,efforts will be made to produce candidate prophylactic anti-HIV and DNA vaccines for tuberculosis.

The Mission programme is being carried out in a network fashion involving scientists at institutions located at various geographical regions of the country. It is expected that at the end of the programme at least 2-3 vaccines will be available for upscaling and use to control/contain the infections while others would reach the stage of the clinical trials. The programme is being co-ordinated by the department and is being implemented at NICED, Calcutta; NIV, Pune, NARI, Pune; NIN, Hyderabad; Institute of Microbial Technology, Chandigarh; NIMHANS, Bangalore; JN Centre for Advanced Studies, Bangalore; AIIMS, New Delhi; NII, New Delhi; NCCS, Pune ; PGIMER, Chandigarh; SGPGIMER, Lucknow; Delhi University(South Campus), New Delhi; Pune University, Pune and Indian Immunologicals, Bangalore.

The activities of the two vaccine production units, namely, Bharat Immunologicals & Biologicals Corpn. Ltd. (BIBCOL) and Indian Vaccines Corpn. Ltd. (IVCOL), set up by the Department for the production of viral vaccines are described below:

BIBCOL: The company is formulating Oral Polio Vaccine (OPV) from imported bulk since January, 1996 and is supplying it to the National Immunisation Programme. During the year, the company has formulated 175 million doses of OPV, a part of which was supplied to UNICEF.

As a follow up of WHO team's visit to BIBCOL facility in Sept., 1998, for WHO-GMP certificate, a joint team of experts inspected BIBCOL production facility and they were satisfied with the set up. BIBCOL has been declared as sick company and the IDBI has been appointed as "Operating Agency" to prepare a "revival package". Under the Integrated Long Term Programme of Co-operation in Science & Technology (ILTPOC) between India and Russia, basic production of OPV as well as BCG and measles vaccines in collaboration with IPVE and MPBP, Moscow, Russia is proposed to be undertaken.

IVCOL: The company is being restructured for the utilisation of its facilities following the withdrawal of French collaborator for the production of vaccines and immuno-diagnostics with the involvement of strategic partner.

Edible Vaccine: At DUSC, New Delhi, vectors for transformation of *V.cholerae* antigen in tomato and tobacco have been designed. At CIMAP and NBRI, Lucknow, rabies viral G-protein has been cloned gene in cabbage.

3.15. ANIMAL BIOTECHNOLOGY

As in the previous year, programmes on development of transgenics, providing better animal nutrition, vaccine development and disease diagnostics were pursued with emphasis.

Two meetings of the Task Force were conducted and the progress of 36 ongoing and completed projects were evaluated and 11 new projects were also considered for funding. A brain storming session on Ovum Pick Up (OPU) technology was also held.

An inactivated vaccine for Infectious Bovine Rhinotracheitis (IBR) was developed by BAIF Development Research Foundation, Pune through DBT funding and this technology was transferred to Hoechst Roussel Vet. Ltd. in April 1999 through the National Research Development Corporation.

Embryo Transfer Technology and related areas : Embryo transfer techniques in camel were standardised. Two ET camel calves were born at the National Research Centre on camel, Bikaner. High cell density fermentation for expression of ovine growth hormone (OGH) in *E.coli* was optimised. The authentic recombinant OGH without histidine tag from the inclusion bodies of *E.coli* has also been purified and refolded. A brain storming session on Ovum Pick Up (OPU) system was held and various aspects of OPU were identified for developing specific research projects.

Nutrition: A multicentric programme on various aspects of lignocellulose degradation has been launched as a follow up action of the brain storming session.

Transgenics: Significant progress has been made towards the development of transgenic mice. Transgenic mice expressing Inter leukin-2 (IL-2) gene under the control of β -casein promoter in pCAGGS vector have been produced and founders are being analysed for the expression of IL-2 gene. Transgenic mice expressing neomycin resistant (neo^r) gene have been produced and immunological responses in neo^r transgenic mice are currently under evaluation. Transgenic mouse for hepatitis B virus X protein, fragile x syndrome and 5HT1A receptor have also been developed.

