10. AUTONOMOUS INSTITUTIONS

10.1 NATIONAL INSTITUTE OF IMMUNOLOGY (NII), NEW DELHI

The National Institute of Immunology (NII) has steadily worked at furthering knowledge of the molecular mechanisms of interaction between the biological systems and their environment while searching for new intervention modalities to combat mortality and morbidity as well as enhancing national competence in modern biology research. The Institute follows the two-pronged strategy of linking excellence in fundamental research with the pragmatic pursuit of application possibilities in entrepreneurial partnerships. The major areas of research at NII are Immunity and Infection, Gene Regulation, Molecular Design and Reproduction and Development. Salient features of the achievements during the year 1999 are highlighted below.

Immunity and infection

The Prime Minister released world's first therapeutic vaccine based on Mw developed at NII in the market under the trade name Leprovac during the year.

The complete genome of an Indian isolate of JEV (GP78) was sequenced revealing its evolutionary closeness to the Chinese SA14 isolate. A method to grow JEV to high titres in tissue culture was successfully adapted to help in large-scale production of the viral vaccine.

A novel immunomodulatory use of an Indian medicinal plant product (NII-60) to prevent septic shock received a US patent and was transferred to an Indian pharmaceutical company for commercialisation. Another product, NII-70, showing positive effects on hemopoiesis also received patent protection in USA and is being developed in collaboration with a French company for commercialisation.

In a major contribution to modulate immune responses for prophylactic and therapeutic purposes, antigens targeted to scavenger receptors were shown to enter an endo-lysosomal antigen processing and MHC class I-restricted presentation pathway independent of cytoplasmic processing and transport steps. This pathway is efficiently used by all antigen-presenting cell lineages including B cells, and the finding is of potential importance towards overcoming the problem of generating class I restricted cytotoxic T cells against subunit vaccines aimed at viral infections. Furthermore, such targeting led to modulation of the cytokine profile of anti-allergen responses indicating the potential of this approach for shifting immune responses for therapeutic utility in allergy.

Reproduction and development

In a project to generate transgenic animal models for some human diseases, transgenic mice expressing the hepatitis B virus X gene were generated that rapidly developed hepatocarcinoma. Such a model has potential for its one in understanding the disease processes and for screening of Chemotherapeutic agents.

In exploring the zona pellucida as a potential contraceptive target, a monoclonal antibody against the bonnet monkey zona pellucida (bmZP) was shown to inhibit human

egg-sperm binding *in vitro*. The motif recognised by this antibody was mapped using overlapping octapeptides. Zona protein B cell epitopes have also been genetically engineered, and efficacy studies of synthetic zona peptide vaccine to inhibit fertility were initiated.

The human testis-specific gene *hi*, fished out from the human testicular cDNA library, has now been shown to be uniquely expressed in testicular tissue. Its homologues in monkeys and mice have also been determined. Meanwhile, another recombinant testicular protein has been used for immunising primates. Antibodies so generated react to the anterior part of the acrosome and inhibit sperm-egg binding in an *in vitro* hamster sperm penetration assay.

Molecular design

Molecular mimicry has been the focus of studies to understand the structural aspects of molecular recognition. The structure of a carbohydrate mimicking-peptide complexed with concanavalin A (ConA) was determined to atomic resolution. Although the peptide-binding site does not overlap with the sugar-binding site, this peptide inhibits T cell stimulation by ConA indicating its functional relevance. Similarly, in order to understand the structural basis of innate immune response, the studies revealed that the molecule achieves functional activation by a conformational transformation.

To exploit the biosynthetic pathways of *Leishmania donovani* as molecular targets for vaccine development or chemotherapeutic attack, attempts are continuing to synthesise the surface lipophosphoglycans (LPG) and glycosyl inositol phospholipids (GIPLs) of the parasite. Several intermediates in the biosynthetic pathway were synthesised to establish the role of GIPL/LPG of the parasite on protein kinase C activity within the macrophages.

