Annual Report

2015-16

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Preface

ICAR-National Research Centre on Plant Biotechnology has completed 30 successful years of existence as a premier Institute of the Indian Council of Agricultural Research. During these years, the Centre kept pace with the changing needs of Indian agriculture and contributed in terms of products, processes, publications, patents, partnerships and human resource development. The current scenario of global climate change has brought high temperatures, frequent and intense bouts of droughts and floods. The vagaries of climate have also altered the spectrum of biotic stresses. Sustaining yield under these adverse conditions with limited land, water and nutrient resources has become a big challenge. Since every crop has specific biotic and abiotic constrains, the centre has six research groups working on rice, wheat, Brassica, pigeonpea and chickpea to address specific issues related to these mandate crops in focused manner. The Centre pursues basic and strategic research in the area of structural and functional genomics; identification of QTLs and molecular markers in various crops; transgenic development for biotic and abiotic stress tolerance in pulses, Brassica and wheat; improvement of nitrogen use efficiency in cereal crops; and bioprospecting of novel genes and promoters.

One of the significant achievements of the Centre in 2015-16 includes designing of a 50K SNP genotyping chip in rice which will expedite molecular breeding and genetic and evolutionary studies in rice. Genome wide analysis of disease resistance (R) and defense response (DR) genes in three rice genomes revealed 'hot spots' of *R*-genes on chromosome 11 and chromosome 1. Complete chloroplast genome sequences of fertile and sterile pigeonpea were finalized, and a draft assembly of pigeonpea mitochondrial genome was generated for the fertile pigeonpea.

Wild species and land races are rich source of agronomical traits for adaptation to environmental stresses. The centre has added 83 new entries in wild rice germplasm collection. Allele mining studies for identification of novel alleles of *HKT2;3* and *HKT5;1* for salt tolerance and *Pi54* gene for blast resistance in rice have been carried out. Thirty two *B. juncea* lines, having desirable agronomic features, were resynthesized from six different crosses of its progenitor species to enhance diversity of mustard.

Transgenic lines of rice, pegionpea, and Brassica were developed and characterized to impart resistance to biotic stresses. A number of abiotic stress responsive genes are being deployed in wheat to enhance tolerance to abiotic stresses. The choice and efficiency of a promoter can be a rate limiting step for success of a transgenic crop. Many stress or tissue specific promoters were validated to address this issue.

Standard Operating Protocols (SOP) for genetic fidelity testing of tissue culture raised plants were developed for potato, sugarcane and banana plants using ISSR marker system. The centre has also embarked on CRISPR-Cas genome editing strategy for crop improvement.

Human resource development is an important pursuit of NRCPB. Seven Ph. D. and six M. Sc. students were awarded with Doctoral and Master's degrees, respectively. The Centre is proud of Dr. Siddanna Savadi for winning the prestigious 'IARI Gold Medal' for Ph. D., and Dr. Shallu Thakur, who worked as SRF at NRCPB, for receiving ICAR-Jawaharlal Nehru Award for best Ph. D. thesis in Biotechnology. The NRCPB fraternity is extremely proud of ICAR-Norman Borlaug Award recipient Prof. N. K. Singh.

I am thankful to all the staff and students of NRCPB for their team efforts in organizing several institute activities including Foundation Day and Swachha Bharat Abhiyaan. The annual report is a reflection of the activities and achievements of entire NRCPB family. I thank all the NRCPB staff for their contributions. My special thanks to Dr. D. Pattanayak, Dr. Monika Dalal, Dr. Rohini Sreevathsa, Dr. Naveen C. Gupta, Dr. Deepak S. Bist and Dr. Rampal S. Niranjan for their help in compiling and editing of the annual report.

I am grateful to Dr. T. Mohapatra, Secretary, DARE and Director General, ICAR; Dr. J. S. Sandhu, Deputy Director General (Crop Sciences); and Dr. J. S. Chauhan, ADG (Seeds), ICAR for their constant support and help in overall functioning of the institute.

Date: May, 2016
New Delhi

(T R Sharma)

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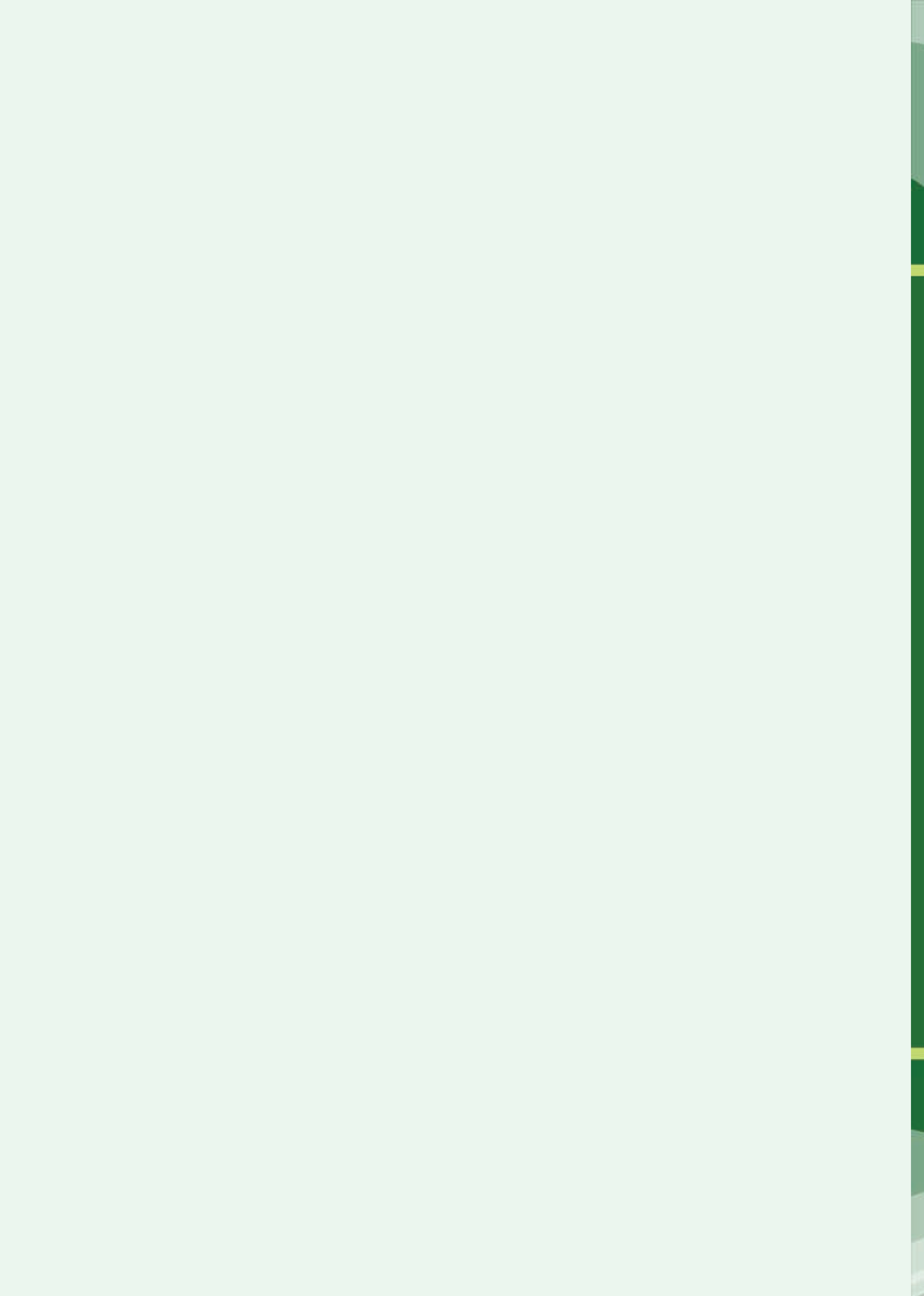


EXECUTIVE SUMMARY

Executive Summary

The committed and sustained efforts of the National Research Centre on Plant Biotechnology (NRCPB) 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 2015-2016. Salient achievements of the Centre in research under six major projects, Genomics and molecular markers in crop plants, Biotechnological applications for pulses improvement, Biotechnological approaches for Brassica improvement, Adaptation of wheat to climate change induced abiotic stresses, Improvement of nitrogen use efficiency in cereal crops, and Bioprospecting of novel genes and promoters; and progress in human resource development (HRD) efforts during 2015-2016 are mentioned below:

- A unique genic-SNP genotyping chip in rice consisting of 50051 SNPs from 18980 different genes, mainly single copy genes, was designed and validated. This would serve as a great tool for molecular breeding, genetic and evolutionary studies.
- A method for rapid identification of QTLs for salt tolerance by BSA approach using the 50K rice SNP chip was experimentally validated. Using 50K SNP genotyping data of CSR11, MI48 and the bulked-tolerant (BT) and bulked-susceptible (BS) pools of 30 RILs, 21 QTLs for grain yield were identified. The method was also validated with 'CSR27/MI48' RILs used earlier for mapping salt tolerant QTLs using low-density SSR markers.
- To the wild rice germplasm collection at NRCPB, 83 new entries had been added. Allele mining of this collection for genes related to salinity tolerance identified novel alleles for *HKT2;3* and *HKT5;1*.
- The molecular basis of oxidative stress management (OSM) under reproductive drought stress was unraveled in drought tolerant rice variety, Nagina22, by using a RIL mapping population generated from Nagina22 and IR64. Nine of the 11 *OSM* genes in rice OSM network had functional alleles in Nagina22.
- Allele mining and mutation profiling of *Pi54* gene by using 92 rice lines revealed that most of the mutational sites showed a constant frequency distribution between the resistant and susceptible group.
- Rice transgenic lines were developed for genes *Pi54* and *Pi54rh*. The site of integration and chromosomal position were identified by inverse PCR. Based on analysis of blast resistant phenotype imparted by the transgene *Pi54* and *Pi54rh* in transgenic lines it was concluded that both the genes can be effectively used for development of broad spectrum durable resistance against blast disease in rice.
- Genome wide distribution of disease resistance *(R)*- genes and defense response *(DR)*- genes study in three rice genomes revealed that most of the *R* genes were present on cluster and distributed across the genome. Maximum *R*-genes (92) consisting of 31 clusters were present on chromosome 11, followed by chromosome 1, indicating chromosomal 'hot spots' for rapid gene duplication.
- Physical positions of 167 defence response genes, such as chitinases, glucanases and thaumatin like proteins, were assigned on the 12 rice chromosomes.
- Overexpression of rice serine hydroxyl methyltransferase (OsSHMT) in *Arabidopsis thaliana* conferred salt tolerance. This is the first report available for characterisation of SHMT gene on salt stress in plant system.
- Sequencing of four TAL effector genes from a *xa*13 compatible *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain was accomplished, and it was identified that these effectors harboured 13-18 repeats. The central repeat region of each *TALe* gene was analysed to delineate the Repeat Variable Diresidues (RVD) of each gene. RVDs define the

functionality and host specificity of a *TALe* gene and hence with these sequences, the DNA region in the host can be decoded.

- An *in vivo* method of panicle blast analysis was developed and evaluated in two panicle blast resistant and susceptible rice lines.
- More than 100 SSR and 100 ISSR markers were screened for polymorphism in Tetep and HP2216, and 3 polymorphic bands associated with panicle blast resistance were identified using ISSR in resistant and susceptible pools.
- *WRKY10, SnRK1* and *SKP1* genes, involved in modulating the terminal heat stress response, were cloned from tolerant wheat cultivars. A number of genes, *viz. TaDREB, PgASR, PgDREB, ZnNAC, ZnLEA, TaWRKY10* and *BcZAT12,* were being deployed in wheat to enhance tolerance to abiotic stresses using tissue culture and *in planta* transformation methods.
- Mapping population was developed to identify QTLs and genes for root traits in wheat. Two full length genes, *viz. TaBRX* (*BRAVIS RADIX*), and *TaMIZ1* (*MIZU-KUSSEI 1*), involved in RSA in wheat (*T. aestivum*), were introduced into *Arabidopsis* for functional characterization.
- Transcriptome analysis in Kharchia local and *Penniseteum glaucum* led to identification of several differentially regulated genes under salt and drought stress, respectively, and several SSR markers.
- To map QTLs for wheat leaf spot disease, crosses between tolerant and susceptible wheat genotypes were made, and backcross populations were generated. The BC_1F_1 plants were analysed for the presence of the tightly linked SSR markers. The foreground markers *Xgwm148-2B* and *Xgwm111-7D* showed polymorphism among the wheat lines and hence could facilitate selection, mapping, cloning genes and QTL analysis.
- Morpho-physiological (biomass measurement; shoot and root length and their fresh and dry weight, root system architecture) and biochemical (activity of nitrate and ammonia assimilation enzymes) studies of two N-responsive wheat genotypes (Kalyansona and NP-890) under controlled condition in N-limited and optimum conditions demonstrated that NP-890 was less sensitive to N-starvation than Kalyansona in seedling stage.
- Nine wheat genotypes, diverse in their nitrogen use efficiency at field level, were analyzed to understand the role of root system architecture in nitrate uptake, and gene expression of different nitrate transporter genes (High and Low Affinity Nitrate Transport System) under optimum and starved N-condition. Significant genotypic differences existed at genotype level, and a few genotypes showed least changes under N starvation condition.
- Drought-responsive miRNAs were identified in chickpea through expression profiling of tissues from drought tolerant cultivar, ICC4958 and drought susceptible cultivar, ICC1882. Differential expression in conserved and novel miRNAs was observed between the two chickpea cultivars.
- Steroidogenic acute regulatory related transfer (START) proteins, involved in transport of lipid molecules, play a myriad of functions in animals and plants. Genome-wide search for all START domain proteins in chickpea identified 36 chickpea genes belonging to the START domain family. Four transmembrane START (TM-START) proteins in chickpea were identified through a phylogenetic tree, reconstructed with *Arabidopsis*, rice, chickpea, and soybean START proteins. Expression analysis in various tissues showed that these genes were predominantly expressed in flowers and roots of chickpea. Three of the chickpea TM-START genes showed induced expression in response to drought, salt, wound and heat stress, suggesting their role in stress response.
- The complete chloroplast genome sequences of fertile and sterile pigeonpea were finalized and characterized. The plastid genome was subjected to comparison with other legumes. Plastid SSR markers were mined and validated by genotyping six wild species.
- Genome wide analysis of PPR genes (candidates for fertility restoration) was carried out in the pigeonpea genome and after comparative analysis with other legumes candidate, putative RFLs were identified.
- A draft assembly of the pigeonpea mitochondrial genome was generated for the fertile pigeonpea.

- Transgenic pigeonpea lines were developed with two *Bt*ICP genes, *cry1AcF* and *cry2Aa*, following a non-tissue culture-based *in planta* transformation protocol for *Helicoverpa armigera* resistance. The putative transformants were selected based on kanamycin resistance and presence of T-DNA by PCR analysis. Bioassay against *H. armigera* identified promising transgenic lines with 80-100% mortality. In T_3 generation, eight stably integrated events with 80-100 % insect mortality and high *cry1AcF* expression were identified. Four events had the T-DNA integrated as a single copy. Ten stably integrated events with high *cry2Aa* expression demonstrated 80-100% insect mortality.
- The effects of varying concentrations of NaCl treatment for 7 days were evaluated on the developmental responses of root system architecture (RSA) and shoots of pigeonpea. Developmental responses of these traits were significantly affected during growth at 150 mM NaCl. A total of 119 differentially expressed (DE) proteins were identified of which 78% of them were represented in both roots and shoots. Interestingly, some of them such as RSA related and RNA-dependent RNA polymerase were detected only in the roots of the seedling that were subjected to salinity stress.
- Developed siRNA distribution profiles for all the segments of *Pigeonpea sterility mosaic virus*-1 (PPSMV-1) and *Mungbean yellow mosaic India virus* (MYMIV) by next generation sequencing.
- Potential strategies, viz. RNAi based resistance, the candidate genes and the genetic elements, were identified for efficient transgenic incorporation in Indian mustard (*Brassica juncea*) for resistance management against hemipteran insect-pest, aphid. Several phloem-specific promoters for RNAi based resistance were characterized.
- Several plant lectins and protease inhibitor genes were assayed for their insecticidal effects against mustard aphid. The fusion protein of protease inhibitor and lectin incorporated in the diet at 0.1% (w/v) showed significant effect at $P < 0.05$ on the survivability of aphid after day 6. Southern analysis of T_2 mustard transgenic lines showed single locus of insertion of chickpea lectin gene in 18 lines. Expression of the inserted gene was confirmed through Real-time PCR analysis.
- CRISPR-Cas genome editing strategy was devised for *B. juncea.* A chimeric sgRNA for *GUS* reporter gene targeting two sites individually and together were designed and cloned into pRGEB31 vector carrying CRISPR/Cas system. These recombinant vectors were mobilized into *Agrobacterium* strains for plant transformation.
- Thirty two *B. juncea* lines, having desirable agronomic features with respect to plant habit, were resynthesized from six different crosses of its progenitor species to enhance the diversity of mustard. About 82 individual events of resynthesized *B. juncea* from different crosses were obtained after amphidiploidization. A *B. rapa* line was identified that supports *in vivo* seed development following crossing with *B. nigra* thereby obviating the need for embryo rescue to obtain interspecific hybrids. This feature was maintained in the F_1 of the cross with other *B. rapa* accessions.
- A number of individual plants was selected from mustard IL's population derived from *Eru-rapa* and *B. juncea* cross which showed high degree of resistance/tolerance against *Alternaria brassicae* on natural as well as epiphytotic screening. The introgressions from wild species were confirmed using *Diplotaxis erucoides*-specific molecular markers.
- Defensin gene of *B. juncea* was cloned by PCR using *Arabidopsis* and *B. rapa* primers and sequenced (600bp, Acc. No. KF578144). Also, 2.5kb long promoter of defensin was cloned and sequenced (GenBank Acc. No. KP300038). Promoter deletion analysis in transgenic *Arabidopsis* with defensin full length (2.5 Kb) and 1Kb promoter showed that full length promoter was pathogen-, jasmonic acid- and wound- inducible but not affected by salicylic acid. However, a truncated version of the promoter (1Kb) lost JA- and pathogen-inducibility. Defensin promoter showed higher activity during development and in a tissue specific manner. It was active in seeds, meristematic tissue and at the base of siliques.
- The phenomics studies in dwarf stature mutant (DSM) line identified from the activation tagged population of *Arabidopsis* has showed significant

deviation from parental wild type plants in terms of plant architectures, and morpho-physiological parameters. Southern analysis revealed single copy of T-DNA insertion in DSM line. For introducing the observed phenotype in *B. juncea* for validation purpose and for reversal of the altered phenotype in DSM lines of *Arabidopsis*, relevant gene constructs of the *dsr* gene were developed. These recombinant vectors were mobilized individually into *Agrobacterium* strains for plant transformation.

- A nematode-responsive-root-specific gene (*AT1G26530*) was identified which was up-regulated in roots of *A. thaliana* after nematode infection. In transgenic plants harboring *AT1G26530*prm::*GUS* fusion construct, reporter gene expression was seen exclusively in galls after nematode inoculation. The strong GUS activity was observed at early stages of nematode infection, starting from 14 days and was sustained up to 30 days post inoculation. The specificity of the activity of the *AT1G26530* promoter, in terms of nematode-responsiveness and root-specificity, makes it a suitable candidate to express dsRNA of nematode genes and engineer plants with resistance against root-knot nematodes using HD-RNAi technology.
- Standard Operating Protocols (SOP) for genetic fidelity testing of tissue culture raised plants were developed for potato, sugarcane and banana plants using ISSR marker system.
- Plant genome database (PGDB) was developed having sequence information for more than 70 plant species and consisting of 31,20,333 sequence entries.
- Fifteen NRCPB staff (9 scientists, 4 technical staff and 2 administrative staff) received training from different national institutes of repute in 2015-16 under HRD programme.
- Twenty six Ph. D. and 12 M. Sc. students were registered in the discipline of Molecular Biology and Biotechnology in 2015-16. Seven Ph. D. and six M. Sc. students were awarded with Doctoral and Master's degrees, respectively. Dr. Siddanna Savadi won the prestigious 'IARI Gold Medal' for Ph. D. More than fifty students from different universities and institutes across the country were trained in various aspects of biotechnology at the Centre.

About the Centre

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

ICAR-NRCPB

National Research Centre on Plant Biotechnology (NRCPB) is the premiere research institution of the Indian Council of Agricultural Research (ICAR), 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 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). NRCPB has acquired, in the past, an excellent infrastructure in terms of equipment 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 and wheat 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 like ICRISAT. The Center is now placing considerable emphasis on the development of products, processes, patents and research publications in journals with high impact factor. It also encourages and practices Public-Private Partnership (PPP) mode for commercializing the products of genetic engineering.

Mandate

- Basic plant molecular biology research for understanding molecular basis of biological processes
- Coordination and capacity building for devising tools and techniques of biotechnology and genetic engineering for crop improvement

Staff Strength of the Centre

Staff	Sanctioned	Filled	Vacant
Scientific	33+1	32+1	01
Technical	14	11	03
Administrative	18	09	09
Skilled Supporting Staff	04	-	04
Total Strength	**70**	**53**	**17**

Financial Statement 2015-16

(Rs. In lacs)

	Plan		Non-plan	
	Allocation	Utilization	Allocation	Utilization
Capital	100.00	99.97	7.00	6.93
Revenue				
Establishment	00	00	625.00	622.76
Pension & other retirement benefits	00	00	84.00	82.46
Travelling Allowances	3.00	3.00	4.00	3.96
Research and Operational Expenses	230.00	229.99	95.00	94.85
Administrative Expenses	260.00	259.90	128.00	127.91
Miscellaneous Expenses	7.00	6.99	4.00	3.95
Total	**600.00**	**599.85**	**947.00**	**942.82**

Resource Generation

Sales of Farm Produce	-
License Fee	5.40
Leave Salary and Pension Contribution	-
Interest Earned on Short Term Deposits	22.79
Income Generated from Internal Resource Generation (Trg. etc)	2.86
Miscellaneous Receipts	12.95
Total	**44.00**

Fund Received through Externally Funded Projects

Externally Funded Projects	544.58
Consultancy Projects	-
Total	**544.58**

Research Achievements

- Genomics and Molecular Markers in Crop Plants
- Biotechnological Applications for Pulses Improvement
- Biotechnological Approaches for Brassica Improvement
- Adaptation of Wheat to Climate Change Induced Abiotic Stresses
- Improvement of Nitrogen Use Efficiency in Cereal Crops
- Bioprospecting of Novel Genes and Promoters
- Honorary Scientists' Projects

1 GENOMICS AND MOLECULAR MARKERS IN CROP PLANTS

Designing and validation of 50K SNP chip in rice based on single copy genes

Single nucleotide polymorphism (SNP) is the most abundant DNA sequence variation present in plant genomes. At NRCPB, a unique genic-SNP genotyping chip for genetic and evolutionary studies as well as molecular breeding applications in rice was designed and validated. The chip designed for Affymetrix platform incorporated 50051 SNPs from 18980 different genes spanning 12 rice chromosomes, including 3710 single-copy (SC) genes conserved between wheat and rice, 14959 SC genes unique to rice, 194 agronomically important cloned rice genes

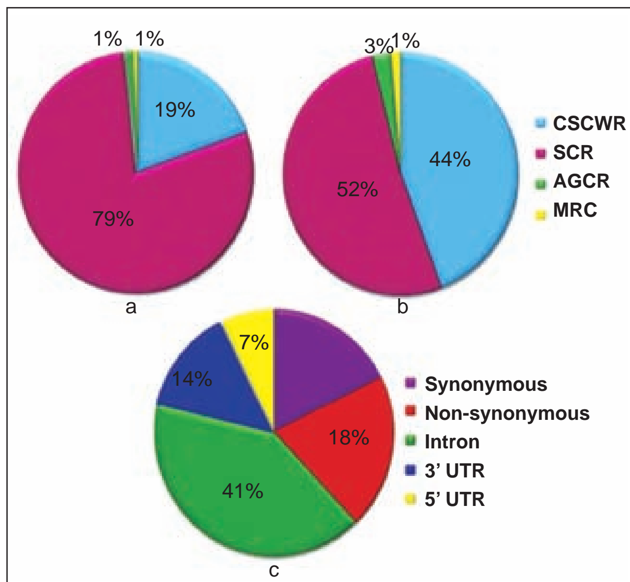


Fig. 1.1: Distribution of different kinds of rice genes and number of SNPs in the Affymetrix 50K rice SNP chip (OsSNPnks). (a) Number of genes in different categories; (b) number of SNPs in different categories of genes; (c) number of SNPs in different regions of the genes.

and 117 multi-copy rice genes (Fig. 1.1). A total of 320 wild rice accessions were genotyped by 50K SNP chip. Assays with this chip showed high success rate and reproducibility. Being a single copy gene based array with no sequence redundancy the chances of cross hybridisation were negligible. The usefulness of the chip in genetic diversity and phylogenetic studies of cultivated and wild rice germplasm was demonstrated. Furthermore, its efficacy was validated for analysing background recovery in improved mega rice varieties with submergence tolerance developed through marker-assisted backcross breeding.

Integration of physical mapping with BSA for identification of QTLs for salt tolerance in CSR11/MI48 RILs

Under this project, a method for rapid identification of QTLs for salt tolerance by BSA approach using a 50K rice SNP chip has been proposed and experimentally validated. Array based SNP genotyping of pools of 10, 20, 30, 40 and 50 RILs was done to identify the optimum pool sizes for maximum heterogeneity (heterozygote calls) of alleles in the pool, so that the bulked tolerant and bulked sensitive pools did not differ for alleles other than those in the associated QTL regions. As expected, pool of 10 RILs showed the minimum heterogeneity (70%), with successive increase in heterogeneity in higher pools (Fig. 1.2). Since the maximum gain was between pool size of 10 to 30 RILs, the pool size of 30 was fixed as the optimum. Bootstrapping results

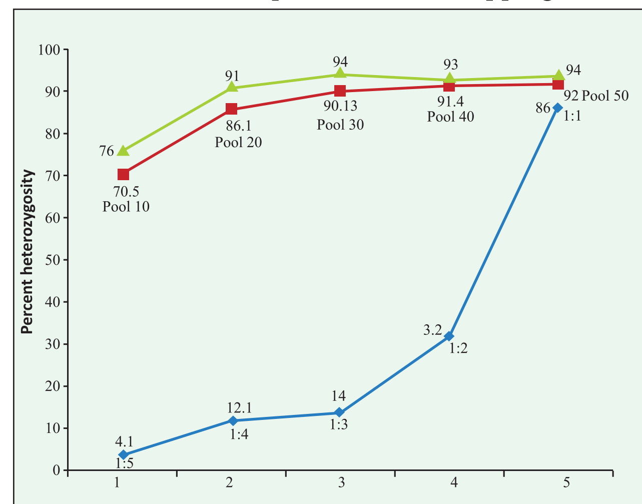


Fig. 1.2: Analysis of heterogeneous loci in different rice RIL pool sizes and mixture of parental DNA samples using 50K SNP chip. Genomic DNA from 10 to 50 individual RILs of CSR11/MI48 mapping population were pooled in equal amounts, with higher pools including all the RILs of lower pools for the analysis of allele heterogeneity (Red line). Computational expectations on Bootstrap analysis of the pools of 10 to 50 lines, showing successive increase in heterogeneity up to 94% (Green line). Observed heterogeneity with mixing of genomic DNA from the two parental lines was in the proportions of 1:5, 1:4, 1:3, 1:2 and 1:1 (Blue line).

also validated the experimental results (Fig. 1.2). Using 50K SNP genotyping data of CSR11, MI48 and the bulked-tolerant (BT) and bulked-susceptible (BS) pools of 30 RILs, 21 QTLs for SSI for grain yield on rice chromosomes 1, 2, 3, 5, 6, 8, 9 and 12 were identified (Fig. 1.3). To minimize the false discovery rate, QTL status was assigned to a SNP or a string of SNP loci only when both the pools were homogeneous for contrasting alleles. The method was validated further with 'CSR27/MI48' RILs used earlier for mapping salt tolerance QTLs using low-density SSR markers. BSA with 50K SNP chip identified 34 QTL regions. This not only confirmed the location of previously mapped QTLs but also identified several new QTLs, and provided a rapid way to scan the whole genome for mapping QTLs for complex agronomic traits in rice.

their validation in the recipient rice cultivars and genotyping. This project was planned to fast track the development of abiotic stress tolerant rice varieties in India. NRCPB designed 50K Affymetrix gene chip assay for the selection of background genome recovery of various stress tolerant lines developed at respective centers. A high-density background selection will ensure minimum alterations in the genome of highly popular recipient variety, which will greatly help in their adoption by farmers. At present the background selection has been completed for four crosses, namely Swarna-Sub1/IR86918-B-305 for the introgression of drought tolerance QTL *qDTY*$_{1.1}$ (20 lines), MTU 1075/Swarna-Sub1 for submergence tolerance (18 lines), MTU 1010/FL478 (20 lines) and Ranjit/Swarna-Sub1 (20

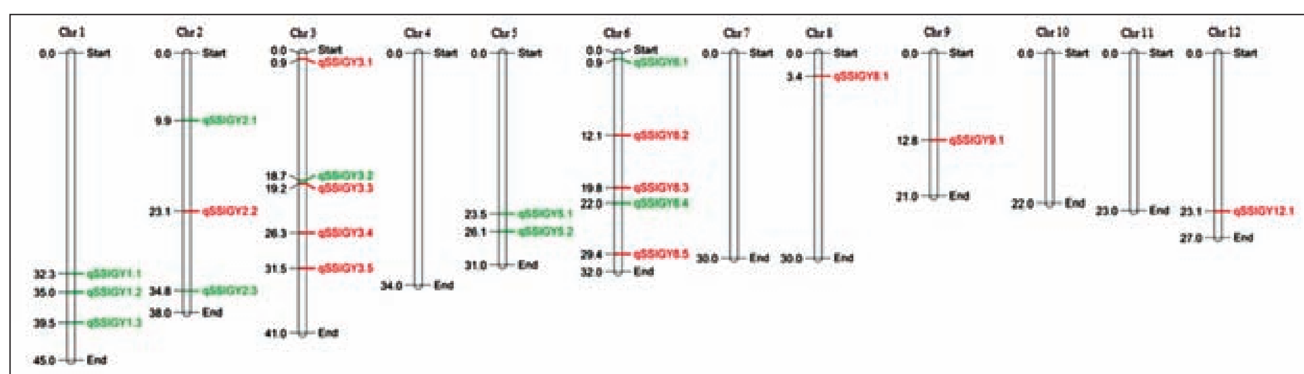


Fig. 1.3: Physical map positions of QTLs identified by BSA of rice CSR11/MI48 RIL population using 50K SNP chip. QTLs shown in green color have salt tolerant allele coming from the tolerant parent CSR11 and those in red color have tolerant allele contributed by the sensitive parent MI48.

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

The DBT India-IRRI collaborative Project "From QTL to Variety" was initiated in 2010 for transferring major genes/QTLs for drought, submergence and salt tolerance into popular varieties of India through marker-assisted backcross breeding (MABB). The network involved fifteen Indian institutions including ICAR Institutes, State Agricultural Universities and Central Universities with National Research Centre on Plant Biotechnology being the lead centre. The main objectives of the project were (i) improved varieties of rice tolerant to drought, submergence and salinity, (ii) advance breeding lines, (iii) trained human resource for MABB and (iv) information on markers flanking the QTL for drought tolerance, lines). These advanced backcross lines from each cross were identified based on agro-morphological, grain yield and foreground trait phenotyping. A range of 82.33% to 94.01% overall recipient genome recovery was observed in the cross Swarna-Sub1/IR86918-B-305 for transferring drought QTL *DTY*1.1. The 18 advanced backcross lines from MTU 1075/Swarna-Sub1 cross for the transfer of *Sub1* gene showed 91.88% to 90.29% overall recipient genome recovery. Similarly, cross of MTU 1010/FL478 for transferring *Saltol* and Ranjit/Swarna-Sub1 for transferring *Sub1* showed range of 97.2 to 98.5% and 89.6 to 96.5% genome recovery, respectively. Among the lines having recombined the dwarf stature with the QTL *DTY*$_{1.1}$, line no. R7 showed the highest recipient genome recovery. Most of the donor lines for aforesaid QTLs used in this study were product of pre-breeding/genetic enhancement activities from

IRRI's breeding programme. These included FL478, a RIL derived from IR29/Pokkali cross harbouring *Saltol* for seedling stage salinity tolerance, Swarna-Sub1, CR1009-Sub1 and IR64-Sub1, as donors for submergence tolerance, since these donors were available in highly adapted genetic background, negative linkage drag was not a problem. Similarly, a study was made to find out the utility of the 50K SNP developed at the centre with submergence tolerant varieties, namely Swarna-Sub1, IR64-Sub1, Savitri-Sub1 and Sambha Mahsuri-Sub1. So far, five entries have been entered AVT 2 trial of AICRIP.

Physiological and molecular basis of drought tolerance in F_1 hybrids and their parental lines

Moisture stress or drought is one of the most severe constraints to rice productivity and a serious threat to global food security. Rice hybrids are known to withstand moisture deficit stress better than their parental purelines. To study the effect of water deficit stress in rice hybrids, in terms of productivity related traits and physiological parameters pertaining to drought stress, 22 rice hybrids and their parental lines were evaluated under well irrigated and water deficit conditions. Sixteen F_1 hybrids showed positive heterosis over their respective mid-parental means for yield and spikelet fertility. Pusa 6A and Vandana were the best cross combination for developing high yielding hybrid rice varieties under drought since they had the highest positive standard heterosis and heterobeltiosis for grain yield, spikelet fertility and membrane stability index (MSI). Among the physiological parameters, MSI was found to reflect the drought tolerance ability of a genotype better. A set of 60 genome-wide SSR markers were used for prediction of heterotic potential of genotypes under well irrigated and water deficit conditions which revealed that genetic distances and spikelet fertility had a positive and significant correlation (Table 1.1).

Table 1.1: Coefficients of correlation between parental genetic distance and heterosis in rice

Trait	Control	Drought Stress
Yield	0.06	-0.06
Filled grain	-0.15	0.04
Unfilled grain	0.18	-0.41
Total grains	-0.04	0.07
Spikelet fertility	-0.17	0.43*
Chlorophyll content	-0.02	0.04
MSI	-0.21	0.18
RWC	0.02	-0.10

* p<0.10

Role of ROS pathway in imparting drought tolerance in rice

To dissect drought, a complex trait, it is vital to understand the complete physiological, biochemical and molecular mechanism of drought tolerance. Osmotic adjustment, oxidative stress management (OSM) and cell membrane stability (CMS) are the major components of cellular tolerance under drought stress. In the current study, the molecular basis of OSM in an internationally well known drought tolerant rice variety, Nagina22, with respect to a drought sensitive variety, IR64, was explored by using a RIL mapping population generated from these two contrasting genotypes and its association with spikelet fertility (SF) which is an important component of drought tolerance in Nagina22. Using biochemical assays of superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX) of the extreme bulks (from the population) made, based on SF under reproductive stage drought stress, the relationship between SF and OSM gene expression was analyzed. Though, the extremes were identified based only on SF, the other productivity related traits also followed the trend presumably because of the in-built trait correlations. This trend was more evident in single plant yield and stress susceptibility index, besides SF. The hierarchical correlation analysis conducted under drought and stress clearly revealed the changes under drought as compared to irrigated condition (Fig. 1.4).

Phenomics of moisture deficit tolerance

Identification and functional characterization of stress responsive genes are important steps towards understanding and improving drought tolerance of rice. A total of 17 T_0 events were generated with a total of 36 transgenic plants involving seven different constructs and eight genes identified as drought responsive from publically available microarray data (Table 1.2). PCR with *hptII* and gene specific primers was done to identify plants, positive for transformation. Subsequently, seeds

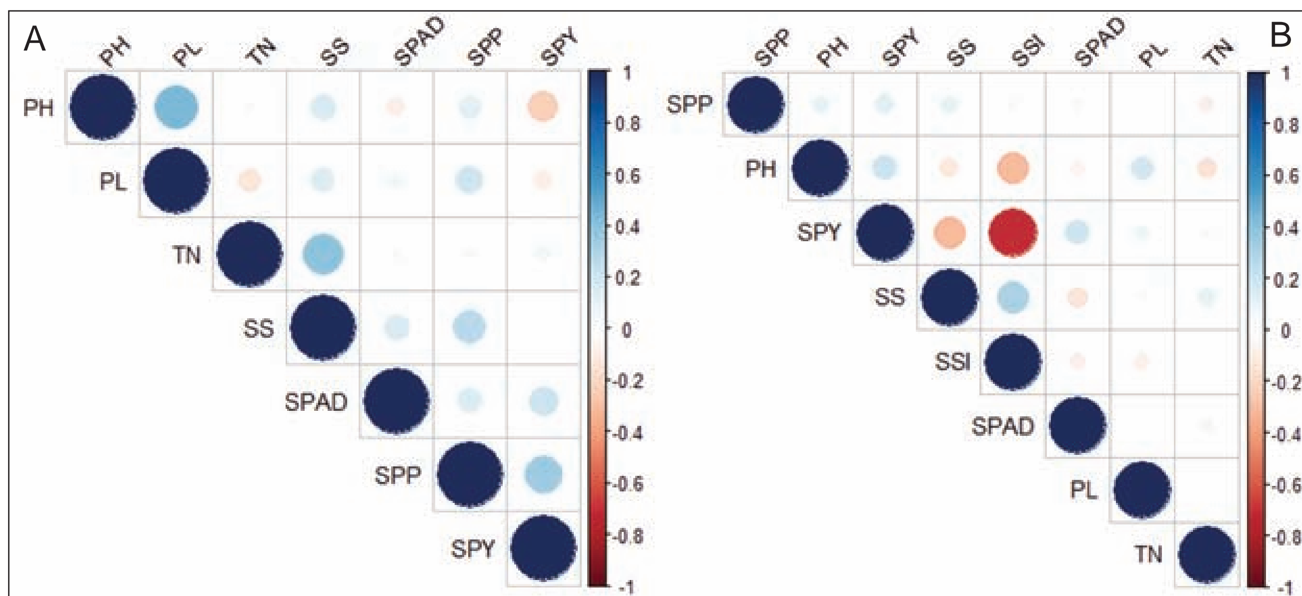


Fig. 1.4: Correlogram of morpho-physio and enzymatic data in (A) irrigated control, and (B) reproductive stage drought stress based on the parents and the rice RILs constituting the bulks.

were harvested from *hptII* positive T_0 plants in order to get T_1 generation which has also been confirmed for transgenic status with *hptII* and gene specific primer. Totally, 18 plants were obtained in T_1 generation from five different constructs and five different genes. Other than for the gene, *Os11g0210100*, transformants have been generated for all other genes with one or the other construct. Other than one plant from Nagina22 transformed with pCXUN *Os11g0670900*, all were found to be PCR positive (Fig. 1.5). The transgenic plants were assessed for their performance for various physiological parameters (chlorophyll content, carotenoid content, RWC and proline content) under normal growth conditions.

Table 1.2: Status of transformation and transgenic development for functional validation of drought responsive genes in rice

S. No	Construct	No. Of Events in T_0	No. Of Events in T_1
1.	pCXUN 670900-N22	4	4
2.	pCXUN 173000-N22	3	3
3.	pCXUN 412800-PSII	2	2
4.	pCAMBIA 690500- N22	3	1
5.	PCXUN 546400-N22	1	1
6.	pCAMBIA 10210.1 N22	3	-
7.	pCAMBIA 10210.1 PS2	4	-
8.	pCAMBIA 690500 PS2	3	-

Fig. 1.5: PCR confirmation of rice T_1 transgenic plants by *hptII* primer. Lane: 1, 100bp ladder; 2, positive control; 3, negative control; 4-5, pCXUN 412800-PSII; 6-8, pCAMBIA 690500- N22; 9-11, pCXUN 173000-N22; 12, pCXUN 546400-N22; 13 to 21, pCXUN 670900- N22.

Allele mining for agronomically important genes in wild rice germplasm and stress tolerant landraces of rice growing in the hot spots

A total of 560 wild rice accessions have been collected and maintained at NRCPB from wide geographical regions from 14 °N to 32 °N latitude and 70 °E to 93 °E longitude. This was a result of multiple expeditions made to around 64 districts belonging to 12 political states of India. To the 477 accessions available till last year, an addition of 83 accessions was made this year (Fig. 1.6). So far, 544 accessions have been characterized based on 46 morphological descriptors whereas 418 accessions have been characterized based on *pSINE1* and SSR molecular markers. A web portal (nksingh.nationalprof.in/iwrdb) was created for 516 wild rice accessions. The database contains information on geographical origin, passport data, morphological evaluation data, photographs and video clips on each of the wild rice samples collected under the project. The database also includes basic information on 24 recognized wild rice species based on literature, including their genome information, distribution, habitat, morphological characters and specific trait values.

Accessions were evaluated for their tolerance to abiotic stresses viz., drought, salinity, submergence and anaerobic germination, from 2012 to 2014. The tolerant accessions identified were re-examined for confirmation of their phenotype in the year 2015. In order to transfer the tolerance source for drought, salinity and flooding from wild relative to cultivated rice mega varieties, 12 genetic crosses were also made.

Of the 299 wild rice accessions collected and evaluated for growth under salt stress, 103 representative accessions were sequenced for members of *HKT* ion transporter family genes by employing Ion Torrent *PGM* sequencing platform. Genes validated for salt tolerance in rice and some probable salt stress responsive genes validated in

Fig. 1.6: Representative ecological habitats and collection sites of the Indian wild rice accessions. A) Large shallow ponds full of wild rice, B) Large wild rice stand on lowland river banks, C) Threatened by building construction in small town, and D) Threatened by upcoming industrial development in large city.

other organisms were taken for allele mining. In total, 30 genes of various classes, viz., transporters (eight *HKT* genes, five *NHX* genes, *SOS1, PIP1*) genes involved in osmotic regulation (two *BADH* genes, two *P5CS* genes, 2 *MIPS* genes, *CMO*), transcription factors (*Hsfc1b, MYB2, bZIP71, bZIP23*) and signalling pathway genes (*SIK1, VTE1, SOS2, SOS3*), were selected for allele mining and sequenced. From 125.5 kb of sequence information, 2043 SNPs with an average density of 16 SNPs per kb were identified. All major and few minor haplotypes of the candidate genes were randomly distributed around all eco-geographic region. A minor haplotype with maximum seven accessions, five from west coast and two from lower Gangetic plain region, were found in 13 genes. Cultivated rice varieties grouped with major haplotype for most of the genes. LD based analysis showed geographic region based association of SNPs. Phenotyping for Na and K concentration in roots and shoots was done in 30 days old accessions which were subjected to 15 days of 150 mM of salt stress in hydroponics (Fig. 1.7). This is the first study of allele mining of eight members of *HKT* gene family from Indian wild rice reporting a salt tolerant allele of *HKT2;3*. Besides, *HKT1;5* also showed a salt tolerant allele from wild rice. Haplotype analysis revealed that haplotypes H5 and H1 of *HKT1;5* and *HKT2;3*, respectively, were associated with high salinity tolerance.

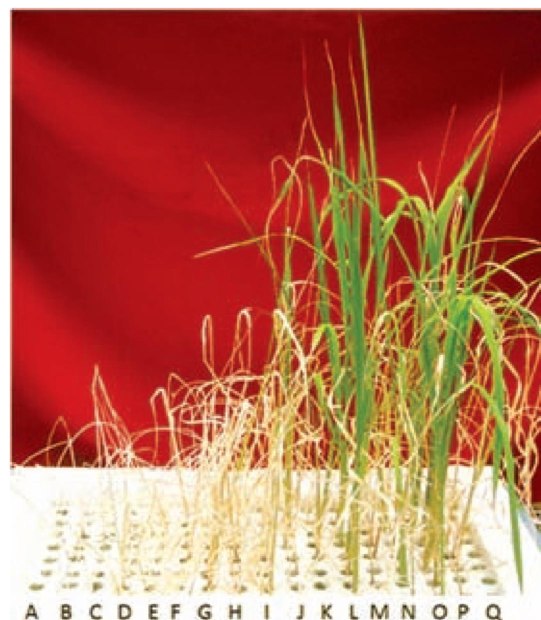


Fig. 1.7: Response of wild rice accessions after 15 days of 150 mM of salt stress in NKSWR104; E. NKSWR092; F. NKSWR119; G. NKSWR085; H. NKSWR115; I. NKSWR132; J. NKSW R097; K. *Oryza nivara*330646; L. NKSWR143; M. NKSWR101; N. NKSWR079; O. FL478; P. VSR156; Q. Border (NKSWR143 is tolerant and other accessions are sensitive, FL478- tolerant check, VSR156- sensitive check).

Maintenance, characterization and use of EMS mutants in an upland variety Nagina22 for functional genomics in rice

The phase I of Nagina22 EMS mutants funded by DBT operated from 2007-2013 with multiple state and central institutions along with NRCPB as the lead centre. Based on the progress made in those six years, the second phase of the project was sanctioned by DBT in Nov 2015 for five years. In this phase, a seed storage facility for maintaining the mutants is to be created, mutant data base is to be developed for the mutants in the mutant garden and causal genes for the few promising mutants identified in the first phase needs to be mapped. In this regard, development of SSR marker set to identify clean EMS mutants from the Nagina22 EMS mutant population is essential. For this, a set of 60 genome-wide SSR markers had been identified (Fig. 1.8). This set will also be used for BSA of mapping population

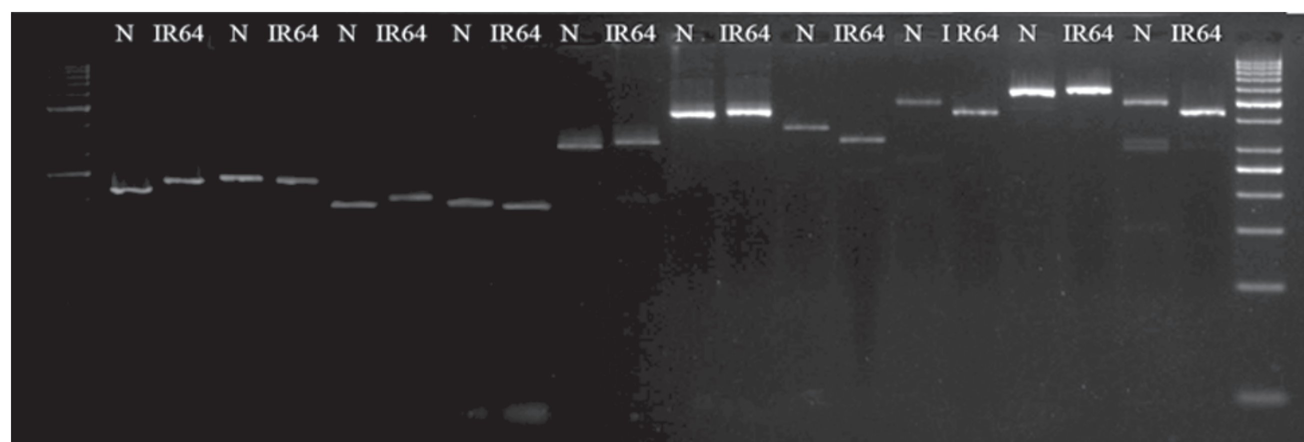


Fig. 1.8: Selection of SSRs showing polymorphism between Nagina22 and IR64. Lane 1 and lane 22: 100bp ladder; N: Nagina22.

developed between the mutant and IR64 in order to identify the causal gene in the mutant.

Chromosomal position of *Pi54* gene in different transgenic events

Rice blast caused by *Magnaporthe oryzae* is an important disease of rice which affects rice production at global level. Use of blast resistance varieties is one of the best options to manage this disease. Different transgenic lines were developed for the validation of *Pi54* and its orthologues *Pi54rh* genes using biolistic approach. Plants which contain single copy insertions in homozygous conditions and also showing resistance reaction to blast were used for finding their chromosomal positions in the rice genome. To determine the location of *Pi54* gene in the genome of transgenic lines inverse PCR was performed using a set of specific primers. Inverse PCR analysis showed that in two transgenic lines, *Pi54* gene was integrated on short arm of chromosome 6 and long arm of chromosome 10 at 12.94 Mb and 22.3 Mb, respectively. Other blast resistance gene, *Pi54rh* was found to be integrated on short arm of chromosome 1 at 16.25 Mb (Fig 1.9). The disease

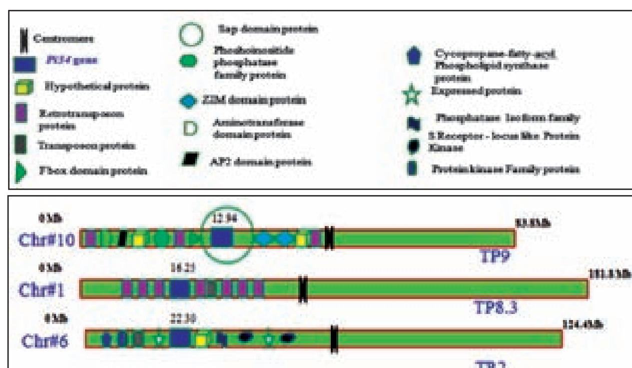


Fig. 1.9: Physical location of *Pi54* gene on different rice chromosomes in transgenic lines.

resistance phenotype was also not affected because of the changed positions of these genes in the rice genome. It was proposed that *Pi54* and *Pi54rh* genes can be effectively used in the breeding programmes for the development of broad spectrum and durable blast resistant rice.

Allele mining of *Pi54* gene and sequence analysis of the variants

The rice lines (92) used in present study were grown in contained condition and the fifteen day old seedlings were challenged with the diagnostic isolate of *M. oryzae* (Mo-nwi-37'-1). After one week of inoculation, all the rice lines were grouped into resistant and susceptible categories based on their reaction to *M. oryzae*. Out of 92 rice lines, 72 were found resistant and the rest of the 20 lines were susceptible (4-5). These lines were used for the allele mining of *Pi54* gene. Percentage of substitutional change at protein level in the *Pi54* gene was calculated and compared within and between disease resistant and susceptible phenotypes of *Oryza* species. The amino acid substitutions were scored for the specific positions. The number of substitution pre site was found to be equal to and greater than 10 (*i.e.* $N_{mut} \geq 10$). Overall, 23 substitutional sites were identified across the alignment of 64 Pi54 protein. The number of mutations per mutational site was calculated in all the aligned 64 predicted sequences implicating 66% of mutational sites having mutation in one site, 19% of the sites having mutations in two to nine sites and rest of the 15% sites having mutation in ten or more than ten sites. The mutational profiling of disease resistance and susceptible phenotypes of *Oryza* species indicated that most of the mutational sites were showing a constant frequency distribution between the resistant and susceptible groups (Fig. 1.10).

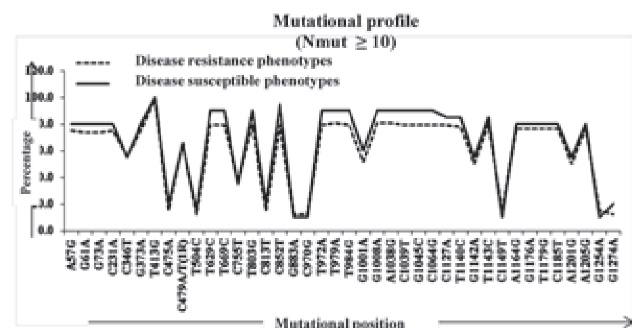


Fig. 1.10: Number of mutational changes in 92 *Pi54* alleles: mutational profiling of disease resistant and susceptible phenotype. Mutational changes were scored for the specific positions only, where the number of mutations per site was found to be equal to and greater than 10 (Adapted from Thakur *et al.*, 2015).

Genome wide distribution of disease resistance and defence response genes in rice

In silico approach was used to identify and physically map 786 resistant (R) -genes and 167 defense response (DR) - genes in the rice genome. This analysis showed that most of the *R-* genes

present in clusters of which 55.6% of the genes are tandemly repeated within the clusters and distributed on all rice chromosomes. Maximum (92) *R*-genes consisting of 31 clusters were present on chromosome 11, followed by chromosome 1 in which 91 genes formed 24 clusters which indicates that there are some chromosomal 'hot spots' in which genes duplicates rapidly. Defense response genes (167) such as chitinases, glucanases and thaumatin like proteins, were physically positioned for the first time on 12 rice chromosomes. It was also shown that among all *R*- and *DR*- genes, 73.45% are expressed having variable frequency of matches with the expressed sequenced tags (EST) in the rice EST database. The genes and their paralogues had been identified across the chromosomes (Fig. 1.11). Exact position of these genes along with their expression levels had been provided in the public domain as a community resource. Structural organization of *R*-genes and their important allelic variants found in this study can be used after validation to understand the molecular mechanism of disease resistance and their evolution in rice and related species.