A repeatable procedure for cloning rabbit embryos through nuclear transfer using both fresh and frozen thawed embryos as nuclear donors was successfully established. An *in vitro* culture system that supports development of cloned rabbit embryos to blastocyst stage was also established.

Several mutations both in the activation and oligomerisation domains of tumour suppresser gene p53, in oral cancer patients have been identified. These mutants were generated, *in vitro* by site specific mutagenesis. The constructs carrying the p53^{mut} genes will be transfected into oral epithelial cells and embryonic stem cells for screening.

Animal Vaccines: The production of rabies vaccines for animals was standardised in micro carrier system and sterility, toxicity, identity, determination of pH and potency tests of this vaccine were carried out as per the WHO guidelines. Challenge test was also conducted in mice. Efforts are on to validate this vaccine for commercialisation.

To generate a recombinant vaccine against anthrax, several varieties of non toxic protective antigen (PA) molecules defective in different functions have been generated, thus leading to a loss of virulence of anthrax toxin. PA purified from pMS1 by conventional chromatographic techniques led to loss and degeneration of PA. Thus, PA was subcloned into expression vector pQE-30 and purification was done using affinity chromatography. The protein was renatured by slow removal of the denaturant. Renatured PA was purified using Ni-NTA affinity chromatography and anion exchange chromatography on FPLC. The biological activity of renatured PA was found to be comparable with PA from *B.anthraxis*. Mutations were done replacing certain amino acids in domain 2 of PA using mutagenic primers.

Recombinant dog zona pellucida glycoproteins were successfully cloned and expressed in *E. coli*. The expression was further optimised in a fermentor to obtain higher yield of the

recombinant protein. The antibodies against recombinant dog zona proteins reacted with native dog zona pellucida. Batches of these have been purified and conjugated to diphtheria toxoid (DT); these would be evaluated for their efficacy to regulate fertility.

Diagnosics: Using residual binding, competitive and displacement binding methods did pregnancy diagnosis in cattle and buffalo from milk. Three alternate methods have been developed for antibody purification, which are required for pregnancy diagnosis.

For development of immunodiagnostic kit for bacterial pathogens in laboratory rats, pathogenic strains of *Streptococcus* α , β , *E.coli* and *Klebsiella pneumoniae* were isolated. Antigens were prepared and used for immunising BALB/C mice for Hybridoma and monoclonal antibody production. In addition, rabbits were immunised and polyclonal antibodies obtained. The polyclonal antibodies were coated to latex particles and a latex diagnostic reagent developed showed strong positive reaction.

For development of diagnostics for Blue tongue virus, PCR of the VP-7 gene was done and restriction enzyme digestion of the product revealed differences between various isolates. Antigens were developed, purified and tested using negative, weak positive and strong positive serum of a commercially available competitive ELISA kit.

Monoclonal antibodies were produced against a local strain of PPRV. All the nine MAbs were found specific to 'N' protein of PPRV and were of IgG 1 isotype. The MAbs were found to cross-react with six other isolates of PPRV but not with the RBOK strain of rinderpest virus in both radioimmunoprecipitate assay and fixed-cell ELISA. All monoclonals were used in epitope mapping using additive effect of the optical density as a criterion to indicate binding to different epitopes. All the MAbs appeared to bind to the same epitope. One of the nine MAbs was produced in bulk, concentrated by ultra filtration and tested in the imported commercial kit for PPRV / RPV diagnosis. MAbs production and characterisation against RBOK strains of RPV is under progress.

Animal By-products: Serum from slaughterhouse waste was studied for the preparation of protein binder, serum based binder and for their ability to form film which were tested for their mechanical properties and thermal behaviour. Studies showed that the serum based protein preparation could serve as a binder in the finishing operation of leather processing to improve aesthetic value. The binder developed was found to be comparable with the casein based commercial binder. However, it was observed that resistance to water needs further improvement.

