ANNUAL REPORT
2013-14

NATIONAL RESEARCH CENTRE ON PLANT BIOTECHNOLOGY

(Indian Council of Agricultural Research)

ANNUAL REPORT

2013-14

National Research Centre on Plant Biotechnology
Lal Bahadur Shastri Building
Pusa Campus, New Delhi-110 012

Published by

Dr. T.R. Sharma
Project Director
National Research Centre on Plant Biotechnology
Pusa Campus, New Delhi- 110012
Tel.: 25848783, 25841787, 25842789
Fax: 25843984
Website: http://www.nrcpb.org

Compiled and Edited by

Dr. T.R. Sharma
Dr. Debasis Pattanayak
Dr. Kanika Kumar
Dr. Monika Dalal
Dr. Subodh K. Sinha
Dr. Amol Kumar U. Solanke
Dr. S.V. Amitha CR Mithra
Dr. Rampal Singh Niranjan

Cover Page Legend

1. Orthologous pairs between different plant species for *PAL* gene family
2. Affinity chromatography purification of recombinant SHMT3 protein
3. Draft genome sequence of mango, *cv.* Amrapali, decoded

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Phase II, New Delhi - 110 028 Ph.: 98110 53617, 45576780; E-mail: nvpnanda@gmail.com

Preface

Indian population is increasing with a growth rate of 1.58 % per year which may surpass China's population and become the largest country in the world by 2030. To feed the millions of growing mouth and to fulfil the pledge of food security, the county will have to produce 350 million tons of food grains by 2030. During post green revolution era, India has made a steady and significant progress in agriculture and a record food grain production of 259 million tons has been achieved in 2012-13. However, the uneven distribution of rain fall and unique geographical features make India prone to floods and drought in different regions. The problems may get exaggerated with the frequent episodes of *El nino* and other vagaries of climate change.

To address these issues, National Research Centre on Plant Biotechnology (NRCPB) has been entrusted with the responsibility of developing new tools and techniques and also embracing breakthrough in biotechnology for the crop improvement. During the year under report, many nematode-, fruit-, root-, and pathogenesis specific genes/ promoters have been isolated and characterized. These will be valuable genomic resources for the regulation of spatial and temporal expression of transgenes in different crops. To overcome the regeneration problems, *in vitro* and *in planta* methods have been standardized for the genetic transformation of pegionpea and wheat with insecticidal and heat stress responsive genes, respectively.

Structural and functional genomics have been flagship programmes of NRCPB. Our genomics team has generated the first draft sequence of the genome of Amrapali variety of Mango. Besides, NRCPB is a part of the International Wheat Genome Sequencing Consortium and was entrusted with fingerprinting and sequencing of the short arm of wheat chromosome 2A. In these programmes, large number of genes and genomics resources are being developed for use in crop improvement.

Commercialization of technologies developed at NRCPB has been an important part of overall activities of the centre. During this year, two patents were filed and five memorandum of understanding were signed for sharing gene constructs with different universities and Institutes of India.

The scientists from the Institute received prestigious awards and fellowships of scientific academies and brought laurels to the centre. Dr. T.R. Sharma was awarded the prestigious J.C. Bose National Fellowship by the Department of Science and Technology, Govt. of India and he also received NASI- Reliance Platinum Jubilee Award. Dr. R.P. Sharma received INSA Silver Jubilee Commemoration Medal and Quality Initiative Mission Education Leadership Award. Dr. A.U. Solanke received C.V. Jacob Award.

NRCPB has also contributed significantly towards human resource development by awarding M.Sc. and Ph.D. degrees in the area of plant molecular biology and biotechnology. Beside organizing short and long term trainings for the scientists of NARS, we also train M.Sc. students from different universities and institutions across the country.

Like previous years, this Annual Report elaborates different activities of our Centre covering research achievements, human resource development and other institutional activities. I sincerely thank Dr. D. Pattanayak, and his team comprising of Dr. Kanika, Dr. Monika Dalal, Dr. S.K. Sinha, Dr. A.U. Solanke, Dr. S.V. Amitha C.R. Mithra and Dr. R.S. Niranjan for their help in compilation and editing of the Annual Report. I also thank all the scientists and staffs of NRCPB for their persistent efforts towards the growth of the Institute.

New Delhi
Date: May 31, 2014

(T.R. Sharma)
Project Director

Contents

Executive Summary

Executive Summary

The National Research Centre on Plant Biotechnology (NRCPB) was established in 1985 as a centre of Indian Agricultural Research Institute, and subsequently it was evolved into its present status of institute in 1993. NRCPB undertakes research and teaching, and imparts training to various clients, students, scientists, NGOs and officers. The committed and sustained efforts of the Centre in conducting basic, applied, strategic and anticipatory research towards crop improvement are culminated into high standard research discoveries, which are aptly reflected by quality publications and patents applications in 2013-2014. Salient achievements of the Centre in research under six major projects, Isolation of plant genes and promoters, Transgenic crops for biotic stress resistance, Adaptation of wheat to climate change induced abiotic stress, Biotechnological approaches for increasing productivity, Genomics and molecular markers, and Improvement of nitrogen use efficiency in cereal crops; and progress in human resource development (HRD) efforts during 2013-2014 are given below:

- Small RNA libraries of chickpea in response to wilt and salt stress were constructed and identification and profiling of miRNAs were achieved by high-throughput sequencing. Identification of several predicted target genes of miRNAs, including transcription factors, DNA replication proteins and genes involved in cellular metabolism and responsive to a number of stress conditions, was done by *in silico* analysis. Eleven conserved and five novel miRNAs were validated using quantitative real time PCR.
- Microarray resources in *Arabidopsis* were used for identifying nematode-responsive and root-specific (NRRS) genes. The combined expression and *cis* element based screening led to the identification of a large number of NRRS genes. Two genes were significantly up regulated in response to nematode infection. The promoters of these selected genes were fused with GUS and the nematode responsive nature of the promoters was validated in *Arabidopsis*.
- Full length cDNAs of antifungal genes *pdf*1.2 (plant defensin) and *hsp* (heat shock protein) were isolated from SA-and JA- induced *Brassica juncea*. Pathogen-inducible glucanase gene promoter was validated in *Arabidopsis*. The promoter was induced during development, wounding, fungal infection and jasmonic acid treatment. Induction of chitinase and defensin genes was studied in *B. juncea, Camelina sativa* and *Sinapis alba* after different hours of post-infection by *Alternaria brassicae*. Higher induction of chitinase and defensin genes in *S. alba*, compared to that in *B. juncea* and *C. sativa,* contributed towards resistance to *A. brassicae*.
- Advanced back cross progenies of the interspecific hybrid and stable amphidiploids with *B. juncea* were screened for resistance to *Alternaria*. Out of 161 lines tested, 34 plants possessed high degree of resistance to *Alternaria* spp. Amphidiploid *Diplotaxis erucoides* x *B. rapa* showed highly resistant reaction.
- The molecular basis of fertility reversion in an improved Ogura CMS line (Og-1) was investigated as an effort towards developing and characterizing genetic stocks for heterosis breeding. Southern and northern hybridization with mitochondrial gene specific probes revealed the loss of male-sterility-inducing mitochondrial orf, *orf138*, in male fertile revertant lines of Og-1. Comparative examination of the mitochondrial gene

organization in Og-1 male sterile and fertility reverted plants indicated intramolecular recombination involving two repeat regions leading to the loss of *orf138* gene. Likewise, mitochondrial genome organization and expression were studied in CMS lines (Cybrids) derived from *Brassica oxyrrhina* cytoplasm. RFLP patterns established that the chlorosis-corrected cybrids had recombinant mitochondrial genome. Northern analysis suggested changes in *atp1* transcripts associated with male sterility/fertility.

- Centromere histone protein (CENH3) genes and cDNAs were cloned from *B. juncea* and related wild species to develop haploid inducer lines in *B. juncea* through silencing of *CENH3*. RNAi construct was designed to silence the native *CENH3* genes of *B. juncea*. Also, a synthetic version of *CENH3* gene was designed to serve as a rescue vector as loss of *CENH3* is lethal to cells. *CENH3* promoters were also cloned from *A. thaliana* and *B. juncea* to drive RNAi and rescue constructs.
- *Bacillus thuringiensis* isolates were recovered from 13 samples collected from diverse locations in India and selected by PCR amplification of the conserved 16S rRNA internal transcribed spacer region and N-acyl homoserine lactonases (*aiiA)* gene. Amplification of *flagellin* gene by PCR using specially designed primers was carried out with known *Bt* strains to explore its utility for classification of *Bt* isolates.
- Transformants in pigeonpea (*Cajanus cajan*) were developed using two *Bt* ICP genes, *Cry1AcF* and *Cry2Aa*, employing *in planta* transformation strategy. The primary transformants were maintained in the poly house, where they grew normally and set seed. Putative transformants were screened based on kanamycin screening, molecular analysis by PCR and bioassay against *Helicoverpa armigera*.
- A vector cassette in the background of pCAMBIA1300 binary vector harbouring the gene construct *Cry1Aabc* under the control of maize ubiquitin promoter was constructed to develop transgenic rice for yellow stem borer resistance. Calli, derived from rice seeds (*var.* IR64) was transformed with the gene construct employing *Agrobacterium*-mediated transformation technique. The putative transformants were screened by PCR using *Cry1Aabc* gene specific primers and tested for the Cry protein expression. Nine *Cry1Aabc* transgenic lines of IR64 were obtained.
- Jasmonate-activated defence genes were elicited and their antibiosis effect on mustard aphids were assayed to understand the potentiality of endogenous defence genes of mustard in manifesting quantitative defence against aphids. Potential targets for developing gene-silencing based aphid resistance in mustard were identified through intensive screening of dsRNAs generated from a large number of insect-genes.
- Differentially expressed genes were identified from cotton after bollworm infestation through comparative transcriptome analysis during boll developmental stages. In total 8,694 transcripts, which accounted for approximately 39% of the total transcripts present on the cotton GeneChip, displayed differential expression under biotic stress at various stages analyzed. Transcripts involved in signalling and defence mechanism were up-regulated and transcripts involved in cell wall and photosynthesis were down-regulated.
- Transcriptome analysis and EST isolation during fruit development stages was done in brinjal to identify promoters from fruit tissues for insecticidal gene expression in tissue specific manner. Fruit specific genes were selected based on both microarray and EST sequencing strategies, and these genes were confirmed by qRT- PCR. Promoter from one of the genes was isolated by genome walking method and cloned into binary vector for fruit specific characterization.

- EST data from *Pennisetum glaucum, Prosopis cineraria*, *Ziziyphus*, and tolerant genotype of *Triticum aestivum* were generated and analysed for prospecting of abiotic stress responsive genes. Differentially expressed genes were validated through expression analysis. *PgCRT and PgP5CS* from *P. glaucu, Pchsp*17 from *P. cineraria, ASR* (Abscisic acid stress ripening) from *Ziziyphus nimmularia* and *CPN60* and *pAPX* from wheat were cloned and characterized in bacterial system.
- Expression of genes, *WRKY10*, *RBP* and *Skp1,* under control and heat stress conditions at different generative stages of wheat was analyzed for isolation and characterization of genes conferring terminal heat stress tolerance in bread wheat. *WRKY10* gene was cloned and sequenced. Besides, three new *annexin* family members were identified from *P. glaucum,* and expression of *annexin* genes was analyzed in rice genotypes under drought stress and heavy metal stress conditions.
- Twenty one heat shock factor genes (*Hsfs*) from the chickpea (ICC4958) genome were identified during heat stress. Expression analysis of a few *Hsfs* was studied using qRT-PCR, and up-regulation as well as down-regulation was observed in different members of the *Hsfs* family of chickpea.
- NRCPB was entrusted to fingerprint and sequence the short arm of wheat chromosome 2A as a partner of International Wheat Genome Sequencing Consortium. Minimum tiling path of chromosome 2A consisting of 4442 clones was identified using High Information Content Fingerprinting of 42488 clones (of the total 55648). The first draft genome of popular mango variety Amrapali was generated using Roche 454 and Illumina sequencing technologies.
- A new 50K SNP genotyping assay, comprising of 51933 SNPs, identified mostly from single copy genes of rice, was designed and validated using Affymetrix GeneTitan Multi Channel platform which could serve as great resource for linkage and LD mapping and in recovering recipient genome in back cross derivatives in rice.
- Eighty isolates of *Magnaporthe oryzae* (causing rice blast) were collected across different geographical regions of India for allele mining of *Avr-Pita* gene. Among the 80 *Avr-Pita* alleles, 82 polymorphic sites and 54 haplotypes including the original *Pita* allele were identified. The maximum haplotype diversity was observed among the *Pita* avirulent isolates compared to the virulent ones.
- Genome-wide identification and comparison of phenylalanine ammonia lyase (PAL), a key player in response to biotic and abiotic stresses in plants, was investigated in nine diverse plant species including dicot and monocot species, tree as well as crop species. The orthology analysis revealed that *Vitis vinifera* had the least orthology with other species.
- Genome wide analysis of heat shock factor (*Hsf*) genes was carried out in pigeonpea in order to understand their structure and function. A total of 23 *Hsfs* were predicted and labelled as *CcHsf*, of which 14 unique sequences were characterized for their presumed structures. Phylogenetic analysis showed that *CcHsf* genes were distributed into eight groups. Their expression analysis revealed that *CcHsfA-1d* and *CcHsfA*-2 were highly up regulated under heat stress and *CcHsfA-1d* acted as an early response factor. *In silico* genome wide characterization of *Hsp 100* family genes [*ClpB*], major and critical genes for acquired thermo tolerance, was carried out in pigeonpea. Out of the five *Hsp100* like gene sequences identified, two genes showed constant expression under heat stress. Twenty more *ClpB* genes were identified in pigeonpea using *Glycine max Hsp 100* sequences.
- Analysis of gene expression profiles associated with drought stress in flax revealed that 183 genes are differentially expressed in shoot and root. These differentially regulated genes belong to twelve diverse functional categories and some of

them displayed co-ordinated expression under stress conditions suggesting functional implications to drought in flax. Although, visible bleaching was not observed in drought stressed shoots, the findings suggest that photosynthetic activity is one of the main regulatory mechanisms affected by drought.

- Development of local database of genomic resources of crop plants database is a continuous process at NRCPB. Plant genome database is developed and maintained at NRCPB which consists of 2,61,45,232 sequence entries from 90 crop plants. It is publically available at www.nrcpb.org.
- Morphological and biomass partitioning studies in different wheat genotypes were undertaken to improve nitrogen use efficiency in wheat. Study of root architecture of four wheat genotypes, Kalyansona, DBW-17, NP-890, PBW-502, showed significant difference among the genotypes and also between starved and optimum nitrogen condition in case of each genotypes. Nitrate reductase, glutamine synthetase and glutamate synthase showed significant difference under N-starved condition. Differential expression pattern of twenty seven microRNAs, which are available in wheat miRBase (microRNA database) depository, showed differential expression under normal and starved nitrate conditions.
- Forty one Ph.D. and 12 M.Sc. students are registered in the discipline of Molecular Biology and Biotechnology at the Centre. Three Ph.D. and four M.Sc. students were awarded with doctoral and masters degrees, respectively, in February 2014. More than fifty students from different universities and institutes across the country were trained in various aspects of biotechnology at the centre. Besides, a ICAR-sponsored winter training programme on "Frontier technologies, in the area of biotechnology, on gene isolation, characterization and breeding with reference to abiotic stress related genes" was conducted and 23 trainees across the countries participated.

About the Centre

- NRCPB and the Mandate
- Staff Position
- Financial Statement
- Resource Generation


लाल बहादुर शास्त्री केन्द्र
LAL BAHADUR SHASTRI CENTRE

NRCPB

National Research Centre on Plant Biotechnology (NRCPB), the premiere research institution of the Indian Council of Agricultural Research (ICAR), is engaged in molecular biology and biotechnology research. The Biotechnology Centre, established in 1985, as part of the Indian Agricultural Research Institute (IARI), was upgraded to a National Research Centre on Plant Biotechnology in the year 1993, with a vision to impart the biotechnology advantage to the National Agricultural Research System (NARS). The NRCPB has acquired, in the past, an excellent infrastructure in terms of equipments and other physical facilities and also a high degree of scientific competence. Development of transgenic crops for biotic and abiotic stress management, exploitation of heterosis through marker and genomic approaches, marker assisted selection and molecular breeding of major crops for productivity and quality enhancement, search for novel genes and promoters for efficient native and transgene expression are the major activities taken up by the centre. There is now considerable emphasis on structural and functional genomics of crop species such as rice, pigeonpea, chickpea, cotton, tomato, wheat and mango in the centre. In addition to research, the centre is contributing significantly to competent human resource development by way of offering regular M.Sc and Ph.D programmes by partnering with PG School, IARI.

In order to develop strong inter-and intra-institutional linkages for promoting and strengthening plant biotechnology research in the ICAR system, the centre has been identified as the lead centre. It has established strong linkages with various research institutes in the country including ICAR, CSIR, State Agricultural Universities and CGIAR institutes. The center is now placing considerable emphasis on the development of products, processes, patents and research publications in the journals with high impact factor. It also encourages and practises Public-Private Partnership (PPP) mode for commercializing the products of genetic engineering.

Mandate

- To undertake basic plant molecular biology research for understanding molecular mechanisms underlying basic biological processes
- To develop capabilities of devising tools and techniques of biotechnology and genetic engineering for crop improvement
- To use the knowledge gained and technologies developed for advancing agricultural development
- To serve as a national lead center for plant molecular biology and biotechnology research
- To create trained manpower in the area of plant biotechnology.

Staff Strength of the Centre

Staff	Sanctioned	Filled	Vacant
Scientific	33+1	31+1	02
Technical	14	11	03
Administrative	18	08	10
Skilled Supporting Staff	04	01	03
Total Strength	70	52	18

Financial Statement 2013-14

(Rs. in Lakhs)

	Plan		Non-plan	
	Allocation	Utilization	Allocation	Utilization
Capital	30.00	29.96	10.00	7.04
Revenue				
Establishment	0.00	0.00	568.00	553.73
Travelling Allowances	1.68	1.68	2.00	1.92
Research and Operational Expenses	200.98	200.98	35.00	35.00
Administrative Expenses	213.85	213.84	75.56	70.56
Miscellaneous Expenses	3.49	3.48	0.00	0.00
Total	**450.00**	**449.94**	**690.56**	**668.25**

Resource Generation

Sales of Farm Produce	0.21
License Fee	0.00
Leave Salary and Pension Contribution	0.00
Interest Earned on Short Term Deposits	22.14
Income Generated from Term Deposits	9.89
Miscellaneous Receipts	3.33
Total	**35.57**

Funds Received through Externally Funded Projects

Externally Funded Projects	563.89
Consultancy Projects	0.00
Total	**563.89**

Research Achievements

1. Isolation of Plant Genes and Promoters
2. Transgenic Crops for Biotic Stress Resistance
3. Adaptation of Wheat to Climate Change Induced Abiotic Stresses
4. Biotechnological Approaches for Increasing Productivity
5. Genomics and Molecular Markers
6. Improvement of Nitrogen Use Efficiency in Cereal Crops
7. Honorary Scientists' Projects

1. Isolation of Plant Genes and Promoters

Cloning and characterization of microRNAs in chickpea

MicroRNAs (miRNAs) are non-coding small RNAs (21 to 24 nt long) that function in many biological processes (development, stress responses, signalling etc.). No miRNA has so far been reported in chickpea using cloning technique. Last year, we reported identification and profiling of miRNAs in response to *Fusarium* wilt and salt stress. Analysis was performed to predict target genes involved in different biological processes under a large number of gene families. The putative miRNA target genes in chickpea were predicted using psRNATarget program. The predicted target genes included transcription factors, DNA replication proteins and genes involved in cellular metabolism and responsive to a number of stress conditions (Fig. 1.1). This year we carried out validation of a few conserved and novel miRNAs using quantitative real time PCR. Expression of eleven conserved and five novel miRNAs has been validated (Fig. 1.2 & 1.3).

Characterization of nematode-responsive promoters

Microarray resources were used for identifying nematode-responsive and root-specific genes. After

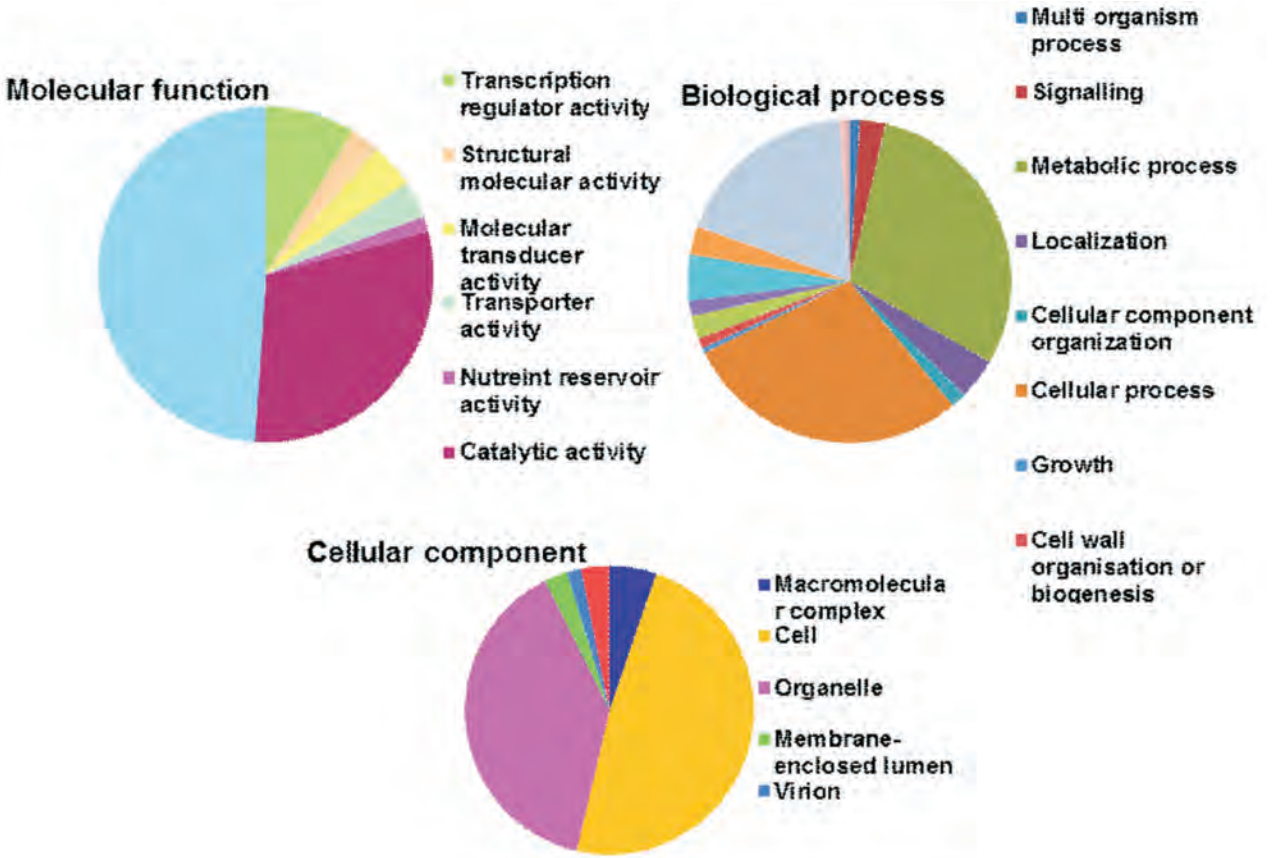

Fig. 1.1: Gene categories of the predicted target genes for chickpea miRNAs.

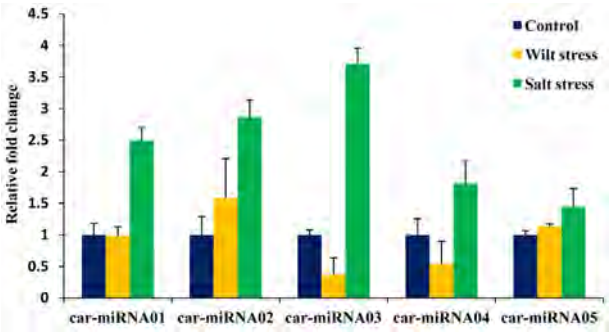

Fig. 1.2: qRT-PCR validation and expression analysis of novel miRNAs in chickpea.

identifying a set of about 50 genes, the genes which had low expression under control conditions were selected (5 nos.). *In silico* analysis revealed the presence of three novel consensus motifs with respect to nematode-responsive root-specific genes in all these genes. Expression of these genes was studied by qRT-PCR. Both the genes were found to be expressed in response to nematode infection. The promoters of two of these selected genes were fused with GUS and checked for expression in transgenic *Arabidopsis* in response to nematode infection and were found to be nematode responsive (Fig. 1.4).

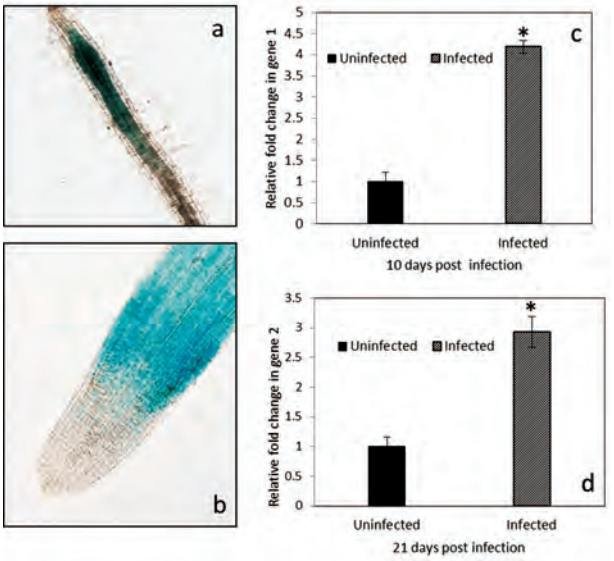

Fig. 1.4: Transformed *Arabidopsis* plants harboring the promoter 1::*GUS* and promoter 2::*GUS* shows expression of GUS in (a) galls and (b) roots, respectively, in response to nematode infection. (c) Changes in expression of Gene1 in root samples containing galls at 10 days post inoculation (d) Changes in expression of Gene 2 in root samples containing galls at 21 days post inoculation. The transcript levels of gene 1 and gene 2 was measured by qRT-PCR relative to the expression of UBQ10. A star indicates the significant difference between expression levels in infected and uninfected wild type *Arabidopsis* root samples.

Identification of heat shock transcription factors in chickpea

In chickpea, 21 Hsfs (Heat shock factors) were identified from the ICC4958 genome. Using these sequences, Neighbor Joining phylogeny was re-constructed along with *Arabidopsis* and *Medicago Hsfs* (Fig. 1.5). The expression analysis of a few *Hsfs* was studied using qRT-PCR and up-regulation as well as down-regulation was observed in different members of *Hsfs* family of chickpea (Fig. 1.6).

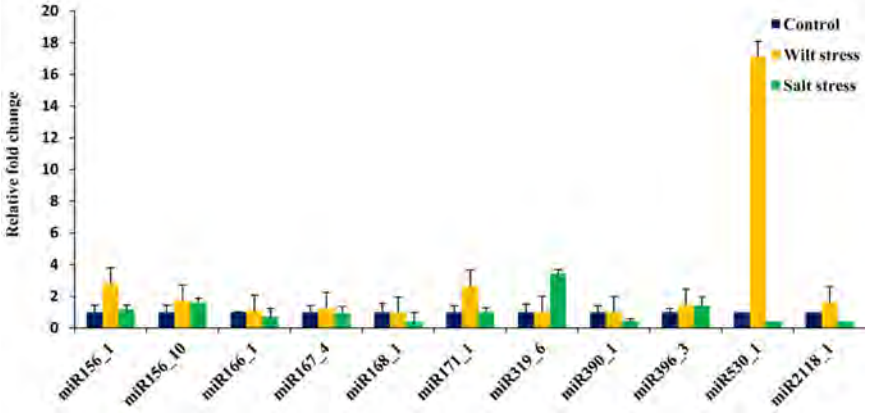

Fig. 1.3: qRT-PCR validation and expression analysis of eleven conserved miRNAs in chickpea.

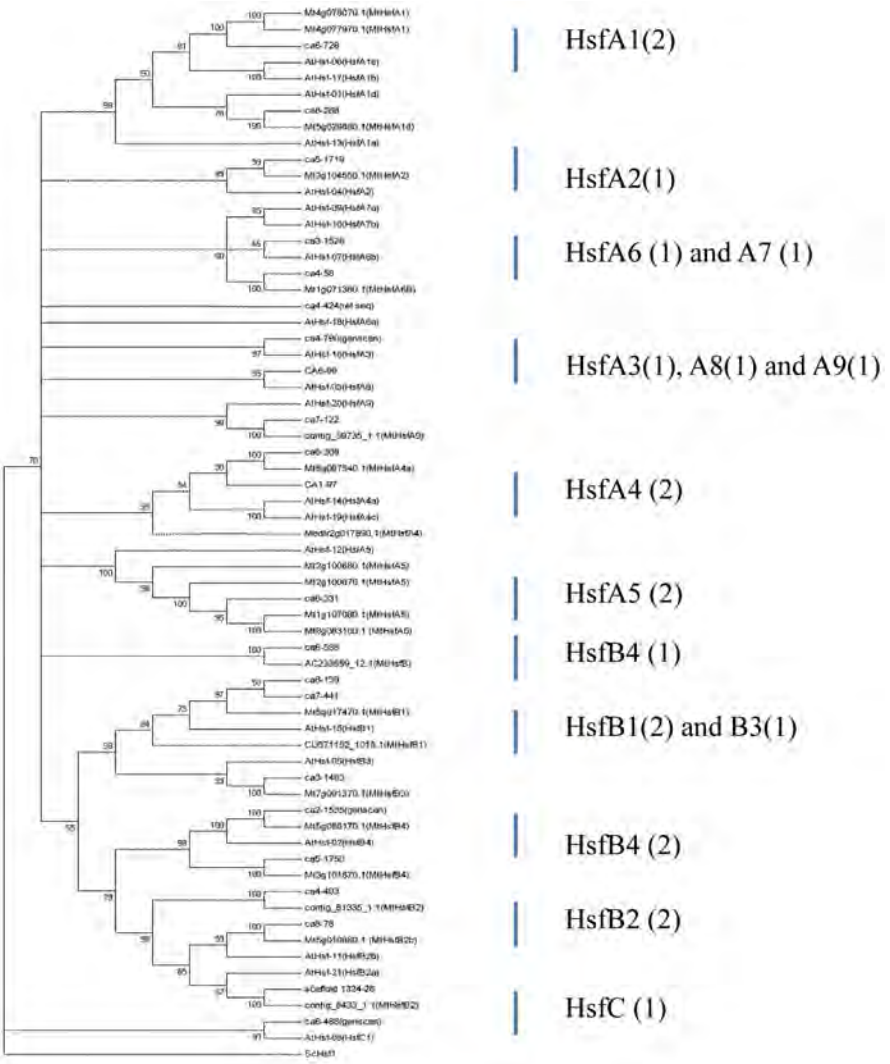

Fig. 1.5: Phylogenetic tree reconstructed using *Arabidopsis*, *Medicago* and chickpea heat shock factors. The number within parentheses indicates the number of *Hsfs* within that particular subgroup.

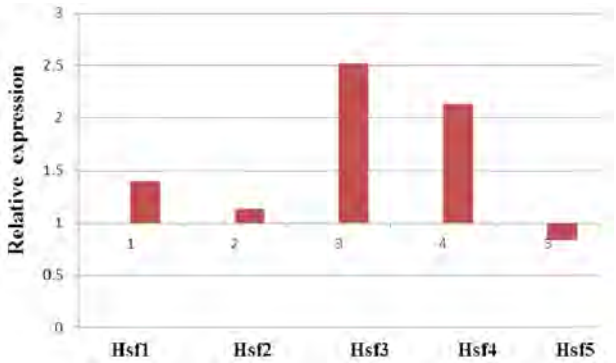

Fig. 1.6: Quantitative real time expression analysis of a few *Hsfs* in chickpea in response to heat stress in shoot tissue.

In silico identification of NAC genes in pigeonpea

Pigeonpea (*Cajanus cajan* L.), a diploid legume crop species (2n = 2x = 22) whose genome was sequenced at our Centre has a genome size of ~858 Mb. NAC transcription factors have been found to play important roles in plant development and responses to environmental stresses. *In silico* analysis led to the identification of 96 unique NAC domain containing proteins in pigeonpea (Fig. 1.7).

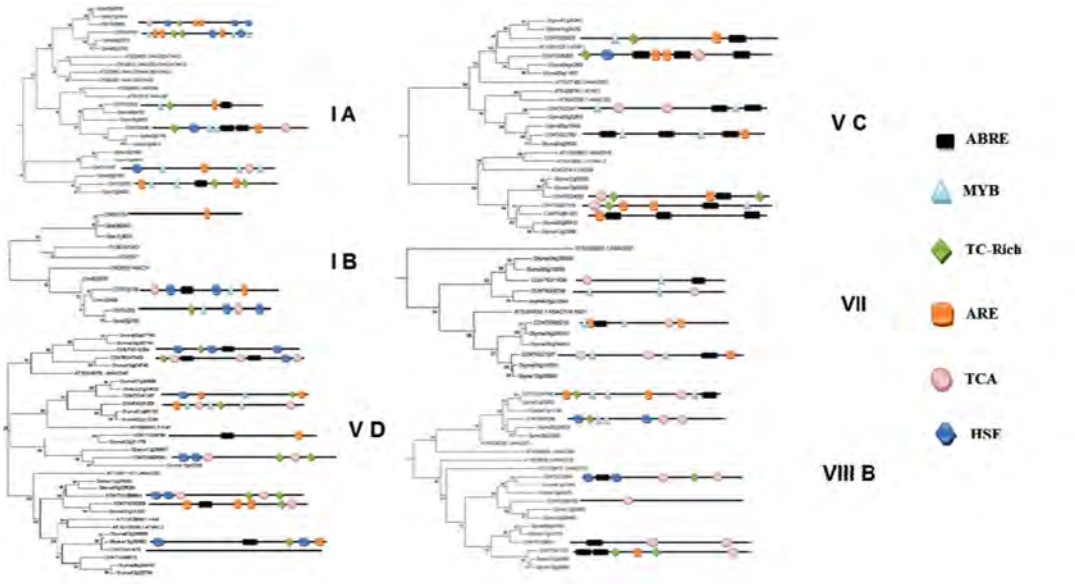

Fig. 1.7: Six clades from the phylogenetic tree reconstructed using *Arabidopsis*, soybean and pigeonpea NAC TFs containing the putative stress-responsive NAC proteins. The promoters for the pigeonpea NAC genes were analyzed for presence of stress-related motifs and represented schematically in the right frame.