Receptor-mediated endocytosis for delivery of pharmacological agents into specific cell types continues to be one of the major themes. Administration of the anti-cancer drug doxorubicin in a conjugated form with maleylated BSA for delivery via scavenger receptors to nude mice transplanted with multidrug-resistant cancer cells showed markedly better survival and significant regression of preformed tumours, establishing the feasibility of this regimen to counter drug resistance.

Gene regulation

Clinical isolates of *Mycobacterium tuberculosis* are being genotyped in order to understand the scale and nature of multidrug-resistant tuberculosis (MDR-TB) in India. Over a hundred isolates were characterised, and a great deal of polymorphism, especially in the MDR-TB strains, was observed. This pattern differs from the West where two strains, W and P, are the predominant ones. At the gyr A locus, most of the resistant strains have been mapped to point mutations between amino acid residues 87 and 98.

A synthetic gene construct encoding multiple B cell, T cell proliferative and CTL epitopes derived from various stages of *Plasmodium falciparum* was expressed in the baculovirus system as a candidate vaccine against malaria in collaboration with the Centres for Disease Control, Atlanta, USA. The resultant protein elicits antibody responses in rabbits against both the vaccine components and all stages of *P. falciparum*.

A 906-bp EcoRI satellite fraction from the genome of a prominent endangered species of India, the one-horned rhinoceros, *Rhinoceros unicornis*, was cloned and sequenced. The contig analysis suggests that this is either a part of or adjacent to a functional gene. The resulting probe was established to be species-specific and useful for assessing intra-species relatedness.

Other activities

As part of industrial interaction, NII is an intellectual partner in the emerging research park being entrepreneurial being developed at Hyderabad. Six technology transfer negotiations with industries for commercial exploitation of research leads are in progress. Similarly, NII continues to protect the commercial potential of its research leads by international patenting, - seven applications were filed during the past year. Furthermore, over fifty research papers originating from the work done in NII were published in highly acclaimed international peer-reviewed journals over the past year.

10.2. NATIONAL CENTRE FOR CELL SCIENCE (NCCS), PUNE

National Centre for Cell Science has been functional in three important areas viz., establishing a national repository of animal cell cultures as a part of the germ-plasm collection programme; manpower development in animal tissue culture and to extend infrastructure facilities to researchers and institutions in biomedical fields; and to carry out state-of-art research and development in the area of cell sciences.

Cell Repository:

The cell repository has a collection of 1127 cell lines derived from 25 different species including hybridomas. During the reporting period, 390 cell cultures comprising of 384 different cell lines were supplied to 384 research institutions across the country.

Human Resource Development:

As a follow-up of the technology transfer effected last year, a specialised training workshop on “Skin Keratinocyte Cultivation and Transplantation” was conducted for the staff of various hospitals. The centre continued to engage in the training programmes at various levels in animal tissue culture and related areas such as hybridoma, molecular biology, etc. Summer training programme was organised for the post-graduate students from the University of Pune and the Devi Ahilya University, Indore. A practical course in “Animal Tissue Culture” was conducted for 8 M.Sc. students of Developmental Biology from University of Pune.

Cell Biology:

Studies on stromal cell biology and signalling in haematopoietic process highlighted the role of CD2 population as cellular target of erythropoietin. CD2 population completely abrogated TGF release, indicating a role of T lymphocytes in the process. Ability of the cytosolic extract from regenerating pancreas to reverse the status of STZ induced diabetes in mice has been evaluated. One of the fractions, FR4D, was most effective. This fraction has been sequenced for 35 amino acids from the N-terminal and homology searches suggest this to be a novel protein. Using the *in vitro* wound healing models; hydroxyurea failed to inhibit cell migration. While protein synthesis