Procedures for the isolation of two hydrolase enzymes, pig liver esterase and pig pancreatic lipase have been developed from slaughterhouse blood.

The leaves of *Calotropis gigantea* extract treated with alcohol showed better clotting of milk. On separation using Sephadex G-200 column, five fractions were obtained. The second fraction showed higher protein value (6.8 mg) and also had a higher milk clotting activity.

Poultry Demonstration: Locally available feed ingredients were used for feed formulation to enhance the broiler productivity in the hilly region. The effect of different dose levels of probiotics on feed conversion efficiency was also analysed. Microbial agents (Lactobacilli and Streptococci) along with yeast and enzymes were administered through drinking water and feed to establish their population in the gastro-intestinal tract. This helped to enhance gain in body weight, feed conversion efficiency and survival. These agents also increased plasma globulins to provide non-specific resistance to withstand physiological as well as climatic stress in hills.

3.16. AQUACULTURE AND MARINE BIOTECHNOLOGY

Sustained efforts were made towards the overall development in aquaculture by developing various technologies for productivity and production enhancement and also in pursuing research in critical areas. The various problems in disease diagnostics and vaccines received attention. Areas like transgenic development and molecular biology in aquaculture and fisheries remained a focus. Several leads were generated towards process and product development.

Feed development: At CFTRI, a fermented silk worm pupae (SWP) silage has been developed which can be incorporated in diet as a source of protein for replacing conventionally used fish meal. Microbiologically safe silage product can be prepared by fermentation of SWP under microaerophilic conditions at ambient temperature. A pilot plant facility was set up for production of about 100-kg offal silage and a process know-how for production of fermented SWP developed. A process patent for the preparation of fermented silkworm pupae silage useful as an ingredient in animal feeds has been obtained.

At CIFA, Bhubaneswar, effect of hormones and essential amino acids (EAA) on growth of catla fry with special reference to lysine and methionine were studied. It was observed that feed without fishmeal but with lysine and methionine supplementation @ 1.0% gave better growth in the fry of *Catla catla* compared to the control. Experiments were conducted in culture ponds with fingerlings of *Catla catla* and *Labeo rohita* at a stocking density of 30,000 per ha. It was observed that the addition of lysine and methionine in the feed significantly enhanced the growth in catla and rohu fingerlings as compared to those maintained on the control diet. An attempt was made to record the effect of varying dietary protein and 17- α -methyl testosterone content on the growth of *Labeo rohita* fry under hatchery condition. It was observed that fish fed with 42.3% crude protein diet registered significantly higher growth as compared to those fed on 30.8% crude protein diet. The fry given 17 α -methyl testosterone incorporated diet recorded higher growth rate in comparison to the control diets.

Synthetic prostaglandins: At NCL, Pune, work on synthetic analogues of prostaglandins was carried out for fish breeding. Cloprostenol, prosolvin and tiaprost were employed for the studies on fish reproductive endocrinology. Prosolvin, tiaprost and three new analogues administered to male *Labeo rohita* showed oozing milt. Tiaprost was found effective in inducing milt and when tested on *L. rohita* complete spawning was reported. A new methodology has been investigated for the preparation of thiaprostaglandins using a heterogeneous catalyst. A process patent has been filed on the investigation made. This study is likely to lead to new fish spawning agents

Transgenic development: Under a project on transgenic fish, cloning of growth hormone cDNA of Indian major carp rohu (*Labeo rohita*) and Indian catfish *Heteropneustes fossilis* was taken up. cDNA clones were developed and libraries constructed from the poly A RNA of rohu and catfish. These libraries were screened and GH cDNA clones were sequenced. Transformation vectors have been constructed with the GH cDNA of rohu and catfish with/without the reporter genes such as GFP, for the expression of rohu and catfish GH cDNA in *E.coli* and fish systems. The study could lead to the development of a series of vectors using the GHcDNA sequences of rohu and catfish. This will be useful in the detection of transgene expression in fish. Indigenous vectors have been developed. These are likely to lead to development of transgenic fish using indigenous gene constructs.