Fig. 1.11: An example of *R*-genes paralogs in rice genome using CIRCOS software. The circle represents rice chromosomes having paralogous genes and their number of paralogous gene matches on different rice chromosomes.

Expression and functional characterization of serine hydroxymethyltransferase (SHMT) gene from salt tolerant rice

The enzyme serine hydroxyl methyltransferase (SHMT) is involved in synthesis of an essential amino acid, which plays important roles in a variety of biological processes including metabolism, purine and pyrimidine biosynthesis, and generation of activated one-carbon (C-1) unit which are utilised in methionine and purine metabolism. No reports are available for characterization of SHMT on salt stress in plant system. This gene was identified through gene and protein expression studies from salt tolerant rice cultivar and overexpressed rice SHMT gene (*OsSHMT*) in *A. thaliana*.

OsSHMT Arabidopsis showed higher salt tolerance and could grow very well under 100 and 200 mM NaCl stress (Fig. 1.13). Plant height and dry weight of *OsSHMT Arabidopsis* showed less reduction in comparison with wild type (WT) (Fig. 1.14). Overexpressed plants could recover well after applying salinity stress (Fig. 1.15). Chlorophyll content depicted less reduction in NaCl stress in comparison with WT (Fig. 1.16). OsSHMT protein is induced by applying salinity stress in root, stem, leaf

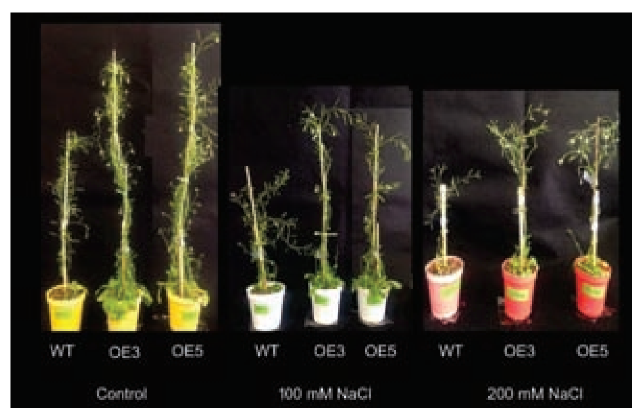


Fig. 1.13: Growth of wild-type and *OsSHMT* overexpressing *Arabidopsis* (OE3 and OE5) after one month of NaCl (100 and 200 mM) stress.

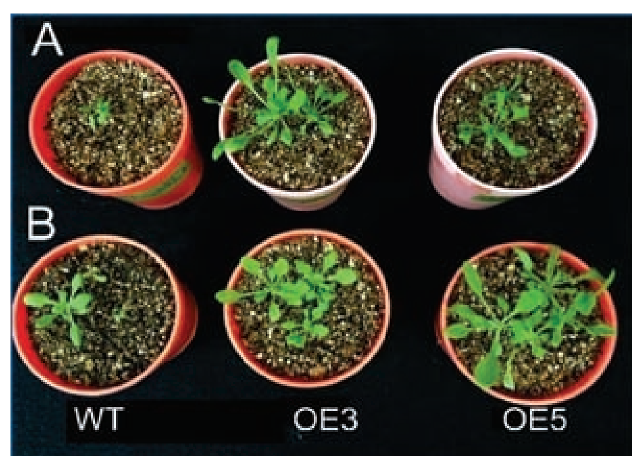


Fig. 1.14: Growth showing recovery of wild-type and *OsSHMT* overexpressing *Arabidopsis* (OE3 and OE5) after 150 and 200 mM of NaCl stress. Seedlings were subjected to 150 and 200 mM NaCl stress for one week and transferred to normal condition.

and siliques (Fig. 1.17). Accumulation of Na^{+} content is less in *OsSHMT Arabidopsis* in compared with wild types. Serine and glycine were significantly higher in *OsSHMT Arabidopsis* after NaCl stress. Overall this study presented the significant role of SHMT gene in conferring salt stress tolerance.

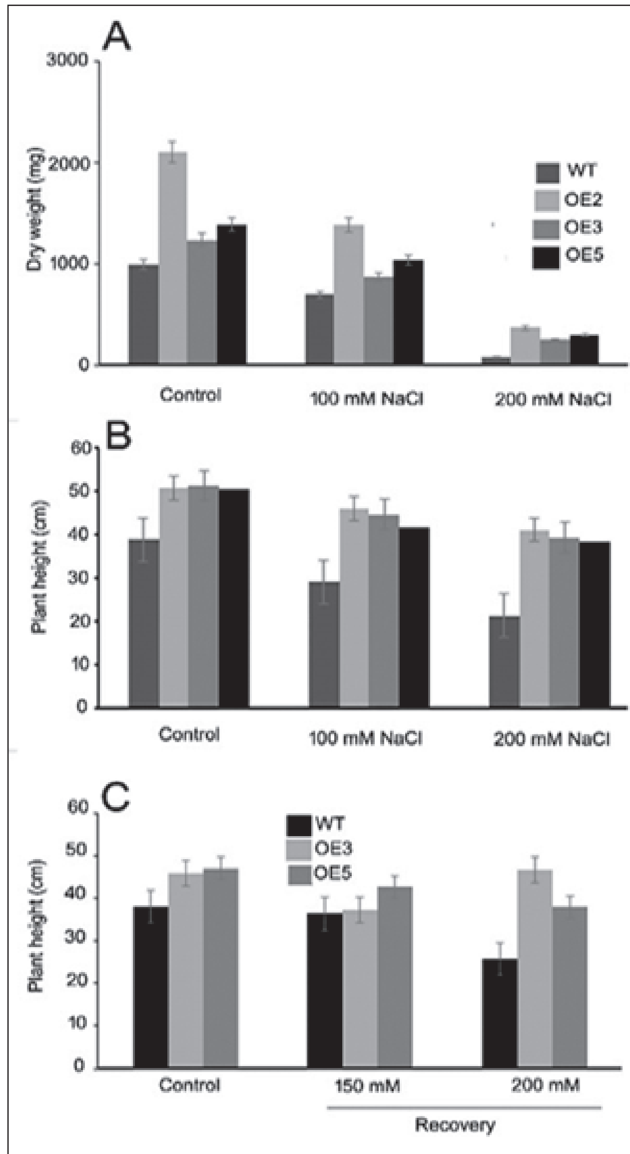
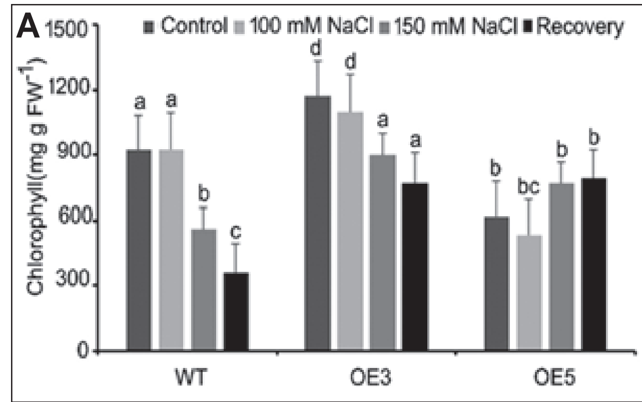


Fig. 1.15: Plant height of wild-type and *OsSHMT* overexpressors: (A) after 4 weeks of 100 mM NaCl stress, (B) after recovery from 150 and 200 mM NaCl stress.



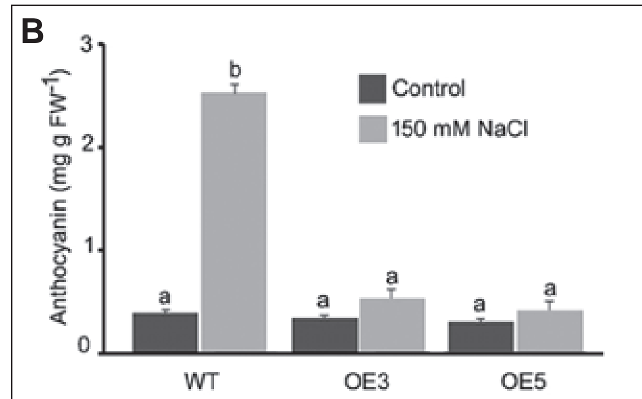


Fig. 1.16: Effect of salinity on (A) chlorophyll, and (B) anthocyanin content of WT and *OsSHMT3* over expressed (OE) *Arabidopsis*. Seedlings (14-d-old) were transferred to 100 and 150 mM NaCl for one week and chlorophyll and anthocyanin were extracted from leaves. For recovery 200 mM stressed (one week) seedlings were again transferred to normal MS-agar media.

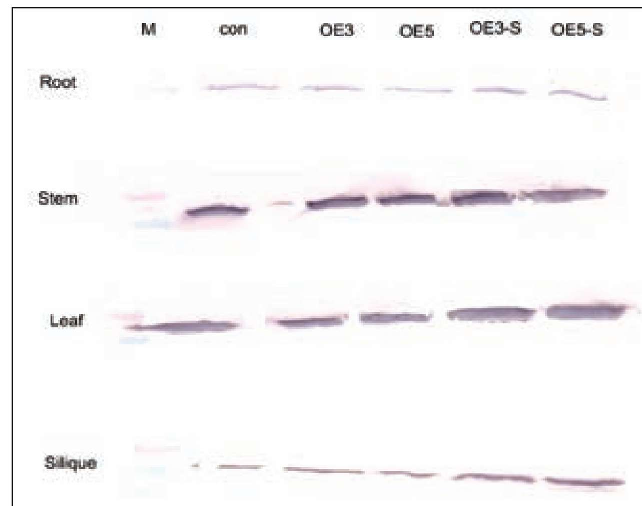
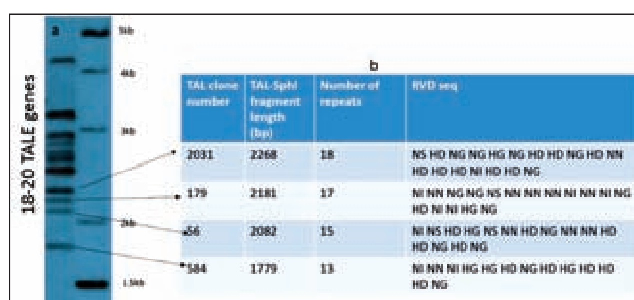


Fig. 1.17: Effect of salinity on OsSHMT protein expression in different tissues of *OsSHMT3* over expressed (OE3 and OE5) *Arabidopsis*.

Cloning of transcription activator like effector genes from bacterial blight pathogen

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 resistant or susceptibility genes. Hence, attempt was made to identify undiscovered TALes and their cognate binding sites in the host by focussing on a *xa*13 compatible strain. To approximate the number of *TALe* genes in the strain RFLP analysis using *SphI* sites were performed. The hybridization result showed 11 clear bands with sizes ranging from 1.8-4.2 kb (Fig. 1.18a). The bands exhibiting stronger intensity and more width indicate presence of more than one *TALe* gene copy of similar sizes. Hence, the *Xoo* strain may contain 16-18 *TALe* genes. For cloning these, a subgenomic library was constructed, and transformants were screened for *TALe* positive clones by PCR. Due to the tandem identical repeats units present in *TALe* genes, sequencing clones with insert size >2.5 kb failed. Nonetheless, four lowest molecular weight *TALe* genes have been sequenced with some ambiguities to be further analysed. Preliminary results identified them to harbour 13-18 repeats. The repeat variable diresidue (RVD) sequence which defines their recognition and binding site in host was further deciphered utilizing *in silico* tools (Fig. 1.18b). Work is in progress to discern the ambiguities and confirm these RVD sequences.



TAL clone number	TAL-SphI fragment length (bp)	Number of repeats	RVD seq
2031	2268	18	NS HD NG NG HG NG HD HD NG HD NN HD HD HD NI HD HD NG
179	2181	17	NI NN NG NG NS NN NN NN NI NN NI NG HD NI NI HG NG
56	2082	15	NI NS HD HG NS NN HD NG NN NN HD HD NG HD NG
584	1779	13	NI NN NI HG HG HD NG HD HG HD HD HD NG

Fig. 1.18: a. RFLP analysis of *TALe* genes in *Xoo* strain RR1. b. Number of repeats and corresponding RVD sequences of four TAL effectors encoded in RR1.

To explore the possibility of this strain harbouring *TALe* gene on a plasmid (s), the strain was checked for the presence of plasmid. The results detected two plasmids, out of which one is major with size 50-60 kb and other small of 3-6 kb (Fig. 1.19). These sizes and number need to be confirmed by restriction profiling after which presence/ absence of *TALe* gene on plasmid will be tested.

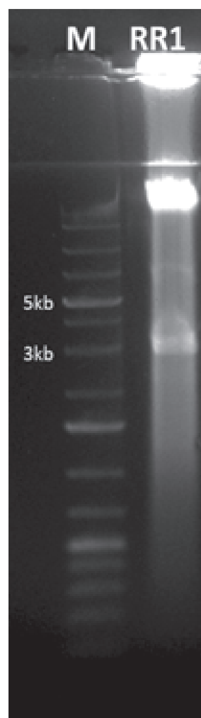


Fig. 1.19: Two plasmids detected in *Xoo* strain RR1.

Enhanced grain-fill under low-light irradiance in rice

Plant B-box proteins (BBX) modulate light-regulated transcription and development of plants through interactions with key regulators of light-signaling pathway. Among all, BBX-32 is a B-box zinc finger protein and specifically involved in modulation of dark to light transition growth in plants. It functions downstream of multiple photoreceptors as a modulator of light responses. Transgenic soybean plants expressing B-box proteins showed higher grain yield in field conditions due to (i) changes in soybean gene expression during the transition from night to day that regulate diurnal processes in soybean at dawn, and (ii) change in timing of reproductive development in transgenic plant with increased duration of pod/seed development leading to higher yield. To explore the possibility of deploying the *Atbbx32* in rice, it was cloned from *Arabidopsis* under 35S promoter (pCAMBIA) and mobilized into rice variety Nagina 22 (Fig. 1.20).

Mapping of panicle blast resistance gene in rice

Rice blast is the most devastating fungal disease caused by *Magnaporthe oryzae*. Although it infects all parts of rice plant, infection at reproductive stage at panicle and neck is the severe form of rice blast. To identify gene(s) associated with panicle blast resistance, a population was developed using cross of Tetep derived RIL4, which is resistant to panicle blast, and susceptible line HP2216. Last year DNA was isolated from F_2 derived F_3 population of this cross and screened known genes to check association with phenotype. From analysis it was observed that in this cross genes related to panicle blast are different from characterized one. Further to identify genes for panicle blast the parents along with two resistant

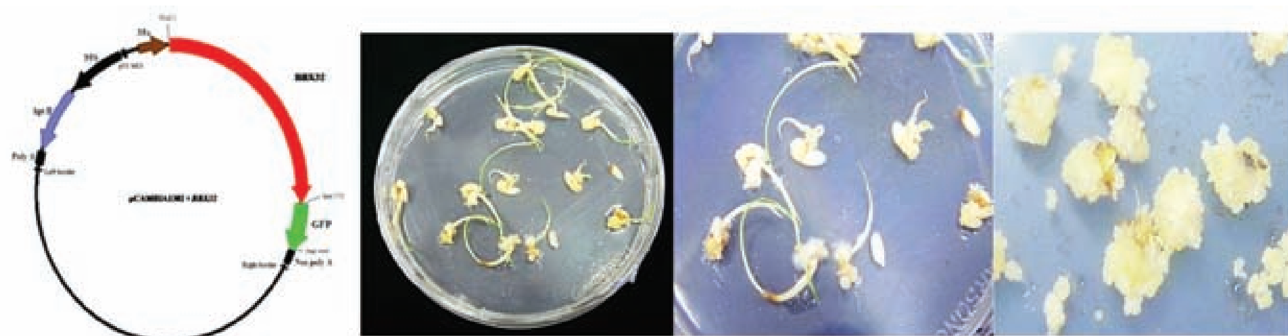


Fig. 1.20: Cloning of *Atbbx32* in pCAMBIA and transformation of rice.

pool and three susceptible pools from three different F_2 derived F_3 lines were used. More than 100 SSR markers were first screened with parents and then with all five pooled samples, but none of the SSR was found associated with phenotype. Then 100 ISSR markers were used to screen parents, out of which amplification was observed in 40 primers. Further three bands from UBC-812, 818 and 856 were showing polymorphism in five resistant and susceptible pooled samples (Fig. 1.21). These PCR amplicons were eluted from gel and cloned in sequencing vector for sequencing. The sequences obtained will help to locate position on the rice chromosomes.

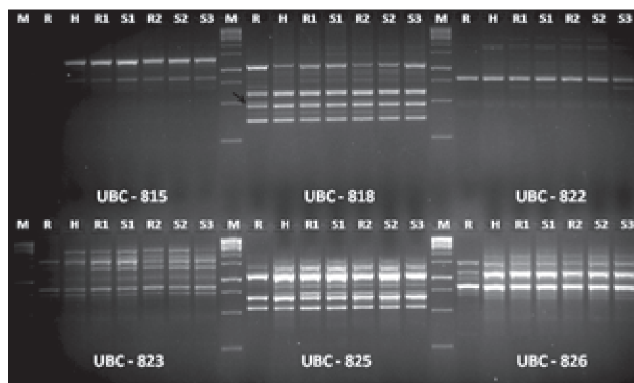


Fig. 1.21: A representative gel electrophoresis image of screening of rice F2 derived F3 plants of RIL4X HP2216 cross with ISSR primers. Sample loading: L, 1Kb DNA ladder; 1, RIL4; 2, HP2216; 3, R1; 4, S1; 5, R2; 6, S2; and 7, S3.

Development of *in vivo* method of panicle blast evaluation

Evaluation of panicle blast resistance at field level is very difficult as this disease can occur at 23-25^{0}C with at least 80% humidity. An *in vivo* method of panicle blast evaluation at controlled condition in living panicle of rice plants was developed. *M. oryzae* strain Dehradun was used for neck infection. The procedure where neck was infected with pathogen solution with spore concentration about 10^5 spores/ml was standardized. The suspension was then injected to the panicle using syringe just before the panicle comes out of flag leaf. The injected portion was covered with moist cotton with double distil autoclaved water to provide moist condition for the pathogen. The infected panicles were observed for the disease occurrence one month after inoculation. Using this method, two resistant lines, Tetep and WR2 and two susceptible lines, HP2216 and HR12, were infected with *Magnaporthe* and samples were collected for transcriptome analysis.

Cloning of intrinsic yield genes from *Arabidopsis*

Cloning of dwarf4

This gene encodes a 22 α-hydroxylase that is involved in various biological processes like biosynthesis of brassinosteroid, response to jasmonic acid and down-stream signaling pathways, leaf development, leaf shaping in plants. Transgenic plants ectopically over-expressing *DWF4* exhibit 35% increase in inflorescence length, bear more than two fold increase in number of branches/inflorescence and siliques/inflorescence with overall yield increase by 59% over wild type (non-transgenic) plants. Thus, it may be plausible to control/enhance yield in crop plants such as mustard by engineering DWF4 biosynthesis. In this endeavour, a full length genomic sequence of *dwarf4* was retrieved from TAIR and amplified from *Arabidopsis* genome using PCR (Fd: CACCAACTAGCTCCATCTTCGAAACAG; Rv: CAGAATACGAGAAACCCTAATAG). High fidelity Phusion *Taq* polymerase was used for error proof amplification of the gene (Fig. 1.22 A) at following conditions for 30 cycles; initial denaturation at 98°C for 30 seconds, denaturation at 98°C for 10 seconds, annealing at 60°C for 20 seconds, extension at 72°C for 1 minute and 45 seconds and final extension at 72°C for 5 minutes. The blunt end PCR product was

directionally cloned in pENTR™/SD/D – TOPO cloning vector for entry in to the Gateway system following the manufacturer's protocol (Fig. 1.22 B). The ligated PCR product was transformed into *E. coli* (XL1 blue) cells using one-shot chemically competent cells and positive clones were screened and confirmed by release of ~500 bp fragment on double digestion with *Bam*HI and *Hin*dIII. Subsequently, the gene was cloned in a destination (binary) vector having 35S promoter and *mGFP* using gateway cloning strategy and currently being deployed in mustard (Fig. 1.22 C,D).

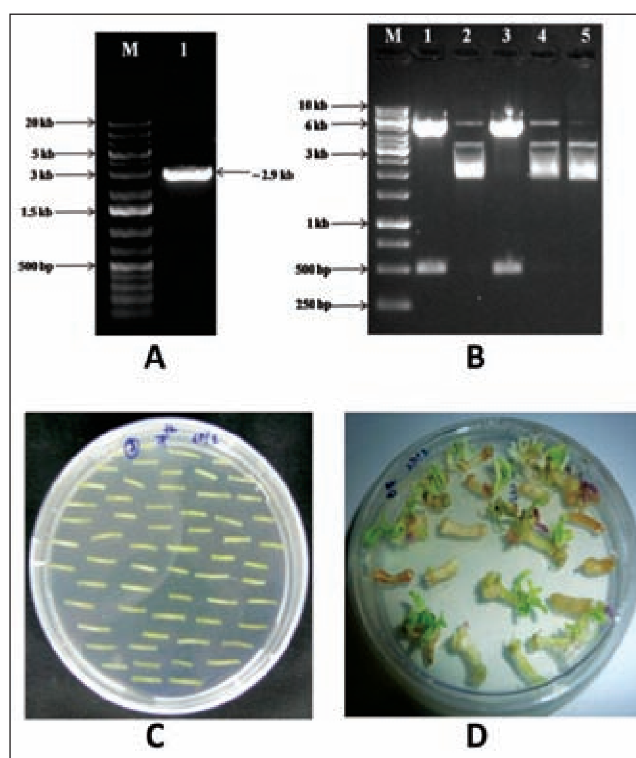


Fig. 1.22: Isolation and cloning of *Arabidopsis dwarf4* gene. (A) PCR amplification of *dwarf4* gene. Lane: M, 1 kb ladder; 1, *Atdwarf4* amplified from *Arabidopsis* genome, (B) Cloning of *dwarf4* gene in pENTR/SD/TOPO vector. Lane: M, 1 kb ladder; Lane 1-5, Putative *dwarf4* gene in pENTR/SD/TOPO vector restricted with *Bam*HI and *Hin*dIII releases ~500 bp fragment of *dwarf4*. (C, D) Transformation and regeneration of Pusa Jaikisan with *dwarf4*.

Cloning of cytokinin oxidase (ckx3)

Cytokinin oxidase genes have their regulatory role in cytokinin production. Root specific over expression of cytokinin oxidase genes showed reduced accumulation of cytokinin in roots apices resulting higher number of primary and secondary root development imparting better nutrients uptake and stress tolerance. Thus, efforts were made to clone the *ckx3* from *Arabidopsis* for genetic transformation of mustard for yield enhancement. Gene specific primers (Fd-5'ATGGCGAG TTATAAT CTTCGTTCAC-3' and Rv-5'ACTCGAGTTTATTTTTTGAAATATATTT TG-3') were used for amplification of *ckx3* gene from genomic DNA and the expected size of 3.2 kb was obtained after successful amplification (Fig. 1.23 A). Amplified gene was ligated to pGEMT easy cloning vector. Recombinant clone was confirmed by colony PCR with similar conditions as performed earlier with genomic DNA with *ckx3* gene specific primers. Amplicon size of 3.2 kb is observed in the colony PCR (Fig. 1.23 B) confirmed cloning of *ckx3* gene. Further confirmation of gene cloning was accomplished through restriction digestion with specific restriction enzymes (Fig. 1.23 C). Two separate restriction enzymes *Eco*RI and *Nde*I were used to check the gene cloning. Presence of three fragments (Fig. 1.23 C, lane 2) and two fragments (Fig. 1.23C, lane 3) confirmed cloning of 3.2 kb *ckx3* gene.

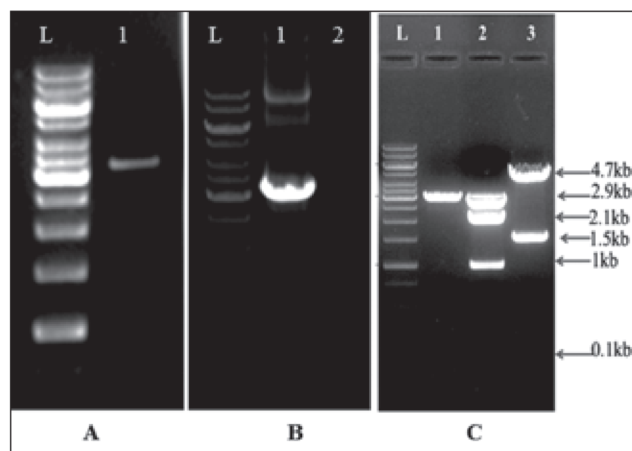


Fig. 1.23: Cloning of *Arabidopsis* cytokinin oxidase gene (*Atckx3*). (A): PCR Amplification of *Atckx3* from *Arabidopsis*. Lane: L, 1 kb plus ladder (Fermentas); 1, *Atckx3* (3.2kb) amplified from *Arabidopsis* genome, (B): Colony PCR of selected colonies. Lane: L, 1 kb plus ladder; 1, amplicon from recombinant colony (3.2kb); 2, No amplification from blue colony, (C): Restriction digestion profile of the cloned gene. Lane: L, 1kb ladder; 1, pGEMT-Easy restricted with *Eco*RI; 2, pGEMT-Easy containing *ckx3* insert restricted with *Eco*RI; 3: pGEMT-Easy containing *ckx3* insert restricted with *Nde*I.

Authenticity of cloned gene was verified by DNA sequencing and homology matching with *ckx3* sequence available in the TAIR database (Fig. 1.24 A,B). Absolute alignment without any mismatch proved that *ckx3* gene has been successfully cloned and the cloned gene is devoid of any PCR induced error. Complete sequencing of the gene and cloning under root specific promoter is being carried out for deploying the gene into mustard for root specific expression.

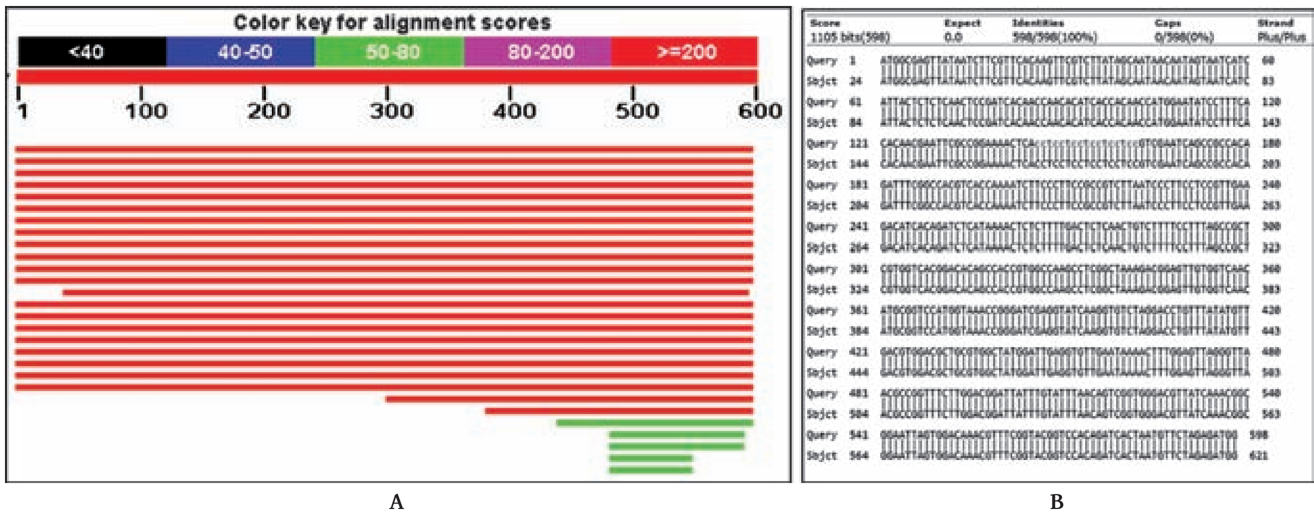


Fig. 1.24: (A) Sequence alignment and (B) blast search showing homology of cloned *Arabidopsis ckx3* gene with available sequence in TAIR data base.

Genome wide analysis of aquaporins in fibre development in flax

Aquaporins are integral membrane proteins (21-34 kD) involved in water translocation and transport of small solutes in plants. Besides transport of water AQPs from different plant species have shown to be involved in abiotic stress tolerance and recently they have also been implicated in cotton fiber development. To understand role of aquaporins in fiber development, genome wide distribution, structure and expression pattern of AQPs were analysed in flax (*Linum usitatissimum*). Genome wide comparative analysis led to identification of 51 non-redundant genes coding for aquaporin (AQP) in flax. Further functional annotation of the sequences using protein database confirmed the function of candidate sequences as aquaporins. Protein domain analysis revealed presence of six transmembranes in 35 of the 51 AQPs identified. Phylogenetic tree of flax AQPs along with the known AQPs from *P. trichocarpa* showed five distinct clusters representing different class of AQPs (Fig. 1.25A). Microarray based expression analysis revealed 33 out of 51 AQPs represented on array showed evidence of expression. Among different AQPs, majority TIPs showed low expression; on the contrary many PIP family members showed higher expression across different tissues (Fig. 1.25B). Role of individual member of these intrinsic proteins (TIP, PIP and NIP) in fibre development is being investigated in flax.

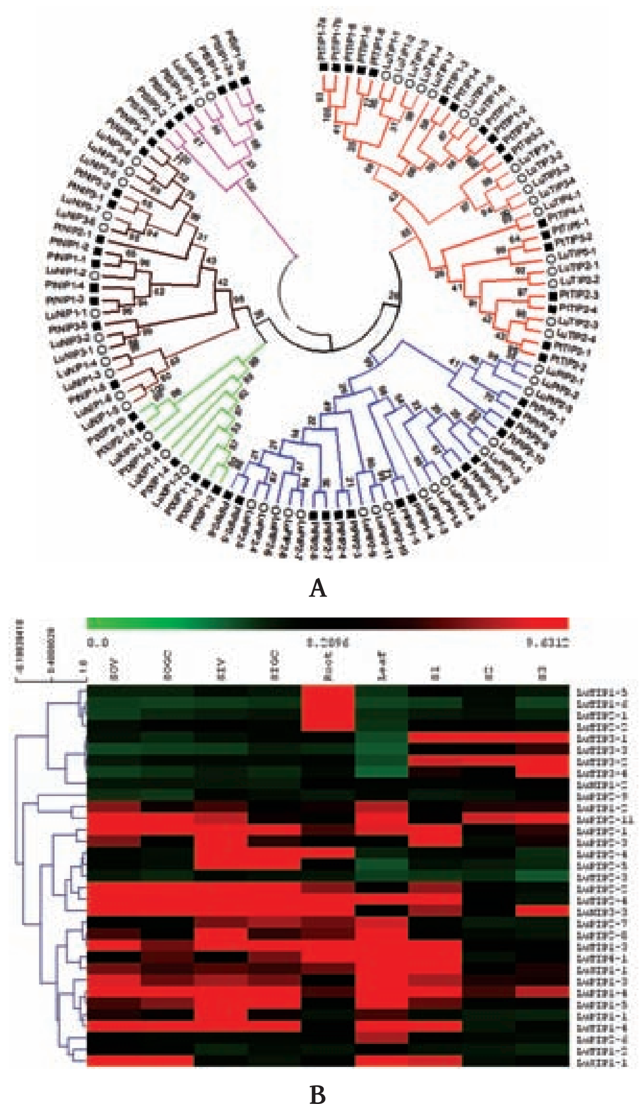


Fig. 1.25: Genome-wide analysis of aquaporins (AQPs). (A) Divergence analysis of AQPs, and (B) their expression pattern showing higher level of expression of majority of PIPs compared to TIPs across the different tissues in flax.

Development of 'Standard Operating Protocols' (SOP) for genetic fidelity testing of tissue culture raised plants

NRCPB is a referral centre for National Certification System for Tissue Culture Raised Plants (NCS-TCP) with the responsibility to identify a set of markers as well as marker systems for unambiguous DNA profiling of the mother plant and its tissue culture multiplied progeny. For this, ISSR markers for genetic fidelity testing of potato, sugarcane and banana were screened and standardized (Fig. 1.26). Out of 60 ISSR primers screened for potato, 22 were found polymorphic with 65 polymorphic bands. In case of sugarcane, 92 ISSR markers were screened and 12 primers were found polymorphic with 28 polymorphic bands. In banana, 48 ISSR primers were used for screening and a total of 32 primers with 103 polymorphic bands were observed. Further, pooling experiment was carried out to reduce cost of evaluation of ISSR primers. The idea of using pooling DNA samples is to reduce the burden of increased number of samples for genetic fidelity testing. Here two differentiating/ contrasting varieties of sugarcane exhibiting explicit banding pattern were pooled in various ratios (A:B - 1:1, 4:1, 9:1, 1:4 & 1:9) and then PCR was run using a specific ISSR marker (Fig. 1.27). Using this it was shown that pooling up to 10 samples with more number of polymorphic bands can reduce the cost of evaluation.

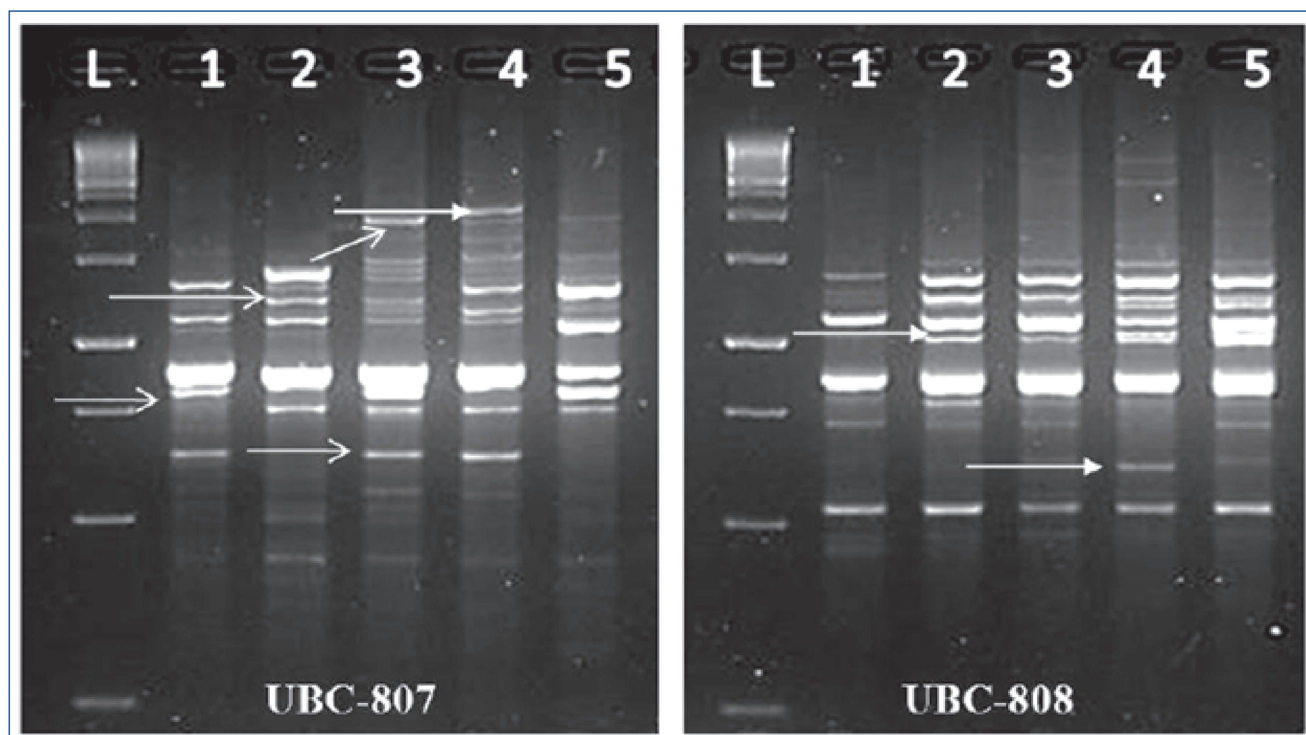


Fig. 1.26: Representative ISSR profiling of five potato varieties with primers UBC-807 and 808. L, 1 Kb Ladder; 1, Kufri Bahar; 2, Kufri Gaurav; 3, Kufri Surya; 4, Kufri Chipsona; 5, Kufri Girdhari.

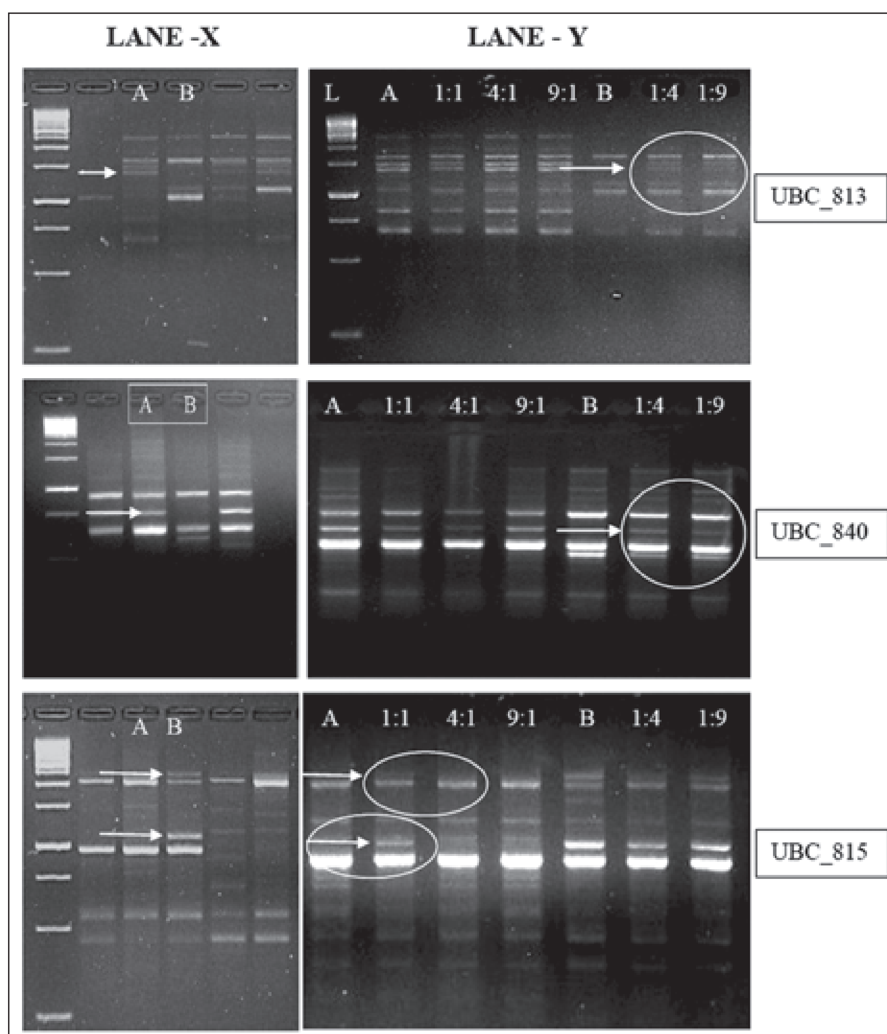


Fig. 1.27: Pooling potato samples in different ratios. Lane: X, Showing normal banding pattern of samples A and B; Y, Showing banding pattern of samples A & B as well as their pooling ratios (A:B).

Plant genome databases

For the use of genomic resources available in the public domain it is very important to collect and collate those information at one place in a database format. Therefore, the data for the different crops is downloaded from the 'www.ncbi.nlm.nih.gov' and from other database sources in FASTA format for different sequences type like EST, GSS, Genomic, cDNA, STS, HTG and Unigene. FASTA format is then converted into the tab delimited format by using the in-house Perl scripts and finally data is uploaded and updated into the local database 'Plant Genome Database' with using Mysql. A total of = 2,95,00,808 entries are present in 'Plant Genome DB' (Table 1.3). Major part of the database is contributed by ESTs (Approximately 57%) and GSS sequences (31%). Least data is available in HTGs and Unigene sequences. The entire crops database was divided in seven categories as Cereals, Vegetables, Oilseeds, Legumes, Fruits, Fiber and Others. Similarly, plant gene database has also been constructed at NRCPB and status of database interms of number of gene sequence entries has been given in Fig. 1.28. As of now gene sequences of more than 70 plant species have been collected in the database containing 31,20,333 sequence entries.

Table 1.3: Status of sequence entries in 'Plant Genome DB'

Sequence Type	Vegetables	Fruits	Cereals	Oilseeds	Legumes	Fibre	Others	Total
cDNA	56487	58736	223193	8364	10814	179	4545	367873
EST	2021731	1800914	5859697	3456151	2216276	403435	660629	16088490
Genomic	661513	204146	242536	191208	92430	78540	116823	1602508
GSS	2373276	598334	4152987	996366	1597935	242674	164913	10168492
HTG	11223	289	26953	1083	3684	227	1078	58832
STS	12655	3038	120051	12318	11968	1148	797	163361
Unigene	287500	89812	355311	121470	76078	21406	46827	1051252
Total	5424385	2755269	10980728	4786960	4009185	747609	995612	2,95,00,808

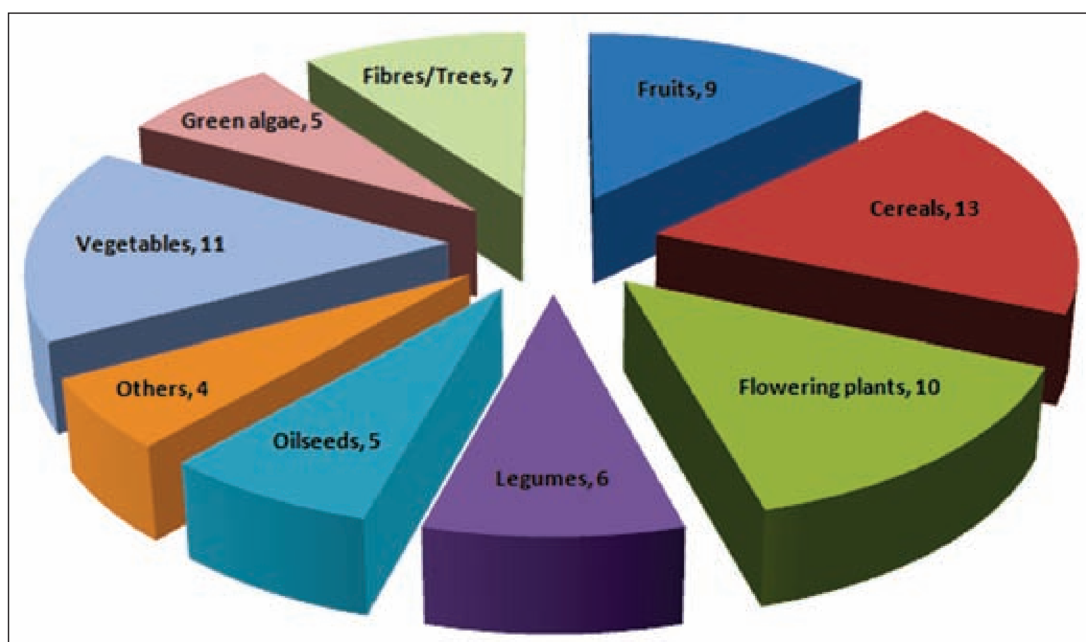


Fig. 1.28: Crops in 'Plant Genome DB'.

2 BIOTECHNOLOGICAL APPLICATIONS FOR PULSES IMPROVEMENT

Functional genomics of the pigeonpea CMS system derived from A_2 cytoplasm (*Cajanus scarabaeoides*)

Development of chloroplast genomes of the fertile and sterile pigeonpea

Pigeonpea [*Cajanus cajan* (L.) *Millspaugh*] is a diploid (2n = 22) legume crop having a genome size of 852 Mbp. It belongs to subfamily Papilionoideae, and family Leguminosae. Sequencing of complete plastid genomes has been made easy by development of next generation sequencing technologies. Genetic features of chloroplast (cp) like uniparental inheritance, low level of recombination and lower substitution rates, has made sequencing of chloroplast genome useful for phylogenetic analysis. The cp genome of sterile and fertile genotypes of pigeonpea was sequenced by Roche 454 technology. The cp genome of sterile genotype was found to be of 152,201 bp having quadripartite structure in which two IRs of 25,402 bp in length separates 83,423 bp of LSC and 17,854 bp of SSC. Similarly fertile genotype of 152,241 bp has a quadripartite structure with LSC of 83,454 bp and SSC of 17,871 bp separated by IR of 25,398 bp. The pigeonpea cp genome (Fig 2.1) contains 118 unique genes, which include 30 tRNA, 4 rRNA, 80 predicted protein coding genes and 5 peudogenes. A 50 kb inversion was observed in the LSC region of pigeonpea cp genome, consistent with other legumes. Comparison of cp genome with other legumes revealed the contraction of IR boundaries as rps19 gene is not included in the IR relative to other legumes at that position. Chloroplast SSRs were analyzed with MISA perl script and a total of 280 and 292 cp SSRs were identified in sterile and fertile pigeonpea respectively. These SSRs were used to genotype pigeonpea and allied species (Fig 2.2). RNA editing was observed in 26 transcripts in both sterile and fertile line. The pigeonpea cp genome sequence would be beneficial in providing informative molecular markers which can be utilized for genetic diversity analysis and phylogenetic and evolutionary studies within *Cajanus* genus and other legumes.

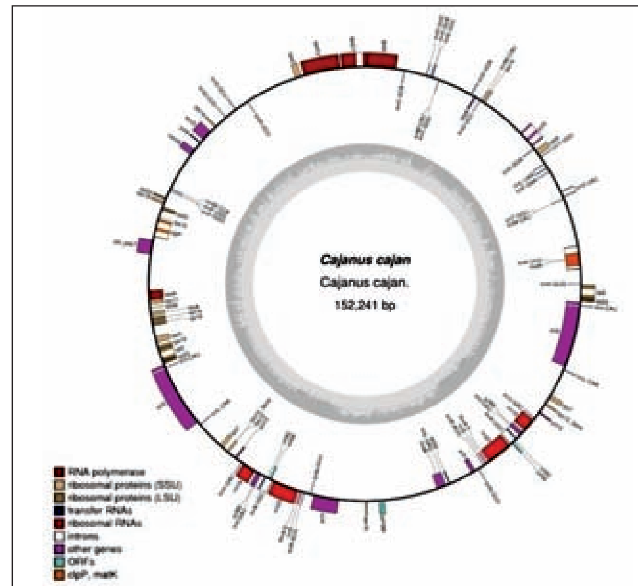


Fig. 2.1: Structural features of the pigeonpea chloroplast genome.

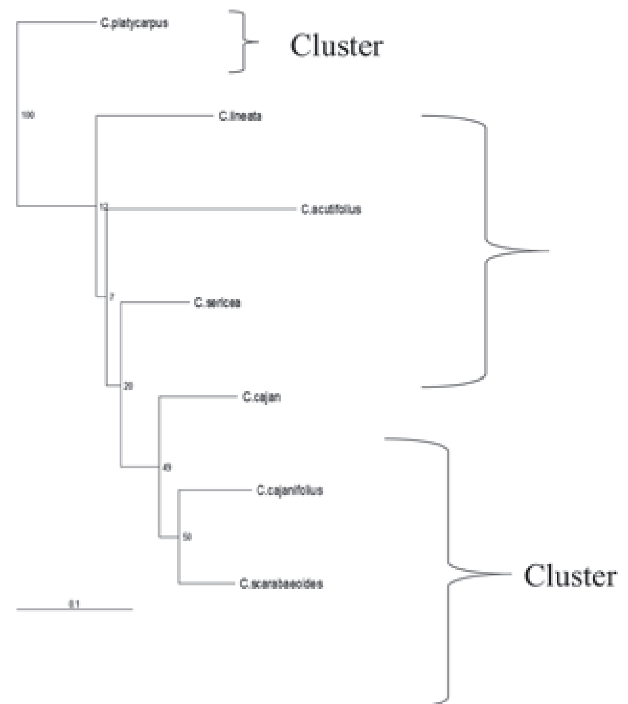


Fig. 2.2: Phylogenetic analysis of pigeonpea with allied species based on plastid SSRs

Development of the draft mitochondrial genome of fertile pigeonpea

Mitochondrial (mt) DNA was extracted from the tissue of the fertile line carrying the fertility restorer gene for the A2 based CMS. The DNA was subjected to library preparation and sequenced on the 454 platform. The reads were filtered and run on NEWBLER for a draft de novo assembly. The contigs thus obtained were then reassembled again using reference guided assembly process. Continous assembly process led to the development of a draft mt genome of approximately 550 kb. This assembly is being further validated by wet lab data.

Genomewide analysis of the PPR genes in pigeopnpea for identification of genes related to fertility restoration (A_2 CMS)

The pigeonpea genome data was collected from various databases and subjected to annotation. Some other data generated inhouse was also added to increase the possibility of finding pentatricopeptide repeat (PPR) motif containing contigs. Fertility restoration is generally dominated by genes targeted to the mitochondria containing signature pentatricopeptide domain. After extensive annotation, Pfam and other analysis a total of 550 plus PPR containing genes were mined from the pigeonpea genome. These sequences were then analyzed for the presence of PPR domains and were further characterized by comparative analysis with other legumes including soybean. These sequences were then classified into various sub-categories namely plastid/mitochondrial targeted (Fig 2.3). These sequences were then mapped on to the available pigeonpea map and it shows a widespread distribution on all the eleven linkage groups (Fig. 2.4). The comprehensive data is now being validated in the sterile and fertile pigeonpea for association with pollen fertility restoration trait.

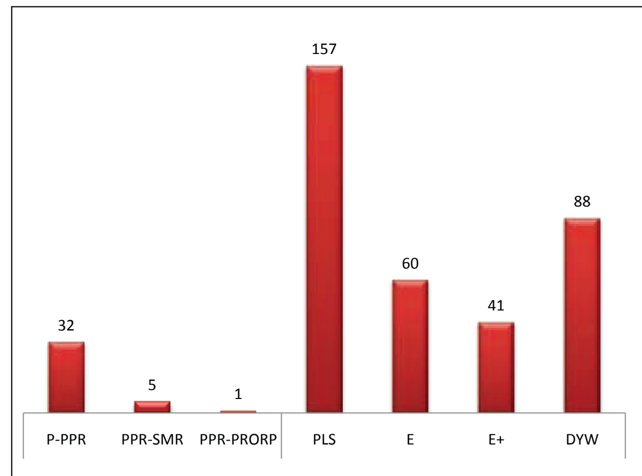


Fig. 2.3: Classification of a subset of PPR containing genes in various categories.

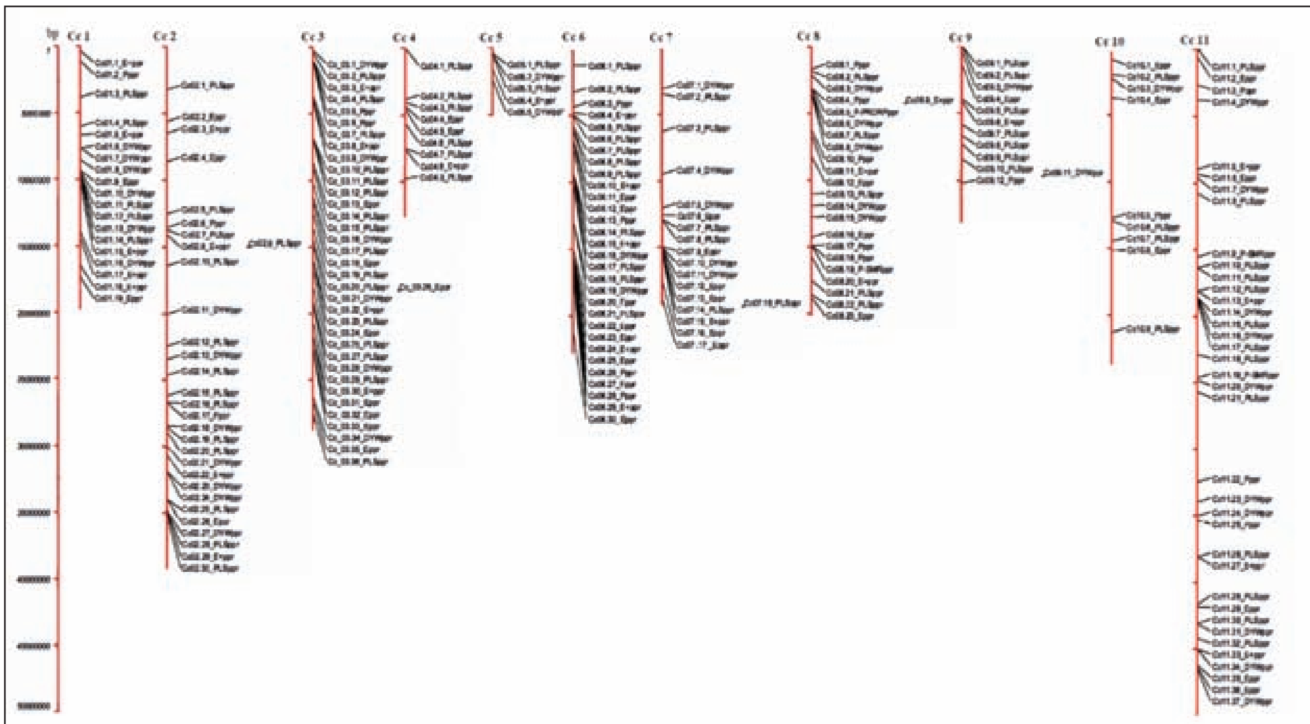


Fig. 2.4: Distribution of the PPR genes on the eleven linkage groups of pigeonpea.