Isolation of pathogen-inducible genes

Alternaria brassicae, which causes black leaf spot in *Brassica juncea*, can lead to great yield loss particularly in favourable conditions. Full length cDNAs of many antifungal genes from Salicylic acid (SA) and Jasmonic acid (JA) induced *B. juncea* libraries were isolated. Two antifungal genes, *pdf 1.2* (plant defensin) and *hsp* (heat shock protein), were isolated and sequenced (Fig. 1.8, 1.9). Defensin gene is induced in macrophages to make holes in the pathogen cells in animal. HSPs present

```
DEFINITION Brassica juncea plant defensin gene, complete cds.

ACCESSION KF578144

Translation="MTKFVSIITLIFLALVLFAAFEASTMVDGQKLCQKPSGTWYGFC
           GNSNTCKNQCINLEGARHGSCNYVFPYHRCICYVPC"

GTTACTACGTTGTTGTGCGATGAACAAGTAAAAGCTAACCTACTAGCATTTCACTATAAATAGAAGTCCA

TCTTACTAAGTGGTGGAGAAACCAGCCATTAACTTTGAATATTGGGCCAGTCTAGAAGTTAACATATGTT

TAATTTGCTGGGCTTATTTCTTCACACTTCACACAAATTCATCGAAAACAAATTACTAATAGTCATGACT

AAGTTTGTTTCCATCATCACCCTTATCTTCCTTGCTCTCGTTCTCTTTGCTGCTTTTGGTGAGTAGTGAT

CTTATCATATGCATGGCAAAATTAGTTTAAATTTTTTATTTCAGAATATTTGATTTCGCCATGATAGATA

TTTATATACAGAAGCATCAACAATGGTGGACGGTCAGAAGTTGTGCCAAAAGCCTAGTGGGACATGGTAC

GGATTTTGTGGAAATAGTAATACTTGCAAGAACCAGTGCATCAACCTTGAGGGAGCACGACATGGATCTT

GCAACTATGTTTTCCCATATCACAGGTGTATCTGCTATGTTCCATGTTAATCTACCAAAACTCATTGGTC

TAACAAAACTCTGTAGTGCTAACGTTCAATAAGTCTGTGTCAC
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Fig. 1.8: Nucleotide sequence of *Brassica juncea* defensin gene.

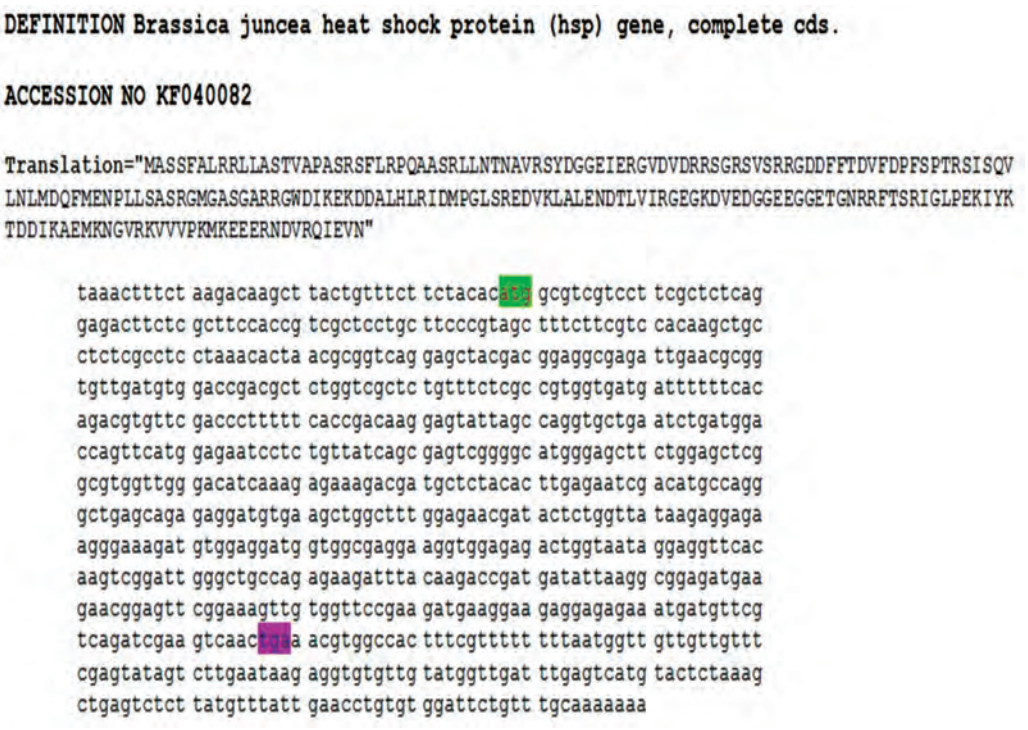


Fig. 1.9: Nucleotide sequence of *Brassica juncea hsp* gene.

the denatured proteins (by pathogen) to proteosome for degradation. Sequence of these genes were deposited to GenBank (Acc. No KF578144 and KF040082 respectively).

Validation of pathogen-induced glucanase promoter

Presently most of the transgenics being developed all over the world are with constitutive promoters. The constitutive expression of the foreign gene taxes unnecessarily the energy demand on the plant. Therefore, it is important to isolate specific promoters which are tissue and development specific. *Arabidopsis* transgenics were developed with pathogen inducible glucanase promoter attached to *GUS* gene. The promoter was found to be induced by development, wounding, fungal infection and jasmonic acid (Fig. 1.10).

Wild relatives of *B. juncea* (*Camelina sativa* and *Sinapis alba*) are much more resistant to *Alternaria* as compared to *Brassica* spp. Therefore, it is important to understand how the defense genes are induced by *Alternaria* in wild relatives as compared to *B. juncea.* Induction of chitinase and defensin genes was studied in *B. juncea, C. sativa* and *S. alba* 2-72 hour after infection by *A. brassicae.*

Chitinase gene was induced in *B. juncea* and *C. sativa* very marginally but to a great extent in *S. alba.* The result was same in both fungus-infected local tissue and distal tissue (Fig. 1.11).

Plant defensin gene was induced to a small extent in *B. juncea* but to a greater extent in *S. alba* and *C. sativa* in fungus-infected local tissue. In fungus-infected distal tissue defensin gene was not induced in *B. juncea* and to a little higher extent in *C. sativa* and still higher extent in *S. alba* (Fig. 1.12). It seems that resistance of *S. alba* might be contributed by higher induction of chitinase and plant defensin genes. Probably a different mechanism of resistance is operating in *C. sativa.*

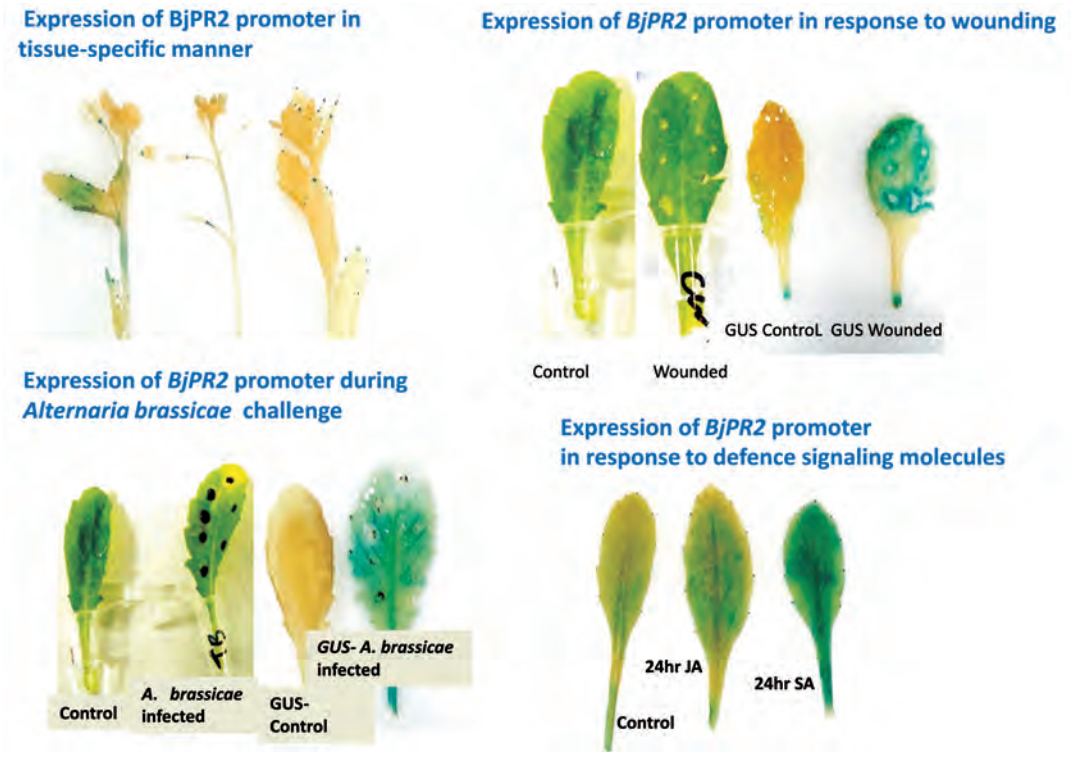


Fig. 1.10: Defense genes induction in *B. juncea* and its wild relatives *C. sativa* and *S. alba* after *Alternaria* infection.

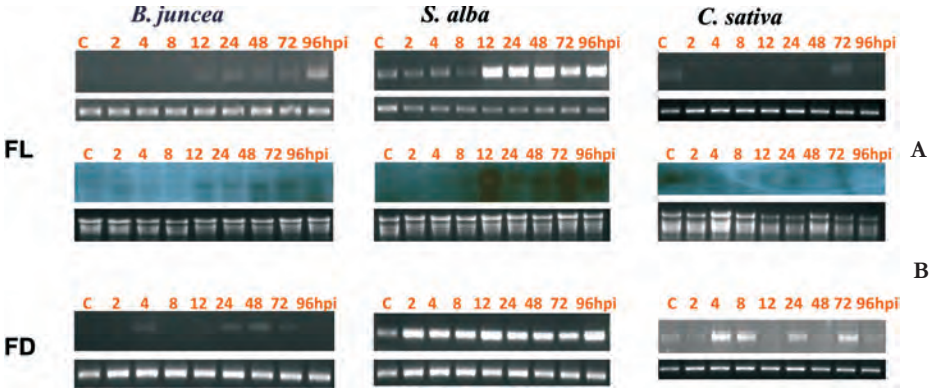


Fig. 1.11: RT-PCR analysis (A) and Northern analysis (B) of the chitinase gene induction in response to *A. brassicae* challenge (fungal local (FL) and fungal distal (FD). C: Control, 2-96hpi: hours post infection.

Cloning and characterization of insecticidal genes from legumes in mustard

B. juncea (mustard) is an important source of edible oil in many countries across the globe. India is one of the largest rapeseed-mustard growing countries in the world. A major constraint on productivity is an aphid species, *Lipaphis erysimi* which causes yield losses from 35.4 to 91.3%. Lectin gene under the control of tissue specific promoter was used to develop transgenic mustard plants to control the damage caused by aphids.

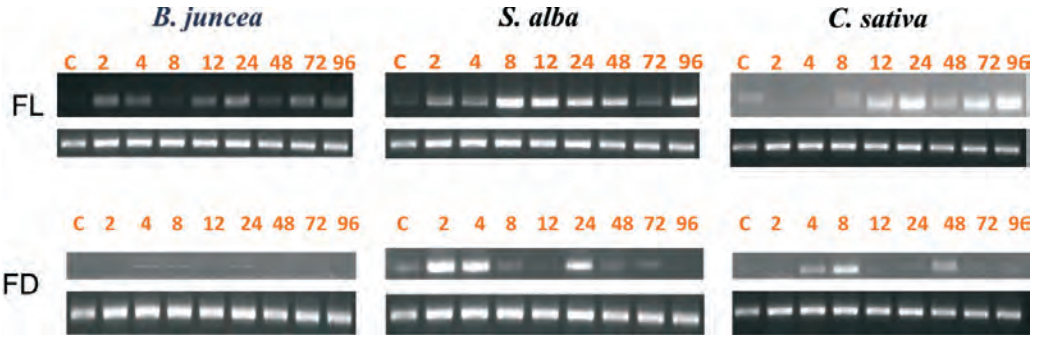


Fig. 1.12: RT-PCR analysis of the *pdf1.2* gene induction in response to *A. brassicae* challenge [fungal local (FL) and fungal distal (FD)]. C: Control, 2-96: hours post infection. β Tubulin was used to normalize the amount of template in PCR reactions.

Development of transformed *B. juncea*

B. juncea (*cv.* Pusa Jaikisan and Varuna) were transformed using chickpea lectin gene construct cloned into pORE O4 binary vector at *EcoRI* and *NotI* restriction sites under the control of phloem specific rolC promoter (Fig. 1.13). Molecular analysis of T0 plants showed successful integration of the gene. T1 seeds of these transformed lines were collected and used for further analysis.

Segregation analysis of T1 transformants

The seeds of T1 generation of *B. juncea* (*cv.* Pusa Jaikisan and Varuna), developed using chickpea lectin construct, were screened by germinating on MS media supplemented with 100 mg/l kanamycin (Fig. 1.14A). The kanamycin resistant plants were hardened (Fig. 1.14B) and transferred to phytotron for further growth and analysis (Fig. 1.14C).

Molecular analysis

The genomic DNA and total RNA from the T1 generation of *B. juncea* (*cv.* Pusa Jaikisan and Varuna) were isolated and analyzed by PCR and RT-PCR using *NPTII* and gene specific primers. The PCR amplification of the isolated genomic DNA by *NPTII* primers gave a product of ~ 750 bp (Fig. 1.15A) and

Fig. 1.13: Construct used for transformation of *Brassica juncea.*

Fig. 1.14: Growth analysis of *B. juncea* T1 lines: (A) Germination (B) Hardening (C) In Phytotron.

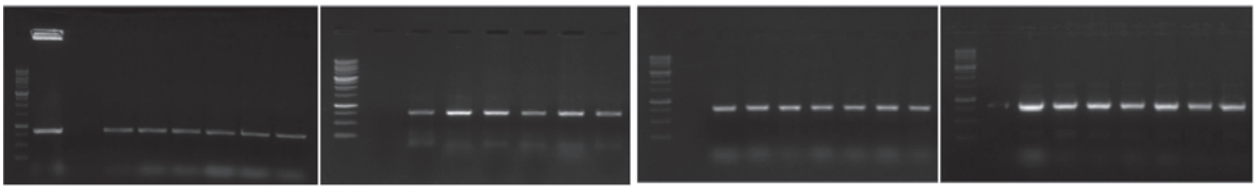


Fig. 1.15: Molecular analysis of *B. juncea* T1 lines by PCR and RT-PCR using *NPTII* and gene specific primers. A, *NPTII* PCR; B, gene specific PCR; C, *NPTII* RT-PCR; D, gene specific RT-PCR.

gene specific primers gave a product of ~828 bp (Fig. 1.15B). RT-PCR also showed the expression of the genes (Fig. 1.15C and D)

Aphid bioassay

Mustard aphids were collected from the field at IARI Campus, New Delhi. Leaves and inflorescence were taken from transgenic as well as control plants. The cut ends of leaves were placed on 1% agar. Ten aphids were placed over each leaf. Three replicates were set for each control and individual transgenic line. Experiment was carried out under controlled conditions in the germinator maintained at 16:8 light:dark photoperiod, 75% relative humidity and 25±1°C temperature. The effect of lectin gene on survivability of aphid was assessed by the total number of insects survived on individual transgenic plants at the end of the entire bioassay period of 5 days. The mortality of aphids was recorded as 40% and 43% in *B. juncea cv.* Pusa Jaikisan and Varuna, along with reduction in fecundity of aphids (Table 1.1).

Construction of *Vigna radiata* lectin gene construct

An amplicon of ~828 bp *V. radiata* lectin gene was cloned in the binary vector pOREO4 with *rolC* promoter at *Eco*RI and *Kpn* I restriction enzymes. The transformed colonies were re-streaked to confirm for resistance. The transformed colonies were subjected to colony PCR. The gene construct was mobilized into *Agrobacterium* strain GV3101 for transformation of *B. juncea*.

Identification of molecular determinants of resistance and disease in bacterial leaf blight of rice

Xanthomonas oryzae pv *oryzae* (*Xoo*), the causal pathogen of bacterial leaf blight of rice mainly relies on the presence of members of the large transcription activator- like effector (TALe) family and their ability to induce specific host genes which may be resistance or susceptibility genes. Hence, in our endeavor to identify undiscovered TALes and their cognate binding

Table 1.1: Aphid bioassay of T1 generation plants

Transgenic line	Surviving aphids	Dead aphids	Mortality percentage	Transgenic line	Surviving aphids	Dead aphids	Mortality percentage
Control	28	2	6.6	Control	28	2	6.6
PJ13	20	10	33.3	VR22	18	12	40.0
PJ20	19	11	36.6	VR25	17	13	43.3
PJ28	18	12	40.0	VR45	19	11	33.3
PJ53	19	11	33.3	VR62	21	9	30.0

site in the host, we had initiated the study last year taking four xa13 compatible *Xoo* strains.

Typing for TALe gene content

We had earlier reported this analysis using the 3.1kb central repeat region flanked by *Sph*I in *avrXa7* gene as probe for Southern hybridization, which needed to be improvised upon. We have added the Phillipine *Xoo* strain PXO99A as reference strain to the four *xa13* compatible strains in our study and using both *SphI* and *BamHI* digests separately, the TALe gene content has been analysed (Fig. 1.16a). Each band represents one TALe gene, so based on the banding pattern, two strains Koul and LUD9, assessed to be different from others have been selected for identification of TAL effectors virulent on *xa13*.

Identification of susceptibility genes

The SWEET gene family of rice holds the best studied examples of TAL effector virulence targets. Hence, transcriptional activation of five SWEET genes (*SWEET 11* to *SWEET 15*) predicted to facilitate *Xoo* in IR24 leaves was analyzed after infection with four *xa13* compatible strains by reverse transcriptase PCR and it was found that all four activate *SWEET 14* (Fig. 1.16b).

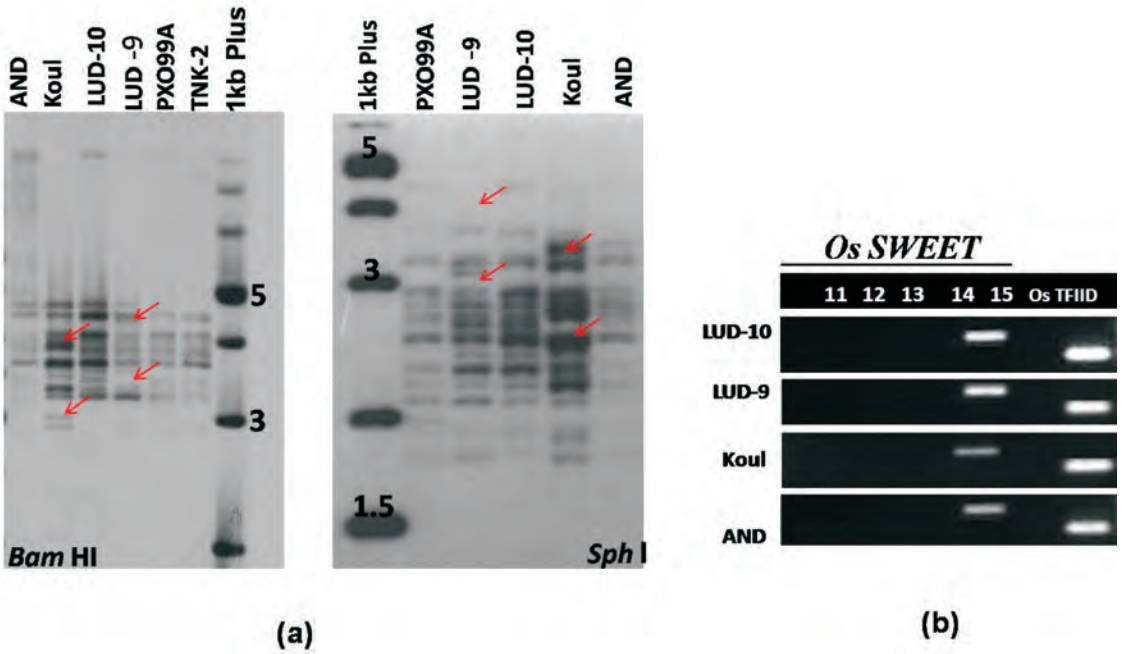


Fig. 1.16: (a) Southern hybridization for analysis of TALe genes in *X. oryzae* strains. (b) Transcriptional activation analysis of *SWEET 11-15* in IR24 leaves by reverse transcriptase PCR.

2. Transgenic Crops for Biotic Stress Resistance

Isolation of *Bacillus thuringiensis* (*Bt*) from different sources and molecular characterization of *Bt* isolates by rRNA-ITS PCR

Bacillus thuringiensis is an aerobic, spore-forming gram-positive bacterium that produces crystal (Cry) proteins having specific toxicity towards a wide range of insects. The *cry* genes have been deployed as biopesticides as well as *Bt*-transgenic plants as a safe alternative to the ecologically harmful pesticides for crop protection. To delay the onset of development of resistance in target insects novel insecticidal genes are required. An effort is being made to isolate novel *cry* genes from native *Bt* isolates from diverse sources in India. Presumptive native *Bt* isolates were recovered from soil from eight agricultural and non-agricultural areas, oil field and insect-infested seeds collected from diverse locations in Tamil Nadu, Gujarat, Uttar Pradesh and Rajasthan. Five samples from diverse locations in Manipur in the North Eastern region of India were also screened for the presence of *Bt* isolates. The samples showed diversity in terms of presence of *Bt* isolates. Presumptive *Bt* isolates were purified to a single colony under selection with the antibiotic penicillin (Table 2.1). Out of 134 presumptive *Bt* isolates, 92 showed amplification of the 234 bp highly conserved 16S rRNA internal transcribed spacer region (Fig. 2.1).

Table 2.1: Prevalence of *Bacillus thuringiensis* in different locations

S.No.	Sample	Location	Presumptive Bt isolates	Bt isolates
1	Urban Area (1)	Tamil Nadu	8	4
2	Oil Field	Tamil Nadu	7	6
3	Urban Area (2)	Tamil Nadu	10	4
4	Cotton Field Soil (1)	Gujarat	7	_
5	Cotton Field Soil (2)	Gujarat	18	18
6	Non-cultivated Soil	Bangalore, Karnataka	9	8
7	Wheat Field Soil	Hardoi, U.P.	10	6
8	Insect Infested Wheat	Dangarpur, Rajasthan	10	6
9	Non-cultivated Soil	Manipur	5	2
10	Forest Soil (1)	Manipur	19	18
11	Paddy Field Soil	Manipur	4	3
12	Forest Soil (2)	Manipur	16	7
13	Kitchen Garden Soil	Manipur	11	10

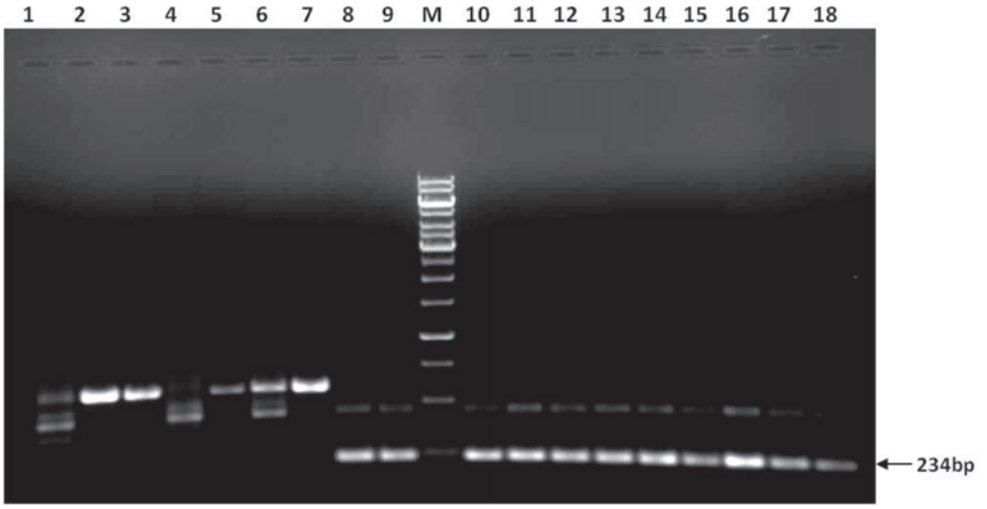


Fig. 2.1: PCR amplification of 16S rRNA ITS. Lanes: 1-18, presumptive *Bacillus thuringeinsis* isolates; M, 1Kb Ladder.

Screening of native *Bt* isolates for the presence of N-acyl homoserine lactonase genes

N-acyl homoserine lactones (AHLs) present in many gram-negative bacteria are quorum-sensing signals which are inactivated by degrading enzymes N-acyl homoserine lactonases. Native *Bt* isolates recovered from diverse habitats in India were screened for the presence of *aiiA* (*autoinducer inactivation),* N-acyl homoserine lactonase genes, borne on chromosomal DNA, by PCR using gene-specific primers. Screening of 38 native *Bt* isolates was carried out for the presence of N-acyl homoserine lactonases (*aiiA)* genes by gradient PCR. Amplified PCR product was observed in 33 *Bt* isolates (Fig. 2.2).

Cloning of *aiiA* gene from native *Bt* isolates

The amplified *aiiA* gene from two *Bt* isolates, 1325 and 1326, was ligated onto p-GEM-T vector and introduced into *E. coli* DH5α cells. The transformants were analyzed by restriction analysis

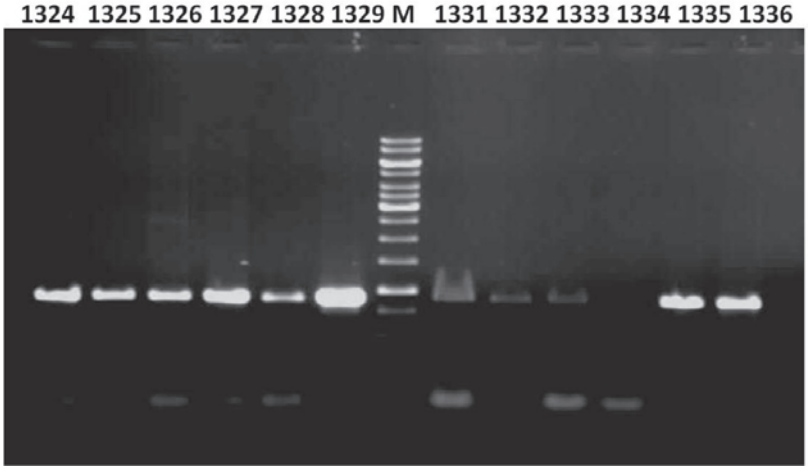


Fig. 2.2: PCR amplification of AHL gene in *Bt* isolates. Lanes: 12 native *Bt* isolates; M=1Kb Ladder.

with *SalI* for linearization of recombinant vector and *NotI* for excision of the insert (Fig. 2.3).

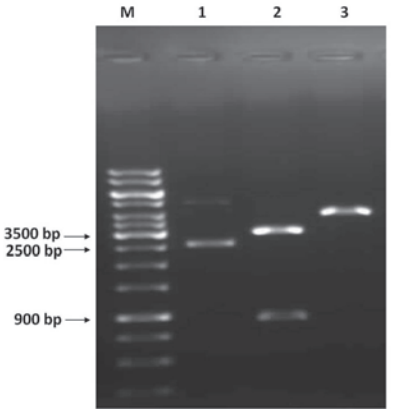


Fig. 2.3: Restriction analysis of recombinant clone pAHL-1325. M: 1Kb Ladder. Lane 1: pAHL-1325 uncut plasmid DNA; Lane 2: pAHL-1325 restricted with *Not I*; Lane 3: pAHL-1325 restricted with *Sal I*.

Phylogenetic analysis of *Bt* strains based on PCR amplified fragment polymorphism of flagellin genes

Phylogenetic analysis of 8 *Bt* strains was performed by PCR amplification of flagellin gene (Fig. 2.4). *Bt* strains, obtained from *Bacillus* Genetic Stock Centre, Ohio State University, OH, U.S.A., were classified into 3 groups based on amplification of PCR products ranging in size from 800 to 1200 bp. This technique can be useful for classification of *Bt* isolates which can not be classified using the H-antigen serotyping method based on H flagellar antigens.

Development of transgenic rice for yellow stem borer resistance

A new vector cassette in the background of pCAMBIA1300 binary vector harbouring the gene construct *Cry1Aabc* under the control of maize ubiquitin promoter was developed (Fig. 2.5). The vector cassette was checked through restriction analysis (Fig. 2.6).

Mature seeds of IR64 were used for callus induction. The *Agrobacterium* strain EHA105 harbouring pCAMBIA1300:: PZmUBi:: *Cry1Aabc::* NosT vector cassette was used for transformation. Twenty day old calli derived from IR64 were transformed with *Cry1Aabc*. The regenerated plantlets

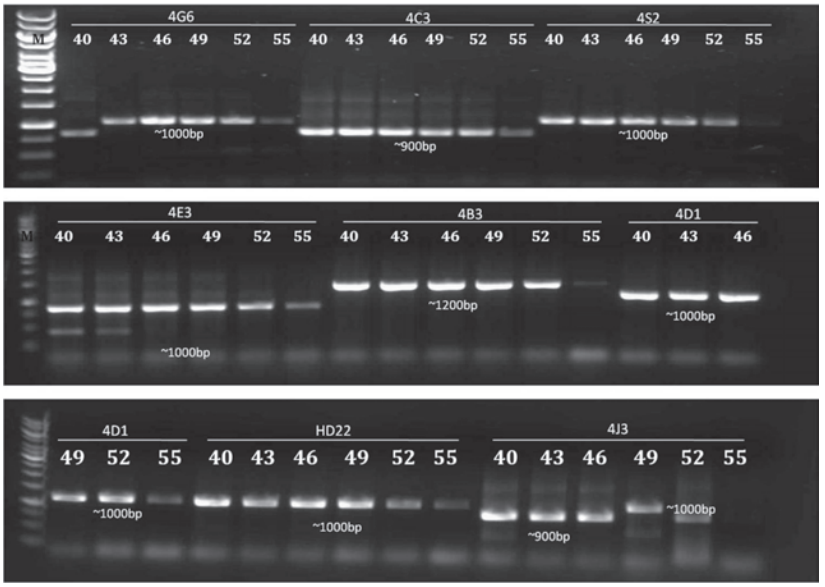


Fig. 2.4: Amplification of flagellin genes using specially designed primers in gradient PCR with different annealing temperatures viz. 40°C, 43°C, 46°C, 49°C, 52°C and 55°C. *Bacillus thuringiensis* strains used as template were: 4G6- *B. thuringiensis galleriae*, 4C3- *B. thuringiensis alesti* , 4S2- *B. thuringiensis Indiana*, 4E3- *B. thuringiensis sotto*, 4B3- *B. thuringiensis finitmus* , 4D1- *B. thuringiensis kurstaki*, HD22- *B. thuringiensis kurstaki HD-73*, 4J3- *B. thuringiensis aizawai*.

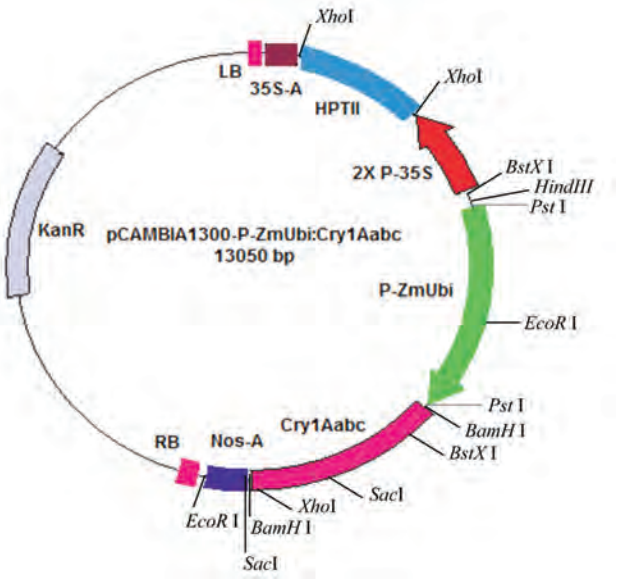


Fig. 2.5: Vector map of pCAMBIA1300::ZmUbi::*Cry1Aabc*.

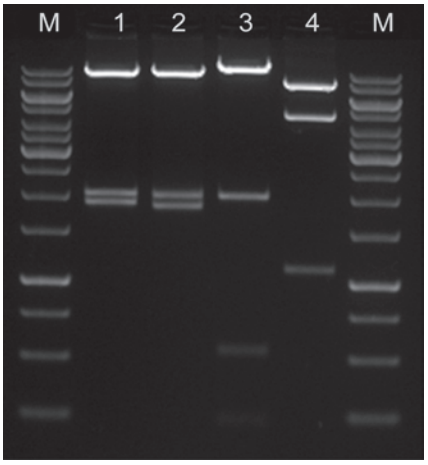


Fig. 2.6: Restriction analysis of pCAMBIA 1300::P ZmUbi::*Cry1Aabc*. Lanes: M, 1 kb ladder; 1, *Hind*III and *Bam*HI digested; 2, *Pst*I digested; 3, *Eco*RI and *Sac*I digested; 4, *Xho*I digested vector.

were separated and kept for rooting. The plants with developed roots were hardened in liquid medium followed by transferring into pots. The putative transformants were analyzed for the presence of transgene through PCR using *Cry1Aabc* gene specific primers. All the putative lines were confirmed positive for the presence of the transgene (Fig. 2.7). The *Cry1Aabc* transgenic lines were tested for the Cry protein expression using Quickstix strips for Cry1Ab/Ac (Envirologix). All the lines were detected positive for Cry1Aabc expression (Fig. 2.8).

Development of aphid resistant plant types of Indian mustard

Rapeseed-mustard (*Brassica spp.*) is the third most important oilseed crop in world agriculture and India is the third largest producer with global contribution

Fig. 2.8: Strip test for Cry1Aabc expression in transgenic rice lines. C, wild type; 1-9, *Cry1Aabc* transgenic lines.

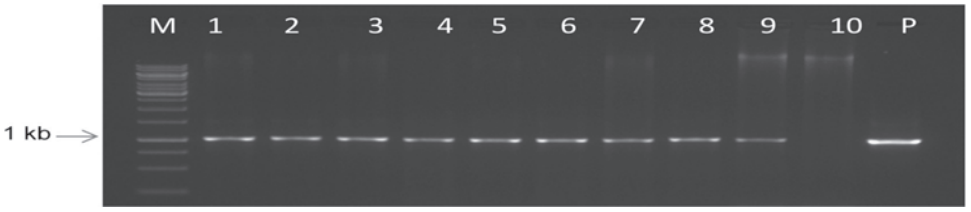


Fig. 2.7: PCR analysis of putative rice transformants. Lanes: M, 100 bp ladder; 1-9, transgenic lines carrying *Cry1Aabc* gene under maize ubiquitin promoter; 10, wild type; P, positive control. Arrow shows 1 kb PCR amplicon of *Cry1Aabc* gene.

of 28.3% acreage and 19.8% production. Among its biotic constraints, hemipteran aphids elicit multitude damaging effects on the productivity of Indian mustard *B. juncea*. The problem is further accentuated due to unavailability of resistance source within the crossable germplasms. As a result, breeding efforts for developing resistant cultivars for minimizing the use of insecticides against aphids has not met with any success. Therefore, it is imperative to develop novel mechanism of genetic resistance against these phloem-feeders. Our ongoing efforts for developing aphid resistant plant types in mustard encompass two strategic areas of research: understanding the endogenous defense systems of *B. juncea* and incorporation of novel mechanisms/pathways to confer aphid-resistance.

Jasmonate elicited defense in *Brassica juncea* and its response to mustard aphids

The innate antiherbivore defense in plants is primarily regulated by jasmonate-mediated signaling. We examined mechanistic differences, if any, in early signalling and activation processes of innate defense responses of *B. juncea* with regard to its elicitation in response to mustard aphid *Lipaphis erysimi* (Kalt.) and the defense elicitor methyl jasmonate (MeJ). Several jasmonate induced unigenes which were orthologous to aphid responsive genes in taxonomically diverse plant-aphid interactions, were identified. A total of 102 unigenes were categorized into thirteen groups as listed in Fig. 2.9. In a functional classification based on Blast2Go analysis, the unigenes were assigned to one or more GO terms and categorized into three GO ontologies viz. biological processes, molecular function, and cellular components. Topical application of MeJ and plant-wounding on leaves of 3-4 weeks grown young plants transcriptionally activated key jasmonate biosynthetic genes (*LOX*, *AOC*, *12-OPDR*), redox genes (*CAT3* and *GST6*), and other downstream defense genes (*PAL*, *ELI3*, *MYR* and *TPI*) by several folds (Fig. 2.10 & 2.11). In order to examine if infestation by mustard aphids (*L. erysimi*) evoke a similar activation of innate defense genes, their relative transcript levels in aphid inoculated plants were assayed in a time course manner. However,

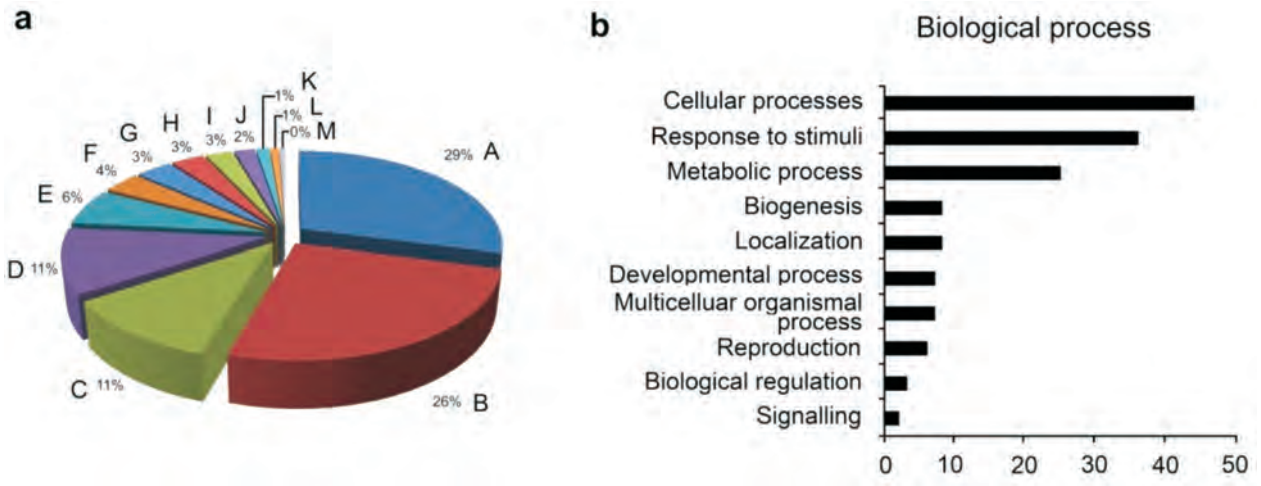


Fig. 2.9: Gene ontology-based functional classification of differentially expressed unigenes in jasmonate treated *B. juncea* plants (a). The functional categories are : A , other cellular processes; B, other metabolic processes; C, response to abiotic or biotic stimuli; D, response to stress; E, other biological processes; F, transport; G, signal transduction; H, cell organization and biogenesis; I, developmental processes; J, protein metabolism; K, electron transport or energy pathway; L, transcription, DNA-dependent; and M, unknown biological processes. Percent distribution of GO terms: Biological process (b).

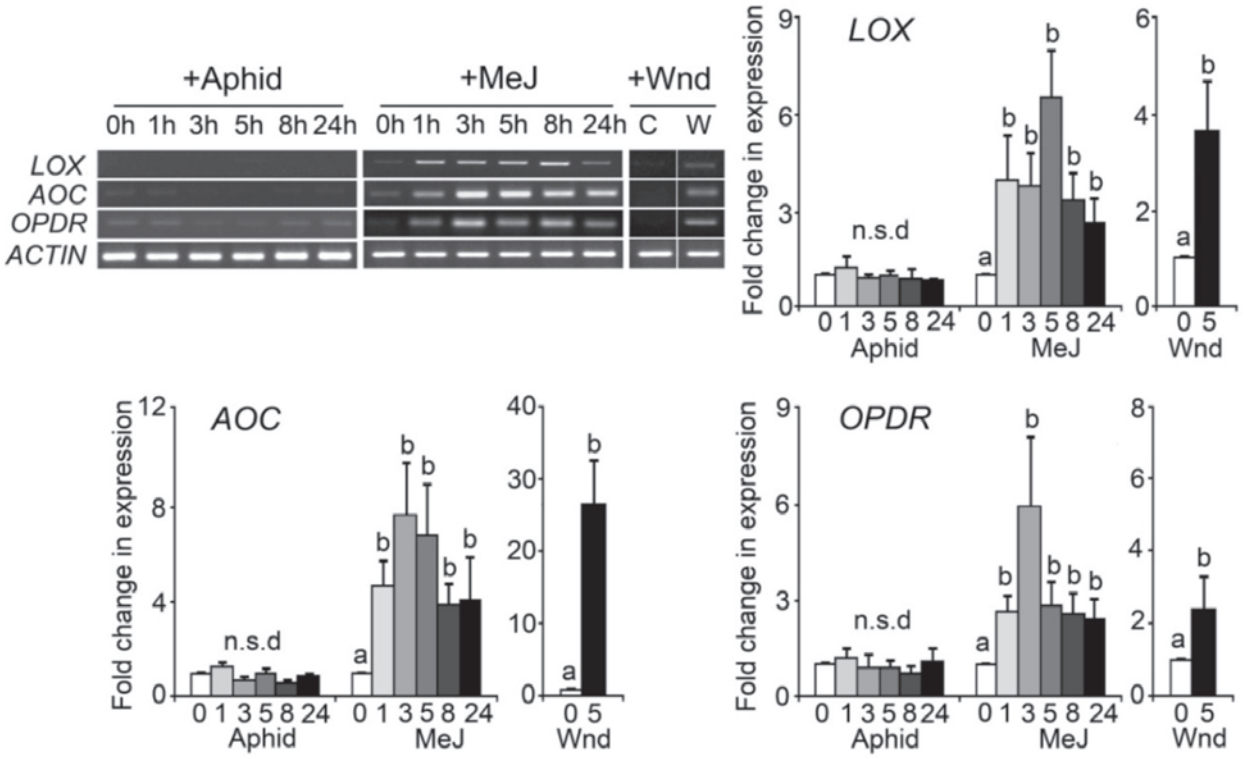


Fig. 2.10: Time course of transcriptional activation of jasmonate biosynthetic genes in *B. Juncea* in response to aphid infestation, MeJ treatment ((+MeJ) and wounding (+Wnd). The wounded (W) sample, showing the highest expression of the genes, was compared to the unwounded control (C) plants. Values represent mean ± SE (*n=3*). *Different letters* indicate significantly different values.

interestingly aphid infestation even after 24 h, did not elicit any activation of these genes. In insect bioassay jasmonate treated plants showed a strong antibiosis effect on the infesting aphids and reduced the growth of aphid populations. The level of redox enzymes CAT, APX and SOD, involved in ROS homeostasis in defense signaling, and several defense enzymes viz. POD, PPO and PAL, remained high in treated plants. The results indicate that in *B. juncea,* the jasmonate activated endogenous-defense has the potential to reduce the population growth of mustard aphids.

dsRNA mediated suppression of aphid-genes: a novel way of developing aphid resistant crop plants

RNA interference (RNAi) is a universally occurring phenomenon across plant, animal and mammalian genera. In recent years, it has been potentially used to knock down important insect genes. In aphids, RNAi and the consequent down-regulation of gene expression has been demonstrated through delivery of custom synthesized siRNA by micro-injection into the insect body. In our earlier report we have demonstrated the feasibility of host-mediated delivery of dsRNA to effect targeted attenuation of gene expression in aphids. However, the study empirically showed that the applicability of this technology will essentially depend on identification of potential target gene in aphid. The target gene must lead to significant insect mortality when its expression is disrupted and such effect should be specific to the target insects. To this direction, we continued to analyse more number of aphid genes as putatively potential targets for gene silencing. In the

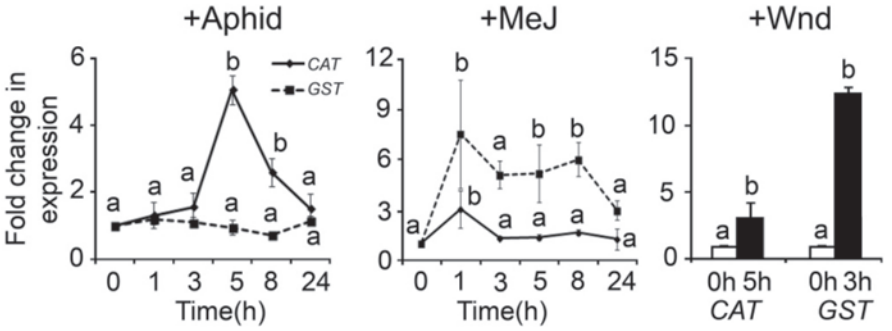


Fig. 2.11: Expression analysis of defense related redox genes in response to aphids, MeJ and wounding. Values represent mean ± SE (*n=3*). *Different letters* indicate significantly different values.

current year five dsRNA molecules (*MpDe2A*, *MpDE3A*, *MpDE4A*, *MpNP6* and *MpNP8*) targeted to aphid-genes encoding vital physiological functions were analyzed for their knock-out-expression effect on aphid-survival. The dsRNAs were synthesized *in vitro* and administered into the aphid through feeding with artificial diet. Interestingly, aphids fed with different dsRNAs showed significant insect-mortality within 48 h compared to the aphids fed with the diet only (RNA minus) (Fig. 2.12). However, the efficacy of different dsRNAs in inducing mortality varied. The target *MpDe4A*, *MpNP6* and *MpNP8* showed more efficacy in causing aphid mortality as compared to *De2A* & *3A*. To ensure sequence-specific effect of the dsRNA, *RUBP* dsRNA was used as a control in

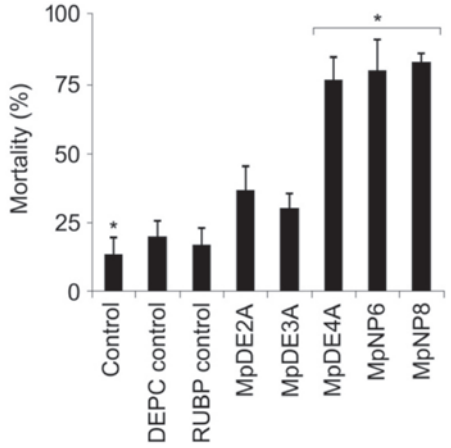


Fig. 2.12: Mortality of aphids (*M. persicae*) after 72 h when fed with dsRNA (0.5 ppm) of different RNAi target genes. Columns represent the mean ± SE (*n=3*). *Asterisk* (*) indicates significant difference in mean compared to the controls (*P ≤ 0.05*).

which no such effect was observed. The results of the feeding bioassay explicitly demonstrated *MpDe4A*, *MpNP6* and *MpNP8* as potential domain to encode dsRNA that can effectively suppress the expression of the target aphid genes so as to induce more than 75% mortality in the feeding aphid population.

Development of *Cre*-expressing lines of Indian mustard for removal of marker genes

One of the major concerns in acceptability of transgenic plants is the presence of selectable marker genes which are used during genetic transformation. Strategy like *Cre/Lox* P site based recombination has been used for successful removal of selectable marker genes from transgenic lines of different plants including *B. juncea*. A binary vector for tapetum specific expression of *Cre* gene is being developed which will be used to generate Cre expressing lines of *B. juncea*. CLAVATA3 (CLV3) and TA29 promoters were selected for tapetum specific expression of *Cre* gene. *Cre* gene (pZAP200), TA29 promoter (Tobacco), CLAVATA3 promoter (pENTR899), and CLAVATA3 terminator (pENTR898) were isolated and cloned in pGEMT-Easy vector system from their respective sources. After their confirmation by sequencing they were cloned and assembled sequentially in pUC19 vector to generate a tapetum specific expression cassette of *Cre* recombinase. The whole cassette with (CLV3 promoter::*Cre* gene::CLV3 terminator) was subsequently cloned in a binary vector pPIPRA560

in its unique *Pac*I site. The recombinant clones were confirmed by restriction digestion and PCR analyses. The recombinant binary vector containing the *Cre* expression cassette was mobilized into *Agrobacterium tumefaciens* GV3101. Floral dip method was employed for the transformation of *B. juncea* plants.

Development of transgenics in pigeonpea for resistance to pod borer with *Bt* insecticidal proteins (ICPs)

The main aim of the programme is to manage infestation of pod borer in pigeonpea with *Bt* ICPs using transgenic technology. In this direction, two ICPs, *cry1AcF* which is a chimeric gene with *cry1Ac* and *cry1F* domains and *cry2Aa* were used independently. Pigeonpea is a recalcitrant crop with poor or no response to *in vitro* regeneration. A tissue culture-independent transformation strategy has been developed for pigeonpea that avoids tissue culture and used to develop transformants for pod borer resistance.

Primary transformants of pigeonpea harbouring *cry1AcF* and *cry2Aa* were developed using the *in planta* transformation protocol and established in polyhouse. The plants established normally, flowered and set seeds. The seeds were harvested for selection of putative transformants.

The putative transformants were primarily selected based on a robust sand screening method in the presence of the selection agent, kanamycin. The concentration of kanamycin detrimental to the wild type was selected. Seedlings with well developed shoot and root system were selected for further analysis.

The selected plants were later subjected to molecular analysis for the integration of the transgenes by PCR and semi quantitative RT-PCR analysis. The positive plants were subjected to *in vitro* challenge by the larvae of *H. armigera.*

It was observed that some of the plants harbouring both *cry1AcF* and *cry2Aa* genes independently were found to resist the attack. There was 100% larval mortality and a minimal 5% leaf damage observed in these plants. These highly resistant plants are being advanced to the further generations to analyse for the stable integration and efficacy of the transgenes (Fig. 2.13).

Genomics of cotton boll developmental stages under bollworm infested biotic stress

Cotton (*Gossypium* spp.) is the leading natural fiber crop worldwide and is also an important source of oilseed and protein meal. *Gossypium hirsutum* L., known as Upland or American cotton is the most widely cultivated species, accounting for more than 95% of the world cotton production and now dominates global cotton commerce. *Helicoverpa armigera* (cotton bollworm) is an important lepidopteran pest responsible for severe yield loss in cotton and other crops. In cotton crops, blooms that have been attacked by insect pest may open prematurely and stay fruitless. When the bolls are damaged, some will fall off and others will fail to produce lint or produce lint of an inferior quality. Consequently the secondary infections by fungi and bacteria are very common and they lead to rotting of fruits. Here we studied comparative transcriptome analysis of field grown cotton during boll developmental stages under bollworm infested biotic stress. The second instar larvae of *H. armigera*, maintained in insect culture room, were released in the days post anthesis flowers and covered with polythene bag to prevent insect escape and collected the sample after 8 hrs infection and labelled as 0 dpa/ days post anthesis, likewise samples were collected after 2 and 5 days post infection and labelled as 2 and 5 dpa, respectively. After 5 days insects were removed from the bolls and allowed to grow up to 10 days and collected the infested bolls and then labelled as 10 dpa (Fig. 2.14A and B). Respectively control boll samples also collected from field grown condition. The collected samples were immediately frozen in liquid nitrogen and stored at -80^{0}C for later use of total RNA extraction.

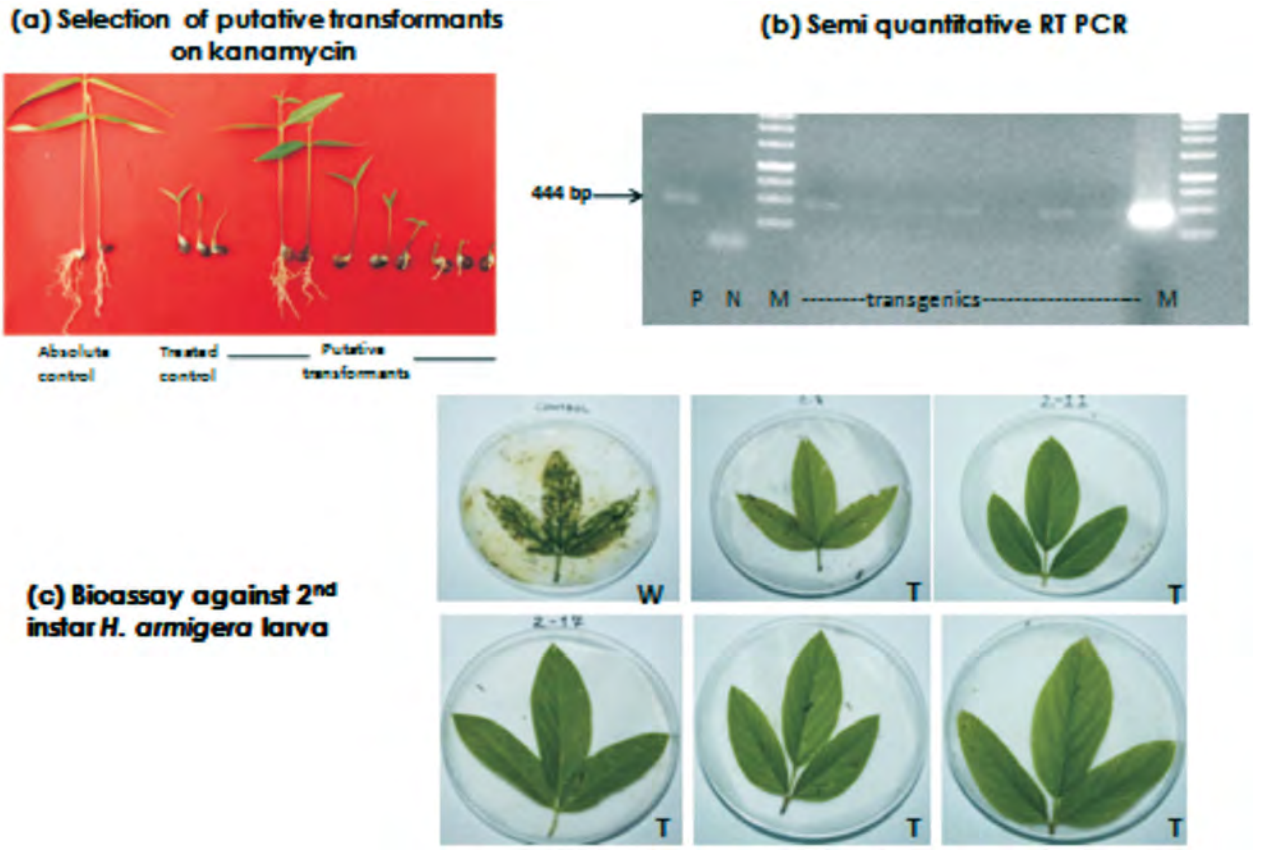


Fig. 2.13: Analysis of the pigeonpea plants for the selection of transformants harboring *cry1AcF* and *cry2Aa* genes. (a). Sand screening for the identification of putative transformants in the presence of kanamycin. Germinating seedlings (overnight) were treated with 50 ppm kanamycin for 4h and put on sand for further growth and selection. Absolute control: wild type seedlings which were not treated with kanamycin; treated control: wild type seedlings treated with kanamycin; putative transformants: T1 generation seeds. (b) Semi quantitative RT-PCR for the expression of the *cry1AcF* transgene (444 bp amplicon) Lane P: positive control (*cry1AcF* plasmid); Lane N: negative control (wild type); Lane M: marker (c) Bioassay of the selected pigeonpea plants against *H. armigera*. Trifoliate leaves from forty-fifty days old plants were subjected to *in vitro* challenging by five second instar larvae and observed for mortality and leaf damage for 96 h. W: leaf from the wild type plant; T: leaves from the selected putative transformants.

Total RNA was extracted from infested and control cotton bolls using Spectrum Plant Total RNA Isolation kit (Sigma, USA). Microarray analysis was performed using Affymetrix cotton GeneChip Genome arrays (Affymetrix, USA) and further hybridization, washing and scanning protocols was followed by as per manufacture instructions and data analysis was performed using GeneSpring GX-12.5 software and identified number of differentially expressed transcripts (DETs) in bollworm infested cotton bolls as compared with their respective control bolls (Fig. 2.14C and D). Functional classification was studied based on MIPS database (http://mips.gsf.de/projects/funcat) and Pathway analysis was performed using MapMan online tool. The transcription factors and phytohormones were also identified using Arabidopsis Transcription Factor (http://plntfdb.bio.uni-potsdam.de) and Phytohormone databases (http://ahd.cbi.pku.edu.cn). qRT-PCR was performed to validate the microarray data which were significantly differentially expressing under bollworm infested biotic stress.

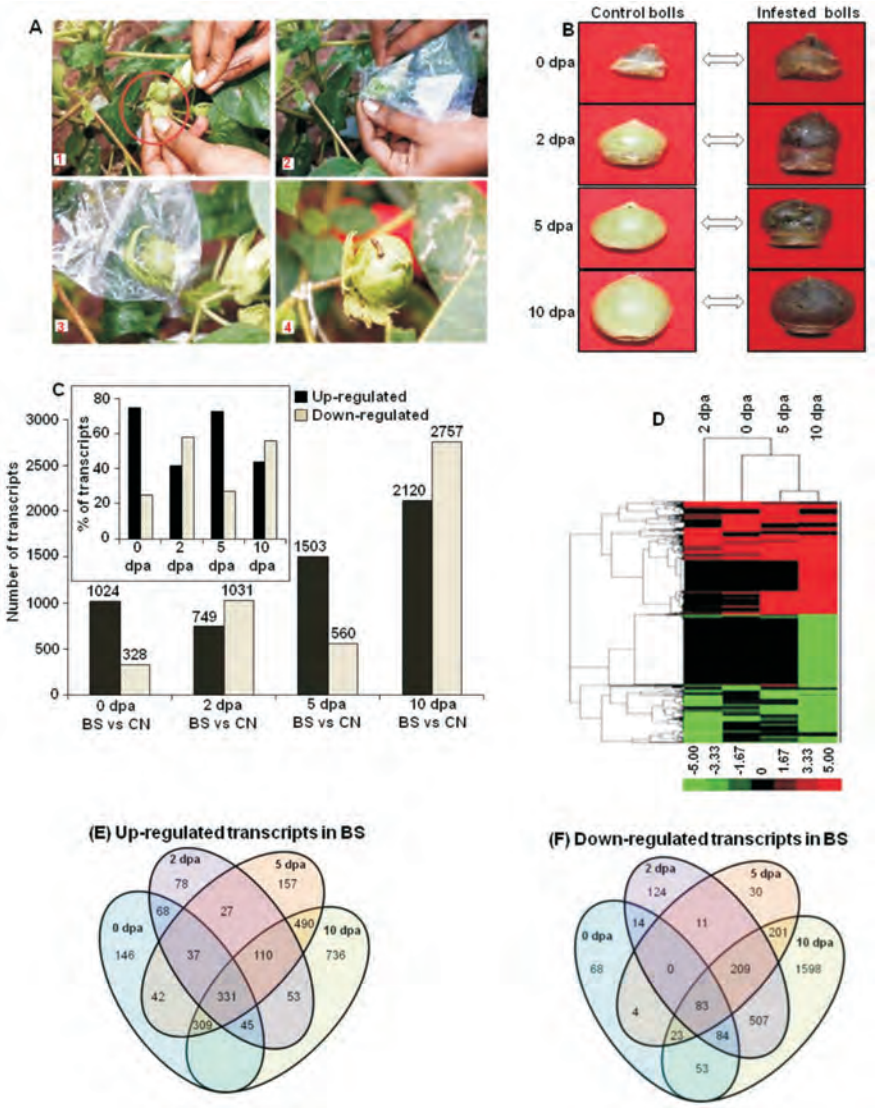