Protoplast Isolation: Experiments were conducted to understand the optimum requirement for the isolation of seaweed protoplasts. The isolation procedure of protoplast from *Gracilaria edulis* has been standardised. Viable protoplasts were successfully isolated from *G. edulis* using enzymes agarase, macerozyme, cellulase and pectinase. Maximum liberation of protoplasts was

achieved within six hours and the liberated protoplasts were separated from the cell wall and other debris by sieving. The protoplasts settled at the bottom were maintained in a protoplast culture medium and fusion attempted either with varying levels of PEG or in an electric cell fusion system.

Biomolecules/bioactive compounds: The species of *Oscillatoria*, *Anabaena*, *Lyngbya*, *Synechocystis*, *Nodulria*, *Nostoc* are potent toxins producers. They were isolated and characterised for toxin production. Optimisation of culture conditions of cyanobacteria for production of secondary metabolites containing toxins are being attempted.

Studies undertaken on Indian horseshoe crab (*Tachypleus gigas*) indicated the presence of a specific type of bioactive compound in female horseshoe crab that prevented settlement of fouling organisms. Screening of bacterial extracts obtained from female horseshoe crabs indicated that bacteria colonising the horseshoe crab have the potential of producing metabolites that can prevent further epibiosis. Antibacterial assays revealed that the bacteria associated with the female horseshoe crab has the potential of controlling bacterial epibiosis by producing low molecular weight bioactive compounds. Larval assay with extracts from three strains of bacteria (*Proteus sp.*, *Micrococcus sp.* *Staphylococcus sp.*) associated with female crabs showed minimum metamorphosis. The results from this indicated that bacteria from the horseshoe crab could also produce toxic compounds helpful in the control of epibiosis. A patent on a novel process for the extraction of antifouling compounds from the horseshoe crab has been filed.

Molecular diagnostics for shrimp disease: Among the various disease problems affecting shrimp, the white spot syndrome virus disease is the most serious one. This disease has spread considerably and it is estimated that there is considerable loss due to this viral disease. A polymerase chain reaction (PCR) based diagnostic method has been developed at the College of Fisheries, Mangalore for detection of white spot syndrome virus (WSSV) in shrimp. The method is useful in screening the virus in brood-stock, larvae and carrier animals. It can detect the presence of virus even in a dormant stage.

Monoclonal antibodies have been developed for diagnosis, serotyping and characterisation of antigen of white spot virus. An ELISA technique has been developed which will be useful for measuring the antibody titre in immunised mice for screening of hybridoma clones and for antigen characterisation. The fusion carried out resulted in a set of stable hybridoma clones, which seem promising. These clones are being characterised by ELISA and Western blot for use in Immunodot /ELISA for diagnosis and antigen characterisation.

Development of fish vaccines: Vaccines against infection by *Aeromonas sp* and *Pseudomonas sp.* have been developed at Bharathidasan University, Tiruchirapalli and at American College Madurai. These are useful in preventing infections in cultured freshwater fishes like *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. The efficacy of the conventionally developed heat killed whole cell and formalised vaccines appear promising as seen through field trial.

Development of bioreactors for shrimp hatcheries: Using isolates of ammonia and nitrite oxidising bacteria, two types of bioreactors such as *in situ* Stringed Bed Suspended Bioreactors (SBSBR) and *ex-situ* packed Bed Bioreactor have been developed. The reactor developed for penaeid larval rearing system was experimented in field conditions and it was found to be useful in reducing the ammonia content in water and enhancing the larval survival. Field trials undertaken at the Regional Shrimp Hatchery, Department of Fisheries, Govt. of Kerala and in private hatcheries indicated promising results. The technology is under patenting.