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

The main aim of the programme is to tackle pod borer in pigeonpea with *Bt* ICPs using transgenic technology. In this direction, two ICPgenes, *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.

The selection of putative transformants in the T_1 generation was mainly based on screening in the presence of selection agent kanamycin. Detailed molecular analysis of the screened plants demonstrated the presence of the T-DNA in the genome of the selected plants. The PCR positive plants were challenged with the larvae of *H. armigera* to analyse the efficacy of the transformed plants. Analysis of plants this way led to the identification of 28 plants with *cry1AcF* gene and 54 plants with *cry2Aa* gene. These plants were advanced to the next generation for the integration and inheritance analysis of the T-DNA.

For analysis in the T_3 generation, 100 plants belonging to 9 events harbouring *cry1AcF* and 310 plants belonging to 32 events harbouring *cry2Aa* were established under nethouse conditions. Leaves of 45-60 days old plants (all the plants) were challenged with 2nd instar *H. armigera* demonstrated stability of performance in some lines. Dot blot analysis demonstrated varied but high levels of expression of the respective Cry proteins using antisera against the specific ICPs (Fig. 2.5). Plants/lines demonstrating least damage (5-10%) against *Helicoverpa* challenge and expressing high amount of cry proteins were shortlisted. Molecular analysis using PCR depicted stable integration and inheritance of the transgenes. Semi quantitative RT-PCR analysis and western blot analysis confirmed the expression of the transgenes in the selected transformants. Bioinformatic allerginicity analysis; comparative proteome and nutritional analysis of seeds of two selected events in comparison with wild type pigeonpea seeds demonstrated absence of unintended effects and lack of allerginicity in the selected *cry* genes.

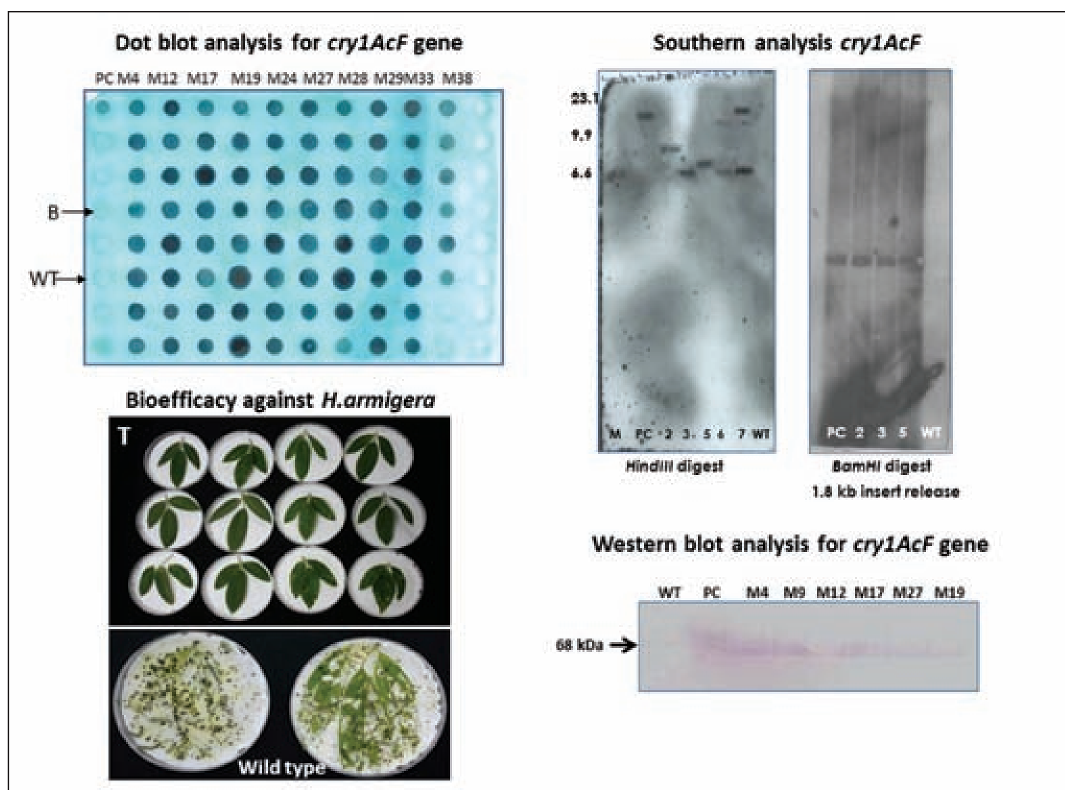


Fig. 2.5: Snapshot of molecular and bioefficacy analysis of the pigeonpea transgenics harbouring *cry1AcF* and *cry2Aa* for the identification of promising transgenics.

Pigeonpea viruses: characterization of viral genome, their genes and transgenic control

Sterility mosaic disease (SMD) of pigeonpea (*C. cajan*), is the economically most important viral disease. Pigeonpea sterility mosaic virus (PPSMV), a species of the genus *Emaravirus* (Tentative Family: *Bunyaviridae*), is the causal agent of SMD. PPSMV is a negative sense RNA virus; consisting of 5-6 RNA segments (Fig. 2.6). In addition to PPSMV, mungbean yellow mosaic India virus (MYMIV) is also known to infect pigeonpea causing yellow mosaic disease, although currently it does not cause significant damage.

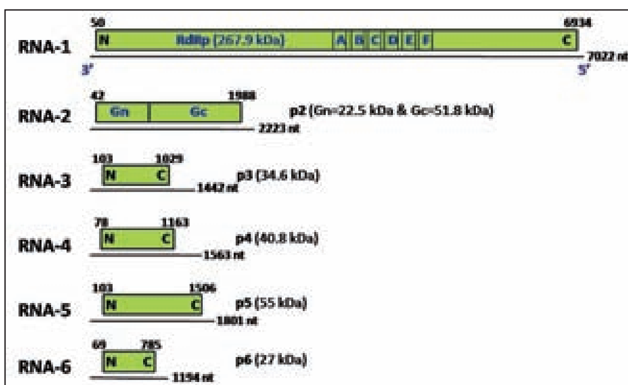


Fig. 2.6: Genome organization of pigeonpea sterility mosaic virus.

In a virus-infected plant, small interfering RNAs (siRNAs) corresponding to the viral genome form a large proportion of the small RNA population. Thus by employing Next Generation Sequencing (NGS) technology one can sequence the small RNA population from a virus infected plant and subsequently use this sequence information for reconstitution of viral genome and also for virus identification. The small RNA population from virus infected pigeonpea leaf samples collected from Gulbarga (Karnataka State, India) and New Delhi were subjected to NGS by using Ion Proton technology. These small RNA sequence reads were subjected to *de novo* assembly and assembled into contigs which represent viral sequences and thus viral genome sequence was reconstituted to near completeness. The data revealed that the virus infected pigeonpea sample from Gulbarga was infected with PPSMV, while the sample from Delhi was infected with a geminivirus, MYMIV. The virus specific filtered small RNA sequences were retrieved and mapped across the PPSMV and MYMIV genomes and the distribution of different size classes of small RNAs (21 nt, 22 nt and 24 nt) across the viral genomes was studied (Fig. 2.7 and 2.8).

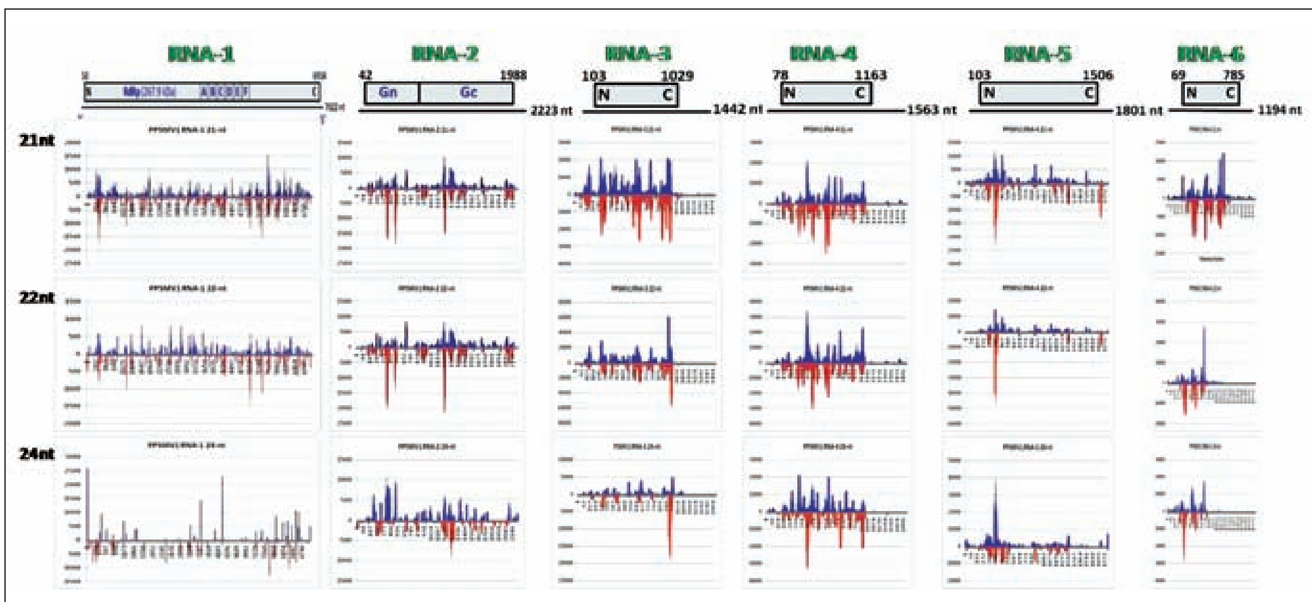


Fig. 2.7: Distribution of siRNA across the genome of pigeonpea sterility mosaic virus.

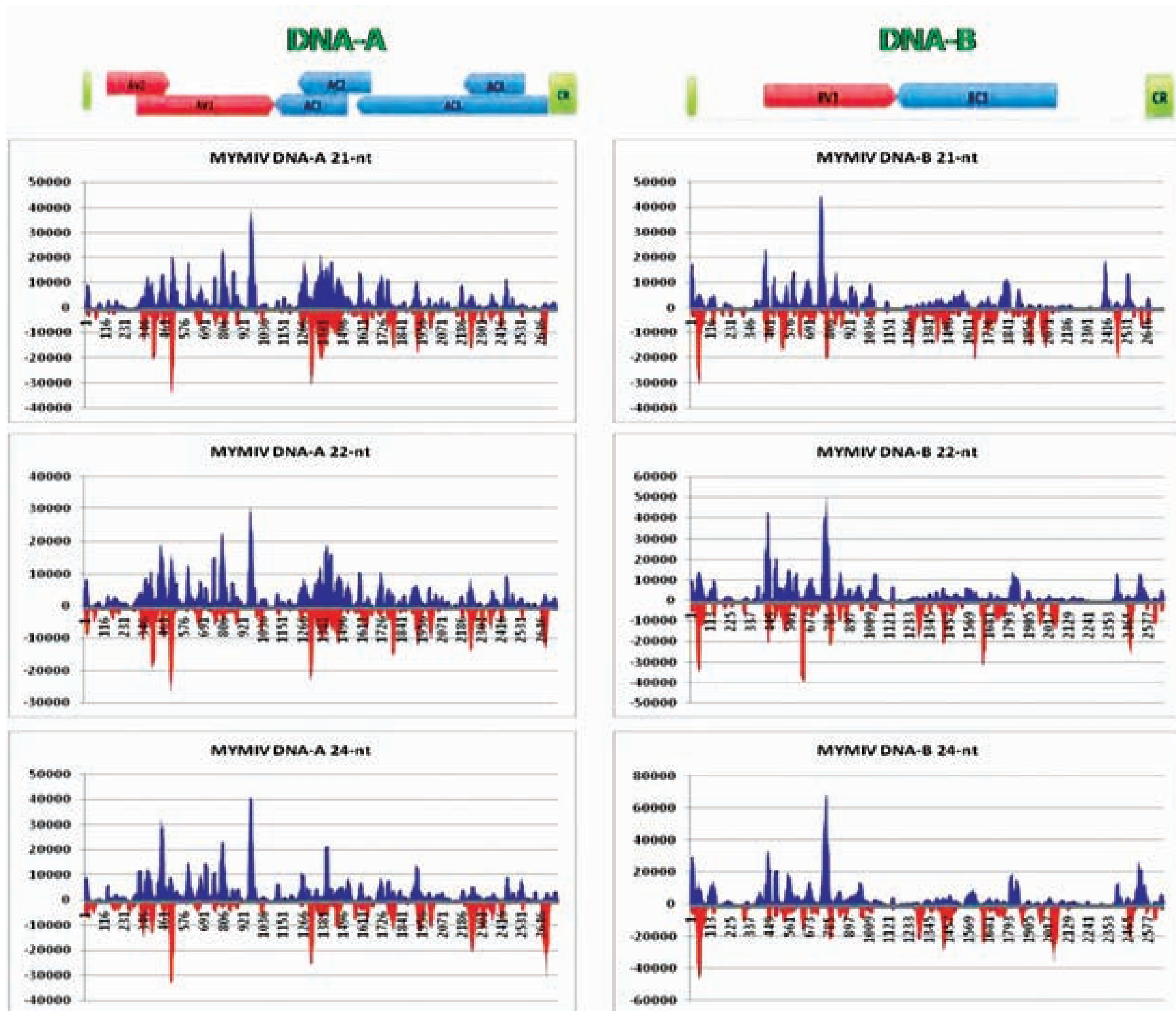


Fig. 2.8: Distribution of siRNA across the genome of mungbean yellow mosaic India virus.

This information will help in identifying viral sequences with high density of small RNA, which can be efficiently targeted by RNA-interference based virus control strategy. To validate the above data, primers were designed to amplify different segments of PPSMV from Gulbarga by RT-PCR and were cloned and sequenced. Thus near complete genome sequence of PPSMV-Gulbarga was obtained. Similarly the DNA of MYMIV was amplified by Rolling Circle Amplification (RCA) and then cloned and sequenced. These clones will be eventually developed into infectious clones with an objective of developing a 'Virus Induced Gene Silencing' (VIGS) vector for pigeonpea functional genomics.

For a long time the genome sequence of PPSMV was not known and very recently the genome sequence of PPSMV from Patancheru (Telangana State) has been published. However PPSMV isolates from different parts of the country involved in SMD are believed to exhibit sequence diversity, as elucidated from their differential reactions in ELISA based diagnostics. Thus field surveys were made and virus infected pigeonpea leaf samples were collected from the following states: Karnataka, Tamil Nadu, Maharashtra, Bihar, Uttar Pradesh and Delhi. Subsequently we have cloned and sequenced different segments of PPSMV for the samples collected from Gulbarga, Bidar, Raichur and Bengaluru in Karnataka state, Coimbatore in Tamil Nadu, Pune from Maharashtra, Pusa in Bihar, Gorakhpur in Uttar Pradesh and New Delhi (Fig. 2.9). Most of the samples were PPSMV-1, the samples from Coimbatore, Bengaluru and Raichur were PPSMV-2 and there were also instances of mixed infection. Hence, this is the first study on molecular biodiversity of PPSMVs involved in SMD of pigeonpea and their small RNA profiling.

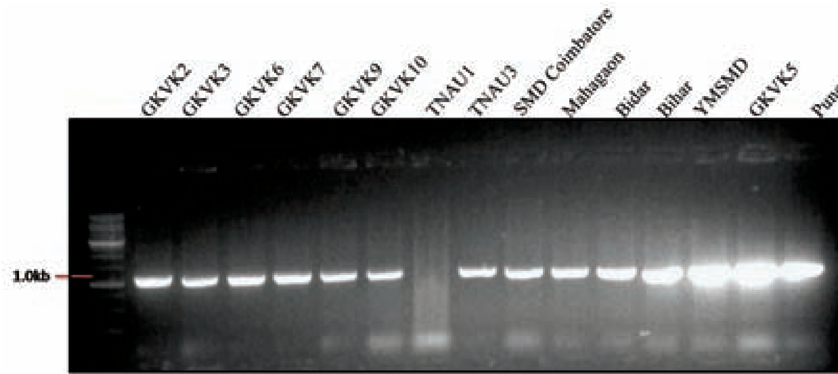


Fig. 2.9: RT-PCR for the segment RNA-6 of pigeonpea sterility mosaic virus.

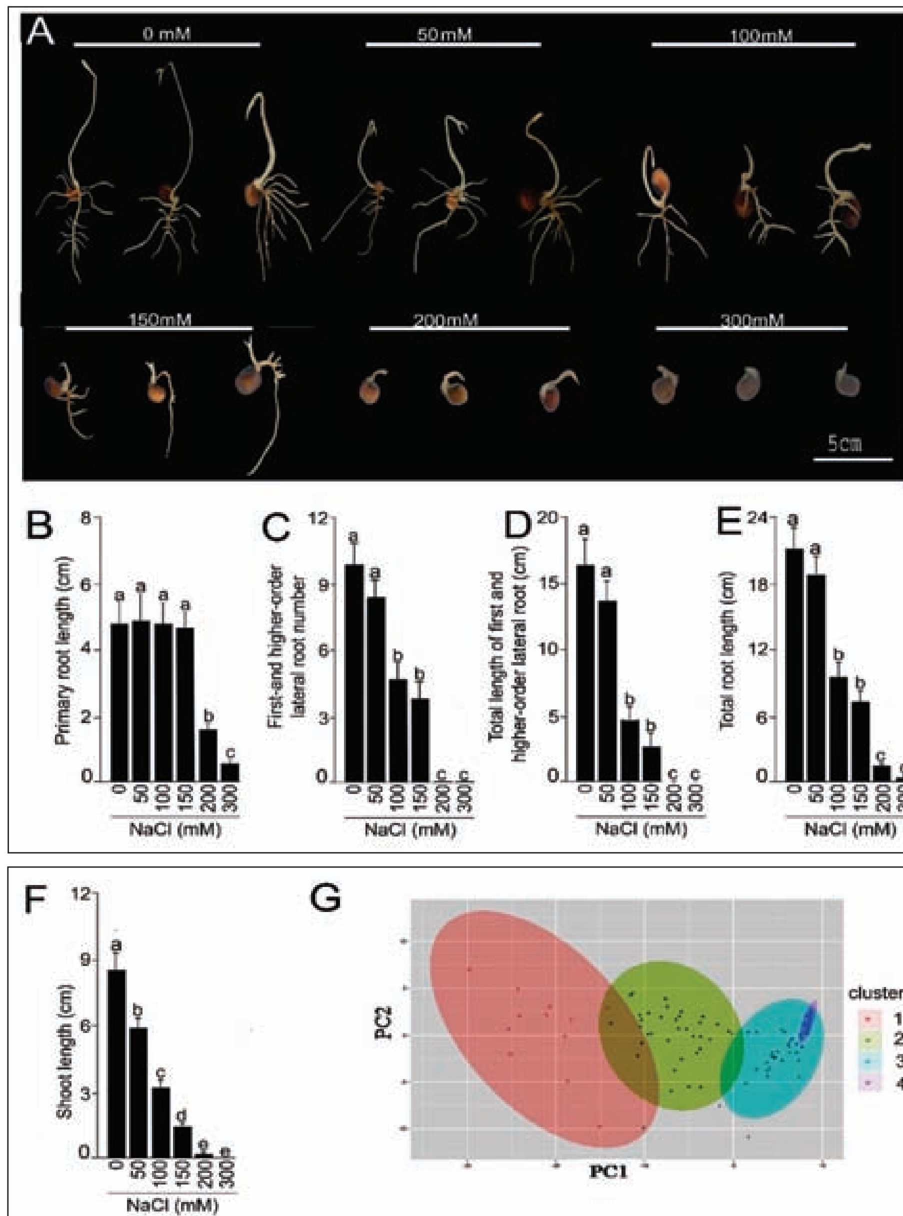


Fig. 2.10: Effect of NaCl availability on the morphological traits. (A) Pigeonpea variety 'Asha' seeds were grown on six concentrations of NaCl – 0, 50, 100, 150, 200 and 300 mM. The 7-d-old seedlings were spreaded on agar plates and documented using ImageJ software. Data are presented for 3 replicates of 10 seedlings each; (B) Primary Root Length; (C) First and higher order lateral root number; (D) Total length of first and higher order lateral root; (E) Total root length; (F) Shoot length. Values are means ± SE. Different letters indicate that the means differ significantly ($P < 0.05$).

Effect of salt stress on pigeonpea

To determine the effect of salt on the root system architecture (RSA) in particular to primary root length (PRL), number and length of lateral roots (NLR, TLLR), a dose responsive experiment was conducted on a popular pigeonpea variety 'Asha'. Various NaCl concentrations (0, 50, 100, 150, 200 and 300 mM) were used to study the salinity triggered response on seed germination and their subsequent growth. It was noted that with an increase in the salt strength there was a gradual decrease in the survival /germination rate of the seeds (Fig. 2.10A). In the absence of salt stress the average germination was 92 % whereas it drastically falls to 40 % when a stress of 300 mM was applied. High salt concentration appeared to be lethal to the seed germination may be due to the osmotic imbalance which inhibits water imbibition required to kick start the cellular metabolism and growth to resume. The seedlings maintained on germination paper for 7 days were scanned and quantified with ImageJ software for their RSA -PRL, NLR, TLLR and also the shoot length (Fig. 2.10 A-F). There was not much significant difference in the primary root length at low doses of salt stress but at higher concentrations of 200 and 300 mM salt the decline was momentous. Interestingly, lower salt concentration of 50 mM NaCl slightly increased the PRL.

Gel-free quantitative proteomic analysis of pigeonpea seedlings after NaCl stress

A comparative proteomic analysis combined with high resolution mass spectrometry and gel-free quantification was performed to identify and determine the profiles of salt responsive proteins in the 7-d-old pigeonpea seedlings after being subjected to salinity stress and without stress. The pigeonpea seedlings were raised under both salt-free environment (control) and in presence of 150 mM NaCl stress. Total soluble proteins were extracted from root and shoot of three independent experiments for both salinity stressed and un-stressed conditions. The samples were digested with trypsin and further subjected to nano-LC–QTOF analysis. The obtained spectral (MS) data were analyzed using uniprot (www.uniprot.org) database. The protein identifications were based on homology patterns with other similar plant species as limited information is available in public domains for the *Cajanus* species. Characterization of proteins in pigeonpea for root and shoot were based on the publicly available databases. The 77% of differentially expressed proteins unique to shoot under salt stress condition were characterized protein while this ratio was quiet higher (96%) in shoot protein (Fig. 2.11A). Similarly, unique to root the characterized protein fraction was 92%, thus for common to root protein the ratio was 10 percent lower than to unique root protein here, 82% proteins were fully characterized (Fig. 2.11B). Thus, we found average 13% of total pigeonpea proteins including both common as well as unique uncharacterized in shoot and root of pigeonpea. Therefore, these were the novel proteins which need to be further characterized for the identification of their role in growth, development and for pigeonpea varietal improvement and also enhancing the salt tolerance ability.

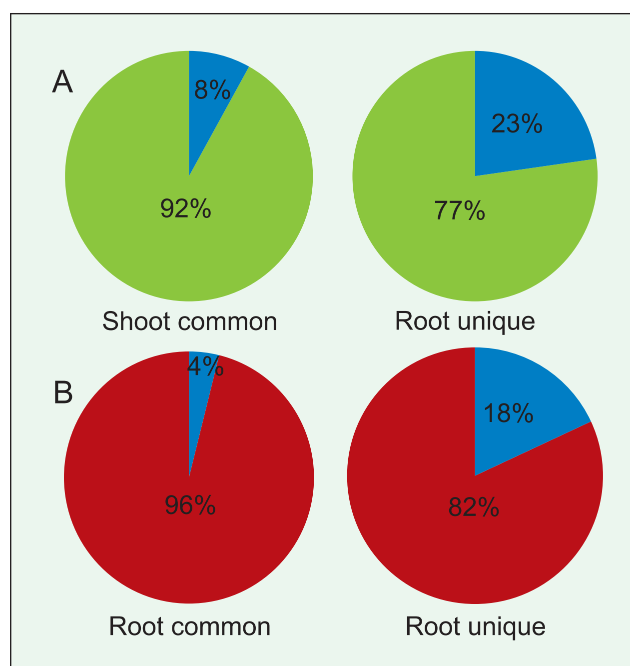


Fig. 2.11: Classification of shoot and root proteins based on their biological annotation (www.uniprot.org). (A) 92% characterized and 8% uncharacterized proteins in shoot common in both control and stress. 77% characterized and 23% uncharacterized proteins in shoot expressed only in 150 mM salt presence. (B) 96% characterized and 4% uncharacterized proteins in root common to both control and stress.82% characterized and 18% uncharacterized proteins in root exclusively present in stress state.

Identification and characterization of drought stress-responsive microRNAs in chickpea

microRNAs, small non-coding RNAs, have emerged as important links between control of plant growth and development during stress responses. The expression of several miRNAs is altered during stress responses (both biotic and abiotic) and included drought, salinity, cold, heat, ABA, oxidative and hypoxia. Chickpea plants are very vulnerable to drought stress and productivity of chickpea is severely affected by drought stress. To have a better understanding of drought stress *per se* and the involvement of genes and microRNAs, small RNA libraries were constructed from the total RNA of control and drought stressed tissues of ICC4958 genotype of chickpea and sequenced (previous work). Majority of the small RNA population comprised of 24 nt long sequences followed by 23 nt sequences (Fig. 2.12).

To know the role of miRNAs in response to drought stress, tissues from drought tolerant cultivar, ICC4958 and drought susceptible cultivar, ICC1882 were used for expression profiling. Differential expression in miRNAs was observed between the two cultivars. miR159 and miR168 showed up regulation in ICC4958 cultivar as compared to ICC1882, a low root biomass cultivar (Fig. 2.13**).** Novel miRNA107 exhibited different expression pattern in both the cultivars (Fig. 2.14). It was down regulated in stressed tissue of ICC4958 and showed up regulation in stressed tissue of ICC1882.

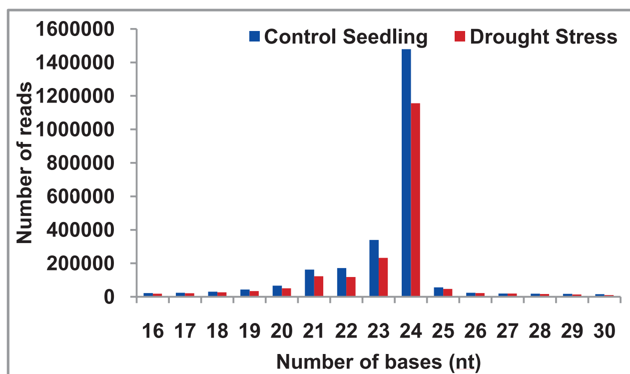


Fig. 2.12: Length distribution of small RNA population in control and drought stressed chickpea tissues. In both, control and drought stress libraries, 24 nt miRNAs are major class as compared to other miRNAs.

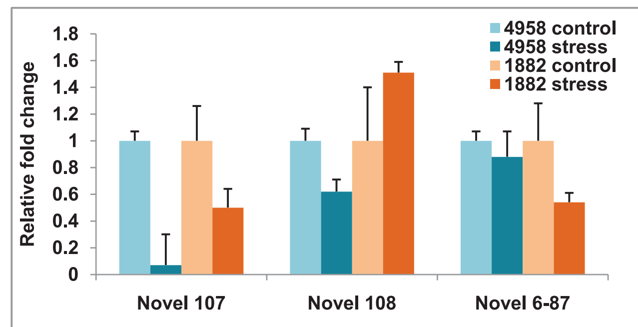


Fig. 2.13: Expression analysis of conserved miRNAs in control and drought stressed samples of ICC 4958 and ICC 1882 chickpea cultivar as evaluated by qRT-PCR.

Identification, characterization and expression analysis of transmembrane START domain proteins under stress conditions in chickpea (*Cicer arietinum* L.)

Steroidogenic acute regulatory related transfer (START) proteins that are involved in transport of

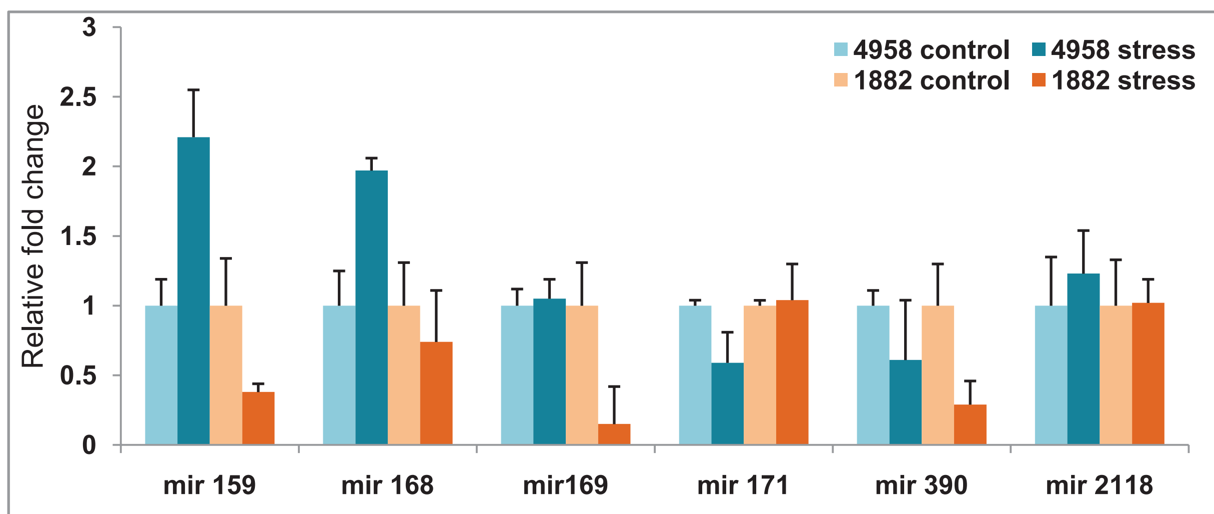


Fig. 2.14: Expression analysis of novel miRNAs in control and drought stressed samples of ICC 4958 and ICC 1882 chickpea cultivar as evaluated by qRT-PCR.

lipid molecules, play a myriad of functions in animals and plants. These proteins consist of a modular START domain of approximately 200 amino acids which binds and transfers the lipids. In the present study we have performed a genome-wide search for all START domain proteins in chickpea and identified 36 chickpea genes belonging to the START domain family (Fig. 2.15). Through a phylogenetic tree reconstructed with *Arabidopsis*, rice, chickpea, and soybean START proteins, we were able to identify four transmembrane START (TM-START) proteins in chickpea. These four proteins are homologous to the highly conserved mammalian phosphatidylcholine transfer proteins. Multiple sequence alignment of all the transmembrane containing START proteins from *Arabidopsis*, rice, chickpea, and soybean revealed that the amino acid residues to which phosphatidylcholine binds in mammals, is also conserved in all these plant species, implying an important functional role and a very similar mode of action of all these proteins across dicots and monocots. This study characterizes a few of the not so well studied transmembrane START superfamily genes that may be involved in stress signaling. Expression analysis in various tissues showed that these genes are predominantly expressed in flowers and roots of chickpea (Fig. 2.16). Three of the chickpea TM-START genes showed induced expression in response to drought, salt, wound and heat stress, suggesting their role in stress response (Fig. 2.17).

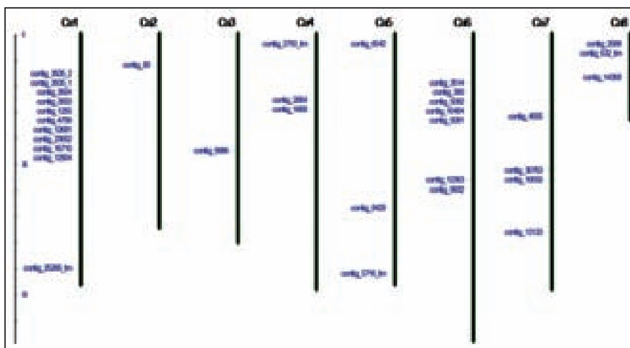


Fig. 2.15: Chromosomal mapping of identified START proteins from chickpea. Ca1 – Ca8 represents the number of chickpea chromosomes distanced by centimorgan.

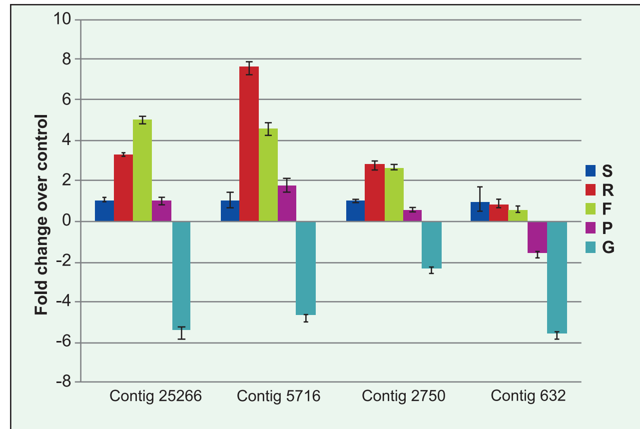


Fig. 2.16: qPCR analysis of TM-START proteins of chickpea genes done in different tissues. Relative fold change values are given with respect to GADPH2 was used as reference genes. S-Shoot, R-Root, F-Flower, P-Young pod and G-Grain. The expression in shoot was taken as 1 and compared to other tissues.

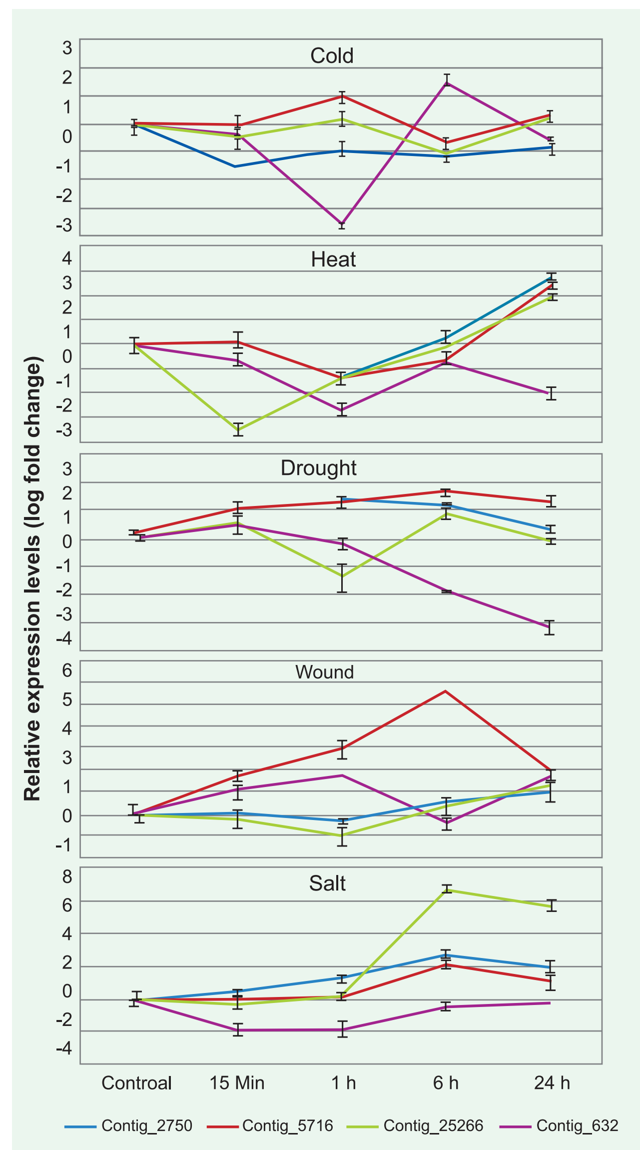


Fig. 2.17: Quantitative real time PCR analysis of the 4 TM-START genes under stresses such as cold, heat, drought, wound, and salt at varying time intervals.

3 BIOTECHNOLOGICAL APPROACHES FOR BRASSICA IMPROVEMENT

Development of aphid resistance in Indian mustard

Rapeseed-mustard (*Brassica spp.*) constitute a major group of oilseed crops in India. In mustard (*B. juncea*) damage caused by the hemipteran insect-pest aphid imposes a severe constrain on the crop productivity. The major challenge in breeding aphid resistance in the cultivars of *B. juncea* has been the lack of resistance source within the crossable gene pool. Even the transgenic means could not be utilized mainly because of lack of effective transgene. Therefore, novel genes and biological mechanisms were explored for developing aphid resistant plant types in Indian mustard. In this pursuit, one of the strategies aims for developing RNAi mediated resistance in which plants are engineered to generate dsRNAs against specific metabolically important aphid genes. As a consequence, attenuation in the expression of the important aphid gene caused by the host-delivered dsRNAs is likely to result either lethality or significant inhibition of the aphid population on the transgenic plants.

Developing RNAi mediated aphid resistance in *B. juncea*: cloning of RNAi targets in binary vector pANDA35HK/pBinAR

Earlier, it was demonstrated that host delivered dsRNA specific to aphid's serine protease gene led to RNAi mediated transcriptional attenuation of serine protease gene and significant reduction in aphid-fecundity. However, from that study it was evident that the efficacy of the strategy will largely depend on identification of appropriate target gene in aphid and identification of appropriate domain(s) within the target gene which are amenable to gene-silencing. Therefore, screening of large number of aphid genes was done through diet-supplementation of dsRNAs specific to at least two domains of 200 bp each from each putative target. In the screening experiments, two potential target domains, named as *MpDE4A* and *MpDE8A* within a metabolic gene of aphid, were identified. dsRNAs specific to these domains when fed into aphid adults led to significant insect mortality and transcript attenuation in the target gene. Binary construct, pBinAR-*MpDE4A*, for generation of the dsRNAs under CaMV35S promoter was developed. Several transgenic tobacco plants (*cv.* Petita) were developed by *Agrobacterium*-mediated transformation to examine if the construct could lead to the synthesis of desired dsRNAs in transgenic plants. *In vitro*- raised transgenic plants were hardened on soilrite and transferred to transgenic glasshouse for further analysis. Transgene integration was confirmed by PCR using different combinations of primer pairs followed by Southern blot analysis (Fig. 3.1). To check for the expression of the desired

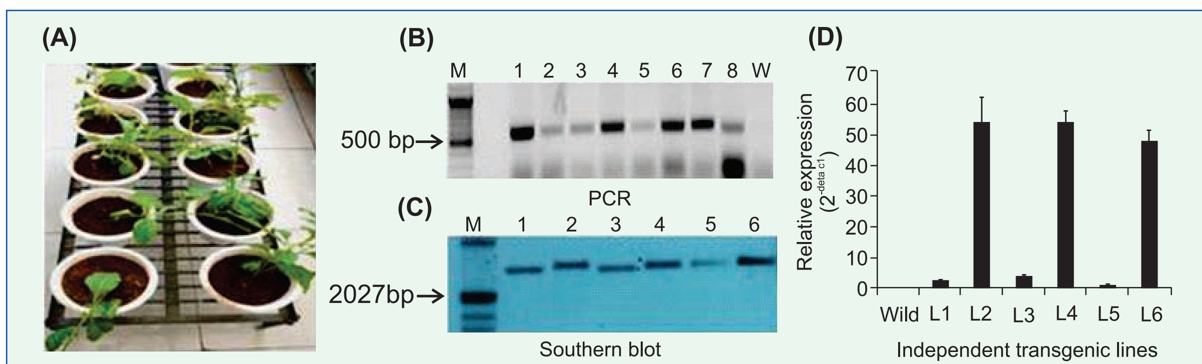


Fig. 3.1: Molecular characterization of *MpDE4A* transgenic tobacco lines. (A) *MpDE4A*-transgenic tobacco lines in transgenic glass house; (B) PCR analysis of the tobacco lines. Lane M, 500 bp ladder (ThermoScientific); Lane 1-8, expected amplicon of ~550 bp using primer pairs specific to the CaMV35S promoter (forward) and the gene (reverse); Lane W, non-transformed tobacco; (C) Southern blot analyses by DIG labeled gene-specific probe. Lane M, molecular marker (Roche); Lane 1-6, inserts of size ~2.1 kb released by *Eco*RI and *Bam*HI restriction enzymes; (D) RT-qPCR analyses of dsRNA expression in independent transgenic lines.

dsRNA, RT-qPCR was performed using RNA from independent transgenic lines. Interestingly, the level of dsRNA was significantly variable among the independent transgenic lines. Based on the RT-qPCR analysis, the lines 2, 4 and 6 were scored suitable for further insect bioassay.

Deciphering endogenous phloem-specific promoters for expressing aphid-resistance genes in *B. juncea*

Transgenic strategies for developing aphid-resistant plant types necessitate phloem-bound expression of the insecticidal genes. A few known phloem-specific promoters, in spite of tissue-specific activity fail to confer high level gene-expression. Based on literature, 39 known phloem-specific promoters were chosen and their sequences were downloaded from NCBI database. These promoters, from monocot as well as dicot plants, ranged from smallest *Arabidopsis* germin-like protein (AtGLP13) promoter of 762 bp to *Cucumis melo* galactinol synthase (CmGAS1) promoter of 3000 bp. Based on putative function of their cognate genes, the promoters were classified in two GO terms viz., biological process and molecular function (Fig. 3.2). Across the ontogenic groups, seven promoters that were associated with host-response against insects and pathogens, either directly or indirectly were identified. Homologues of the seven promoters were predicted in *Brassica* spp. and identified either in *B. rapa* or in *B. juncea*.

RT-PCR based detection of the cognate mRNAs in the phloem exudates of *B. juncea* and their RT-qPCR based quantitative analysis validated activity of the *in silico* identified promoters and their relative strength, respectively in the phloem exudates of *B. juncea* (Fig. 3.3A & 3.3B). Specific amplification of the cognate genes in the cDNA sample indicated phloem-bound activity of the seven orthologous *B. juncea* promoters. The relative transcript levels of the cognate genes were estimated in RT-qPCR analysis and expressed as multifold ratio of their normalized level to the least abundant transcript level of *GAS1* (Fig. 3.3C). The results empirically showed significant variation among the transcript levels indicating significant differences in strength of the promoters. Amongst the variability, *GS3A* showed the highest transcript level followed by *PP2*, *GLP13* and *SULTR2*.

DNA-motifs globally associated with phloem-specific promoters were identified analyzing a comprehensive set of 39 promoter sequences from diverse origin. The over-represented motifs in them were discovered through string based (oligoanalysis of RSAT) and position weight matrix-based (info-gibbs of RSAT, AlignACE, and MEME) motif discovery programmes. Oligo-analysis revealed the commonly occurring over-represented motifs in all the promoter sequences. Among the identified motifs, the two with lowest expectation (E)-values were scored as significant and their frequency of occurrence per promoter has been shown in Fig. 3.4A. Independent analysis based on info-gibbs identified a CT-rich signature motif (Fig. 3.4B) specifically present in the promoters of phloem-specific transcripts.

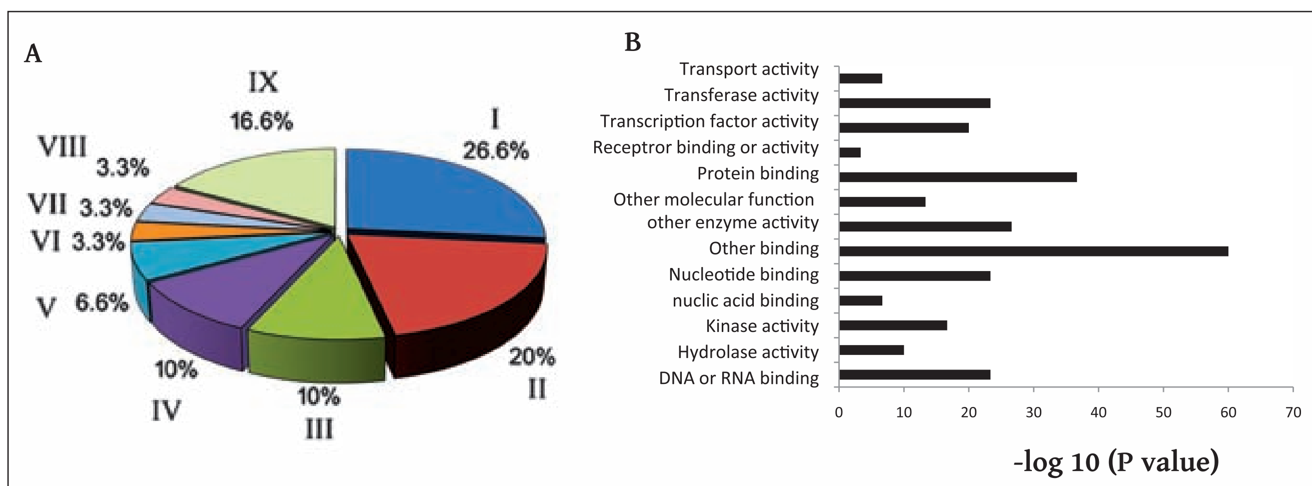


Fig. 3.2: Gene ontology of phloem-specific transcripts. (A) Nine functional categories of the transcripts are represented by *different letters* in the pie chart. *I.* Response to biotic and abiotic stimulus; *II.* Developmental processes; *III.* Transcription; *IV.* Transport; *V.* Signal transduction; *VI.* Electron transport; *VII.* Cellorganization and biogenesis; *VIII.* Protein metabolism; and *IX.* Unknown biological processes. (B) Distribution of Go terms based on their molecular function.

Promoter-activity of the *in silico* identified *B. juncea* promoters at different growth stages of the plant were analyzed by assessing their cognate-transcript levels at the vegetative, bud initiation

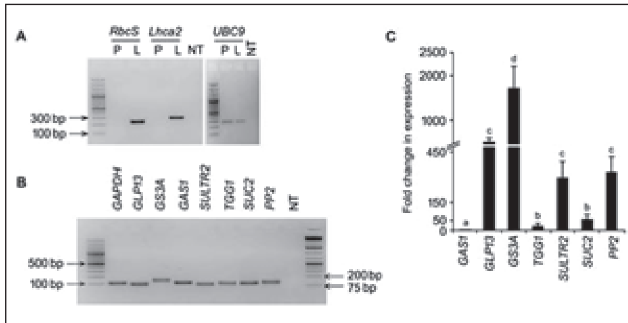


Fig. 3.3: RT-PCR analysis for the cognate-transcripts of the phloem-specific promoters. (A) RT-PCR amplification of *RbcS*, *Lhca2* and *UBC9* in phloem exudates (P), and leaf tissues (L) along with non-template (NT) control. (B) RT-PCR amplification of the cognate-transcripts in phloem exudates. (C) RT-qPCR based analysis of the cognate-transcript levels in phloem exudates.

and flowering stage (Fig. 3.5A). Irrespective of strength, the consistency in promoter-activity across different growth stages was compared. For that the gene-expression data were analyzed by four Excel based statistical methods, BestKeeper, deltaCt method, geNorm, and NormFinder. The output of the individual statistical method indicated different ranking of the promoters in terms of consistent activity (Fig. 3.5B–E). In conclusion, the signature-motifs identified in this study can be used for quick identification of potential phloem-specific promoters across the species based on the available sequence

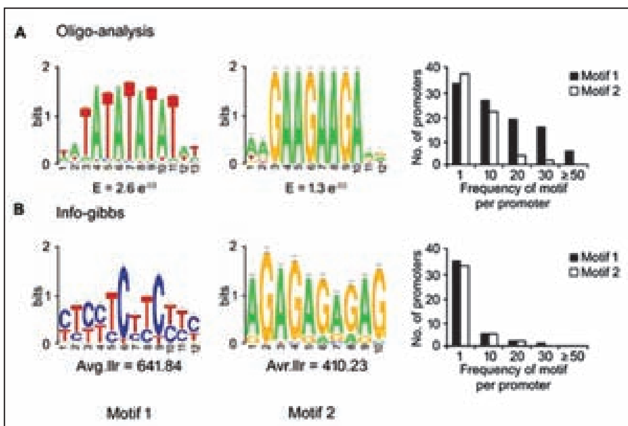


Fig. 3.4: Significant *cis*-motifs identified by RSAT oligo-analyzer and info-gibbs. The motifs were identified by RSAT oligo-analyzer (A) and info-gibbs (B) motif discovery tool. Frequency distributions of significant motifs were searched by using FIMO program of MEME with $p < 0.0001$. Two motifs showing lowest expectation (*E*)-values and high log likelihood ratio (Avg. llr) in each case and their frequencies in the phloem-specific promoters have been shown. The *X*- and *Y*-axis show the position of nucleotides and the bits score, respectively.

data. In future perspective, it will be intriguing to examine whether complementation of the phloem-specific motifs to a promoter can endow phloem-specific activity to it.

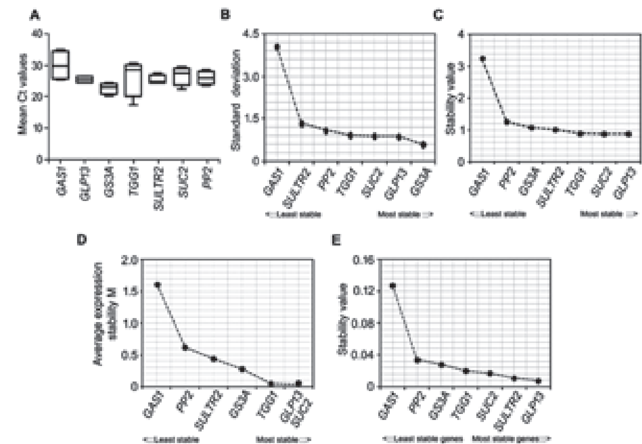


Fig. 3.5: RT-qPCR based analysis of expression-stability of the *B. juncea* phloem-specific promoters. (A) Mean Ct values of the cognate-transcripts analyzed by RT-qPCR in phloem-cDNA samples collected at different growth stages of *B. juncea* plants. (B–E) Ranking of the *B. juncea* phloem-specific promoters in terms of expression-stability measured by four Excel based statistical methods, BestKeeper (B), deltaCt method (C), geNorm (D), and NormFinder (E) and plotted in increasing manner from left to right.

Identification and characterization of an endogenous elicitor peptide in Indian mustard *B. juncea*

Innate-immunity in plants is a receptor-mediated defense system, which in response to herbivore/pathogen associated molecular patterns (PAMP/HAMP) activate host-defense. Even in case of specific *R*-genes the same defense machinery is deployed to mount a resistance response. In mustard while studying jasmonate responsive ESTs we have identified a gene *proBjEli1*. It codes for 113 amino acids proBjEli which is processed likely into a smaller peptide BjEli1. *proBjEli1* is transcriptionally activated by plant hormones viz., methyl jasmonate (MeJ), salicylic acid (SA), and by artificial wounding (Fig. 3.6A-B), indicating a possible role of the gene product in plant defense response. Interestingly, BjEli1-peptide activated the production of H_2O_2 and several genes of ROS pathways in *B. juncea* leaves when a synthetic version of it was supplied through cut petioles (Fig. 3.7). The initial studies indicated potential role of BjEli1 in activation of endogenous chemical defense in mustard.

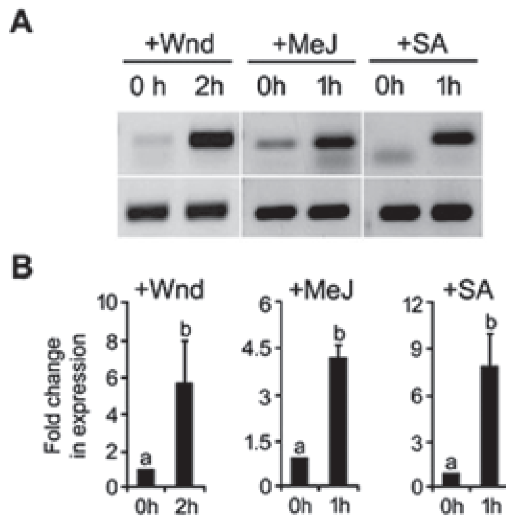


Fig. 3.6: Defense elicitor BjEli1 in *B. juncea*. (A) Semi-quantitative RT-PCR of *proBjEli1* expression in response to mechanical wounding and treatment of leaves with methyl jasmonate (MeJ) and salicylic acid (SA). Relative abundance of the *proBjEli1* transcript was estimated using *Actin* as an internal control. (B) Fold change in expression was derived based on integrated density values (IDV) of the amplicons.