inhibited by cycloheximide also inhibited cell migration. Immunocytochemical analysis of migrating cells by confocal laser scanning microscopy (photographs 2,3a and 3b) was made. Studies have indicated that the bone marrow derived factor induces cellular migration of breast adenocarcinoma cells *in vitro*. An active component, a 55-kDa protein, has been identified. Locations of cyclin D 1 loci were identified in randomly growing population of SiHa cells by fluorescent *in situ* hybridisation. These loci localised near the nuclear membrane and/or the nucleoli. Utilising, two-colour immunofluorescence and two-colour FISH, preparations studied with the confocal laser-scanning microscope helped in simultaneous identification of nuclear and extranuclear structures. The relative positions of the cyclin D 1 loci in chromosome 11 territories were assessed in 3-dimensional reconstruction. A cDNA library of possible oncogene generated from Clone M3: mouse melanoma cells could be expressed in NIH 3T3 cells by transfection. One of the clones (G418) was remarkable in its ability to induce large tumours in nude mice at the sites of injection, to induce angiogenesis and to display propensity to metastasise. This property of the cloned gene to induce the transformation and metastases are significant since most of the known oncogenes require co-operation from two or more oncogenes from supporting transformation. The cDNA clone has been recently sequenced and shows no homology to any of the known oncogenes implying it to be a novel oncogene. Molecular studies on haemolytic bacteria obtained from mosquito midguts led to identification of these bacteria as *Aeromonas jandei*. Complete sequencing and PCR amplified 1.5kb rRNA gene confirmed this finding. It appears to be first report of isolation of this organism from the mosquito midgut. However the significance of the finding is still unclear.

Infection and Immunology:

Studies being carried out on: (a) immunosuppression during experimental leishmaniasis to establish role of non-T cell CD28 expression/function; costimulatory molecules and parasite specific receptors on macrophages; (b) molecular characterisation of SMAR 1 protein: to identify DNA binding motifs, protein-protein interaction and construction of knock-out and transgenic mice of this protein and (c) molecular and cellular basis of HIV pathogenesis: to identify differentially expressed molecules during HIV induced apoptosis and to elucidate the interaction of those molecules in the signalling cascade leading to cell death.

Others:

The Experimental Animal Facility has acquired BALB/cJ, C57BL/6j,DBA/2J and NOD/LtJ mouse from Institute of Microbial Technology, Chandigarh. A single male mouse with “spontaneous congenial cataract” was detected in a production colony of BALB/c mice. This mutant colony is currently at F7 level of inbreeding. A true breeding mutant strain is being attempted. Based on the work being carried out at NCCS, 29 publications have appeared in reputed international journals while another 15 have been communicated during the previous year.

10.3. CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS (CDFD), HYDERABAD

The major service components that CDFD has been to provide DNA typing and related analyses on exhibits referred to the Institute by crime investigating agencies, CDFD has also been assisting police personnel, forensic scientists, lawyers and the judiciary the evidential value of DNA profiling and related techniques in crime investigation and family matters.

During the, reporting year, CDFD received 155 cases comprising paternity disputes (83 cases), identification (44 cases), rape (18 cases), murder (6 cases) and others (4 cases). DNA fingerprinting was the only answer to many of the cases referred above.

With DNA fingerprinting becoming more and more acceptable, the demands on CDFD to carry out such examinations have been increasing. CDFD has entered into a Memorandum of Understanding with two State Forensic Science Laboratories of Tamil Nadu and Gujarat respectively for proving. The technical know-how for fingerprinting of routine cases referred to the State Forensic Science Laboratories

The techniques of DNA typing have moved into an era of detective and predictive medicine. The burden of genetic disorders on Indian Society is becoming more and more evident as better medical care and better immunization facilities against various infectious diseases are available. The DNA diagnostic group at CDFD has been making a concerted effort towards developing the molecular diagnoses of a number of common genetic disorders such as Fragile X Syndrome, beta-Thallasaemia, Sickle Cell Anemia, Duchenne and Becker Muscular Dystrophy, Huntigton's disease, Neural tube defects and other triplet repeat associated pathologies like Friedriech Ataxia, Myotonic Dystrophy, Muscular Atrophy and Alzheimer's disease and disorders like Hemophilia A and B, male infertility, Spinal Muscular Atrophy and various inborn errors of metabolism. Of the total 409 cases received by CDFD during the last year, the largest number was for male fertility (135 cases) followed by Hemophilia A (67 cases), Fragile X syndrome (43 cases), Duchenne and Becker Muscular Atrophy (32 cases), Hemophilia B (23 cases), Spinal Muscular atrophy (21 cases) and beta -Thallasaemia (11 cases).