Fig. 2.14: Bollworm infested biotic stress induction in cotton, collection of cotton boll tissues and transcriptome analyses during boll development stages in *G. hirsutum* L. *cv.* Bikaneri narma control and its biotic stress induced samples. (A) Method of biotic stress induction in cotton bolls under field condition. (B) Boll developmental stages of control and bollworm infected tissues used for microarray study (0, 2, 5 and 10 dpa/days post anthesis). (C) Number of differentially expressed transcripts (DETs) during boll development stages under BS as compared to their respective stages of CN and inset represents the percentage of up- and down-regulated transcripts. (D) Cluster analysis showing the differentially expressed transcripts related to biotic stress. E, F Venn diagrams showing the up- and down-regulated transcripts among the boll development stages in the BS, respectively. DETs with p value ≤ 0.01 and fold change ≥ 3 were considered for the experiment.

After statistical data analysis, transcripts with false discovery rate (FDR) adjusted P value ≤ 0.01 and fold change ≥ 3 were considered as significantly DETs. In total 8,694 transcripts which accounted for approximately 39% of the total transcripts present on the cotton GeneChip showed differential expression under biotic stress at various stages analyzed. Maximum number of transcripts [4,877 (56.09%) of total DETs] showed differential expression at 10 dpa and numbers of differentialy expressed transcripts [1,352 (15.55 %) of total DETs] were the least at 0 dpa.

Commonly up- and down-regulated stress responsive transcripts were identified among the boll development stages to find out the degree of overlap (Fig. 2.14E and F). Maximum number of commonly up-regulated transcripts (931) was observed between 5 and 10 dpa whereas maximum number of commonly down-regulated transcripts was observed between 2 and 10 dpa (516). A total of 414 differentially expressed (331 up-regulated and 83 down-regulated) transcripts were identified among the boll development stages. To profile the gene expression patterns in response to biotic stress during boll development, the 8,694 DETs were classified using hierarchical clustering. Transcripts and pathways which are involving in signalling and defence mechanism were up-regulated and transcripts and pathways which are involved in cell wall and photosynthesis were down-regulated, significantly.

Fruit specific promoter isolation from brinjal

To identify promoters from fruit tissue for insecticidal gene expression in tissue specific manner, transcriptome analysis of brinjal during fruit development was carried out. A genome-wide expression analysis was carried out during fruit development stages (0, 5, 10, 20 and 50 dpa/days post anthesis) in brinjal using Affymetrix tomato GeneChip genome array. About 1395 transcripts were shown to be differentially expressed at various stages of fruit development analyzed. Maximum number of transcripts (858) showed differential expression at 50 dpa while least number of transcripts (383) showed differential expression at 5 dpa as compared to 0 dpa. MYB and MADS box family TFs were mainly down regulated while IAA/AUX family TFs were up-regulated at all stages except at ripening stage. Genes involved in photosynthesis, carbon and energy metabolism and redox responsive genes were up-regulated throughout the fruit development stages where as genes involved in brasinosteroid signaling, cell cycle and growth and cell wall biosynthesis were up-regulated during early fruit development stages (5, 10 and 20 dpa). Further, genes involved in ethylene biosynthesis were up-regulated at 20 dpa. Several stage specific differentially expressed transcripts involved in various metabolic pathways were identified. After understanding expression pattern, to get actual gene sequences from brinjal, ESTs were generated from brinjal cultivar PPL and KKM1 by reciprocal SSH generation from various fruit developmental stages. Quality of libraries was checked by mobility shift of colonies and sequencing. About 4100 good quality ESTs were generated from ten different libraries, and assembled into contigs. ESTs were annotated against *Arabidopsis* protein database TAIR.

Fruit specific genes were selected based on both microarray and EST sequencing strategies and these genes were again confirmed for expression by quantitative real time PCR in PPL and KKM1 varieties of brinjal (Fig. 2.15). On the basis of qRT-PCR results seven fruit specific genes were selected for promoter isolation. Recently 1Kb promoter region is isolated from brinjal Sm2034, a fruit specific gene using genome walking method. This promoter is first cloned into pUC19 vector and confirmed by sequencing. A sequence of the promoter is submitted to NCBI. The promoter was introduced into pBI121 for plant transformation to express *β-glucuronidase* (GUS) gene (Fig. 2.16).

Gene prospecting for thermotolerance from Finger millet

Subtractive cDNA libraries were prepared from Finger millet cultivar MR1 ten-days-old seedlings subjected to early heat stress (15, 30, 45 and 60 min) and late heat stress (2, 4, 8 and 24 h) at 42^{0}C. Total 4 libraries were prepared, two for early sample i.e. Early Forward (pool A Forward) and Early Reverse (pool A Reverse), and two for late sample i.e. Late Forward (pool B Forward) and Late Reverse (pool B Reverse). These heat stress libraries were sequenced with 454 pyrosequencing method. Sequencing reads were assembled into unique molecules for each library and

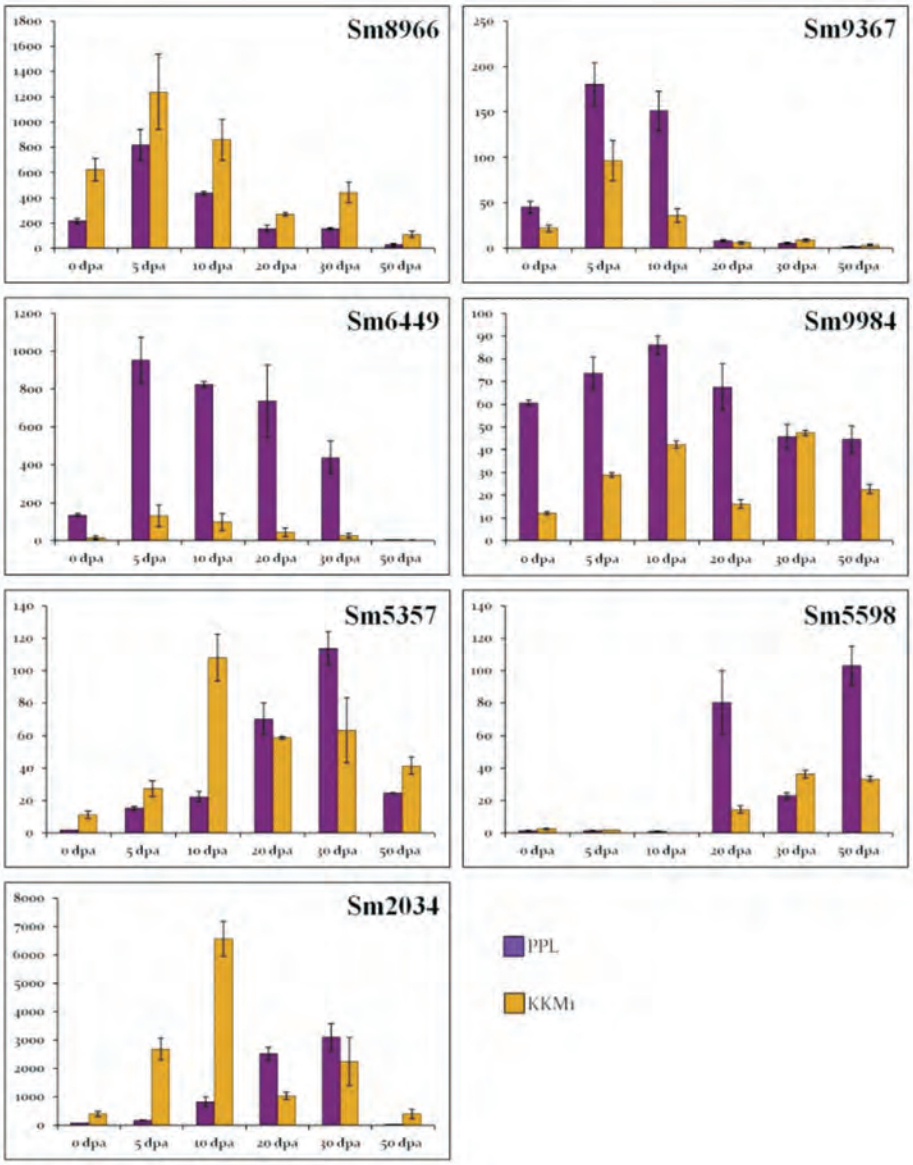


Fig. 2.15: Expression analysis of seven fruit specific genes in PPL and KKM1 varieties of brinjal at 0, 5, 10, 20, 30 and 50 dpa fruit developmental stages (Y axis - Fold change).

1073 transcripts were obtained for early forward library, 2822 for early reverse library, 903 for late forward library and 5254 for late reverse library. GO terms were obtained for around 80% transcripts in each library (Fig. 2.17).

After sequencing, genes were selected for qRT-PCR analysis and *BCL-2-associated athanogene a* and *b*, *heat shock protein 1*, *PP2C*, *xylanase inhibitor*, *putative bZIP transcription factor*, *DREB* and *citrate synthase* showed high expression under heat stress (Fig. 2.18). These genes were selected for further characterization.

As ragi is tetraploid species (2n=36) with 9 basic chromosomes, it is difficult to get amplification of desired genes because of its ploidy level. To get complete CDS of selected genes RACE (Rapid Amplification of cDNA Ends) analysis was

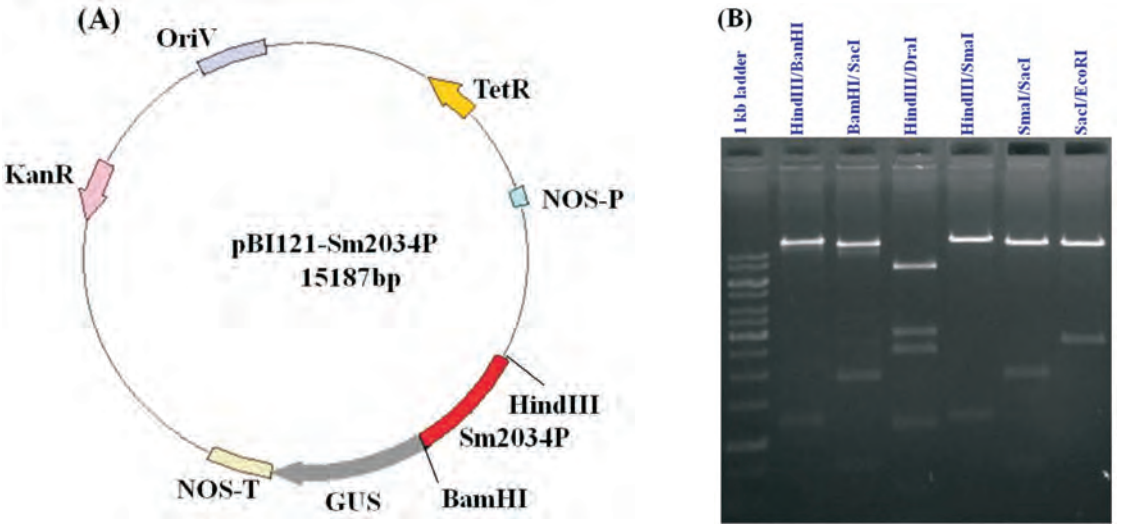
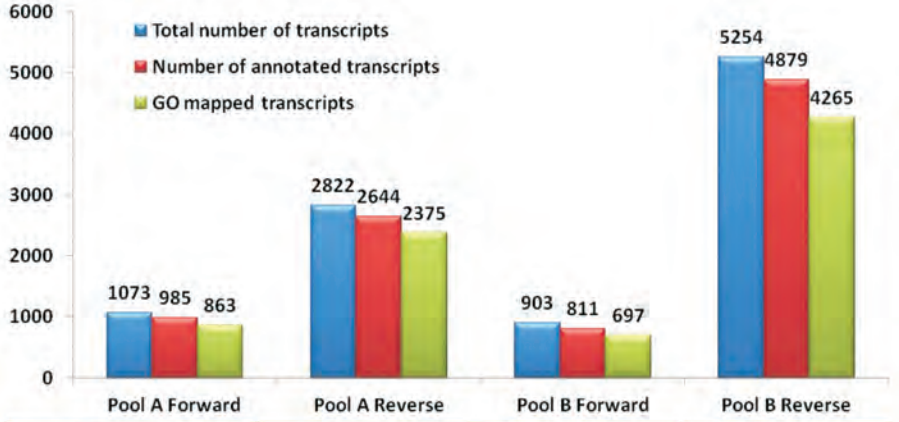


Fig. 2.16: (A) Cloning of brinjal Sm2034 promoter into binary vector pBI121 and (B) its restriction digestion analysis.



Sample Name	Biological processes	Molecular functions	Cellular components
Pool A Forward	370	399	458
Pool A Reverse	1228	1334	1430
Pool B Forward	322	322	350
Pool B Reverse	1962	2241	2279

Fig. 2.17: Distribution of transcripts mapped on GO database from ragi heat up- and down-regulated libraries.

carried out. *EcDREB*, *EcbZIP* and *EcBAG6* genes were amplified for 5' as well as 3' ends. These three genes were cloned and confirmed for correct sequences in pGEM-T vector. These gene sequences are submitted to NCBI and further transferred into binary vector for plant transformation.

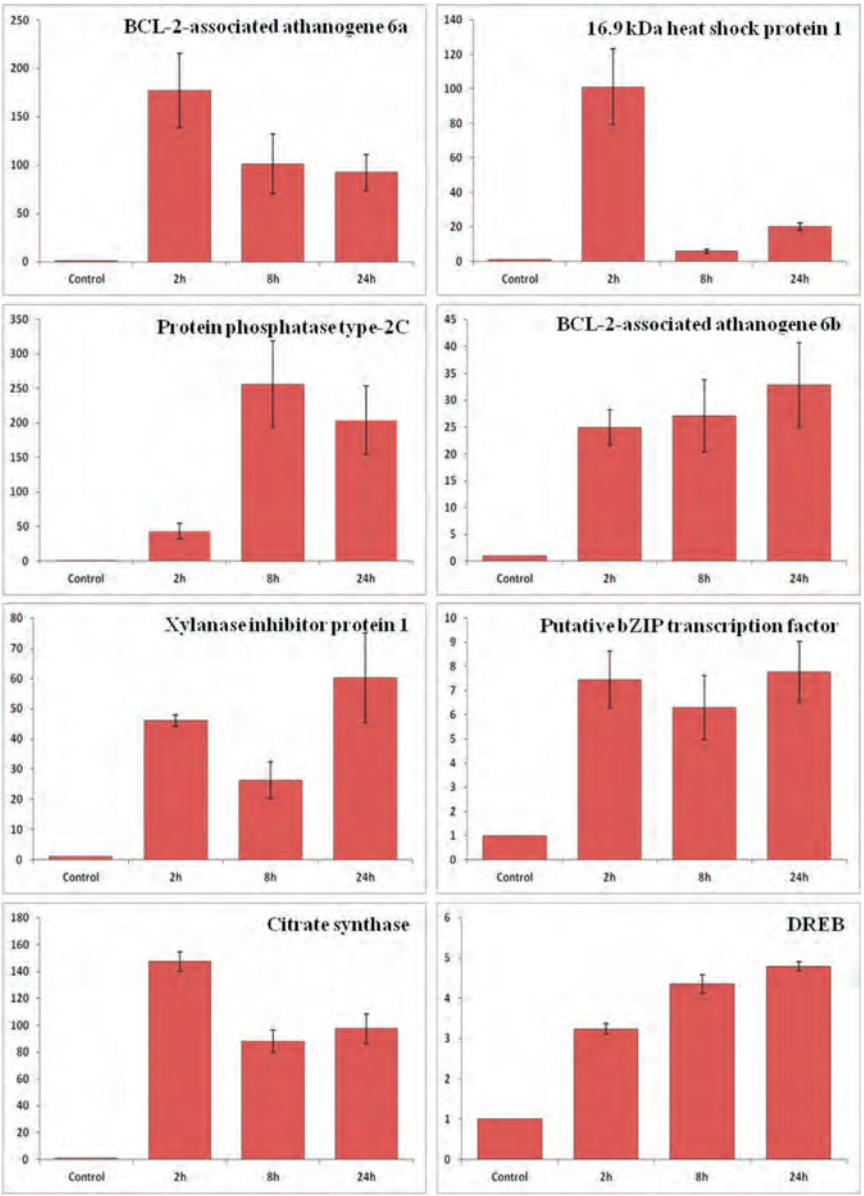


Fig. 2.18: Validation of selected genes from heat stressed cDNA libraries by qRT-PCR analysis in ragi seedling samples subjected to heat stress at 42 ^{0}C (Y axis- Fold change).

3. Adaptation of Wheat to Climate Change Induced Abiotic Stresses

Identification and characterization of heat responsive genes from tolerant plant species

The project aimed at prospecting of abiotic stress responsive genes from tolerant plant systems such as *Pennisetum glaucum*, *Prosopis cineraria*, and stress tolerant cultivars of wheat. The potential genes would be exploited for genetic transformation into elite cultivars of wheat for enhancing abiotic stress tolerance. Previous year, heat responsive EST libraries were generated from *P. glaucum, P. cineraria*, and tolerant cultivars of wheat, and drought responsive EST libraries were constructed from *Zizyphus*. Sequencing, annotation and assembly of the available ESTs led to identification of a number of abiotic stress responsive genes. Based on the results, a few differentially expressed genes were selected and validated through qPCR analysis. Genes such as *PgP5CS*, *PgCRT*, *TaApx*, *TaCpn*60, *Tahsp87*, *PcHSP*17.9, and *TaHSP-ST1* that were highly upregulated under stress were taken for further studies. *PgCRT* (*calreticulin;* CDS:1287bp) from *P. glaucum* was characterized in prokaryotic system with regard to expression and function under heat stress. Full length CDS (2155 bp) of *PgP5CS* (Δ^1-pyrroline-5-carboxylate synthetase) from *P. glaucum* was isolated, sequenced and mobilized in expression vector. Using RACE approach, a full length CDS (474 bp) of *Pchsp*17.9 (heat shock protein 17.9) from *P. cineraria* was amplified and sequence was submitted to NCBI GenBank. *Pchsp*17.9 had also been mobilized in expression vector. Heat stress responsive gene *CPN60* was isolated, cloned and sequenced from bread wheat (*Triticum aestivum*). *CPN60* and *pAPX* genes from wheat were cloned in pET 28 vector and expression of these genes in *E.coli* was confirmed by SDS-PAGE, western blotting and MALDI (Fig. 3.1). Functional validaton of drought responsive *Abscisic acid stress ripening (ASR)* gene from *Zizphus nimmularia* was carried out in prokaryotic system.

Genetic transformation of wheat

Genetic transformation protocol (*in vitro* and *in planta* methods*)* in elite wheat cultivars is being standardized. For this purpose gene construct pCAMBIA1301 harbouring herbicide tolerance gene

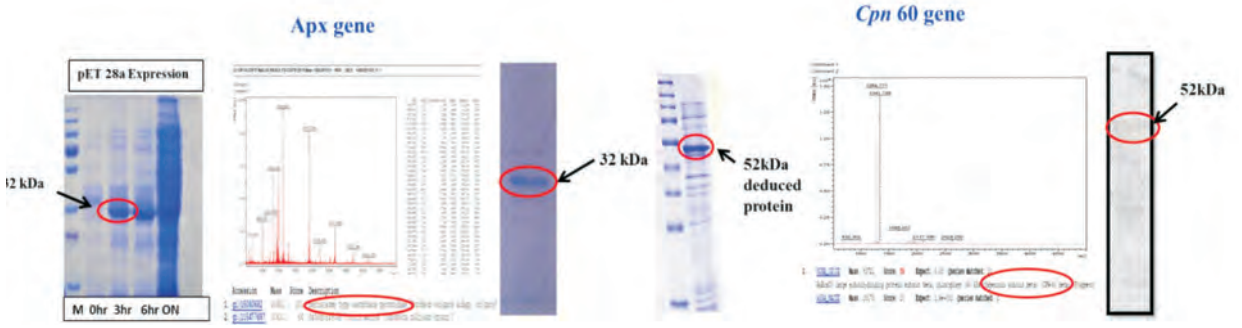


Fig. 3.1: Overexpression of *APX* and *Cpn60* genes in *E. coli*, peptide mass finger printing of the over-expressed protein and western blotting.

Fig. 3.2: *In planta* transformation of wheat *cv.* HD2894 with pCAMBIA1301 harboring herbicide tolerance gene (*EPSPS*) A) One month old T_1 seedlings before glyphosate spray, B) Seedlings after glyphosate spray, survived plants are circled, C) Survived glyphosate resistant plants in pots, D) Plants at maturity stage.

(*EPSPS*) under control of maize ubiquitin promoter is being used.

In planta transformation method was standardized in elite Indian wheat cultivar HD2894. Wheat transgenics harbouring herbicide tolerance gene (*EPSPS*) were screened for presence of transgene by spraying glyphosate at one month old seedling stage (Fig. 3.2). The positive transformants developed by i*n vitro* (T_2) and *in planta* (T_3) methods were confirmed by PCR analysis and Southern hybridization (Fig. 3.3). Furthermore, transformation of wheat, *cv.* HD2894, HD2967, HD2932 and HD2987, with constructs harboring heat stress responsive genes, *csp*B, *mpgp*, *cspA*, *TaAPX* and *EcDreb,* has been initiated to develop thermotolerant transgenic wheat.

Isolation and characterization of candidate genes for terminal heat stress tolerance from wheat at different generative stages

To isolate and characterize genes conferring terminal heat stress tolerance in bread wheat, initially a few genes viz. transcription factor *WRKY10*, RNA binding protein (RBP) and *S-phase kinase prorein1* (*Skp1*) were selected from available microarray and transcriptomic database of wheat as well as other plant species. The expression of these genes was analyzed in twelve diverse genotypes under control (natural field conditions) and high temperature stress (37°C) at various generative stages from flag leaf to mature grain filled spikes (Fig. 3.4).

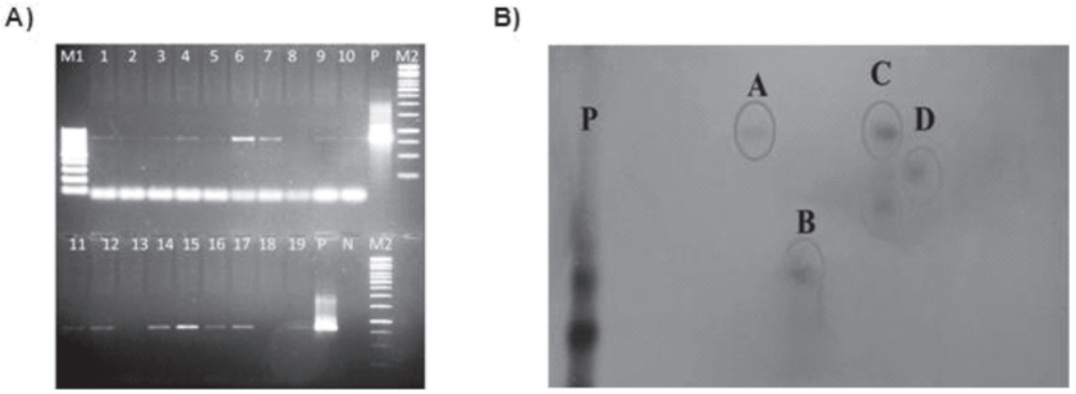


Fig. 3.3: PCR confirmation of *EPSPS* gene in transgenic (T_3) lines of HD2894 developed by *in-planta* transformation method. A) M1: 100bp marker, M2: 1kb marker, 1-19: T_3 Transgenics, N: -ve control, P: +ve control, B) Southern hybridization analysis of T_3 plants (A, B) obtained by *in planta* method & T_2 plants (C, D) obtained by mature embryo transformation method (HD2967).

Fig. 3.4: Developmental stages of common bread wheat *Triticum aestivum* utilized for high temperature stress candidate gene analysis.

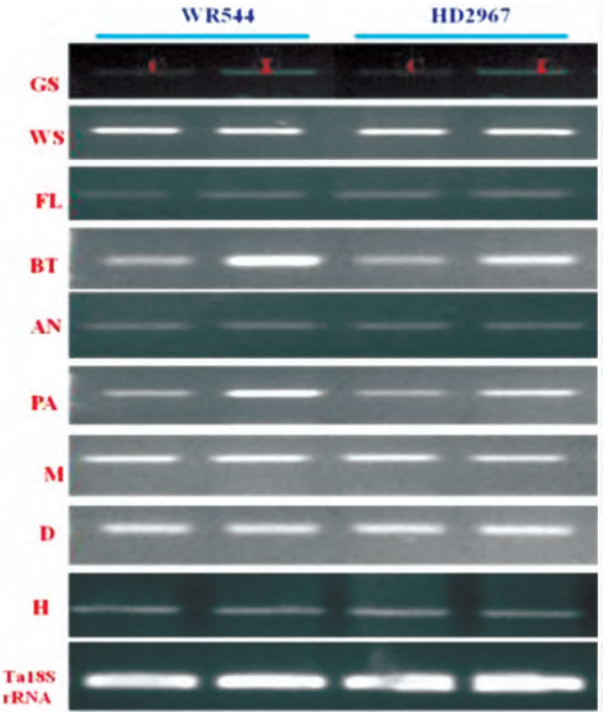


Fig. 3.5: Transcript expression profile of *TaWRKY10* under high temperature stress and control condition at 9 different stages from thermotolerant (WR544) and sensitive (HD2967) wheat cultivars. GS- germinating seeds; WS-whole seedling; FL-flag leaf; BT-boot; AN-anthesis; PA- post anthesis; M-milky; D-dough; H- hardening; C-control; T-treatment.

WRKY transcription factors are one of the largest families of transcriptional regulators in plants and form integral parts of signaling network that modulates many plant processes. Transcript expression profile of *WRKY10* was carried out in a heat tolerant, late sown variety WR544 and a popular variety HD2967 (Fig. 3.5). Steady state expression of *WRKY10* was observed in both the cultivars, however higher induction was seen in tolerant (WR544) as compared to the sensitive (HD2967) variety. Highest induction was seen at boot stage and post anthesis spikelets. A 650bp full length cDNA of *WRKY10* was cloned in a cloning vector and sequenced. Amino acid analysis confirmed the presence of WRKY domain and C2H2 zinc finger domain (Fig. 3.6).

RNA-binding proteins (RBPs) control RNA fate from synthesis to decay. Post transcriptional regulation of RNA metabolism is increasingly being recognized as a key regulatory process in plant response to environmental stress. This is largely achieved by diverse RNA binding proteins (RBPs). Hence identification and characterization of RBPs from different plant species is an indispensable step to better understand a variety of cellular processes. A transcript expression profile of a RBP from bread wheat was carried out in 10 diverse cultivars grown under rainfed condition at five different generative stages from flag leaf to mature grain filling stage. The highly modulated expression of the RBP was observed during grain development (Fig. 3.7).

Global expression profile under high temperature stress during anthesis

To identify changes in global expression profile under high temperature stress during anthesis, SSH libraries were constructed from pre and post anthesis inflorescence of wheat genotype WR544. After sequence analysis, around 400 ESTs that showed up regulation were categorized into different classes by RGAP (Rice Genome Annotation Project) BLASTx analysis. About 22% ESTs belonged to energy metabolism, 3% to phytohormone synthesis (Fig. 3.8). Selected genes from the libraries are being validated by real time RT-PCR.

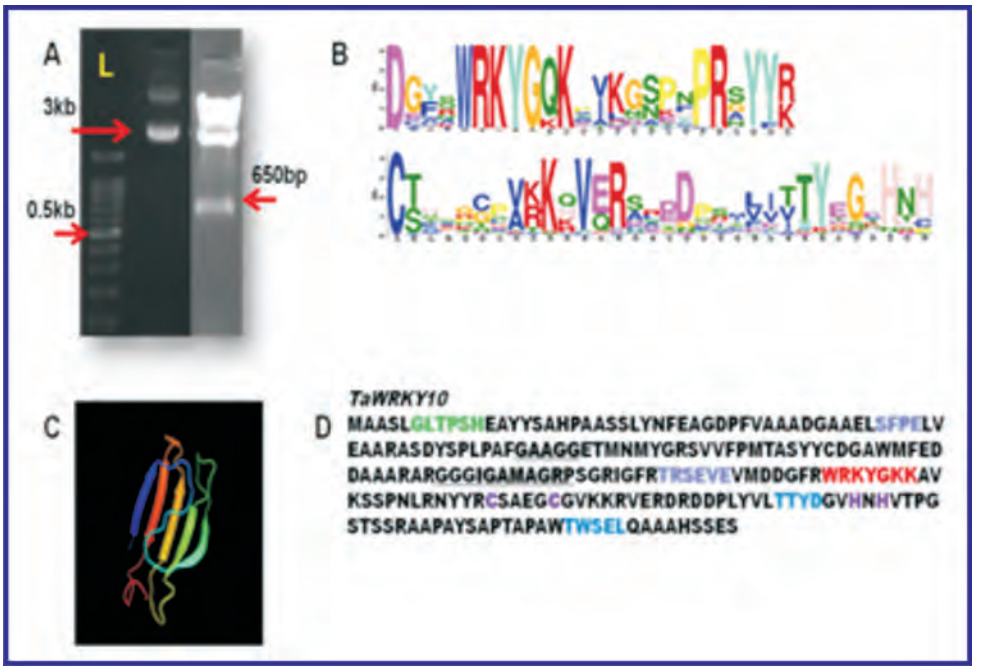


Fig. 3.6: A) Amplification and cloning of *TaWRKY10* full CDS in a cloning vector. B) Predicted domains in the WRKY protein. The conserved domains were carried out by MEME using the protein sequences of *TaWRKY*s and other known WRKYs to create the logo representations of the WRKY domain and the zinc finger motif. The Y axis depicts the overall height of the stack indicating the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position. C) 3D- strucrure of *TaWRKY10* deduced using Phyre². D) *TaWRKY10* amino acid sequence. Color representation: Blue-casein kinase II phosphorylation sites, Green- N-myristoylation, Red- WRKY domain, Purple- zinc-finger motif.

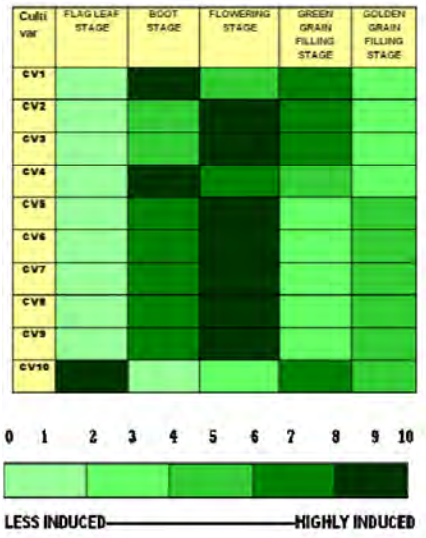


Fig. 3.7: Relative expression profile of *TaRBP* in 10 divergent wheat cultivars in various generative tissues. Lower panel indicates color scale expression potential measured by semiquantitative RT-PCR.

Plant annexins

Annexins act as targets of calcium signals in eukaryotic cells and play an important role in plant stress responses. Hence annexin genes from various crop species are being isolated and their functional characterization is being done for their further deployment in plant stress management. Last year, cloning of two full length cDNAs of annexin from *P. glaucum* was reported. Three more annexins were identified from a *P. glaucum* namely *AnnPg3, AnnPg4* and *AnnPg5* by transcript expression analysis. Partial cDNAs of these genes were amplified by RT-PCR, and subsequently cloned and confirmed by sequencing (Fig. 3.9). *AnnPg 2* was cloned into a binary vector pBI121 and transformed into *Arabidopsis thaliana*. T2 seeds have been collected and are now functionally characterized under stress environment (Fig. 3.10).

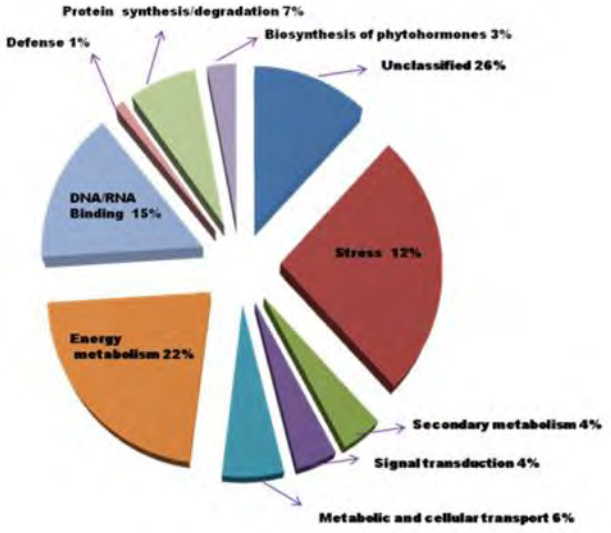


Fig. 3.8: Functional categorization of ESTs from a FSSH library of post anthesis inflorescence of wheat genotype WR544.

Ten genotypes of rice were selected for expression profile studies of seven different annexins viz.*AnnOsI1, AnnOsI2, AnnOsI3, AnnOsI4, AnnOsI5, AnnOsI6, and AnnOsI7* earlier cloned in the lab. The expression was analyzed at 6 different stages viz. seedling, tillering, panicle initiation, heading, flowering and grain filling in rice. Seeds were germinated in pots containing autoclaved soilrite under 25^{0}C with 16 hrs light/ 8hrs dark photoperiod in a culture room and used for expression studies in seedling stage. For long term analysis in further stages, plants were grown in the controlled environmental condition of phytotron facility (Fig. 3.11). Water stress was imposed

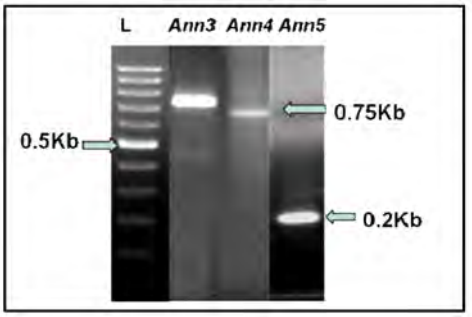


Fig. 3.9: Amplification of partial cDNA of *AnnPg3* (767bp), *AnnPg4* (750bp) and *AnnPg5* (211bp) from *Pennisetum glaucum* cultivar WGI126 by RT-PCR.

Fig. 3.10: Transgenic *Arabidopsis* plants over expressing *AnnPg2,* and control plants grown at National Phytotron Facility, IARI, New Delhi.

by withholding water for 48 h before tissue harvest and relative water content was measured. RT-PCR expression profiling of rice annexins gene family was carried out under drought stress at various developmental stages (Fig. 3.12).

In another experiment, induction of *annexins* in *indica* rice IR65 was analysed under various heavy metal exposure. Fifteen day old seedlings of IR64 were treated with salts of five heavy metals namely cadmium, nickel, chromium, copper and zinc for 5h along with water as control and used for expression studies. Differential modulation of annexins indicates that these annexins have potential role under different heavy metal stresses (Fig. 3.13).

Phenotyping of wheat germplasm for root traits under drought stress conditions

In India, majority of the irrigated wheat receives less than the 5 recommended irrigations. Hence, wheat crop suffers moisture deficit stress at one or more important stages of plant development. Therefore, to sustain production, genetic improvement in drought tolerance of wheat is necessary. In India, C306, a variety released in the year 1969, is still considered the best wheat variety under drought stress, and is used as a standard check throughout the country. In order to explore the genetic variability in wheat germplasm for root traits under drought stress

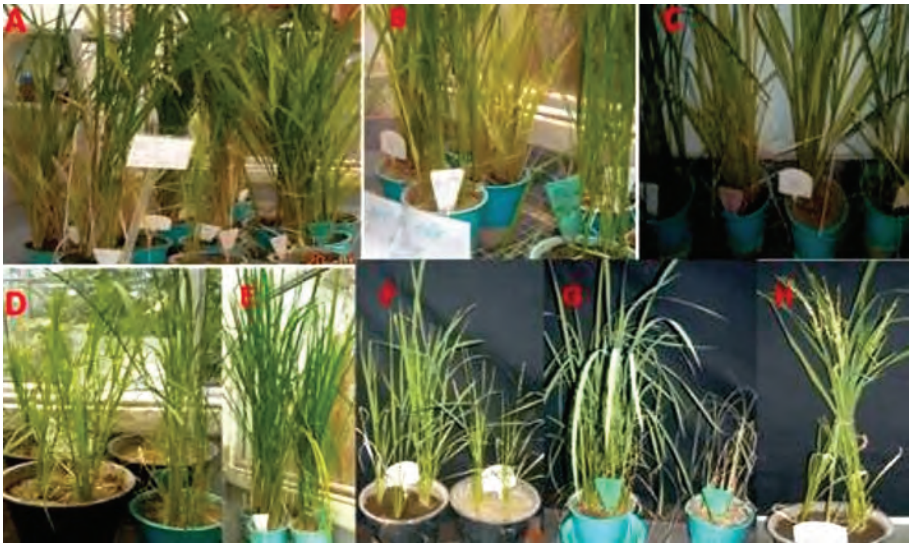


Fig. 3.11: A representation of diverse rice varieties grown under control and water stressed conditions.

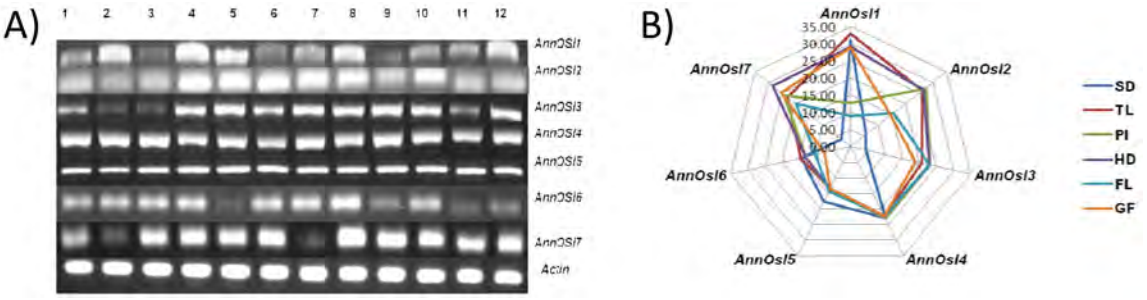


Fig. 3.12: A) A representative gel picture showing transcript expression of seven different *Annexin* genes after 48 h drought stress and control rice plants at different critical growth stages. Lane 1 and 2: Seedling stage control and treated, Lane 3 and 4: Tillering stage control and treated, Lane 5 and 6: Panicle initiation stage control and treated, Lane 7 and 8: Heading stage control and treated, Lane 9 and 10: Flowering stage control treated, Lane11 and 12: Grain filling stage control and treated, B) Graphical representation of gene expression in rice cultivar Nagina22. SD-seedling; TL-tillering; PI-panicle initiation; HD-heading; FL-flowering; GF-grain filling stage respectively.

conditions, total 60 wheat genotypes were analyzed for root traits under non-stress and drought stress conditions in seedling stage. The genotypes exhibited large variation for constitutive and drought induced root growth. Based on the total root length under drought stress, eight genotypes were identified which were performing better than the drought tolerant check C306 (Fig. 3.14). The contrasting set of genotypes that are identified will be used for genetic and molecular dissection of drought induced root growth. To characterize the genes involved in root system architecture, homolog of *NAC1* which play an important role in development of root system architecture in *Arabidopsis* was identified in wheat. The cDNA of *TaNAC1* was cloned from wheat genotype C306 and confirmed by sequencing (Fig. 3.15).

Procurement and multiplication of *Aegilops*

Aegilops species have been considered a genetic resource for increasing the genetic potential of cultivated wheat to withstand biotic and abiotic stresses. A detailed study of the heat tolerance mechanism in this wheat species will help in improving

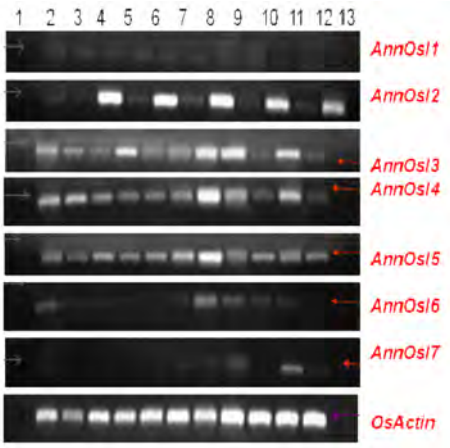


Fig. 3.13: Differential induction of annexins in *indica* rice cultivar IR64. Lane 1: 50 bp DNA ladder, Lane 2: expression in control (water treated) seedlings. Lane 3, 5, 7, 9, 11: seedlings treated with 10 ppm of Cd, Zn, Cu, Ni and Cr respectively, Lane 2, 4, 6, 8, 10, 12: expression in seedlings treated with 20 ppm of Cd, Zn, Cu, Ni and Cr respectively, Lane 13: RT- PCR reaction without cDNA (negative control).

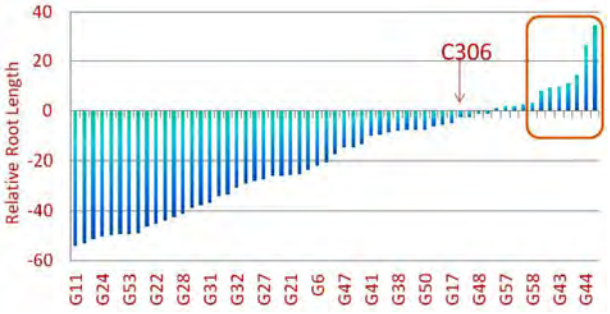


Fig. 3.14: Genetic variation in drought induced root traits in wheat germplasm.

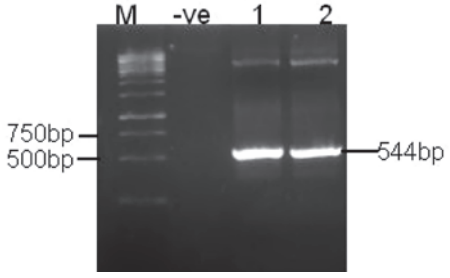


Fig. 3.15: Cloning of *TaNAC1* from wheat genotype C306.

our understanding of the intricacies of heat tolerance mechanism in plants and developing a strategy for improving the high temperature tolerance in crop plants especially wheat. Accessions of *Aegilops genicultata* and *A. speltoides* has been procured from NBPGR and successfully multiplied in National Phytotron Facility, New Delhi (Fig. 3.16).

Screening of Finger millet germplasm for abiotic stress tolerance

Accessions of ragi were obtained from ACRIP on small millets and screened for abiotic stress tolerance (Fig. 3.17). Contrasting genotypes will be used for cloning of stress responsive genes.

Transcriptomics of Finger millet

As Finger millet has very less genomic information in public database, a transcriptome analysis was carried out for Finger millet cultivar MR-1 at seedlings stage. Normalized cDNA library was constructed and sequenced using GS-FLX 454. Total 539 Mb data was generated with 11,73,571 reads. These reads were assembled into 21,118 transcripts. These 21,118 transcripts were further used for GO annotation. Gene ontology distribution can be used to better understand the distribution of annotated

Fig. 3.16: *Aegilops* accessions at different growth stages in National Phytotron Facility, New Delhi.

Fig. 3.17: Screening of Finger millet genotypes for drought stress tolerance.

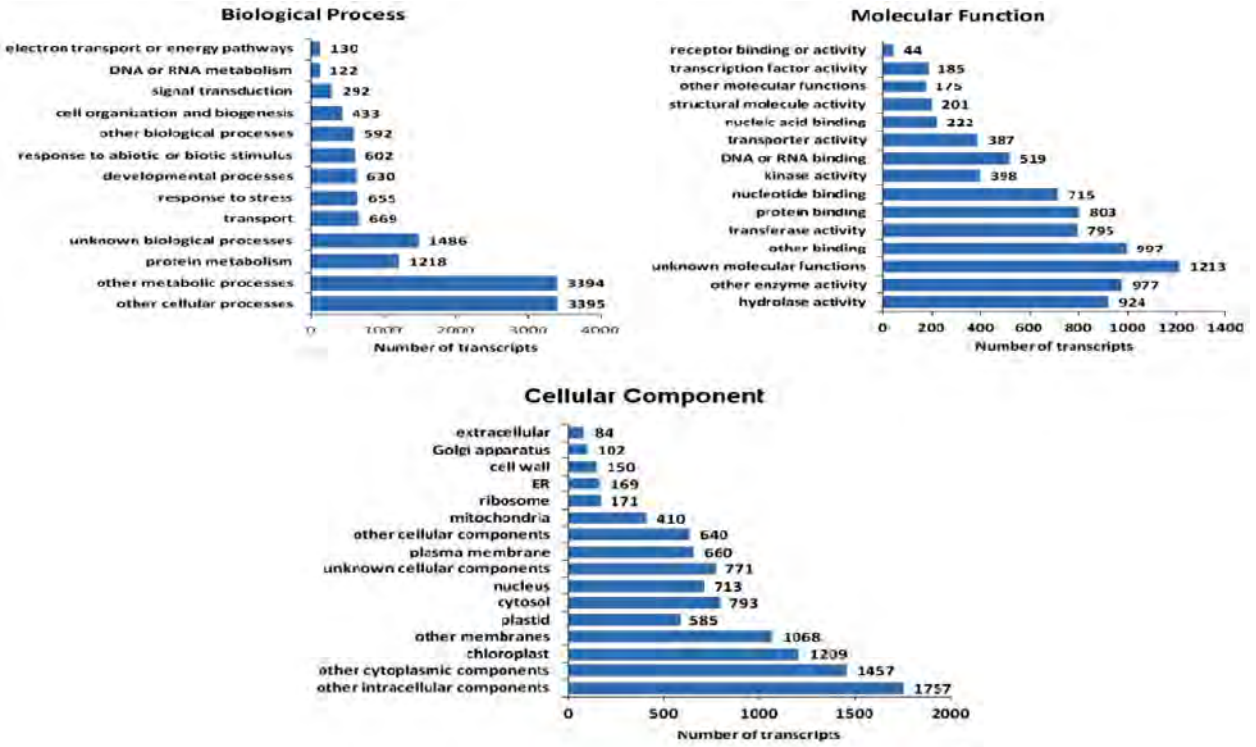


Fig. 3.18: GO annotation of Finger millet transcripts.

transcript contigs in specific ontology domains such as Biological Process, Molecular Function or Cellular Component (Fig. 3.18).

Isolation and characterization of genes for abiotic stress tolerance from extremophiles

Previous year, microbes from extreme environments including hot springs and cold regions were collected and isolated. Using a PCR based approach; these microbes were screened for the presence of candidate genes (*CspA*, *CspB, mpgp* etc.) which are known to impart tolerance against various abiotic stresses. Full length native *Csp A* and *CspB* genes were amplified and cloned from the identified bacteria (Fig. 3.19). These bacteria will be submitted to Culture Collection Facility, NBAIM, Mau Nath Bhanjan, Uttar Pradesh. The codon optimized C*sp A*, *Csp B* and *mpgp* have been cloned in binary plant transformation vectors (Fig. 3.20) and being transformed in *Arabidopsis* and rice.

Isolation of novel *Bt* strain

A variant allele of *CryIAa* was functionally validated in *E. coli* by insect bioassay. *CryIAa* has been submitted to *Bt* nomenclature, UK. A novel *Bt* isolate harbouring 9 *Cry* genes and 7 other insecticidal genes

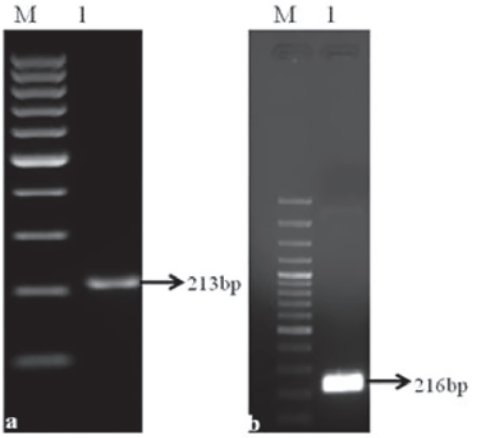


Fig. 3.19: PCR amplification of bacterial *Csp*A and *CspB* gene a) M: 100 bp DNA Ladder , 1: PCR amplified *Csp*A gene (213bp) b) M: 100 bp DNA Ladder , 1: PCR amplified *CspB* gene (216bp).

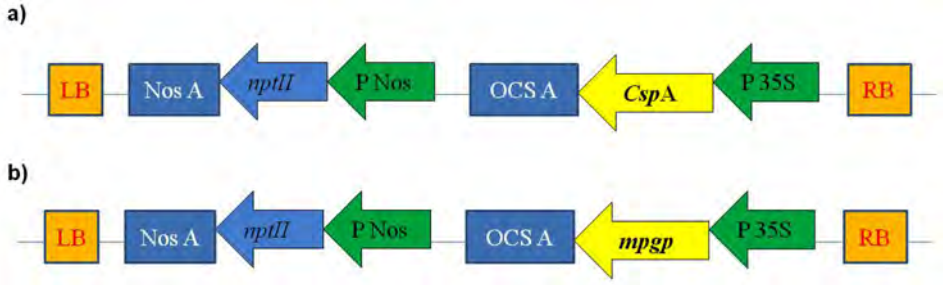


Fig. 3.20: a) Map of construct carrying *Csp*A gene cloned in pBinAR, b) map of construct carrying *mpgp* gene cloned in pBinAR.

were identified and submitted to IMTECH, Chandigarh under Budapest treaty.

Characterization of synthetic promoter for overexpression of *nif* gene in *Azotobacter*

Two independent constructs were developed having a synthetic promoter designed based on bioinformatic tools and a *tet* (from pBR322) promoter, respectively, upstream of promoter less β-galactosidase gene (from pSD5B). The constructs were found to be stable in both *E. coli* and *Azotobacter vinelandii*. The results established that the constructed plasmid vectors are suitable for determination of activity of promoters and the synthetic promoter was found to be more active than the *tet* promoter.

4. Biotechnological Approaches for Increasing Productivity

Development and characterization of CMS and fertility restorer stocks

Although improved, chlorosis-corrected *Brassica juncea* CMS lines based on *B. oxyrrhina* and *Raphanus sativus* (Ogura) cytoplasm have been generated long back, they have not been well characterized at the molecular level. One of the improved Ogura CMS lines named Og-1 was found to give rise to male fertile progeny at quite high frequency. Such male fertile lines neither yielded male sterile progeny nor restored fertility suggesting that the male fertile lines are permanent revertants. To investigate the molecular basis of reversion, Southern and northern hybridizations were carried out with mitochondrial gene probes. Both Og-1 and Og2 CMS lines were found to carry recombinant mitochondrial genome involving *B. juncea* and Ogura (Fig. 4.1a, b). In the Og1-revertant lines, abundance of the CMS-associated Ogura *orf138* gene was greatly reduced (Fig. 4.1c). Also, transcripts of *orf138* were not detectable by RT-PCR in the revertants. Comparison of *orf138* flanking sequences in the Og-1 and revertant lines suggested intramolecular recombination in the mt-genome of Og-1 male sterile line giving rise to new mt-genome lacking *orf138* in the revertant lines. This proves that *orf138* causes CMS in Og1 and its loss through intramolecular recombination leads to reversion.

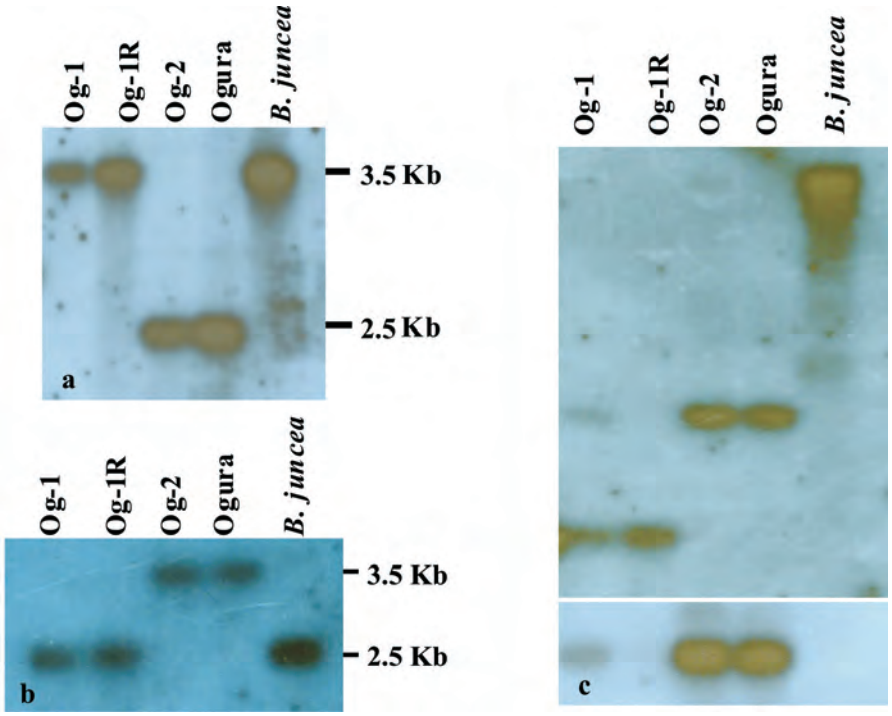
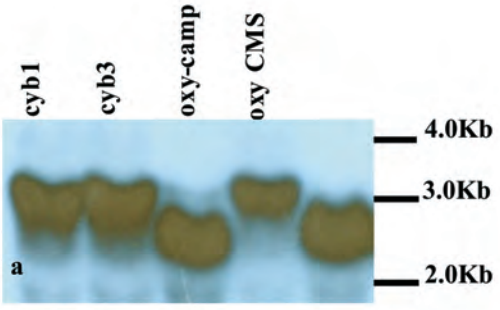


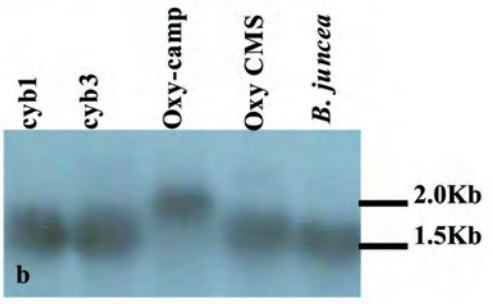
Fig. 4.1: RFLP analysis of Ogura based CMS and normal *B. juncea* lines using mitochondrial gene probes. a-c. Southern blot with *atp6* with *Bam*H I (a), *rps7* with *Eco*RI (b), *atp8* with *Bam*HI (upper panel), *orf138* with *Bam*HI (lower panel)(c).

Chlorosis corrected *B. juncea* CMS cybrids carrying *B. oxyrrhina* cytoplasm show stable male sterility and carry no cytoplasmic penalty. Lack of fertility restorer lines has prevented its commercial use. Therefore, attempts are on to introgress male fertility restorer genes through interspecific hybridization. To identify the mitochondrial genes responsible for male sterility, comparisons were made for the RFLP patterns among Oxy CMS (i.e. chlorotic CMS line), cybrids 1, 3 (chlorosis corrected CMS lines), amphidiploid *B. oxyrrhina-B. rapa* (Oxy-camp) and normal *B. juncea* (cv. RLM-198) lines. Northern hybridization revealed variations for *atp1, atp4, nad4L* and *cox3* transcripts (Fig. 4.2a, b). Further, Southern hybridization showed differences in RFLP pattern for the *atp1* gene between chlorotic CMS and cybrids (Fig. 4.2c) indicating that cybrids have recombinant mitochondrial genome, contradicting earlier view that they carry unaltered mt-genome from *B. oxyrrhina*.

Genetic engineering of *CENH3* to develop haploid inducer lines of *Brassica juncea*

In order to engineer centromere histone proteins (CENH3) for selective chromosome elimination, *CENH3* genes and cDNAs were cloned from *B. juncea* and related wild species such as *Diplotaxis erucoides, D. catholica* and *Orychophragmus violaceous*. *B. juncea* was found to carry two copies of *CENH3* one each corresponding to the progenitor species *B. nigra* and *B. rapa*. Further, both the copies were expressed. In *D. catholica* and *D. erucoides*, a single copy of *CENH3* was found. By comparing genomic and cDNA sequences of different species, target sequences for RNAi were identified and vector for silencing of native *CENH3* genes of *B. juncea* was designed (Fig. 4.3). Since loss of *CENH3* is lethal, *CENH3* rescue vector was constructed wherein a synthetic version *CENH3*





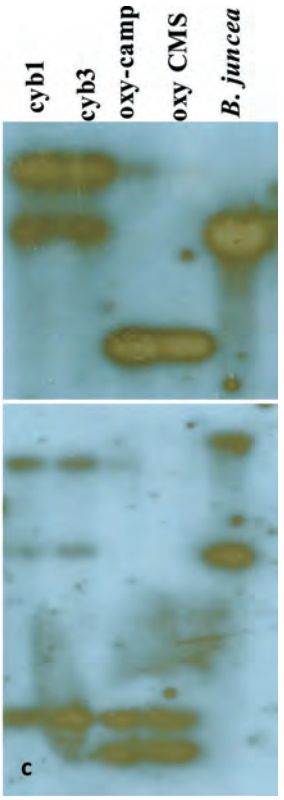


Fig. 4.2: Northern and Southern blots of oxyrrhina-based CMS lines. (a-b) Northern blots probed with *atp1* and *nad4L* gene probes,(c) Southern blot cut with *Eco*RI (upper panel) and *Bam*HI(lower panel) and probed with *atp1* gene.

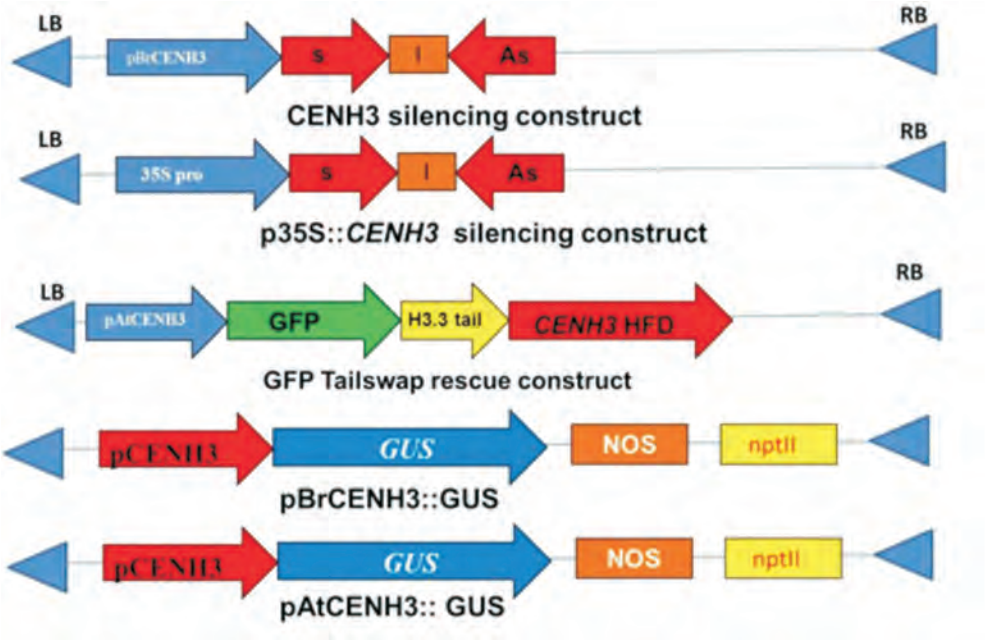


Fig. 4.3: Schematic representation of the gene constructs for *CENH3* engineering in *B. juncea*.

gene was designed which would not be targeted by the RNAi. *CENH3* promoters from *Arabidopsis thaliana* and *B. juncea* were amplified and used to develop RNAi and rescue constructs. Also, the promoter sequences were cloned upstream to the reporter gene for verification of promoter activity.

Introgression of genes for *Alternaria brassicae* tolerance from *Diplotaxis erucoides*

Our previous studies have shown that *D. erucoides* is highly resistant to *A. brassicae*. In order to introgress genes for *Alternaria* resistance into cultivated *Brassica*, interspecific hybrid (*D. erucoides* x *B. rapa*) was developed and stable amphidiploid was established. The amphidiploid is highly vigorous and fully male fertile. However, the seeds abort after about 10-15 days of anthesis and the hybrid shows <1 seed/silique. Seed set is also low when the pollen from amphidiploid is used to cross with *B. rapa* or *B. juncea*. After two generations of back crossing with *B. juncea* we have generated 161 lines which are similar to *B. juncea* and show full seed set upon selfing. These lines along with the amphidiploid (*D. erucoides* x *B. rapa*) were raised and screened for *Alternaria* tolerance under natural infection conditions in Pantnagar. Control *B. juncea* lines showed severe *Alternaria* infection (Disease score 8-9) on leaves and silique whereas the amphidiploid showed resistance (2-3 disease score). From the segregating backcross progenies, 34 plants showing resistant reaction (Score 3-4) (Fig. 4.4) were selected. These and susceptible progenies are being tested for alien gene introgression through molecular markers.