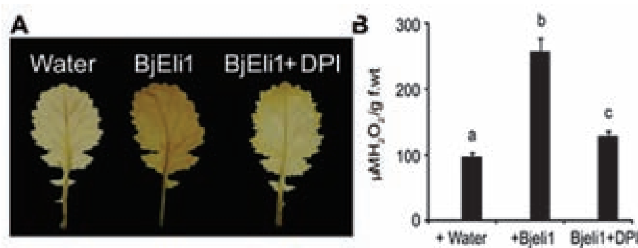


Fig. 3.7: H_2O_2 production by BiEli1. (A) Detection of H_2O_2 in *B. juncea* leaves supplied with 1 µM BiEli1, 1 µM Bjeli1 with 100 µM DPI for 2 h in diaminobenzidine (DAB) assay. (B) Quantification of H_2O_2, generated in leaves fed with 1 µM BiEli1, 1 µM Bjeli1 with 100 µM DPI and water. Data are mean values ± SE (n = 3) of three independent set of experiments performed in triplicate. Significant difference ($P < 0.05$) between the water fed control and the peptide fed samples is represented by *different letters.*

Isolation and characterization of pathogen-inducible genes and promoters and using them for developing fungus (Alternaria)-resistant transgenic *B. juncea*

Alternaria causing Alternaria blight is an important pathogen of *B. juncea* and leads to a great yield loss of this crop. Therefore, it is important to study defense mechanisms in Brassica against Alternaria. It was previously shown that defensin gene is induced in *B. juncea* in response to Alternaria infection (Defensin gene is also induced in macrophages to make holes in the pathogen cells in animals). Promoter of defensin can probably be used as pathogen-inducible promoter after validation. Presently most of the transgenics being developed all over the world are with constitutive promoters. This 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- specific, development specific, and pathogen-inducible.