Complementing the DNA diagnostic services, the chromosomal diagnostic group at CDFD has been providing services in the area of molecular cytogenetics. Of the 129 cases referred to CDFD, those for premature ovarian failure (30 cases) comprised the single largest number followed by primary amenorrhea (9 cases), abnormal sexual development (8 cases) and ambiguous genitalia (6 cases) among others. The primary cause of childhood blindness and the contribution of genetic elements to this abnormality continues of genetic elements to this abnormality continues to be another area of interest to CDFD.

Other service-related activities at CDFD include automated genome analysis and a DNA synthesis facility. Over the last year, CDFD has been able to generate a 1.5-megabase-sequence databank for use by research scientists. 500 oligonucleotide primers, with or without modifications, were also synthesized on demand. The Instrumentation group at

CDFD has been active for routine maintenance of equipment as well as providing advice on related issues.

The Bioinformatic group of CDFD is fully functional with several database and adequate application software, with utilities related to sequence analyses, molecular modeling and visualization. The Bioinformatics group has also initiated fundamental research on analysis of protein sequences and structures with respect to the role of proline residues. With a 64 Kbps leased line from VSNL the entire system at CDFD has been efficiently networked. EMBnet is a computer network of the European Molecular Biology and Biotechnology Research Community consisting of 37 members from different member countries of the world. CDFD is the India node of the EMBnet and besides China, which is the only other node in Asia. The Homepage of CDFD EMBnet (http://salarjung.embnet.org.in) can be accessed. Several data banks, data bases and the complete genome sequences of *Helicobacter pylori, Borrelia burgdorferi, Haemophilus influenzae, Mycoplasma gentitalium, Methanococcus jannaschii, Mycobacterium tuberculosis and Mycoplasma pneumoniae* can be accessed from the Web server.

CDFD has also initiated fundamental research in the areas of relevance to it. CDFD is an intellectual partner in a global effort to sequence the entire genome of the silkworm *Bombyx mori.* A very large number of simple sequences repeats (SSRs) have been identified for use in SSR-based molecular mapping of the silkworm genome. A comparative genome analysis, with respect to the SSR loci, of the six species of the silkworm viz. *B. merdarina, Antherea mylitta* (Tassar silk moth), *a, assama* (Muga silkmoth) and *Philosamia ricini* (Eri silk moth), is being carried out to highlight basic and applied issues of possible interest. Development of the *mariner* transposable element from the Tassar silkworm as putative vector for genetic transformation is another of research. Another relatively conserved sequence, GATA, originally derived from the banded krait minor satellite sequences has also been found to be present in the silkworm. These sequences, which have been predominantly associated with the sex chromosomein eukaryotes are anticipated to be involved in sex determination. The importance of these sequences and the proteins binding to them in determinig sex in silkworm is another area of basic research at CDFD.

Molecular analysis of the genetic epidemiology of drug resistance in clinical isolates of *Mycobacterium tuberculosis* and the rapid detection of *M. tuberculosis* in clinical samples are areas of research which have been initiated at CDFD. Molecular genetic and immuno- epidemiologic analysis of the malarial parasite is another focus of investigation, results from which will complement the development of a multi-stage candidate vaccine against *Plasmodium falciparum* malaria.

Genetic fluidity as evidenced by genomic alteration in the human genome leading to meningioma, a form of brain cancer, has been a subject of investigation at CDFD. In collaboration with the All India Institute of Medical Sciences (AIIMS), New Delhi. three different novel genetic loci which are believed to be associated with human meningioma have been identified. One such locus shows significant homology with a bacteriophage

protein, raising the possibility of the association of the bacteriophage gene with human meningioma and its role in promoting tumorigenesis in humans.

Research at CDFD has also been initiated on understanding basic cellular and molecular processes using gene expression in Baculovirus-infected insect cells as a model.