Resynthesis of *B. juncea*

Eighty five plantlets from 15 cross combinations obtained from ovary culture and embryo rescue during the previous year were hardened and transferred to net house, and true hybrids were identified on the basis of morphological and molecular analysis. Only 14 plants from seven different crosses were identified as true hybrid (Fig. 4.5). In addition, seeds obtained *in vivo* from *B. rapa* x *B. nigra* crosses were also tested for hybridity. Out of 28 crosses only 11 crosses gave true hybrids and all were sterile. Axillary buds of the hybrids were treated with colchicine to obtain amphidiploids. Only 10 plants from three crosses

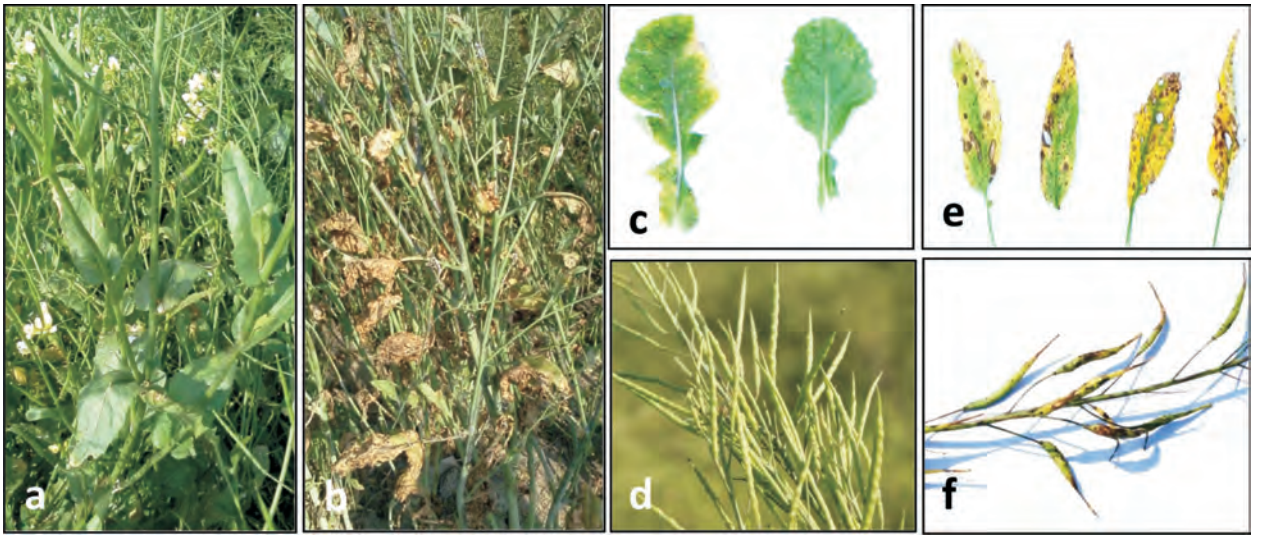


Fig. 4.4: Introgression of *Alternaria* resistance into *B. juncea*. (a) Amphidiploid (*D. erucoides* x *B. rapa*) showing resistant reaction,(b) *B. juncea* showing highly susceptible reaction, (c & d) Leaf and silique of resistant progeny, (e & f) Leaf and silique of susceptible progeny.

Fig. 4.5: Hardening of embryo rescued plants of *B. rapa* x *B. nigra* crosses and transfer to soil.

showed some fertility. Seeds were harvested from these synthetic *B. juncea* plants.

Activation tagging in *Arabidopsis thaliana* to discover new genes of agronomic importance

Activation tagged lines were developed using constitutive (pBin-35S) or green tissue specific (pBin-rbcS) promoter at the right border of the T-DNA. About 850 lines were screened in the T1 generation to identify mutants for various morphological traits. Several interesting mutants such as plants with increased number of branches/plant, high number of silique per branch, dwarf stature, sterile flowers were identified (Fig. 4.6). These lines are being investigated for stability of mutant phenotype in the T2 generation and for identifying the T-DNA insertion site for further characterization.

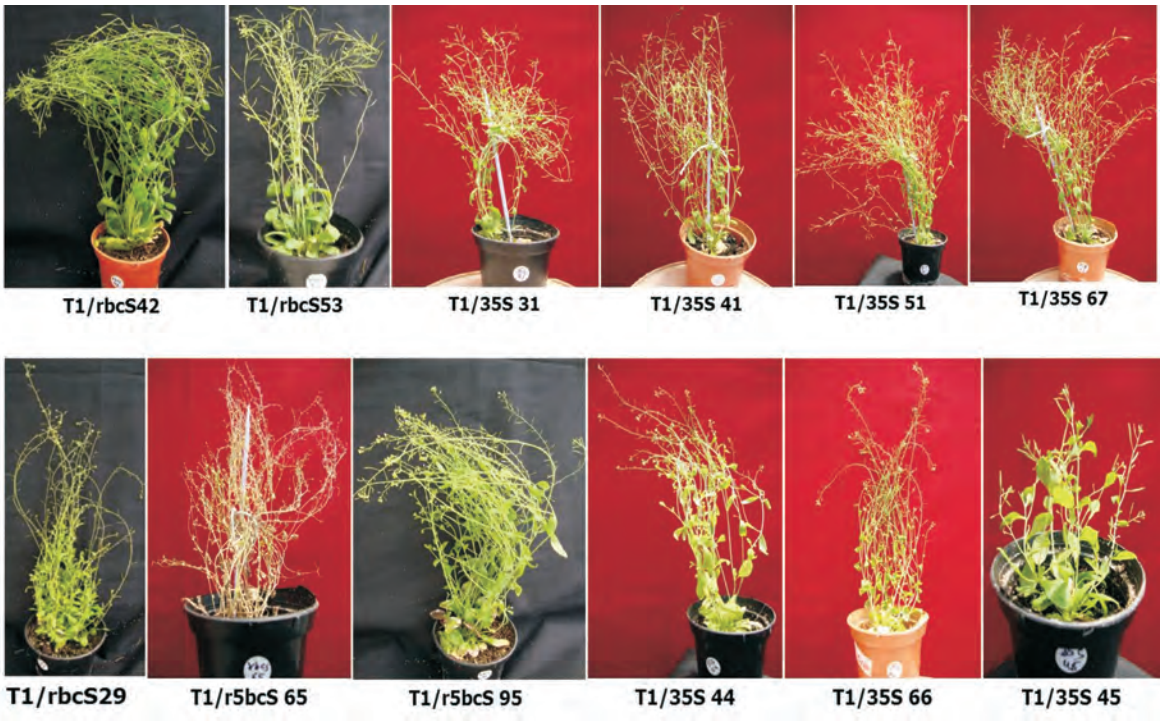


Fig. 4.6: Activation tagged mutants of *A. thaliana* identified in the T1 generation.

5. Genomics and Molecular Markers

Genome sequencing of the wheat chromosome2A under International Wheat Genome Sequencing Consortium (IWGSC)

Wheat, the most important cereal, next to rice, has a very huge genome size of 17 Gbp. Unravelling the wheat genome sequence using clone by clone approach, thus generating a pseudomolecule of high quality, is expected to greatly assist in wheat improvement. National Research Centre on Plant Biotechnology is a partner in IWGSC with the responsibility to fingerprint and sequence the short arm of wheat chromosome 2A which was estimated to be 391 Mbp long. To enable this, 2AS specific BAC library consisting of 55648 clones was received from the consortium. High Information Content Fingerprinting (HICF) of 42488 BAC clones was carried out using SNaPShot technique in DNA fragement analyzer (ABI 3700xl). These fingerprinting data were processed using bioinformatics software GeneMapper 4.1, FPB, GenoProfiler 2.1 and FPB to generate the BAC fingerprinted contigs (Fig. 5.1). The summary results obtained from the analysis of 42488 BAC clones are given in Table 5.1. With the moderate stringency parameter of 1.00E-35, MTP consisting of 4442 clones was identified from 42448 clones.

Fig. 5.1: A screen shot of FPC assembly of the BACSNaPShot.

Table 5.1: SNaPShot fingerprint assembly summary using software FPC

Chromosome size (Mb)	391
BAC library size (number of clones)	55648
Fingerprinting technique	SnaPShot HICF
No. of clone with fingerprints	42488
FPC assembly stringency	1.00E-35
No. of clones in assembly	31826
No. of clones in contigs	23207
No. of singeltons	8619
Total number of contigs	1075
No. of contigs with >5 clones	687
No. of clones in MTP	4442

First draft of the mango genome sequence

Mango (*Mangifera indica* L.) is called "king of fruits" in India due to its sweetness, richness of taste, huge variability, large production volume and variety of end usage. It is a member of the family Anacardiaceae and is an allotetraploid (2n= 40) fruit tree with small genome size of about 450 Mbp. India is the largest producer of mango in the world, with an annual production of 15.03 million tons from an area of 2.31 million hectares, contributing about 56% of the total world production. More than 1,000 varieties of mango exist in India today that contributes 39.5% of the total fruit production in the country. Despite its huge economic importance the genomic resources in mango is very limited and genetics of useful horticultural traits are poorly understood. We have generated more than 40 Gbp (~88.5X) shotgun and pair-end next generation genomic sequence data of mango genome from popular mango variety Amrapali using Roche 454 and Illumina (MiSeq, HiSeq and Mate pair) sequencing technologies. We have also generated RNA sequence data using SOLiD and MISeq sequencing technology. As first draft assembly a total of 211,141 (~492 Mbp) contigs were generated. A total of 11,448 unigene contigs were generated and 78,831 genes were predicted (Fig. 5.2) with an average gene length of 701 bps. We also mined 185,763 SSR in the assembled draft genome. Generation of additional PacBio sequencing data is in progress which will be further used for improving the mango genome assembly. The information on mango genome will provide valuable resource for mango variety improvement.

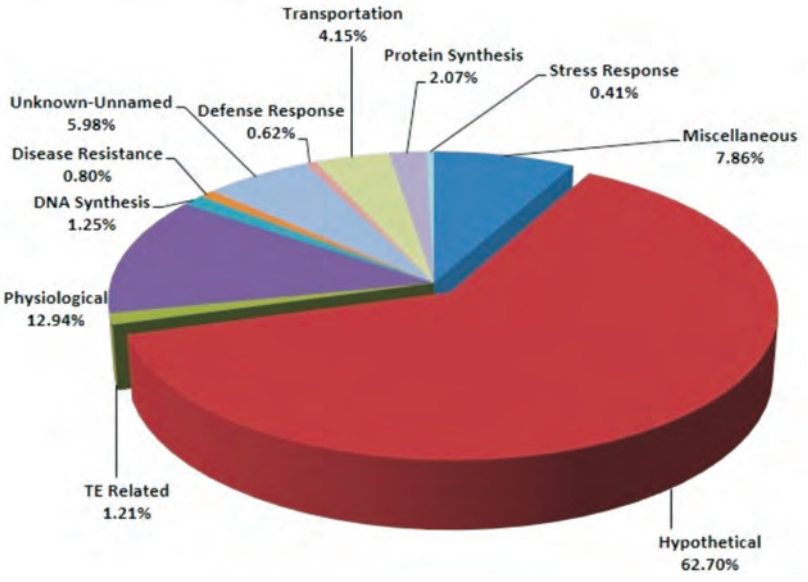


Fig. 5.2: Functional categorization of predicted genes from mango genome.

Identification of genes for bread making quality in wheat

Thirty one near isogenic lines (NILs) in the background of wheat variety HD2329 were developed to understand the contribution of individual seed storage protein alleles on the end use quality and are being maintained. BC_3 F_{12} seeds from homozygous NILs were sown for field trials in Div. of Genetics, IARI for large scale multiplication and data collection. These NILs had been evaluated over four years for different tests related to dough rheological properties and bread making quality. The seeds collected from these NILs were tested for quality parameters in the Quality Lab, Div. of Genetics, IARI. This led to the identification of NILs specific for HMW and LMW glutenins and Triticin and their effect on bread making quality. *Glu-A1a, Glu-B1b, Glu-B1b*, Glu-B1i, Glu-D1d, Glu-A3d* and *Tri-D1a* and *Tri-A1a* show consistent positive and Glu-A1c, *Glu-A3c, Gli-B1c*, Gli-A1g* and *Gli-A1h* show consistent negative effect on bread making quality when compared with HD2329. All the gliadin alleles were observed to show negative effect on end product quality while albumin alleles did not show any significant effect. Triticin alleles were found to have same effect as that of HD2329 alleles which were already known for their prominent effect on end product quality. A brief summary of average data of quality traits in selected NILs is given in Table 5.2. For mapping chapatti making quality traits in wheat, 206 RILs (C306 /WL711) and 60 DHs are being devevloped.

Table 5.2: Grain and flour quality scores of selected near-isogenic lines of wheat variety HD2329

Protein class	NIL allele	SDS-SV (ml)*	Farinograph		Bread loaf volume(cc)**	Chapati quality score**
			DDT (min)**	Stability (min)**		
	HD2329	35.3 ^{c}	4.8 ^{a}	10.5 ^{c}	550 ^{c}	60 ^{c}
HMW-glutenin subunits 80 ^{a}	Glu-A1a	38.8 ^{b}	4.3 ^{a}	13.4 ^{b}	610 ^{a}	
	Glu-A1c	32.8 ^{d}	4.6 ^{a}	8 ^{d}	520 ^{d}	65c
	Glu-B1b	40 ^{b}	5.2 ^{a}	13.3 ^{b}	570 ^{b}	79 ^{a}
	Glu-B1b*	39 ^{b}	5.3 ^{a}	13.3 ^{b}	580 ^{b}	79 ^{a}
	Glu-B1i	39.5 ^{b}	4.5 ^{a}	14.8 ^{b}	600 ^{a}	79 ^{a}
	Glu-D1d	45 ^{a}	4.5 ^{a}	16.3 ^{a}	620 ^{a}	79 ^{a}
LMW- glutenin subunits 70 ^{b}	Glu-A3c	32 ^{d}	4.3 ^{a}	7.8 ^{d}	460 ^{e}	
	Glu-A3e	38.8 ^{b}	4.3 ^{a}	12.5 ^{b}	540 ^{d}	60 ^{c}
	Glu-A3d-1	37 ^{b}	4.7 ^{a}	14.8 ^{b}	570 ^{b}	70 ^{b}
	Glu-A3d-2	46 ^{a}	5.8 ^{a}	12.8 ^{b}	600 ^{a}	70 ^{b}
	Glu-A3a	35.5 ^{b}	4.4 ^{a}	10.4 ^{c}	540 ^{d}	65 ^{c}
	Glu-D5a	39 ^{b}	3.8 ^{b}	12.1 ^{b}	580 ^{b}	65 ^{c}
	Glu-B3	37 ^{b}	5.5 ^{a}	12.2 ^{b}	500 ^{e}	69 ^{b}
Triticin	Tri-D1a	46 ^{a}	4.5 ^{a}	17.3 ^{a}	630 ^{a}	79 ^{a}
	Tri-A1a	44 ^{a}	4.8 ^{a}	15.5 ^{a}	610 ^{a}	79 ^{a}

Identification and fine mapping of QTLs for salt tolerance in rice

To map QTLs for salt tolerance, polymorphism was surveyed between a salt tolerant rice variety CSR11 and a salt sensitive variety MI48, the two parents of a RIL population comprising of 206 lines. Out of 750 SSR markers surveyed 80 were polymorphic. In the current study, we followed bulk segregant analysis (BSA) which is based on marker analysis of pooled genomic DNA samples from extereme bulks to identify association between marker and trait phenotype and thereby designate a probable location for the QTL. Phenotyping for salt tolerance was done in controlled microplots under moderate (pH 9.5) and high sodicity (pH 9.9) conditions at CSSRI Karnal (Fig. 5.3a & 5.3b). Ten extreme tolerant and ten extreme sensitive RILs were identified on the basis of their stress susceptibility index for grain yield from three years phenotyping data (Fig. 5.4). DNA was extracted from each of the extreme RILs and combined in equal quantities to prepare extreme tolerant and sensitive bulks and analyzed along with the parents using all the polymorphic markers. By this approach, we have identified eight markers located at 5 regions on chromosomes 1, 2, 8, 9 and 10 showing tight linkage with the trait. Two of these locations match with QTLs previously mapped on chromosomes 1 and 8. Genetic map of the three novel QTLs on chromosomes 2, 9 and 10 has been prepared to identify their precise location and effect.

Earlier we have mapped a consistent quantitative trait locus (QTL) for salt sensitivity index (SSI) for spikelet fertility at high salt concentration (*qSSISFH-8.1*) on rice chromosome 8 in marker interval HvSSR08-25 -RM3395 with a LOD score of 4.17 and explaining 8.0% of the phenotypic variance in CSR27 X MI48 derived RILs. The two markers flanking the QTL *qSSISFH-8.1* lie in 12.3 cM distance. To narrow down the map interval and to identify the salt tolerance genes, fine mapping of QTL qSSIFH-8.1 was undertaken. A cross was made between two RILs, with similar background but contrasting QTL markers. Out of 4285 F_2 plants 68 homozygous and 486 heterozygous recombinant lines were identified. Out of 486 heterozygous recombinants 160 homozygous recombinants were identified in F_3 generation using high throughput genotyping approach in ABI 3570 fragment analyzer. To saturate the QTL region 18 primers (1 KB size) were amplified in all homozygous recombinants (Fig. 5.5a). Sequencing of these barcoded amplicons was done using Ion Torrent (PGM) (Fig. 5.5b). For finding the breakpoints in recombinants, phenotyping for salt stress traits will be done in next season at CSSRI, Karnal.

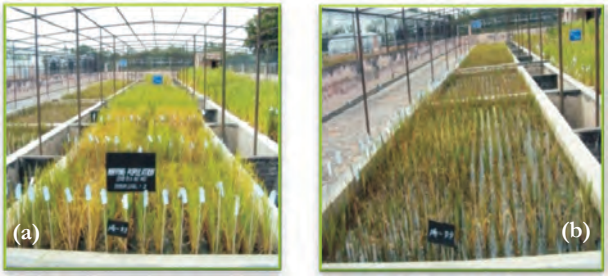


Fig. 5.3: Salt tolerance in rice (a) Moderate stress; (b) High stress.

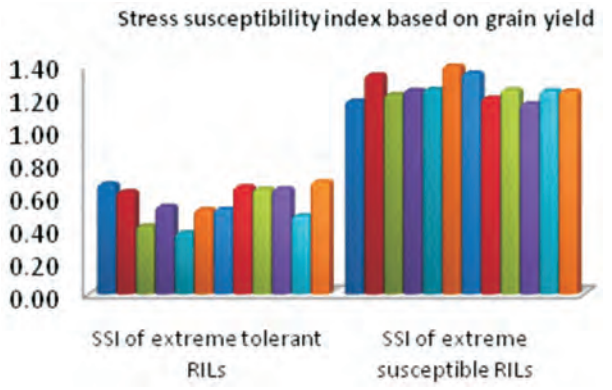


Fig. 5.4: Bar diagram showing SSI of extreme tolerant and susceptible RILs of rice.

Collection and characterization of wild rice germplasm

Expeditions were made to eight states namely, Uttar Pradesh, Uttarakhand, Bihar, Himachal Pradesh, Assam, Gujrat, Goa and Chhattisgarh in the reporting year, taking the tally of wild rice accessions to 367,

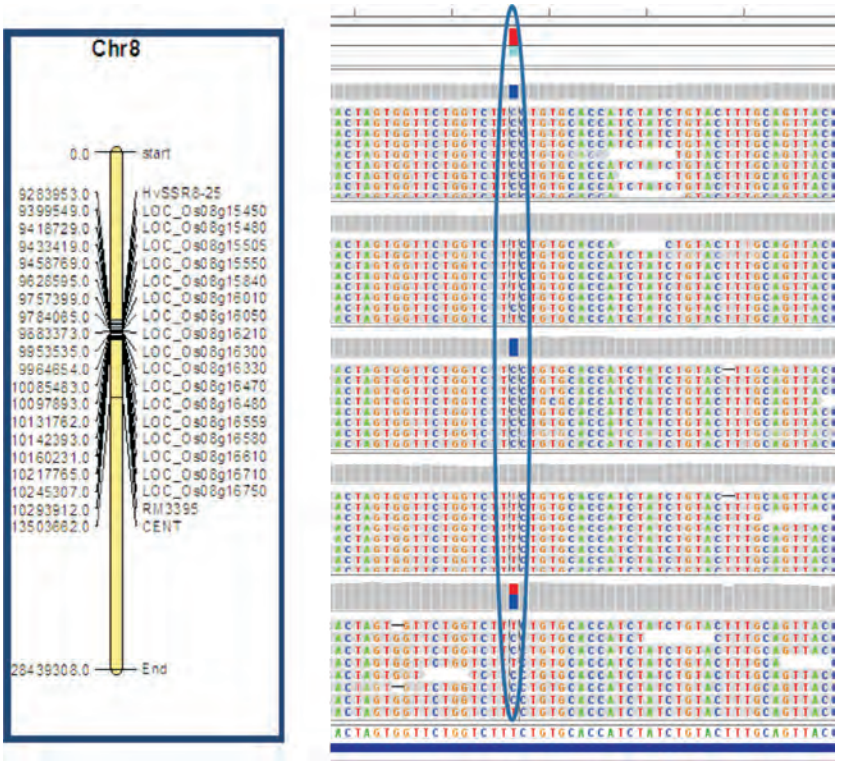


Fig. 5.5: QTL for salt tolerance in rice. (a) Genes targeted for amplification in QTL region of Chr8; (b) Pattern of SNPs present in homozygous and heterozygous recombinants.

from the 248 collected till the previous year (Fig. 5.6). Along with the seed samples, complete geographical location information, passport data and photographs of the samples and habitat were recorded for these accessions. A web portal database has been created (nksingh.nationalprof.in) for the entire 426 accessions of wild rice, of which 367 accessions were collections made under this project and the rest 58 accessions were obtained from NBPGR, New Delhi. These accessions were evaluated for 46 phenotypic traits and characterized with 26 *p-SINE* markers. Based on *pSINE1* markers, the accessions could be classified into annual, perennial and intermediate type. For further characterization, a set of 48-plex Illumina GoldenGate genome-wide SNP assay was developed and 186 wild rice accessions were genotyped. Based on the SNP assay the wild rice genotypes were categorized into two subpopulations using STRUCTURE analysis (Fig. 5.7).

One of the objectives of the project is to screen the wild rice accessions for tolerance to drought, submergence and salinity. For submergence tolerance, a total of 193 accessions were screened and based on elongation during flooding 125 were found to be elongating type while the rest 68 were non-elongating type. For salt stress tolerance, 160 wild rice accessions were screened in hydroponics with 150mM NaCl and four highly tolerant, 16 moderately tolerant and 140 sensitive accessions were identified. Allele mining efforts for HKT2;4 and HKT2;1 transporters in these accessions identified 65 haplotypes.

Marker-assisted breeding of abiotic stress tolerant rice varieties with major QTLs for drought, submergence and salt tolerance

The overall objective of the project 'QTL to variety' funded by DBT is to develop improved

Fig. 5.6: India map showing collection sites of wild rice accessions.

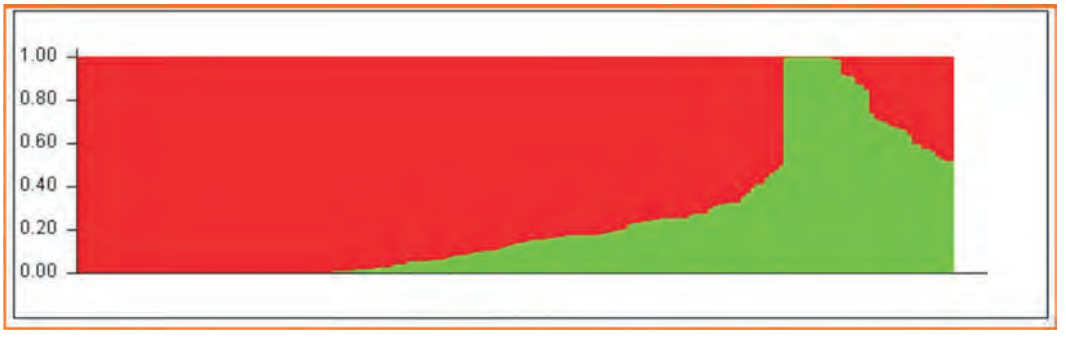


Fig. 5.7: Population stratification of 186 Indian wild rice accessions as revealed through STRUCTURE based on genome wide 48-plex SNP genotyping assay.

varieties of rice tolerant to different abiotic stresses with marker-assisted backcross breeding approach (MABC), evaluate advance breeding lines and find new sources of flood and salt tolerance. NRCPB coordinates the activities among fifteen participating institutions from India and IRRI for transfer of major QTLs for drought, submergence and salinity tolerance. The status report of the transfer of different QTLs for abiotic streses is given in the Table 5.3.

Table 5.3: Trait wise status report of the progress in introgression of QTLs into popular Indian varieties

A. Drought tolerance

S. No.	Recipient Variety	QTL	Donor Line	Original Source	Status *March 2013*	Recombinant selection	Institution
1	IR 64-Sub 1	***DTY1.1***	IR 86918-B-305	Nagina 22	BC3 F1 seeds	Fixed both sides	CRURRS, Hazaribagh
		DTY2.2	IR 87728-367-B-B IR 87728-75-B-B	Aady sel	BC3F1 seeds	Fixed both sides	
		DTY3.1	IR 81896-B-B-142	Apo	BC3F1 seeds	Fixed one side	BAU, Ranchi
		DTY9.1	IR 87728-59-B-B	Aady sel	BC3F1 seeds	Not successful	
2	Swarna- Sub 1	***DTY1.1***	IR 81896-B-B-195 IR 81896-B-B-142	Nagina 22	BC1F3 lines		IGKV, Raipur
		DTY12.1	IR 87728-75-B-B IR 87728-367-B-B	WayRarem	F1 seed		
		DTY2.1	IR 81896-B-B-195 IR 81896-B-B-142	Apo	BC3 F2	Fixed both sides	CRRI, Cuttack
		DTY3.1	IR 81896-B-B-195 IR 81896-B-B-142	Apo	BC3 F2	Fixed both sides	
3	Sambha Mahsuri-Sub1	***DTY1.1***	IR 86918-B-305	Nagina 22	BC1F1 seeds	Not done	CAU, Umiam
		DTY2.2	IR 87728-367-B-B	Aady sel	BC2F1 seeds	Not done	
		DTY2.1	IR 88286-830-1	Apo	BC3 F1 seeds	Not done	DRR, Hyderabad
		DTY3.1	IR 81896-B-B-195	Apo	BC23F1 seeds	Not done	

Introgression of *DTY3.2* in Swarna-Sub1 and Sambha Mahsuri-Sub1 Initiated

B. Submergence tolerance

S. No.	Recipient Varieties	QTL	Donor Line	Original Source	Status *Kharif* 2011	Recombinant selection	Institution
4	Pooja	***SUB 1***	IR 88286-830-1	FR13 A	BC2F1 seeds	Fixed one side	CRRI, Cuttak
5	Pratikshya	***SUB 1***	IR 88286-830-1	FR13 A	BC2F1 seeds	Fixed both sides	
6	Sarjoo 52	***SUB 1***	Swarna -Sub1	FR13 A	BC2F1	Fixed one side	BHU, Varanasi
7	HUR 105	***SUB 1***	IR64 -Sub1	FR13 A	BC2F1	Fixed both side	
8	Rajendra Mahsuri	***SUB 1***	Swarna Sub1	FR13 A	BC2F1 seeds	Fixed on one side	RAU, Samastipur
9	ADT 39	***SUB 1***	CR1009 -Sub1	FR13 A	BC2F1 seeds	Not fixed	AU, Annamalai Nagar
10	Ranjit	***SUB 1***	Swarna -Sub1	FR13 A	BC2F1	Fixed one side	AAU, Assam
11	Bahadur	***SUB 1***	Swarna -Sub1	FR13 A	BC2F1	Fixed one side	AAU, Assam
12	MTU 1075	***SUB 1***	Swarna -Sub1	FR13 A	BC3F1	Fixed both sides	ANGRAU, Maruteru
13	ADT 46	***SUB 1***	Swarna Sub1	FR13 A	BC2F1	Fixed both sides	PAJANCOA, Kararikal

C. Salinity tolerance

S. No.	Recipient Varieties	QTL	Donor parent	Original Source	Status *Kharif* 2011	Recombinant selection	Institution
14	Sarjoo 52	**SALTOL**	FLA478	Pokkali	BC2F1	Not done	CSSRI, Karnal
15	Pusa 44	**SALTOL**	FLA478	Pokkali	BC2F1	Not done	
16	PR 114	**SALTOL**	FLA478	Pokkali	BC2F1	Not done	
17	Gayatri	**SALTOL**	FLA478	Pokkali	BC2F1 seeds	Fixed both sides	CRRI, Cuttak
18	Savitri	**SALTOL**	FLA478	Pokkali	BC2F1 seeds	Not fixed	AU, Anna Nagar
19	MTU 1010	**SALTOL**	FLA478	Pokkali	BC2F2	Fixed one side	ANGRAU, Mauteru
20	ADT 45	**SALTOL**	FLA478	Pokkali	BC2F1	Fixed one side	PAJNCOA, Karaikal

Allele mining in *Sub1* locus of rice

A set of 215 rice accession collection from different countries were evaluated for finding additional sources of submergence tolerance. *Sub1* locus consisting of all the three genes, viz. *Sub1A*, *Sub1B* and *Sub1C*, were amplified in the accessions and sequenced on Ion-Torrent PGM platform (Fig. 5.8). A total of 8, 9 and 68 haplotypes were identified in *Sub1A*, *B* and *C* locus respectively. The association analysis is in progress using the phenotype data received from partner institutes involved in phenotyping.

Development of a new high density SNP genotyping assay in rice

A new 50K SNP assay, comprising of 51933 SNPs identified mostly from single copy genes of rice and some more from agronomically important cloned genes was designed and used for genotyping using Affymetrix GeneTitan Multi Channel platform. This would serve as great resource for both coarse and fine mapping biparental association mapping studies, to assess recipient genome recovery in back cross derivarives and to select near isogenic RILs for fine mapping stuides. Using this assay, the percent recovery of background genome in Sambha Mahasuri *Sub1* was studied and it was observed that on an average only 80% of the background genome was recovered ranging from of 79 to 86% as against the popular belief of 95% and above genome recovery (estimated using SSR markers) (Fig. 5.9).

Analysis of coexpression network under abiotic stress tolerance in rice

Co-expression network analysis for drought and heat tolerance in rice was performed using WGCNA package in R, using the transcriptome data generated in the laboratory and publically available microarray data, to divide the stress responsive genes into tightly co-expressed modules thus revealing organization of stress transcriptome. The blockwise Modules function of WGCNA package in R was used to generate the modules with powers 8 and 6 for drought and heat tolerance, respectively, which gave the best approximate scale-free topology (model fit >0.8) of the resultant network (Fig. 5.10). The long length of

VARIETY	(T) 4341	(C) 4471	(G) 4621	(G) 4627	(G) 4870	(T) 5070	(A) 5192
SINGHARA	C	T	G	C	C	C	G
KARIYWA	C	T	G	C	C	C	G
SUGAPANKH	C	T	G	C	C	C	G
MOROBEREKAN	C	T	G	C	G	C	G
MADHUKAR	C	T	G	C	G	C	G
ARC 614	C	T	G	C	G	C	G
JAYMIS	C	T	G	C	G	C	G
KALUWEE	C	T	G	C	G	C	G
JALDUNGI	C	T	G	C	G	C	G
VADAI	C	T	G	C	G	C	G
CR 1009	C	T	G	C	G	C	G
KAOLACK	C	T	G	C	G	C	G
IR 64	C	T	G	C	G	C	G
BARA SALI	C	T	G	C	G	C	G
IR 36	C	T	G	C	G	C	G

Fig. 5.8: *Sub1A* sequence in a few accessions of rice.

CHR NO.	1	2	3	4	5	6	7	8	9	10	11	12
% recipient	80.7508	76.82545	82.16922	78.38983	79.79986	68.37514	72.02228	84.71683	78.44968	86.29283	79.2009	83.3996
	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1	Sambha Mah Sub1
	TT	CC	CC	AA	CC	AA	TT	GG	TT	GG	TT	AA
	CC	TT	CC	GG	AA	AA	AA	TT	TT	GG	CC	GG
	CC	GG	AA	GG	GG	GG	TT	CC	AA	GG	TT	AG
	GG	AA	AA	AA	AA	AA	CC	GG	CC	GG	CC	TC
	GG	CC	GG	GG	TT	GG	CC	AA	GG	TT	GG	AG
	TT	CC	TT	CC	TT	CC	GG	GG	TT	CC	TT	---
	GG	TT	AC	AA	TT	CC	AA	CC	TT	AA	CC	CG
	CC	AA	TT	CC	GG	CC	CC	AA	GG	CC	CC	AG
	CC	GG	GG	CC	AA	GG	CC	AA	CC	GG	GG	TG
	TT	AA	GG	TT	GG	AA	AA	AA	CC	TT	GG	CG
	CC	TT	AA	GG	TT	AA	GG	GG	AA	CC	TG	CC
	GG	GG	AA	CC	GG	GG	GG	CC	GG	AA	GG	AA
	GG	AA	GG	CC	GG	CC	AA	CC	GG	CC	CC	AA
	AA	CC	GG	AA	AA	CC	TT	AA	GG	AA	GG	CC
	GG	AA	GG	CC	TT	CC	CC	GG	CC	GG	CC	CC
	GG	CC	GG	TT	CC	CC	AA	TC	AA	AA	GG	CC
	GG	GG	AG	CC	CC	GG	TT	CC	TT	GG	AA	GG
	CC	GG	CC	GG	AA	CC	CC	CC	GG	TT	CC	AA
	GG	TT	TT	TT	TT	AT	TT	GG	CC	GG	GG	AA
	AG	GG	AA	CC	GG	TT	CC	CC	CC	CC	CC	CC
	CC	TT	CC	AA	CC	GG	GG	TT	GG	GG	AA	CC
	AA	TT	GG	AA	GG	---	GG	AA	TT	CC	GG	GG
	TT	CC	CC	AA	CC	CC	CC	TT	GG	TT	AT	GG
	GG	AA	GG	GG	TT	GG	GG	CG	CC	AA	GG	CC
	AA	GG	GG	GG	CC	AA	CC	AA	GG	CC	GG	CC
	TT	GG	CC	CC	AA	GG	CC	GG	GG	AA	GG	AA

Fig. 5.9: Percent background recovery in Sambha Mahsuri *Sub1.*

the dendrogram branches and corresponding intensity of colour in the heat maps of co-expression modules illustrate high co-expression of stress regulatory genes (SRGs) within modules and less co-expression outside the module.

The DEGs common to drought and heat stress were analyzed to find consensus modules showing co-expression patterns across stresses using the function blockwise Consensus Modules with the following settings: powers 7 and 10, minimum module

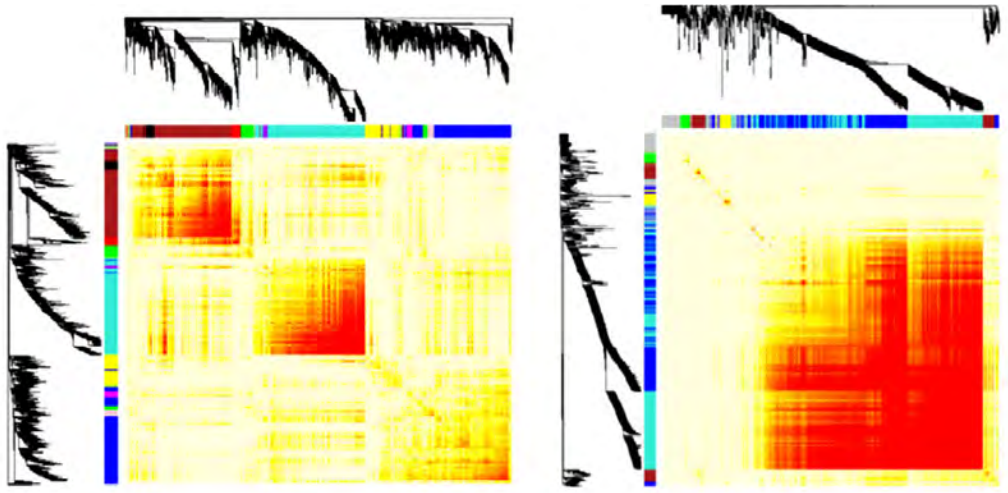


Fig. 5.10: The differentially expressed genes (DEGs) in rice were clustered based on co-expression patterns as represented by the dendrogram and correlation heat map. Intensity of red colour in the heat map indicates strength of correlation between pairs of genes on a linear scale, (A) Drought and (B) Heat.

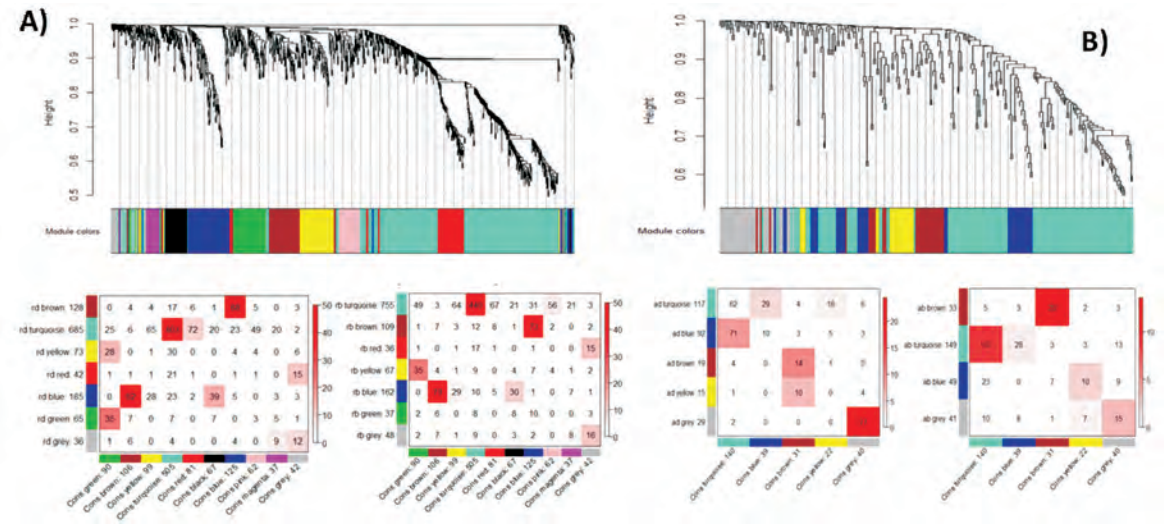


Fig. 5.11: Clustering dendrogram of genes and consensus modules. The correspondence between consensus modules and modules found individually in drought and heat stress in rice based on the expression values of the common genes are also shown as a table. Each row of the table corresponds to individual stress specific module (labeled by color as well as text along with the number of genes in the module), and each column corresponds to one consensus module. Numbers in the table indicate gene counts in the intersection of the corresponding modules. Coloring of the table encodes -log(p), with p being the Fisher's exact test p-value for the overlap of the two modules. The stronger red color indicates more significant overlap.

size 30 and 15 for rice drought and heat, respectively, with the merge cut height set at 0.15 (Fig. 5.11). Further analysis was carried to identify the consensus co-expression modules by constructing a network based on co-expressed genes with high absolute Pearson correlation coefficient (r >0.8) in both drought and heat stresses (Fig. 5.12). One of the top edges was between LOC_Os02g43790, an ethylene responsive TF and LOC_Os02g41510, a MYB TF with r >0.98 in both stresses. Colour coding of nodes

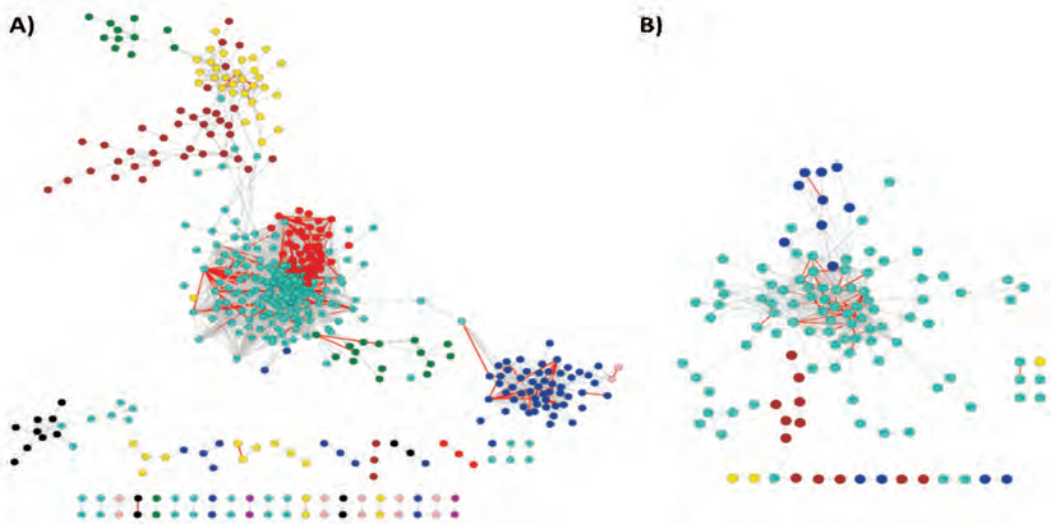


Fig. 5.12: Network based analysis of (A) co-expressed modules in drought stress, and (B) co-expressed modules in heat stress in rice. The dominant modules under drought stress are Turquoise and Blue, dominated by photosynthetic and water response genes, respectively. In heat stress, the dominating module is Turquoise dominating *HSP* and *HSF* genes.

Table 5.3: Number of Up-regulated and Down-regulated genes in rice

Sample Name	Up regulated	Significantly Up-regulated	Down regulated	Significantly Down-regulated	Total number of significant genes showing differential expression
K198 Control Vs. K198 Salt Stress	1,313	122	315	10	132
K478 Control Vs. K478 Salt Stress	690	52	487	23	75

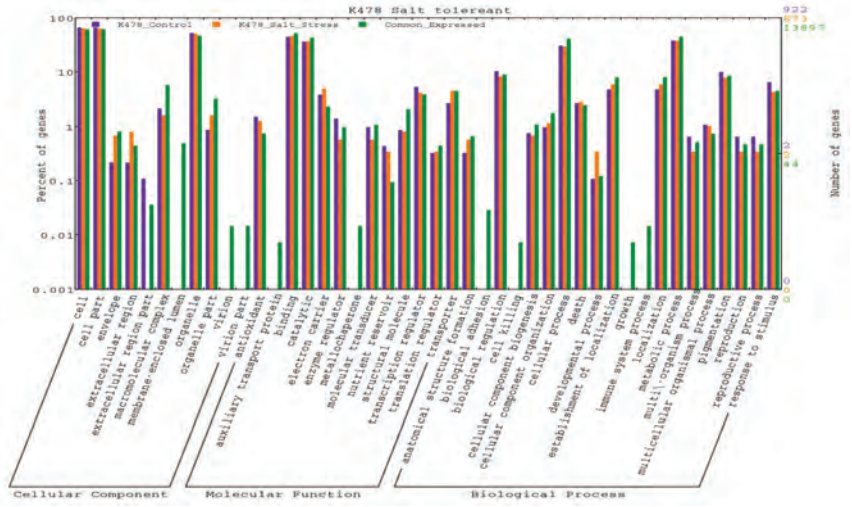


Fig. 5.13: Functional categorization of differentially expressed genes under salinity stress in the salt tolerant rice introgression line K478.

in network with their consensus module colour showed clear grouping of genes from the same module with high number of intra-modular edges.

Expression analysis of salt tolerance using introgression lines in rice

A total of 60,870,091 and 51,316,836 reads were generated from SOLID sequencing of salt tolerant introgression line (K478) under control and salt stressed (150 mM NaCl) condition. Similarly 64,815,738 and 57,775,129 reads were generated from salt sensitive introgression line (K198) under control and salt stressed condition. Reads were aligned against *O. sativa* reference genome using Tophat (v.2.0.7) and Bowtie (v.0.12.9). The details of differentially expressed genes identified in both the samples are given in Table 5.3. A total 7,537 transcripts in control and 8,596 transcripts in salt stressed conditions in K478 were identified as novel transcripts. Similarly, a total of 18,685 junctions in control sample and 18,446 junctions in salt sample were identified as novel splice junctions from K478. GO analysis and GO enrichment was performed in both the samples using the Singular Enrichment Analysis (SEA) of agriGO (Fig. 5.13). The KEGG Orthology database was used for pathway mapping.

Characterization of EMS induced drought tolerant mutant in rice

The screening of EMS induced mutants under soil moisture-deficit stress in pots, drought structure and PVC tubes identified a mutant N22-D-131

showing enhanced tolerance to stress than Nagina22. It showed less percentage of leaf rolling, leaf drying and higher recovery rate after six days of stress at seedling stress (Fig. 5.14). This mutant has shorter roots and shorter grain size as compared to its parent Nagina22 but found to be genetically similar to the wild type as revealed by SSR genotyping. The transcriptome of this mutant incomparison with the wildtype has already been reported. To study the genetic inheritance of the mutant, it was crossed with its wild type Nagina22 and F_2 segregating population consisting of 100 plants was developed. F_2 individuals were grown in pots last year along with their parents. Irrigation was withheld at late vegetative stage for six days and phenotyped for leaf rolling, leaf drying and drought recovery for individual F_2s (Fig. 5.15) apart from plant stature, root length and grain size. The population segregated in 3:1 ratio (Nagina22 type: Mutant type) for all the mutant specific traits. This result suggested that the drought tolerance of the identified mutant along with other muatnt specific traits were governed by single recessive gene. Efforts are underway to map and clone the candidate gene responsible for mutant type.

Characterization of unknown candidate genes for drought tolerance in rice

In the network project on "Phenomics of moisture deficit and low temperature tolerance in rice" initiated in 2011-12 with funding from National fund, ICAR, eight target genes were identified for functional characterization and 24 gene constructs were made in pCXUN, pCAMBIA and RNAi vectors in the previous year. Seven out of the nine plants, reported last year to be PCR positive for transformation of Pusa Sugandh 2 with Os08g0412800 under ubiquitin promoter, flowered and set seeds in National Phtotron Facility. The seeds have been sown this year in Phytotron to raise T_1 generation. Seeds of 17 T_0 plants

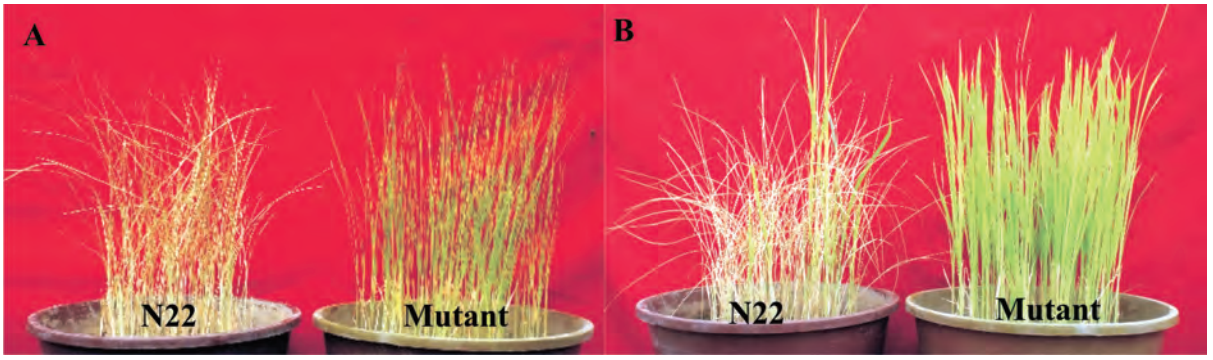


Fig. 5.14A: Rice plants under stress of six days; B: Plants after recovery.

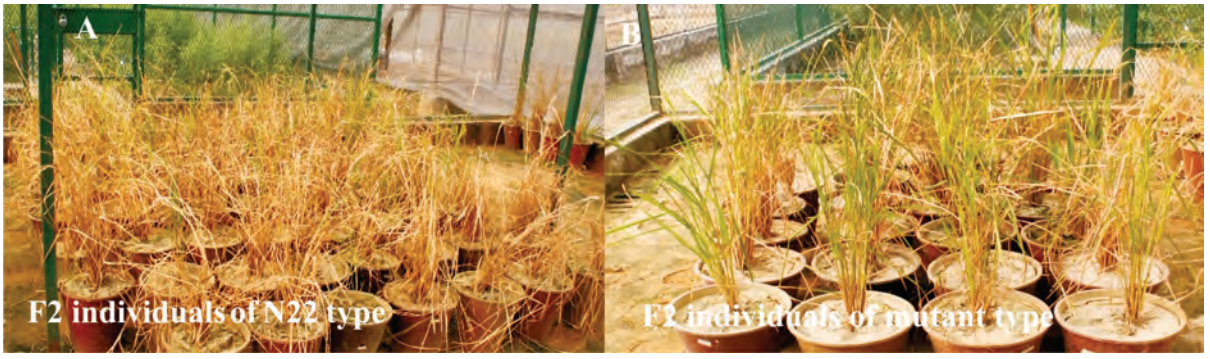


Fig. 5.15A and B: Screening of F_2 segregating rice plants under drought stress.

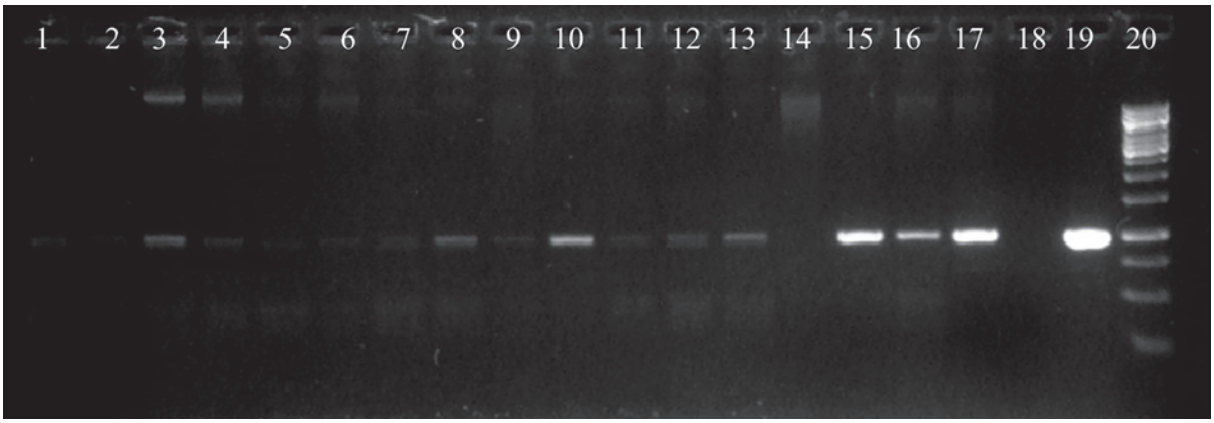


Fig. 5.16: PCR confirmation of rice T_0 transgenic plants by *hpt II* primer (lane 1–4, pCXUN412800-PS II; Lane 5-11, pCXUN670900-N22; lane 12-13, pCAMBIA 690500-N22; lane14, pCXUN 690500-N22; Lane 15, pCXUN17300–N22; lane 16, pCXUN54640-N22; lane 17, pCXUN 21060–N22; lane 18, Negative control; lane 19, Positive control; lane 20, 1Kb DNA Ladder).

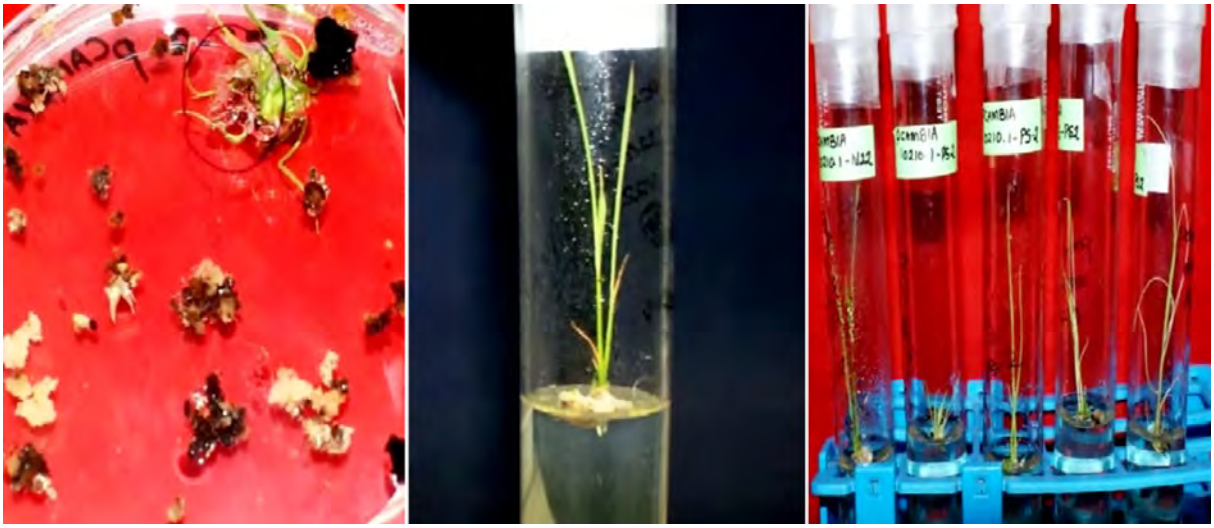


Fig. 5.17: Shoot induction of T_0 callus of Pusa Sugandh2 and N22 genotypes transformed with pCAMBIA Os06g10210.1 and Os06g 10210.3.

were obtained from 36 transgenic plants (Table 5.4). PCR with *hptII* primer was done to identify plants positive for transformation (Fig. 5.16). *Agrobacterium*-mediated transformation of pCAMBIA constructs has been initiated in different genotypes (IR64, Nagina22 and Pusa Sugandh2) and they are in different stages of transformation (Fig. 5.17). A total of forty seven insertion mutant lines pertaining to candidate genes for drought tolerance have been procured from Crop Biotech Institute, Kyung Hee University, Korea.

Out of the eight candidate genes being studied, five code for proteins that have not yet been characterized. To initiate characterization, the positive clones of pGEMT plasmids harbouring these five genes were selected and digested with restriction enzyme (*Kpn*1 and *BamH*1) which were further sub-cloned in pET43.1 expression vector. A positive clone of Os10g0546400 was transformed into BL21 (DE3) cells for protein expression and characterization. The cloned gene was induced by 0.5mM IPTG and

Table 5.4: Details of T_0 transgenic rice plants that flowered in phytotron

S. No.	Gene Constructs	Genotype background	Number of T_0 Transgenic Plants that set seeds
1.	pCXUN Os10g0546400	Nagina22	1
2.	pCXUN Os11g0670900	Nagina22	6
3.	pCXUN Os08g0412800	Pusa Sugandh 2	4
4.	PCXUN Os04g0690500	Nagina22	1
5.	pCXUN Os10g0173000	Nagina22	2
6.	pCXUN Os03g210600	Nagina22	1
7.	pCAMBIA Os04g0690500	Nagina22	2

confirmed by using 10% SDS-PAGE for protein expression.

Functional characterization of serine hydroxy methyltransferase (*SHMT*) gene from salt tolerant rice accession