Isolation of defensin gene from *B. juncea*

Defensin gene of Brassica was isolated by PCR using *Arabidopsis* and *B. rapa* primers. To confirm, the PCR product was cloned and sequenced (Fig. 3.8, 600bp, Acc. No. KF578144).

```
  1 gttactacgt tgttgtgcga tgaacaagta aaagctaacc tactagcatt tcactataaa
 61 tagaagtcca tcttactaag tggtggagaa accagccatt aactttgaat attgggccag
121 tctagaagtt aacatatgtt taatttgctg ggcttatttc ttcacacttc acacaaattc
181 atcgaaaaca aattactaat agtcatgact aagtttgttt ccatcatcac ccttatcttc
241 cttgctctcg ttctctttgc tgcttttggt gagtagtgat cttatcatat gcatggcaaa
301 attagtttaa atttttattt tcagaatatt tgatttcgcc atgatagata tttatataca
361 gaagcatcaa caatggtgga cggtcagaag ttgtgccaaa agcctagtgg gacatggtac
421 ggattttgtg gaaatagtaa tacttgcaag aaccagtgca tcaaccttga gggagcacga
481 catggatctt gcaactatgt tttcccatat cacaggtgta tctgctatgt tccatgttaa
541 tctaccaaaa ctcattggtc taacaaaact ctgtagtgct aacgttcaat aagtctgtgt
601 cac
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Fig. 3.8: Nucleotide sequence of B. *juncea d*efensin gene (Accessions No. KF 578144.)

Expression analysis of *BjDef* gene

PCR amplification was done using forward primer from *Arabidopsis thaliana* defensin-like protein 17 (PDF1.2c; accession no NM123810.2) and reverse primer from *Brassica rapa subsp. pekinensis* (AF528180.1; defensin mRNA). Defensin gene *was* induced by Alternaria, JA and wounding but not by SA (Fig. 3.9).

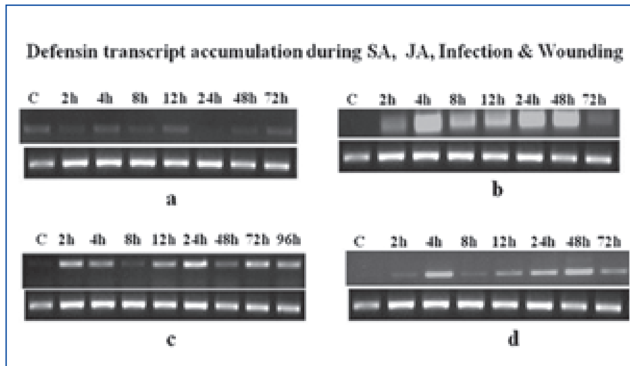


Fig. 3.9: Semi-quantitative PCR analysis of Brassica *BjDef* genes in response to SA, JA, Alternaria infection and wounding. α-tubulin gene was used to normalize the sample. (A) Salicylic acid treatment, (B) Jasmonic acid treatment, (C) Pathogen infection, (D) Wounding.

Cloning of defensin promoter from *B. juncea*

A 2.5kb long promoter of defensin was isolated by using Genome Walker kit (Clonetech), sequenced (Fig. 3.10) and submitted to GenBank (Acc. No. KP300038).

Characterization and validation of defensin gene promoter in *Arabidopsis*

Full length and deleted promoter were used for

Fig. 3.10: *B. juncea* chitinase promoter sequences (2.5kb) depicting basic elements like TATA and CAT boxes, and motifs responsive to various stresses and defense signaling hormones.

developing transgenic *Arabidopsis* plants. To know what length of promoter is actually important for pathogen- inducibility, 5' upstream of 1.5 kb was deleted. Full length promoter was pathogen-, JA- and wound- inducible. Promoter was not induced by SA (Fig. 3.11). Defensin promoter was also induced during development and in specific tissues. Promoter was seen to be active in seeds, meristematic tissue and at the base of siliques (Fig. 3.12).

Validation of *BjDef (Del-3)* promoter

It was found out that 1kb promoter results in loss of JA- and pathogen-inducibility (Fig. 3.13). Further deletions will give the information about which area is specifically required for pathogen-inducibility.

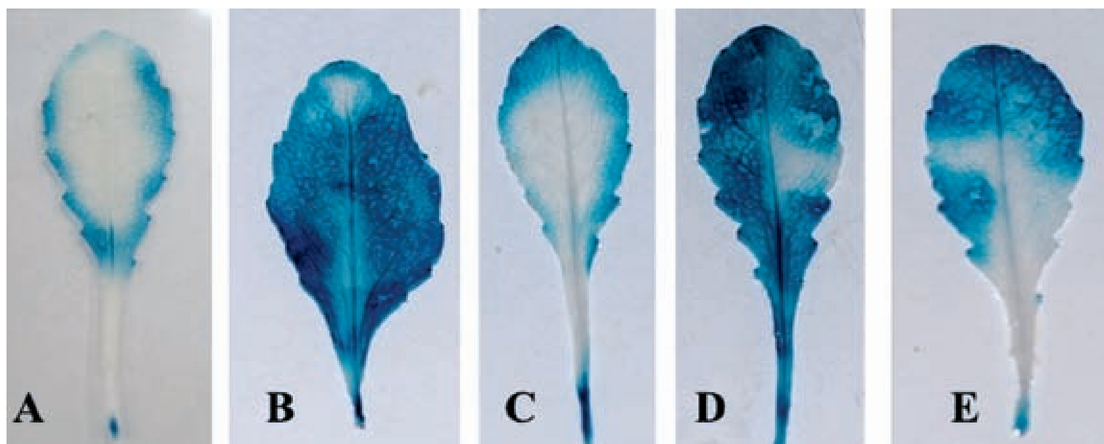


Fig. 3.11: GUS expression in *Arabidopsis* lines containing the *BjDef* promoter 24 hrs after *Alternaria brassicae* infection, SA treatment, MeJA treatment. (A) GUS expression of untreated transgenic leaf; (B) expression in inoculated transgenic leaf; (C) after 2mM SA treatment of transgenic leaf; (D) after 100μM MeJA treatment of transgenic leaf; (E) after wounding.

Fig. 3.12: Histochemical GUS analysis of *BjDef* T_2 *Arabidopsis* plants in different tissues and stages of plant development. (A) Transgenic *Arabidopsis* seed showing green colour; (B) GUS expression in the meristematic tissues of transgenic seedlings; (C) GUS expression seen in the receptacle part of flower and seen in the base of the siliques.

the 5´ end of the forward primer and Phusion DNA polymerase enzyme. The amplicons were ligated into pENTR-D-TOPO vector and transformed. The plasmid DNA isolated from the positive colonies was ligated with the 'Destination Vector' pET300/301 using LR recombinase enzyme. Colony PCR of the transformed colonies was done to confirm the presence of the genes. 100ng of the plasmid DNA isolated from the selected positive colonies was transformed into BL21 DE3 pLysS competent cells for *in vitro* expression of the proteins. Colony Blot of the transformed colonies was done using nitrocellulose filter membrane by placing on the colonies which were incubated for 4 hrs at 37^{0}C for induction of protein expression. The positive (purple colored, Fig. 3.14) colonies were then selected from the master plate and streaked on a LA plate with kanamycin (50µg/ml) for protein expression studies against *Lipaphis erysimi.*

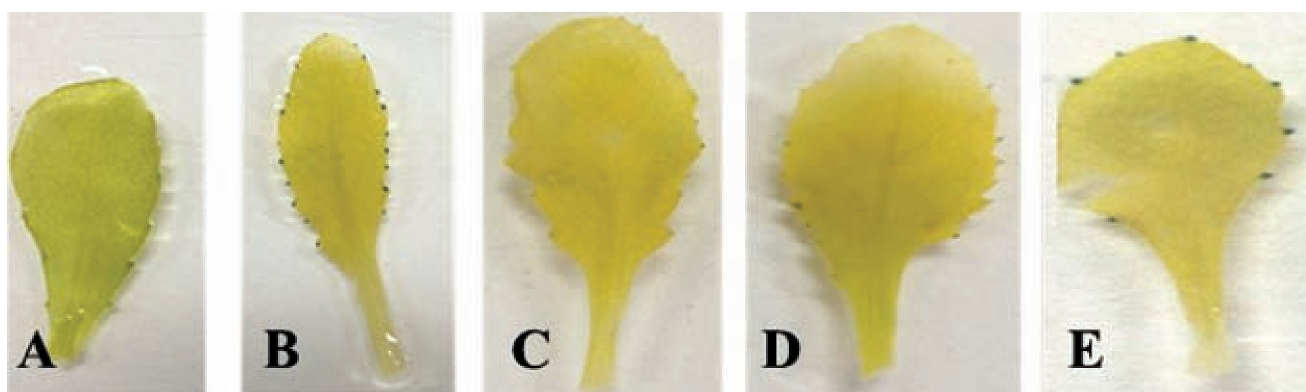


Fig. 3.13: GUS expression in *Arabidopsis* plants containing the *BjDef* deletion -3 promoter 24 hrs after *Alternaria brassicae* infection, SA treatment, MeJA treatment. (A) GUS expression of untreated transgenic leaf; (B) expression in inoculated transgenic leaf; (C) after 2mM SA treatment of transgenic leaf; (D) after 100µM MeJA treatment of transgenic leaf; (E) after wounding.

Cloning, validation and characterization of insecticidal genes from legumes in mustard

The use of chemical pesticides has led to harmful effects on the environment, human health, water pollution and non-target organisms. Therefore, new control methods are being used to protect plants against insect pests. Plants and insects interact in a complex way by activating metabolic pathways and developing secondary metabolites such as lectins, protease inhibitors and amylase inhibitors etc. These proteins have shown insecticidal effects towards Lepidopteran, Coleopteran and Hemipteran insects by expression in transgenic plants.

Cloning of protease inhibitor and lectin genes in an expression vector

The *Vigna mungo* protease inhibitor gene (HQ629949), chickpea lectin (HM235917) and *Vigna radiata* lectin gene (HM3487150) were amplified using gene specific primers with the addition of CACC at

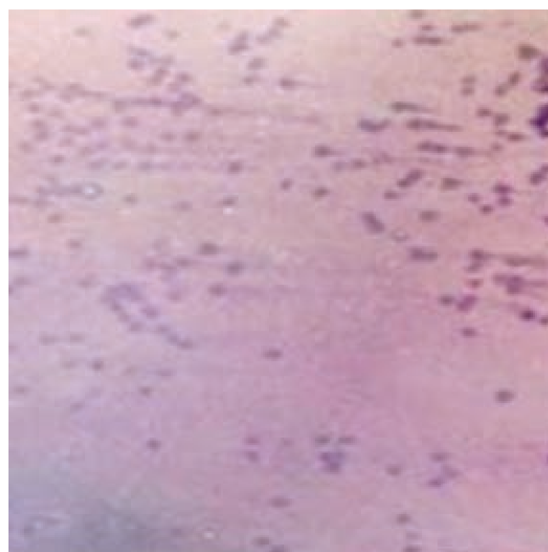


Fig. 3.14: Colony blot of the chickpea lectin gene expressed in pET300/301 vector.

Artificial diet bioassays

The purified protease inhibitor and lectin fusion protein was incorporated at 0.1% (w/v) of the diet. Controls containing no protein and no diet were set up for each trial. Ten second instar aphids (*Lipaphis erisimi*) were transferred from the host plant and the petri dishes sealed with a stretched parafilm membrane. An aliquot of 200 µl diet was added on the parafilm membrane and the diet was covered with another layer of stretched parafilm membrane to form a feeding sachet through which the aphids could imbibe the diet. Three replicates were set up for treatment and controls. The feeding chambers were maintained in incubator, illuminated with a 16/8 h light/ dark at a temperature of 25±2°C. Diets were changed every other day to ensure a fresh nutrient supply. Starting two days after the treatment, aphid mortality and the numbers of new-born nymphs in each aphid group were checked daily for 10 days. The number of surviving aphids and nymphs produced were recorded every 2 days. All the aphids fed without diet died on day 6. The mean number of aphids fed with diet containing fusion protein was significantly less than that fed only with diet constantly throughout the assay period with the differences significant at $P < 0.05$ after day 6, and all the aphids fed with diet containing fusion protein died on day 12 (Fig. 3.15).

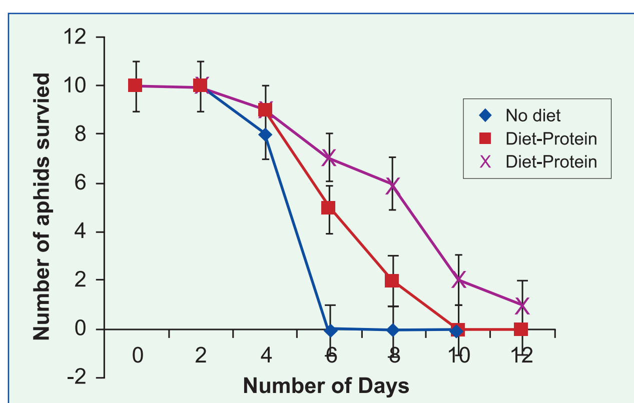


Fig. 3.15: Artificial diet bioassay of the chickpea lectin fusion protein against *Lipaphis erisimi*.

Transformation of *B. juncea* using urdbean protease inhibitor gene construct

The hypocotyls were excised from 5 days old seedlings of *B. juncea* cv. Varuna and co-cultivated with *Agrobacterium* harboring urdbean protease inhibitor gene construct for 10-15 minutes. The explants were finally transferred on selection medium augmented with kanamycin (Fig. 3.16A, B) and incubated at 25±2°C under cool fluorescent light with 16:8 hr photoperiod. The multiple shoots developed were excised and cultured on medium containing IBA (2.0 mg/l) for rhizogenesis (Fig. 3.16C, D, E). The putative transgenic plants with well-developed roots were hardened, acclimatized and shifted to pots with soil and agropeat in the ratio of 2:1. After hardening the putatives were shifted to phytotron (Fig. 3.16F). The hypocotyls showed 6-7 % transformation efficiency.

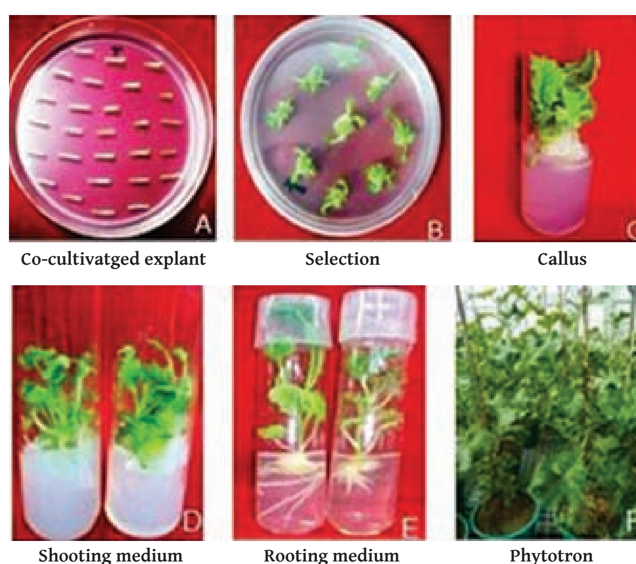


Fig. 3.16: Transformation of *B. juncea cv.* Varuna with urdbean protease inhibitor gene construct using hypocotyls as explant.

Southern analysis of T_2 transgenics of *B. juncea* having chickpea lectin gene

The seeds of T_1 generation of *B. juncea cv.* Pusa Jaikisan developed using chickpea lectin gene under the control of *rolC* promoter were screened by germinating them on 1/2 MS medium supplemented with 100 mg/l kanamycin and shifted to pots in the Phytotron. Genomic DNA from the leaves of T_2 plants was isolated using CTAB method and purified using RNAse A. Purified DNA (10 µg) was restricted using *Eco*RI and separated on 0.8 % (w/v) agarose gel. The gel was depurinated, denatured and neutralized and the DNA was transferred from agarose gel onto the positively charged Hybond N^+ nylon membrane by capillary blotting method. The blot was then hybridized with DNA fragment of chickpea lectin gene and *nptII* gene with [α-32 ρ] dCTP radiolabel.

Eighteen T_2 generation transgenic lines showed single locus for chickpea lectin gene which confirmed integration of the transgene into the *B. juncea* (Fig. 3.17).

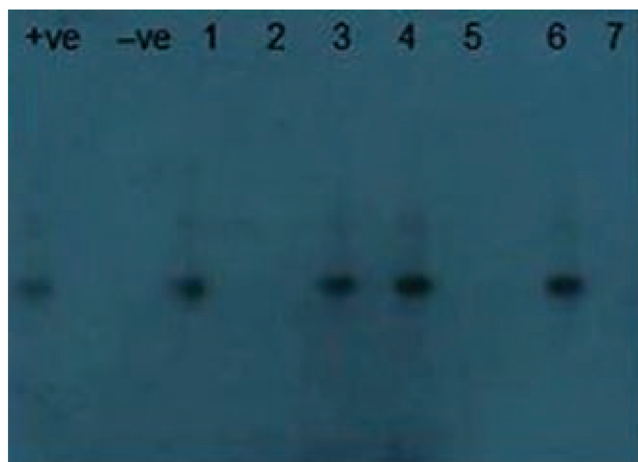


Fig. 3.17: Southern blot of transgenic *B. juncea cv* Pusa Jaikisan (T_2) transformed with chickpea lectin gene construct. +ve, DNA of lectin gene; -ve, DNA from untransformed *B. juncea* plant; 1-7, DNA from transformed *B. juncea* plants

Real-time PCR analysis of T_1 transgenics of *B. juncea* for expression of genes

Total RNA was isolated from the leaves of *B. juncea* cv. Pusa Jaikisan and Varuna using Trizol method. RNA (100 ng) was used for first strand synthesis using cDNA synthesis kit. cDNA (30ng) was used in triplicates for Real-time PCR using SYBR Green flouresence detection dye. *B. juncea* β-actin was used as a reference gene. From the Southern positive Pusa Jaikisan lines 5 lines showed about 15 fold expression of the lectin gene, while in 6 lines of Varuna, about 12 fold expression was recorded (Fig. 3.18 A and B).

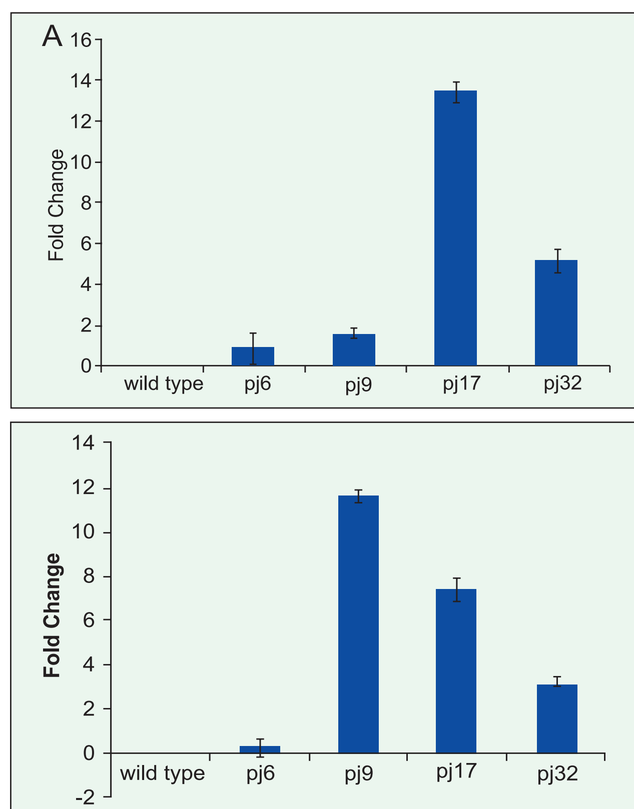


Fig. 3.18: Real-time PCR of non-transformed and transformed *B. juncea* lines (T_2) with chickpea lectin gene. (A) *cv.* Pusa Jaikisan, (B) *cv.* Varuna

Optimizing genome editing technique in *B. juncea*

Genome editing tools like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein (Cas)/single guide RNA (sgRNA) system have enabled efficient and precise genetic modifications. Amongst these techniques of genome editing CRISPR/Cas has recently emerged as a potentially facile and efficient alternative for inducing targeted genetic alterations like gene disruption, gene activation/repression and genome editing in several cell types and organisms across the kingdoms.

Construction of GUS-specific CRISPR/Cas cassette

For optimizing the CRISPR/Cas system in genome editing of *B. juncea* a binary vector was developed for introducing targeted lesions in two regions of the GUS (*uidA*) - reporter gene. The 1809 bp long *GUS* sequences were analyzed in different CRISPR designing tools viz., CRISPR-MultiTargeter/CRISPR-PLANT/ DNA2.0 etc., and two sgRNA (20 bp; Fig. 3.19a) of score 99 and above having PAM (Protospacer Adjacent Motif), NGG at 3' end were selected. These sgRNAs were first duplicated and then ligated into pRGEB31 binary vector at *Bsa*I site (Fig. 3.19b). The recombinant construct had been mobilized into *Agrobacterium* for initiating plant transformation in *B. juncea*.

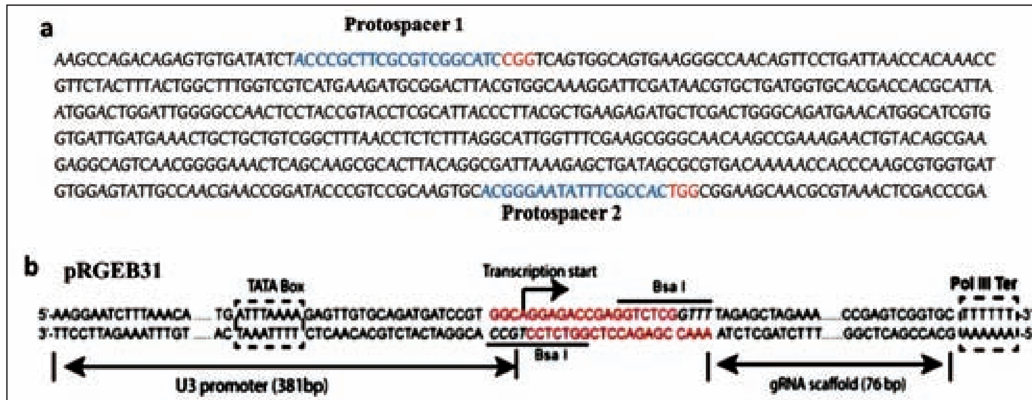


Fig. 3.19: CRISPR/Cas target region of the GUS gene and sgRNA cloning vector backbone. (a) Chimeric guide RNA (cgRNA) for targeting GUS (*uidA*) reporter gene. Target region is given in blue font and PAM region in red font, (b) Schematic view of the CRISPR/Cas vector backbone for cloning sgRNA.

Activation tagging in *A. thaliana* for identification of genes for plant architecture

As the complete genome sequences of various plant and animal species are being readily available, focus of research has also moved gradually from structural to functional genomics studies especially in case of *Arabidopsis* and rice. The ingenious use of T-DNA based vectors and various tagging strategy has led to identification of number of genes and promoters that are fundamental to plant development, metabolism and disease resistance. Using T-DNA or transposons as tags, significant progress has been made by using "Knock-in" approaches ("gain-of-function" or "activation tagging") in different plant species. The present study utilizes the T-DNA based tissue-specific activation tagging technique where, tissue-specific/ inducible/ constitutive promoter sequences were used at the right border of the T-DNA cassette. Depending on the position of T-DNA insertion, the downstream gene either ectopically expressed or overexpressed resulting in gain-of-function phenotype.

Phenomics studies of the 'Dwarf Stature' mutant line of A. thaliana

The dwarf stature mutant line (DSM) identified last year after screening of the activation tagged population of *Arabidopsis* was critically analyzed for various phenotypic parameters in two subsequent T_3 and T_4 generations. The comparative analysis of the DSM lines from wild type *Arabidopsis* showed significant differences in plant architectures, morphological and physiological parameters. The DSM lines as compared to wild type were scored and found to demonstrate diverse variant-phenotype (Fig. 3.20) viz. shorter height, more numbers of primary and secondary branches with dense canopy, robust root morphology, compact inflorescence with reduced internodal length etc.

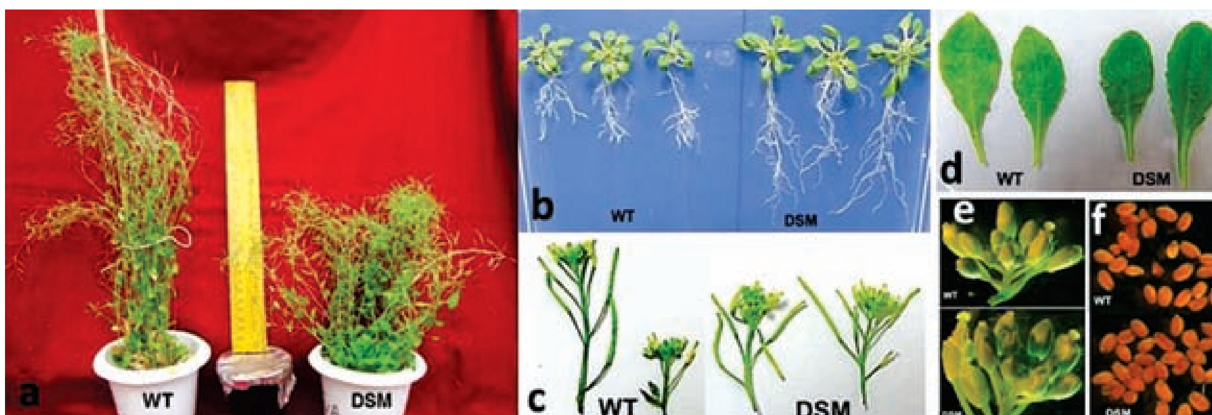


Fig. 3.20: Comparative studies of the phenomics in dwarf stature mutant (DSM) lines of *Arabidopsis*. (a) Full-grown plants of 75 days showing reduced height in DSM; (b) Seedlings of WT and DSM (30 days old) grown on vertically placed MS plate showing robust and long root morphology in DSM; (c, e) Inflorescence of the DSM are more compact and bears large numbers of floral buds; (d) Rosettes leaves from the DSM plants are comparatively more serrated and dark green; (f) Seed morphology of WT and DSM lines.

Copy number identification and construction of the overexpressing and RNAi constructs of DSR gene in binary vector

The dwarf stature of the identified line may be due to overexpression of the nearby gene because of the presence of CaMV35S promoter in T-DNA cassette of the activation tagging construct. The insertion flanking sequence has already been cloned and mapped on chromosome 1 of *Arabidopsis* and the same was reported last year. Before characterizing the tagged gene it is important to know the copy number of T-DNA insertion in the DSM line. Therefore, in continuation with characterization of the DSM line, Southern analysis was performed. The Southern blot with Digoxigenin (Non-radioactive) labeled *nptII* probe showed a single and contrasting hybridization signal in sample DNA digested with different restriction endonucleases and not in wild type sample (Fig. 3.21).

Validation of the gene-function of the identified gene was attempted by developing overexpressing lines in *B. juncea* and expression knock-down through RNAi in the mutant DSM line. It remains interesting to verify whether the transgenic *B. juncea* lines demonstrate the similar phenotype as observed in *Arabidopsis* and reversal of the wild phenotype in RNAi-DSM lines. For that purpose the gene from *A. thaliana* was cloned (Fig. 3.22a) under a constitutive

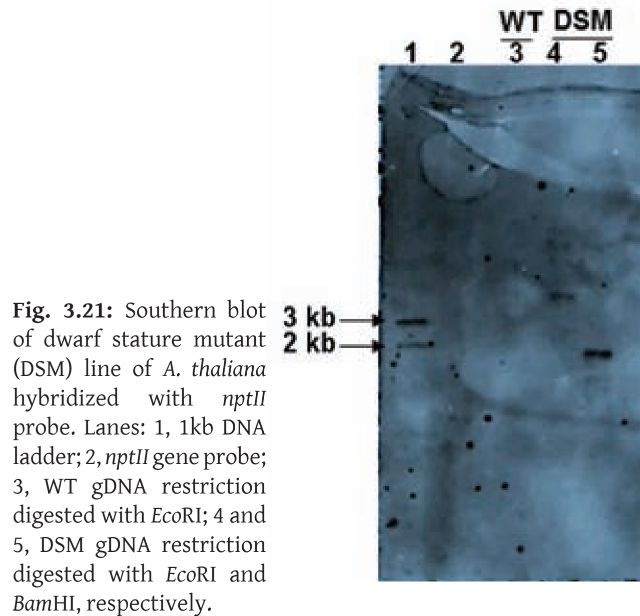


Fig. 3.21: Southern blot of dwarf stature mutant (DSM) line of *A. thaliana* hybridized with *nptII* probe. Lanes: 1, 1kb DNA ladder; 2, *nptII* gene probe; 3, WT gDNA restriction digested with *Eco*RI; 4 and 5, DSM gDNA restriction digested with *Eco*RI and *Bam*HI, respectively.

promoter CaMV35S in pBI121 vector at *XbaI* and *SacI* cloning site (Fig. 3.22b) and named as DSR-OEXP (Fig. 3.23a). For RNAi construct, 447 bp region of the *dsr* gene was cloned from *Arabidopsis* and placed downstream to the CaMV35S promoter in antisense orientation at *Sal*I and *Sac*I cloning site in pOREO4 vector (Fig. 3.22c) and named the construct as DSR-ANS (Fig. 3.23b). The recombinant vectors carrying overexpressing and antisense constructs for the *dsr* gene were individually mobilized into *Agrobacterium* (GV3101) (Fig. 3.22d).

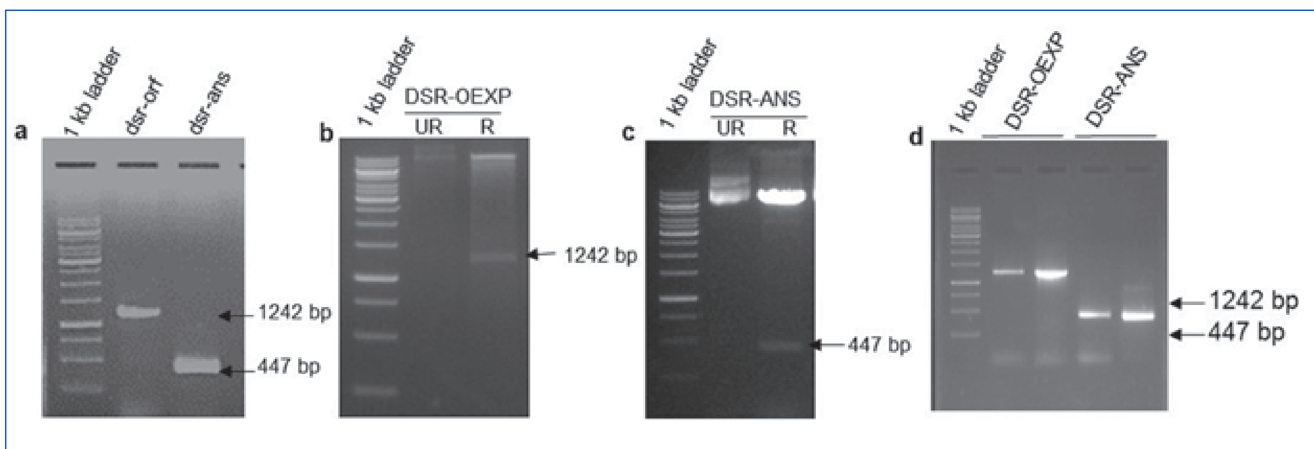


Fig. 3.22: Development of overexpressing and RNAi constructs of the *dsr* gene. (a) PCR amplification of the full length (1242 bp) and partial (447 bp) *dsr* gene sequences for cloning into binary vector; (b & c) Restriction digestion of the recombinant clones of DSR-OEXP (with *XbaI* and *SacI*) and DSR-ANS (with *Sal*I and *SacI*); (d) C-PCR of the recombinant *Agrobacterium* clones of DSR-OEXP and DSR-ANS. UR-unrestricted and R-restricted pDNA.

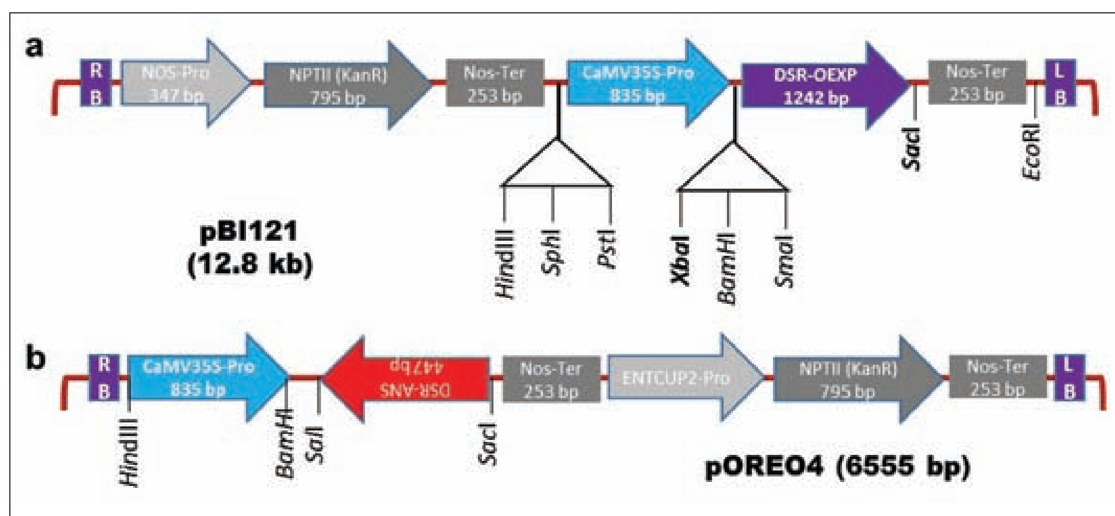


Fig. 3.23: Schematic view of the T-DNA region of the binary vector. (a) Overexpressing gene construct of the *DSR-OEXP* in pBI121 vector; (b) Antisense construct of the *dsr* gene (DSR-ANS) in pOREO4 vector.

Introgression of genes for Alternaria tolerance from wild species in *B. juncea*

About 110 individual plant progenies, selected in 2014-2015 at NRCPB, Delhi based on artificial screening and at GBPUAT, Pantnagar under natural hot spot conditions to introgress gene(s) for tolerance to *Aletrnaria brassicae* from wild species, were grown with control (resistant) and susceptible genotypes and some wild species at both the locations during 2015-2016 (Fig. 3.24). The natural screening at Pantnagar was conducted in collaboration with Department of Genetics and Plant Breeding, GBPUA&T. Disease scoring was done on a score of 0-9 (where 0 is resistant and 9 is most susceptible) for each line and individual resistant plants were selected. Fifty three individual plants and four individual lines were selected which showed high degree of resistance/tolerance. Leaf samples were collected from these plants for molecular analysis. In general, resistance level in progenies of this year was higher than those recorded in the previous year's suggesting that the selection has been effective.

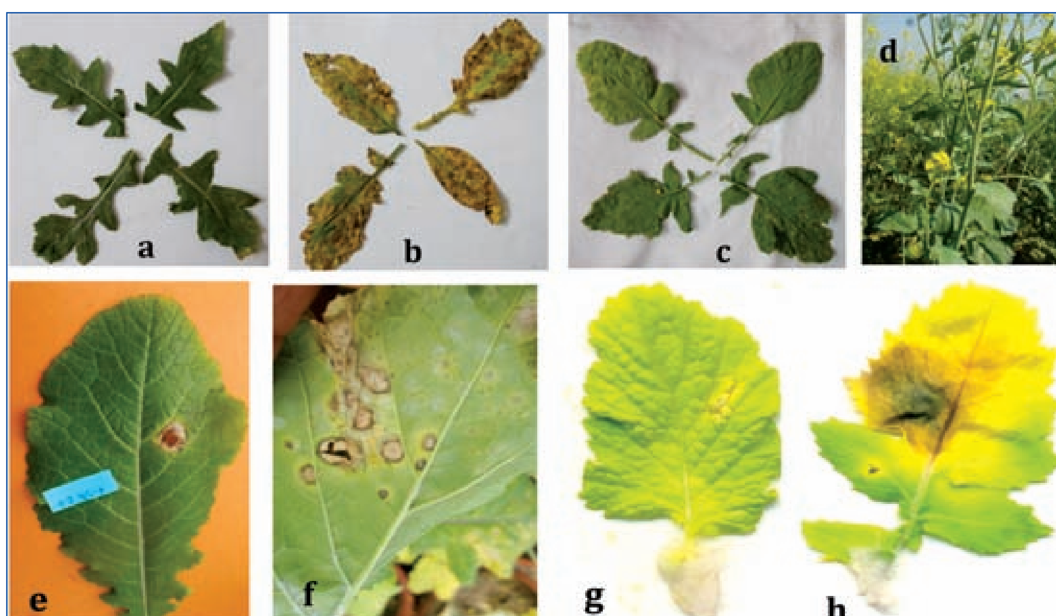


Fig. 3.24: Introgression of Alternaria resistance into *B. juncea*. (a-d) Screening from GBPUAT, Pantnagar on natural infection; a. resistant control (*Erurapa*), b. susceptible control (*B. juncea*), c. leaves from IL's, d. IL's line. (e-h) Artificial screening at ICAR-NRCPB, New Delhi; e-f. Leaf of IL's plant showing resistant and susceptible reaction in *in vivo*, g-h. Leaf of IL's plant showing resistant and susceptible reaction in *in vitro.*

For artificial screening, the same set of progenies were grown at NRCPB, New Delhi in polyhouse and *in vivo* and *in vitro* screening was performed using two isolates of *A. brassicae* (*AC-9* & *AC-7*) in 2015-2016. Out of these two isolates, one was isolated from Pantnagar (*AC-9*) and another one from IARI, New Delhi (*AC-7*). *In vivo* inoculations were performed by leaf wounding (three leaves per plant) and the scoring was done on 0-5 scale (where 0 is highly resistant and 5 is highly susceptible) on the basis of size and number of fresh spots developed on whole plant. In *in vitro* condition, detached leaf method was used and inoculation was done by disc method. After seven days of inoculation disease scoring was done on the basis of spot diameter developed. Samples were collected from each and every plant for molecular analysis.

Samples collected last year were tested for introgression of gene (s) from wild species using *Diplotaxis erucoides*- specific molecular markers developed in-house. Out of sixty primers tested, about 49 primers showed polymorphism between the parents. These selected polymorphic primers were used to check the introgression in IL's and introgression from wild species were confirmed (Fig. 3.25).

Also, the back crosses were performed using F_1 of *Eru-rapa* x *B. juncea* with *B. juncea* followed by embryo rescue. Flower samples from F_1 were collected for cytogenetic study. Crossing and embryo rescue were started using *D. catholica* and *B. rapa* with the objective to transfer the gene(s) conferring *Alternaria* resistance and a few plantlets from this cross were obtained. The F_1 will be further established by chromosome doubling and later it will be used as bridge species to transfer the resistant gene (s) to *B. juncea* followed by embryo rescue.

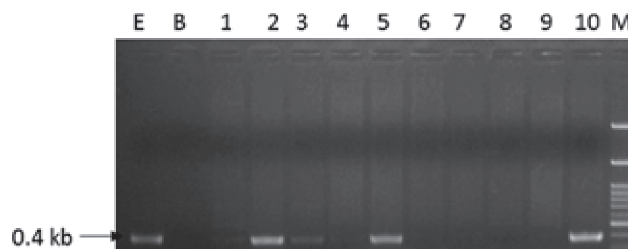


Fig. 3.25: Gel electrophoresis confirming introgression from wild species to IL's of mustard using ESSTS57 marker. Lanes: E, *D. Erucoides*; B, *B. juncea*; 1-10, IL's; M, 100 Kb DNA ladder.

Resynthesis of *B. juncea*

The experiment started in the year 2012-13 for widening of genetic resource of *B. juncea* through resynthesis using the progenitor species has made significant headway. This year 32 individual events of resynthesis of *B. juncea* from six different crosses using embryo rescue and amphidiploidization were obtained. The characterization of newly resynthesized *B. juncea* lines was initiated. These new lines showed high pollen viability (≥85%). Some of these new lines have desirable agronomic features with respect to plant architecture like, number of secondary and tertiary branches, number of siliques on main axis, number of siliques *per* plant, plant height, leaf colour, seed size etc. (Fig. 3.26).

Fig. 3.26: Different lines of resynthesized *B. juncea* and pollen viability test.

In addition 74 individual events from four different crosses using selected *B. rapa* and *B. nigra* were obtained after chromosome doubling in 2015-2016. These lines were developed using the special *B. rapa* line selected at NRCPB, New Delhi which had an *in vivo* seed setting property when it was crossed with *B. nigra,* i.e. there was no need of embryo rescue during resynthesis of *B. juncea*. Also, in 2012-13, the *B. rapa* lines were intercrossed and the F_1'S were crossed with *B. nigra* to tap the maximum diversity in resynthesized *B. juncea,* and total eight individual events from two cross combinations were obtained this year after embryo rescue and amphidiplodization. The fertility of these plants was low, so bud pollination was performed and seed from these plants will be harvested separately.

Based on experiments on resynthesis of *B. juncea* over the past four years, a *B. rapa* line was identified that supports *in vivo* seed development following crossing with *B. nigra* thereby obviating the need for embryo rescue to obtain interspecific hybrids (Fig. 3.27). This feature was maintained in the F_1 of the cross with other *B. rapa* accessions. Thus this is unique resource and could facilitate efficient resynthesis of *B. juncea*. Using such F_1 *B. rapa* plants, *in vivo* seeds from eight cross combinations were obtained for amphidiploidization.

Fig. 3.27: *B. rapa* line screened and identified for resynthesis of *B. juncea*.

4 ADAPTATION OF WHEAT TO CLIMATE CHANGE INDUCED ABIOTIC STRESSES

Identification and characterization of heat responsive genes from tolerant plant species

The project involves 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. Based on data generated by SSH method, in the previous years in thermotolerant plant systems (*Ziziphus, P. glaucum, P. cineraria* and tolerant Wheat genotypes), a number of heat responsive genes were identified and their differential expression under heat stress was validated. Complete CDS of a few heat responsive genes (*Tahsp-sti-TaGPX, TaMYB, TaDREB, PgASR, PgDREB, ZnNAC, ZnLEA*) were isolated and binary constructs were developed for genetic transformation of wheat (Fig. 4.1)

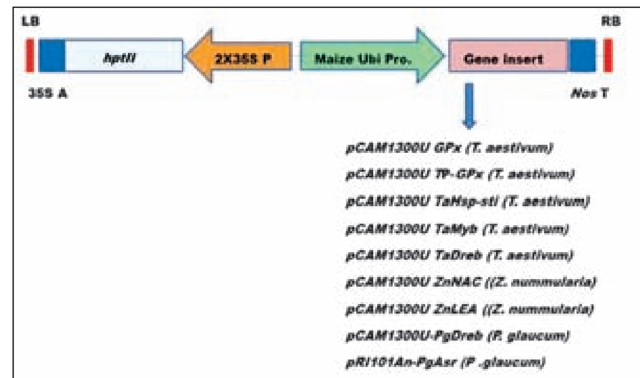


Fig. 4.1: Schematic presentation of binary vector pCAMBIA1300:Zm*Ubiquitin* promoter with different heat responsive genes

Wheat *cv.* HD2894, HD2967, HD2932 and HD2987 were transformed using the newly developed as well as previously developed gene constructs (*ZnASR, ZnGolS1, Pchsp17.9, PgCRT, PgP5CSTapAPx, TaCpn60, TaGPx, Ta-TP-GPx, Tahsp-sti, TaMyb, TaDREB, EcDREB, EcBAG, Csp-A*) using *in planta* methods. Putative transgenics at T_1 & T_2 stage were screened using selection marker and PCR analysis.

Among the selected genes, *PgP5CS* (Δ1-pyrroline-5-carboxylate synthetase) from Pearl millet (*P. glaucum*) was functionally validated in *E. coli* as well as tobacco. Leaf disc explants of tobacco (*Nicotiana tabacum* var. Petit Havana) were transformed with pCAMBIA1300 harbouring *PgP5CS* gene (Fig 4.2). Putative transgenic plants were screened by gene specific PCR analysis (Fig. 4.3). The integration and expression of *PgP5CS* in tobacco was confirmed by Southern hybridization analysis (Fig. 4.4), T-DNA flanking sequence and qRT-PCR analysis.

Fig. 4.2: Transformation of *Nicotiana tobacum* with *PgP5CS*. Different stages of tobacco transformation- a) explants, b) Callusing and selection, c) multiple shooting, d) plantlet.

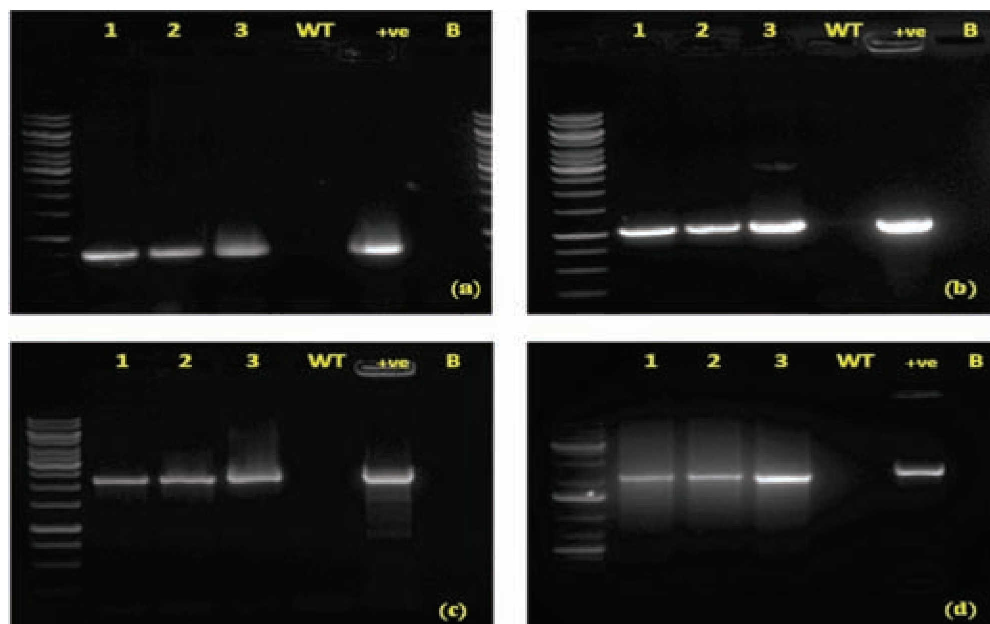


Fig. 4.3: Confirmation of *PgP5CS* transgenic tobacco lines with different set of primers. PCR with a) *HptII* primer; b) Gene specific (*P5CS*) internal primers, c) Gene specific (*P5CS*) full length primers and d) Promoter & gene specific primers (Ubi-Pro-F and P5CS-R, 1742nt); Lanes 1 to 3 different transgenic lines; WT, Wild type; +ve, control (plasmid); B, blank control.

The *PgP5CS*- transgenic (TR) lines of tobacco were assessed for tolerance to heat (35 °C and 42 °C for 6 hr) and drought stress (25% PEG-6000 for 5 days) tolerance based on different physiochemical parameters such as proline, relative water content (RWC), chlorophyll content and malondialdehyde (MDA) (Fig. 4.5).

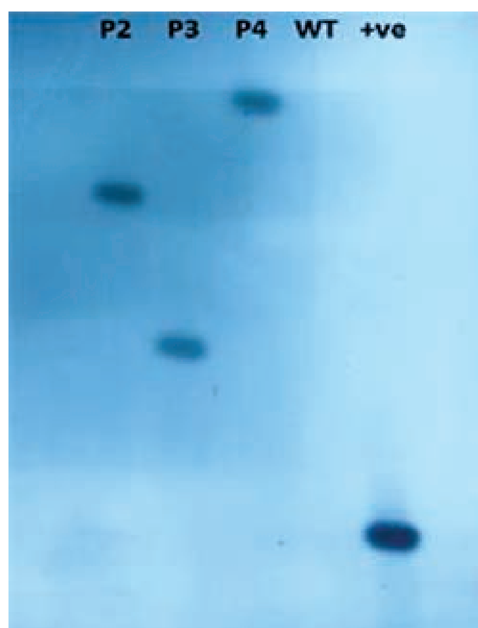


Fig. 4.4: Southern blot analysis of *PgP5CS* transgenic tobacco lines. Genomic DNA of transgenic events was digested with *EcoRI*. The blot was hybridized with PCR-DIG-labelled *HPTII* probe.

The high temperature tolerance of the tobacco transgenic plants was assayed by leaf disc assay. Leaf discs from transgenic tobacco and wild type plants were floated on water and subjected to 45 °C treatment. Leaf discs were analysed for per cent reduction in chlorophyll content in tobacco transgenic lines over wild type control after 72 hr (Fig. 4.6). Transgenic lines showed better chlorophyll retention than the wild type control thus indicating that *PgP5CS* enhanced the high temperature tolerance of the tobacco plants.

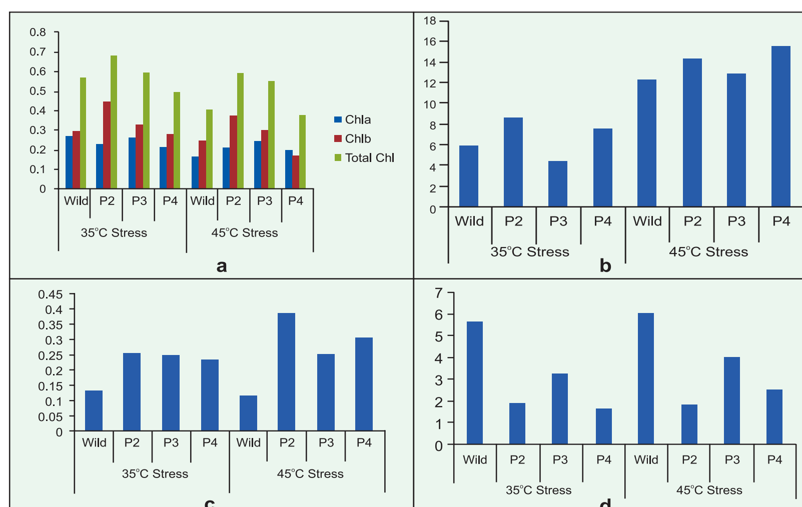


Fig. 4.5: Physiological analysis of the *PgP5CS* transgenic tobacco lines (P2, P3 and P4) under heat stress conditions. a) Chllorophyll, b) RWC, C) Proline and d) MDA.

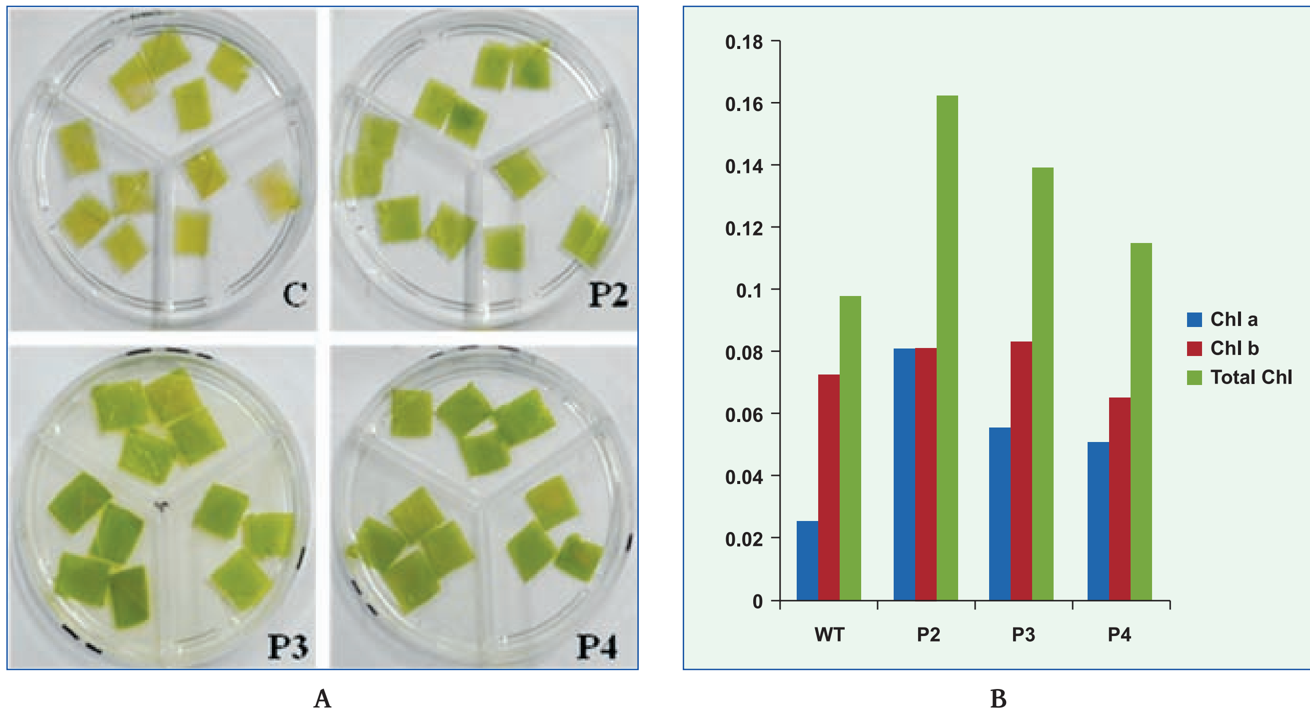


Fig. 4.6: Response of *PgP5CS* expressing transgenic tobacco plants to heat stress. Leaf discs from wild type and transgenic plants were floated on distilled water. A) Photograph taken 72 h after exposure to heat at 45 °C treatment, B) Reduction in chlorophyll content (%) in tobacco transgenic lines over control.

Analysis of drought responsive genes through transcriptomics

To prospect genes responsive to drought stress, transcriptome data was generated in *P. glaucum* using Roche454 sequencing platform. The pre-processed high quality single end reads from each file were combined separately by *de novo* assembly tool of CLC genomics. Maximum numbers of assembled sequences were of 300-400 bp length. Mean of the GC content of the reads was between 50-60 implying stability of the reads whereas mean of the Phred score was around 30 indicating 99.9% accuracy.

Functional annotation and characterization of *P. glaucum* transcript revealed that 43% transcripts were annotated. To detect the molecular functions, biological processes and cellular components, 'Gene Ontology' (GO) database (AmiGO 2) was utilized to assign GO term for millets transcript and 78% transcripts were found to be having GO terms. DNA integration and metabolic process were highly dominating in biological processes while nucleic acid binding proteins were highly represented compared to other molecular function (Fig. 4.7). Total of 12883 sequences were examined and Misa tool was used for the identification SSRs (Fig. 4.8). The SSR markers are being validated for abiotic stress tolerance in 1500 genotypes.

Combating terminal heat stress in wheat

High temperature is becoming an important limiting factor of yield and quality and crops will have to adjust to even higher temperatures in the near future. The grain-filling stage is critical to the yield and quality of wheat and is also very sensitive to high temperatures. High-temperature fluctuations decrease grain plumpness, starch content, and protein accumulation, impacting both yield and quality. Thus heat stress, specifically terminal heat stress is one of the top most research priorities in wheat crop today. Therefore, efforts were made to isolate and functionally characterize novel genes involved in modulating heat stress response.

SnRK1 (sucrose non fermenting-1- related protein kinase1): Molecular cloning and transcript expression profiling

SnRK1 (*Sucrose non-fermenting-1-related protein kinase 1*) is a key metabolic regulator which plays an important role in plant's carbon, nitrogen metabolism and development. These serine/threonine kinases respond to cellular energy change through relative AMP and ATP concentrations. The AMP/ATP ratio allosterically regulates mammalian AMPK and the plant SnRK1 is regulated by phosphorylated sugar.

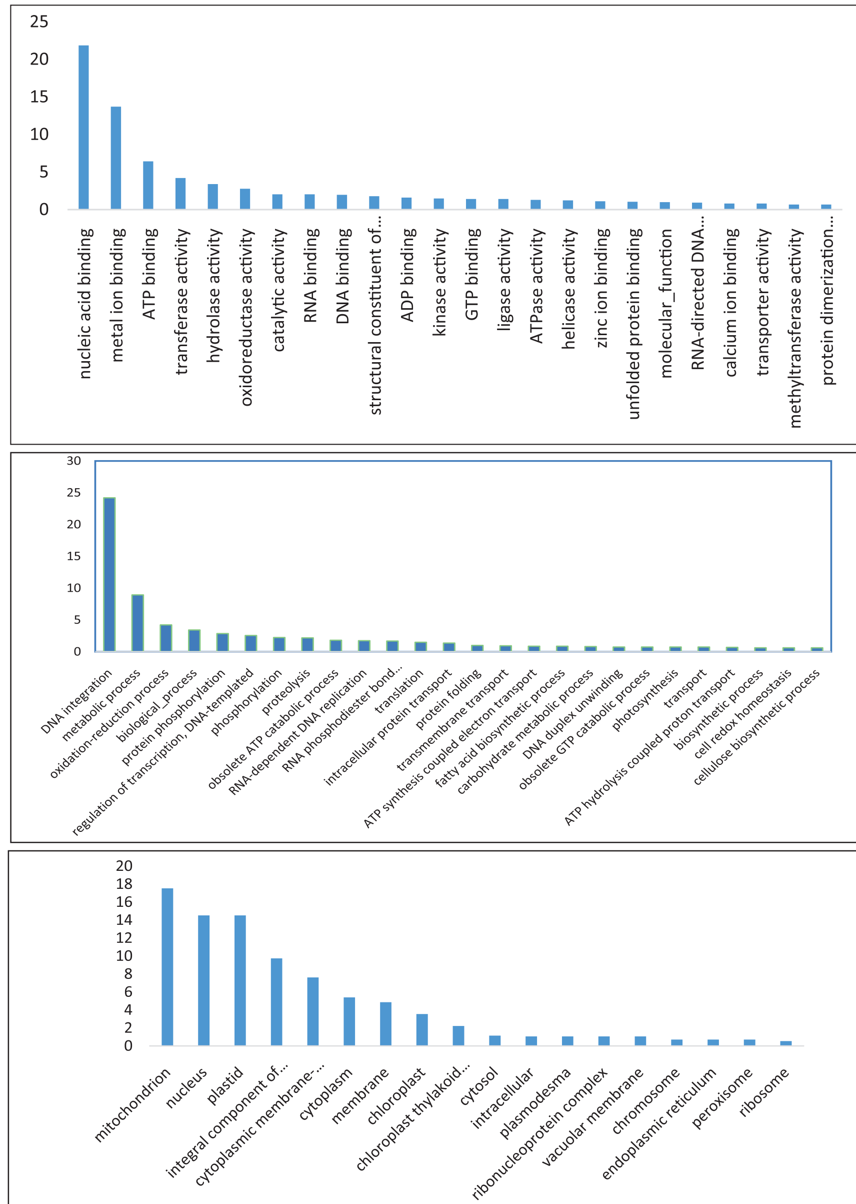


Fig. 4.7: Number of contigs involved in significant biological process. (A), molecular; (B) biological; and (C) cellular functions.

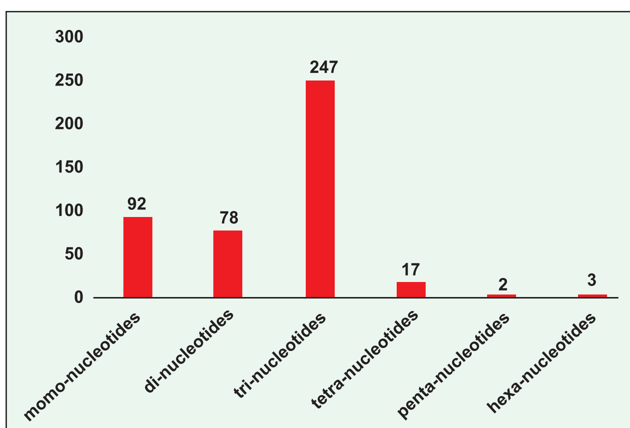


Fig. 4.8: Distribution of different SSR markers identified in the contigues.

Two cultivars, viz. HD2967, a highly popular variety and C-306 known for superior quality, were grown in pots in the phytotron facility and exposed to high temperature stress at 37ºC for two hours at various vegetative and reproductive stages. The expression analysis of *TaSnRK1* revealed a differential and positive modulation in both the cultivars during early as well as terminal heat stress and also under regular growth and development conditions. In C-306, *TaSnRK1* expression was higher in early vegetative stages while in HD2967 the expression was higher during generative stages (Fig. 4.9). As a whole, these transcript expression findings show that SnRK1s are involved in diverse physiological processes throughout the life cycle of wheat

plants, beginning from seedling establishment to senescence as well as under high temperature stress. A full coding region of *TaSnRK1* catalytic subunit was cloned and confirmed by various *in silico* analyses.

Transient expression of pCAMBIA1302:: *TaWRKY10-GFP* in *Nicotiana benthamania*

A WRKY transcriptional activator family member from wheat *TaWRKY10* was reported by us in previous year is highly modulated under heat stress in generative stages. *TaWRKY10* full length coding region was cloned and heterologous expression was carried out in *E. coli.* This year *TaWRKY10* had been mobilized into binary vectors and transient expression of protein fused to GFP had been analyzed in *N. benthamenia* (Fig. 4.10). The construct was transformed into *Arabidopsis* and T_1 generation plants were raised.

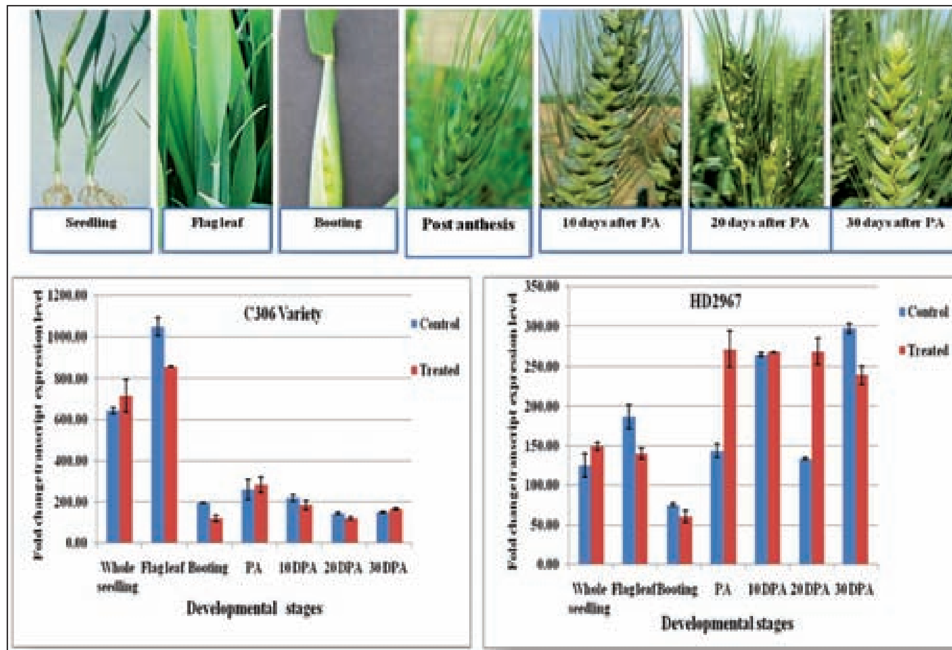


Fig. 4.9: Different developmental stages of wheat cultivar at which samples were collected and expression profiling of *TaSnRK1 gene* was analysed in wheat genotypes, C306 and HD2967.

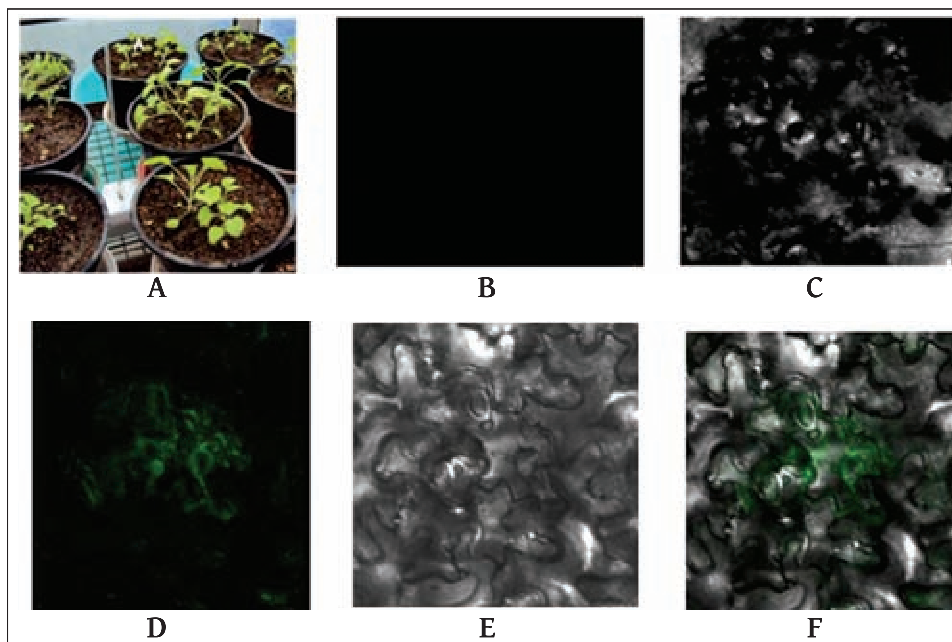


Fig. 4.10: Transient expression of pCAMBIA1302::*TaWRKY10-GFP* in leaves of *N. benthamania.* A. Infiltered leaves; B. GFP image of wild type tissue; C. DIC image of wild type tissue; D. GFP image of leaves infiltrated with *TaWRKY10-GFP*; E. DIC image of leaves infiltrated with *TaWRKY10-GFP;* F. Merged image of D&E (GFP-DIC).

Phenotyping under heat stress and expression and cloning of full length *S-phase kinase protein1* (*Skp1*) gene

To analyze the involvement of specific phytohormones under heat stress, a series of morpho-physiological analysis had been carried out at various developmental stages in selected cultivars of wheat. Plants were exposed to different hormones alone and in combination with heat stress in growth chambers and in field using portable heat traps. The time course experiments showed differential effect on plant growth and development and modulation of heat induced gene expression. Fig. 4.11 and Fig. 4.12 show the germination percentage, and shoot and root length of germinating seedlings, respectively.

SKP1 proteins are essential component of the SCF ubiquitin ligase complex, which mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription. These are induced and upregulated under abiotic stresses including heat. After comprehensive transcript profiling, two full length coding regions of wheat *Skp1* family members were cloned, confirmed and are being functionally characterized. Fig. 4.13 depicts steps in the isolation and sequence analysis of *TaSkp1*.

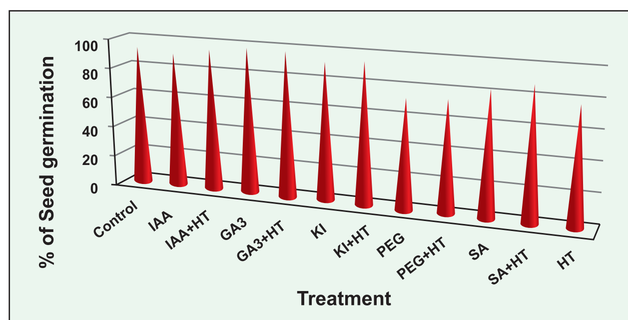


Fig. 4.11: Effect of exogenous application of various hormones and PEG individually, and in combination with heat stress on germination percentage in wheat cultivar HD2967.

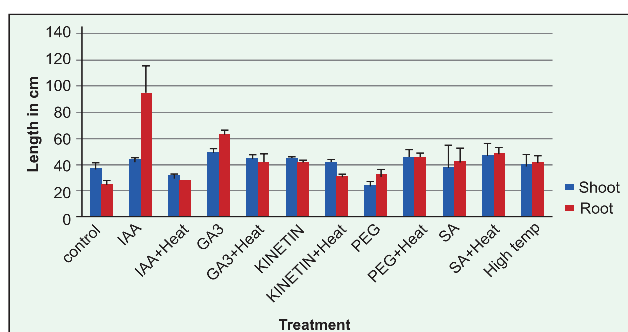


Fig. 4.12: Effect of exogenous application of various hormones and PEG individually, and in combination with heat stress on root and shoot length of one week old seedlings of cultivar HD2967.

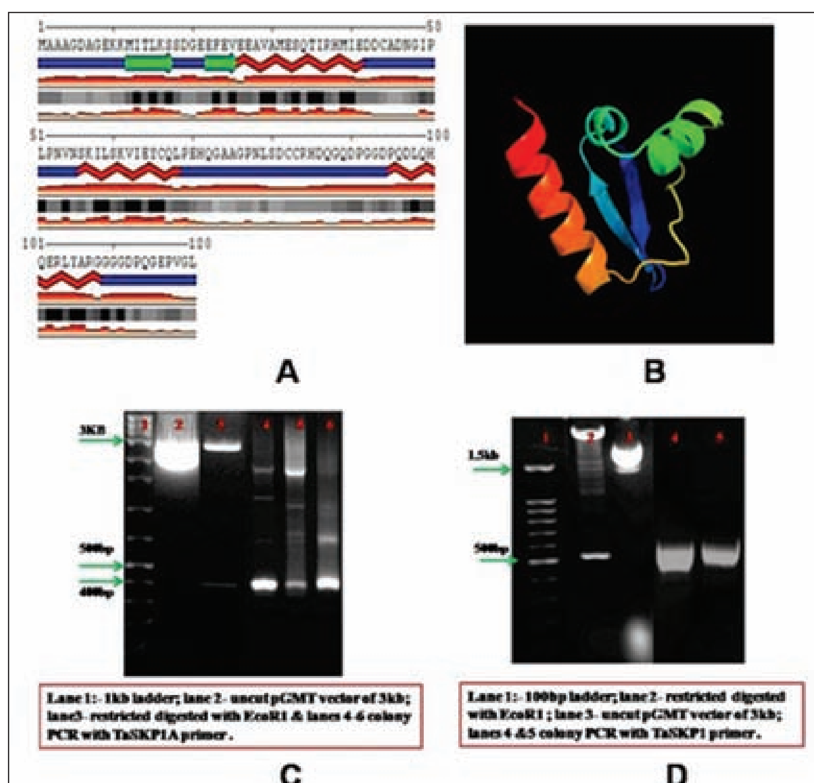


Fig. 4.13: Isolation, cloning and amino acid sequence analysis of two family members of *S-phase kinase protein 1* from bread wheat. A. Secondary structure prediction of SKP1 motif using SABLE; B. 3-D structure using Phyre2; C. Cloning of *Skp1* CDS in pGMT vector; D. Cloning of *Skp1A* CDS in pGMT vector.

Genetic transformation of wheat for terminal heat stress tolerance

For developing wheat with enhanced heat tolerance, work was initiated for *in planta* transformation of germinating wheat seeds with a few selected genes, viz. *TaWRKY10* and *Osannexin4* (*OsAnn4*). The transformation protocol is shown in Fig. 4.14. Seeds of putative transformant lines confirmed by amplification of a 450 bp fragment of *HptII* gene had been collected.

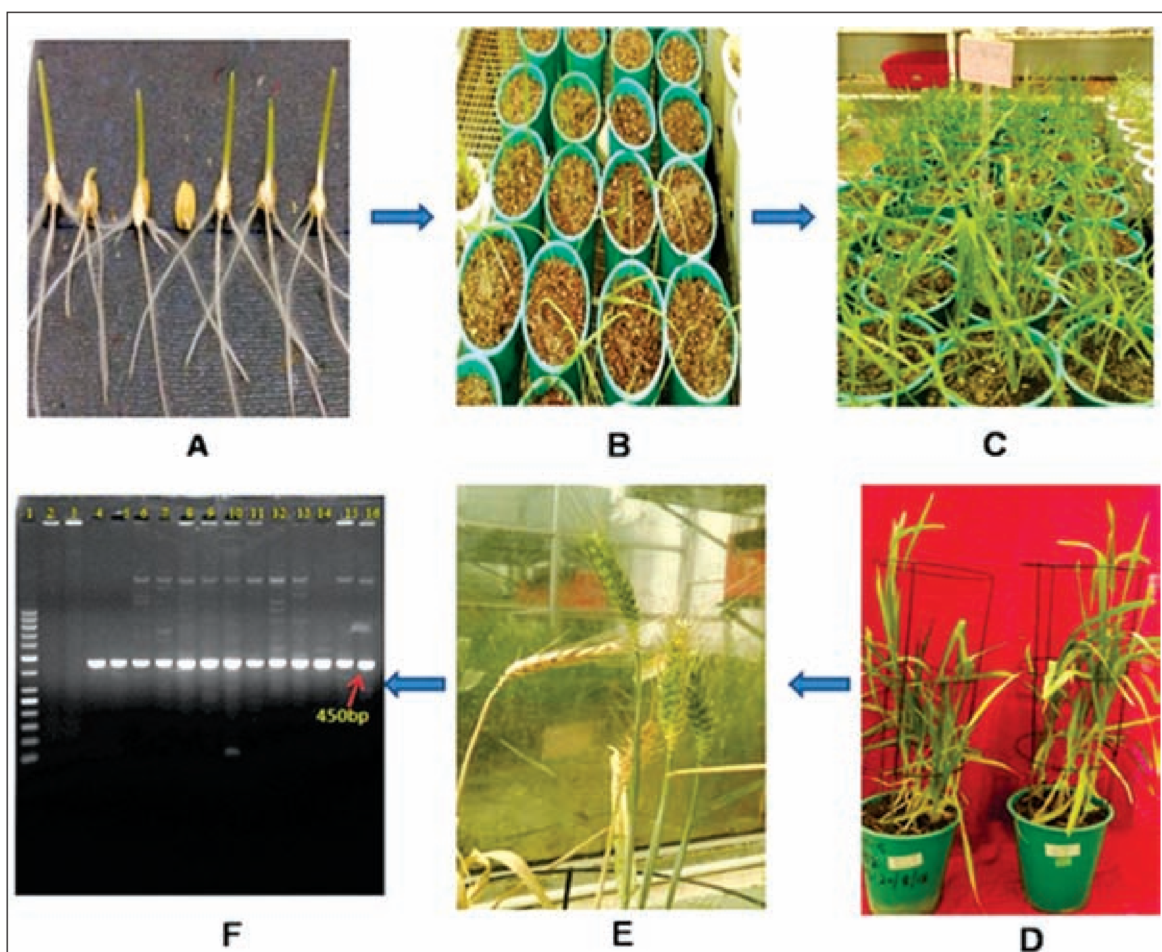


Fig. 4.14: A representation of various steps involved in *in planta* transformation of wheat using germinating seeds.

Transcriptome analysis for understanding salt tolerance mechanism in Kharchia local

Kharchia local wheat is known for its ability to tolerate salinity stress. Salt tolerance trait in Indian wheat cultivars has been derived from this land race. So it was used as a resource for deciphering the mechanism of salt tolerance using NGS technology. Total RNA was isolated from different tissues of control and salt stressed seedlings grown hydroponically. Libraries were constructed and sequencing of transcriptome was performed using Illumina Hiseq. Bioinformatic analysis of the transcriptome data of the Kharchia local wheat under salinity stress was carried out. Raw reads obtained after sequencing were assembled using Trinity RNA-Seq *de novo transcriptome* assembly tool (Table 4.1).

KEGG pathways were assigned to the assembled sequences using the online KEGG automatic annotation server (KAAS). Molecular markers play an important role in plant biology. These are commonly used for the analysis of plant genomes and identification of association between genetic variation and heritable traits. The sequence information was used for identifying SSR markers. Tri-nucleotides were the most abundant SSRs in the transcriptome (Fig. 4.15).

Table 4.1: Annotation statistics of the *T. aestivum* transcriptome assembly

TRINITY	CT_ROOT	TR_ROOT
No. of Contigs	78310	79415
Total Bases	48138667	44568639
No. of contigs >1kb	13365	11372
Max Contig Length	2322	2057
Mean Contig Length	346	526.08
N50	889	751
No. of Contigs in N50	811	681

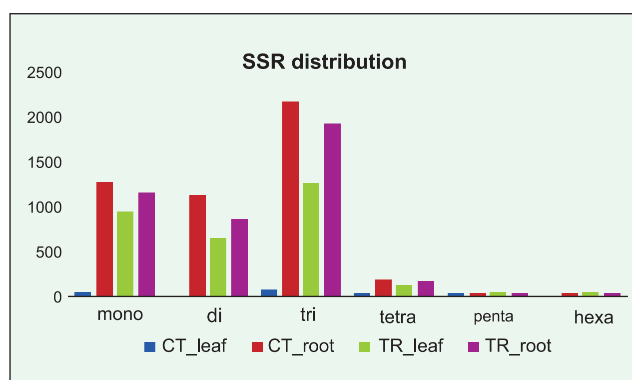


Fig. 4.15: Distribution of SSRs. Number of SSRs classified based on motif type.

Molecular genetic analysis of root system architecture in wheat