Human Resource Development, is of special interest to CDFD, and the training of personnel for providing state-of-the-art services in DNA fingerprinting is area of strengths. CDFD conducts an advanced short and long term training courses every year, in collaboration with CCMB and ADNAT. The last workshop was organised from February 23-6 March , 1999 and experts from India and abroad were invited to impart theoretical as well as laboratory training to the participants. An active summar training programme attracts the attention of young scholars about to enter the Ph.D programme in different institutions in the country. CDFD is affiliated to the University of Hyderabad and has enrolled Ph.D students under this programme.

The centre has been functioning from the space leased to it by CCMB, Hyderabad. The Centre has been alloted 13 acre land at Gandipet for setting up its own laboratories and other facilities for its full-fledged activities. During the year, the Centre had finalized its plans and design for its building and other infrastructure facilities

10.4. NATIONAL BRAIN RESEARCH CENTRE

National Brain Research Centre (NBRC), an autonomous centre of the Department of Biotechnology, New Delhi was registered as a society under the Societies of Registration Act, New Delhi in June 1999. National Brain Research Centre has been established by the Department of Biotechnology as a national apex centre for brain research, to consolidate, network, and undertake basic research of high calibre in neuroscience and establish linkages with national/international organisations involved in neuroscience research. Thus, NBRC would not only have infrastructural facilities and a coordinated multidisciplinary team of scientists working at the frontier of neuroscience, but also to support and network the existing groups in the country. The major objectives of NBRC are to undertake basic research to understand brain function in disease and normal conditions. The networking would involve either forming new linkages or augmenting existing linkages between various laboratories working in the field of neuroscience. While the major focus of networking would be within the country, efforts will be also made to set up collaborations outside the country, since research activities are of international nature.

The research laboratories of NBRC will be located on 38 acres of land at Manesar near Gurgaon, Haryana. A formal lease agreement between the transfer of land from IVCOL to NBRC has been completed and NBRC has acquired a long term lease on 38 acres of land in which, two buildings are located. The procedures are being initiated for the completion of these two buildings to serve as a laboratory and animal house of NBRC while the main building is being constructed. In addition to this, an interim

laboratory is also being set up at ICGEB in New Delhi. A Distributed Information Centre (DIC) has been sanctioned by the Department of Biotechnology for NBRC and it is being readied at ICGEB laboratories of NBRC. One of the major goals of the DIC of NBRC is to provide access to scientific literature to all the scientists working in neuroscience in the country.

One of the other major activities of NBRC is to develop human resource in the area of neuroscience. As a primary initiative in this regard, seven post-doctoral fellowships in different disciplines of neuroscience were initiated in several centres in India. In addition six workshops have also been held so far. These workshops include i)"Infections of the Nervous System"; ii) International Neuroscience Up-date; iii) "Immunohistochemistry, quantitative imaging techniques and confocal microscopy"; iv) "Emerging Trends in Neurophysiology; v) "Computational Neuroscience" and vi) "Morphometric evaluation of brain for neurotoxicological studies". These workshops were highly successful. On one hand they provided an introduction to the state-of-art techniques in some areas of neuroscience to investigators and on the other hand they helped to introduce the challenging areas of neuroscience to young investigators.

As a part of the inaugural activities of the NBRC, an "International Colloquium on Brain Research" was organised by NBRC at INSA, New Delhi. Scientists from several countries participated in this colloquium which focused on the emerging areas of neuroscience such as, computational neuroscience, neuroimaging, cognitive neuroscience, neurogenetics, etc. These institutes Japan participated in the symposium and presented research papers in frontier areas that are pursuing official delegations from National Institute of Mental Health, USA and RIKEN Brain Science Institute. An important feature of the colloquium was the large participation by young students and 120 poster presentation were made by the young scientists from all over the country. This is perhaps one of the largest gathering of neuroscientists in the country so far. The Hon'ble Minister for Science and Technology, Dr. Murli Manohar Joshi addressed the scientists and gave a special lecture on 'Consciousness' wherein he brought out the interdisciplinary linkages between physics, metaphysics in relation to mind and brain function.