Salt is one of the most important environmental constraints that limit plant growth and agricultural productivity. Identification and characterization of salt responsive genes is the first step in understanding the responses to salinity. Serine hydroxymethyltransferase (SHMT), catalyzes the transfer of the hydroxymethyl group of serine to tetrahydrofolate. All the five *SHMT* genes present in rice genome were profiled in salt-tolerant (CSR27) and salt-sensitive (MI48) *indica* rice varieties grown under control as well as 150mM NaCl at different time intervals (3h, 6h, 12h, and 24h). Maximum expression was observed at 24hrs (Fig. 5.18) for *SHMT3* under salinity. *SHMT* expression profiling in other rice varieities revealed that CSR27 root tissues had maximum expression (Fig. 5.19). The full length *SHMT3* cDNA was amplified from 150mM NaCl stressed roots of CSR27, cloned in pET29a expression vector and expressed in *E. coli* cells. The recombinant protein was purified with Ni-NTA affinity chromatography and 57 KDa purified protein was used for catalytic reaction and antibody production (Fig. 5.20). The transformed clones could grow well even in 200mM NaCl supplemented media confirming the role of *SHMT3* in providing salt tolerance (5.21). For functional characterization, *Arabidopsis thaliana* was genetically transformed with *SHMT3* cloned in plant transformation vector.

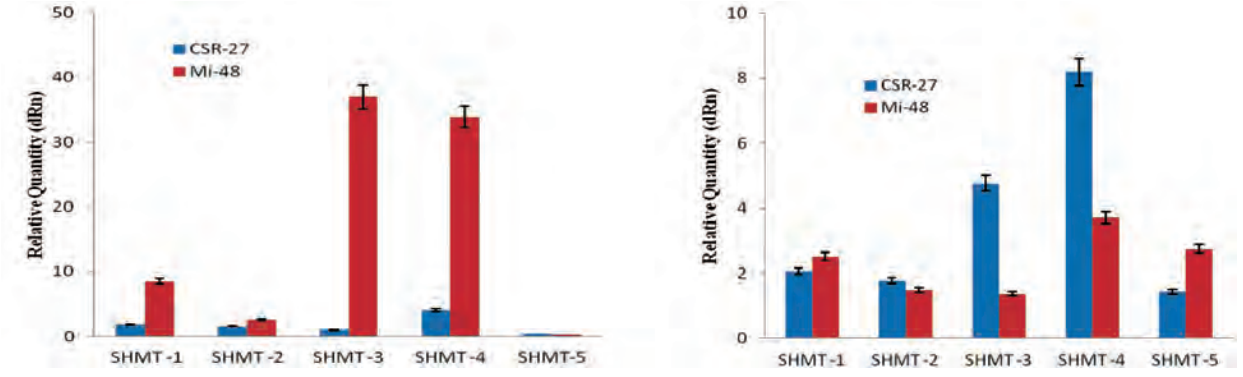


Fig. 5.18: Relative expression of SHMT genes at control and after 24hrs of NaCl (150 mM) stress in salt tolerant CSR 27 and salt sensitive MI 48 rice geneotypes.

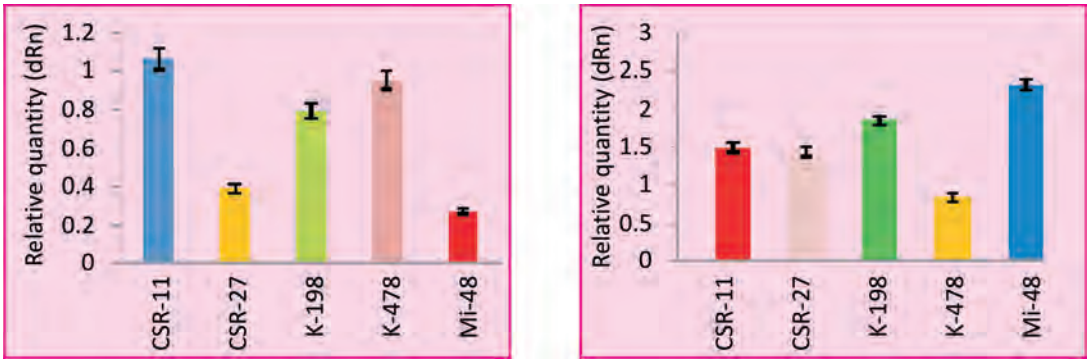


Fig. 5.19: Expression of *SHMT-3* in different rice varieties in control and after 24 hrs NaCl (150 mM) stress.

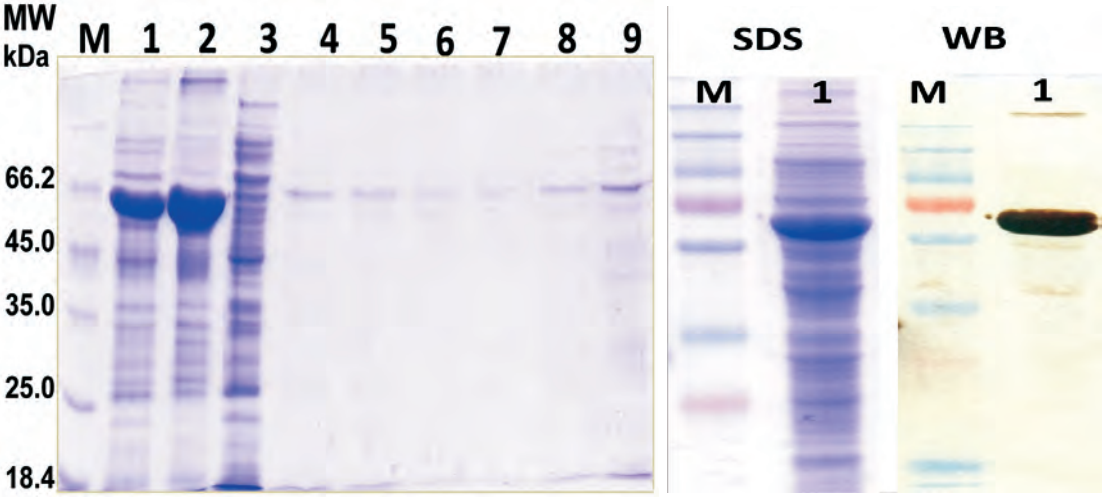


Fig. 5.20: Purification of rice SHMT with Ni-NTA Affinity Chromatography. SDS-PAGE analysis of pET29a-SHMT Purification with Ni-NTA Affinity Chromatography Protein expression of SHMT3 using anti-6-His (6X-His tag) secondary antibody; Lane M: Protein ladder; Lane 1: Total protein of induced sample; Lane2: sonicated pellet; lane 3: sonicated supernatant; Lane 4: Flow through; Lane 5-9: Elution 1-5.

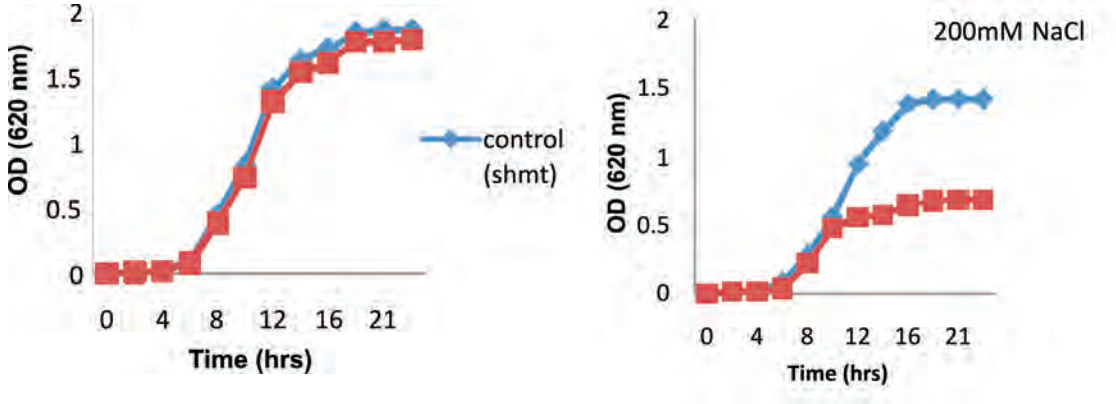


Fig. 5.21: Growth of SHMT3 *E. coli* (BL21) in control and 200 mM NaCl.

Mining of *Avr-Pita* alleles *Magnaporthe oryzae* populations

Rice blast is one of the important diseases of rice caused by the fungus *Magnaporthe oryzae*. The fungus is highly variable in nature leading to breakdown of resistance in many rice varieties. To determine the nucleotide diversity at *Avr-Pita* locus in Indian isolates of *M. oryzae*, 1082 bp long fragments of *Avr-Pita* gene were amplified and sequenced from 80 isolates. The obtained nucleotide sequences of *Avr-Pita* alleles were deposited in the EMBL database. A total of 82 polymorphic sites were identified in the *Avr-Pita* alleles of the isolates collected from the different geographical regions of India. Based on observed mutational changes, a haplotype network depicting inter- and intra-linkage relationships was constructed to identify genealogical relationship among different *Avr-Pita* alleles (Fig. 5.22). Fifty-four haplotypes including the original *Pita* allele were identified among 80 *Avr-Pita* alleles. In the network, 54 haplotypes were clustered in three major haplogroups (contained five or more *Avr-Pita* alleles) and 51 minor haplogroups. The maximum haplotype diversity was observed among the *Pita* avirulent isolates compared to the virulent ones.

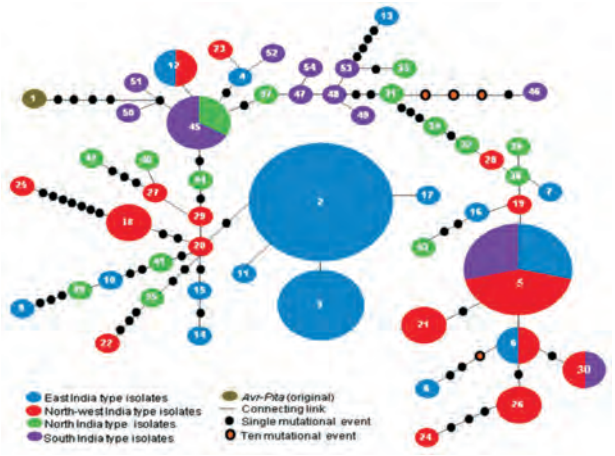


Fig. 5.22: Minimum spanning network of 54 haplotypes of *Avr-Pita* alleles from 80 isolates of *M. oryzae* collected from geographically different regions. Area of circles is proportional to haplotype frequency in the dataset. Geographical region of each haplotype is colour coded. Steps among haplotypes are shown with lines. Small black circles represent missing haplotypes in the populations. Haplotype number 1-54 represents H1 to H54.

Differential gene expression during compatible interactions in *Magnaporthe* - rice interaction

Rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases causing extensive yield loss throughout the world. The present study deals with identification of stress responsive genes in susceptible rice cultivar HR 12 after challenge with *M. oryzae*. Transcript profiling using the Affymetrix 57 K GeneChip revealed a total of 72 differentially expressed genes whose expression level was significantly altered under diseased condition (Fig. 5.23). Molecular function enrichment analysis suggested that the differentially regulated genes were mainly related to protein degradation and modification, cell signalling and stress-related mechanisms (Fig. 5.24). This study reveals that majority of the genes of protein degradation and modification, transport, signalling and transcription factors are repressed at the initial stages of biotrophic interaction. The host genes that were induced in response to pathogen infection included hormonal

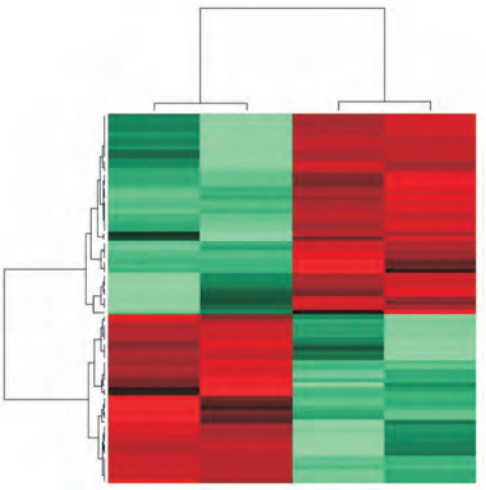


Fig. 5.23: Heatmap of differentially expressed genes generated by using heatmap function of R package. Hierchical Clustering was done using Pearson and Spearman correlation as distance method.

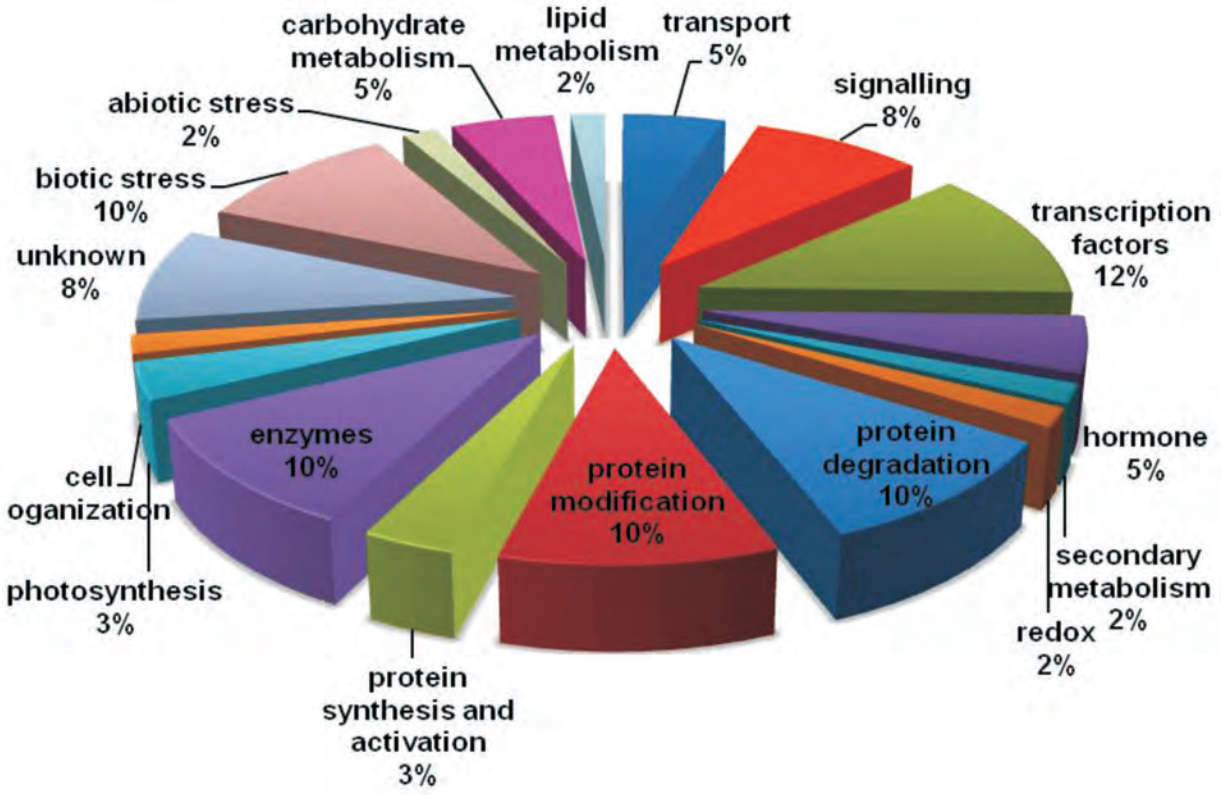


Fig. 5.24: Functional categorizations of differentially expressed genes at 6 h after inoculation with *M. oryzae* isolate, Mo-si-63.

signalling, cell wall defense and transcription factors belonging to the WRKY super family, however, it does not affect susceptible phenotype.

Comparative analysis of defence response genes in different plants

Phenylalanine ammonia lyase (PAL) is a key enzyme in plant phenylpropanoid pathway, which catalyses the first step in the pathway and not only leads to the accumulation of phytoalexins but also contributes in growth and development of plants and responses to biotic and abiotic stresses. The plant peroxidase (*POX*) genes present in large numbers in higher plants. These genes are involved in defense against pathogen infection or insect attack and several other physiological functions. We investigated the conservation and divergence of these two gene families in plants by their genome-wide identification and comparison in 9 plant species representing very diverse groups like legumes (*Glycine max* and *Medicago truncatula),* fruits (*Vitis vinifera*), grains (*Sorghum bicolor*, *Zea mays* and *Oryza sativa*), trees (*Populus trichocarpa*) and model dicot and monocot species (*Arabidopsis thaliana* and *Brachypodium distachyon*). The orthology analysis revealed that for *PAL* genes, *Vitis vinifera* having the least orthology with other species (Fig. 5.25). As each ribbon arising from a species (shown as clades) corresponds to the percentage of orthologous pairs with the destined species, *Vitis* (red clade) found to have the minimum orthology with other plant species. The orthologous pairs of *Vitis* were very low with the dicots and the monocots. Comparison of *Vitis PAL* genes with the dicot genomes indicates that orthologous relationships are conserved.

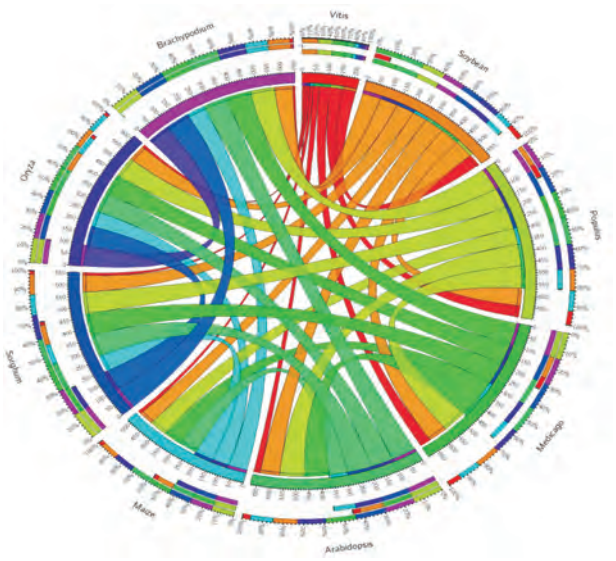


Fig. 5.25: Orthologous pairs between different plant species for PAL gene family. Each ribbon arising from a species (shown as clades) corresponds to the percentage of orthologous pairs with the destined species. PAL *Vitis* (red clade) found to have the minimum ortholgy with other plant species. Orthologous pairs between different plant species for PAL gene family.

Genomewide analysis of heat shock factors and heat shock proteins from pigeonpea genome

Genome wide analysis of heat shock factor (*Hsf*) genes was carried out in pigeonpea in order to understand their structure and function. A total of 23 *Hsfs* were predicted and labeled as *CcHsf*. Out of the 23 genes, 14 unique sequences were selected and characterized for their presumed structures such as protein domain and motif organization. The phylogenetic relationships and expression profiling of *CcHsf* genes under heat-stress was studied. Phylogenetic analysis showed that *CcHsf* genes were distributed into eight groups (Fig. 5.26). In this study, classes A, B and C were further subdivided into subclasses such as A1, A2, A3, A4, A5, A6, A8, A9, B1, B2, B3, B4 and C1. Expression profiling of all 14 genes was carried out by semi-quantitative PCR, among which *CcHsfA-1d* and *CcHsfA*-2 were observed to be highly upregulated during heat-stress. Relative

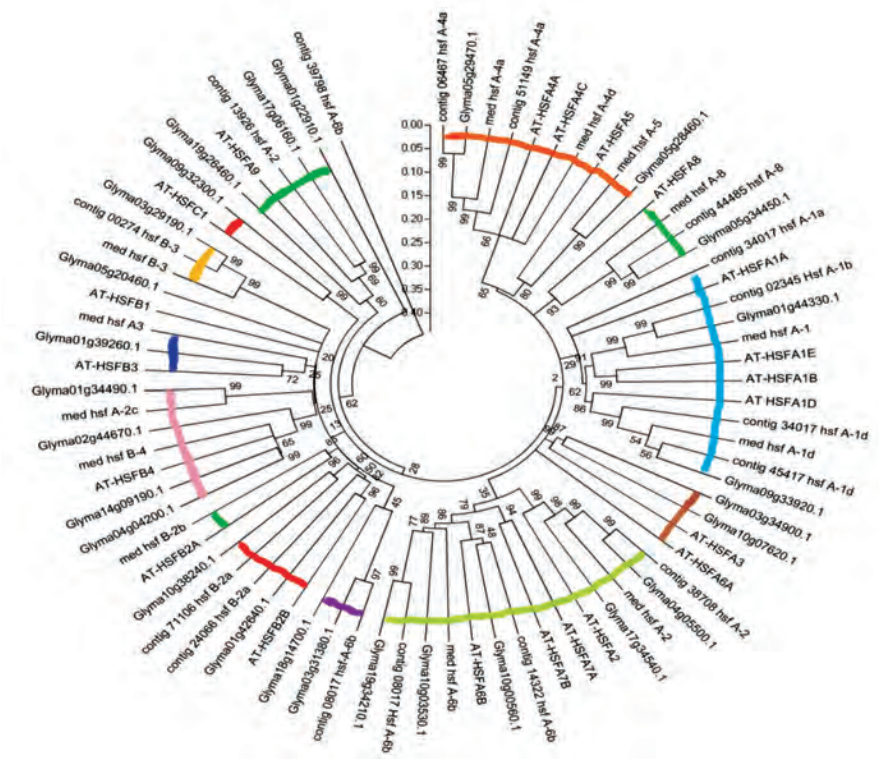


Fig. 5.26: Phylogenetic relationships among pigeonpea, soybean, medicago and Arabidopsis *Hsfs* using MEGA 5.

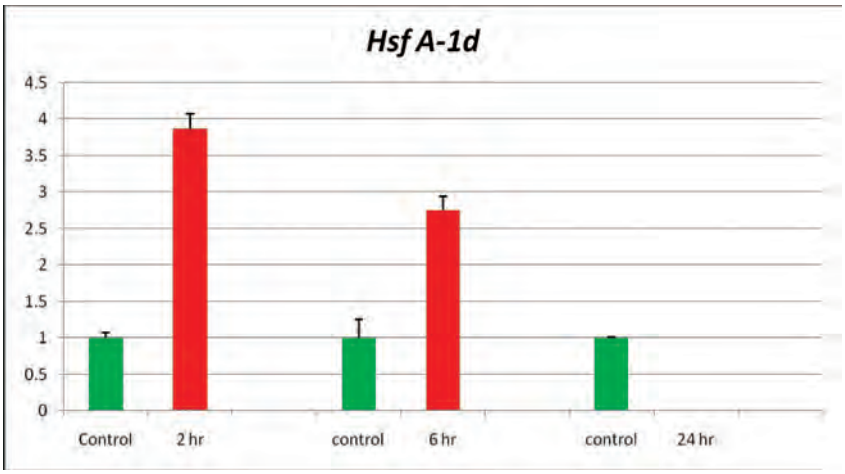


Fig. 5.27: Relative quantification of *CcHsf A-1d* gene expression during heat stress.

quantification with qRT-PCR showed that *CcHsfA-1d* was upregulated 2-6 hrs after heat-stress indicating its role as an early response factor (Fig. 5.27). The study provides a first glimpse into the *Hsf* gene family in pigeonpea and the information can be utilized to gain more insight into the heat-response mechanism in pigeonpea.

The *hsp100* family genes [ClpB] are one of the major and critical genes required for acquired thermo tolerance. The current study was taken up to identify and characterize the *Hsp100* family genes in pigeon pea as not much information is available for this thermotolerant plant. *In silico* genome wide characterization was carried out and 5 *Hsp100* like gene sequences were identified and found to be aligned neatly with related legume sequences (Fig. 5.28). Domains were predicted and three proteins were found to possess Class I type ClpB domains for only three proteins. Expression of these genes was observed at control 2, 6 and 24h post heat stress conditions indicating their heat inducible nature. Out of the 5 genes predicted two *Clp* genes show constant expression under heat stress and could be important components of the heat stress response in pigeonpea as they are targeted to the chloroplast. To identify additional members, candidate gene approach was taken up. *Glycine max hsp100* gene sequences were downloaded and their expression studied under heat stress conditions in Asha, Pusa dwarf and *C. platycarpus* genotypes. Out of 23, 20 genes were expressed in pigeonpea indicating the presence of more *hsp100* family genes in the genome that need further investigation. This study would help in understanding the molecular components governing thermo tolerance in pigeonpea, since *Hsp100* family genes are the key factors governing the response to high temperatures.

Establishment of national rice resource database: upgradation of the Vanshanudhan rice gene database

Vanshanudhan is a relational rice gene database built on a UNIX Sun Solaris version 5.9 platform and is housed at the National Research Centre on Plant Biotechnology, New Delhi, India. Rice gene data are stored in MySQL. Java Server page has been used as scripting language and deployed in JBoss 4.0 web server. The data represented in the first version of Vanshanudhan rice gene database was based on the rice chromosomal pseudomolecules version 3.0 which is released by TIGR Rice Genome Annotation Project. Web interface of Vanshanudhan provide gene information in relation to the molecular function, gene ID and genetic location along with unique gene ID

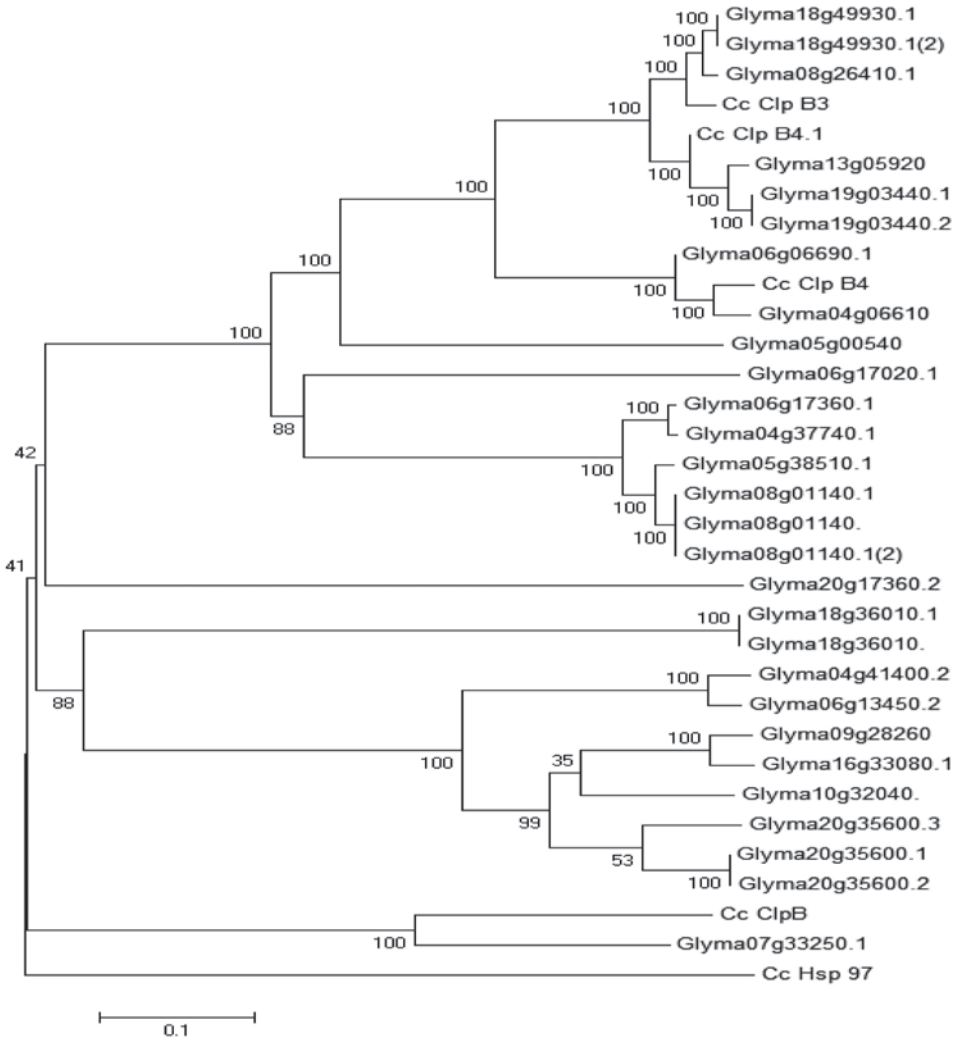


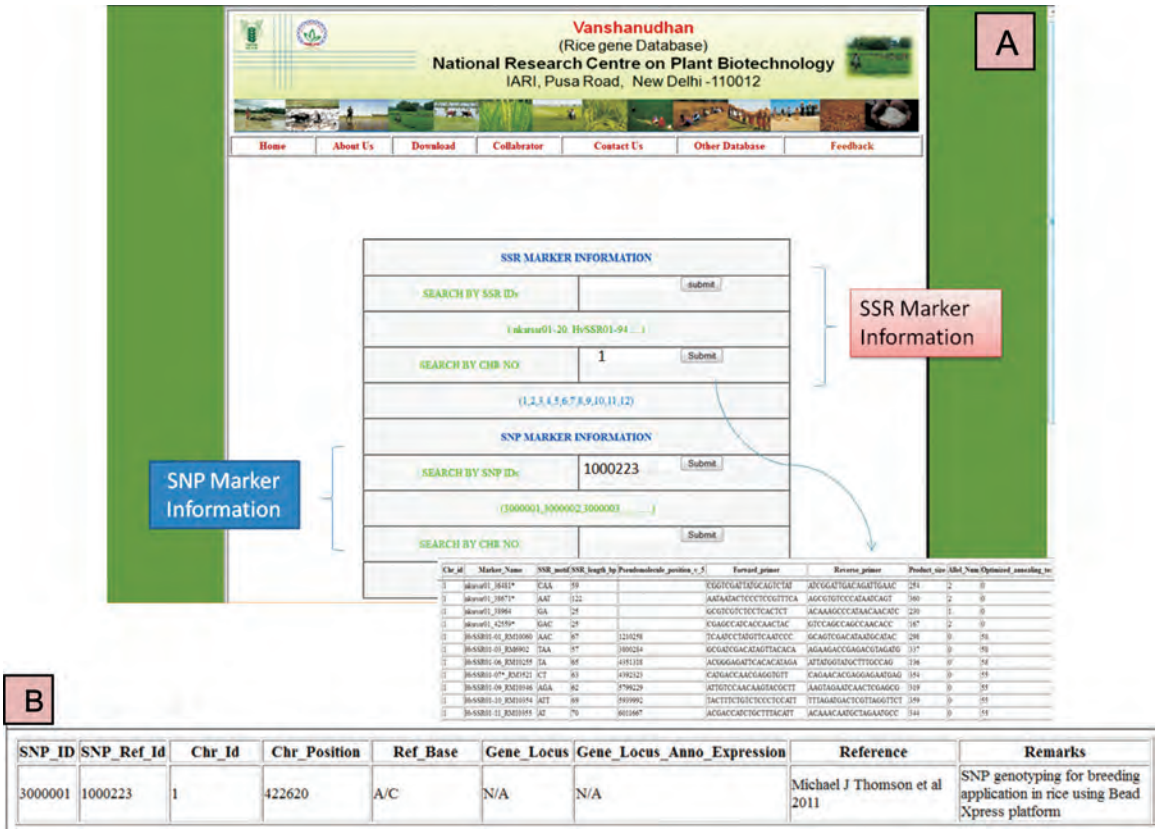
Fig. 5.28: Phylogenetic analysis of *Glycine max* and *Cajanus cajan Hsp 100* family genes.

e.g. 01-0001, for the first gene on rice chromosome 1. NRCPB Rice Marker and Trait Information System are also added in the database. At present 637 SSR and 384 SNP markers are uploaded in the database. This database has been created to help rice breeders.

A user-friendly web interface has been developed within this database to facilitate data retrieval according to specific marker needs from the NRCPB Rice Marker Information System. User can search for markers and related information by using two different query forms (Search by SSR or SNP IDs and Search by SSR or SNP chromosome number). The search results are displayed in the form of a table providing general information about the markers (Fig. 5.29). User can easily download the related information from the web interface.

Plant Genome Databases

Development of local database of genomic resources of crop plants is a continuous process and database named as Plant Genome Database is developed and maintained at NRCPB. Basic data on genomic resources such as EST, GSS, Genomic, cDNA, STS, HTG and Unigene are continously



SNP_ID	SNP_Ref_Id	Chr_Id	Chr_Position	Ref_Base	Gene_Locus	Gene_Locus_Anno_Expression	Reference	Remarks
3000001	1000223	1	422620	A/C	N/A	N/A	Michael J Thomson et al 2011	SNP genotyping for breeding application in rice using Bead Xpress platform

Fig. 5.29: Screen Shot of NRCPB Rice Marker information system. (A) SSR and SNP Marker query page resulting from searching for the Chromosome number 1 marker. The result page provides general information about the SSR marker (SSR motif, SSR length, product size, allele number) as well as corresponding marker sequence (B). Similarly in SNP information, resulting page shows SNP ref ID, chromosomal position, reference basepair and SNP source.

uploaded in MySql database for future use in comparative genome analysis. The plant genome database of NRCPB consists of 2,61,45,232 sequence entries (Table 5.5). Besides, we have also prepared plant gene information system which consists of 19,95,473 gene sequences from 50 crop plants. Percentage of databases of each species has been depicted in Fig. 5.30. All these databases are publically available at *www.nrcpb.org*.

Analysis of gene expression pattern in flax/linseed (*Linum usitatissimum*) under drought stress

Hydropenia, caused by drought, is a global phenomenon that affects growth, accumulation of biomass, performance and productivity of crops. In combination with other form of stresses it reduces yield potential of crops up to 70%. While priority crops like corn, rice, and wheat along with *Arabidopsis* have witnessed significant advances in drought research including the application of molecular breeding tools in varietal development; limited progress has been made in flax/linseed crop. To address this gap a genome-wide expression analysis was carried out to identify genetic programs associated with drought in flax. The present study reports a suite of gene regulation and metabolic changes to drought stress in flax obtained from microarray based genome-wide gene expression analysis. Since response of plants to drought stress differs between tissues, duration and degree of stress, the experiment was carried out in

Table 5.5: Different types of genome sequence entries stored database

Sequence Type	Vegetables	Fruits	Cereals	Oilseeds	Legumes	Fibre	Others	Total
cDNA	42804	48562	200894	8331	10775	173	4160	315699
EST	1813974	1787742	5335412	2945460	2201986	297570	639200	15021344
Genomic	310477	75156	242042	109760	33369	4071	5093	779968
GSS	1730550	437739	4099676	617247	1398294	62806	84080	8430392
HTG	7193	289	26896	966	3662	196	952	40154
STS	9571	1485	1149487	11601	10534	1148	185	1184011
Unigene	278509	89812	355311	121470	79812	21406	61883	1008203
Total	4193078	2440785	11409718	3814835	3738432	387370	795553	2,61,45,232

Fig. 5.30: Percentage of share of different crops in Gene Information System database.

two different tissues, i.e., root and shoot; and at two different time points of four and five days after treatment.

Hydropenia induced five-fold gene expression changes in flax tissues (Fig. 5.31). Genes encoding protein kinase APK1A, brassinosteroid insensitive1

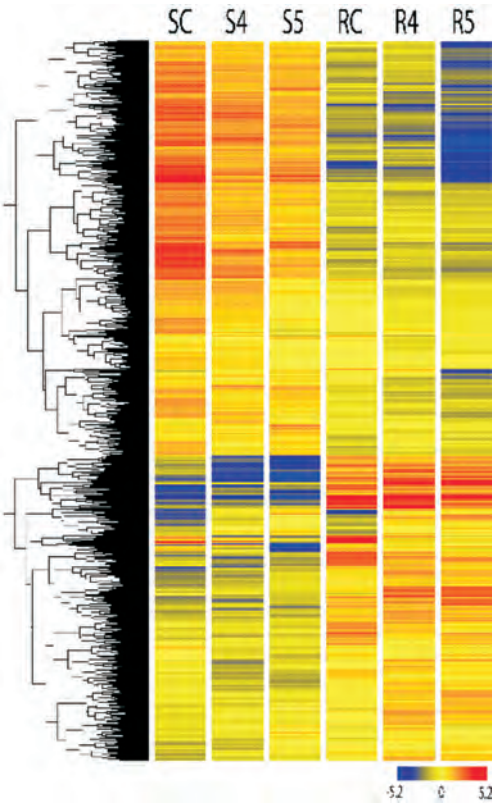


Fig. 5.31: Differentially expressed genes in flax at different time points (SC-shoot control, S4 and S5-shoot four day and five day after stress, RC- root control, R4 and R5- root four day and five day after stress).

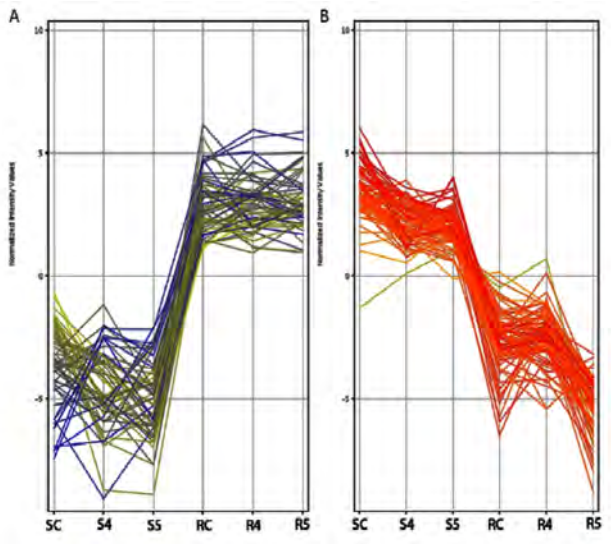


Fig. 5.32: Flax genes that are expressed at low levels (A) and high levels (B) in the shoot compared to roots.

and cytochrome P450 were differentially regulated in shoot and root. Similarly, genes encoding geranyl-geranyl reductase involved in chlorophyll biosynthesis, chlorophyll A/B binding protein, involved in chlorophyll stabilization, and trans-ketolase, involved in oxidative/reductive pathway, were highly expressed in shoot compared to root. Simultaneous comparison of gene expression in different tissues of flax (Fig. 5.32) at different time points revealed interesting commonalities in transcription responses. Ninety percent of genes up-regulated in shoot after 4 day stress were same as in root while ninety-three percent of the genes down-regulated in root were same as those identified to be down-regulated in shoot. Additionally, comparison of gene expression profiles between 4d and 5d shoot and root tissues revealed that fifty-one percent and eighty-two percent genes were commonly up-regulated and down-regulated in shoot and forty-eight percent and sixty-two percent were up and down-regulated in root respectively. This implies that simultaneous up/down regulation of genes in shoot and root is required to coordinate shoot-root ratio of water homeostasis.

Genes inducible under drought stress are classified into three classes such as functional proteins, regulatory proteins and proteins of unknown function. The microarray data identified genes belonging to all the three classes (Fig. 5.33). Among the first class, prominent genes that are identified belonged to late embryogenesis proteins, carbohydrate metabolism, amino acid metabolism, lipid transfer proteins, photosynthesis and chloroplastic proteins, heat shock proteins, and developmental proteins. This suggests that normal developmental processes were significantly affected in flax during drought and there is a massive transition of anabolic metabolism to catabolism. Although, drought induces cellular damage to vital organs such as cell membrane, tolerant plants exhibit immediate closure of stomata to avoid loss of water. But, this results in decreased photosynthetic efficiency due to reduced CO_2 availability. In addition to stomata closure, we found genes involved in

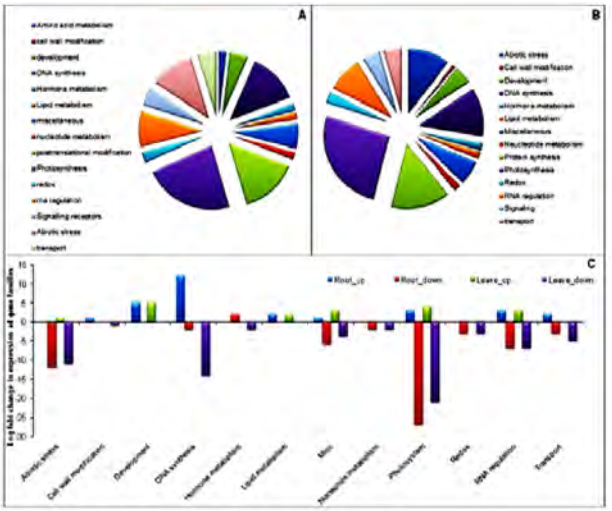


Fig. 5.33: Functional classification of stress inducible gene families in flax.(A) Up- and down-regulated gene families in shoot tissue after 4d and 5d drought. (B)Up- and down-regulated gene families in root tissue after 4d and 5d drought.(C) Families of genes showing differential expression with log –fold change values.

photosynthetic light reaction were severely dampened during drought stress and our results are in agreement with similar findings in rice and barley.

In root and shoot, five DEGs encoding transcription factors such as NAC012, NAC002 and NAC043 involved in developmental process were up-regulated while two of them are continuously up-regulated (Table 5.6). NAC transcription factors are reported to impart drought stress tolerance by triggering a cascade of signaling pathway. In the signaling sub-group, out of five DEGs, two were up-regulated in shoot while all were up-regulated in root. Two of the DEGs were leucine-rich repeat trans-membrane protein kinase having higher expression level in root compared to shoot. Typically, 16 candidate genes including dehydrin, cytochome P450, lipid transfer proteins, amino acid transporters and protein phosphatase 2c expression were found to be in agreement with published literature. Dehydrin belongs to a class of late embryogenesis proteins that are most abundant during water stress. Similarly, cytochrome P450 involved in the synthesis of fatty acids, phenols, terpenols and secondary metabolites, suberin and protective tissue like cutin may be involved in protecting water loss by forming a protective covering and these are previously shown to be induced during drought. Lipid transfer proteins, involved in the deposition of cuticular wax and important in drought tolerance, were also found to be up-regulated in our study. It has been reported that mono-oxygenases are highly up-regulatedduring drought stress. But current data for the expression of mono-oxygenase is different and shows that this gene is down-regulated in both shoots and roots.

Table 5.6: Continuously up/ down-regulated genes in drought stress in flax

Group	No. of genes down regulated	co-down-regulation	No. of genes up-regulated	co-up-regulation
4d L vs. Control	62	22	27	4
5d L vs. Control	69	Ribulosebiphosphate	20	
4d R vs. Control	49	carboxylase/ oxygenase	45	NAC-domain containing
5d R vs. Control	61	activase-2, Lipid transfer protein, Photosystem I reaction centre, Elongation factor-TU, Cell wall synthesis genes etc	33 20	proteins (2), r2r3-MYB transcription factor (1) and Protein kinase APK1A (1)

6. Improvement of Nitrogen Use Efficiency in Cereal Crops

Improving the nutrient use efficiency to reduce the use of chemical fertilizer is one of the priority areas of agriculture in the world. This would not only reduce the cost of cultivation, but also reduce the environmental pollution, energy consumption for production of these chemical fertilizers, improve soil health and ultimately help in mitigating climate change. Among the different nutrients, nitrogen is the most important one. This project has been initiated to identify nitrogen use efficient wheat genotype and understand biochemical and molecular basis of nitrogen use efficiency in wheat.

Morphological and biomass partitioning studies in different wheat genotypes

We have reported last year the standardization of growth conditions for growing wheat seedlings under hydroponics. The seedlings were grown in *phytajar* with sterile media. The culture solution was refreshed after every three days. 4.0 mM and 0.04 mM concentration of nitrate in growth media was considered as control (N-optimum) and N-starved (-N) respectively for growing 15 days old wheat seedlings under hydroponics.

Four wheat varieties (viz. Kalyansona, DBW-17, NP-890, PBW-502) were selected among several based on preliminary study on nitrogen use traits carried out at field level. These genotypes were grown under hydroponic condition for 15 days. Morphological differences in terms of root and shoot length, fresh and dry weight of roots and shoots were observed between N-optimum and N-starved condition for each genotypes (Fig. 6.1). Statistical analysis showed significant genotypic differences in these morphological parameters (Fig. 6.2). Study of root architecture of these genotypes also showed significant difference among the genotypes and also between starved and optimum nitrogen condition in each genotype (Fig. 6.3).

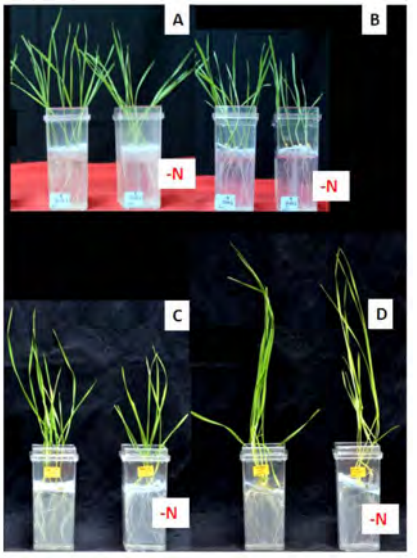


Fig. 6.1: Wheat seedlings of four genotypes growing in optimal and starved N condition in hydroponics.

Assay of enzymes involved in nitrogen metabolism

Assay of five enzymes, viz. pyruvate kinase (PK); nitrate reductase (NR); glutamine synthetase (GS); glutamate synthase (GOGAT); and glutamate dehydrogenase (GDH) were standardized and subsequently assayed in the leaf tissue of these four genotypes. NR, GS and GOGAT showed significant difference under N-starved condition. For all the enzymes except GOGAT and GDH, genotypic

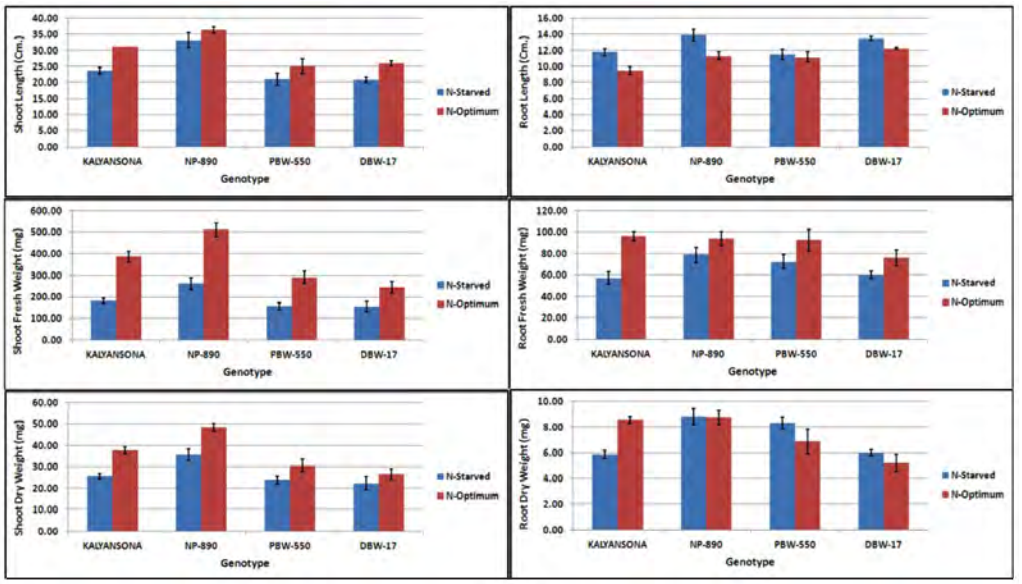


Fig. 6.2: Morphological differences of four wheat genotypes grown in optimal and starved N condition in hydroponics.

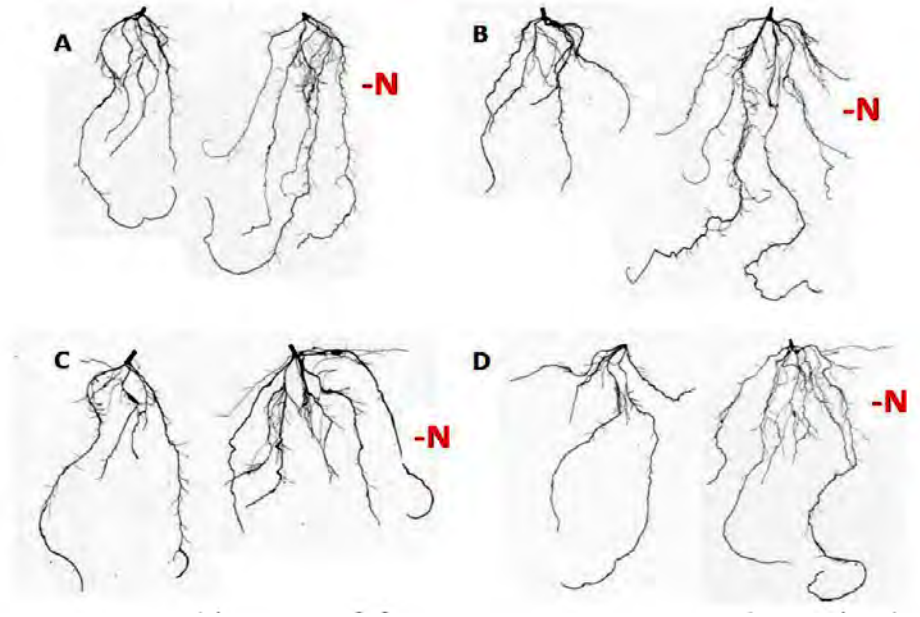


Fig. 6.3: Root system architecture of four wheat genotypes grown under optimal and starved N condition in hydroponics.

variations were observed with respect to the four genotypes under study. When the analysis was carried out among the genotypes in combination with N-concentration, it was observed that NR and GS activity significantly decreased in all the genotypes under N-starved condition. NR activity increased (significantly in case of Kalyansona) in all genotypes except for PBW-502 (where it was significantly decreased) under N-starved condition. GOGAT activity also increased in all the genotypes (significant only in case PBW-502). In case of GDH and PK, enzyme activity reduced significantly in case of Kalyansona and PBW 502 respectively and all others were on par under N-starved condition (Fig. 6.4).

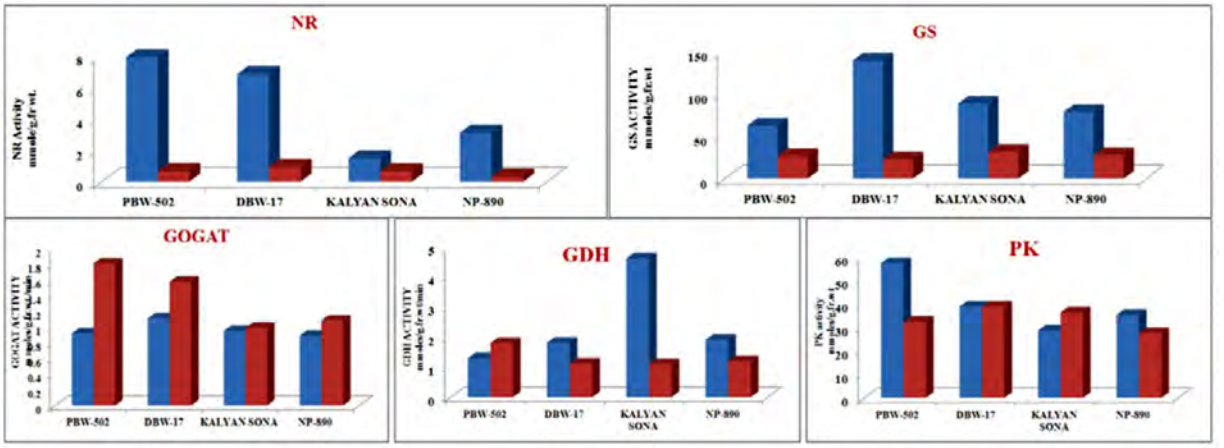


Fig. 6.4: Enzymatic assay of five enzymes involved in nitrogen related metabolism in four different genotypes of wheat grown under optimal and starved N conditions.

Expression profiling of microRNAs in N-deficiency conditions

The regulatory roles of miRNAs in response to nitrate and N-deficiency have recently been explored in some of the crops. In order to understand the possible role of miRNAs in nitrate sensing and deficiency in wheat, the expression pattern of twenty seven microRNAs, available in wheat miRBase (microRNA database) depository, were studied in a wheat genotype (HD-2967) growing in optimum (4.0 mM NO_3^-) and starved nitrate condition (0.04 mM NO_3^-) in hydroponics. Differential expression of these microRNAs were studied in both chronic (15 days starvation) as well as transient (24 hrs.) nitrate starvation conditions in root tissues. Some of the miRNAs (under present study) which showed differential expression under normal and starved nitrate conditions have also been observed in other crop species while others are for the first time. The differential expression in chronic and transient starved conditions were observed in some of the conserved micro RNAs i.e. miR159b, miRNA160, miRNA399 and miRNA408 while others such as miRNA 159a, miRNA164, miRNA167 and miRNA444a showed their presence under these conditions. Other miRNAs such as 1117, 1120, 1124, 1130, 1134 and 1139 also showed differential expression in root tissues under these conditions (Fig. 6.5).

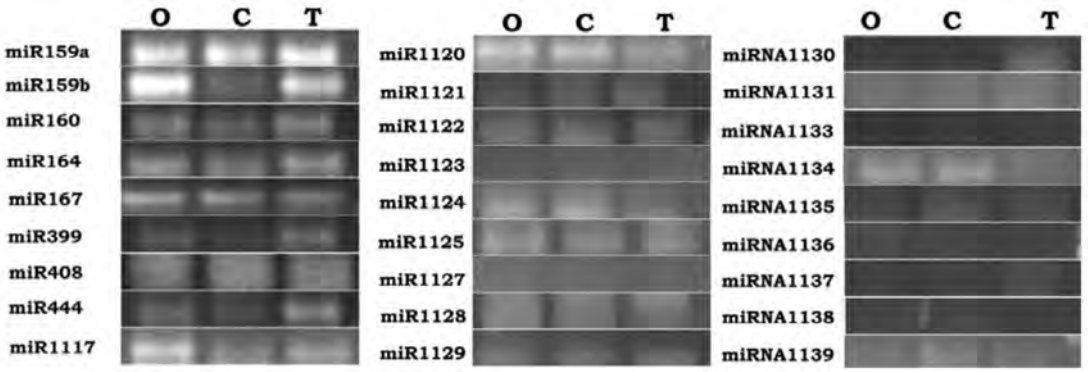


Fig. 6.5: Expression patterns of 27 TaMIRs in root tissues of wheat genotype (HD-2967) grown in optimal and starved N condition in hydroponics.

7. Honorary Scientists' Projects

Generation, characterization and use of EMS induced mutants of upland variety Nagina22 (Prof. R. P. Sharma, INSA Honorary Scientist)

This multi-institutional project sponsored by DBT involving 6 partner institutes including NRCPB initiated in 2007 came to a closure in Nov. 2013. The second phase of this project has been submitted to DBT for funding involving more traits and more research institutes. To enable distribution of seeds to the partners, for screening them for multiple traits such as yield and yield components, phosphorus use efficiency, nutritional content in terms of protein and micronutrients, resistance to blast and nematode, tolerance to drought and the component traits and plant architecture based traits, 7000 mutant lines were already multiplied and the rest 15,000 are being sown for multiplication with the help of CRRI, Cuttack and TNAU, Coimbatore. To augument the mutant population, a fresh batch of mutangenesis was initiated and the M1 seeds were harvested with the help of TNAU, Coimbatore.

At NRCPB, two early flowering mutants, both of them maturing in 45 days (Fig. 7.1) as compared to the wildtype which is maturing in 105 days, one semidwarf mutant with narrow and erect leaves, a spreading type mutant with anthocyanin pigmentation have been crossed with the wildtype Nagina22 and IR64 in order to study inheritance and develop mapping population for mapping the mutant traits. Mutants with increased biomass have been identified and they were also crossed with Nagina22 and among themselves for inheritance studies. However, the seed set was poor in these crosses and hence they will be attempted again next year. The herbicide tolearnt mutant identified by one of the institutes was grown

Fig. 7.1: Early flowering rice mutants M1359-1 and M1377-1 are shown along with the wildtype Nagina22 at 45 days after sowing.

at NRPCB (IARI farms) and characterized for 46 DUS traits and agronomic characteristics. The data revealed that the mutant had all the characteristics identical to the wildtype Nagina 22. This has also been crossed with the biomass mutants and IR64. Mutant garden comprising of 800 genotypes was grown this year in the IARI farms for maintenance and data recording so as to update the mutant database created and maintained at NRCPB. The database has complete details of 382 mutants. Since this data have already been published, with the revival of the project, the database will be made public.

Gene stacking approach for development of aphid resistant transgenic mustard (*Brassica juncea*) (Prof. K. R. Koundal, ICAR Emeritus Scientist)

Construction of stacking gene constructs using OVERLAP EXTENSION PCR-based fusion of two genes (*LL+CPPI*)

Fusion of multiple genes can be performed by using overlap extension PCR. Overlapping extention PCR is a precise procedure for the assembly of two or more multiple genes using chimeric primers. We have attempted to assemble two genes lentil lectin (*LL*) and Chickpea protease inhibitor (*CPPI*) using four chimeric primers. Chimeric primers were designed and at the same time the forward primer is same as first gene and reverse primer of first gene (*LL*) is reverse complementary having flanking sequences of second gene (*CPPI*). Similarly Primers for the second gene were designed. Forward primer was designed having Sequences same as second gene (*CPPI*) but having flanking sequences same as first gene (*LL*) for overlapping during hybridization and reverse primer same as the second gene (*CPPI*). (Overlapping regions shown clearly in bold).

I. LL-Kp-F- 5' GCG**GGTACC**ATGGCTTCTC TTCAA 3'

II. Fu-LLPI-R- 5'TACAATGGATTTCATTGCATC TGCAGCTTG3'

III. Fu-**LL**PI-F- 5'**CAAGCTGCAGATGCA**ATGAA ATCCATTGTA**3'**

IV. PI-Xb-R- 5'GCG**TCTAGA**CTAAACTGACG CATC3'

PCR amplification of lentil lectin gene (*LL*) using chimeric primers

For fusion gene preparation, the lentil lectin gene was PCR amplified from pGEMT-Easy vector with gene specific primers having *KpnI* restriction site incorporated in the forward primer and chimeric reverse primer (Fu-LLPI-F) having flanking sequences of LL gene and *XbaI* restriction site incorporated in the reverse primer respectively. PCR was performed for 30 cycles with initial denaturation for 3 min at 94°C, annealing for 45 sec temperature at 60°C and final extension at 72°C for 1min using *Pfu* DNA Polymerase(Recombinant). The PCR product was checked on 1% agarose (Fig. 7.2).

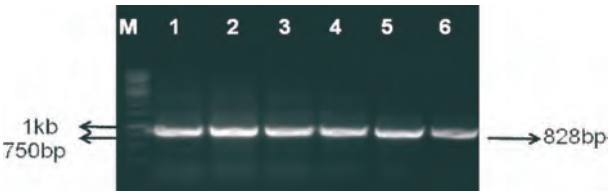


Fig. 7.2: PCR amplification products of lentil lectin gene (828bp). Lane M; 1kb DNA ladder, Lanes 1-6: PCR amplification of LL gene.

PCR product was excised from the gel and purified using Gel extraction kit (Fermentas) and concentration was checked on 1% agarose gel as well as on Nanodrop.

PCR amplification of chickpea protease inhibitor gene (*CPPI*) using chimeric primers

The CPPI gene was PCR amplified from pCR2.1 TOPO vector with gene specific primers having chimeric forward primer (Fu-LLPI-R) having flanking sequences of *CPPI* gene respectively. PCR was performed for 30 cycles with initial denaturation for