Wheat is grown in rabi season and depends on irrigation and stored soil moisture. Deep root system can significantly enhance water mining by crop and its yield under long dry spells. Therefore, two approaches have been adapted to understand the molecular genetic mechanism of root traits under drought stress in wheat. These include functional characterization of genes related to root system architecture in wheat and to map QTLs for drought and/or root traits in wheat. To characterize the genes involved in RSA from wheat (*T. aestivum*), two full length cDNAs of root related genes i.e. *TaBRX* (*BRAVIS RADIX*), and *TaMIZ1* (*MIZU-KUSSEI 1*) were cloned into binary overexpression vectors (Fig. 4.16). These binary vectors were transformed into *Agrobacterium* strain *GV3101* and were used for transformation into *A. thaliana* ecotype Columbia by floral dip transformation method.

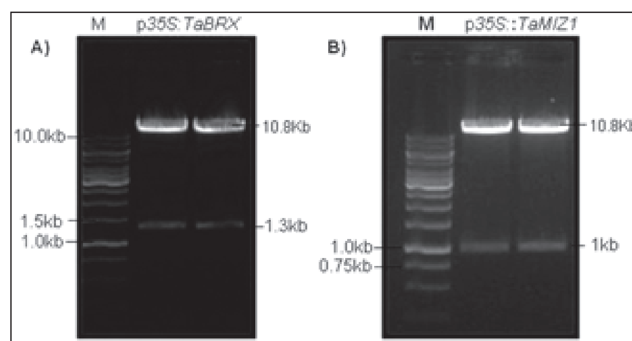


Fig. 4.16: Confirmation of binary vectors harboring A) *TaBRX* and B) *TaMIZ1*, respectively, by restriction digestion. M, 1kb DNA marker.

To analyze the spatiotemporal regulation of *TaBRX* (*BRAVIS RADIX*), two promoter fragments (2381bp and 639bp) of *TaBRX* were isolated from wheat genotype Raj3765, confirmed by sequencing and subcloned into binary vector harboring *GUS* and *GFP* reporter genes (Fig. 4.17).

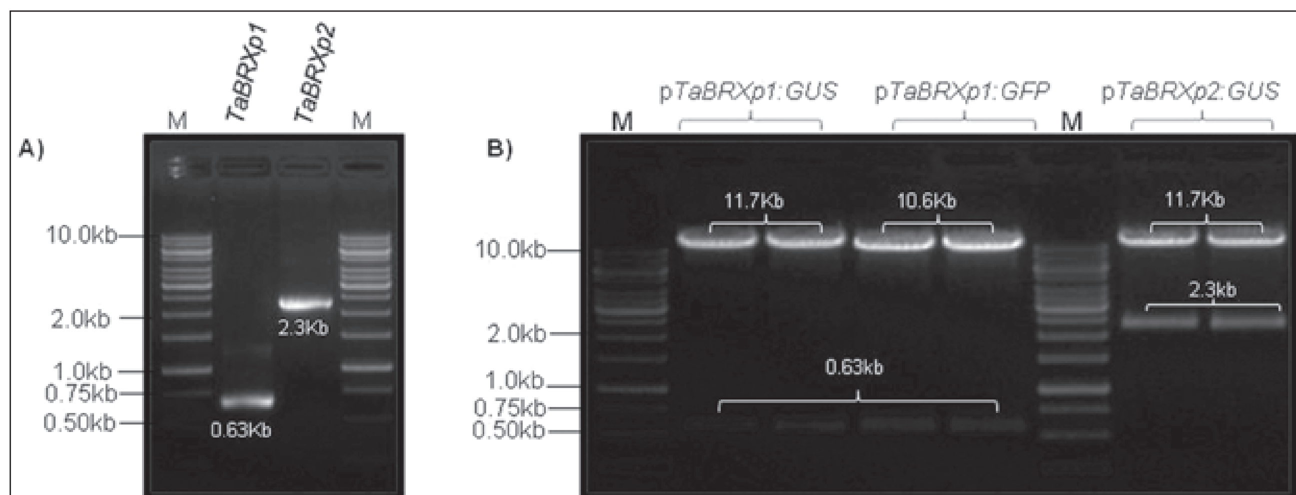


Fig. 4.17: Isolation and cloning of *TaBRX* promoter fragments from wheat. A) Amplification of promoter fragments of *TaBRX* by PCR; B) Confirmation of binary vector harboring *TaBRX* promoter fragments (p1 and p2) by restriction digestion; M, 1kb DNA marker.

To understand the transcriptional regulation of genes in roots under drought stress, comparative transcriptome analysis was carried out in two genotypes, viz. Raj3765 and HD2329, with contrasting root traits under drought stress. The transcriptome data was generated on Illumina HiSeq 2000 platform. The pre-processed reads were aligned to the reference wheat genome. The differentially expressed transcripts were identified using cufflinks program and total 1324 genes were upregulated while 3203 genes were down regulated (p-value £ 0.01) in roots under drought stress. To map the QTLs for root and drought tolerance traits, a mapping populations viz. Raj3765xHD2329 is being developed and about 2400 F_2 plants were grown in field during rabi 2015-16.

Development of transgenic wheat with enhanced drought tolerance

ZAT12 is a zinc-finger protein. Transgenic plants expressing *ZAT12* have been found to be tolerant to oxidative stress and drought. For developing over expression construct for wheat transformation, *ZAT12* (486 bp) was isolated from *B. carinata* genotype NPC-9 and cloned into a binary vector under the control of stress inducible RD29a promoter (Fig. 4.18). The binary construct *pC12RD::ZAT12* was transformed into *Agrobacterium* and used for genetic transformation of wheat genotypes HD2329.

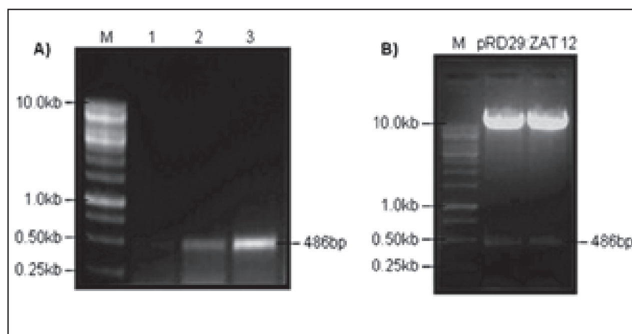


Fig. 4.18: Isolation and cloning of *ZAT12* gene from *B. carinata*. A) Amplification of *ZAT12* gene by RT-PCR; B) Confirmation of binary vector harboring *BcZAT12* by restriction digestion; M, 1kb DNA marker.

Molecular breeding for leaf spot disease in wheat

Leaf spot disease mostly occurs in eastern parts of India. To develop tolerant varieties against the leaf spot, three wheat varieties, viz. HD 2967, HD 2733 and PBW343, which are highly adapted but susceptible to leaf spot disease, were individually crossed with three tolerant varieties, Chiyra 3, SW89-5422 and Shangai. The F_1 plants of all the four crosses (Chiyra 3 x HD 2733, SW89-5422 x HD 2733, Shangai x PBW343 and Shangai x HD 2733) were backcrossed with the recurrent parents using F_1 plants as male (Fig. 4.19). The BC_1F_1 seeds were grown during rabi season at IARI, New Delhi. The BC_1F_1 plants were analysed for the presence of the tightly linked SSR markers *Xgwm148* and *Xgwm111* (foreground selection). All the tested plants contained expected size of bands (Fig. 4.20). The summary of the analysis with these two tightly linked markers is given in Table 4.2. Further, a set of 50 pairs of polymorphic SSR primers distributed across the wheat genomes were used for the background selection (Fig. 4.21). The BC_1F_1 plants were screened for resistance to leaf spot disease by artificial inoculation method (Fig. 4.22).

On the basis of field screening and foreground selection, 47 to 55 BC_1F_1 lines were selected from all four crosses for further recombinant and background selection to minimize 'linkage drag' while recovering more genetic background of recurrent parent. Twenty seven to thirty BC_1F_1 plants showed the resistance gene specific bands at 1000 bp, in between 450 to 550 bp and 160 bp. Two foreground markers *Xgwm148-2B* and *Xgwm111-7D* showed polymorphism among the wheat lines and hence could facilitate selection, mapping, cloning genes and QTL analysis.

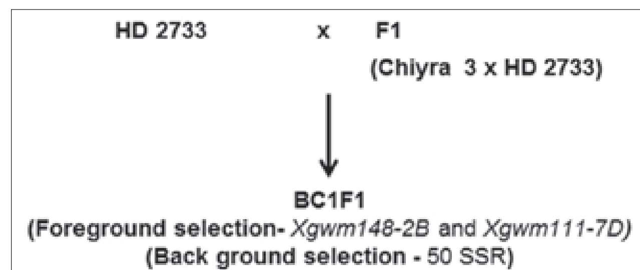


Fig. 4.19: Schematic presentation for development of BC_1F_1 seeds from different cross combinations.

Table 4.2: BC_1F_1 wheat populations with respect to the alleles amplified by the microsatellite primers i.e., *Xgwm148-2B* and *Xgwm111-7D*

BC1F1 population	No. of Plant BC_1F_1 lines	Name of primers (Xgwm148-2B)			Name of primers (Xgwm111-7D)		
		Susceptible type	Tolerant type	Heterozygous	Susceptible type	Tolerant type	Heterozy-gous
Chiyra x HD 2733	152	65	35	52	75	24	53
SW89-5422 x HD 2733	156	72	32	52	67	29	60
Shangai x PBW343	158	78	27	53	76	19	63
Shangai x HD 2733	155	56	30	67	72	20	63

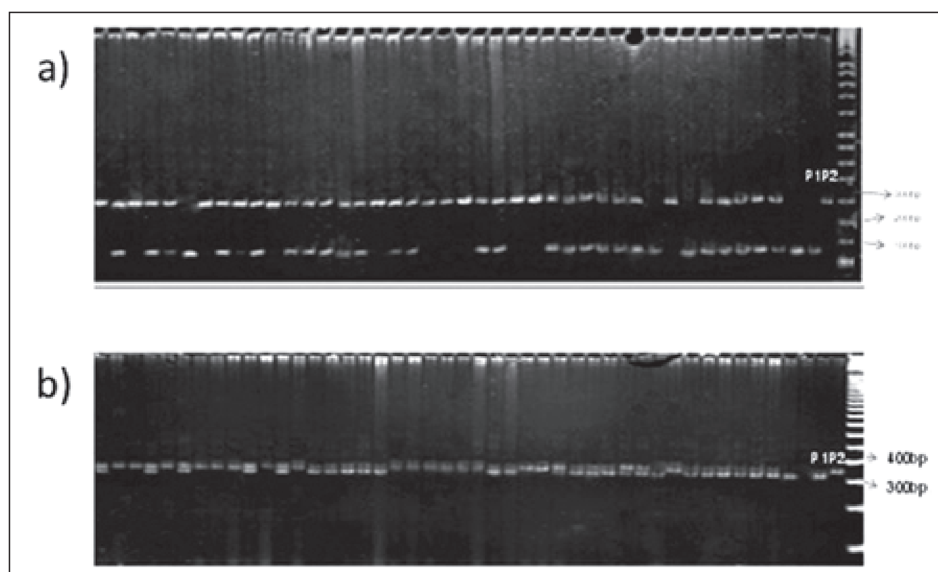


Fig. 4.20: Foreground selection of BC_1F_1 wheat population of Chiyra 3 x HD 2733 using SSR markers, a) *Xgwm148-2B* and b) *Xgwm111-7D*.

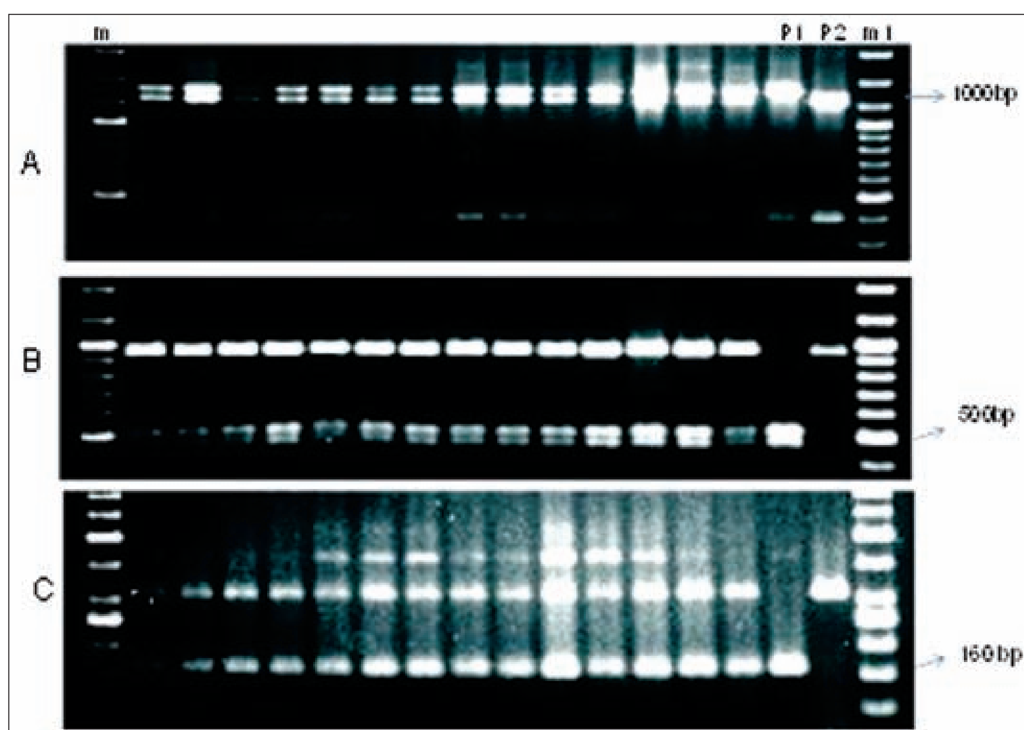


Fig. 4.21: Background selection of BC_1F_1 with PCR amplification of markers, A) *Xgwm63-7A*, B) *Xgwm136-1A*, and C) *Xgwm234-5B.* A) *P1* - Chiyra 3 and P2- HD 2733, B) P1 SW89-5422, P2 – HD2733, C) P1 - Shangai and P2- PBW343.

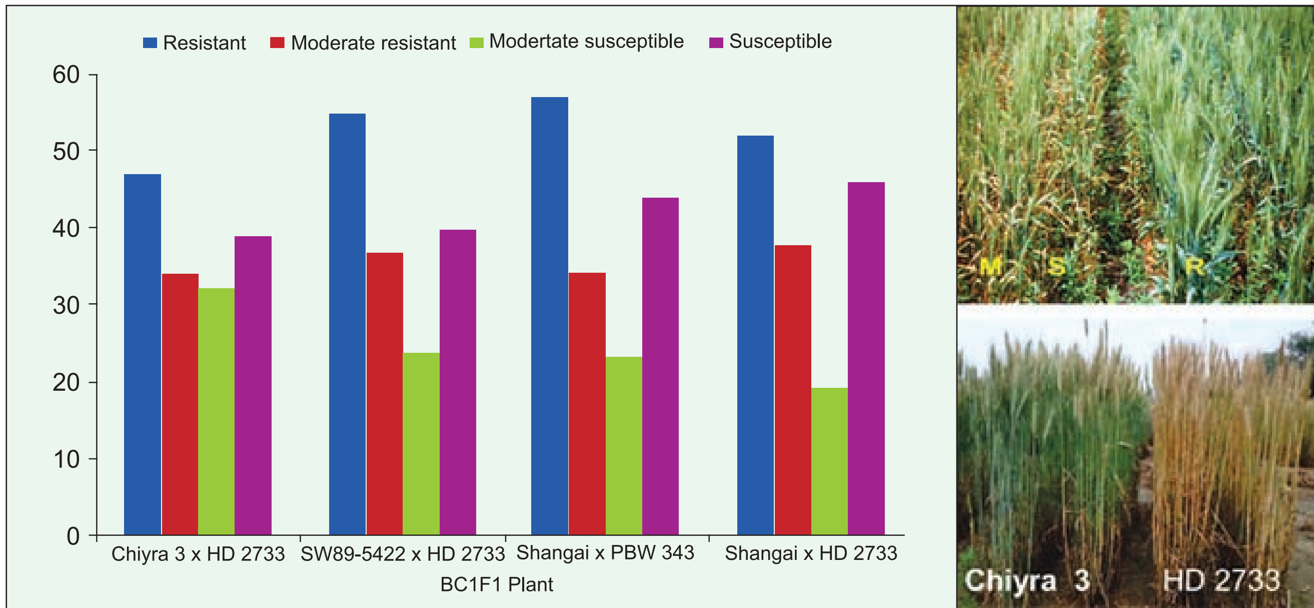


Fig. 4.22: Screenings of BC_1F_1 generation plants of the four crosses of wheat varieties through artificial inoculation show the reaction of resistant and susceptible plants against *Bipolaris* leaf spot.

5 IMPROVEMENT OF NITROGEN USE EFFICIENCY IN CEREAL CROPS

Nitrate starvation induced changes in nitrogen-responsive wheat genotypes

Morphological (with special emphasis on root system architecture) and biochemical responses (in terms of assay of the key enzymes involved in N assimilation) of two N-responsive wheat genotypes, Kalyansona (KS) and NP-890 (NP), at the seedling stage under nitrate-optimum and nitrate-starved conditions grown in hydroponics, were studied (Fig. 5.1). Total root size (TRS), primary root length (PRP), and first - and second - order lateral root numbers responded significantly under nitrate-starved condition (Fig. 5.2). Morphological parameters in terms of root and shoot length, and fresh and dry weight of roots and shoots were significant between N-optimum and N-starved condition for each genotypes (Fig. 5.3). Nitrate reductase (NR), glutamine synthatase (GS), and glutamate dehydrogenase (GDH) activity significantly decreased under N-starved condition. Glutamine oxoglutarate amino transferase (GOGAT) and pyruvate kinase (PK) activity was found to be genotype dependent (Fig. 5.4).

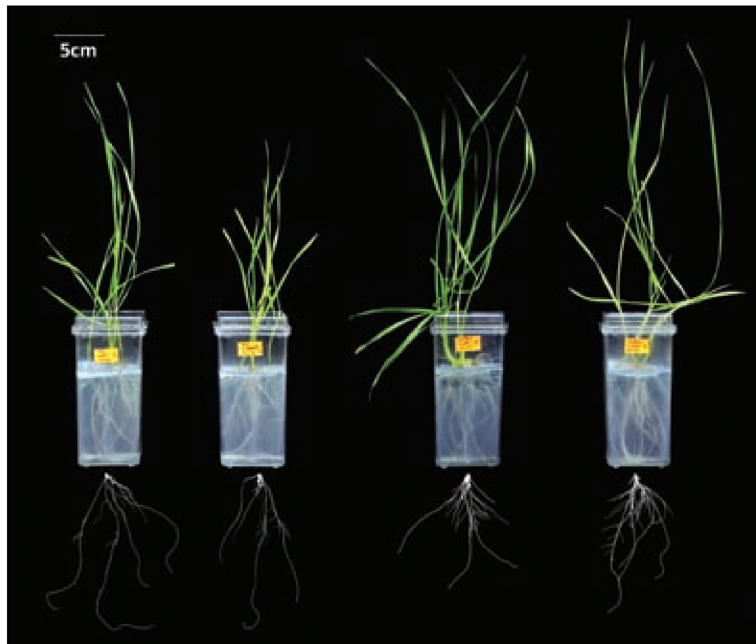


Fig. 5.1: Wheat seedlings of KS and NP growing in N-optimum and N-starved conditions in hydroponics.

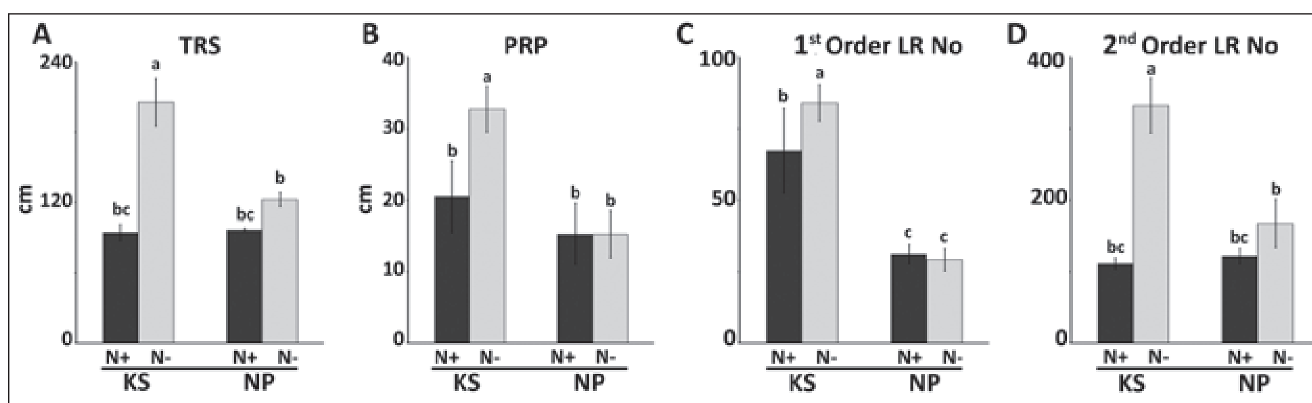


Fig. 5.2: Variation in TRS (A), PRP (B), first-order LR no. (C), and second-order LR no. (D) in KS and NP genotypes under different nitrate concentrations.

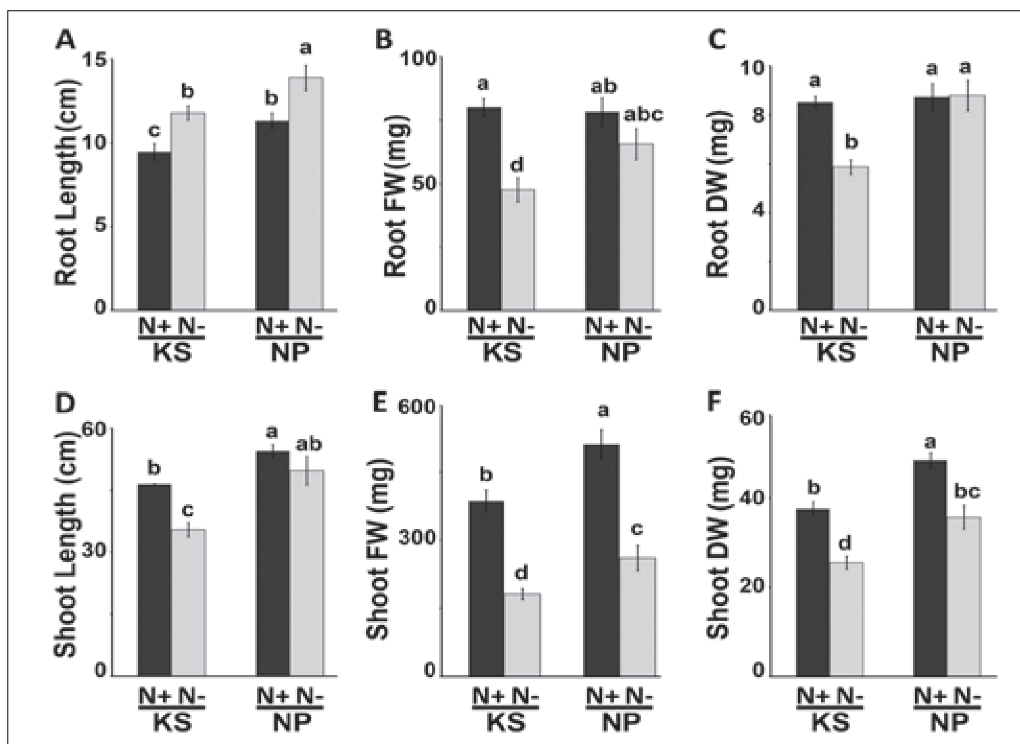


Fig. 5.3: Morphological variation of two wheat genotypes grown under N-optimum and N-starved conditions in hydroponics.

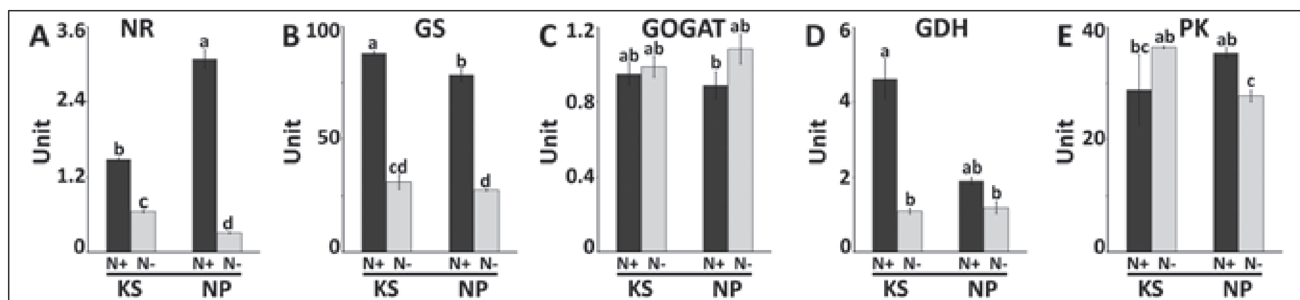


Fig. 5.4: Enzymatic assay of NR (A), GS (B), GOGAT (C), GDH (D), and PK (E) enzymes involved in nitrogen assimilation in KS and NP genotypes of wheat grown under N-optimum and N-starved conditions.

Root study of diverse wheat genotypes under N-starved conditions

Nitrogen use efficiency is comprised of N-uptake by the root and then assimilation, utilization, remobilization by the shoot. However, utilization is primarily dependent on available resources, i.e. amount of N-uptake by the root system. Root phenotype, mainly the architecture, and the transporters are key factors which determine the amount of nitrogen taken up by a genotype at different level of soil nitrogen. In the present study, wheat seedlings for nine diverse genotypes (in terms for NUE at field level experiment) were grown in vermiculite, perlite media with N-optimal and N-stressed condition, to study the root parameters including 'root system architecture' (RSA) and gene expression of some of the high as well as low affinity transporters.

Root Phenotyping

When root biomass was analysed, two of the genotypes HD-2967 and Karchia showed significant increase in root dry weight under N-stress. In general, shoot biomass decreased under N-stress (Fig. 5.5). This is mainly due to resource allocation under nutrient stress.

RSA parameters showed increase in root length for all the genotypes except Kharchia and HD-2967. Root length increased in combination with root diameters, projected area, surface area and root volume. This study reveals that mainly 1st order length contributed significantly towards the other parameters (Fig. 5.6 & 5.7).

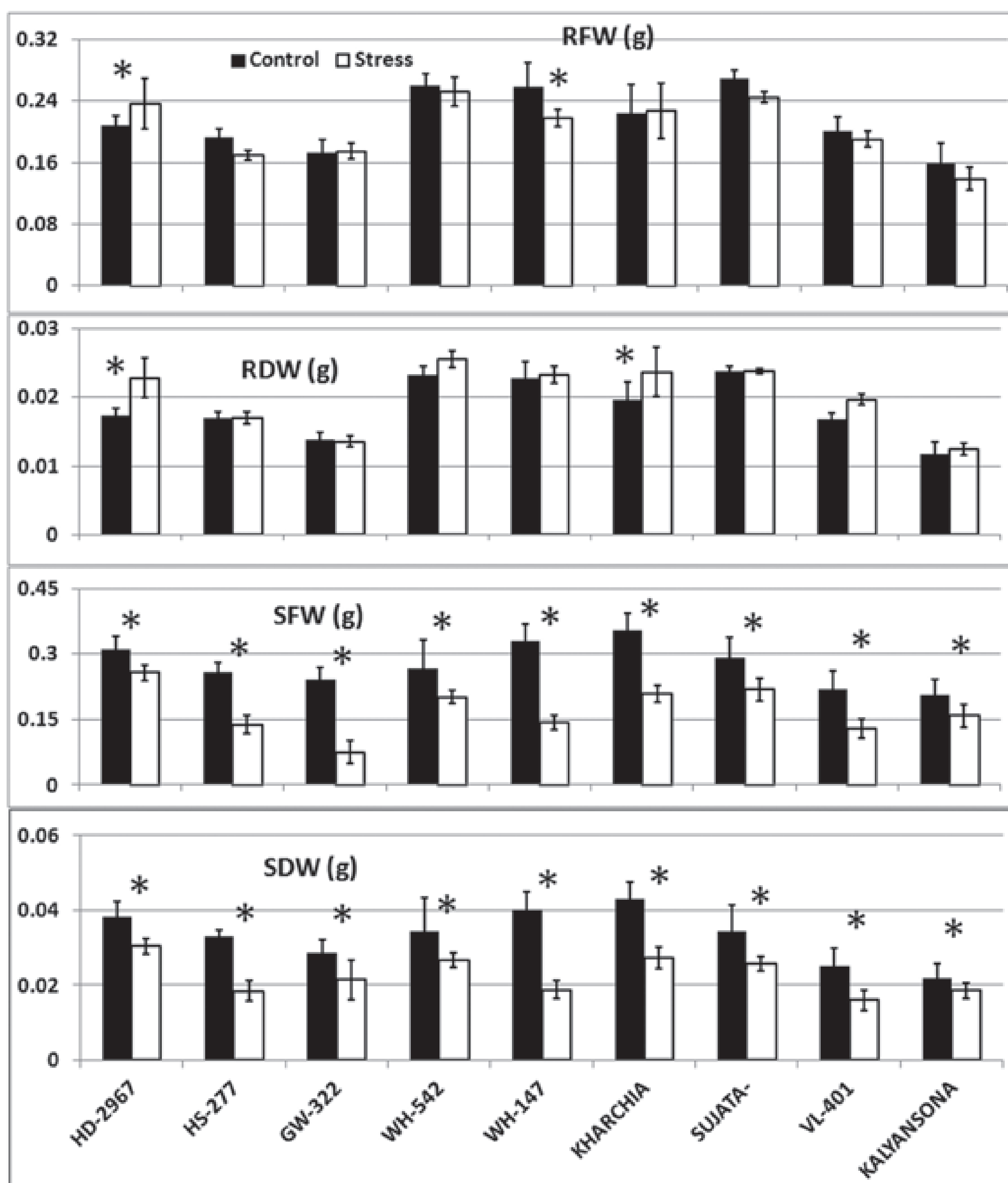


Fig. 5.5: Comparison of fresh and dry root and shoot biomass under both N optimum and N stress condition.

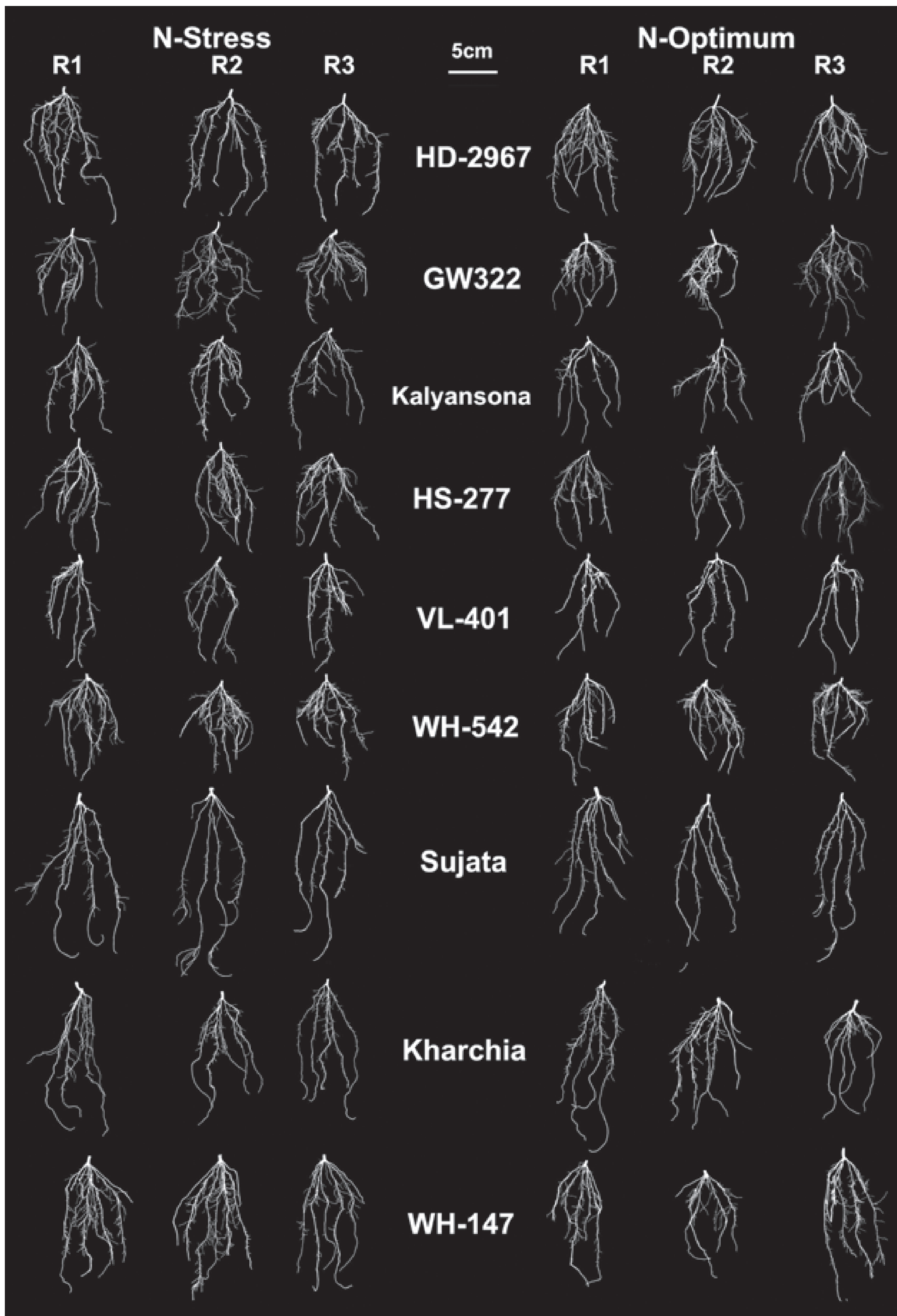


Fig. 5.6: Scanned pictures of RSA for all the genotypes under study with three replications.

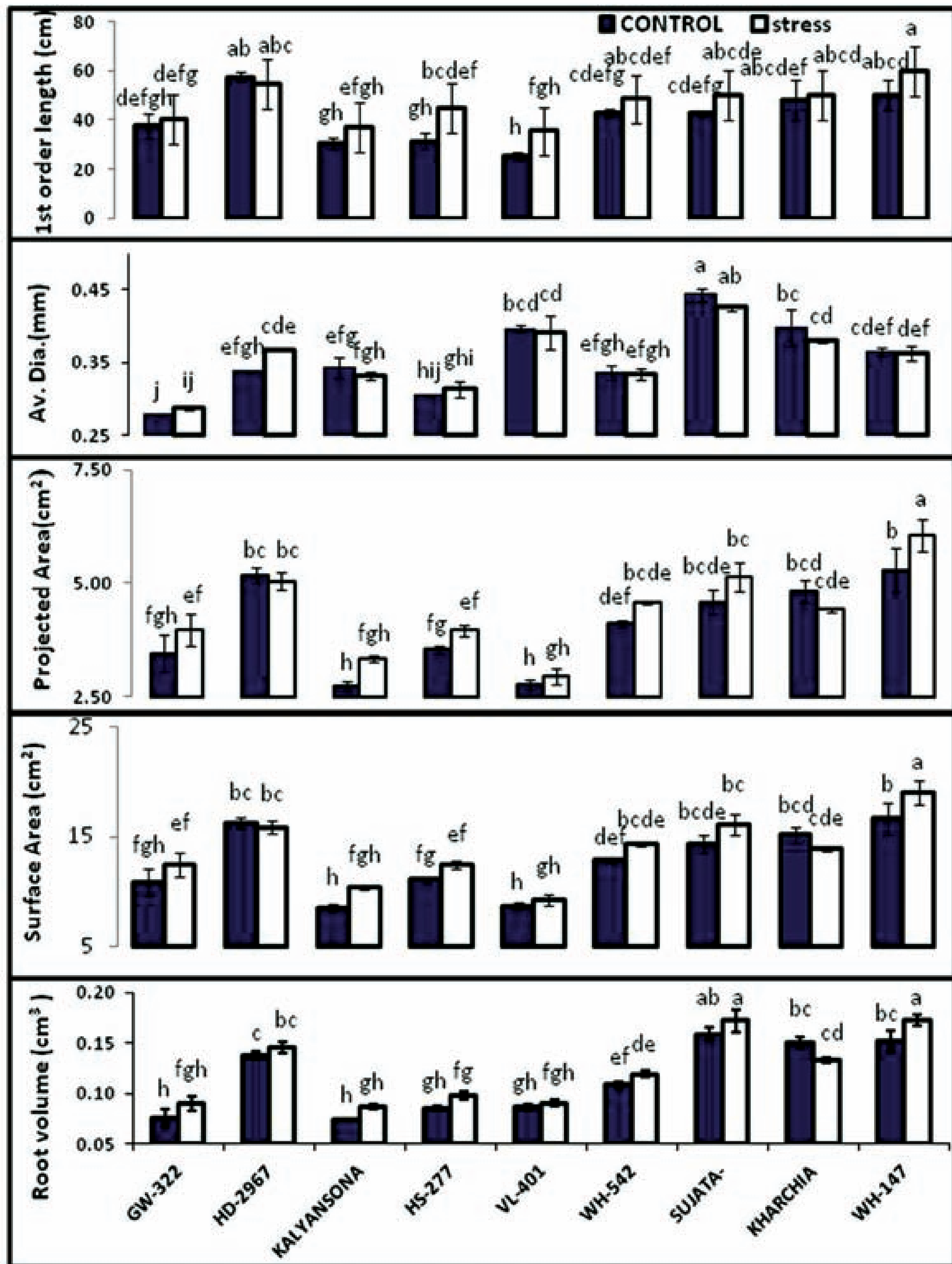


Fig. 5.7: 1st order root length, root diameter, projected area, surface area, and volume of diverse wheat genotypes.

In general, all the RSA parameters increased under N-stress in comparison to N-optimum condition except Forks and 2nd Order length, which decreased significantly. However, only seven parameters, namely length, projected area, surface area, root volume, total length, 1st order length and total root system, increased significantly (Fig. 5.8).

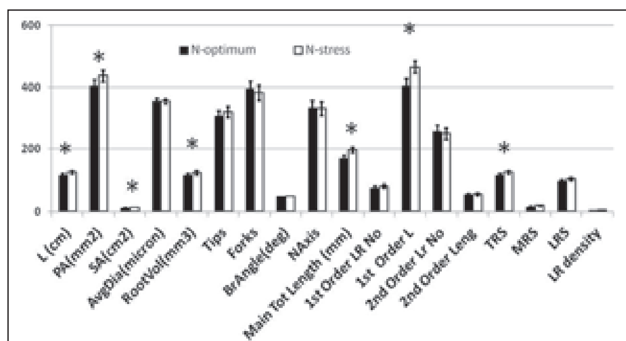


Fig. 5.8: Different parameters of RSA of wheat seedlings under N-stress.

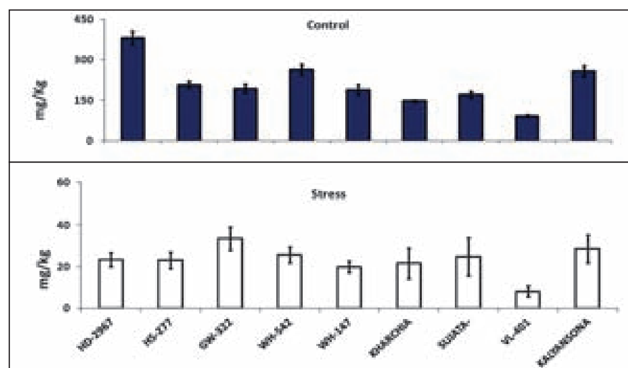


Fig. 5.9: Comparision of residual nitrate for diverse wheat genotypes in N-optimum and N-stress condition.

Residual nitrate

Residual nitrogen in the growing media (vermiculite/ perlite mixture) was measured to get an idea about the nitrate uptake by the different genotypes under the experimental condition (Fig. 5.9). HD2967 showed maximum residual nitrate followed by WH-542 and Kalyansona, and least in case of VL-401 under optimum condition. Under N-stress condition maximum residual nitrate was reported in GW-322 followed by Kalyansona and least in case of VL-401.

Gene expression of nitrate transporter

Nitrate transporters are classified into low and high affinity transporters. The low affinity transport system (LATS) is used preferentially at high external nitrate concentrations above 1 mM, while the high affinity transport system (HATS) works at low concentrations (1μM–1 mM). LATS is constitutive in nature and possibly has a signaling role to induce the expression of HATS and nitrate assimilatory genes, presumably playing a nutritional role only above a certain threshold. Kharchia, showed minimum change in expression, where as VL-401 and Kalyansona were distinctly different from the rest of the genotypes for LATS. Kharchia showed its distinct character by significantly down regulating HATS under N-stress condition (Fig. 5.10).

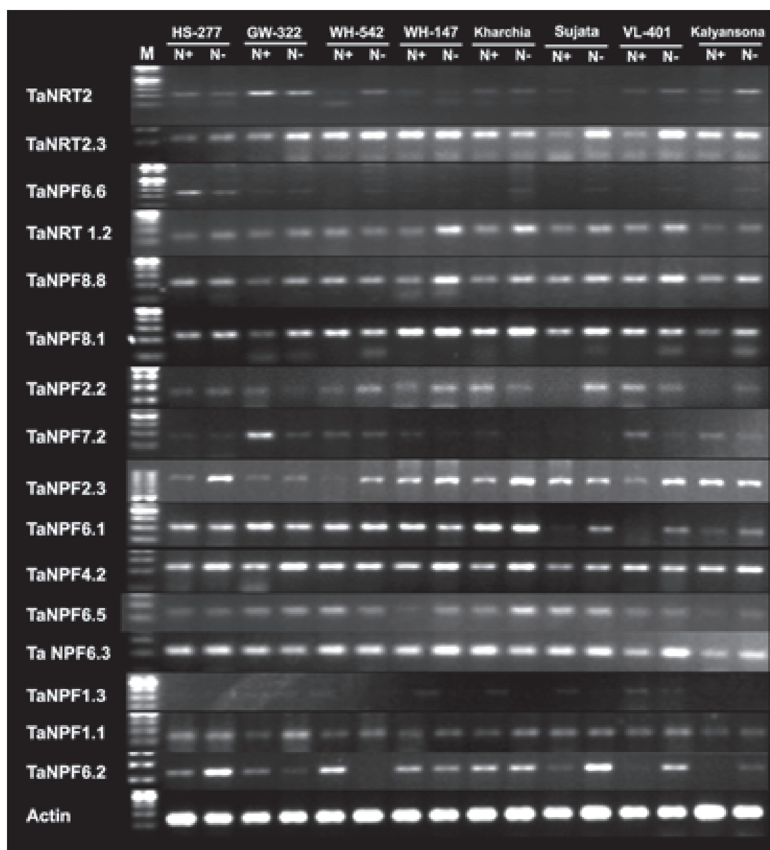


Fig. 5.10: Semi-quantitative PCR to study the comparative gene expression of different nitrate transporters in wheat seedling root tissues.

Effect of different induction systems on expression of high affinity nitrate transporter genes in wheat

In order to understand the contribution of high affinity nitrate transporter genes in nitrate uptake under nitrate limited conditions, the expression pattern of different high affinity nitrate transporters genes was studied in root tissue of wheat seedling (HD-2967) after induction in different level of nitrate concentration ranging from low to high external concentration under hydroponic condition. The effect of external carbon (sucrose) and nitrogen source (glutamine and asparagine; the end products of nitrate and ammonia assimilation) on gene expression was also studied. All high affinity nitrate transporter genes were invariably up-regulated under lower external nitrate concentration (0.2 mM, 0.02 mM, 0.04 mM) in root tissues as compared to non induced (NI) root tissues except HA3 at 4 mM, HA1 at 4 mM and 1 mM external nitrate condition which gets down-regulated than NI. The maximum up-regulation was observed in case of HA2 at 1 mM followed by HA3 at 0.2 mM, HA2 at 4 mM, HA1 at 0.2 mM external nitrate concentration in decreasing order of log fold change. As far as external C and N sources are concerned, sucrose in case of HA1 and HA3 was found to be stimulatory in up-regulation when added in 1 mM nitrate solution whereas it was inhibitory in case of HA2. Except in one case (glutamine in HA3 expression) both the amino acids (glutamine and asparagine) were inhibitory in up-regulation of all three high affinity transporter genes (Fig. 5.11).

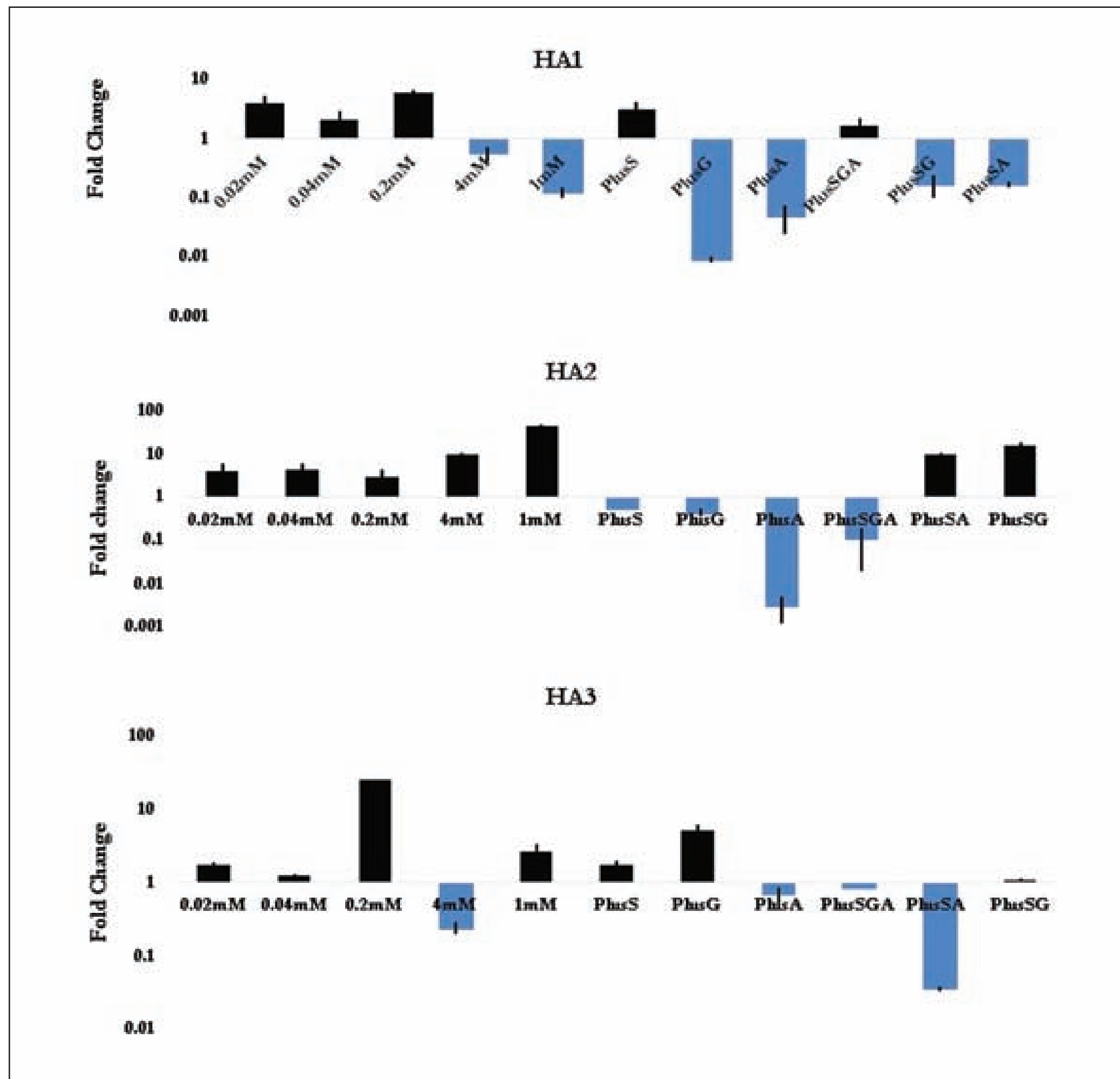


Fig. 5.11: Expression of high affinity nitrate transporter genes under nitrate induction.

6 BIOPROSPECTING OF NOVEL GENES AND PROMOTERS

Isolation and characterization of insecticidal genes from native *Bacillus thuringiensis* isolates

Bacillus thuringiensis (*Bt*) is an aerobic, spore-forming gram-positive bacterium that produces insecticidal crystal proteins (Cry) and vegetative insecticidal proteins (Vip), which have specific toxicity towards target insect pests of different insect orders. The *cry*- and *vip*-type genes have been deployed in *Bt*-transgenic plants for crop protection. Transgenic cotton expressing *cry1Ac* gene has shown good level of protection from *H. armigera*. The area under cultivation of transgenic crops has been increasing steadily. Since, there is possibility of development of resistance to currently deployed genes, novel insecticidal genes are required to delay the onset of development of resistance in target insects. *Bt* isolates have been recovered from diverse habitats in India and *cry*- and *vip*-type genes have been isolated.

PCR amplification of candidate insecticidal protein (ip) genes

PCR amplification of candidate *ip* genes, identified on different scaffolds in the data of Illumina sequencing of *Bt* isolates, was standardized with specially designed nested primers under different annealing conditions and 60^{o}C was found to be the optimum annealing temperature. Therefore, PCR amplification of six *ip* genes was carried out at this annealing temperature. PCR products corresponding to *ip5*, *ip9*, *ip11*, *ip12* and *ip18* genes were observed while no amplification was seen in case of *ip19* gene with pooled DNA sample of 36 *Bt* isolates as template.

Identification of *Bt* isolates carrying candidate *ip* genes

Identification of *Bt* isolates carrying candidate *ip* genes was done using 12 primary pooled samples of DNA from 6 *Bt* isolates each, named as A- F and 1- 6, and high fidelity polymerase at annealing temperature 60^{o}C (Fig. 6.1-6.5). The *ip9* gene was found to be most abundant, present in six *Bt* isolates, followed by *ip12* and *ip18* present in four *Bt* isolates each, while *ip5* was present in two and *ip11* was present in only one isolate (Table 6.1). *Bt* isolate SK855 was found to have three and isolates SK792 and SK850 showed the presence of two *ip* genes, while all other isolates had one *ip* gene each (Table 6.2).

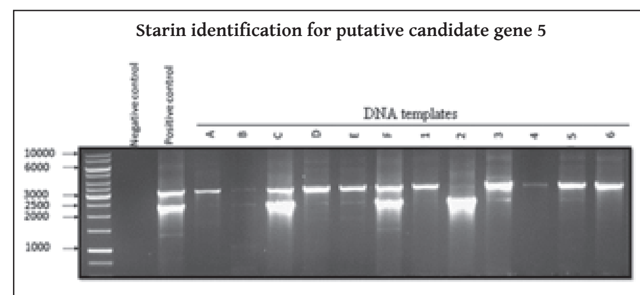


Fig. 6.1: Identification of *Bt* isolates carrying candidate *ip* gene 5 by PCR with primary pool DNA templates. Negative control: no template, Positive control: DNA pool from 36 *Bt* isolates. A-F and 1-6 are primary pool DNA samples from 6 *Bt* isolates each. Nested primers for candidate *ip* gene 5 were added to each PCR reaction. Expected gene size is 2390 bp. *Bt* isolates carrying candidate *ip* gene are C-2 (SK792) and F-2 (SK798). First lane is DNA marker, 1 Kb ladder (MBI Fermentas).

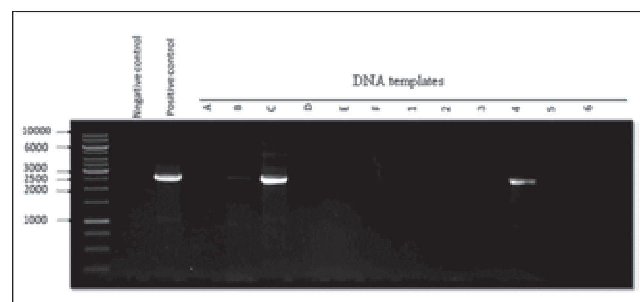


Fig. 6.2: Identification of *Bt* isolates carrying candidate *ip* gene *11* by PCR with primary pool DNA templates. Negative control: no template, Positive control: DNA pool from 36 *Bt* isolates. A-F and 1-6 are primary pool DNA samples from 6 *Bt* isolates each. Nested primers for candidate *ip* gene *11* were added to each PCR reaction. Expected gene size is 2748 bp. *Bt* isolate carrying candidate *ip* gene *11* is C-4 (SK855). First lane is DNA marker 1 Kb ladder (MBI Fermentas).

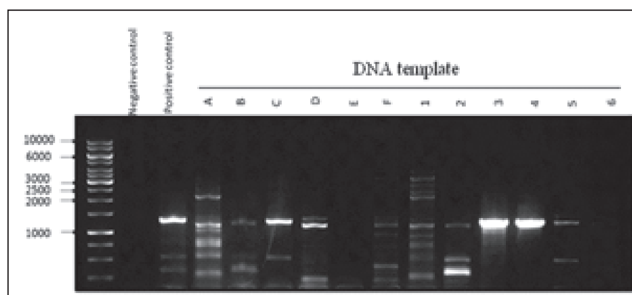


Fig. 6.3: Identification of *Bt* isolates carrying candidate *ip* gene *12* by PCR with primary pool DNA templates. Negative control: no template, Positive control: DNA pool from 36 *Bt* isolates. A-F and 1-6 are primary pool DNA samples from 6 *Bt* isolates each. Nested primers for candidate *ip* gene *12* were added to each PCR reaction. Expected gene size is 1346 bp. *Bt* isolates carrying candidate *ip* gene are C-3 (SK850), C-4 (SK855), D-3 (SK936) and D-4 (SK959). First lane is DNA marker 1 Kb ladder (MBI Fermentas).

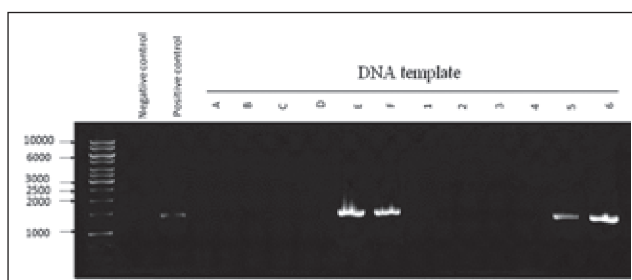


Fig. 6.4: Identification of *Bt* isolates carrying candidate *ip* gene *18* by PCR with primary pool DNA templates. Negative control: no template, Positive control: DNA pool from 36 *Bt* isolates. A-F and 1-6 are primary pool DNA samples from 6 *Bt* isolates each. Nested primers for candidate *ip* gene *18* were added to each PCR reaction. Expected gene size is 1392 bp. *Bt* isolates carrying candidate *ip* gene are E-5 (SK28), E-6 (SK82), F-5 (1069) and F-6 (SK56). First lane is DNA marker 1 Kb ladder (MBI Fermentas).

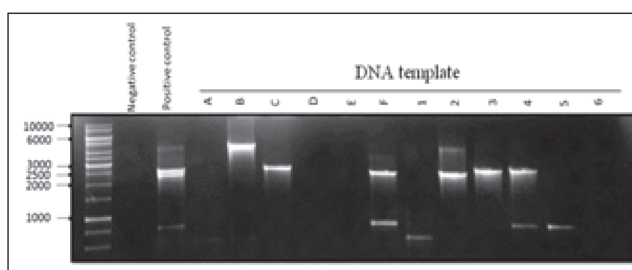


Fig. 6.5: Identification of *Bt* isolates carrying candidate *ip* gene *9* by PCR with primary pool DNA templates. Negative control: no template, Positive control: DNA pool from 36 *Bt* isolates. A-F and 1-6 are primary pool DNA samples from 6 *Bt* isolates each. Nested primers for candidate *ip* gene *9* were added to each PCR reaction. Expected gene size is 2530 bp. *Bt* isolates carrying candidate *ip* gene are C-2 (SK792), C-3 (SK850), C-4 (SK855), F-2 (SK798), F-3 (SK 1047) and F-4 (SK1067). First lane is DNA marker 1 Kb ladder (MBI Fermentas).

Table 6.1: PCR amplification of candidate *ip* genes with pooled DNA sample of 36 *Bt* isolates as template at an annealing temperature of 60 °C.

S. No	Candidate ip gene	PCR Product (bp)	*Bt* isolates
1	*ip5*	2390	SK792, SK798
2	*ip9*	2530	SK792, SK850, SK855, SK798, SK 1047, SK1067
3	*ip11*	2748	SK855
4	*ip12*	1346	SK850, SK855, SK936, SK959
5	*ip18*	1392	SK28, SK56, SK82, SK1069,

Table 6.2: Candidate *ip* genes profiles of *Bt* isolates

S. No.	Bt isolate	Source	Candidate ip genes profile
1	SK28	Soil, Cotton field, Malout, Punjab,	*ip18*
2	SK56	Soil, Orchard, Shimla, Himachal Pradesh	*ip18*
3	SK82	Grain dust, grain mill, Shimla, Himachal Pradesh	*ip18*
4	SK792	Chilli seeds, Warehouse, Guntur, Andhra Pradesh	*ip5, ip9*
5	SK798	Chilli seeds, Warehouse, Guntur, Andhra Pradesh	*ip9*
6	SK850	Soil, Wheat field, Burdwan, West Bengal	*ip9, ip12*
7	SK855	Soil, fallow land, Burdwan, West Bengal	*ip9, ip11, ip12*
8	SK936	Soil, Desert, location 3, Sriganganagar, Rajasthan	*ip12*
9	SK959	Soil, Desert, location 1, Sriganganagar, Rajasthan	*ip12*
10	SK1047	Soil, field, Baramulla, Jammu and Kashmir	*ip9*
11	SK1067	Soil, Maize field, Dungarpur, Rajasthan	*ip9*
12	SK1069	Soil, Maize field, Dungarpur, Rajasthan	*ip18*

PCR amplification of *ip5* and *ip11* genes from individual *Bt* isolates

The candidate *ip* genes were subsequently amplified using gene-specific primers from the individual *Bt* isolates that were identified to carry these genes. The *ip5* gene (2390bp) was amplified from *Bt* isolates SK792 and SK798 and *ip11* gene (2748 bp) was amplified from *Bt* isolate SK855 (Fig. 6.6).

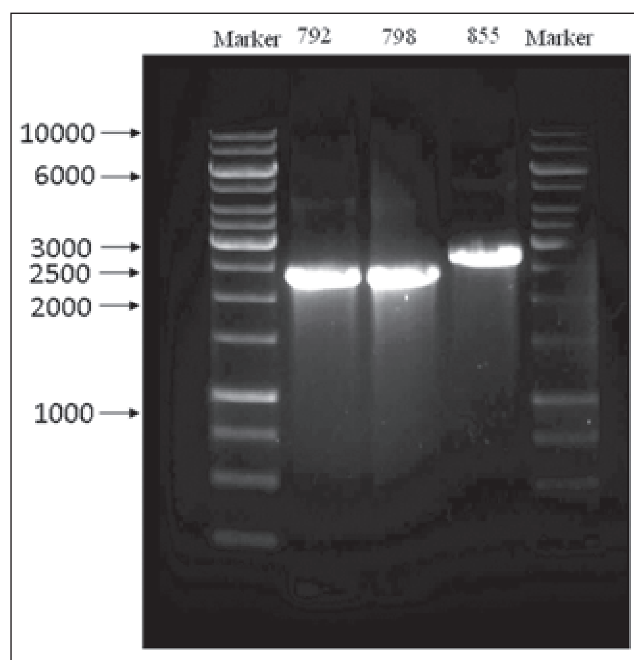


Fig. 6.6: PCR amplification of candidate *ip* gene 5 and gene 11 from individual *Bt* isolates carrying these genes. Lane M: 1 Kb ladder (MBI Fermentas). Lane 792, 798 and 855 correspond to plasmid DNA of SK792, SK798 and SK855, respectively, used as template with gene-specific primers in PCR reaction. Expected PCR product was observed in all three *Bt* isolates. First lane is DNA marker 1 kb ladder (MBI Fermentas).

Phylogenetic typing of *Bt* isolates by 16S ribosomal RNA sequencing

Four *Bt* isolates, from which *cry*-type genes have been isolated in our laboratory and have been found to be toxic to *H. armigera,* were confirmed by 16S rRNA sequencing (Table 6.3). Per cent toxicity of *Bt* isolates at 5 ppm concentration in laboratory bioassays with artificial diet using 30 laboratory raised neonates of *H. armigera* per treatment was determined earlier in Division of Entomology, ICAR-IARI, as part of a collaborative DBT project. *Bt* isolate SK798 was found to have highest toxicity. *Bt kurstaki* strain HD1 was used as a control.

Table 6.3: Native *Bt* isolates toxic towards *H. armigera*

S. No.	Bt Isolate	Source	Gene isolated	Toxicity (per cent)
1	SK798	Chilli seeds warehouse, Nallapadu, Guntur, Andhra Pradesh	*cry1Aa20*	46.43
2	SK793	Chilli seeds warehouse, Nallapadu, Guntur, Andhra Pradesh	*cry1Ac37, cry2Ab16*	17.85
3	SK958	Desert soil, Location 1, Khyaliwala, Sriganganagar, Rajasthan	*cry1Ac36*	14.28
4	SK758	Sorghum grain dust, Guntur, Andhra Pradesh	*cry2Af2*	10
5	HD1	BGSC, OSU, Columbus, USA	-	10

Characterization of root-knot nematode (*Meloidogyne incognita*) responsive and root-specific promoter (containing PIN domain) from *A. thaliana*

Root-knot nematodes (RKN) are obligate plant parasites, causing significant economic loss, that alter expression of host genes in order to establish and maintain their feeding sites in the roots of host plants. A nematode-responsive-root-specific gene (*AT1G26530*) was identified which expressed in roots of *A. thaliana* after nematode infection. Quantitative RT-PCR analysis of this gene revealed maximum (~2.58 fold) up-regulation at 21 days post inoculation of nematode (Fig. 6.7). A 1580 bp region upstream of the translational start site of *AT1G26530* was isolated and transformed into *Arabidopsis* through floral dip method. On analysis of *Arabidopsis* transgenic plants harbouring *AT1G26530*prm::*GUS* fusion, reporter gene expression was seen exclusively in galls after nematode inoculation. The strong GUS activity was observed at early stages of nematode infection, starting from 14 days and was sustained up to 30 days post inoculation (Fig. 6.8). Furthermore, 85 to 93 % galls exhibited GUS activity in the nematode feeding sites. The specificity of the activity of the *AT1G26530* promoter, in terms of nematode-responsiveness and root-specificity, makes it a suitable candidate to express dsRNA of nematode genes and engineer plants with resistance against root-knot nematodes using HD-RNAi technology.

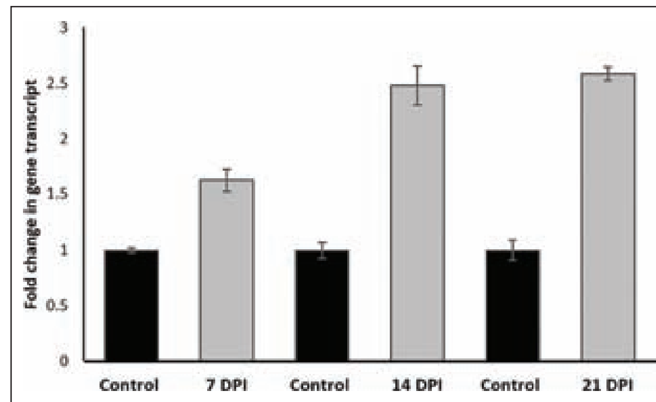


Fig. 6.7: Expression analysis of *AT1G26530* gene using qRT-PCR in uninfected (control) and *Meloidogyne incognita* infected *Arabidopsis* roots at 7, 14, and 21 dpi. *Ubiquitin* gene of *Arabidopsis* was used as a control. Three biological and three technical replicates were used for experimental as well for control (uninfected) conditions.

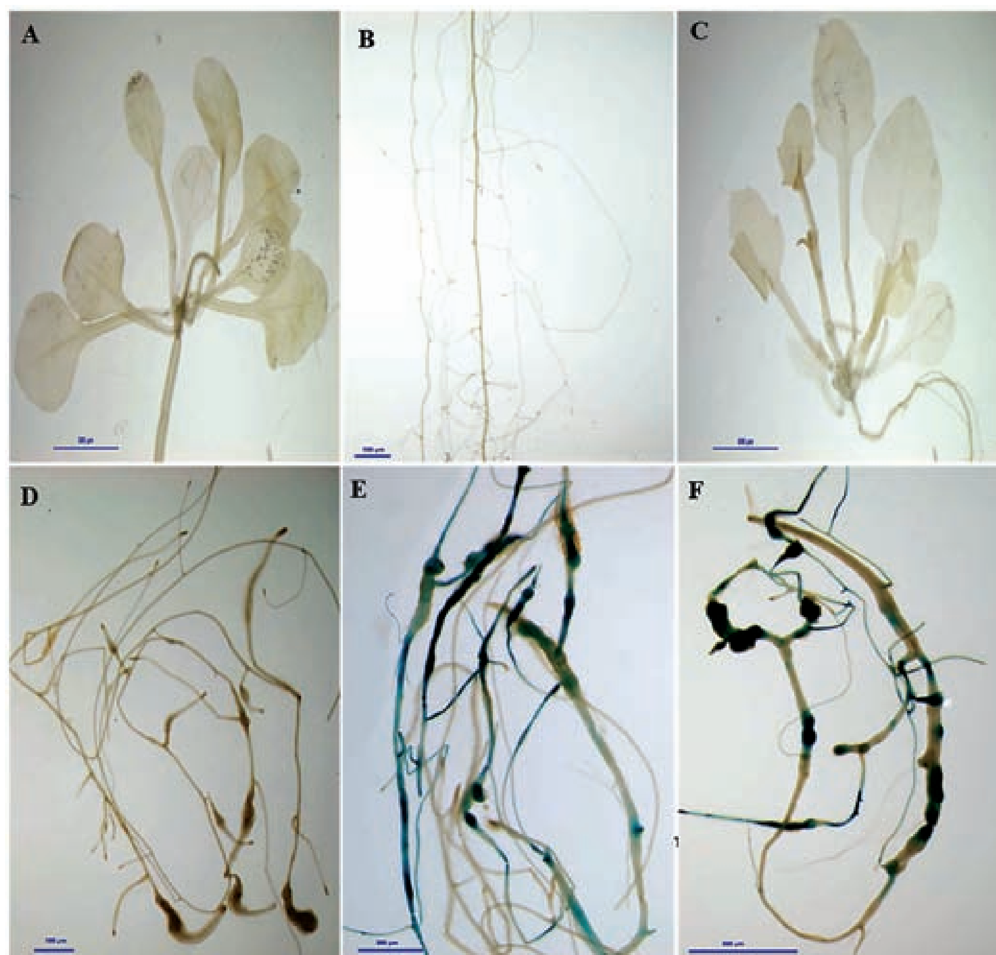


Fig. 6.8: Histochemical GUS analysis of *AT1G26530 prm-GUS* transgenic *Arabidopsis* plants. A) Aerial (shoot and leaves) parts of plant showing absence of GUS staining under control conditions. B) Roots of plant showing absence of GUS staining under control conditions. C) *Arabidopsis* plant 7 days after infection showing absence of GUS staining in either shoot or root. D) Roots of *Arabidopsis* plant 7 days after infection showing the presence of galls but no GUS staining. E) Roots of *Arabidopsis* plant 14 days after infection showing GUS staining in galls. F) Roots of *Arabidopsis* plant 21 days after infection showing GUS staining in galls.

7 HONORARY SCIENTISTS' PROJECTS

Maintenance, characterization and use of EMS induced mutants of upland variety Nagina22 for functional genomics in rice – Phase II (Prof. R. P. Sharma, INSA Honorary Scientist)

This multi-institutional project, sponsored by DBT for six years from 2007 to 2013, was sanctioned for the second phase in Nov 2015. A project meeting was conducted in 8 Jan 2016 at TNAU for finalizing the project activities and assigning responsibilities for phase-II (Fig. 7.1). The major activities for the phase II would be (a) to map a few promising mutants using MutMap approach; (b) to rejuvenate the entire mutant resource and its maintenance; (c) to introgress herbicide tolerance identified in the phase-I to six rice varieties; and (d) to explore the genetic basis of component traits of yield, drought tolerance, P use efficiency and photosynthetic efficiency in rice.

Mutant garden comprising of 600 genotypes was grown this year in the IARI farm for maintenance and data recording. The mutants have been grouped based on their height at maturity and days to 50% flowering into 6 groups. Three of these groups have been sent to TNAU, IARI and UAS-B. The rest of the three groups have been sent to NRRI, IIRR for multi-location data. This exercise is a pre-requisite for registration of mutants. The new batch of mutants generated has been distributed to IARI, NRCPB, UAS-B, TNAU and CRRI for rejuvenation and maintenance. The old batch of mutants is being maintained by NRRI. Specifications for establishing a mutant seed storage facility which would function as "National Repository of Rice EMS mutants" have been drawn.

Fig. 7.1: Nagina22 EMS mutagenesis Project Launch meet at TNAU, Coimbatore on 8th Jan 2016. From L to R: Dr . S Manonmani, Assoc. Prof, TNAU; Dr MK Kar, PS, NRRI, Cuttack; Dr MP Rajanna, Assoc. Prof, Mandiya, UAS-B; Dr S Gopalakrishnan, Sr Scientist, IARI; Prof. RP Sharma, INSA Honorary Scientist, NRCPB; Prof. S. Robin, Head, Dept of Rice, TNAU; Prof. N Sarla, National Prof, IIRR; Dr. SV Amitha CR Mithra, Scientist, NRCPB; Dr. Padmini Swain, PS, NRRI, Cuttack; Dr. AK Mukherjee, NRRI, Cuttack.

Study on the emerging issues on biosafety and socio-economic aspects related to genetically modified crops in India (Prof. K. R. Koundal, UGC Emeritus Scientist)

It is important that we should enhance farm productivity per unit land, water and capital without harming the ecosystem. During green revolution period, 87% of the improved seeds of wheat varieties were moved from farmers to farmers. Therefore, it is important to release genetically modified (GM) crops varieties (not necessarily hybrids) by public sector so that farmers can produce same and exchange seeds without paying any price. There is also a need to ensure that farmers got better remunerative price for their produce so that transgenic crops can bring economic benefits to farmers' community by reducing the input cost and saving crops from damage by biotic & abiotic stresses giving socio-economic benefit. More R&D of agricultural biotechnologies should be focused on the needs of smallholder farmer orphan crops and producers for their benefits. The development of transgenic crops expressing a variety of novel traits such as insect resistance, disease resistance, herbicide tolerance, hybrid production, improved oil quality and post harvest technology etc. have led to large scale cultivation of GM crops globally. In recent years, development of transgenic crops has led to large scale cultivation of GM crops which currently occupy 179.7 million hectares (2015) on a global scale in 28 countries. India became the highest cotton producer

in the world, with much of the success attributed to *Bt* cotton hybrids. India in 2015 continued to be the largest biotech cotton country in the world with 11.6 million hectares planted by 7.7 million small farmers with an adoption rate of 95%, similar to 2014. Thus, the application of plant biotechnology will go a long way for enhancing the crop productivity.

In democratic societies, public perceptions can either promote or hamper commercial adoption of new technologies and is seen as an important component in scientific and technological development. Biotechnology has diverse stakeholders, differing in their knowledge and requirements. There are several constraints and apprehensions regarding biosafety of human health and environmental safety of GM food crops. Therefore, strategy for promoting public awareness should provide specific 'information capsules for each of the identified target groups. The DBT and BCIL (Biotech Consortium India Limited) have been conducting trainings and workshops to create awareness among the different stakeholders in the country. They have identified target groups such as farmers, consumers, social groups, entrepreneurs, exporters, scientists, extension agents, managers and policy makers and also specific information capsules. However, an inter-ministerial collaboration should be strengthened to disseminate these capsules to the target groups. Recommendation of biotechnological techniques and products including release for transgenic crops should be based on both biosafety and value of cultivation and use. There is need to ensure that the guidelines on GMOs are well advertised to increase public awareness in different languages and that technology is well received. It is important that the decision making processes are totally transparent and take all shades of stakeholders into confidence. There is a need for inclusion of women, especially women farmers in trait selection and decision making in GM crops, greater involvement of women in farmers association, equal access to women for biotech resources and GM crop cultivation. A participatory approach is required if the biotechnological products are to be accepted by the farmers and consumers. Knowledge sharing and communicating with active participation of the public is very important component for commercialization of GM crops.

The socio-economic impact of GM crops should include the overall cost and benefit analysis, and impact of seed price and IPRs involved. A famous crusader against GM crops, Mark Lynas, has now reversed his GM stand as unscientific and untenable and given growing evidences that the GM crops are potential savior of human race. Similarly, Patrick Moore, a former co-founder and 15 years leader of Green Peace has founded Allow Golden Rice Society for golden rice campaign. In India, policy makers hailed *Bt* cotton as a success story. Based on Indian study Agriculture Ministry had in 2010 concluded that cultivation of *Bt* cotton had resulted in 31% increase in yield, 39% decrease in pesticide usage and more than 80% increase in profitability. However, recently there have been cases of *Bt* cotton failure in Panjab and other states in India due to white flies attack. This aspect requires detailed study. A study conducted by Klumper and Quaim (2014), Department of Agricultural Economics, Rural Development, George-August University Goetting in Germany published a Meta- analysis on impact of GM crops mainly soybean, maize and cotton as yield, pesticide use and farmer profit. The analysis highlights robust evidence of GM crops benefits based on 147 global studies during the last 20 years (1995-2014) examined and combined data from number of sources, studies field trials, research papers & statistical analysis. On average, this Meta -analysis showed that GM technology adoption has reduced chemical pesticides use by 37%, increased crop yield by 27% and increased farmers' benefits by 68% for all the farmers having different size of land holdings. Yield gains and pesticides reductions are larger for insect resistant crops than for herbicide tolerant crops. These studies should be treated as definitive and conclusive findings by the scientists and policy experts who have often caught in the GM debate. The government's transgenic development policy should be promotional and not inhibitory to the growth/development and commercialization of GM crops. In order to avoid IPR issue, it is important to isolate the genes and promoters from our own germplasms which can be expressed in crop plants for strengthening the development of transgenics resistant to biotic and abiotic stresses and post harvest losses. The future course of Indian agriculture will depend on the farmers' acceptance

or reservations for the adoption of biotechnological applications.

Iron availability affects phosphate deficiency-mediated responses, and evidences of cross talk with auxin and zinc in *Arabidopsis* (Dr. Ajay Jain, Ramalingaswamy Fellow)

Deciphering phosphate deficiency-mediated temporal effects on different root traits in rice grown in a modified hydroponic system.

Phosphate (Pi), an essential macronutrient for growth and development of plant, is often limiting in soils. Plants have evolved an array of adaptive strategies including modulation of root system architecture (RSA) for optimal acquisition of Pi. In rice, a major staple food, RSA is complex and comprises embryonically developed primary and seminal roots and post-embryonically developed adventitious and lateral roots. Earlier studies have used variant hydroponic systems for documenting the effects of Pi deficiency largely on primary root growth. Here, we report the temporal effects of Pi deficiency in rice genotype MI48 on 15 ontogenetically distinct root traits (Fig. 7.2) by using easy-to-assemble and economically viable modified hydroponic system (Fig. 7.3). Effects of Pi deprivation became evident after 7 days-treatments on eight different root traits (Fig. 7.4). The effects of Pi deprivation for 7 days were also evident on 14 different root traits of rice genotype Nagina 22 (N22). There were genotypic differences in the responses of primary root growth along with lateral roots on it and the number and length of seminal and adventitious roots. Notably though, there were attenuating effects of Pi deficiency on the lateral roots on seminal and adventitious roots and total root length in both these genotypes. The study thus revealed both differential and comparable effects of Pi deficiency on different root traits in these genotypes. Pi deficiency also triggered reduction in Pi content and induction of several Pi starvation-responsive (PSR) genes in roots of MI48 (Fig. 7.5 and 7.6). Together, the analyses validated the fidelity of this modified hydroponic system for documenting Pi deficiency-mediated effects not only on different traits of RSA but also on physiological and molecular responses.

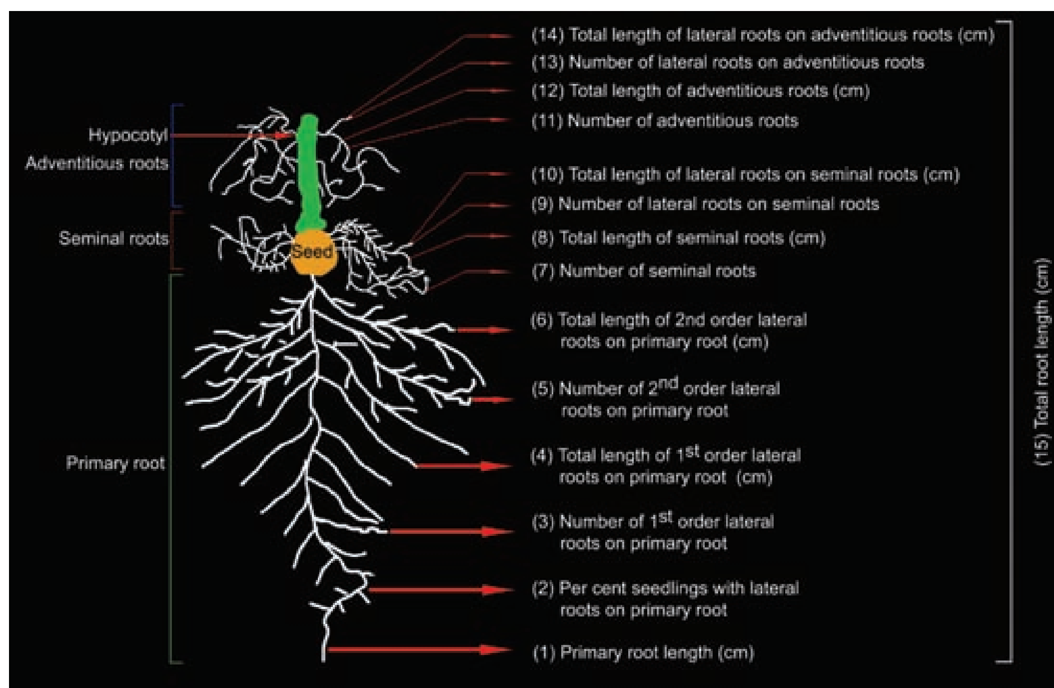


Fig. 7.2: Schematic overview of rice RSA. Temporal effects of Pi deficiency was quantified on developmental responses of 15 roots traits comprising primary, seminal and adventitious roots and lateral roots on each of them.

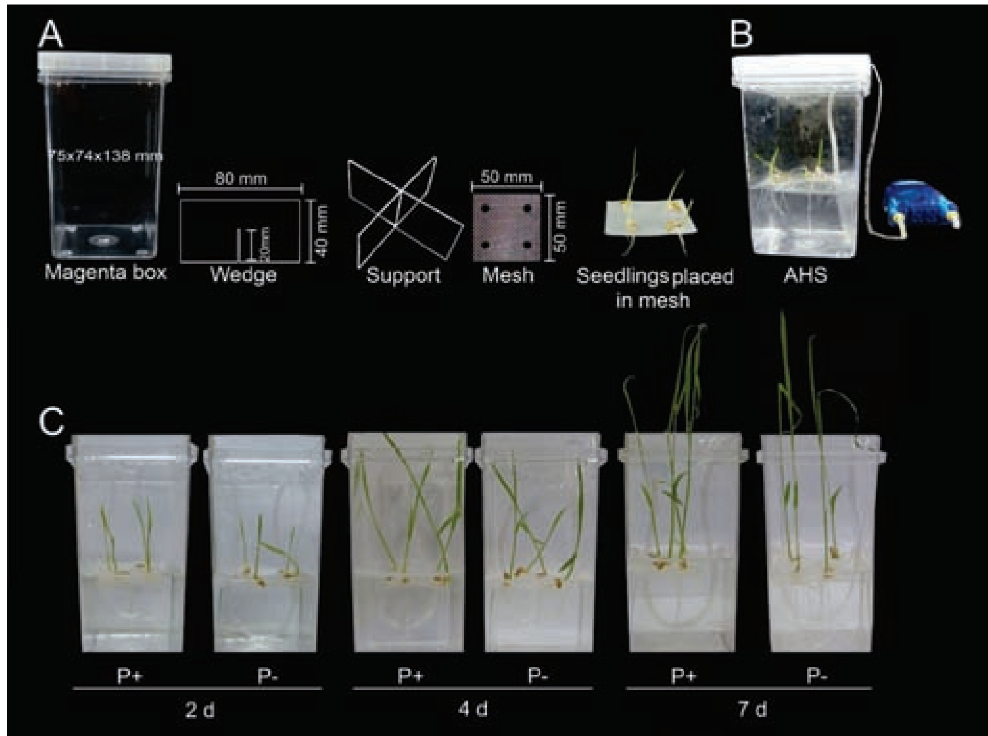


Fig. 7.3: Modified hydroponic system. (A) Modified hydroponic system made of autoclavable Magenta box, polycarbonate wedge support, polypropylene mesh and germinated rice seedlings placed on the mesh with radicle traversing through the hole punched around its perimeter. (B) Complete aerated hydroponic system (AHS). (C) Seedlings were grown in AHS under P+ (0.3 mM NaH_2PO_4) and P- (0 mM NaH_2PO_4) conditions for 2, 4, and 7 days.

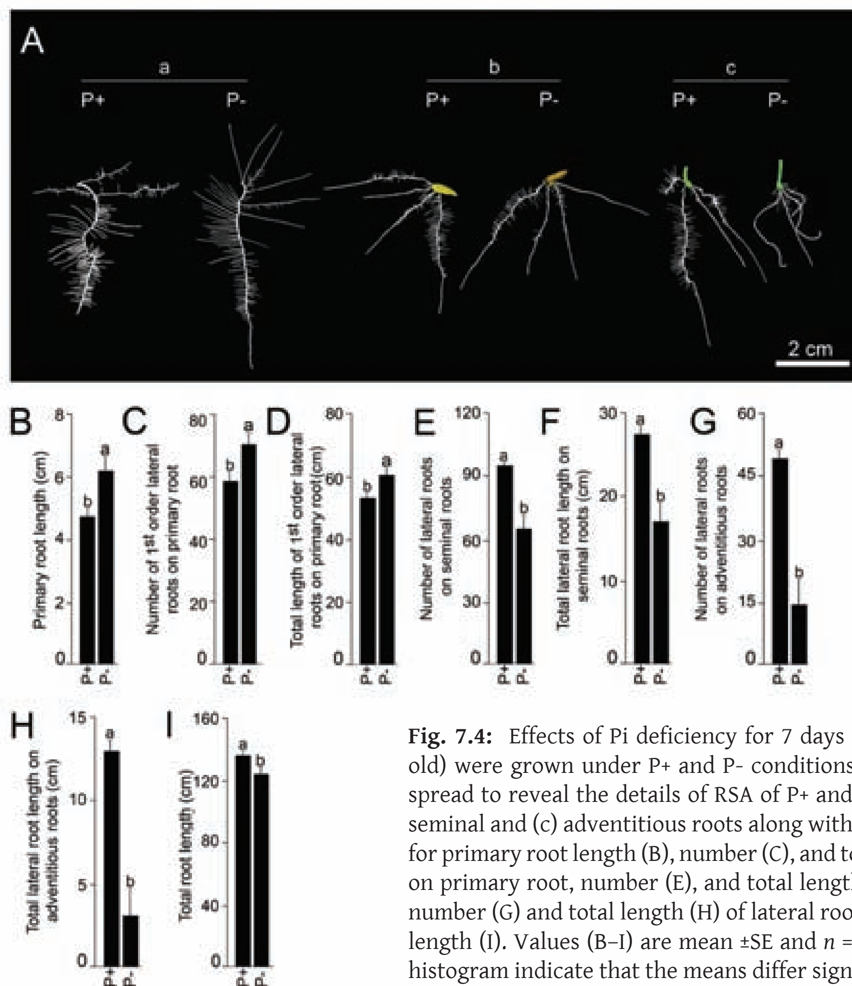


Fig. 7.4: Effects of Pi deficiency for 7 days on MI48 RSA. MI48 seedlings (4-days-old) were grown under P+ and P- conditions for 7 days. (A) Harvested roots were spread to reveal the details of RSA of P+ and P- seedlings showing (a) primary, (b) seminal and (c) adventitious roots along with their lateral roots. Data are presented for primary root length (B), number (C), and total length (D) of 1st order lateral roots on primary root, number (E), and total length (F) of lateral roots on seminal roots, number (G) and total length (H) of lateral roots on adventitious roots and total root length (I). Values (B–I) are mean ±SE and *n* = 12 replicates. Different letters on the histogram indicate that the means differ significantly ($P < 005$).

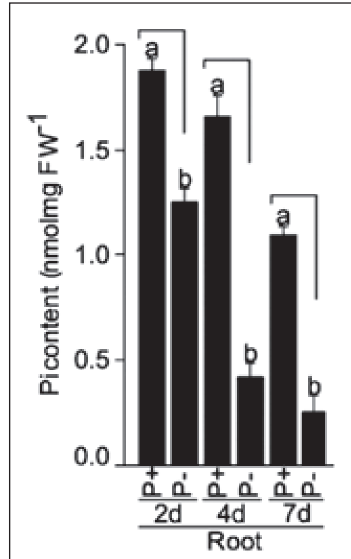


Fig. 7.5: Effects of Pi deficiency on soluble Pi content. MI48 seedlings (4-days-old) were grown under P+ and P- conditions for 2, 4, and 7 days. Roots were harvested for determining soluble Pi content. Values are mean ± SE and *n* = 12 replicates. Different letters on the histogram indicate that the means differ significantly (*P* < 005).

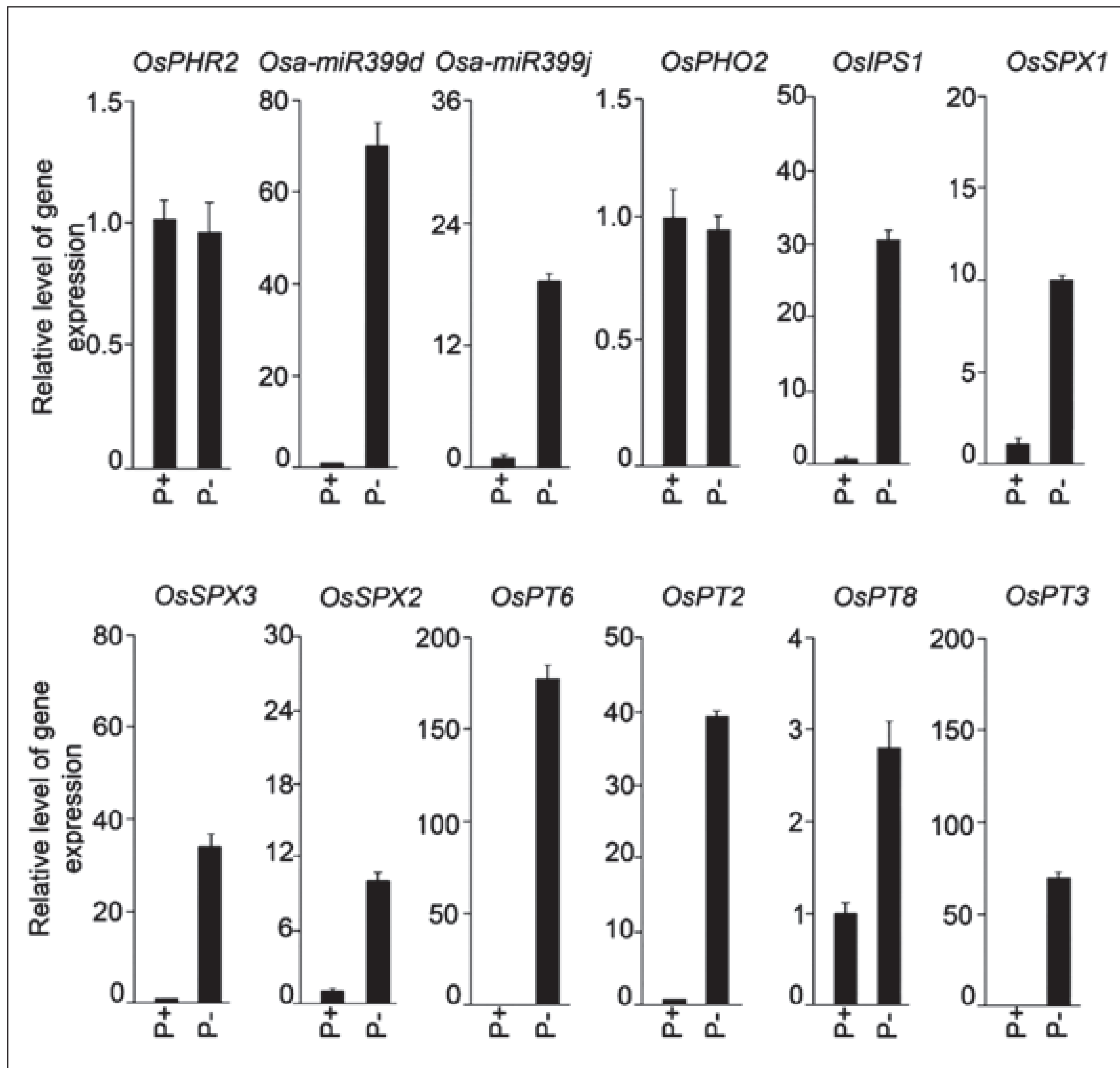


Fig. 7.6: Effects of Pi deficiency-mediated responses of genes involved in Pi homeostasis. Real-time PCR analysis of relative expression levels in roots of MI48 seedlings grown under P+ and P- conditions for 7 days. *OsRubQ1* was used as an internal control. Data presented are means of six technical replicates ±SE.