Development of culture technologies in new species: Attempts were made to produce seeds of black lip pearl oyster *Pinctada margaritifera* in onshore tanks. These specimens collected from the pearl banks of Gulf of Mannar were successfully transplanted to Tuticorin. The survival rate of pearl oyster in various environmental and hydrological conditions was studied.

Studies on the development of technology for mud crab larval hatchery indicated that only, *Scylla oceanica* and *S. tranquibarica* are suitable for hatchery development. The egg takes 7-20 days for embryonic development and hatching at the ambient temperature. A hatching of about 95-97% was reported with careful monitoring of water quality parameters. The zoea larvae took about 10 days to transform to the megalopa stage. The megalopa larvae are the ideal stages for stocking them in the culture ponds.

Attempts were made to produce seed of groupers and breams at Mandapam. The brooders collected from wild were studied for induced maturation and for their acclimatisation to the local conditions. Various parameters were studied for improving their survival through monitoring physico-chemical parameters and antibiotic treatment at regular intervals.

Mission Mode Demonstration projects: At CIFA, the prawn complex, hatchery, feed mill and prawn farm was functional. About 1.08 lakhs freshwater prawn (*Macrobrachium rosenbergii*) seed were produced. Nearly 50 tonnes of feed was also produced. Both these inputs have been supplied to the farmers for growout operations. Around 15 farmers were adopted who have produced 1.0 to 1.5 t/ha/7 months. In Madhya Pradesh, freshwater prawn culture technology has been developed and 2.5 ha water area has been used for demonstration. Both monoculture and polyculture experiments were undertaken to produce about 1.0 to 1.5 t/ha.

At NIO, R&D activities were undertaken to study the white spot baculovirus infection in *P. monodon*. Encouraging results were shown with selected plant extracts which were found effective in controlling and treating WSSV infected ponds. An alternate live feed (*Diaphanosoma celebensis*) for shrimp larvae has been identified in place of *Artemia nauplii*, which is being used in shrimp hatcheries. Techniques have been developed to culture them in the laboratory. Probiotic bacterial strains isolated from various marine niches gave better growth and survival of the test animals (*Artemia nauplii* and *Cladocera*) in comparison to control.

At Salem, culture operations for the second crop commenced and 2,04,000 PL 30 of scampi stocked in nursery ponds. A survival of about 51.2% was obtained from the nursery ponds and satisfactory growth was reported. The growth in growout culture ponds is progressing well. The harvest is likely in March 2000. At MSSRF Chennai, growout freshwater prawn aquaculture was established at Chidambaram in Cuddalore district. Ecologically sustainable aquaculture was adopted at the project site with integrated farming system. The usage of rainwater harvesting, zero-water exchange without any application of chemicals/antibiotics has been practised. Nearly 0.5 ha water body yielded a harvest of about 1.02 t/ha. Prawn seeds were produced and supplied to the farmers. Brooders were distributed to nearby hatcheries for seed production. A training programme was organised to cover different aspects of prawn culture, feed formulation, method of harvesting for various target groups like farmers, women folk and vocational students.

Several freshwater prawn farmers meet and extension programmes were organised by CIFA and MPCST to disseminate the technology of giant freshwater prawn culture to the farmers and entrepreneurs. The technologies have been disseminated and transferred through various training programmes to the interested farmers and entrepreneurs in different states. 23 persons from different states have been trained on breeding and hatchery management. Grow out culture technology was popularised in various rural and tribal regions. Publications in regional

languages were brought out as extension and training material for the benefit of the prawn farmers.

The growth and survival of hatchery reared *Marobrachium rosenbergii* were studied at different salinities. Though this species can survive and grow in low saline brackishwater areas, the best growth performance occurs in freshwater medium. Based on the performance, this species can be considered as a candidate species for polyculture in brackishwater areas also, leading to diversification in aquaculture.