3 min at 94°C, annealing for 45 sec temperature ranging between 60°C and final extension at 72°C for 1 min using *Pfu* DNA Polymerase. The PCR product was checked on 1% agarose gel as shown in Fig. 7.3.

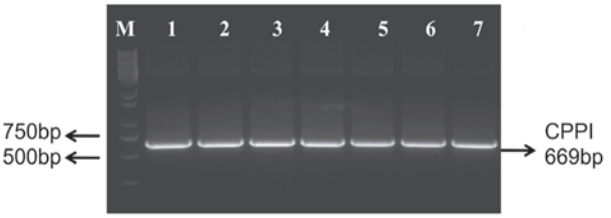


Fig. 7.3: PCR amplification products of CPPI using chimeric primers. Lane M: 1kb DNA ladder, Lanes1-7: PCR amplification product of CPPI gene.

PCR product was excised from the gel and purified using Gel extraction kit (Fermentas) and concentration checked on 1%agarose gel as well as on Nanodrop.

Construction of fusion gene constructs

Fusion Reaction step A. Equimolar aliquots of both the gel eluted gene fragments (LL & CPPI) having overlapping sequences were taken in a PCR tube and rest of the components of PCR were added (water, buffer, DNA Polymerase, dNTPs, $MgCl_2$) except the primers in the reaction volume of 50µl each in five different PCR tubes kept at five different annealing temperatures ranging between 50-55°C. Fragments carrying sequence overlaps were fused at different annealing temperatures.

Cycling parameters: Initial denaturation 94°C 2 min, subsequent steps 94°C 15 s, annealing at 50-55°C 1min-20 min, extension 72°C 2 min, for10 cycles total, hold at 4°C. This hybridization /fusion product was utilized for the PCR amplification of whole fusion product of both the genes.

Fusion reaction step B. 5-10 ul of unpurified PCR fusion product of step A was taken as template and rest of the components of PCR (water, buffer, dNTPs, $MgCl_2$, Pfu DNA Polymerase) added and forward primer of LL gene(I) and reverse primer of CPPI gene(IV) was also added.

Cycling parameters: Initial denaturation 94°C 2 min, subsequent steps 94°C 15s, annealing at 62°C 20 s, extension 72°C 2 min 30 s, 30-35 cycles total, and additional Extension 72°C 3 min, hold at 4°C.

The resulting ~1494bp PCR product was analyzed by electrophoresis in 1% agarose gel as shown in the Fig.7.4. PCR product was checked on 1% agarose gel and purified using Qiagen PCR cleanup kit method.

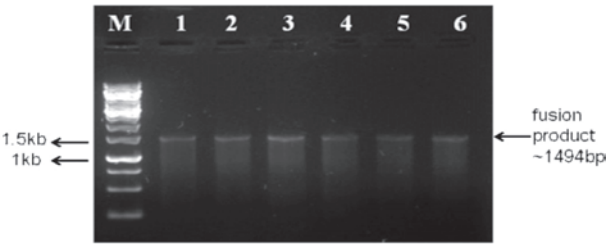


Fig. 7.4: PCR amplification fusion product of LL and CPPI. Lane M: 1kb DNA ladder, Lanes 1- 6: PCR amplification of fusion product.

Cloning of fusion gene product in pCAMBIA1300 (*NOS-T*) construct

The eluted fusion PCR product was restricted with *Kpn*I and *Xba*I restriction enzymes. These restriction sites were already incorporated in forward primer of *LL* gene and reverse primer of *CPPI* gene for cloning in pCAMBIA1300 construct. Restriction was performed for 3hrs at 37°C and 1µl was checked on agarose gel. Plasmid DNA of pCAMBIA1300 wih *nos T* was isolated from the overnight grown culture at 200 rpm and temperature set at 37°C. Plasmid DNA of overnight grown culture was isolated by G-Biosciences Miniprep kit method and was quantified on 0.8% agarose gel. Plasmid DNA was linearised by restricting with *Kpn*I and *Xba*I restriction enzymes and was checked on agarose gel and purified by PCR cleanup kit method. Concentration of purified vector was checked on 1% agarose gel (Fig. 7.5) as well as on Nanodrop before ligation.

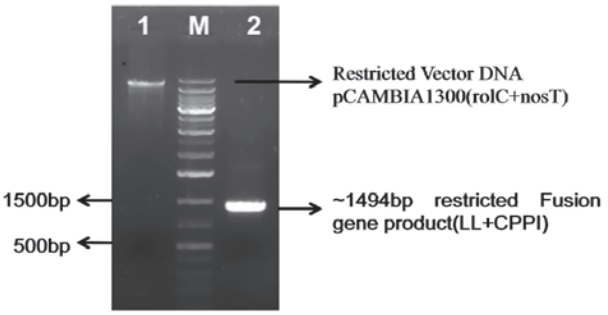


Fig. 7.5: Agarose gel electrophoresis of purified restricted Fusion gene product (*LL+CPPI*) and restricted pCAMBIA(*rolC+nosT*) plasmid DNA. Lane M: 1 kb DNA ladder, Lane 1: Linearised pCAMBIA constuct DNA, Lane 2: Restricted fusion gene product (*LL+CPPI*).

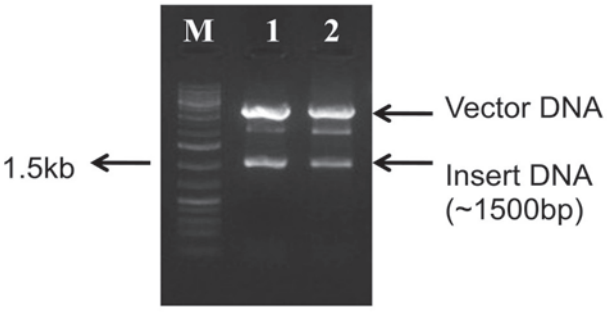


Fig.7.6: Agarose gel electrophoresis of restricted recombinant clones pCAMBIA (*rolC* +fusion gene+*nos T*) .Lane M: 1kb DNA ladder , Lanes 1-2 Restricted clones showing insert and vector DNA.

Ligation of fusion gene (LL+CPPI) with linearized pCAMBIA 1300 binary vector construct

Ligation was performed overnight at 16°C keeping 1:1, 3:1 and 5:1 insert to vector molar ratio in 10µl reaction volume using 50-100ng/µl of vector DNA,15/20 ng/µl of insert DNA(as per the insert ratio to vector DNA), 1X buffer and T_4 DNA ligase (5U/µl). Transformation was performed with standard protocol using *E. coli* XL1-Blue competent cells which was already prepared by $CaCl_2$ method and plated on LA plate having Kanamycin (50µg/ml) as antibiotic selection marker. Plates were kept for growth at 37°C for 18hrs.

Selection and restriction analysis of recombinant clones: Colony PCR was performed with 10 colonies and master plate was also prepared of the transformed colonies. Out of 10 colonies, only 2 transformed colonies were found to be positive. These two recombinant clones were also confirmed by restriction for the presence of fusion gene construct (Fig. 7.6).

Cloning of phloem specific promoter (*rolC*) into pCAMBIA1300 binary vector construct (fusion gene + *NOS-T*)

For cloning of phloem specific promoter (*rolC*), plasmid DNA of *rolC* in pGEMT-easy vector was isolated and restricted with *Eco*RI and *Kpn*I. The restricted *rolC* was purified and ligated to linearised pCAMBIA1300 having *LL* gene in 3:1 insert to vector molar ratio. Ligation was performed overnight at 16°C using T_4 DNA ligase (Fermentas). Ligated product was transformed in competent cells of *E. coli* XL1-Blue stain and transformation was performed with the standard protocol and was plated on LA plate with kanamycin (50mg/ml) as antibiotic selection marker and was kept overnight for growth at 37°C in incubator. Next day distinguished colonies were selected and master plate was prepared. Colony PCR of ten colonies was performed with *rolC* specific primers which confirmed the presence of *rolC* in 6 colonies when resolved on 1% agarose gel. Plasmids DNA of selected colonies were isolated from overnight grown culture by kit method and restriction analysis confirmed the presence of desired ~1kb *rolC* promoter in pCAMBIA1300 construct (Fig.7.7).

Now the whole construct prepared was confirmed by restriction of the recombinants with *Eco*RI and *Sal*I which gave fall out of ~2.6kb fragment which included *rolC*+fusion gene +*NosT* and backbone of binary vector pCAMBIA1300 as shown in Fig.7.8. Also schematic representation of *fusion* gene construct is shown in Fig.7.9. All the three constructs in the pCAMBIA1300 binary vector were mobilized to *Agrobacterium* strain *GV3101* for genetic transformation of brassica for aphid resistance.

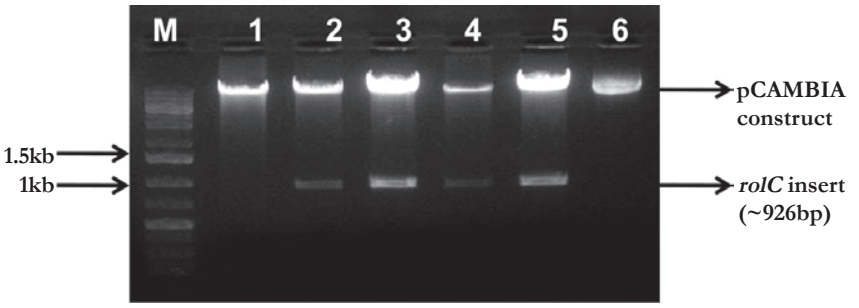


Fig. 7.7: Agarose gel electrophoresis of restricted recombinant plasmid. Lane M: 1kb DNA ladder, Lanes 2-5- showing the presence of *rolC* promoter.

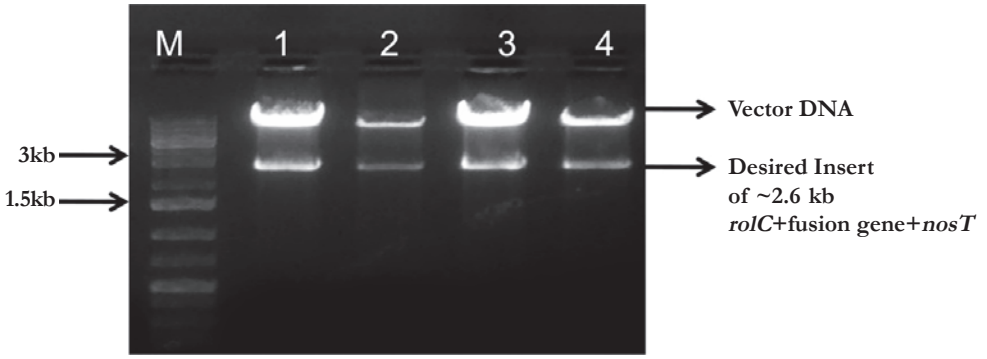


Fig. 7.8: Agarose gel electrophoresis of restricted recombinant plasmid DNA. Lane M: 1kb DNA ladder, Lane 1-4, Recombinant clones showing ~2.6 kb cassette of *rolC* +fusion gene+*nosT* gene construct.

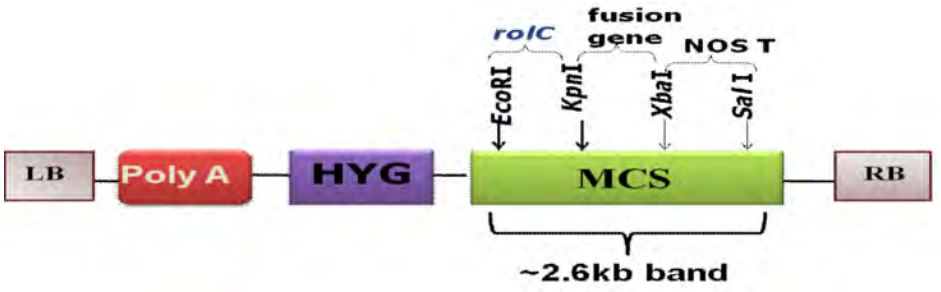


Fig. 7.9: Schematic representation of the cloning sites of *rolC*, fusion gene (*LL+CPPI*) and *NOS* terminator in the MCS of pCAMBIA1300 binary vector.

Mobilization of three gene constructs into *Agrobacterium*

Three genes i.e. lentil lectin gene (LL), chickpea protease inhibitor gene (CPPI) and fusion gene (FuG) were mobilized into *Agrobacterium tumefaciens* and confirmed for the presence of desired construct by gene specific PCR and by restriction. Further work is under progress.

A comparative study on the effects of iron availability on phosphate deficiency-mediated responses in *Arabidopsis* and rice (Dr. Ajay Jain, Ramalingaswamy Fellow)

Inorganic phosphate (Pi) availability to plants is one of the key limitations to its growth and development. Pi-deprived plants trigger an array of species-specific and spatiotemporally regulated

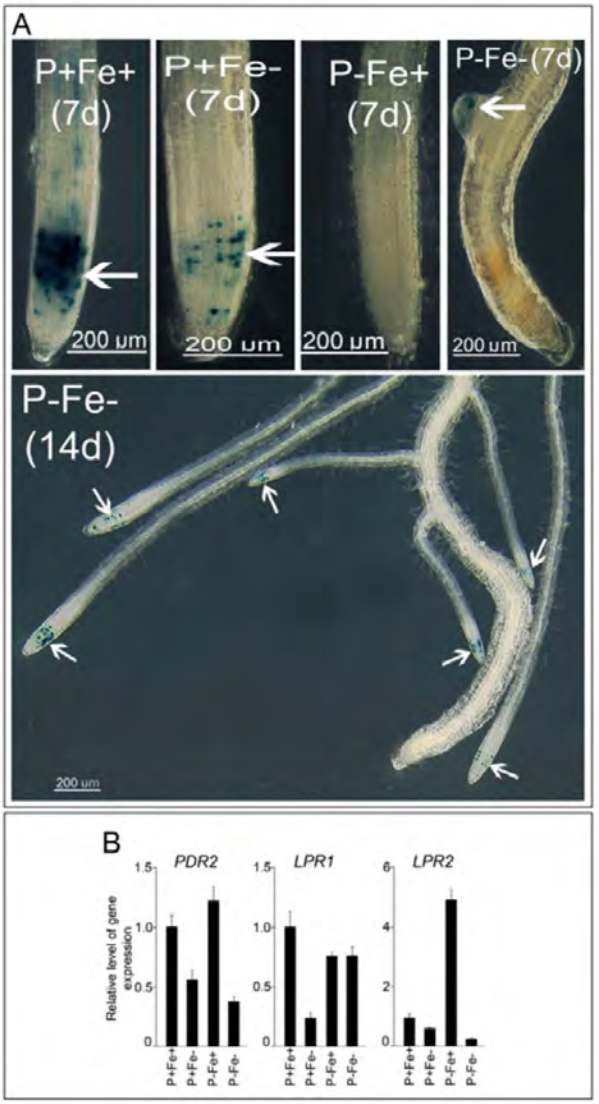


Fig. 7.10: Effect of Pi and/or Fe deficiency on meristematic activity of *Arabidopsis* root system. *CycB1;1*::*uidA* (A), and wild-type (B) *Arabidopsis* seedlings were grown under different nutrient regime as described in legend to Figure, A, Representative photographs of 10-15 histochemical GUS stained primary root tips of *CycB1;1*::*uidA* seedlings grown under different nutrient conditions for time intervals indicated in parenthesis. Arrows indicate *expression* of cell cycle marker *CycB1;1::uidA in primary and lateral roots. B,* Real-time PCR analysis of relative expression levels in wild-type roots of genes involved in local Pi sensing. *ACT2* was used as an internal control. Data presented are means of six technical replicates ± SE.

responses. Although antagonistic cross talk between Pi and iron (Fe) is well documented, the effects of Fe availability on Pi deficiency-mediated morphophysiological and molecular responses in taxonomically diverse model species *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*) are far from being elucidated. To decipher this interaction, *Arabidopsis* and rice seedlings were subjected to Pi and/or Fe deficiency. P-Fe+ *Arabidopsis* seedling showed determinate primary root growth due to loss of meristematic activity. However, on set of determinate growth of primary root was either delayed or remained indeterminate when grown under P-Fe- (Fig. 7.10) and P-Zn++ conditions, respectively. The study provided evidence towards the role of both Fe and Zn in eliciting differential aberration in primary root's ability to sense local Pi deficiency. Retarded lateral root development in P-Fe+ seedlings and its accentuated growth in P-Fe- and P-Zn++ seedlings further suggested the effects of these micronutrients on auxin-mediated Pi deficiency responses on lateral root development. Expression analyses of auxin-responsive genes, reporter gene assays, and root phenotyping of *arf7arf19* together provided evidences toward altered auxin response in roots of P-Fe- seedlings compared to that of P-Fe+ seedlings. Pi and/or Fe deficiency also exerted differential effects on the expression of the members of cyclophilin gene family in *Arabidopsis*. Relatively the effects of these different nutrient conditions were less dramatic on the root system architecture of rice seedlings. Further, analysis of the roots of *Arabidopsis* and rice seedlings grown under Pi and/or Fe-deprived condition revealed differential effects of Fe availability on the expression of systemically regulated genes involved in Pi homeostasis that are regulated via *PHR2*-miR399-*PHO2* dependent or independent Pi signaling pathway in *Arabidopsis* and rice, respectively. Together these results clearly showed variable and species-specific effects of Fe availability on Pi deficiency-mediated local and systemic responses of *Arabidopsis* and rice.

Institutional Activities

- Human Resource Development
- Personnel
- Other Activities
- Recruitments/Promotions
- Institutional Projects
- Externally Funded Projects
- Technology Commercialization and IPR
- Awards and Honours
- Visits Abroad
- Linkage and Collaboration
- List of Publications
- Distinguished Visitors
- Important Committees

Human Resource Development

NRCPB has been actively engaged in human resource development in the area of plant molecular biology and biotechnology since its inception. Currently 41 Ph.D. and 12 M.Sc. students are registered in the discipline of Molecular Biology and Biotechnology at the Centre. In the previous year, three Ph.D. and four M.Sc. students were awarded with doctoral and masters degrees, respectively. The list of students currently enrolled for their post graduate studies is given below:

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
Ph.D.			
01.	9562	Mr. Rama Prashat G*	Dr. N. K. Singh
02.	9563	Ms. Neetu Singh Kushwah	Dr. S. R. Bhat
03.	9564	Mr. Devanna+	Dr. T. R. Sharma
04.	9566	Mr. Viswanathan Satheesh*	Dr. Srinivasan
05.	9567	Mr. Rajiv Kumar Singh*	Dr. P. A. Kumar
06.	9568	Mr. Vinod Kumar*	Dr. K. C. Bansal
07.	9710	Mr. Soham Ray*	Dr. T. R. Sharma
08.	9711	Ms. Deepika Singh	Dr. N. K. Singh
09.	9712	Mr. Parameswaran, C	Dr. Srinivasan
10.	9713	Mr. Deepak Singh Bisht	Dr. S. R. Bhat
11.	9715	Mr. Siddanna Savadi	Dr. S. R. Bhat
12.	9716	Ms. Charagonda Revathy	Dr. Srinivasan
13.	9766	Mr. N. M. C. Nayankantha*	Dr. Anita Grover
14.	9858	Mr. Anshul Watts	Dr. S. R. Bhat
15.	9860	Mr. Shanmugavadivel P.S.*	Dr. N. K. Singh
16.	9861	Mr. Senthilkumar, K.M.	Dr. K. C. Bansal
17.	9862	Mr. Chandra Prakash	Dr. Srinivasan
18.	9863	Ms. Archana Kumari	Dr. T. R. Sharma
19.	9864	Mr. Kumaraswamy H.H.	Dr. N. K. Singh
20.	9865	Ms. Suman Lata	Dr. S. R. Bhat
21.	9867	Mr. Jagannadham Prasanth Tej	Dr. Srinivasan
22.	9869	Ms. K. N. Poornima*	Dr. Anita Grover

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
23.	10012	Mr. Neeraj Anand	Dr. N. K. Singh
24.	10013	Mr. Ravi Prakash Saini	Dr. P. A. Kumar
25.	10015	Mr. Hemant Balasaheb Kardile	Dr. K. C. Bansal
26.	10016	Mr. Chandra Shekar, N	Dr. Anita Grover
27.	10017	Mr. Chet Ram	Dr. R. C. Bhattacharya
28.	10018	Mr. Rajendra Prasad Meena	Dr. J. C. Padaria
29.	10059	Mr. Bhupendra Singh Panwar	Dr. Sarvjeet Kaur
30.	10152	Mr. Rakesh Bhowmick	Dr. N. K. Singh
31.	10153	Mr. Deepak V. Pawar	Dr. T. R. Sharma
32.	10154	Ms. Antara Das	Dr. P. A. Kumar
33.	10155	Mr. Prashant Yadav	Dr. Anita Grover
34.	10217	Ms. Mamta Gupta	Dr. Sarvjeet Kaur
35.	10313	Mr. Lianthanzauva	Dr. R. C. Bhattacharya
36.	10314	Mr. Alim Junaid	Dr. Kishor Gaikwad
37.	10315	Mr. Albert Maibam	Dr. J. C. Padaria
38.	10316	Mr. Mahesh Mohanrao Mahajan	Dr. Kanika
39.	10317	Mr. Rakesh Kumar Prajapat	Dr. Rekha Kansal
40.	10318	Mr. Vinod Kumar Jangid	Dr. Anita Grover
41.	10319	Ms. Anupma	Dr. Sarvjeet Kaur
M.Sc.			
01.	20228	Ms. Priyanka	Dr. R. C. Bhattacharya
02.	20317	Mr. Sudhir Kumar	Dr. Rekha Kansal
03.	20318	Mr. Manoj, M.L.	Dr. Kishor Gaikwad
04.	20319	Ms. Shruti Sinha	Dr. P. K. Mandal
05.	20320	Mr. Mainkar Pawan Sitaram	Dr. P. K. Jain
06.	20321	Mr. Kishor Prabhakar Panzade	Dr. P. K. Dash
07.	20441	Mr. Sandeep Jaiswal	Dr. Debasis Pattanayak
08.	20442	Mr. Lalbahadur Singh	Dr. P. K. Jain
09.	20443	Mr. Chetan Kumar Nagar	Dr. P. K. Mandal
10.	20444	Ms. Neeta Pargi	Dr. Sharmistha Barthakur
11.	20445	Mr. Ningombam Ravichandra Meitei	Dr. Rhitu Rai
12.	20481	Mr. Duong Van Hay	Dr. P. K. Dash

*Taken relief from P. G. School pending submission of thesis
+Thesis submitted

Degrees awarded in the discipline of Molecular Biology and Biotechnology during the Convocation held in February, 2014

S. No.	Name and Roll No. of the student	Chairperson, Advisory Committee	Thesis Title
Ph.D.			
01.	Joshitha Vijayan (9441)	Dr. T. R. Sharma	Cloning and functional validation of pathogen-responsive promoter from rice
02.	Ali Salari (9606)	Dr. K. C. Bansal	Genetic transformation of wheat (*Triticum aestivum* L.) for enhanced thermotolerance
03.	Israr Ahmad (9866)	Dr. T. R. Sharma	Transcriptome proteome study of bringal (*Solanum melongena* L) during fruit development
M.Sc.			
01.	Mr. Lianthanzauva (20188)	Dr. Rekha Kansal	Development of transgenic Indian mustard (*Brassica juncea*) cv. Pusa Jaikisan with pigeonpea lectin gene driven by *rolC* promoter
02.	Mr. Albert Maibam (20189)	Dr. Kishor Gaikwad	Genome wide identification and characterization of heat shock factor (*hsf*) genes from pigeonpea (*Cajanus cajan*) (L.) Millsp.
03.	Ms. Denkar Priya Balkrishna (20191)	Dr. Kishor Gaikwad	Molecular characterization of *Hsp 100* family genes from pigeonpea and related wild species
04.	Mr. Mahajan Mahesh Mohanrao (20230)	Dr. Kanika	Metagenomic approach for microbial diversity analysis of hot water spring

Personnel

Scientific staff	Area of interest
Dr. T. R. Sharma trsharma@nrcpb.org	Major areas of research include structural, functional and comparative genomics of plant and plant pathogens; concentrating on mapping, cloning and functionally validating disease resistance genes and QTL in rice; cloned rice blast resistance gene *Pi-kh* (*Pi54*) from rice line Tetep and *Pi54rh* from a wild relative of rice *Oryza rhizomatis*; development of DNA markers and various databases.
Dr. N. K. Singh nksingh@nrcpb.org	Working on different aspects of structural, functional and comparative genomics with special emphasis on rice, wheat, pigeonpea, tomato and Rhizobium; contributed to genome sequencing of rice, tomato and chickpea *Rhizobium M. ciceri*; mapping genes for yield and quality traits in rice, wheat and pigeonpea; high resolution mapping of loci for salinity tolerance and grain characteristics in rice.
Dr. Srinivasan srinivasan53@gmail.com	Structure-function relationship in enzymes; molecular biology and genetic transformation of plants and functional genomics of drought tolerance are the main areas of interest; developed T-DNA mutagenized populations of Arabidopsis with promoter less GUS; isolated and characterized novel genes and tissue specific promoter elements. Current interest is on functional genomics of abiotic stress tolerance in chickpea.
Dr. S. R. Bhat srbhat22@rediffmail.com	Working on development and molecular/genetic characterization of cytoplasmic male sterility systems in Brassica, molecular tagging of fertility restorer genes, development of transgenic Brassica for improved seed and oil yield, wide hybridization and gene introgression, and cloning and characterization of plant promoters.
Dr. (Mrs.) Sarvjeet Kaur dr_sarvajeetkaur@yahoo.com	Working on isolation and characterization of quorum quenching genes from *Bacillus thuringiensis* and other bacteria for plant disease control; also working on isolation of *cry1*- and *cry 2*-type genes from native *Bacillus thuringiensis* isolates from diverse habitats in India for development of transgenic crops.
Dr. (Mrs.) Anita Grover anitagrover@hotmail.com	Research includes exploring plant-fungus interaction with the system of *Brassica juncea-Alternaria brassicae* leading to the isolation of defense genes and development of transgenics of *Brassica juncea* for increased resistance to *Alternaria* blight.
Dr. (Mrs.) Rekha Kansal rekhakansal@hotmail.com	Isolating genes (lectins, protease inhibitors and amylase inhibitors) from legumes like chickpea, pigeonpea, peas and mothbean for imparting improved tolerance to sucking pests like, *Lipaphis erysimi*; development of transgenic mustard with lectin gene driven by tissue specific promoter is underway.
Dr. Sanjay Singh sanjay_singh777@yahoo.com	Molecular basis of nitrogen use efficiency in wheat.

Scientific staff	Area of interest
Dr. (Mrs.) J. C. Padaria jasdeep_kaur64@yahoo.co.in	Major area of research is biotechnology and climate change with emphasis on cloning and characterization of abiotic stress responsive genes from different tolerant plant system as Penniseteum, Zizyphus and Prosopis for development of transgenic wheat adaptable to climate change induced abiotic stresses. Work with microbial resources primarily focuses prospecting of genes for biotic stress tolerance (fungicidal and insecticidal) from *Bacillus* sp. and for development of genetically engineered efficient nitrogen fixing Azotobacter.
Dr. Pranab Kumar Mandal pranabkumarmandal@gmail.com	Working on molecular and biochemical basis of nitrogen use efficiency in wheat. Current interest include identification of differentially expressed gene(s) govening this trait in different wheat genotypes.
Dr. Debasis Pattanayak debasispattanayak@yahoo.co.in	The main area of research interest is gram pod borer resistant management employing host-delivered RNAi and Bt vegetative insecticidal protein. Development of RNA-based gene silencing tools for crop improvement and functional genomics is another area of interest. Long-term research interest includes deciphering RNAi pathways as development and stress regulators.
Dr. R. C. Bhattacharya ramcharan99@yahoo.com	Identification and mobilization of genes conferring resistance to insect herbivores and fungal pathogens into the elite crop cultivars through development of transgenics is the major area of research. Long term objective is identification of defense related signal peptides which initiate the signaling pathways and the associated signaling cascades so as to fully understand systemic wound signaling in plants.
Dr. Pradeep Kumar Jain jainpmb@gmail.com	Broad area of research is functional genomics including isolation of plant genes and promoters. Contributed to isolation of anther and trichome specific promoters and nematode-responsive root-specific promoter. Also involved in identification and characterization of candidate genes associated with a few important traits like drought, temperature, blight and wilt in chickpea.
Dr. (Ms.) S. Barthakur sbthakur@yahoo.com	Research interest is molecular biology and genetic engineering for abiotic stress tolerance in plants; working on isolation and functional characterization of genes and promoters involved in various abiotic stress responses.
Dr. K. S. Gaikwad kish2012@yahoo.com	Contributed to the sequencing of the rice and tomato genomes; pursuing the development of genomic resources including deep transcriptome analysis in crops like pigeonpea; also focusing on abiotic stress particularly on salinity and thermotolerance in rice and pigeonpea.
Dr. (Mrs.) Kanika Kumar kanika@rediffmail.com	Research focus is on abiotic stress management in plants through the microbes associated with them by exploiting PGPRs. Currently studying the role of microbial genes involved in reducing the concentration of stress ethylene in plants; also involved in structural and functional genomics studies of *Mesorhizobium ciceri*.
Dr. (Mrs.) Vandana Rai vandnarai2006@gmail.com	Working on salinity and drought stress in rice and wheat with the objective of discovering new genes and allele for drought and salinity using transcriptomics, proteomics and metabolomics.
Dr. (Mrs.) Monika Dalal monika@nrcpb.org	Functional genomics for abiotic stress tolerance especially drought and salinity stress in wheat.

Scientific staff	Area of interest
Dr. Subodh Kumar Sinha subsinha@gmail.com	Working on molecular aspects of nitrogen use efficiency in wheat with special emphasis on regulatory role of microRNA in nitrogen use metabolic pathway. Current interests include identification and understanding expression behavior of nitrogen responsive microRNA to unravel the details of regulatory role of miRNA in nitrogen use pathway.
Dr. Rohini Sreevathsa rohinisreevathsa@rediffmail.com	Involved in crop improvement programmes for biotic stress tolerance. The core of these programmes has been the meristem-based in planta transformation strategy developed by me. Presently, working on pod borer resistance in pigeonpea. This will be dealt with by using different insecticidal proteins (ICPs) from *Bacillus thuringiensis* using the tissue culture-independent transformation strategy.
Dr. Prasanta K. Dash prasanta@nrcpb.org	Worked on isolation of insecticidal genes and promoters from Indian legumes. Has isolated lectin genes from chickpea and lentil and protease inhibitor genes from mung bean. Current focus is on manipulating pathways for yield enhancements.
Dr. B. L. Patil blpatil2046@gmail.com	The major research interest is on study of diversity in plant viruses infecting different crop plants, wild species and weeds, and their evolution across space and time; behaviour & movement of plant viruses across different species, their molecular basis, and their ecological & economical importance; characterization of the plant viral genes and their role in viral pathogenicity, host-pathogen interaction and insect vector transmission; studies on RNAi and epigenetics, and their role in manifestation and management of plant viral diseases; developing and employing different transgenic technologies for the control of plant viral diseases.
Mr. R. S. Jaat rsjaat@gmail.com	The current area of research is genomics and markers.
Dr. Rhitu Rai rhitunrcpb@yahoo.com	Research focus is on plant pathogenesis and plant disease resistance mechanisms. Aim to functionally characterize *R*/*Avr* genes, gene expression profiling of pathogen and host plant during pathogenesis and understand mechanism of pathogenicity.
Dr. N. C. Gupta guptanc@nrcpb.org	Use of molecular and bioinformatics approaches to comprehend the novel genes and promoters and their function with a view to utilize them in crop improvement program; also working on development of transgenic mustard with high oil content.
Dr. S. V. Amitha CR Mithra amitha@nrcpb.org	QTL mapping specifically for grain traits and abiotic stress tolerance in rice by using SSR and SNP markers is the current area of research.
Dr. Amolkumar U. Solanke amolsgene@nrcpb.org	Main research interests are genomics and transgenic development with special emphasis on understanding biotic and abiotic stress in plants through various genomic tools; molecular biology of fruit development; development of transgenics for stress tolerance.
Mr. Ramawatar ram_nrcpb@nrcpb.org	Functional genomics particularly understanding role of non-coding RNA in plant pathogen interaction and disease development is the current area of research.
Mr. Dinabandhu Behera aumreetam@rediffmail.com	Main research interest is in the area of structural, functional and comparative genomics of crop plants.
Mr. Mahesh Rao amritkushinagar@gmail.com	Improvement of *Brassica spp.* using tissue culture, molecular marker, wide hybridization and gene introgression approach and enlarging gene pool of *Brassica juncea* using different parental lines.
Mrs. Nimmy M. S. nimmybiotech@gmail.com	Isolation and characterization of abiotic stress responsive gene(s) and promoter(s) from chickpea.

ICAR Emeritus Scientist
Dr. K. R. Koundal

DBT Ramalingaswamy Fellow
Dr. Ajay jain

INSA Honourary Scientist	
Prof. R. P. Sharma	Dr. H. K. Das

Technical staff	Administrative staff
Ms. Suman Bala	Sh. Vampad Sharma
Sh. H. C. Upreti	Sh. Mohan Singh
Sh. Ram Niwas Gupta	Sh. Krishan Dutt
Sh. R. K. Narula	Sh. A. K. Jain
Dr. Krishan Pal	Smt. Anima Lugun
Smt. Sandhya Rawat	Sh. B. S. Dagar
Smt. Seema Dargan	Smt. Sangeeta Jain
Dr. Rohit Chamola	Sh. Vipin Kumar
Dr. Pankaj Kumar	Smt. Rekha Chauhan
Sh. Ravinder Rishi	Rajesh Kumar Pal
Dr. Ram Pal Singh Niranjan	

Other Activities

Vigilance Awareness Week

NRCPB, New Delhi, observed Vigilance Awarness Week during October 2013. Dr. Srinivasan, Project Director (acting), NRCPB, administered the pledge to the staff.

Hindi Week

Competitions in Hindi Essay writing, technical work writing, declamation, poetry recitation and noting and drafting were conducted for the staff of the NRCPB as part of Hindi week 2013 from 07.09.2013 to 13.09.2013.

Agricultural Exhibition

An agricultural exhibition, Krishi Vasant, was organized by ICAR at CICR, Nagpur, on February 9-13, 2014. NRCPB participated in this grand event and showcased the achievements.

Sports

NRCPB participated in Central Zonal Sports Comptetion held at Bhopal, MP, from 24-09-2013 to 28-09-2013.

Dr. Rhitu Rai received 1st Prize in 100 m Race.

Different activities of Hindi Week 2013

Recruitments/Promotions/Retirements

Recruitments

1. Dr. Sanjay Singh, joined as Principal Scientist in September, 2013.

Promotions

1. Dr. P. K. Jain, Sr. Scientist was promoted as Principal Scientist from October, 2012.
2. Dr. Prashanta K. Dash, Scientist (SS) was promoted as Sr. Scientist in September, 2009.
3. Ms. Suman Bala, ACTO (T-7/8) was promoted as CTO (T-9) from February, 2012.
4. Dr. Kamlesh Batra, ACTO (T-7/8) was promoted as CTO (T-9) from February, 2013.
5. Sh. Ravinder Rishi, T-6 was promoted as ACTO (T-7/8) from July, 2012.
6. Sh. H. C. Upreti, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
7. Sh. R. N. Gupta, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
8. Dr. Krishanpal, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
9. Sh. R. K. Narula, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
10. Smt. Sandhya Rawat, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
11. Smt. Seema Dargan, ACTO (T-7/8) was promoted as CTO (T-9) from July, 2013.
12. Smt. Sunita Srivastva, ACTO (T-7/8) was promoted as CTO (T-9) from February, 2013.
13. Smt. Rekha Chauhan, LDC was promoted as UDC from April, 2013.

Retirement

1. Dr. Kamlesh Batra, CTO (T-9) retired in August, 2013.
2. Smt. Sunita Srivastva, CTO (T-9) retired in January, 2014.

Institutional Projects

Project Title	Date of Start	Date of Completion	Principal Investigator	Name of Associates
Isolation of plant genes and promoters	1st April, 2012	31st March, 2017	Dr. Srinivasan	Dr. Anita Grover Dr. R. Kansal Dr. P. K. Jain Dr. B. Patil Dr. Rhitu Rai Mrs. Sandhya Rawat
Biotechnological approaches for increasing crop productivity	1st April, 2012	31st March, 2017	Dr. S. R. Bhat	Dr. P. K. Dash Dr. N. C. Gupta Mr. Mahesh Rao Mrs. Seema Dargan Dr. Rohit Chamola
Genomics and molecular markers in crop plants	1st April, 2012	31st March, 2017	Dr. N. K. Singh	Dr. T. R. Sharma Dr. N. Kanaka Durga Dr. Kishor Gaikwad Dr. Vandana Rai Mr. R. S. Jaat Dr. S V A C R Mithra Mr. R. A. Nagar Dr. D. Behera Dr. Pankaj Kumar Mr. H. C. Upreti Dr. R. S. Niranjan
Transgenic crops for biotic stress resistance	1st April, 2012	31st March, 2017	Dr. Sarvjeet Kaur	Dr. D. Pattanayak Dr. R. C. Bhattacharya Dr. Rohini Sreevathsa Dr. A. U. Solanke Dr. K. P. Singh Mr. Rakesh Narula
Adaptation of wheat to climate change induced abiotic stresses	1st April, 2012	31st March, 2017	Dr. J. C. Padaria	Dr. S. Barthakur Dr. Kanika Dr. Monika Dalal
Improvement of nitrogen use efficiency in cereal crops	1st April, 2012	31st March, 2017	Dr. P. K. Mandal	Dr. Sanjay Singh Dr. Subodh Sinha Mr. R. N. Gupta

Externally Funded Projects

S. No.	Funding Agency	Project Title	Principal Investigator	Budget (Rs. in Lakhs)
1.	DBT	From QTL to variety: Marker assisted breeding of abiotic stress tolerant rice varieties with major QTLS for drought, submergence and salt tolerance	Dr. N. K. Singh	588.98
2.	DBT	Physical mapping and sample sequencing of wheat chromosome 2A-International Wheat Sequencing Consortium(India)	Dr. N. K. Singh	871.92
3.	ICAR	Genomics for augmenting fibre quality improvement in jute	Dr. N. K. Singh	49.04
4.	DBT	Establishment of National Rice Resource Database	Dr. N. K. Singh	92.708
5.	CIMMYT	Molecular breeding seletion strategies to combine and validate QTLs for improving WUE and heat tolerance in wheat	Dr. N. K. Singh	USD 154560
6.	ICAR	Allele mining for agronomically important genes in wild rice germplasm and stress tolerant landraces of rice growing in the hot spots	Dr. N. K. Singh	250
7.	DBT	Identification and functional analysis related to yield and biotic stresses	Dr. N. K. Singh	93.1
8.	NAIP	Bioprospecting of genes and allele mining for abiotic stress tolerance	Dr. N. K. Singh	2356.17
9.	DBT	Generation, characterization and use of EMS induced mutants of upland variety Nagina 22 for functional genomics in rice	Dr. N. K. Singh	177.76