Arabidopsis MYB-related HHO2 exerts regulatory influence on a subset of root traits and genes governing phosphate homeostasis.

Phosphate (Pi), an essential macronutrient required for growth and development of plants, is often limiting in soils. Pi deficiency modulates the expression of Pi starvation-responsive (PSR) genes including transcription factors (TFs). Here, we elucidated the role of MYB-related TF *HYPERSENSITIVITY TO LOW PHOSPHATE-ELICITED PRIMARY ROOT SHORTENING1 HOMOLOG2* (*HHO2*, *At1g68670*) in regulating Pi acquisition and signaling in *A. thaliana*. *HHO2* was specifically and significantly induced in different tissues in response to Pi deprivation (Fig. 7.7A). Transgenic seedlings expressing *35S::GFP::HHO2* confirmed the localization of HHO2 to the nucleus (Fig. 7.7 B, C). Knockout mutants of *HHO2* (Fig. 7.8 A, B**)** showed significant reductions in Pi content of different tissues (Fig. 7.8 C**)** and number and length of first-and higher-order lateral roots (Fig. 7.8 D). Whereas, *HHO2*-overexpressing lines exhibited augmented lateral root development, enhanced Pi uptake rate and

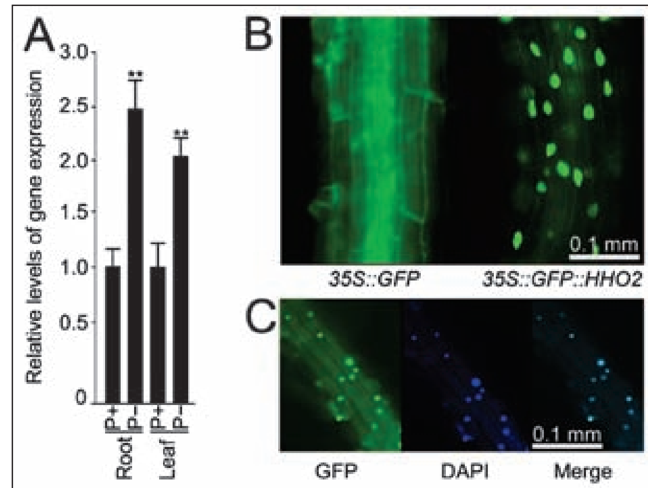


Fig. 7.7: Pi deficiency-induced expression of *HHO2* and nuclear localization of its fusion protein. (A) Wild-type plants were grown hydroponically in modified Hoagland's nutrient solution for 7 d and transferred to P+ (250 µM Pi) and P- (0 µM Pi) for 7 d. Relative expression levels of *HHO2* in root and leaf was determined by qRT-PCR. Asterisks on the histogram indicate means that differ significantly (***P* < 0.001). (B and C) Wild-type plants transformed with empty vector *35S::GFP* and *35S::GFP::HHO2* were grown on vertically-oriented agar-solidified plates containing one-half-strength MS for 5 d. (B) Microscopic images of root cells of empty vector *35S::GFP* (left panel) and *35S::GFP::HHO2* (right panel) showing nuclear localization of GFP::HHO2 fusion protein. (C) Microscopic images of root cells of *35S::GFP::HHO2* transgenic plants stained with nuclear-specific dye DAPI showing GFP fluorescence (left panel), nuclear staining by DAPI (center panel) and their merged images.

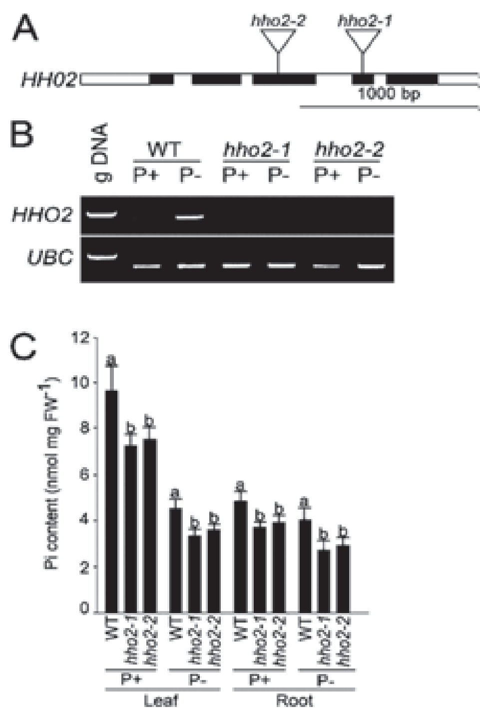


Fig. 7.8: Knockout mutants of *HHO2* showed attenuated Pi content and lateral root development. Schematic representation of *HHO2* indicating location of T-DNA inserts (triangles) in *hho2-1* and *hho2-2*. White and black boxes represent untranslated regions and exons, respectively. (B) RT-PCR analysis for qualitative assessment of *HHO2* amplification in wild-type and mutants (*hho2-1* and *hho2-2*). Wild-type genomic DNA (gDNA) and *UBC* (*At5g25760*) were used as indicators of gDNA contamination, if any, during cDNA synthesis and loading on the gel, respectively. (C) Data are presented for Pi contents of wild-type and mutants in leaf and root of seedlings grown on agar plates. Values (*n* = 5 replicates of 20 plants each) are means ± SE and different letters on histograms indicate that the means differ significantly (*P* < 0.05). (D) Lateral roots of wild-type and mutants, grown under P+ and P- conditions for 7 d on vertically oriented agar-solidified plates, were spread for revealing architectural details and are representative of 10-12 seedlings each.

higher Pi content in leaf compared with wild-type (Fig. 7.9 A-F). Expression levels of PSR genes involved in Pi sensing and signaling in mutant and overexpressors were differentially regulated as compared with wild-type (Fig. 7.10). Attenuation in the expression of *HHO2* in *phr1* mutant suggested a likely influence of PHR1 in *HHO2*-mediated regulation of subset of traits governing Pi homeostasis (Fig. 7.11).

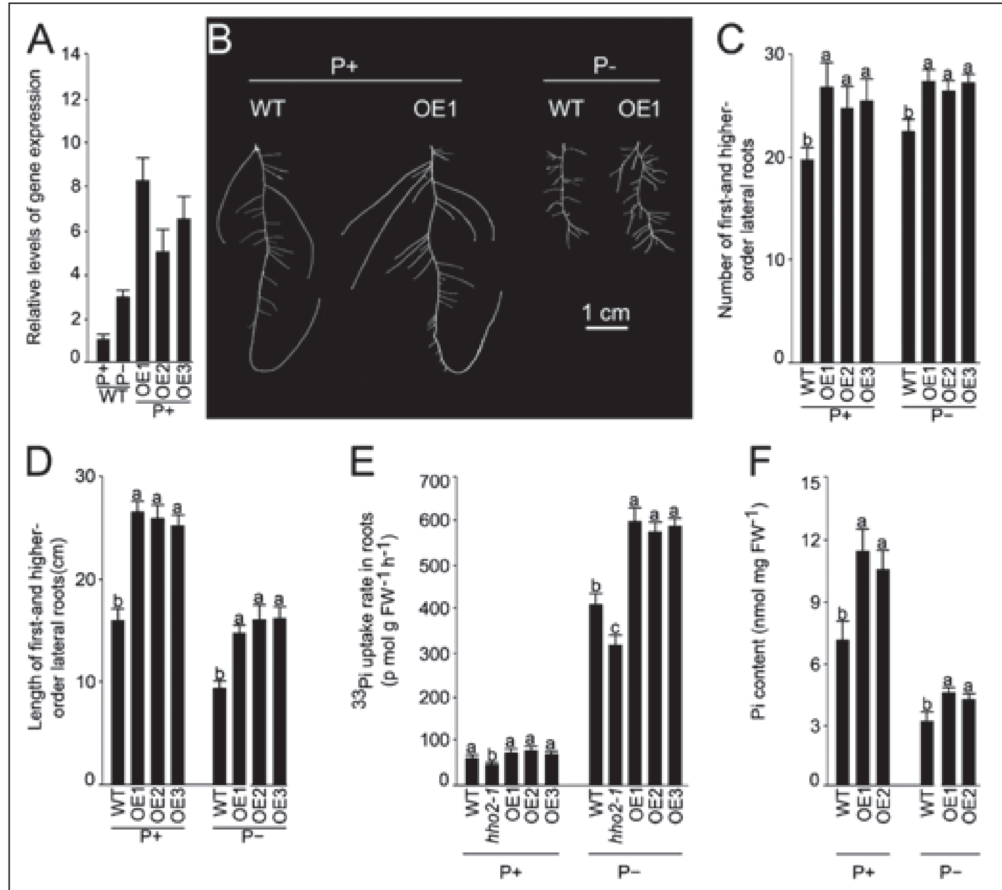


Fig. 7.9: Overexpression of *HHO2* triggered accentuated lateral root development, increased root Pi uptake rate and Pi content. (A-D and F) Wild-type and *HHO2* overexpressing transgenic plants (OE1-OE3) were grown on vertically oriented agar-solidified P+ and P- media for 7 d. (A) qRT-PCR analysis for determining relative expression levels of *HHO2* in wild-type and overexpressing seedlings. (B) Lateral roots of wild-type and OE1 seedlings grown under P+ and P- conditions were spread for revealing architectural details of root system and are representative of 12 seedlings each. Data are presented for (C) number and (D) total length of first-and higher-order lateral roots. (E) ^{33}Pi uptake rate in roots. Five-day-old seedlings of wild-type, *hho2-1* and OE1-OE3 were grown hydroponically in P+ and P- media for 7d and then transferred to respective media supplemented with ^{33}Pi for 2 h. Roots were analyzed for ^{33}Pi uptake rate. (F) Pi content in leaf. Values (A-D, n = 12; E and F, n = 4 replicates of 20-25 seedlings each) are means ± SE and different letters on histograms indicate that the means differ significantly ($P < 0.05$).

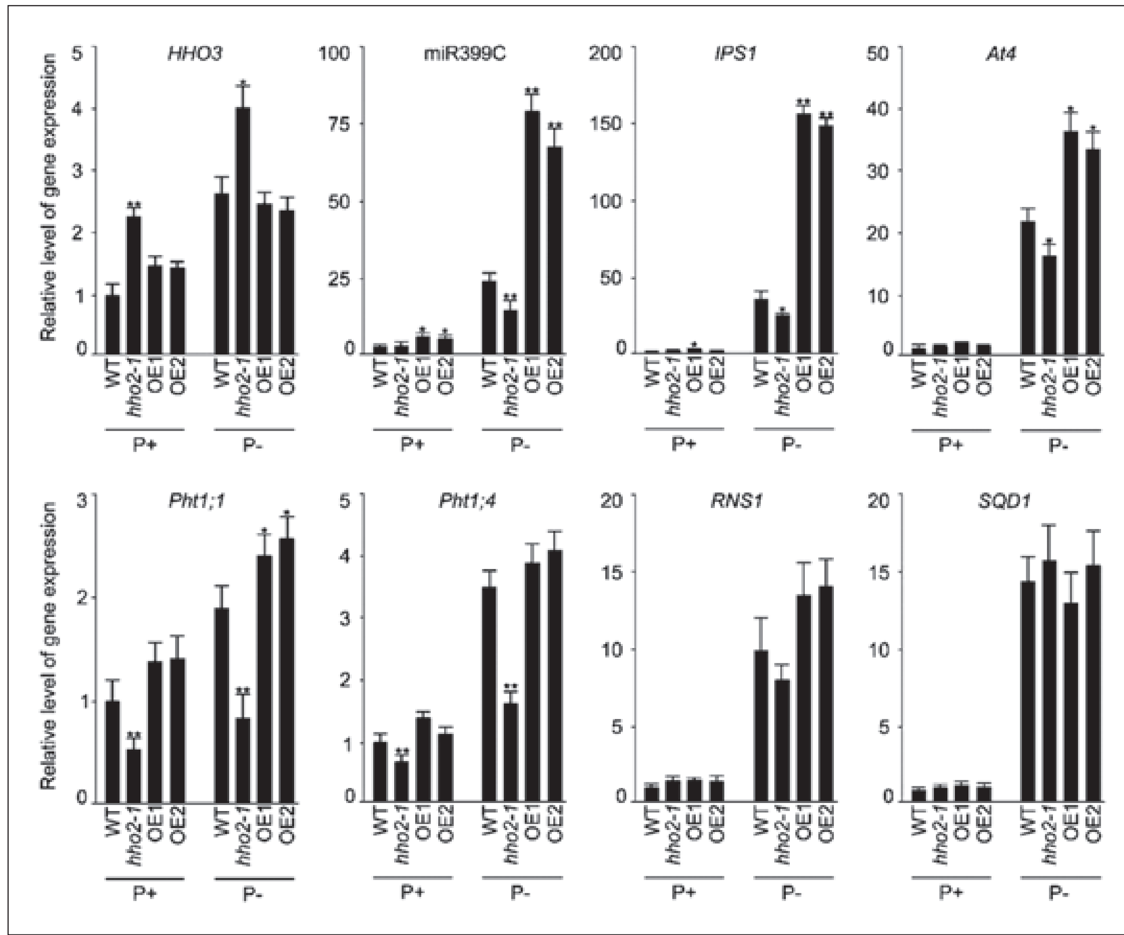


Fig. 7.10: Loss-of-function mutation and overexpression of *HHO2* differentially affects the expression of some of the PSR genes. Relative expression levels of PSR genes in P+ and P- seedlings as determined by qRT-PCR. Asterisks on the histogram indicate means that differ significantly (*$P < 0.05$; **$P < 0.001$).

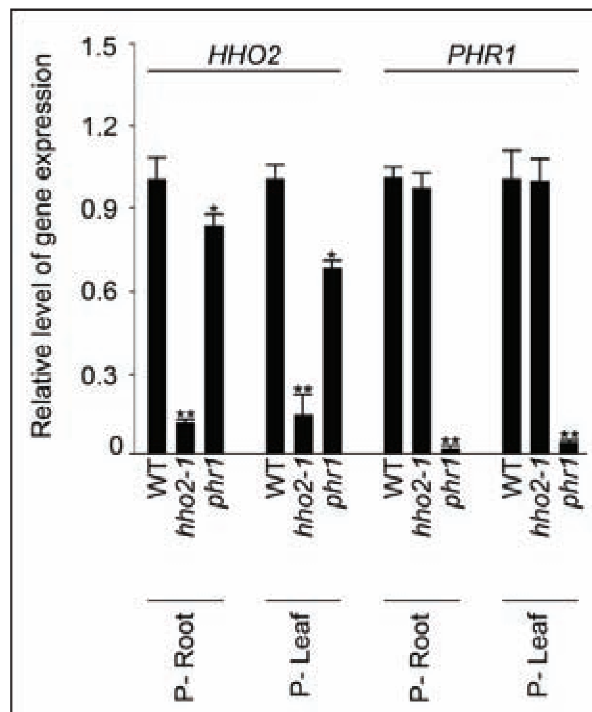


Fig. 7.11: Mutation in *PHR1* attenuates the expression of *HHO2* under Pi-deprived condition. Wild-type, *hho2-1* and *phr1* were grown on vertically oriented agar-solidified P- medium for 7 d. Relative expression levels of *HHO2* and *PHR1* in leaf and root of Pi-deprived seedlings as determined by qRT-PCR. Asterisks on the histogram indicate means that differ significantly (*$P < 0.05$; **$P < 0.001$).

Do non-symbiotic nitrogen fixing soil bacteria contribute to nitrogen nutrition of wheat seedlings? (Prof. Hiren K. Das, INSA Honorary Scientist)

Inoculation of wheat seeds by different wild type species of the nitrogen fixing soil bacteria *Azotobacter* usually results in about 8% to 10% enhancement of yield of wheat grain. It is not certain if this enhancement is due to biological reduction of nitrogen by the bacteria, or due to mobilization of unavailable nitrogen in the soil, or due to the plant growth substances elaborated by *Azotobacter.* Experiment was conducted to settle this issue by sowing wheat seeds into pots containing an inert solid medium (vermiculite and perlite) that did not contain any nitrogen or any other nutrient. Negative control pots had Hoagland's medium devoid of any nitrogen after sowing wheat seeds (HD2967). In the positive control pots complete Hoagland's medium (which contained fixed nitrogen) was added after sowing wheat seeds. In the experimental pots in which wheat seeds were inoculated with *Azotobacter chroococcum* CBD15, Hoagland's medium devoid of any nitrogen was added. Ten seeds were sown per pot and five pots were there per treatment. The number of seedlings alive and not withered was periodically counted (Fig. 7.12). The height of the seedlings was also measured periodically (Fig. 7.13).

In the positive control pots, where complete Hoagland's medium (which contained nitrogen) was applied after sowing wheat seeds, 46 seeds germinated and all the 46 seedlings survived and thrived well throughout the duration of the experimental period (Fig. 7.12). These seedlings grew vigorusly (Fig. 7.13). In the negative control pots, where Hoagland's medium devoid of any nitrogen was applied after sowing wheat seeds, 48 seeds germinated, but by seven days after sowing, only 41 seedlings survived, which dwindled to only 19 seedlings alive at eighteen days after sowing. The growth of these seedlings was the worst (Fig. 7.13). In the experimental pots, where wheat seeds that were inoculated with *A. chroococcum* CBD15, but Hoagland's medium devoid of any nitrogen was applied, 46 seeds germinated and all the seedlings thrived after seven days of sowing. Thirty four seedlings survived after eighteen days of sowing (Fig. 7.12). The growth of these seedlings was better than that of negative control pots (Fig. 7.13).

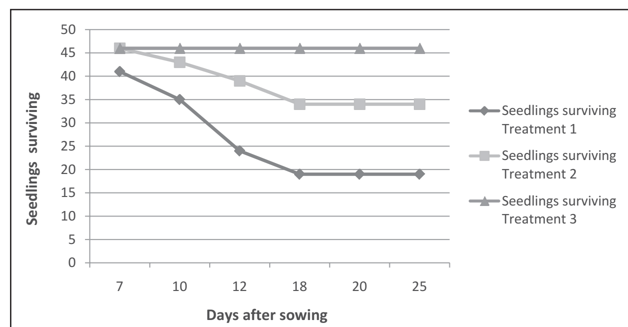


Fig. 7.12: Wheat seedlings surviving and not withered. Wheat seeds (HD2967) were sown in pots containing sterile Vermiculite – Perlite. Treatment 1 (♦): Seeds were not inoculated with any *Azotobacter.* Sterile Hoagland's medium devoid of any nitrogen was added as nutrient. Treatment 2 (■): Seeds were inoculated with *Azotobacter chroococcum* CBD15. Sterile Hoagland's medium devoid of any nitrogen was added as nutrient. Treatment 3 (▲): Seeds were not inoculated with any *Azotobacter.* Sterile complete Hoagland's medium (containing fixed nitrogen) was added as nutrient.

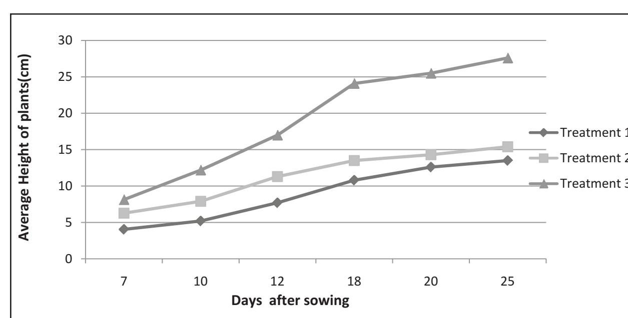
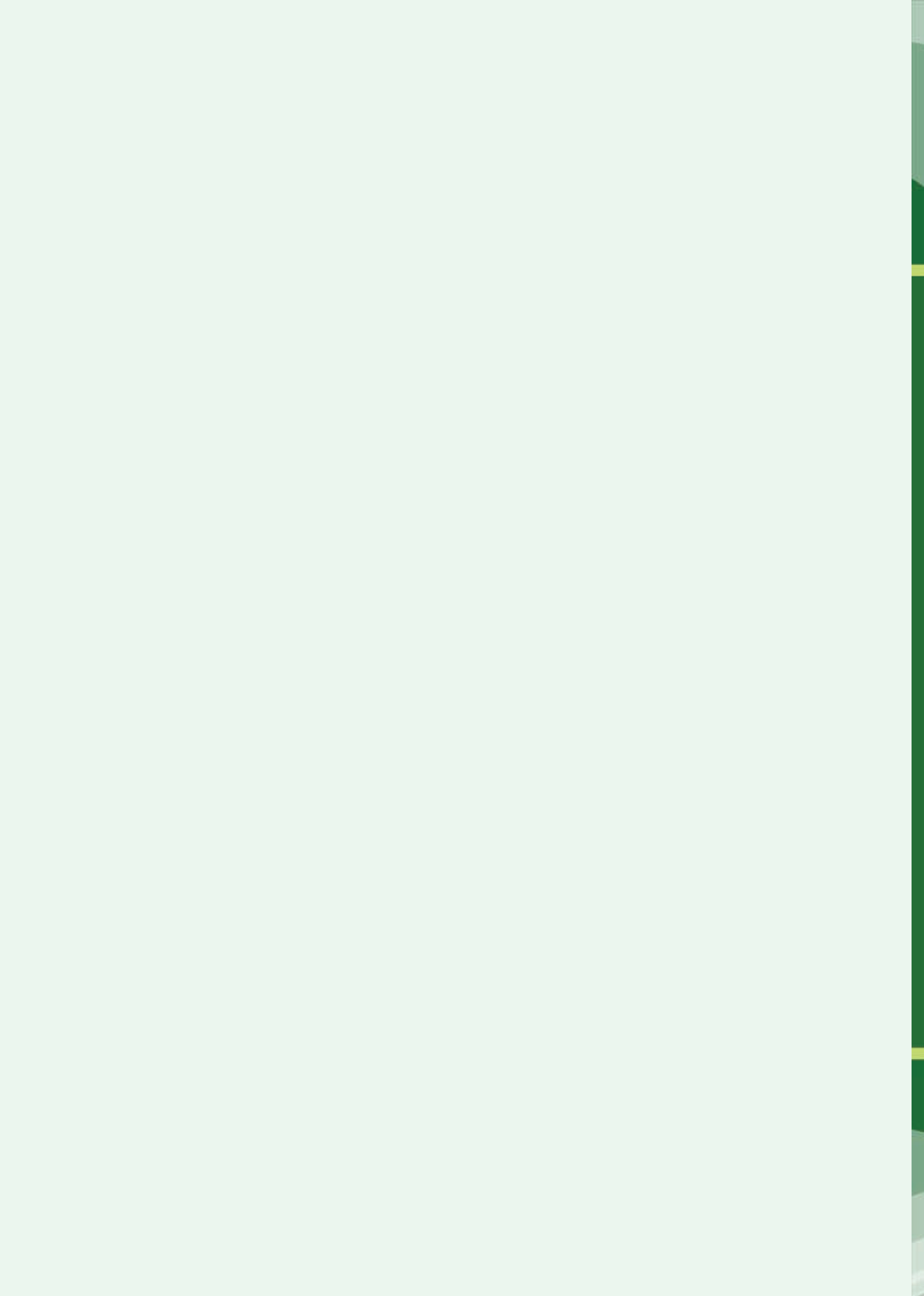


Fig. 7.13: Average height (cm) of wheat plants. Wheat seeds (HD2967) were sown in pots containing sterile Vermiculite – Perlite. Treatment 1 (♦): The seeds were not inoculated with any *Azotobacter.* Sterile Hoagland's medium devoid of any nitrogen was added as nutrient. Treatment 2 (■): The seeds were inoculated with *A. chroococcum* CBD15. Sterile Hoagland's medium devoid of any nitrogen was added as nutrient. Treatment 3 (▲): The seeds were not inoculated with any *Azotobacter.* Sterile complete Hoagland's medium (containing fixed nitrogen) was added as nutrient.

Since there was no nitrogen in vermiculite or perlite, there was no question of unavailable nitrogen in the medium being made available by *Azotobacter.* Again since there was no nitrogen in vermiculite or perlite, any incremental growth of seedlings inoculated with *Azotobacter* can not be because of growth substances elaborated by *Azotobacter.* The growth substances can not enhance growth of the seedlings in the absence of fixed nitrogen supply. It was concluded that beneficial effect of inoculation of wheat seeds with the nitrogen fixing soil bacteria, *A. chroococcum* CBD15, was very likely due to fixed nitrogen being made available by reduction of atmospheric nitrogen.



Institutional Activities

- Human Resource Development
- Personnel
- Training and Capacity Building
- Other Activities
- Recruitments/Promotions/Retirements
- 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 26 Ph. D. and 12 M. Sc. students are registered in this discipline. Seven Ph. D. and six M. Sc. students were awarded with Doctoral and Master's degrees, respectively, in the previous year. Dr. Siddanna Savadi has won the prestigious 'IARI Gold Medal' for Ph. D. Students enrolled in the discipline of Molecular Biology and Biotechnology for their post graduate studies in the Academic Session 2015-16 are listed below-

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
Ph. D.			
1	9862	Mr. Chandra Prakash	Dr. N. K. Singh
2	10013	Mr. Ravi Prakash Saini	Dr. T. R. Sharma
3	10018	Mr. Rajendra Prasad Meena	Dr. Jasdeep C. Padaria
4	10059	Mr. Bhupendra Singh Panwar	Dr. Sarvjeet Kaur
5	10153	Mr. Deepak Pawar	Dr. T. R. Sharma
6	10313	Mr. Lianthanzauva	Dr. R. C. Bhattacharya
7	10314	Mr. Alim Junaid	Dr. Kishor Gaikwad
8	10315	Mr. Albert Maibam	Dr. Jasdeep C. Padaria
9	10316	Mr. Mahajan Mahesh Mohanrao	Dr. Kanika
10	10317	Mr. Rakesh Kumar Prajapat	Dr. Rekha Kansal
11	10318	Mr. Vinod Kumar Jangid	Dr. Anita Grover
12	10319	Ms. Anupma	Dr. Sarvjeet Kaur
13	10495	Mr. Manoj M.L.	Dr. N. K. Singh
14	10496	Mr. Sudhir Kumar	Dr. R. C. Bhattacharya
15	10497	Ms. Shruti Sinha	Dr. T. R. Sharma
16	10498	Ms. Shikha Dixit	Dr. Anita Grover
17	10549	Mr. Pawan S. Mainkar	Dr. Jasdeep C. Padaria
18	10500	Mr. Kishor Panzade P	Dr. Kanika
19	10501	Ms. Priyanka	Dr. Rekha Kansal
20	10550	Mr. Kamble Viraj Gangadhar	Dr. Sarvjeet Kaur
21	10639	Mr. Sandeep Jaiswal	Dr. Debasis Pattanayak
22	10640	Ms. Sarita Kumari	Prof. N. K. Singh
23	10643	Ms. Neeta Pargi	Dr. Anita Grover
24	10696	Mr. Lalbahadur Singh	Dr. P. K. Jain
25	10697	Ms. Priyanka Singh	Dr. T. Mohapatra
26	10720	Mr. Chetan Kumar Nagar	Dr. P. K. Mandal
M. Sc.			
27	20569	Ms. Parichita Priyadarshini	Dr. P. K. Jain
28	20570	Ms. Alka Bharati	Dr. P. K. Mandal

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
29	20571	Ms. Sharani Choudhury	Dr. Amol Kumar U. Solanke
30	20572	Ms. Sreeshma N	Dr. S. V. A. C. R. Mithra
31	20573	Ms. Jyotsana Tilgam	Dr. Debasis Pattanayak
32	20574	Mr. Awakale Pramod Atmaram	Dr. Monika Dalal
33	20704	Ms. Bablee Kumari Singh	Dr. S. V. A. C. R. Mithra
34	20705	Mr. Soham Choudhary	Dr. Monika Dalal
35	20706	Mr. Sumit Jha	Dr. Amol Kumar U. Solanke
36	20707	Mr. Sougata Bhattacharjee	Dr. S. Barthakur
37	20708	Ms. Loitongbam Ashakiran Devi	Dr. Rhitu Rai
38	20709	Mr. Abinash Biswajit Shetty	Dr. P. K. Dash

Degrees awarded in the discipline of Molecular Biology and Biotechnology during the Convocation held in February, 2016.

S No.	Name of the Student and Roll No.	Chairperson, Advisory Committee	Thesis Title
		Ph. D.	
1	Rama Prashat (9562)	Dr. N. K. Singh	Molecular characterization of FISH mapping of major repetitive DNA in pigeon pea (*Cajanus cajan* L. Mills.)
2	Nimmy M.S. (9565)	Dr. R. Srinivasan	Isolation and characterization of salt inducible gene(s)/promoter(s) from chickpea
3	Soham Ray (9710)	Dr. T. R. Sharma	Functional characterization and validation of *AvbrPi54* gene cloned from *Magnaporthe oryzae*
4	Deepika Singh (9711)	Dr. N. K. Singh	Allele miming for salt tolerance zenes in landraces and improved varieties of rice (*Oryza sativa L.*)
5	Deepak Singh Bisht (9713)	Dr. S. R. Bhat	Towards mapping and cloning of male fertility restorer gene of CMS (*Moricandia arvensis) Brassica juncea*
6	Siddanna Savadi * (9715)	Dr. S. R. Bhat	Effect of altered expression of key genes involved in fatty acid biosynthesis and seed development on productivity of Indian mustard (*Brassica juncea*)
7	Chandrashekar (10016)	Dr. Anita Grover	Isolation and molecular characterization of PR gene pathogen-inducible promoter from *Arabidopsis thaliana* in response to *Alternaria*
		M.Sc.	
8	Mr. Sandeep Jaiswal (20441)	Dr. Debasis Pattanayak	Host-Delivered RNAi-mediated silencing of *Ace-1* gene for gram pod borer resistance

S No.	Name of the Student and Roll No.	Chairperson, Advisory Committee	Thesis Title
9	Mr. Lal Bahadur Singh (20442)	Dr. P. K. Jain	Identification and characterization of tissue specific miRNAs in chickpea
10	Mr. Chetan Kumar Nagar (20443)	Dr. P. K. Mandal	Expression studies of nitrate transport system gene(s) in contrasting wheat genotypes for nitrogen use efficiency
11	Ms. Neeta Pargi (20444)	Dr. Sarmishtha Barthakur	Molecular cloning and transcript expression profiling of *SnRk1* (Sucrose non-fermenting-1 related protein Kinase 1) under terminal heat stress in wheat (*Triticum aestivum L*)
12	Mr. Ningombam Rabichandra Meitei (20445)	Dr. Rhitu Rai	Molecular characterization of virulent *Xanthomonas osyzae pv. oryzae* (Xoo) isolates and their responsive susceptibility genes in rice
13	Mr. Hay Van Duong (20481)	Dr. P. K. Dash	Cloning and transformation of mustard (*Brassica juncea L.*) with *At-DWARF4*

* IARI Gold Medal in 2016

9 PERSONNEL

Scientific Staff	Area of Interest
Dr. T. R. Sharma trsharma@nrcpb.org	Structural, functional and comparative genomics of plant and plant pathogens; mapping, cloning and functional validation of disease resistance genes and QTL in rice; development of DNA markers and various databases.
Prof. N. K. Singh nksingh@nrcpb.org	Sructural, functional and comparative genomics of rice, wheat, pigeonpea, tomato, mango, jute and *Rhizobium*; mapping and fine mapping of genes and QTLs for salinity tolerance, yield and quality traits in rice, wheat and pigeonpea; molecular breeding to create climate resilient rice varieties; exploration and molecular characterization of Indian wild rice.
Prof. S. R. Bhat srbhat22@gmail.com	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. Sarvjeet Kaur dr_sarvjeetkaur@yahoo.com	Bioprospecting of novel genes and promoters; identification and molecular characterization of genes of potential interest from *Bacillus thuringiensis* by PCR and NGS-based approaches; functional validation of synthetic codon-optimized novel *cry-* and *vip-* type genes *in planta*.
Dr. Anita Grover anitagrover@hotmail.com	Plant-fungus interaction using *Brassica juncea- Alternaria brassicae* system, understanding defence signaling pathways, isolation of defence genes and development of transgenics of *B. juncea* for increased resistance to *Alternaria* blight.
Dr. Rekha Kansal rekhakansal@hotmail.com	Isolation of genes encoding lectins, protease inhibitors and amylase inhibitors from legumes, like chickpea, pigeonpea, peas and mothbean, for imparting improved tolerance to sucking insect pests (*Lipaphis erysimi*)
Dr. Sanjay Singh sanjay_singh777@yahoo.com	Marker assisted introgression of gene(s)/QTLs for resistance to Bipolaris leaf spot (*Bipolaris sorokiniana*) in wheat
Dr. J. C. Padaria jasdeep_kaur64@yahoo.co.in	Cloning and characterization of abiotic stress responsive genes from different tolerant plant systems as Pennisetum, Zizyphus and Prosopis for development of transgenic wheat adaptable to climate change induced abiotic stresses.
Dr. Pranab Kumar Mandal pranabkumarmandal@gmail.com	Molecular and biochemical basis of nitrogen use efficiency in cereals.
Dr. Debasis Pattanayak debasispattanayak@yahoo.co.in	Gram pod borer resistance management employing host-delivered RNAi, development of RNA-based gene silencing tools for crop improvement and functional genomics, and deciphering RNAi pathways as development and stress regulators.
Dr. R. C. Bhattacharya ramcharan99@yahoo.com	Identification and mobilization of genes for conferring resistance to insect herbivores and fungal pathogens into the elite crop cultivars, elucidation of plant triggered immunity and defense signaling in plants.
Dr. Pradeep Kumar Jain jainpmb@gmail.com	Isolation of plant genes and promoters for important traits like drought, temperature, blight and wilt in chickpea employing functional genomics.
Dr. S. Barthakur sbthakur@yahoo.com	Isolation and functional characterization of genes and regulatory elements towards genetic engineering for abiotic stress tolerance in crops, particularly terminal stress in wheat using various functional genomics tools.

Dr. K. S. Gaikwad kish2012@yahoo.com	Development of genomic resources including deep transcriptome analysis in crops; molecular analysis of cytoplasmic male sterility and fertility restoration and thermo-tolerance in pigeonpea.
Dr. Kanika Kumar kanika@rediffmail.com	Abiotic stress management in plants through the associated microbes by exploiting PGPRs, role of microbial genes in reducing the concentration of stress ethylene in plants, structural and functional genomics of *Mesorhizobium ciceri*.
Dr. Vandana Rai vandnarai2006@gmail.com	Discovering new genes and alleles for salinity and submergence stress tolerance in rice and pigeonpea using transcriptomics, proteomics and metabolomics.
Dr. Monika Dalal monika@nrcpb.org	Functional genomics for abiotic stress tolerance especially drought and salinity stress in wheat.
Dr. Subodh Kumar Sinha subsinha@gmail.com	Molecular analysis of nitrogen use efficiency in cereals and microRNA-mediated regulation of nitrogen metabolism.
Dr. Rohini Sreevathsa rohinisreevathsa@gmail.com	Meristem-based *in planta* transformation for biotic stress tolerance using different insecticidal proteins (ICPs) from *Bacillus thuringiensis*.
Dr. Prasanta K. Dash pdas@nrcpb.org	Understanding the mechanism of light-regulated gene functioning in rice.
Dr. B. L. Patil blpatil2046@gmail.com	Molecular analysis of plant viruses infecting different crop plants, RNAi and epigenetics for manifestation and management of plant viral diseases.
Dr. Rhitu Rai rhitunrcpb@yahoo.com	Identification and molecular characterization of pathogenicity factors, their targets in rice and delineation of underlying mechanism of interaction involved in bacterial leaf blight and false smut diseases of rice.
Dr. Navin Chandra Gupta guptanc@nrcpb.org	Molecular analysis of plant pathogen interaction, identification and utilization of genes and pathways for development of stem rot resistance and genome editing in Indian mustard (*Brassica juncea*) for quality improvement.
Dr. S.V. Amitha CR Mithra amitha@nrcpb.org	QTL mapping for grain traits and abiotic stress tolerance in rice using SSR and SNP markers.
Dr. Amolkumar U. Solanke amolsgene@nrcpb.org	Genomics and transgenics to understand biotic and abiotic stress tolerance, molecular biology of fruit development.
Mr. R. S. Jaat rsjaat@gmail.com	Genomics and molecular markers.
Mr. Ramawatar ram_nrcpb@nrcpb.org	Functional genomics to understand the role of non-coding RNA in plant pathogen interaction and disease development.
Dr. Dinabandhu Behera aumreetam@rediffmail.com	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 approaches.
Ms. Nimmy M. S. nimmybiotech@gmail.com	Isolation and characterization of abiotic stress responsive gene(s) and promoter(s) from chickpea.
Dr. Deepak Singh Bisht deebisht@gmail.com	Molecular identification of resistance and susceptibility genes in rice-*Rhizoctonia solani* system.
Mr. Anshul Watts watts_086@yahoo.com	Development and maintenance of genetic stocks for heterosis breeding in *Brassica juncea*.
Dr. Era Vaidya Malhotra vaidya.era@gmail.com	Identification and characterization of microRNAs in gram pod borer.

INSA Honourary Scientist	Prof. R. P. Sharma
	Dr. H. K. Das
ICAR Emeritus Scientist	Prof. K. R. Koundal
	Prof. Srinivasan
DBT Ramalingaswamy Fellow	Dr. Ajay Jain

Technical Staff	**Administrative Staff**
Ms. Suman Bala	Smt Piyush Malyan
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	Sh. B. S. Dagar
Smt. Sandhya Rawat	Smt. Sangeeta Jain
Smt. Seema Dargan	Sh. Vipin Kumar
Dr. Rohit Chamola	Smt Rekha Chauhan
Dr. Pankaj Kumar	Sh. Rajesh Kumar Pal
Sh. Ravinder Rishi	
Dr. Rampal Singh Niranjan	

10 TRAINING AND CAPACITY BUILDING

Details of training by the NRCPB staff during 2015-16

S. No.	Name	Subject Area	Duration (Month of the year)	Host Institute
Scientific staff				
1.	Dr. Amitha C. Mithra	TILLING in crop plants	15 days (12-26 April 2015)	University of Hyderabad, Hyderabad
2.	Mr. Mahesh Rao	Cytogenetic Techniques in Crops	25 days (1-25 October 2015)	North Eastern Hill University, Shillong
3.	Dr. D. Pattanayak	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
4.	Dr. J. C. Padaria	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
5.	Dr. P. K. Mandal	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
6.	Dr. P. K. Jain	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
7.	Dr. R. C. Bhattacharya	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
8.	Dr. N. C. Gupta	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
9.	Dr. Nimmy M. S.	Scientific Writing/ Presentation	Two Days (12-13 October 2015)	NRCPB, New Delhi
Technical staff				
1.	Dr. R. S. Niranjan	Intellectual Property Rights in Agriculture	21 Days (12 August to 1 September 2015)	ZTM&BPD unit, IARI, New Delhi
2.	Dr. K. P. Singh	Competency Enhancement Program for Technical Officers of ICAR	10 Days (14-23 December 2015)	NAARM, Hyderabad
3.	Dr. Pankaj Kumar	Competency Enhancement Program for Technical Officers of ICAR	10 Days (14-23 December 2015)	NAARM, Hyderabad
4.	Ms. Seema Dargan	Competency Enhancement Program for Technical Officers of ICAR	Ten Days (01-10 March 2016)	NAARM, Hyderabad
Administrative staff				
1.	Smt. Sangeeta Jain	Implementation of NIC's e-Procurement Solution Through CPP Portal	Two days (18-19 February 2015)	ICAR Krishi Bhavan, New Delhi
2.	Smt. Rekha Chauhan	Implementation of NIC's e-Procurement Solution Through CPP Portal	Two days (18-19 February 2015)	ICAR Krishi Bhavan, New Delhi

11 OTHER ACTIVITIES

Vigilance Awareness Week

NRCPB observed Vigilance Awareness Week from 26th October, 2015 to 31st October, 2015. Project Director, NRCPB administered the pledge to the staff on 26th October, 2015. A guest lecture on "Vigilance Awareness" by Sh. SK Behra, Deputy Secretary, ICAR and an essay writing competition on anticorruption were organised. All NRCPB staff participated in these activities.

Hindi Activities 2015-16

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 2015 from 14-19th September, 2015.

Hindi Workshop

Following four different Hindi workshops were conducted in 2015-16.

S.No.	Date	Quarter	Chief Lecturer
1.	31-01-2015	January-March	Dr. Ramesh Sapra
2.	27-04-2015	April- June	Prof. Srinivasan
3.	30-11-2015	October-December	Prof. N. K. Singh
4.	05-03-2016	January-March	Dr. Nimesh Kapur

National Unity Day- (Rashtriya Ekta Divas)

Rashtriya Ekta Diwas was celebrated at NRCPB on 31st October, 2015 at NRCPB Auditorium. Project Director administered the national unity pledge to all the staff.

Swachha Bharat Abhiyaan

Institute observed a special drive on Swachha Bharat Abhiyan from 25th September to 31st October 2015. During the occasion 'Swachhta' awareness campaign was made among public including children and facilitated 'Swachhta' by distributing dustbins to the local schools. All staff of the centre also participated in cleaning of their respective laboratories and offices on 8th October, 2015.

Foundation Day Celebration

Centre celebrated its second Foundation Day on 15th January, 2016. Prof. V. L. Chopra, Former Member, Planning Commission and Former Director General, ICAR & Secretary, DARE, was the Chief Guest of the function. Dr. J. S. Sandhu, Deputy Director General (Crop Sciences), ICAR, was the Guest of Honour on this occasion. The other distinguished invitees included Dr. H. S. Gupta (Director General, BISA), Dr. Renu Swarup (Senior Advisor, Department of Biotechnology, Govt. of India), Prof. Anupam Varma, Prof. R. P. Sharma, Dr. K. V. Prabhu (Jt. Director, Research, IARI), Dr. J. S. Chauhan (ADG, Seed, ICAR), Heads of Divisions of IARI and retired employees of NRCPB.

The function started with welcome address and presentation by the Project Director, Dr. T. R. Sharma, on centre's achievement. During this occasion, Padma Bhushan Prof. G. Padmanaban, Former Director, Indian Institute of Sciences, Bengaluru, delivered foundation day lecture on "Biotechnology in Health and Agriculture Sectors – Relevance for India". NRCPB staff members who have completed their thirty years of service at the centre were also felicitated on this occasion. During the function, three publications of NRCPB, viz. NRCPB News Letter (both English and Hindi version), NRCPB Video Film (both English and Hindi) and NRCPB compendium of Product & Technologies, were released by the dignitaries.

Farmers Day

The Centre organized Farmers Day on 3rd October, 2015 in order to adress the farmers need, their problems and biotechnological intervention in agricultural research. Thirty progressive farmers

of village Nekpur and its surrounding villages of Bulandashar, U.P. participated in the function. On this occasion a packet of seed of mustard variety “Pusa Jaikisan”and important reading materials were distributed to all the farmers.

Sports

A team of seven staff members from NRCPB participated in ICAR-Zonal Sports of Central Zone hosted by ICAR-IIWSR, Jabalpur, Madhya Pradesh from 7-11 December, 2015. Dr. PK Dash received Gold Medals in Shot-put and Discus-throw.

12 RECRUITMENTS/PROMOTIONS/ RETIREMENTS

Recruitments

1. Dr. Deepak Singh Bisht joined as Scientist in April 2015.
2. Mr. Anshul Watts joined as Scientist in April 2015.
3. Dr. Era Vaidya Malhotra joined as Scientist in September 2015.

Promotions

1. Sh. Rajesh Kumar Pal, SSS promoted to the post of LDC in June, 2015.

Retirement

1. Prof. S. R. Bhat, retired in Feb., 2016.
2. Mrs. Suman Bala, retired in Feb., 2016.
3. Mrs. Piyush Malayan, resigned in Feb., 2016.

13 INSTITUTIONAL PROJECTS

Project Title	Date of Start	Date of Completion/ ongoing	Principal Investigator	Name of Associates
Genomics and molecular markers in crop plants	1st April, 2012	31st March, 2017	Dr. Kishor Gaikwad	Prof. N. K. Singh
				Dr. T. R. Sharma
				Dr. Vandana Rai
				Dr. P. K. Dash
				Dr. Rhitu Rai
				Dr. S. V. A. C. R. Mithra
				Dr. Amol U. Solanke
				Mr. R. S. Jaat
				Mr. R. A. Nagar
				Dr. Deepak S. Bisht
				Dr. Pankaj Kumar
				Mr. H. C. Upreti
Biotechnological applications for pulses improvement	1st April, 2012	31st March, 2017	Dr. Debasis Pattanayak	Dr. P. K. Jain
				Dr. Rohini Sreevathsa
				Dr. B. L. Patil
				Dr. M. S. Nimmy
				Dr. Era Vaidya Malhotra
				Dr. K. P. Singh
				Ms. Suman Bala
				Dr. R. S. Niranjan
Biotechnological approaches for Brassica improvement	1st April, 2012	31st March, 2017	Dr. R. C. Bhattacharya	Dr. Anita Grover
				Dr. Rekha Kansal
				Dr. Navin C. Gupta
				Dr. Mahesh Rao
				Mr. Anshul Watts
				Mrs. Seema Dargan
				Dr. Rohit Chamola
				Mrs. Sandhya Rawat
Adaptation of wheat to climate change induced abiotic stress	1st April, 2012	31st March, 2017	Dr. J. C. Padaria	Dr. Sanjay Singh
				Dr. S. Barthakur
				Dr. Kanika
				Dr. Monika Dalal

Project Title	Date of Start	Date of Completion/ ongoing	Principal Investigator	Name of Associates
Improvement of nutrient use efficiency in cereal crops	1st April, 2012	31st March, 2017	Dr. P. K. Mandal	Dr. Subodh Sinha
				Mr. R. N. Gupta
Bioprospecting of novel genes and promoters	1st April, 2012	31st March, 2017	Dr. Sarvjeet Kaur	Dr. A. Dinabandhu
				Mr. Rakesh Narula

14 EXTERNALLY FUNDED PROJECTS

S. No.	Funding agency	Project Title	Principal Investigator	Budget (Rs. in lakh)
1.	ICAR-NAIP	Maintenance, characterization and use of EMS mutants in an upland variety Nagina22 for functional genomics in rice – Phase II	N. K. Singh	263.16
2.	DBT	Physical mapping and sample sequencing of wheat chromosome 2A- International Wheat Genome Sequencing Consortium (India) (IWGSC)	N. K. Singh	871.92
3.	ICAR-NASF	Genomics for augmenting fibre quality improvment in jute	N. K. Singh	49.04
4.	DBT	Establishment of National Rice Resource Database	N. K. Singh	92.708
5.	IRRI	Stress tolerent rice for Africa and South Asia (STRASA)	N. K. Singh	$1,17,000
6.	DBT	Identification and functional analysis related to yield and biotic stresses Phase-II	N. K. Singh	153.67
7.	DBT	From QTL to variety: marker assisted breeding of abiotic stress tolerant rice varieties with major QTLs for drought, submergence and salt tolerance	N. K. Singh	588.00
8.	ICAR	Allele mining for agronomically important genes in wild rice germplasm and stress tolerant landraces of rice growing in the hot spots	N. K. Singh	250.00
9.	ICAR	Network project on transgenic crops (Multi-institutional)	T. R. Sharma (Coordinator)	11200.00
10.	DBT	Referral lab for NCS-TCP project on genetic fidelity testing	T. R. Sharma	85.00
11.	ICAR	CRP on genomics for guar and black gram	T. R. Sharma	1350.00
12.	ICAR	Molecular genetic analysis of resistance to panicle blast and sheath blight of rice	T. R. Sharma	195.00
13.	ICAR-NPTC	Bioinformatics and comparative genomics	T. R. Sharma	167.00
14.	DBT	*Puccinia triticina* genomics network on *de novo* genome sequencing, fitness, variation and pathogenicity	T. R. Sharma	571.47
15.	ICAR- NFBSFARA	Molecular and genetic analysis of guggul for the identification of genes governing adventive embryony	S. R. Bhat	240.90
16.	DBT	Development of haploid-inducer lines of *Brassica juncea* through genetic engineering of centromere histone protein	S. R. Bhat	64.10
17.	ICAR- NPTC	Functional genomics of Alternaria blight and drought tolerance in Brassica	S. R. Bhat	232.96
18.	ICAR-NAIP	Unraveling molecular processes involved in adventive polyembryony towards genetic engineering for fixation of heterosis	S. R. Bhat	772.1
19.	ICAR- NPTC	Development of Alternaria-resistant mustard	Anita Grover	50.00

S. No.	Funding agency	Project Title	Principal Investigator	Budget (Rs. in lakh)
20.	ICAR- NPTC-	Development of aphid resistant transgenic Brassica	Rekha Kansal	58.33
21.	DBT	Cross talk between phosphorus and iron in the maintenance of nutrient homeostasis in plants	Ajay Jain	63.90
22.	ICAR-NICRA	National initiative for climate resilient agriculture	J. C. Padaria	186.00
23.	DBT	Design and construction of a strong promoter for constitutive overexpression of *Nif*A gene in *Azotobacter vinelandii*	J. C. Padaria	36.704
24.	ICAR-NFBSFARA	Phenomics of moisture deficit and low temperature tolerance in rice	P. K. Mandal	405.00
25.	CIMMYT	Improving productivity of wheat through enhanced nitrogen use efficiency	P. K. Mandal	26.60
26.	DBT	Identification and characterization of peptides in selected wheat cultivars which are less immunogenic to patients with celiac disease	P. K. Mandal	24.508
27.	ICAR- CRP on Biofortifcation	Exploration for development of a wheat with low immunogenic gluten	P. K. Mandal	138.00
28.	ICAR- Incentivizing Research in Agriculture	Genetic modification to improve biological nitrogen fixation	P. K. Mandal	1290.00
29.	ICAR-NPTC	Amelioration of cold induced sweetening in potato	D. Pattanayak	65.03
30.	DST	Crop plants which remove their own major biotic constraints	R. C. Bhattacharya	140.72
31.	ICAR-NFBSFARA	Common basis of defence induction in rice and mustard against sucking and gall insect pests	R. C. Bhattacharya	63.55
32.	ICAR-NFBSFARA	Understanding plant nematode interaction: identification of plant and nematode genes involved in disease development	P. K. Jain	50.33
33.	ICAR-NPTC	Development of transgenic wheat for terminal heat stress tolerance and grain quality	Sharmishtha Barthakur	49.00
34.	ICAR-NASF	Modeling network of gene response to abiotic stress in rice	Kishor Gaikwad	69.00
35.	ICAR-NPTC	Finishing of pigeonpea genome and functional genomics of fertility restoration	Kishor Gaikwad	10.00
36.	ICAR-CRP on Genomics	Decoding the genomes of guar and black gram for the identification of important genes	Kishor Gaikwad	110.00
37.	ICAR-NASF	Understanding cellular and genetic mechanisms and identifying molecular markers for seed viability in soybean	Kishor Gaikwad	59.84
38.	DBT	Phenomics and genomics of ragi	Kanika	107.348
39.	ICAR-NPTC	Development of wheat transgenics with enhanced tolerance to drought	Monica Dalal	14.25
40.	ICAR-NASF	Transgenics for pod borer resistance in chickpea and pigeonpea	Rohini Sreevathsa	142
41.	ICAR-NPTC	Herbicide tolerance in cotton	Rohini Sreevathsa	46.1
42.	DBT-ISCB	Improvement of pigeonpea for plant type, early maturity, pod borer resistance and moisture stress tolerance	Rohini Sreevathsa	95.90

S. No.	Funding agency	Project Title	Principal Investigator	Budget (Rs. in lakh)
43.	DBT	Root specific reduction of cytokinin to enhance root biomass and seed yield in rapeseed (*Brassica campestris*)	P. K. Dash	28.40
44.	ICAR-NPTC	Bast fibre yield in flax and low light tolerance in rice	P. K. Dash	250.00
45.	ICAR-NASF	Understanding the mechanisms of tolerance to low-light intensity in rice	P. K. Dash	138.95
46.	DBT	Identification and functional characterization of key virulence determinants from Indian Xoo strains knocking down xa13 mediated resistance	Rhitu Rai	39.30
47.	DBT	Sequencing of *Xanthomonas oryzae* pv *oryzae* pathotypes for better management of bacterial leaf blight disease in rice	Rhitu Rai	64.87
48.	ICAR	Study on *Sclerotinia sclerotiorum* with emphasis on management of Sclerotinia rot	N. C. Gupta	27.87
49.	ICAR-NPTC	Jute genomics	S. V. Amitha C. R. Mithra	10.27
50.	DBT	Referral centre for genetic fidelity testing of tissue culture raised plants (NCS-TCP)	Amol Kumar U. Solanke	71.37
51.	ICAR-CABin	RiceMetaSys: Understanding rice gene network for blast resistance and drought tolerance through system biology approach	Amol Kumar U. Solanke	21.39

15 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 2015-16.

I. Patent applications filed: Six

a. Complete Patent Applications

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

1. A Single copy gene based 50k SNP rice chip and uses thereof (Application No. 2280/DEL/2014).
2. Polynucleotide fragments for directing expression of genes in plant roots in response to pathogens (Application No. 2246/DEL/2015)

b. Provisional Patent Application

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

1. Polynucleotide fragments for directing expression of genes in plant roots in response to pathogens, and wounding (Application No. 2245/DEL/2015).
2. Targets genes in aphids for host-delivered RNAi mediated gene silencing and their application for development aphid resistance in plants (Application No. 1817/DEL/2015).
3. Host-delivered siRNA-mediated resistance to *Helicoverpa armigera* (Application No. 3241/DEL/2015).
4. Host-delivered artificial microRNA-mediated resistance against *Helicoverpa armigera* (Application No. 3242/DEL/2015).

II. MoUs/MTAs signed

1. Memorandum of Understanding (MoU) was signed on 08.07.2015 by NRCPB and M/S Nirmal Seeds Pvt. Ltd, Jalgaon for licensing of Mori base CMS and restorer system.

Memorandum of Understanding (MoU) was signed by NRCPB and M/S Nirmal Seeds Pvt. Ltd, Jalgaon for licensing of Mori base CMS and restorer system

2. Material Transfer Agreement (MTA) was signed on 27.05.2015 by NRCPB and Head, Division of Vegetable Science, IARI, New Delhi for material transfer of CMS system in cauliflower background.
3. Material Transfer Agreement (MTA) was signed on 25.06.2015 by NRCPB and ICAR-Indian Institute of Pulses Research, Kanpur for material transfer of pBinAR: CaMV 35S: *cry2Aa:* OcsT.
4. Material Transfer Agreement (MTA) was signed on 08.07.2015 by NRCPB and Assam Agricultural University, Jorhat, Assam for material transfer of *VIP* Gene.