During the colloquium, a Joint Statement was signed between the National Institute of Mental Health, USA and NBRC, India for undertaking scientific collaborations including training, conducting of workshops and joint research projects. A similar agreement was also signed with the RIKEN Brain Science Institute, Japan. As the follow up of the Joint Statements signed between NIMH and NBRC, NBRC now been invited to join the BMAP project for mapping specific transcripts expressed in brain from embryogenesis to adulthood in mice. It is also proposed to hold joint workshops in neuroinformatics and DNA microarrays (which are emerging areas in neuroscience) in the coming year. NBRC is also co-ordinating and organising the activities for conducting the "World Brain Awareness Week 2000" to be held in March 2000. This activity will be conducted all over the country to bring greater public awareness about brain function in health and disease.

10.5. NATIONAL CENTRE FOR PLANT GENOME RESEARCH (NCPGR), New Delhi

The National Centre for Plant Genome Research (NCPGR), an autonomous research institution entered its second year of existence on Ist April 1999. As the Centre's own building is yet to come up, for the time being its research activities are being carried out from the building of erstwhile Centre for Plant Molecular Biology (CPMB) of Jawaharlal Nehru University. The Centre has so far only a skeleton scientific and other staff. The University has provided 15 acre of land on its campus, adjoining the campus of ICGEB and Indian Council for Social Science Research. Master plan for developing the campus. The design of the building of the NCPGR has already been prepared. The Hon'ble Minister for Science & Technology and Human Resource Development Dr. Murli Manohar Joshi laid the foundation stone for the Centre's building on November 30th, 1999. The 'Foundation Stone Laying Ceremony' was chaired by the Hon'ble Minister of State for Science & Technology Shri Bachchi Singh Rawat.

Presently, chickpea (*Cicer arietinum*) has been identified as a mandate crop of the Centre for genomic studies. In India, the chickpea ranks first in both area and production amongst pulses and it accounts for approximately 75% of the world production. It is a major source of protein for human diet and animal feed. In respect to the studies on chickpea, the basic objectives of the Centre are (i) Structural genomics: Development of molecular markers; mapping and positional cloning of genes; Sequences and mapping of ESTs; Identification transposons and retrotransposons. (ii) Functional genomics: Isolation of new genes and promoters such as stress inducible genes, genes involved in nutrition, plant-pathogen interactions, signalling and encoding transcription factors, and genes controlling plant development and differentiation. (iii) Application genomics: Improvement of nutritional quality; Developments of cultivars resistant to pathogens and abiotic stress. In addition, various ongoing research projects started under the CPMB Programme will also be continued till the end of IXth Plan.

Highlights of the achievements made under different ongoing projects are as follows: -

(i) Nutritive value added transgenic potato populations:

The expression of amaranth seed protein, AmA1 in potato resulted in a striking increase in growth and production of tubers in transgenic lines. A significant increase was also observed in total protein content with broad correlation in increase in most essential amino acids.

(ii) Transgenic Crop Plants Resistant to Fungal Pathogen:

Over expression of oxalate decarboxylase (OXDC), the oxalate-catabolizing enzyme from Collybia *velutips* in transgenic tomato plants showed resistance against fungal pathogen, Sclerotinia *sclerotiorum*. The data suggest that the transgenic tomato plants with the ability to degrade oxalic acid can resist the infection and thus established that oxalic acid is an important determinant of pathogenesis.

(iii) Regulatory controls in expression of stress-inducible and stress-modulated plant genes and development of transgenics:

Regulatory controls in expression of two plant genes, phenylalanine ammonia-lyase (PAL) and calmodulin (CaM) of *Arabidopsis thaliana* are being studied. The *PAL*1

and *PAL2* genes are inducible in response to a variety of stress factors whereas CaM genes are stress-modulated. Tobacco plants containing Arabidopsis *CaM* gene in the sense orientation exhibit higher chlorophyll content and tolerance to high concentration of salt indicating that transgenic plants over-expressing CaM may have a potential to be used for producing plants that can be grown in moderately saline soil

(iv) Regeneration and Transformation of Chickpea

The genetic transformation and regeneration studies are being undertaken in order to improve nutritional quality of the Chickpea. A standard protocol of plant regeneration has been developed using the best-selected explants from different varieties and is currently being used to regenerate complete plants after introduction of foreign genes. In *Cicer*, transformation has been carried out in decapitated embryo axis using gene gun initially with plasmid harbouring kanamycin selection marker and GUS reporter gene followed by gene constructs containing desensitized forms of aspartate kinase (AK) and threonine deaminase (TD).