10.	DBT	*Puccinia triticina* genomics network on *De Novo* genome sequencing, fitness, variation and pathogenicity	Dr. T. R. Sharma	571.47
11.	DBT	Development of rice and tomato varieties resistant to *Rhizoctonia solani*, the causal agent of rice sheath blight and tomato root and crown rot diseases (Indo-Australia Collaborative)	Dr. T. R. Sharma	86.39
12.	DBT	Molecular cloning and functional characterization of rice blast resistance genes – Second Phase	Dr. T. R. Sharma	80.41
13.	NAIP	Allele mining and expression profiling of resistance and avirulence genes in rice-blast pathosystem for development of race non-specific disease resistance	Dr. T. R. Sharma	1300.1
14.	ICAR	Bioinformatics and comparative genomics	Dr. T. R. Sharma	167.00
15.	ICAR-NPTC	Fusarium wilt resistance and drought tolerance in chickpea	Dr. Srinivasan	180
16.	NFBSFARA (ICAR)	Molecular and genetic analyses of guggul for the identification of genes governing adventive embryony	Dr. S. R. Bhat	240.9
17.	DBT	Development of haploid-inducer lines of *Brassica juncea* through genetic engineering of centromere histone protein	Dr. S. R. Bhat	64.1

S. No.	Funding Agency	Project Title	Principal Investigator	Budget (Rs. in Lakhs)
18.	NAIP (ICAR)	Unraveling molecular processes involved in adventive polyembryony towards genetic engineering for fixation of heterosis	Dr. S. R. Bhat	772.1
19.	NTPC (ICAR)	Functional genomics of *Alternaria* blight & drought tolerance in *Brassica*	Dr. S. R. Bhat	232.96
20.	NPTC (ICAR)	Development of aphid resistant transgenic Brassica	Dr. Rekha Kansal	110.76
21.	DBT	Design and construction of a stong promoter for constitutive overexpression of a *nifA* gene in *Azotobacter vinelandii*	Dr. J. C. Padaria	36.7
22.	ICAR	National initiative for climate resilient agriculture	Dr. J. C. Padaria	1046.00
23.	NFBSFARA (ICAR)	Phenomics of moisture deficit and low temperature tolerance in rice	Dr. P. K. Mandal	405.60
24.	CIMMYT	Improving productivity of wheat through enhanced nitrogen use efficiency	Dr. P. K. Mandal	26.60
25.	NPTC (ICAR)	Development of transgenic rice tolerant to drought and resistant to yellow stem borer	Dr. D. Pattanayak	140
26.	DST	Crop plants which remove their own major biotic constraints (Indo-Australia Grant Challenge Programme)	Dr. R. C. Bhattachrya	140.72
27.	NFBSFARA (ICAR)	Common basis of defense induction in rice and mustard against sucking and gall insect pests	Dr. R. C. Bhattachrya	63.55
28.	PPV&FRA	Establishment of Referral Laboratory for conducting special test	Dr. R. C. Bhattachrya	73.33
29.	NAIP	Understanding plant nematode interactions using RNAi	Dr. P. K. Jain	167
30.	NFBSFARA	Understanding plant nematode interaction: Identification of plant and nematode genes involved in disease development	Dr. P. K. Jain	50
31.	DBT	Molecular cloning and functional characterization of annexin multigene family from *Pennisetum glaucam*	Dr. S. Barthakur	45
32.	ICAR	Modeling network of gene response to abiotic stress in rice	Dr. K. Gaikwad	71.082
33.	DBT	Genomics and Phenomics of Ragi	Dr. Kanika	107.34
34.	DBT	Prospecting of RNA chaperon in microbes and development of osmotolerant rice	Dr. Kanika	24.17
35.	NPTC (ICAR)	Resistance to pod borer in pigeon pea	Dr. Rohini Sreevathsa	124.17
36.	NFBSFARA (ICAR)	Development of pod borer resistant transgenic pigeonpea and chickpea	Dr. Rohini Sreevathsa	88.93
37.	DBT	Translational research on root knot nematode tolerant RNAi transgenics based on vital parasite gene targets from validation to proof-of-concept to selection of event(s) in the field under confined conditions	Dr. Amolkumar U. Solanke	10.92
38.	NAIP	Genomics of cotton boll and fibre development	Dr. Amolkumar U. Solanke	250.81

Technology Commercialization and IPR

The mandate of the Institute Technology Management Unit relates to registration of patents, facilitation of contract research projects and commercialization of IPR enabled technologies of the centre through Public- Private Partnership.

The following activities were undertaken by the ITMU during the year 2013-14.

I. Patents applications filed

The following two applications were filed for India patent rights by NRCPB.

Nucleotide sequence of rice responsible for resistance to *Magnaporthe oryzae* and uses thereof (Application No. 783/DEL/2014)

This invention is related to the identification, cloning and characterization of *Pi54* gene, an isolated and cloned nucleic acid fragment from rice blast resistant wild rice *Oryza officinalis*. The Pi54 protein encoded by this gene is a unique disease resistance protein and its mechnasim of interaction with AVR-PI54 protein indicates that this protein might have different resistance mechanism as compared to *Pi54* and most of the cloned rice blast resistance proteins. Our complementation analysis reveals that cloned *Pi54* gene confers resistance to virulent strain of *Magnaporthe oryzae* isolate RML21. The cloned Pi54 gene can be used for resistance breeding either alone or through gene pyramiding approach.

Pathogen inducible promoter from rice and uses thereof (Application No. 782/DEL/2014)

This invention is related to the identification, cloning and characterization of *M. oryzae*-inducible promoter fragment, an isolated and cloned nucleic acid fragment from rice (*O. sativa* L.) indica cultivar HR 12. The *OsCYP76M7* gene (LOC_Os02g36110) which encodes a ent-cassadiene C11 α- hydroxylase involved in the production of antifungal phytocassanes was selected as a suitable candidate for promoter validation based on microarray analysis and quantitative PCR due to its higher expression after infection with *M. oryzae*. *In silico* analysis of upstream region (2004 bp) from TSS showed the presence of pathogen-responsive *cis* elements such as GT-1, ASF-1, W box and a BIHD1OS binding site. Functional validation of putative promoter region was carried out by constructing deletions of promoter fused with GUS reporter gene and transformation of Arabidopsis (Columbia ecotype) to obtain stable transgenic plants. GUS histochemical assay performed to check pathogen inducibility of the promoter showed that the promoter fragment of 2004 bp, now named Os-PiP01, (*Oryza sativa*-Pathogen Inducible Promoter) induced GUS expression in response to *M. oryzae* at 24hpi. The results obtained in transient expression assay of rice were found similar to that of Arabidopsis plants. Thus the *M. oryzae*- responsive promoter, *Os-PiP01*, identified in our study would be useful in the development of blast resistant cultivars.

II. MoUs/MTAs signed

S.No.	With whom	Purpose	Date of Signing MoUs/MTAs
1.	Guru Jambeshwar University of Science and Technology, Hisar	To facilitate and support the research of Ph.D students registered at the University who are either working as Project Fellows in NRCPB or have their own source of funding like the CSIR-UGC fellowship.	25.10.2013
2.	Indian Institute of Pulses Research, Kanpur	Transfer of *cry1Ac* gene	07.06.2013
3.	Centre for Biotechnology, M.D. University, Rohtak	Transfer of Codon modified Bt *cry2Aa* and *cry1AcF* genes.	25.10.2013
4.	Dept. of Genetics & Plant Breeding, Annamalai University, Annamalai Nagar	Transfer of Codon modified Bt *cry2Aa* and *cry1AcF* genes.	25.10.2013
5.	Panjabrao Deshmukh Krishi Vidyapeeth, Akola	Transfer of *cry1Abc* gene	01.03.2014

Awards and Honours

Prof. R. P. Sharma received INSA Silver Jubilee Commemoration Medal – 2013

Prof. R. P. Sharma bagged Quality Initiative Mission Education Leadership Award - 2013 by KRD Welfare Group

Dr. T. R. Sharma elected as fellow of Indian National Science Academy, New Delhi

Dr. T. R. Sharma was awarded J. C. Bose National Fellowship by DST, GoI

Dr. T. R. Sharma was awarded NASI Reliance Platinum Jubilee Award 2013 for application oriented research in Biological Sciences

Dr. T. R. Sharma was elected Chief Editor, Journal of Plant Biochemistry and Biotechnology, Jan 2009 till date

Dr. P. K. Mandal was awarded the Fellowship of the Indian Society of Agricultural Biochemists

Dr. Kanika received second best paper award in National Conference on Changing Scenario of Agriculture in Madhya Pradesh: Prospects and Challenges, held at Bhopal, Madhya Pradesh, Sept 1-2, 2013

Dr. Kanika was elected as Fellow Society of Applied Biotechnology, India

Dr. Kanika was elected as member of Editorial Board of Universal Journal of Microbiological Research

Dr. Kanika was invited as a panelist at the Plant Genomics Congress Asia, held on February 24-25, 2014, Kuala Lumpur, Malaysia

Dr. Rhitu Rai and her group received Best Poster award in National Seminar in Plant Biotechnology: Challenges and opportunities in 21st Century held at Jamia Hamdard, New Delhi from 3rd-4th Mar, 2014

Dr. Amolkumar U. Solanke received C. V. Jacob Award (2013) from Society for Biotechnologists (India), Cochin, Kerala for Best original research paper presentation in plant biotechnology

Visits Abroad

- Dr. N. K. Singh participated the 7th International Rice Genetics Symposium on 05-07 November, 2013 held in Philippines
- Dr. N. K. Singh attended IWGSC workshop meeting on 11-15th January, 2014 at San Diego, USA
- Dr. N. K. Singh attended "Physical Mapping & Sample sequencing of wheat Chromosome 2A- International Wheat Genome Sequencing Consortium (India)" on 16-20 January, 2014 at CIMMYT, Mexico
- Dr. Vandna Rai visited Meijo University, Nagoya, Japan for study/ training programme in International Centre for Green Biotechnology Program by Research Centre of Meijo University on 10th September, 2013 to 9th October, 2013
- Dr. Rhitu Rai visited USA to participate in the Plant & Animal Genome (PAG) XXII Conference on 11-15th January, 2014 at San Diego California, USA
- Dr. Ajay Jain visited USA to participate in National Science Foundation meeting on 6th August, 2013 to 30th October, 2013 at West Kentucky, University, USA

Linkages and Collaboration

The centre has an active and close collaboration with other ICAR institutes and State Agricultural Universities and is instrumental in providing gene constructs for transgenic development targeting biotic and abiotic tolerance and quality improvement. It also plays a pivotal role in developing genomic resources, both functional and structural, for a variety of crop species. The scientists at the centre work in close collaboration with different disciplines of the Indian Agricultural Research Institute like Biochemistry, Plant Physiology, Genetics, Entomology, Pathology, Nematology, Vegetable Science, Horticulture, Microbiology and Water Technology Centre. With the ever-increasing need of computational and statistical intervention in the area of modern molecular biology and biotechnology, the centre has also developed active collaboration with the Indian Agricultural Statistics Research Institute. Over the years the centre has developed working linkages with the international laboratories.

A. Inter-Institutional Linkages

- Delhi University, New Delhi
- Jawaharlal Nehru University, New Delhi
- Chaudhary Sarwan Kumar Krishi Vishwavidyalaya, Palampur
- Central Rice Research Institute, Cuttack
- Directorate of Medicinal and Aromatic Plants, Anand
- University of Agricultural Sciences, Dharwad
- Punjab Agricultural University, Ludhiana
- Institute of Himalayan Bioresources and Technology, Palampur
- Tamil Nadu Agricultural University, Coimbatore
- DWR-Flowerdale Shimla
- National Bureau of Plant Genetic Resources, New Delhi
- Indian Agricultural Statistical Research Institute, New Delhi
- Bose Institute, Kolkata
- Indian Institute of Technology, Kanpur
- International Centre for Genetic Engineering and Biotechnology, New Delhi
- Twenty three ICAR institutes under the Network Project on Transgenics
- Twenty seven ICAR institutes, SAUs and others IITs in NAIP Mega Project entitled "Bioprospecting of genes and allele mining for abiotic stress tolerance"

B. International Linkages

- Division of Plant Pathology, UC Davis, California
- International Rice Research Institute, The Phillipines
- Sixteen institutes under International Wheat Genome Sequencing Project

List of Publications

Research Article

- Ahmed I, Solanke AU, Deshmukh DP, Kanakachari M, Sreevathsa R, Pattanayak D, Sharma TR & Kumar PA. 2014. Isolation and characterization of ethylene responsive factor (ERF) genes from brinjal. *Indian J Hort* **71**: 49-54.
- Barthakur S. 2013. Efficient *in vitro* regeneration and *Agrobacterium* mediated transformation protocol of an elite tomato cultivar (*Solanum lycopersicum* cv Pusa Gaurav). *Annl Agric Res New Series* **34:** 106-113.
- Bhattacharya R, Koramutla MK, Negi M, Pearce G & Ryan CA. 2013. Hydroxyproline-rich glycopeptide signals in potato elicit signalling associated with defense against insects and pathogens. *Plant Sci* **207**: 88-97.
- Chitra N, Gupta V, Kumar K & Prasanna R. 2013. Molecular Characterization of a fungicidal endoglucanase from the cyanobacterium *Calothrix elenkinii*. *Biochem Genet* **51**: 766-779.
- Choudhary P, Khanna SM, Jain PK, Bharadwaj C, Kumar J, Lakhera PC & Srinivasan R. 2013. Molecular characterization of primary gene pool of chickpea based on ISSR markers. *Biochem Genet* **51**: 306-322.
- Danekar P, Tyagi A, Mahto A, Singh A, Raje RS, Gaikwad K & Singh NK. 2014. Genomewide characterization of Hsp 100 family genes from pigeon pea. *Indian J Genet Plant Breed* (Accepted).
- Dash PK**.** 2013. High quality RNA isolation from polyphenol-, polysaccharide- and protein-rich tissues of lentil (*Lens culinaris*). *Biotech* **3**: 109-114.
- Dogra T, Priyadarshini A, Kanika, Kumar A & Singh NK. 2013. Identification of genes involved in salt tolerance and symbiotic nitrogen fixation in chickpea rhizobium *Mesorhizobium ciceri* Ca181. *Symbiosis* **61**:135-143.
- Dutta S, Mehto AK, Sharma P, Raje RS, Sharma TR & Singh NK. 2013. Highly variable 'Arhar' simple sequence repeat markers for molecular diversity and phylogenetic studies in pigeonpea *Cajanus cajan* (L.). *Plant Breeding* **132**: 191-196.
- Geetha KA, Kawane A, Bishoyi AK, Phurailatpam A, Ankita C, Malik SK, Srinivasan R & Bhat SR. 2014. Characterization of mode of reproduction in *Commiphora wightii* [(Arnot) Bhandari] reveals novel pollen-pistil interaction and occurrence of obligate sexual female plants. *Trees Struct Func* **27**: in press.
- Goyal E, Singh RS & Kanika. 2013. Isolation and functional characterization of *Salt overly sensitive 1* (*SOS1*) gene promoter from *Salicornia brachiata*. *Biol Plant* **57**: 465-473.
- Jain A, Sinilal B, Dhandapani G, Richard B. Meagher RB, Shivendra V & Sahi SV. 2013. Effects of deficiency and excess of zinc on morphophysiological traits and spatiotemporal regulation of zinc-responsive genes reveal incidence of cross talk between micro- and macronutrients. *Environ Sci Tech* **47**: 5327-5335.
- Jayakumar S & Kaur S. 2013. Occurrence of *cry* genes in *Bacillus thuringiensis* (Bt) isolates recovered from phylloplanes of crops growing in the New Delhi region of India and toxicity towards Diamond-back moth (*Plutella xylostella*). *J Biol Sci* **13**: 463-473.
- Jayanthi M, Sarika N, Sujatha G, Mathur RK, Rao CS & Mandal PK. 2013. Evaluation of SSRs (microsatellites) for detecting genetic variability

in oil palm (*Elaeis guineensis*) clone. *Curr Hort* **1**: 3-6.

- Karthikeyan AS, Jain A, Nagarajan VK, Sinilal B, Sahi SV & Raghothama KG. 2014. *Arabidopsis thaliana* mutant *lpsi* reveals impairment in the root responses to local phosphate availability. *Plant Physiol Biochem* **77**: 60-72.
- Katara J, Deshmukh R, Singh NK & Kaur S. 2013. Diversity analysis of *Bacillus thuringiensis* isolates recovered from diverse habitats in India using random amplified polymorphic DNA (RAPD) markers. *J Biol Sci* **13**: 514-520.
- Keshavareddy G, Rohini Sreevathsa, S. V. Ramu, Sundaresha S., A. R. V. Kumar, P. Ananda Kumar & Udayakumar M (2013). Transgenics in groundnut (*Arachis hypogaea* L.) expressing *cry1AcF* gene for resistance to *Spodoptera litura* (F.). *Physiol Mol Biol Plants* DOI 10.1007/s12298-013-0182-6.
- Khanna SM, Choudhary P, Saini R, Jain, PK & Saini R. 2014. Specific activities and transcript levels of glycolytic enzymes under dehydration in chickpea (*Cicer arietinum* L.) seedlings. *Legume Res* (Accepted).
- Khanna SM, Choudhary P, Saini R**,** Jain, PK & Srinivasan R. 2014. Effect of water deficit stress on growth and physiological parameters in chickpea cultivars differing in drought tolerance. *Annals Biol* **30**: 77-84.
- Khomdram S**,** Barthakur S & Devi GAS. 2013. Water stress inducible proline transporter from *indica* rice. *Annl Agric Res New Series* **34**: 189-196.
- Khomdram S, Barthakur S & Devi GAS. 2014. Biochemical and molecular analysis of wild endemic fruits of the manipur region of India. *Intl J Fruit Sci* **14**: 1-14.
- Koramutla MK, Kaur A, Negi M, Venkatachalam P & Bhattacharya RC. 2014. Elicitation of jasmonate mediated host defense in *Brassica juncea* (L.) attenuates population growth of mustard aphid *Lipaphis erysimi* (Kalt.). *Planta* (DOI 10.1007/s00425-014-2073-7).
- Kuhar K, Kansal R**,** Subrahmanyam B, Koundal KR, Miglani K & GuptaVK. 2013. A Bowman–Birk protease inhibitor with antifeedant and antifungal activity from *Dolichos biflorus*. *Acta Physiol Plant* **35**: 1887-1903.
- Kulkarni KP, Vishwakarma C, Sahoo SP, Lima JM, Nath M, Dokku P, Gacche RN, Mohapatra T, Robin S, Sarla N, Seshashayee M, Singh AK, Singh K, Singh NK & Sharma RP. 2013. Phenotypic characterization and genetic analysis of dwarf and early flowering mutants of rice variety Nagina22. *Oryza* **50**:18-25.
- Kumar M, Prasanna R, Lone S, Padaria JC & Saxena AK. 2014. Cloning and expression of *dnaK* gene from *Bacillus pumilus* of hot water spring origin. *Appl Trans Genom* 3: 14-20.
- Kumar PP, Prasad NG, Kamaraju D, Banakar P, Rohini Sreevathsa & Rao U. 2013. Utility of host delivered RNAi of two FMRF amide like peptides, *flp-14* and *flp-18*, for the management of root knot nematode, *Meloidogyne incognita*. *PLOS ONE* **8**: e80603.
- Kumar S, Malik SK, Uchoi A, Chaudhury R & Bhat SR. 2014. A new wild type of Citron (*Citrus medica* L., Rutaceae) identified through morphology and *psb*M-*trn*D spacer region of chloroplast DNA. *Trees* (Accepted).
- Kumar T, Bharadwaj C, Tara Satyavathi C & Jain PK. 2013. A high throughput, improved rapid and reliable genomic DNA extraction protocol from chickpea (*Cicer arietinum* L.). *Vegetos* **26**: 185-190.
- Kumar V, Malik SK, Pal D, Srinivasan R & Bhat SR. 2014. Comparative transcriptome analysis of ovules reveals stress related genes associated with nucellar polyembryony in citrus. *Tree Genet Genom* **10**: DOI 10.1007/s11295-013-0690-0.

- Kumari A, Das A, Devanna BN, Thakur S, Singh PK, Singh NK & Sharma TR. 2013. Mining of rice blast resistance gene *Pi54* shows effect of single nucleotide polymorphisms on phenotypic expression of the alleles. *Eur J Plant Pathol* **137**: 55-65.
- Liu Z-L, Li Y-J, Hou H-Y, Zhu X-C, Rai V, He X-Y & Tian C-J. 2013. Differences in the arbuscular mycorrhizal fungi-improved rice resistance to low temperature at two N levels: Aspects of N and C metabolism on the plant side. *Plant Physiol Biochem* **71**: 87-95.
- Manjulatha M, Rohini Sreevathsa, Manoj K, Sudhakar C, Prasad TG, Tuteja N & Udayakumar M. 2013. Overexpression of a pea DNA helicase (PDH45) in peanut (*Arachis hypogaea* L.) confers improvement of cellular level tolerance and productivity under drought stress. *Mol Biotechnol* (DOI 10.1007/s12033-013-9687-z).
- Manjunath KC, Mahadeva A, Rohini Sreevathsa, Ramachandra N, Prasad TG. 2013. *In Vitro* screening and identification of putative sunflower (*Helianthus annuus* L.) transformants expressing *ECNAC1* gene by salt stress method. *Trends Biosci* **6**: 108-111.
- Meena RK & Kaur S. 2014. Screening of *Bacillus thuringiensis* isolates recovered from diverse habitats in India for the presence of insect and nematode-active *cry* genes. *Intl J Agri Environ Biotech* **7**: 55-62.
- Mittal A, Nagar S, Kansal R & Gupta VK. 2013. Standardization of trypsin inhibitor extraction from kidney bean and cumulative effect of temperature, incubation time and pH on its activity. *Indian J Agric Biochem* **26**: 118-124.
- Mohapatra T, Robin S, Sarla N, Sheshahsayee M, Singh AK, Singh K, Singh NK. Amitha Mithra SV & Sharma RP. 2014. EMS induced mutants of upland rice variety Nagina22: generation and characterization *Proc Indian Natn Sci Acad* **80**: 163-172.
- Mukesh Sankar S, Tara Satyavathi C, Singh MP, Bharadwaj C, Singh SP & Barthakur S. 2013**.** Genetic vaiability and association studies in pearl millet for grain yield and high temperature stress tolerance. *Indian J Dryland Agric Res Dev* **28**: 71-76.
- Nepolean T, Hossain F, Shiriga K, Mittal S, … Amitha Mithra SV, Mohapatra T & Gupta HS. 2013. Unraveling the genetic architecture of subtropical maize (*Zea mays* L.) lines to assess their utility in breeding programs. *BMC Genomics* **14**: 877.
- Padaria JC, Choudhary M & Tarafdar A. 2013. An efficient protocol for genomic DNA isolation from field grown mature leaves of *Penniseteum gluacum* [(L) R. Br.]. *Res J Biotechnol* 8: 30-34.
- Panigrahy M, Rao DN, Yugandhar P, Sravan Raju N, Krishnamurthy P, Voleti SR, Reddy GA, Mohapatra T, Robin S, Singh AK, Singh Kuldeep, Sheshshayee M, Sharma RP & Sarla N. 2013. Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. *Arch Agro Soil Sci*, DOI:10.1080/03650340. 2013.821197, Francis & Taylor UK,
- Patil M, Ramu SV, Jathish P, Rohini Sreevathsa, Reddy PC, Prasad TG, Udayakumar M. 2013. Overexpression of AtNAC2 (ANAC092) in groundnut (*Arachis hypogaea* L.) improves abiotic stress tolerance. *Plant Biotechnol Rep* (DOI 10.1007/ s11816-013-0305-0)
- Pattanayak D, Solanke A U & Kumar PA. 2013. Plant RNA interference pathways: diversity in function, similarity in action. *Plant Mol Biol Rep* **31**: 493-506.
- Pradhan A, Shahi VK & Sinha SK. 2013. Evaluation of genetic diversity in Faba bean (*Vicia faba* L.) genotypes using seed protein and isozymes electrophoresis. *Natl Acad Sci Lett:* (Accepted).
- Prasad NG, Kumar PP, Kumar PT, Kamaraju D, Rohini Sreevathsa & Rao U. 2013. Selection and

validation of reference genes for quantitative gene expression studies by real-time PCR in eggplant (*Solanum melongena* L). *BMC Res Notes* **6**: 312.

- Pratibha P, Singh SK, Sharma I, Kumar R, Srinivasan R, Bhat SR, Ahuja PS & Sreenivasulu Y. 2013. Characterization of a T-DNA promoter trap line of *Arabidopsis thaliana* uncovers a cryptic bi-directional promoter. *Gene* **524**: 22-27.
- Praveena D, Chowdappa KR, Mandal PK & Kumar N. 2013. Development of molecular diagnostic kit for early detection of basal stem rot in oil palm (*Elaeis guineensis* Jacq). *Intern J Oil Palm* **8**: 23-26.
- Pushpalatha G, Sreenu K, Jain A, Suresh T, Subrahmanyam D, Ram T, Subbarao LV, Giri A, Sarla N & Rai V. 2014. Allele mining of salt tolerance genes in *Oryza sativa* x *O. rufipogon* introgression lines and functional analysis of *cis*-acting regulatory elements. *World Res J Bioinfo* **2**: 25-32.
- Pushpalatha G, Subrahmanyam D, Sreenu K, Ram T, Subbarao LV, Parmar B, Giri A, Sarla N & Vandna Rai. 2014. Effect of salt stress on seedling growth and antioxidant enzymes in two contrasting rice introgression lines. *Indian J Plant Physiol* **18**: 360-366.
- Ram K, Padaria JC & Singh A. 2013. Isolation and characterization of symbiotically defective pyrimidine and amino acid auxotrophs of *Mesorhizobium ciceri* in chickpea. *J Environ Biol* **34**: 793-797.
- Ramaiah M, Jain A & Raghothama KG. 2014. *ETHYLENE RESPONSE FACTOR070* regulates root development and phosphate starvation-mediated responses. *Plant Physiol* **164**: 1484-1498.
- Rawal HC, Singh NK & Sharma TR. 2013. Conservation, divergence, and genome-wide distribution of PAL and POX A gene families in plants. *Intl J Genomics* ID 678969, 10.
- Sah P, Kalia P, Sonah H & Sharma TR. 2013. Molecular mapping of black rot resistance locus *Xca1bo* on chromosome 3 in Indian cauliflower (*Brassica oleracea* var. botrytis L.). *Plant Breeding* doi:10.1111/pbr.12152.
- Shanmugavadivel PS, Amitha Mithra SV**,** Prasad D, Anand RKK, Rao GJN, Singh VP, Singh AK, Singh NK & Mohapatra T. 2013. Mapping quantitative trait loci (QTL) for grain size in rice using a RIL population from basmati x indica cross showing high segregation distortion. *Euphytica* **194**: 401-416.
- Sharma R, Arya S, Patil SD, Sharma A, Jain PK, Navani, NK & Pathania R. 2014. Identification of novel regulatory small RNAs in *Acinetobacter baumannii*. *PLoS ONE* **9**: e93833 (doi:10.1371/journal.pone.0093833).
- Sharma RP. 2013. Wingless to Wnt: discovery of conserved cell signaling gene family in the animal kingdom. *Curr Sci* **104**: 1140-1141.
- Shikari AB, Khanna A, Gopala Krishnan S, Singh UD, Rathour R, Tonapi V, Sharma TR, Nagarajan M, Prabhu KV & Singh AK. 2013. Molecular analysis and phenotypic validation of blast resistance genes *Pita* and *Pita2* in landraces of rice (*Oryza sativa* L.). *Ind J Genet* **73**: 131-141.
- Sindhu R, Singh Y, Sundaresha S, Pattanayak D, Singh BP & Rawat S. 2013. Homology modeling and structural analysis of potato vacuolar acid invertase. *Intl J Compu Bioinfo In Silico Model* **2**: 167-172.
- Singh A, Chand D & Pattanayak D. 2013. Purification and characterization of potato ribonuclease P. *J Plant Biochem Biotechnol* **22**: 425-433.
- Singh S, Sharma SR, Kalia P, Sharma P, Kumar V, Kumar R, Meena BL, Kumar S & Sharma TR. 2013. Screening of cauliflower germplasm for resistance to downy mildew and designing appropriate multiple resistance breeding strategies *J Hort Sci Biotech* **88**: 103-109.

- Sinha SK, Kumar M, Kumar A, Bharti S & Shahi VK. 2013. Antioxidant activities of different tissue extract of Faba bean (*Vicia faba* L.) containing phenolic compounds. *Legume Res* **36**: 496-504.
- Thakur S, Gupta YK, Singh PK, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Chand D, Rana JC, Singh NK & Sharma TR. 2013. Molecular diversity in rice blast resistance gene *Pi-ta* makes it highly effective against dynamic population of *Magnaporthe oryzae*. *Funct Integr Genom* **13**: 309-322.
- Thakur S, Singh PK, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Chand D, Singh NK & Sharma TR. 2013. Positive selection pressure on rice blast resistance allele *Piz-t* makes it divergent in Indian land races. *J Plant Interact* **8**: 34-44.
- Tiwari JK, Poonam, Chakrabarti SK, Kumar V, Gopal J, Singh BP, Pandey SK & Pattanayak D. 2013. Identification of host gene conferring resistance to *Potato virus Y* using *Ry* gene-based molecular markers. *Indian J Hort* **70**: 373-377.
- Tula S, Ansari MW, Prasad Babu A, Pushpalatha G, Sreenu K, Sarla N, Tuteja N & Rai V. 2013. Physiological assessment and allele mining in rice cultivars for salinity and drought stress tolerance. *Vegetos* **26**: 219-228.
- Vijayan J, Jain S, Jain N, Devanna BN, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Singh NK & Sharma TR. 2013. Identification of differentially expressed genes in rice. *Indian J Genet* **73**: 233-243.
- Yamagishi H & Bhat SR. 2014. Cytoplasmic male sterility and mitochondrial genes in Brassicaceae crops. *Breeding Science* (Accepted).

Popular Article

- Dalal M. 2013. An important gene conferring significant increase in grain yield to *indica* rice discovered. *Agbiotech Digest* (Oct-Dec) 4 (3).
- Dalal M. 2013. Genes for resistance to stem rust identified in wheat. *Agbiotech Digest* (July-Sept) 4(2):
- Dash PK & Rai R. 2013. Artificial photosynthesis. *Sci Horizon* **3**: 21.
- Dash PK & Rai R. 2013. Healthy like a horse. *Sci Rep* **50**: 32-34.
- Dash PK, Gupta P & Rai R .2013. Father of engineered babies passes away. *Sci Rep* **50**: 26-27.
- Dash PK, Jain PK, Gaikwad K & Rai R. 2013. Are cloned animals healthy? *Sci Rep*: 16-17.
- Gaikwad K. 2013. Forward and reverse genetics to determine gene function in plants. Epub: http://www.biotecharticles.com, 8th Aug, 2013.
- Gupta NC. 2013. Biotechnological approaches for isolating the genes of agronomic significance. *http://www.biotecharticles.com/Biotech-Research-Article/Biotechnological-Approaches-for-Isolating-the-Genes-of-Agronomic-Significance-2990.html.*
- Gupta NC. 2013. Isolation of a plant promoter by T-DNA insertional mutagenesis. *http://www.biotecharticles.com/Biotech-Research-Article/Isolation-of-a-Plant-Promoter-by-T-DNA-Insertional-Mutagenesis-2982.html.*
- Gupta NC. 2013. Characterization of a plant RNA Pol-II promoter sequence. *http://www.biotecharticles.com/Biotech-Research-Article/Characterization-of-a-Plant-RNA-Pol-II-Promoter-Sequence-2983.html.*
- Kanika. 2013. Transgenics: Challenges and achievements. *J Rur Agril Res* **13**: 9-12.
- Kanika, Singh DP, Singh AK and Kumar S. 2013. Kam pani mein lahlaha sakti hain faslein. *Krishi Samvad*: 30-32.
- Kanika and Shiv Kumar. 2013. Concerns of Indian agriculture to climate change. *http://www.biotecharticles.com/Agriculture-Article/Concerns-of-Indian-Agriculture-to-Climate-Change-2999.html.*
- Kanika and Shiv Kumar. 2013. Way out to manage climate change in Indian agriculture. *http://*

www.biotecharticles.com/Agriculture-Article/Way-Out-to-Manage-Climate-Change-in-Indian-Agriculture-3001.html.

- Kanika and Shiv Kumar. 2013. Bt cotton in India icebreaking for transgenics. *http://www.biotecharticles.com/Agriculture-Article/Bt-Cotton-in-India-Icebreaking-for-Transgenics-3004.html.*
- Kanika and Shiv Kumar. 2013. Synchronization of sources of growth with agricultural research priorities in India. *http://www.biotecharticles.com/Agriculture-Article/Synchronization-of-Sources-of-Growth-With-Agricultural-Research-Priorities-in-India-3011.html.*
- Kanika and Shiv Kumar. 2013. Dynamics of agricultural research and development system in India. *http://www.biotecharticles.com/Agriculture-Article/Dynamics-of-Agricultural-Research-and-Development-System-in-India-3019.html.*
- Kanika and Shiv Kumar. 2014. Transgenics-concerns and prospects. *http://www.biotecharticles.com/Biotech-Research-Article/Transgenics-Concerns-and-Prospects-3189.html.*
- Kumar S, Kanika, Singh DR & Kumar S. 2013. Sushk kshetron mein Karagr hain fawarra sinchai. *Krishi Samvad*: 18-21.
- Mithra Amitha SV & Solanke AU. 2013. Role of functional markers in plant breeding. (*http://www.biotecharticles.com*).
- Mithra Amitha SV & Solanke AU. 2013. Random and insertional mutagenesis for functional genomics in plants (*http://www.biotecharticles.com*).
- Mithra Amitha SV & Solanke AU. 2013. SNPs in complex plant genomes-discovery and genotyping. (*http://www.biotecharticles.com*)
- Mithra Amitha SV & Mandal PK. 2013. The discovery of shell gene will significantly boost palm (*Elaeis guineensis*) breeding in plantation. *Agbiotech Digest* **4** (October-December 2013). Published by DKMA, ICAR.
- Rai R, Dash PK, Gaikwad K & Jain PK. 2013. Phenotypic and molecular profiling of indigenous chickpea rhizobia in India. Epub: *CIBTech Journal of Microbiology* **2**: 33-38.
- Sinha SK. 2014. Agricultural nitrogen cycle and mechanism of N Loss: need for nitrogen use efficient plants. *Biotecharticles.com*
- Sinha SK. 2014. Micro RNA homeostasis: its stability and degradation. *Biotecharticles.com*
- Solanke AU & Mithra Amitha SV. 2013. Tomato genome for comparative genomics. (*http://www.biotecharticles.com*).
- Solanke AU & Mithra Amitha SV. 2013. Genetic and genomics resources in 'Finger Millet- A climate resilient Nutri-Millet'. (*http://www.biotecharticles.com*).
- Solanke AU & Mithra Amitha SV. 2013. Omics approaches for identification of stress responsive genes. (*http://www.biotecharticles.com*).
- Vinutha T, Rama Prashat G, Veda Krishnan, Rohini Sreevathsa A, Singh A & Dahuja A. 2013. Soybeans: A journey from consumption towards health benefits. *Agrobios New letter* **12**: 76-77.

Book Chapters/Manuals

- Behera DB, Solanke AU & Ramawatar N. 2013. Gene prediction, annotation and primer designing. In: *DBT Sponsored Short Course/Training on Advances in Plant Genetic Engineering,* (28th Oct – 2nd Nov, 2013), (Srinivasan, PK Dash, R Rai, AU Solanke & DB Behera, eds.), NRCPB, New Delhi-110012, pp 99-109.
- Chinnusamy V, Dalal M & Zhu J-K. 2013. Epigenetic regulation of abiotic stress responses in plants. In: *Plant Abiotic Stress,* Second Edition, (MA Jenks & PM Hasegawa, eds). John Wiley & Sons, Inc.
- Dalal M & Chinnusamy V. 2014. Molecular genetic approaches for improvement of drought tolerance in rice. *In Abiotic Stress Tolerance in Crop*

Plants, (M Prakash & K Balakrishnan, eds.). Satish Serial Publishing House, Delhi. Pg 1-22, ISBN. 978-93-81226-92-6.

- Gupta NC & Jain PK. 2013. Cloning and characterization of a wound-inducible promoter from *Arabidopsis thaliana*. In: *Recent Adv Biotechnol, pp.*69-78, *ISBN-978-81-928063-1-0.*
- Gupta NC & Rao M. 2013. *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* by floral dip method" in ICAR Winter school training manual on "*Frontier Technologies, in the Area of Biotechnology on Gene Isolation, Characterization and Breeding with Reference to Abiotic Stress Related Genes",* 10th - 30th Dec, 2013. pp 42-48.
- Gupta NC, Kumar K, Kansal R & Padaria JC. 2013. A Teaching manual for the PG course MBB510: "Biotechnology LAB-I". pp 1-73.
- Kanika, Singh AK, Solanke AU & Dogra T. 2013. Role of microbes in mitigating effect of abiotic stress in agriculture. In: *Abiotic stress: Challenges and prospects* (in press).
- Kumar K, Goyal E, Singh AK, Singh DP & Nain L. 2013. Genomic approaches for identification of genes from PGPR involved in plant growth and antibiosis. *In: Fertilizer Technology* Vol.1, Studium Press LLC, USA (in press).
- Kumar K, Mahesh MRM, Singh DP, Etika G & Singh AK. 2014. Exploitation of extremophilic microbes from hot water springs of India to mitigate the effect of climate change on agriculture. In: *Proc. National Symposium on Emerging trends in Biotechnology* 2014. Organized by Department of Biotechnology, CUSAT, Cochin, Kerala, on 24-25 January, pp. 119-130.
- Lone SA, Malik A & Padaria JC. 2014. Application of *Bacillus thuringiensis* for prevention of environmental deterioration. In: *Environmental Deterioration and Human Health* (A Malik, E Grohmann & R Akhatar, eds.), Springer Publishing House, Heidelberg, New York pp: 73-95.
- Padaria JC. 2013. Polyacrylamide-SDS gel electrophoresis. In: *Training manual of ICAR sponsored winter school on frontier technologies in the area of biotechnologies on gene isolation, characterization and breeding with reference to abiotic stress related genes.* (PK Jain, K Gaikwad, R Rai & NC Gupta, eds.), 10th-30th Dec 2013, National Research Centre on Plant Biotechnology, Pusa campus, New Delhi-110012.
- Rethinam P, Mandal PK. Mathur RK & Murugesan P. 2013. Oil palm, In: *Palms and Palm Wilt*, (HP Singh, GV Thomas & V Krishnakumar, eds.), Westville Publishing House, New Delhi. pp 95-132.
- Rohini Sreevathsa A, Pattanayak D & Kumar PA. 2013. Impact of genetically engineered insect resistant crops in developing countries,. In: *Genetically Engineered Crops in Developing Countries*, (DV Reddy, P Ananda Kumar, G Loebenstein & P Lava Kumar, eds.), Studium Press, Texas (USA), New Delhi (India).
- Solanke AU & Kumar PA. 2013. Tomato phenotyping. In: *Phenotyping for Plant Breeding, Applications of Phenotyping methods for Crop Improvement*, (Siva Kumar Panguluri & Ashok Kumar Are, eds.), Springer publications, pp 169-204.
- Solanke AU, Raghavendrarao S, Kanika & Pattanayak D. 2013. Heat stress responses in plants: insight into physiological and molecular mechanisms. In: *Abiotic stress: Challenges and prospects* (in press).
- Solanke AU, Sonam & Kanakachari M. 2013. Gene expression analysis through quantitative real time PCR. In: *DBT Sponsored Short course/Training on Advances in plant genetic engineering* (28th Oct – 2nd Nov 2013), (Srinivasan, PK Dash, R Rai, AU Solanke & DB Behera, eds.), NRCPB, New Delhi-110012, pp 111-120.
- Solanke AU, Sonam, Kanakachari M & Ramakrishna Ch. 2013. Expression analysis of heat responsive finger millet genes by qRT-PCR.

In: *Training Manual of ICAR Sponsored Winter School on Frontier Technologies in the Area of Biotechnologies on Gene Isolation, Characterization and Breeding with Reference to Abiotic Stress Related Genes* (10th-30th Dec 2013), (PK Jain, K Gaikwad, R Rai & NC Gupta, eds.), NRCPB, New Delhi-110012.

- Solanke AU. 2013. Molecular insights into fruit size, shape and colour of eggplant (*Solanum melongena* L.), In: *Recent Adv Biotechnol*, Vol. 2, (SK Mishra ed.), pp 40-46, ISBN-978-81-928063-1-4.
- Solanke, AU, Tribhuvan KU & Kanika. 2013. Genomics: An integrative approach for molecular biology. In: *Vistas in Biotechnology: Possibilities and potentials*. Studium Press LLC, USA (in press).
- Yadav RC, Solanke AU, Kumar P, Pattanayak D, Yadav NR & Kumar PA. 2013. Genetic engineering for tolerance to climate change-related traits. In: *Genomics and Breeding for Climate-Resilient Crops*, Vol. I, (Kole C, ed.), Spinger-Verlag, Berlin, Heidelberg 2013, pp 285-330.

Papers in Seminar/Symposia/ Conference

- Amitha Mithra SV, Shanmugavadivel PS, Kumawat G, Mohapatra T & Singh NK. 2013. Molecular dissection of complex traits in plants using molecular markers with special emphasis on microsatellites and SNPs. In: *National Seminar on Biotechnology, Biodiversity and Environment*, held at Govt. New College, Rewa, M. P. on 19.04.2013.
- Arora K, Rai AK, Narula A & Sharma TR. 2013. Analysis of positional effect of blast resistance gene *Pi54* on its phenotypic expression in transgenic rice lines. Presented at 11th *International symposium on rice functional genomics: sustaining food and nutritional security*, New Delhi. pp 124 (PVI-149).
- Barthakur S, Deka S, Kumar R & Pandey N. 2013. Annexins from indica rice: an analysis under abiotic stress, In: *First international and third national conference on Biotechnology, Bioinformatics and Bioengineering* 28-29 June, Tirupati, India.
- Barthakur S. 2014. Molecular prospecting for terminal heat stress tolerance in bread wheat (*Triticum aestivum*). In: *National Conference Science of Omics for Agricultural Productivity: Future Perspectives,* March 4-6, G. B. Pant University of Agriculture & Technology, Pantnagar, India.
- Bhattacharya RC. 2014. Peptide signals of broad spectrum resistance in plants. *The National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* 4-6 March, G. B. Pant University of Agriculture & Technology, Pantnagar.
- Biswas K, Prabu R, Vishwakarma H, Bhatt D, Raipuria R, Tarafdar A, Kamle M, Yadav R, Lone SA, Choudhary M, Bhati M, Prakash A, Chauhan H, Kumari P, Jasrotia R, Srivastava, S, Singh G, Singh R & Padaria JC. 2013. Transcript profiling of heat stress responsive genes from bread wheat (*Triticum aestivum* L.) under different developmental stages. In: *Proceeding of Indraprastha International Conference on Biotechnology,* organized by University School of Biotechnology, GGS Indraprastha University, New Delhi, February 22-25th.
- Dalal M. 2013. Oral presentation on Genome-wide identification and expression analysis of genes encoding ABA core signaling components in sorghum. In *National conference of plant physiology on current trends in plant biology research*, Directorate of Groundnut Research Junagadh, Gujarat. Dec 13-16, 2013
- Devanna BN, Rai AK, Vijayan J & Sharma TR. 2013. Sub-cellular localization and molecular characterization of rice blast resistance gene *Pi54* cloned from *Oryza officinalis*. Presented at 11th *International symposium on rice functional genomics: sustaining food and nutritional security*, New Delhi. pp 123 (PVI-146).
- Gaikwad K. 2013. Pigeonpea genomics In: International Conference on "*Impact of Technological*

Tools on Food Security under Global Warming Scenario (ITTFS 2012)" at Shobhit University, Modipuram, Meerut, India, on 11-12, May.

- Gothandapani S, Sankar P, Sekar S, Das H K & Padaria JC. 2014.Construction of a plasmid vehicle for evaluation of promoter suitable for use in gram negative bacteria In: *Proceeding of National Conference on Science of Omics for Agricultural Productivity: Future Perspectives,* organized by G. B. University of Agriculture and Technology, Pantnagar, Uttarakhand, May 4-6th March.
- Grover A, Ali S, Rawat S & Chamil N. 2014. Emerging strategies to develop fungus-resistant transgenic plants. *National Conference on Science of Omics for Agricultural Productivity: Future Prospective.* in Pantnagar. Organized by Dept of Molecular Biology and Genetic Engineering, G.B Pant University, pp 84 on March 4-6.
- Gupta NC. 2013. Cloning and characterization of a wound-inducible promoter from *Arabidopsis thaliana*. VIIIth National Conference on Biotechnology, Biodiversity and Environmental Management Research, April 19th–20th, Department of Botany and Biotechnology, Govt.New Science College, Rewa, (M.P). *Abs.*: 20.
- Gupta NC. Jain PK, Bhat SR & Srinivasan R. 2014. Molecular characterization and expression analysis of a wound-responsive promoter cloned from *Arabidopsis thaliana. International Symposium on Plant Signaling and Behavior*, *07th-10th March,* Department of Botany, University of Delhi, Delhi-07. Abs. **P9.2**: 138.
- Gupta RK, Dalal M, Sinha SK, Thomas G, Mahendru A, Choudhury PR & Mandal PK 2014. Celiac disease : wheat gliadin protein as a key player. Pg 44. In *National Conference Science of Omics for Agricultural Productivity: Future Perspectives,* March 4-6, Pantnagar, India.
- Hasan M & Barthakur S. 2013. Modulation of a wheat RNA binding protein (TaRBP) under drought stress, In: *International Conference on Biotechnology*, Indraprastha International Conference on Biotechnology, New Delhi October 22-25.
- Hasan M & Barthakur S. 2014. Cloning and functional analysis of annexins from a drought tolerant wheat (*Triticum aestivum*) cultivar. In *National Conference Science of Omics for Agricultural Productivity: Future Perspectives,* March 4-6, Pantnagar, India.
- Hasan M & Barthakur S. 2014. Expression profiling of wheat SKP1 gene during terminal drought stress in various generative stages. In: *4th International Conference on Climate Change and Sustainable Management of Natural Resources,* February 12-14, Gwalior, India.
- Kanika, Goyal E, Singh RS, Singh DP & Singh AK. 2013. Oral presentation on isolation, identification and expression profiling of salt-induced genes in wheat cv. Kharchia Local, using suppression subtractive hybridization. In: *International Conference Biotechnology, Bioinformatics and Bioengineering* Tirupati, Andhra Pradesh, 28-29 June.
- Khomdram S & Barthakur S. 2013. Transcript expression profiling of a WRKY transcription factor *TaWRKY10* from *Triticum aestivum* under high temperature stress, In: *Indraprastha International Conference on Biotechnology*, organized by School of Biotechnology GGS Indraprastha University, New Delhi October 22-25.
- Khomdram S & Barthakur S. 2014. Transcript expression profiling of two regulatory genes under high temperature stress in bread wheat. In: *4th International Conference on Climate Change and Sustainable Management of Natural Resources,* February 12-14, Gwalior, India.
- Kumar A, Verma G, Dwivedi A, Abdin MZ, Sirohi A, Subramaniam K, Srinivasan R & Jain PK. 2014. Host mediated RNAi of a parasitic gene for eliciting root knot nematode resistance. In: *International Symposium on Plant Signaling and*

Behavior, March 7-10, 2014 at Department of Botany, University of Delhi.

- Kumar K, Singh D P Singh AK & Goyal E. 2013. Oral presentation on metagenomic study for the diversity analysis of extremophiles from hot water springs of India. In: *International Conference on Agriculture, Food Technologies and Environment- New Approaches,* held at Jawaharlal Nehru University, New Delhi on October 19-20.
- Kumar K, Singh DP, Singh AK & Goyal E. 2013. Oral presentation on Isolation and characterization of thermophillic microbes from hot water springs for cloning of thermal tolerance genes. In: *National Conference on Changing Scenario of Agriculture* in Madhya Pradesh: Prospects and Challenges, held at Bhopal, Madhya Pradesh, Sept 1-2.
- Kumar K, Singh DP, Etika G & Singh AK. 2013. Oral presentation on utilization of thermophilic microbes biodiversity from hot water springs of India to mitigate the effect of climate change on agiculture In: *International Conference on Biodiversity, Bioresources and Biotechnology,* held at Mysore, Karnataka on 30-31 January.
- Kumar K, Mahesh MRM, Singh D P, Etika G & Singh AK. 2014. Oral presentation on exploitation of extremophilic microbes from hot water springs of India to mitigate the effect of climate change on agriculture. In: *National Symposium on Emerging trends in Biotechnology* 2014, organized by Department of Biotechnology, CUSAT, Cochin, Kerala, on Jan. 24-25.
- Kumar K, Singh D P, Goyal E & Mahajan M. 2013. Microbial diversity analysis of hot water spring Tattapani, Himachal Pradesh, using metagenomic approach. In: *International Conference on Biotechnology,* University School of Biotechnology Guru Gobind Singh Indraprastha University, New Delhi on October 22-25.
- Kumari A, Kamboj R & Sharma TR. 2013. Identification of sheath specific *Rhizoctonia solani* responsive promoter from rice. Presented at 11th *International symposium on rice functional genomics: sustaining food and nutritional security*, New Delhi, pp 123 (PVI-147).
- Kumari P, Bhati M, Chauhan H, Prakash A, Choudhary M, Kamle M, Jasrotia RS, Vishwakarma H, Yadav R, Lone SA, Biswas K, Prabu R, Bhatt D, Tarafdar A, Raipuria, R, Srivastav S, Singh GP, Singh R & Padaria JC. 2013. *Agrobacterium tumifaciens* mediated genetic transformation in Indian bread wheat (*Triticum aestivum* L.) for development of herbicide tolerance. In*: Proceeding of Indraprastha International Conference on Biotechnology,* organized by University School of Biotechnology, GGS Indraprastha University, New Delhi, February 22-25th.
- Kumari P, Tarafdar A, Bhatt D, Biswas K, Prabu R, Kamle M, Lone SA, Yadav R, Choudhary M, Bhati M, Prakash A, Chauhan H, Vishwakarma H, Jasrotia RS, Singh GP, Srivastav S & Padaria JC. 2013. Biotechnological approach for adaptation of plants to changing global climate. In: *Proceeding of National Seminar on Climate Change and Indian Horticulture,* organized by Bihar Horticultural Society and Bihar Agricultural University, Sabour, Bhagalpur, Bihar, May 25-27th.
- Lima JM, Nath M, Mandol N, Kulkarni KP, Dokku P, Viswakarma C, Sahu S, Kumar P, Chinnusamy V, Robin S, Sarla N, Seshashayee M, Singh K, Singh AK, Mohapatra T, Sharma RP, Amitha Mithra SV & Singh NK. 2013. Isolation and characterization of EMS induced mutants for chlorophyll biosynthesis process in upland rice variety Nagina22. *11th ISRFG*, New Delhi, India, 20-23 Nov. pp 104.
- Lone SA, Boopalakrishnan G, Kumar S, Srivastava AK & Padaria JC. 2014. Isolation and characterization of novel cryI related protein from *Bacillus thuringiensis*. In: *Proceeding of National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* organized by G. B.

Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, March 4-6th.

- Lone SA, Kumar S, Gupta A, Gujar GT, Saxena AK, Malik A & Padaria JC. 2013. Genetic diversity of crystalline protein encoding genes in native *Bacillus thuringiensis* strains isolated from diverse extreme environments of India. In: *Proceedings of The International Conference on Bacillus anthracis, B. cereus, and B. thuringiensis* organised by Lawrence Livermore National Laboratory, USA, Sept 1-5th, Canada.
- Lone SA, Padaria JC & Malik A. 2013. Diversity of insecticidal coding genes in *Bacillus thuringiensis* isolated from diverse locations in Kashmir Valley. In: *Proceeding of 9th J&K Science Congress* organized by University of Kashmir, Srinagar.
- Mandal PK, Sujatha G & Jayanthi M. 2013. Characterization of oil palm (*Elaeis guineensis* Jacq) germplasm introcuced from Africa for oil content, oil quality and genetic diversity. *International Conference on Role of Plant Biochemistry and Biotechnology in Food and Nutritional Security*. Held at Tirupati on December 11-14, pp 69-70.
- Mohapatra T, Ngangkham U, Kulkarni K, Lima JM, Amitha Mithra SV, Robin S, Sarla N, Seshashayee M, Singh AK, Singh K, Singh NK & Sharma RP. 2014. Development and use of mutants induced by EMS in the background of upland variety Nagina22 for rice functional genomics. *4th NGGIBCI*, ICRISAT, Hyderabad, India, 19-21, pp 19.
- Nagar R, Kumari A & Sharma TR. 2014. NGS based small RNA transcriptome profiling of sheath blight resistance and susceptible rice lines. *International Symposium on Plant Signaling and Behavior*, March 7-10, 2014, Delhi University.
- Padaria JC. 2013. Prospecting of genes for development of thermotolerant transgenic wheat. In: *Proceeding of Indraprastha International Conference on Biotechnology,* organized by University School of Biotechnology, GGS Indraprastha University, New Delhi-110078, February 22-25th.
- Pandey N, Tara Satyawathi C & Barthakur S. 2014. Evaluation of annexin gene in an abiotic stress tolerant pearl millet (*Pennisetum glaucum*) cultivar, In *4th International Conference on Climate Change and Sustainable Management of Natural Resources*, February 12-14, Gwalior, India.
- Prabha A, Raghavendrarao S, Sonam, Kumar K, Pattanayak D, Kumar PA. and Solanke AU. 2013. Evaluation of Finger millet (*Eleucine Coracana*. L) genotypes for heat and drought stress. *7th Annual Convention of ABAP and International Conference on Plant Biotechnology, Molecular Medicine and Human Health* held at the Department of Genetics University of Delhi, South Campus, New Delhi, on October 18-20.
- Prakash A, Chauhan H, Kumari P, Choudhary M, Bhati M, Tarafdar A, Vishwakarma H, Biswas K, Prabu R, Jasrotia RS, Yadav R, Lone SA & Padaria JC. 2014. *Agrobacterium* mediated transformation in Indian elite wheat cultivar. In: *Proceeding of National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* organized by G. B. University of Agriculture and Technology, Pantnagar, Uttarakhand, May 4-6th March.
- Rakshit S, Swapna M, Dalal M, Ganapathy KN, Talwar HS, Ghorade RB, Shivani D, Rajendrakumar P & Patil JV. 2014. Creation of reference set for post-flowering drought response study and whole genome SNP detection using Gbs in Sorghum. pp. 45, In: *Procd. 101 Indian Science Congress*, Feb 3-7th Jammu.
- Rakshit S, Swapna M, Sushma G, Ganapathy KN, Dalal M, Karthikeyan M, Sunita G, Talwar HS & Patil JV. 2013. Allele mining in pyrabactin resistance 1-like 5 (PYL5) gene in sorghum, *Sorghum bicolor (L.) Moench . Pg 171, In Global millet meet*, organized by Society for millet research, Dec18-20 at Hyderabad.

- Ramakrishna Ch, Sonam, Rana R, Raghavendrarao S, Kanika, Padaria JC, Kumar PA & Solanke AU. 2013. Isolation of heat responsive bZIP transcription factor from Finger millet (*Eleucine Coracana*. L). *7th Annual Convention of ABAP and International Conference on Plant Biotechnology, Molecular Medicine and Human Health* held at the Department of Genetics University of Delhi, South Campus, New Delhi, on October 18-20.
- Ramakrishna Ch, Sonam, Rana R, Raghavendrarao S, Kanika, Padaria JC, Kumar PA & Solanke AU. 2013. Isolation and characterization of Finger millet (*Eleusine coracana* L.) bZIP transcription factor. *National Seminar on Recent Advances of Varietal Improvement in Small Millets,* held at Madurai, TN on September 12.
- Rani S, Bhattacharya R, Sharma V & Koundal KR. 2014. Preparation of constructs harbouring lectin and protease inhibitor gene individually as well as their fusion product under *rolC* promoter for genetic transformation of *Brassica juncea cv.* Varuna. *The National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* 4-6 March, G. B. Pant University of Agriculture & Technology, Pantnagar.
- Rani S, Koundal KR & Sharma V. 2013. Construction of fusion gene construct of lectin and protease inhibitor genes under roleC promoter for phloem specific expression and durable resistance to insect—pests. In Indraprastha International Conference on Biotechnology (IICB-2013), Guru Govind Singh Indraprastha University, New Delhi. Oct 22-25.
- Reddy A, Gorthy S & Dalal M. 2013. Molecular characterization of *Sgr* gene from Sorghum (*Sorghum bicolor* L. Moench) In: *International Conference on Biotechnology*, University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi on 22-25 October.
- Richa, Tiwari IM, Kumari A, Nagar R, Bottella J, Sharma V, Singh NK & Sharma TR. 2014. Rice sheath blight resistance QTL qSBR11-1 contains tandem repeats of defense response genes. Presented at *Indraprastha International Conference on Biotechnology*, New Delhi.
- Sanagala RR, Rai V, Yadav S, Mishra V, Sarkar A, Kumar PA & Jain A. 2013. A comparative study on the effects of iron availability on phosphate deficiency-mediated responses in Arabidopsis and rice. In: *11th International Symposium on Rice Functional Genomics (ISRFG11)*, organized by NIPGR, University of Delhi & USDA held at New Delhi, India, 20-23 Nov.
- Sarvjeet Kaur. 2013. Vectors for gene isolation. In: *ICAR Winter School on Frontier Technologies in the Area of Biotechnology, on Gene Isolation, Characterization and Breeding with Reference to Abiotic Stress Related Genes,* 10th-30th Dec, at NRCPB, New Delhi-110012.
- Sharma TR. 2014. Invited lecture delivered on Rice Genome Dissection for the Cloning and Characterization of Disease Resistance Genes and QTLs at the International conference at IP University, Delhi 23-10-13.
- Singh NK, Mahato AK, Sharma N, Gaikwad K, Srivastava M, Tiwari K, Dogra V, Rawal HC, Jayaswal P, Singh A, Rai V, Mithra ASV, Bajpai A, Dinesh MR, Ravishankar KV, Rajan S, Rai A, Singh AK & Sharma TR. 2014. A draft genome of the king of fruit, Mango (*Mangifera indica* L.), In: *XXII Plant and Animal Genome Conference*, January 10-15, San Diego, CA, USA.
- Singh P, Tiwari P, Gupta RN & Kansal R. 2013. Development of transgenic *Brassica juncea* resistant to aphids In: *International Conference on Environment, Health and Industrial Biotechnology* (BIOSANGAM 2013), held at Department of Biotechnology, MNNIT, Allahabad from November 21-23, pp 127.
- Singh P, Tiwari P, Gupta RN & Kansal R. 2013. Transformation of *Brassica juncea* for increased resistance to aphids. In: *National Conference on Recent*

Advances in Biotechnology and Nanobiotechnology (BIONANO 2013) held at Amity University, Gwalior from October 29-30, 2013, pp 22.

- Singh PK, Thakur S, Jain P, Dwivedi M, Mahato AK, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Singh NK & Sharma TR. 2014. Molecular diversity analysis of *Avr-Pita* gene in Indian field isolates of *Magnaporthe oryzae*. Presented at *11th International Symposium on Rice Functional Genomics: Sustaining Food and Nutritional Security*, New Delhi. pp 126 (PVI-153).
- Sinha SK, Bansal N, Gayatri, Gupta RK & Mandal PK. 2013. Genotypic variation of wheat seedlings in response to low N condition on growth under hydroponics. *International Conference on Role of Plant Biochemistry and Biotechnology in Food and Nutritional Security* (ICBBFN 2013 and XIIth Indian Society Agricultural Biochemist Convention). December, 11-14, Tirupati, Andhra Pradesh.
- Sinha SK. 2014. Plant micro RNAs-biogenesis and their role in nutrient sensing. National Seminar on "*Recent Advances in Biotechnological Tools for Sustainable Developments*". Organized by Department of Biotechnology, IMS Engineering College, Ghaziabad, 29th March, 2014.
- Solanke AU, Kanakachari M, Ahmed I, Sonam, Srivathsa R, Pattanayak D & Kumar PA. 2013. Gene characterization related to fruit size, shape and colour in eggplant (*Solanum melongena* L.). *8th National Conference on Biotechnology, Biodiversity and Environment* held at Rewa (MP) from April 18-19, 2013. pp 24.
- Solanke AU. 2013. Food security, agricultural biotechnology and environment (Keynote talk). In UGC sponsored '*National Conference on Effects of Biotechnological Advancements on Environment*' at Ajmer from November 22-23, 2013.
- Sonam, Ramakrishna Ch, Rana R, Raghavendrarao S, Kanika, Padaria JC, Kumar PA & Solanke AU. 2013. Isolation and functional characterization of *EcDREB* gene from Finger millet. *National Seminar on Recent Advances of Varietal Improvement in Small Millets* held at Madurai, TN September 12.
- Sonam, Ramakrishna Ch, Rana R, Raghavendrarao S, Kanika, Padaria JC, Kumar PA & Solanke AU. 2013. Isolation and functional characterization of *EcDREB* gene from Finger millet (*Eleucine Coracana*. L). *7th Annual Convention of ABAP and International Conference on Plant Biotechnology, Molecular Medicine and Human Health* held at the Department of Genetics University of Delhi, South Campus, New Delhi, October 18-20.
- Srinivasan R. 2013. Relevance of Promoters in Transgenic Research. *CSIR Foundation Day lecture* entitled at CSIR-Institute of Himalayan Bioresource Technology, Palampur (H.P.) on 26th September, 2013.
- Sushma Rani, Koundal KR, Bhattacharya R & Sharma Vinay. 2014. Preparation of constructs harbouring lectin and protease inhibitor gene individually as well as their fusion product under rolC promoter for genetic transformation of *Brassica juncea cv* Varuna. In: *National conference on Omics for Agricultural Productivity: Future Perspectives*. March 4-6, 2014. G. B. Pant University of Agriculture and Technology, Pantnagar.
- Tiwari IM, Richa Bottella J, Jesuraj A & Sharma TR. 2014. Genetic transformation of rice with RNAi construct for sheath blight resistance. Presented at *Indraprastha International Conference on Biotechnology*, New Delhi. In*: Proceeding of Indraprastha International Conference on Biotechnology,* organized by University School of Biotechnology, GGS Indraprastha University, New Delhi-110078, February 22-25th.
- Tiwari P, Singh P, Gupta RN & Kansal R. 2013. Controlling of insect pest using insecticidal lectin gene of plant origin In: *International Conference on Environment, Health and Industrial Biotechnology* (BIOSANGAM 2013), held at Department of

Biotechnology, MNNIT, Allahabad, pp 128. November 21-23.

- Tiwari P, Singh P, Gupta RN & Kansal R. 2013. Genetic transformation of insecticidal gene in *Nicotiana tabaccum*. In: *National Conference on Recent Advances in Biotechnology and Nanobiotechnology* (BIONANO 2013) held at Amity University, Gwalior, pp 14. October 29-30.
- Vandana Rai. 2013. A comprehensive study for salt tolerance in high yielding rice introgression lines derived from *Oryza rufipogon* and *Oryza nivara*. *11th ISRFG*, New Delhi. Nov 20-23.
- Vandana Rai. 2013. Validation of differentially expressed candidate genes for salinity tolerance in introgression lines derived from the cross KMR3 x *O. rufipogon*. *7th International Rice Genetics Symposium* 5-8 November, in Manila, Philippines.
- Vandana Rai. 2014. Designing and validation of a 48-plex GoldenGate SNP assay for determine the population structure in wild rice. *National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* at Pantnagar. March 4-6.
- Vandana Rai. 2014. Proteome profiling of seeds and young germinated tissues of *Cajanus cajan*. *National Conference on Science of Omics for Agricultural Productivity: Future Perspectives* organized at G. B. Pant University of Agriculture and Technology, Pantnagar, March 4-6.
- Vishwakarma H, Biswas K, Prabu R, Bhatt D, Jasrotia RS, Tarafdar A, Bhati M, Kamle M, Choudhary M, Kumari P, Chauhan H, Prakash A, Yadav R, Lone SA & Padaria JC. 2014. Cloning, characterization and expression study of heat stress induced ascorbate peroxidase gene from Indian bread wheat *T. aestivum*. In: *Proceeding of National Conference on Science of Omics for Agricultural Productivity: Future Perspectives,* organized by G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, March 4-6th.
- Yadav R, Sivalingam PN, Kanika, Patel VB, Singh AK, Padaria JC & Singh NK. 2013. Characterization of *MDHAR* gene from *Ziziphus mauritiana*. In: *Proceeding of National Seminar on Climate Change and Indian Horticulture,* organized by Bihar Horticultural Society and Bihar Agricultural University, May 25-27th, Sabour, Bhagalpur, Bihar.
- Yadav R, Tarafdar A, Raipuria R, Bhatt D, Prabu R, Biswas K, Kamle M, Lone SA, Choudhary M, Bhati M, Prakash A, Chauhan H, Kumari P, Vishwakarma H, Jasrotia RS, Srivastav S, Singh R, Kanika & Padaria JC. 2013. Characterization of abscisic acid stress ripening (ASR) protein isolated from *Ziziphus nummularia*. In*: Proceeding of Indraprastha International Conference on Biotechnology,* organized by University School of Biotechnology, GGS Indraprastha University, New Delhi-110078, February 22-25th.

Distinguished Visitors

22.08.2013	Visit of three member delegation team led by Dr. Mohd. Nazir Basiran, Director General of Agro-biotechnology, Malaysia.
25.10.2013	Visit of an international delegation from George Spencers Academy, UK
13.12.2013	Visit of Dr. Malali Gowda, Facility Director, Next Generation Genomics facility at C-CAMP, NCBS campus, Bangalore.

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National Research Centre on Plant Biotechnology
Lal Bahadur Shastri Centre, Pusa Campus
New Delhi-110012, India
Telephone : +91-11-25848783, Fax : +91-11-25843984
Website: http://www.nrcpb.org

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