(v) Characterization and cloning of components of calcium-mediated signalling pathway/s with an aim to develop plants tolerant to unfavourable conditions

The major interest has been to characterise the various components of calcium-mediated signalling in *Brassica* and *maize.* These components include calcium dependent kinases and calcium binding proteins. Continuing the work on similar lines a cPKC kinase homologue has been purified to homogeneity for the first time from a plant system (maize) and polyclonal antibodies have been raised. This kinase has been shown to be involved in regulating NR (nitrate reductase) activity. Various PKC isoforms also have been identified in *Brassica* and a PKC kinase has been partially purified. A maize calcium/calmodulin kinase homologue and its immuno-homologue from pea have also been purified and characterised. This kinase is further shown to have a role in abiotic (salt and cold) stress signalling. Our studies on cold stress signalling further suggest that besides kinases, phosphatases also have an important role to play in these events.

Recently, a novel CaBPk (calcium binding protein kinase) has been purified from *Brassica.* This kinase seems to be very interesting and important as its presence in plants has opened up the possibility of existence of novel calcium signalling pathway, besides the normal calcium/calmodulin-signalling pathway. Another novel CaM binding, lipid stimulated kinase has been partially purified and characterised from Brassica. Further studies are under progress.

New initiatives on chickpea genomics:

(i) Detection of polymorphism among chickpea cultivars using molecular markers:

The identification of chickpea varieties by AFLP and SSR molecular markers will be helpful in assessing the purity and stability of the genotypes entering into the breeding programme. In order to identify the polymorphism present between the major groups and to standardize the AFLP technique, AFLP reactions were performed on DNA isolated from one kabuli variety (Pusa 362) and one desi variety (Pusa 232). The technique of

AFLP has been demonstrated to detect substantial degree of polymorphism on chickpeas. Between kabuli and desi almost 45% AFLP bands are polymorphic.

In order to map and identify genes that confer or influence early flowering, two chickpea varieties were used for crosses: kabuli type, late flowering Hadas and desi type, early flowering ICC5810. Having determined the favourable primer pair combinations, these were then used to detect polymorphism between the parents (Hadas and ICC5810). Out of the nine combinations, four combinations displayed high degree of polymorphism between Hadas and ICC5810. These four primer pair combinations were used for bulk segregant analysis of the F4 progeny. A number of AFLP bands have been found to be associated with early flowering.

Sequencing of cDNAs and characterization of ESTs:

A normalized cDNA library was constructed in λZAP vector-using mRNA isolated from the tissues consisting all the plant parts of chickpea variety, Hadas. One hundred randomly selected clones of this library were partially sequenced from both ends. These will serve as ESTs for producing probes for RFLP analysis besides the possibility of identifying useful genes. Eighty of the cDNA clones were analyzed for sequence homology from the database using BLAST search programme of NCBI. Some of the sequences show homology with several known plant genes. Few of them possess stretches of protein sequence motifs but some of them do not match with any sequences in the database. An EST clone, pC131, shows strong homology with a known nematode resistance gene (*Hspro*[1]) from sweet potato. At least three of its protein sequence motifs are highly conserved. Northern hybridization experiment using various tissues of uninfected chickpea shows its high expression in leaves as compared to the other tissues. Genomic Southern indicates the presence of a single copy in the chickpea genome.

Construction of subtraction libraries of chickpea:

A tissue specific subtraction library has been constructed for chickpea in order to screen tissue specific genes and the construction of subtraction library against *Fusarium* wilt is in progress.