16 AWARDS AND HONOURS

1. Prof. N. K. Singh, National Professor, BP Pal Chair, was conferred ICAR-Norman Borlang Award on 25th July 2015.
2. Dr. T. R. Sharma was nominated as Chief Editor, Journal of Plant Biochemistry and Biotechnology, Jan 2009 till date; Sectional Editor (Biological Sciences), Current Science; Editor, Indian Journal of Agricultural Sciences.
3. Dr. T. R. Sharma was nominated to the Board of Directors, Biotech Consortium India Ltd. New Delhi; and Member, Academic Council, University of Horticulture and Forestry, Nauni, Solan (HP).
4. Dr. Rekha Kansal received Best Paper Award by the Committee of TJPRC Journals in recognition for research paper quality, originality and significance in modeling and technical flow for the paper "Use of Vigna radiata Lectin Gene in Development of Transgenic Brassica juncea Resistant to Aphids".
5. Dr. Sharmistha Barthakur elected as Councilor North Zone 2015-2017, Indian Society of Agricultural Sciences, New Delhi.
6. Rohini Sreevathsa was elected the member of National Academy of Sciences, India.
7. Dr. Amolkumar U. Solanke received Fellow Award from 'Society for Applied Biotechnology', Krishnagiri, Tamil Nadu in recognition of outstanding achievements and contributions to the field of plant biotechnology.
8. Dr. Shallu thakur who worked as SRF in the NAIP project of Dr. T. R. sharma got ICAR-Jawaharlal Nehru Award 2014 for best Ph. D. Thesis entitled 'Analysis of allelic variants of *Pi5A* and *Piz(t)* blast resistance genes from rice land races'.
9. Mr. Showkat Lone received Young Scientist Award for poster presentation on 'Selection and characterization of *Bacillus thuriengiensis* strains from North-west Himalayas toxic to *Helcoverpa armigera* at the 7th International Conference on *Bacillus anthracis, B. cereus & B. thuringiensis* (*Bacillus* ACT 2015) held in Delhi from October 27-31, 2015.

17 VISITS ABROAD

1. Dr. Ajay Jain visited Purdue University, USA (June 10 - July 24 2015) and Washington University at St Louis, USA (July 26 - August 27, 2015) as a Visiting Scientist.
2. Dr. Sharmistha Barthakur visited Sydney, Australia to attend 9th International Wheat Congress from 20-25 September, 2015.
3. Dr. Vandna Rai visited Meijo University, Nagoya, Japan in November 2015 for a period of two and half months availing Asian Research Fellowship

18 LINKAGES AND COLLABORATION

The centre has an active and close collaboration with other ICAR institutes and State Agricultural Universities. NRCPB 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, 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
- Punjab Agrciultural University, Ludhiana
- Tamil Nadu Agricultural University, Coimbatore
- Central Rice Research Institute, Cuttack
- Directorate of Medicinal and Aromatic Plants, Anand
- University of Agricultural Sciences, Dharwad
- Institute of Himalayan Bioresources and Technology, Palampur
- National Bureau of Plant Genetic Resources, 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, USA
- International Rice Research Institute, Manila, The Phillipines
- Sixteen Institutes under International Wheat Genome Sequencing Project

19 LIST OF PUBLICATIONS

Research Papers

1. Amitha Mithra SV, Kar M, Mohapatra T, Robin S, Sarla N, Seshashayee M, Singh K, Singh AK, Singh NK & Sharma RP. 2016. DBT propelled national effort in creating mutant resource for functional genomics in rice. *Curr Sci* **110**: 543-548.
2. Arora K, Rai AK, Gupta SK, Singh PK, Narula A, & Sharma TR. 2015. Phenotypic expression of blast resistance gene *Pi54* is not affected by its chromosomal position. *Plant Cell Rep* **34**: 63-70.
3. Bhati J, Chaduvula KP, Rai A, Gaikwad K, *et al.* 2016. *In-silico* prediction and functional analysis of salt stress responsive genes in rice (*Oryza sativa*). *J Rice Res* **4**: 164.
4. Chaduvula KP, Bhati J, Rai A, Gaikwad K, *et. al.* 2015. *In-silico* expressed sequence tag analysis in identification and characterization of salinity stress responsible genes in *Sorghum bicolor. Aus J Crop Sci* **9**: 799-806.
5. Chandrashekar N, Ali S, Rawat S & Grover A. 2015. Gene expression profiling of *Arabidopsis thaliana* chitinase genes in response to *Alternaria brassicae* challenge. *Indian Phytopathol* **68**: 106-111.
6. Chhapekar S, Raghavendrarao S, Pavan G, Ramakrishna C, Singh VK, Phanindra MLV, Dhandapani G, Sreevathsa R & Kumar PA. 2015. Transgenic rice expressing a codon-modified synthetic *CP4-EPSPS* confers tolerance to broad-spectrum herbicide, glyphosate. *Plant Cell Rep* **34**: 721-731**.**
7. Chidambaranathan P, Jagannadham PTK, Satheesh V, Jain PK & Srinivasan. 2016. Expression analysis of six chromatin remodeling complex genes (*SWR1*) in chickpea in different tissues and during heat stress *Indian. J Genet Plant Breeding* **76**: 47-56.
8. Choudhary M, Jayanand & Padaria JC. 2015. Transcriptional profiling in pearl millet (*Pennisetum glaucum* L.R. Br.) for identification of differentially expressed drought responsive genes. *Physiol Mol Biol Plants* **21**: 187-196.
9. Dash PK, Gupta P & Rai R. 2015. Hydroponic method of halophobic response elicitation in flax (*Linum usitatissimum*) precise down-stream gene expression studies. *Intl J Trop Agril.* **5**: 1-7.
10. Deka S & Barthakur S. 2015. Impact of biorational pesticides and response of *annexin* gene expression in tomato *(Solanum lycopersicum)* and capsicum (*Capsicum annum* L.) in biotic stress management under greenhouse condition. *Ann Agric Res* **36**: 233-242.
11. Deokar AA, Kondawar V, Kohli D, Aslam M, Jain PK, Karuppayil M, Varshney RK & Srinivasan R. 2015. The *CarERF* genes in chickpea (*Cicer arietinum* L.) and the identification of CarERF116 as abiotic stress responsive transcription factor. *Funct Integr Genomics* **15**: 27-46.
12. Ganeshan P, Jain A, Parmar B, Rao AR, Sreenu K, Mishra P, Mesapogu S, Subrahmanyam D, Ram T, Sarla N & Vandna Rai. 2016. Identification of salt tolerant rice lines among interspecific BILs developed by crossing *Oryza sativa* × *O. rufipogon* and *O. sativa* × *O. nivara. Aust J Crop Sci* **10**: 220-228.
13. James D, Tarafdar A, Biswas K, Sathyavathi TC, Padaria JC & Kumar PA. 2014. Development and characterization of a high temperature stress responsive subtractive cDNA library in Pearl Millet (*Pennisetum glaucum* L.R. Br.). *Ind J Exp Biol* **50**: 543-550.
14. Kanakachari M, Solanke AU, Prabhakaran N, Ahmad I, Dhandapani G, Jayabalan N & Kumar PA. 2015. Evaluation of suitable reference genes for normalization of qPCR gene expression studies in brinjal (*Solanum melongena* L.) during fruit developmental stages. *Appl Biochem Biotechnol* DOI: 10.1007/s12010-015-1884-8.

15. Khanna A, Sharma V, Ellur RK, Shikari AB, Gopala Krishnan S, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M, Prashanthi SK, Nagarajan M, Vinod KK, Bhowmick PK, Singh NK, Prabhu KV, Singh BD & Singh AK. 2015. Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. *Theor Appl Genet* DOI: 10.1007/s00122-015-2502-4.

16. Khomdram S & Barthakur S. 2015. *In situ* evaluation of high temperature stress in wheat (*Triticum aestivum* L.) - validation of a portable heat trap chamber, *Ann Agric Res* **36**: 123-131.

17. Koramutla MK, Bhatt D, Negi M, Venkatachalam P, Jain PK & Bhattacharya RC. 2016. Strength, stability, and *cis*-motifs of *in silico* identified phloem-specific promoters in *Brassica juncea* (L.). *Front Plant Sci* DOI: 10.3389/fpls.2016.00457.

18. Kumar A, Kohli D, Joshi I, Satheesh V, Abdin MZ, Sirohi A, Srinivasan R & Jain PK. 2016. Characterization of root-knot nematode responsive and root-specific promoter containing PIN domain from *Arabidopsis thaliana* (L.) Heynh. *Ind J Genet Plant Breeding* **76**: 75-83.

19. Kumar R, Sreenu K, Singh N, Jain N, Sigh NK & Rai V. 2015. Effect of drought stress on contrasting cultivars of rice. *Intl J Trop Agric* **33**: 1559-1564.

20. Kumar S, Kanakachari M, Dhandapani G, Kumar K, Prabhakaran N, Padmalatha KV, Solanke AU, Gamanagatti S, Hiremath V, Katageri IS, Leelavathi S, Kumar PA & Reddy VS. 2015. Genome wide transcriptomic and proteomic analyses of bollworm-infested developing cotton bolls revealed the genes and pathways involved in the insect pest defence mechanism. *Plant Biotechnol J* DOI: 10.1111/pbi.12508.

21. Lone SA, Yadav R, Malik A & Padaria JC. 2016. Molecular and insecticidal characterization of Vip3A protein producing *Bacillus thuringiensis* strains toxic against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Can J Microbiol* **62**: 179-190.

22. Madhav MS, Siva Raju K, Gaikwad K, Vishalakshi B, Murthy TGK & Umakanth B. 2015. Development of new set of microsatellite markers in cultivated tobacco and their transferability in other *Nicotiana spp*. *Mol Plant Breeding* **6:** 1-13.

23. Mishra S, Singh B, Panda K, Singh BP, Singh N, Misra P, Rai V & Singh NK. 2016. Association of SNP haplotypes of *HKT* family genes with salt tolerance in Indian wild rice germplasm. *Rice* **9**: 1-13.

24. Mohanrao MM, Singh DP, Kanika K, Goyal E & Singh AK. 2016. Deciphering the microbial diversity of Tattapani hot water spring using metagenomic approach. *Internat J Agric Sci Res* **6**: 371-382.

25. Muthusamy SK, Dalal M, Chinnusamy V & Bansal KC. 2016. Identification and expression analysis of class I *caseinolytic protease* (*Clp*) genes in wheat. *Front Plant Sci* (Accepted).

26. Nagarajan VK, Satheesh V, Poling MD, Raghothama KG & Jain A. 2016. *Arabidopsis* MYB-related HHO2 exerts regulatory influence on a subset of root traits and genes governing phosphate homeostasis. *Plant Cell Physiol* (in press).

27. Negi M, Sanagala R, Rai V & Jain A. 2016. Deciphering phosphate deficiency-mediated temporal effects on different root traits in rice grown in a modified hydroponic system. *Front Plant Sci* (in press).

28. Nimmy MS, Kumar V, Singh AK, Jain PK & Srinivasan R. 2015. Expression analysis of a MATE-type transporter gene of *Arabidopsis* and its orthologues in rice and chickpea under salt stress. *Indian J Genet Plant Breed* **75**: 478-485.

29. Padaria JC, Thuy NT, Tarafdar A & Yadav R. 2015. Development of a heat-responsive cDNA library from *Prosopis cineraria* and molecular characterisation of the *Pchsp17.9* gene. *J Hort Sci Biotechnol* **90**: 318-324.

30. Padaria JC, Yadav R, Yadav A & Kumar K. 2015. Cloning, expression and characterization of drought responsive NAC transcription factor from *Ziziphus nummularia. Internat J Tropical Agric* **33**: 2987-2998.

31. Pandey S, Gulati S, Goyal E, Singh S, Kanika K, Nain L & Saxena AK. 2016. Construction and

screening of metagenomic library derived from soil for β-1, 4-endoglucanase gene. *Biocat Agric Biotechnol* DOI: 10.1016/j.bcab.2016.01.008.

32. Panwar BS, Narula RK & Kaur S. 2015. Theoretical 3D modelling of a novel Cry toxin isolated from native *Bacillus thuringiensis* isolate SK-711. *Ind J Entomol* (in press).

33. Patil BL, Bagewadi B, Yadav JS & Fauquet CM. 2016. Mapping and identification of cassava mosaic geminivirus genome sequences for efficient siRNA expression and RNAi based virus resistance by transient agro-infiltration studies. *Virus Res* **213**: 109-115.

34. Pattnaik S, Kumar V, Tiwari KK, Chandra Prakash, Singh A, Singh AK, Behera N, Panigrahi J, Mithra SVA, Mohapatra T. 2016. Physiological and molecular basis of water deficit stress tolerance in F_1 hybrids and their parental lines. *Indian J Genet* (In Press).

35. Phukan D, Goyal V, Palit P, Kalia R, Koundal M, Mithra SVA, Ravi I, Yadava DK, Chinnusamy V & Mohapatra T. 2016. Expression analysis of candidate genes for abiotic stress tolerance in oilseed Brassica genotypes with contrasting osmotic stress tolerance. *Indian J Expt Biol* (In Press).

36. Phukan D, Singh B, Ravi I, Mithra SVA, Yadava DK, Chinnusamy V & Mohapatra T. 2016. Cloning and characterization of drought stress-induced NAC transcription factors from *Brassica juncea* and *Sinapsis alba. Indian J Genet* (In Press).

37. Pragya Mishra P, Mishra V, Takabe T, Rai V & Singh NK. 2016. Elucidation of salt-tolerance metabolic pathways in contrasting rice genotypes and their segregating progenies. *Plant Cell Rep* DOI: 10.1007/s00299-016-1959-1.

38. Rakshit S, Swapna M, Dalal M, Sushma G, Ganapathy KN, Dhandapani A, Karthikeyan M & Talwar HS. 2016. Post-flowering drought stress response of post-rainy sorghum genotypes. *Ind J Plant Physiol* **21**: 8-14.

39. Reddy SVR, Sharma RR, Barthakur S & Srivastava M. 2015. An efficient and rapid method for the isolation of RNA from different recalcitrant tissues of mango (*Mangifera indica* L.). *J Hort Sci Biotechnol* **90**: 285-290.

40. Savadi S, Vasupalli N, Kumar V, Dargan S, Gupta NC, Chamola R & Bhat SR. 2015. Effect of overexpression of *Arabidopsis thaliana SHB1* and *KLUH* genes on seed weight and yield contributing traits in Indian mustard *B. juncea* L. (Czern). *Indian J Genet* **75**: 349-356.

41. Sharma TR, Das A, Thakur S, Devanna BN, Singh PK, Jain P, Vijayan J & Kumar S. 2015. Oscillating transcriptome during rice-*Magnaporthe* Interaction. *Curr Issues Mol Biol* **19**: 99-120.

42. Singh A, Singh DP, Tiwari R, Kanika K, Singh RV, Singh S, Prasanna R, Saxena AK & Nain L. 2015. Taxonomic and functional annotation of gut bacterial communities of *Eisenia foetida* and *Perionyx excavates. Microbiol Res* DOI: 10.1016/j.micres.2015.03.003.

43. Singh BP, Singh B, Mishra S, Kumar V & Singh NK. 2016. Genetic diversity and population structure in Indian wild rice accessions. *African J Crop Sci* **10**: 144-151.

44. Singh K, Vaibhav, Mathuria RC, Singh GP, Singh PK, Singh S, Gogoi R & Aggarwal R. 2015. Characterization of yellow rust resistance genes by using gene postulation and assessment of adult plant resistance in some Indian wheat genotypes. *Res Crop* **16**: 741-750.

45. Singh N, Jain N, Kumar R, Jain A, Singh NK & Rai V. 2015. A comparative method for protein extraction and 2-D gel electrophoresis from different tissues of *Cajanus cajan*. *Front Plant Sci* DOI: 10.3389/fpls.2015.00606.

46. Singh N, Jayaswal PK, Panda K, Mandal P, Kumar V, Singh B, Mishra S, Singh Y, Singh R, Rai V, Gupta A, Sharma TR & Singh NK. 2015. Single-copy gene based 50 K SNP chip for genetic studies and molecular breeding in rice. *Nat Sci Rep* DOI:10.1038/srep11600.

47. Singh N, Majumder S, Singh ON, Vikram P, Singh AK & Singh S. 2015. A large-effect QTL for grain weight in rice on chromosome 10. *Aus J Crop Sci* **9**: 372-377.

48. Singh P, Tiwari P, Prajapat RK, Sabatta BC & Kansal R. 2016. Use of *Vigna radiata* lectin gene in development of transgenic *Brassica juncea* resistant to aphids. *Intl J Agri Sci Res* **6**: 199-209.

49. Singh RK, Singh VK, Raghavendrarao S, Phanindra MLP, Venkat Raman K, Solanke AU, Kumar PA & Sharma TR. 2015. Expression of finger millet *EcDehydrin7* in transgenic tobacco confers tolerance to drought stress. *Appl Biochem Biotechnol* **177**: 207-216.

50. Singh S, Chand S, Singh NK & TR Sharma. 2015. Genome-wide distribution, organisation and functional characterization of disease resistance and defence response genes across rice species. *PLoS ONE* **10**: e0125964.

51. Singh WH, Kapila RK, Sharma TR & Rathour R. 2015. Genetic and physical mapping of a new allele of *Pik* locus from *japonica* rice 'Liziangxintuanheigu. *Euphytica* DOI: 10.1007/s10681-015-1437-9.

52. Sinha SK, Rani M, Bansal N, Gayatri, Venkatesh K & Mandal PK. 2015. Nitrate starvation induced changes in root system architecture, carbon-nitrogen metabolsim and miRNAs expression in nitrogen responsive wheat genotypes. *Appl Biochem Biotechnol* **177**: 1299-1312.

53. Sundaresha S, Sreevathsa R, Appanna VK, Arthikala M-K, Shanmugam NB, Shashibhushan NB, Hari Kishore CM, Pannerselvam R, Kirti PB & Udayakumar M. 2016. Co-overexpression of *Brassica juncea NPR1 (BjNPR1)* and *Trigonella foenum-graecum defensin (Tfgd)* in transgenic peanut provides comprehensive but varied protection against *Aspergillus flavus* and *Cercospora arachidicola*. *Plant Cell Rep* DOI: 10.1007/s00299-016-1945-7 .

54. Thakur S, Singh PK, Das A, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Chand D, Singh NK & Sharma TR. 2015. Extensive sequence variation in rice blast resistance gene *Pi54* makes it broad spectrum in nature. *Front Plant Sci* DOI:10.3389/fpls.2015.00345.

55. Tiwari M, Krishnamurthy S, Shukla D, Kiiskila J, Jain A, Datta R, Sharma N & Sahi SV. 2016. Comparative transcriptome and proteome analysis to reveal the biosynthesis of gold nanoparticles in *Arabidopsis*. *Sci Rep* **6**: 21733.

56. Tiwari P, Singh P, Prajapat RK, Sabatta BC & Kansal R. 2016. Cloning of *Vigna mungo* protease inhibitor gene and its efficacy against aphids. *Res J Agri Sci* (Accepted).

57. Tiwari S, Krishnamurthy SL, Singh B, Amitha Mithra SV, Atmakumari R Rao & Singh NK. 2016. Mapping QTLs for salt tolerance in rice (*Oryza sativa* L.) by bulked segregant analysis of recombinant inbred lines using 50K SNP chip. *PLoS ONE* **11**: e0153610.

58. Vemanna VS, Swetha TN, Sheela SH, Babitha CK, Sreevathsa R, Reddy MK, Tuteja N, Reddy CP, Prasad TG & Udayakumar M. 2016. Simultaneous expression of regulatory genes associated with specific drought adaptive traits improves drought adaptation in peanut. *Plant Biotech J* **14**: 1008-1020.

59. Vijayan J, Devanna BN, Singh NK & TR Sharma. 2015. Cloning and functional validation of early inducible *Magnaporthe oryzae* responsive CYP76M7 promoter from rice. *Front Plant Sci* **6**: 371.

60. Waditee-Sirisattha R, Kageyama H, Fukaya M, Rai V & Takabe T. 2015. Nitrate and amino acid availability affects glycine betaine and mycosporine-2-glycine in response to changes of salinity in a halotolerant cyanobacterium *Aphanothece halophytica*. *FEMS Microbiol Lett* **362**: DOI: 10.1093/femsle/fnv198.

61. Wagaba H, Patil BL, Mukasa S, Alicai T, Fauquet CM & Taylor NJ. 2016. Artificial microRNA-derived resistance to Cassava brown streak disease. *J Virol Methods* **231**: 38-43.

Review Articles

1. Farooq M, Gogoi N, Barthakur S, Baroowa B, Bharadwaj N, Alghamdi SS & Siddique KHM. 2015. Drought stress in grain legumes during reproduction and grain filling. *J Agro Crop Sci* DOI:10.1111/jac.12169.

2. Prajapat RK, Pawar D, Singh P, Tiwari P, Kumar S & Kansal R. 2016. Legume lectins: a promising

candidate for confronting a plethora of biotic stresses. *Ind Res J of Gen and Biotech* (Accepted).

Book Chapters

1. Barthakur S. 2016. Plant derived vaccines: progress, innovations, opportunities and perspectives. In: *Biotechnological Tools for Identification and Utilization of Genetic Resources* (Deka PC, ed.), pp 124-140, Astral International, New Delhi.
2. Dalal M. 2016. Sorghum: transformation and regeneration. *In: Compendium of Plant Genomes: Sorghum* (Rakshit S, Campbell B & Godwin I, eds.) Springer-Verlag New York Inc.
3. Hema M, Patil BL, Celia ChalamV & Kumar PL. 2015. Soybean mosaic. In: *Virus Diseases of Tropical and Subtropical Crops* (Tennant P & Fermin G, eds.), CAB International, Nosworthy Way Wallingford Oxfordshire, UK.
4. Sharma M & Kansal R. 2016. Crop improvement methods: a walk through. In: *Biotechnology: Progress and Application* (Saif H & Zeeshan F, eds.), pp181-206, Daya Publishing House, New Delhi.
5. Solanke AU, Tribuvan KU & Kanika. 2015. Genomics: an integrative approach for molecular biology. In: *Biotechnology: Progress & Prospects* (Khurana S M Paul & Singh M, Eds.), pp 238-274, Studium Press LLC, USA.
6. Venkat Raman K, Aggarwal D, Solanke AU, Sreevathsa R & Pattanayak D. 2015. Transgenic technology: applications in agriculture and plant sciences. In: *Biotechnology: Progress & Prospects* (Khurana S M Paul & Singh M, Eds.), Studium Press LLC, USA.

Papers in Seminar/Symposia/ Conference

1. Ali S, Chandrashekar N, Rawat S, Nayankantha NMC, Mir ZA & Grover A. 2015. Expression analysis of *Brassica juncea PR2* gene and its promoter in response to fungal infection, plant defense hormones and wounding. In: *11th JK Science Congress 2105 on Scientific, Social and Economic Dimensions of Climate Change*, held on October 12- 14, 2015, Srinagar, India.
2. Ali S, Nayankantha NMC, Rawat S, Mir ZA & Grover A. 2015. Development of *NPR1* overexpressing *Brassica juncea* transgenic plants for improved resistance to Alternaria blight. In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.
3. Awakale P & Dalal M. 2015. Genetic variability for inductive root traits under osmotic stress in progenitors of bread wheat (*T. aestivum* L.) (S551). In: *National Symposium on "Germplasm to Genes: Harnessing Biotechnology for Food Security and Health"* held on August 9-11, 2015, New Delhi.
4. Awakale P, Tiwari S & Dalal M. 2015. Transcriptional regulation of *BREVIS RADIX* (*BRX*) in progenitors of bread wheat with contrasting root traits, (PB150). In: *3rd International Plant Physiology Congress (IPPC) "Challenges and Strategies in Plant Biology Research"* held on Dec 11-14, 2015, New Delhi.
5. Barthakur S, Hasan M & Khomdram S. 2015. Terminal stress induced *S-phase kinase associated protein1* (*Skp1*) from bread wheat: regulation and characterization. In: *International Wheat Congress*, pp 146, held on September 20-25, 2015, Sydney.
6. Bhattacharya RC, Bhatt D, Jain V, Dhatwalia D, Singla M, Kalia V, Gujar GT, Kalia P, Russel lD & Bhatnagar RK. 2015. Development of transgenic Indian mustard against mustard aphid. In: *The VII International Workshop on Management of the Diamond Backmoth and Other Crucifer Insect Pests,* held on March 23-27, 2015 at UAS, Bengaluru.
7. Boopalakrishnan G, Kumar A, Solanke AK, Kumar K & Padaria JC. 2015. Transformation of elite Indian wheat cultivar for thermotolerance using *CSPA* gene. In: *Global Research Initiatives for Sustainable Agriculture and Allied Sciences,* held on December 12-13, 2015 at Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior.
8. Chandra Prakash, Pattnaik S, Ramkumar MK, Viswanathan C, Mohapatra T, Amitha Mithra SV & Singh NK. 2015. Global expression profiling of rice miRNAs at flowering stage under drought stress. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology*

for Food Security and Health, held on August 9-11, 2015, New Delhi.

9. Chaturvedi U, Prakash A & Padaria JC. 2015. Embryo targeted *in planta* transformation in elite cultivars of wheat for developing thermotolorant trasngenics. In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health*, pp 17, held on August 9-11, 2015, New Delhi.

10. Deka S & Barthakur S. 2016. Overexpression of indica rice *annexin4* (*AnnOsI4*) imparts drought and heat tolerance in *Arabidopsis thaliana*. In: *Arabidopsis 2016 Emerging Challenges in Plant Biology*, pp 50, held on March 20-22, 2016, Mohali.

11. Gayatri, Sinha S, Sinha SK, Dalal M, Choudhary A & Mandal PK. 2015. Differential gene expression of N-metabolizing enzymes in diverse wheat genotypes under N-starvation. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, pp 46, August 9-11, 2015, New Delhi.

12. Gothandapani S, Das HK, Sekhar S & Padaria JC. 2015. *In vitro* manipulation of *nif LA* operon in *Azotobacter chroococcum* for efficient nitrogen fixation. In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health,* pp120, held on August 9-11, 2015, New Delhi.

13. Gupta NC, Waldia R, Tiwari P & Rao M. 2016. Activation tagging in *Arabidopsis*: a powerful tool for gene discovery. In: *National Seminar on Plant Genomics and Biotechnology*, pp. 25, held on January 23-24, 2016, OUAT, Bhubaneshwar, Odhisa.

14. Gupta RK, Dalal M, Sinha SK, Thomas J, Choudhury PR, Ahlawat AK, Singh AM & Mandal PK. 2015. A modified protocol for sequential extraction of gliadins, glutenins and other proteins from wheat seeds (S607). In*: National Symposium on "Germplasm to Genes: Harnessing Biotechnology for Food Security and Health"* held on August 9-11, 2015, New Delhi.

15. Jain P, Singh PK, Singh AK, Gopala KS, Sharma V, Sharma TR. 2015. *RNA-Seq* study reveals candidate genes for blast resistance in rice- *Magnaporthae* pathosystem. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

16. Jain PK, Bhat SR & Srinivasan R. 2015. *Arabidopsis* promoters for directing need-based gene expression in plants. In: *The National Symposium on 'Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*', organized by Society for Plant Biochemistry and Biotechnology, held on August 9-11, 2015, New Delhi.

17. Khomdram S & Barthakur S. 2016. Terminal heat stress induced transcriptional activation of a WRKY transcription factor from Indian bread wheat. In: *BITS Conference on Biotechnology and Genomics,* pp 46, held on February 18-20, 2016, Pilani.

18. Khomdram S & Barthakur S. 2016. Terminal heat stress induced transcriptional activation of a WRKY transcription factor (*TaWRKY10*) from bread wheat: regulation and characterization. In: *Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, pp 46, August 9-11, *2015, New Delhi.*

19. Kohli D, Kumar A, Bharadvaja N, Subramaniam K, Sirohi A, Srinivasan A & Jain PK. 2015. Identification of a vital development gene, *glp-1*, and its utilization in RNAi mediated silencing for conferring resistance against root-knot nematode in *Arabidopsis* (S124). In: *The National Symposium on 'Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*', pp. 115, organized by Society for Plant Biochemistry and Biotechnology, held on August 9-11, 2015, New Delhi.

20. Kumar V, Tiwari KK, Parida SK, Singh A, Jain S, Singh NK, Mohapatra T & Amitha Mithra SV. 2015. Evolutionary significance, genetic structure and linkage disequilibrium pattern in a diverse set of rice germplasm. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health,* held on August 9-11, 2015, New Delhi.

21. Kushwaha AK, Gagandeep G, Nagarajan M, Bhowmick PK, Amitha Mithra SV, Singh AK & Gopala Krishnan S. 2015. Marker assisted

identification of potential restorers and assessment of their restoration potential in rice germplasm. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi.

22. Lone S, Malik A & Padaria JC. 2015. Selection and characterization of *Bacillus thuringiensis* strains from North-west Himalayas toxic to *Helcoverpa armigera*. In: *7th International Conference on Bacillus anthracis, B. cereus & B. thuringiensis (Bacillus ACT 2015),* held on October 27-31, 2015, New Delhi.

23. Mandeep K, Rai AK, Singh PK, Devanna BN, Arora K, Singh J, Kapoor R & Sharma TR. 2015. Gene stacking: a most imposing approach to get durable resistance in rice against blast disease. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

24. Meena RP, Gaikwad K, Vishwakarma H, Rao AK, Sahu S, Satyavati CT, Pal M & Padaria JC. 2015. Comparative transcriptome analysis of differentially expressed genes in perl millet *Pennisetum glaucum* (L.) during drought stress condition. In: *3rd International Plant Physiology Congress*, pp 216, held on December 11-14, 2015, New Delhi.

25. Meena RP, Yadav R & Padaria JC. 2015. Development of SSH Library from *Pennisetum Glaucum* for identification of drought stress responsive gene. In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health*, pp 65, held on August 9-11, 2015, New Delhi.

26. Mohanrao MM, Singh DP, Goyal E, Singh AK & Kanika K. 2015. Microbial diversity analysis of hot water spring of Tattapani, Himachal Pradesh, through metagenomic approach. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, pp 31, held on 8-10 August, New Delhi.

27. Mohanrao MM, Singh DP, Kanika K, Goyal E & Singh AK. 2016. Metagenomic approach to explore microbial diversity of hot spring, Tattapani, India. In: *2nd International Conference on New Challenges in Biotechnology and Molecular Biology in the Context of 21st Century*, pp 63, held on February 26-27, 2016, Agra.

28. Negi M, Sanagala R, Rai V & Jain A 2015. An efficient element-contamination-free hydroponic system for dissecting phosphate deficiency-mediated response of the root system and molecular traits in rice. In: 3rd *International Plant Physiology Congress* (IPPC-2015), held on December 11- 14, 2015 at JNU, New Delhi.

29. Negi M, Sanagala R, Rai V & Jain A 2015. Elucidation of the effects of iron availability on phosphate deficiency-mediated morphophysiological and molecular responses of the root system of rice using redesigned, aerated hydroponic system. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health'* held on August 9-11, 2015, at NRCPB, New Delhi.

30. Padaria JC. 2015. *In planta* transformation for development of thermotolerent transgenic wheat In: *Global Research Initiatives for Sustainable Agriculture and Allied Sciences*, pp 260-261, held on 12-13 December, 2015 at Rajmata vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior.

31. Pargi N & Barthakur S. 2015. Modulation of *SnRK1* under terminal heat stress in bread wheat *Triticum aestivum.* In: *ISPP West Zonal Seminar on Enhancement of Crop Productivity through Physiological Intervention,* pp 31, held on May 11, 2015, Navsari.

32. Patil BL. 2015. Mapping and identification of Geminivirus genome sequences for efficient RNAi expression and resistance by transient agro-infiltration studies. In: *24th National Virology Conference,* held on October 8-10, 2015, NEIGRIHMS, Shillong.

33. Patil BL. 2015. Reconstitution of pigeonpea sterility mosaic virus genome sequences by next generation sequencing (NGS) of small RNAs, using Ion Proton Technology. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held during August 9-11, 2015, New Delhi.

34. Pattnaik S, Amitha Mithra SV, Panigrahi J, Behera N, Singh NK & Mohapatra T. 2016. Drought responsive miRNA expression profiling in rice F1 hybrids and their parental lines. In: *International Conference on Emerging Trends in Biomedical Sciences* (ETBS-2016), held on March 6-8, 2016, Aligarh Muslim University, Aligarh.

35. Pattnaik S, Tiwari KK, Chandra Prakash, Kumar V, Sandhu M, Amitha Mithra SV, Panigrahi J, Behera N, Singh NK & Mohapatra T. 2015. Physiological and molecular basis of water deficit stress tolerance in F1 hybrids and their parental lines. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health,* held on August 9-11, 2015, New Delhi, India.

36. Ramakrishna Ch, Sonam, Kanika, Padaria JC, Solanke AU & Sharma TR. 2015. Molecular characterization of bZIP transcription factor from finger millet (*Eleusine coracana* L.) under abiotic stresses. In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health,* pp 53, held on August 9-11, 2015, New Delhi.

37. Rani M, Mandal PK & Sinha SK. 2015. Natural variation in nitrate starvation induced root system architecture in wheat genotypes. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, pp 39, held on August 9-11, 2015, New Delhi.

38. Rao M, Kaushik M, Gupta NC, Chamola R & Bhat SR. 2015. Resynthesis of *B. juncea*: enriching diversity for mustard improvement. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, pp 78, held on August 9-11, 2015, New Delhi, India.

39. Rawal HC, Kiran K, Dubey H, Jaswal R & Sharma TR. 2015. Application of PERL scripts in accelerating genome data analysis. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

40. Richa, Tiwari IM, Nagar R, Sharma V, Botella J & Sharma TR. 2015. Genetic transformation of rice with chitinase gene, *LOC_Os11g47510,* for sheath blight resistance. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

41. Shankar P, Gothandapani S, Das HK & Padaria JC. 2015. *In vitro* manipulation of *Azotobacter vinelandii* use as biofertilizer In: *Crop Production in Global Research Initiatives for Sustainable Agriculture and Allied Sciences,* pp 268-269, held on December 12-13, 2015 at Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior.

42. Shankar P, Gothandapani S, Das HK & Padaria JC. 2016. Genetic manipulation of *Azotobacter vinelandii* for efficient nitrogen fixation under changing global climate In: *International Conference on Climate Change and Food Security: Ethical Perspective*, pp 208, held on February 11-13, 2016 at Hyderabad.

43. Sharma L, Verma R, Vinjamuri S, Kushwaha SR, Dalal M & Chinnusamy V. 2015. Auxin plays a critical role in spikelet fertility of rice under drought and high temperature stresses, (PG329). In: *3rd International Plant Physiology Congress (IPPC) "Challenges and Strategies in Plant Biology Research"* held on Dec 11-14, 2015, New Delhi.

44. Sharma M, Devanna BN, Singh NK & Sharma TR. 2015. Comparative genome analysis reveals species-specific genes involved in the different life styles of fungi. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

45. Singh NK, Mahato AK, Jayaswal PK, Singh A, ... Rai A & Sharma TR. 2015. Assembly of highly heterozygous mango (*Mangifera indica* cv. Amrapali) genome using PacBio long sequence reads. In: *Plant and Animal Genome XXIV Conference,* held on Jan 8-13 2016, Sandiego, California, USA.

46. Singh PK, Thakur S, Ray S, Jain P, Kumar S, Rathour R, Sharma V & Sharma TR. 2015. Co-evolutionary dynamics of rice-*Magnaporthe oryzae* host-pathogen system. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

47. Singh S, Mithra ACR, Singh GP, Singh PK, Singh VK, Singh AK & Padaria JC. 2015. Marker-assisted breeding for developing wheat variety to resistance for Bipolaris leaf spot (*Bipolaris sorokiniana*). In: *National Symposium on Germplasm to Gene: Harnessing Biotechnology for Food Security and Health,* pp 53, held on August 9-11, 2015, New Delhi.

48. Sinha S, Gayatri, Dalal M, Sinha SK & Mandal PK. 2015. Differential expression of enzymes involved in organic acid metabolism in relation to nitrogen use efficiency in wheat (S606). In*: National symposium on "Germplasm to genes: Harnessing Biotechnology for Food Security and Health"* held on August 9-11, 2015, New Delhi.

49. Sinha SK, Rani M & Mandal PK. 2016. Identification and characterization of nitrate starvation induced novel microRNAs in wheat. In: *BITS Conference on Gene and Genome Regulation (BCGGR-2016),* pp 42, held on February 18-20, 2016, Birla Institute of Technology & Sciences, Pilani Rajasthan.

50. Solanke AU, Kanakachari M, Ahmed I & Sonam. 2015. Use of genomics approaches for understanding the diversity in fruit size, shape and colour in brinjal (*Solanum melongena* L.) varieties. In: *1st National Conference on "Approaches towards Protection of Biological Resources*" held on May 8-9, 2015, Shobhit University, Meerut, India.

51. Solanke AU, Kumar V, Singh PK & Sharma TR. 2015. Identification of new panicle blast resistance genes from wild rice accessions. In: *National Symposium on Nanotechnology: Environmental, Economic, Social and Health Perspectives*, held on January 22-23, 2016, Savitribai Phule Pune University, Pune, India.

52. Solanke AU, Kumar V, Singh VK, Rthour R & Sharma TR. 2015. Mapping of panicle blast resistance genes in rice. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

53. Tiwari I, Jesuraj A, Kamboj R, Nagar R, Solanke AU, Bottella J & Sharma TR. 2015. Use of host delivered RNA interference for increasing tolerance to sheath blight pathogen (*Rhizoctonia solani*) in transgenic rice. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

54. Tiwari T, Mir ZA, Monga P, Solanke AU & Padaria JC. 2015. Transformation of elite Indian bread wheat with *DREB* gene from finger millet (*Eleusine coracana*). In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

55. Tomar RS, Kumar V, Mahato A, Kaushik S, Yadav B, Sharma N, Chaudhary C, Yadav IS, Sharma P, Gupta OP, Amitha Mithra SV, Gaikwad K, Sharma TR, Khurana P, Khurana JP, Simkova H, Dolezel J, Rogers J, Gill BS, Singh K & Singh NK. 2015. Physical mapping and sample sequencing of chromosome 2AS specific BACs in wheat using SNaPshot technology. In: *National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health*, held on August 9-11, 2015, New Delhi, India.

56. Tyagi A, Gayatri, Mandal PK & Sinha SK. 2016. Effects of carbon and nitrogen sources on nitrate uptake system in wheat at seedling stage. In: *BITS Conference on Gene and Genome Regulation (BCGGR-2016),* pp 53, held on February 18-20, 2016, Birla Institute of Technology & Sciences, Pilani Rajasthan.

57. Vishwakarma H & Padaria JC. 2015. Microarray analysis of heat stress responsive gene in thermotolerant wheat cultivar. In: *International Conference on Plant Genomics*: *Agri Genomics India*, held on August 20-21, 2015.

58. Vishwakarma H, Shekhar S & Padaria JC. 2016. Isolation and characterization of heat inducible promoter from wheat. In: *International Conference on Climate Change and Food Security*: *Ethical Perspective,* held on February 11-13, 2016 at Hyderabad.

59. Yadav S, Lekshmy S, Verma R, Vinjamuri S, Dalal M & Chinnusamy V. 2015. Isolation and characterization of *UDP-glucose pyrophosphatase* (*UGPase*) promoter from potato (*Solanum

tuberosum L.) (PI414). In: *3rd International Plant Physiology Congress (IPPC) "Challenges and Strategies in Plant Biology Research"* held on Dec 11-14, 2015, New Delhi.

Popular Articles

1. Barthakur S. 2015. Sweet potato: the first ever naturally transgenic food crop. *Agbiotech Digest* **6**(1): 1-2
2. Gupta NC & Rao, M. 2016. An Arabidopsis phenomics study in T-DNA insertion mutant population. *Biotech-Research, http://www.biotecharticles.com/Biotech-Research-Article/An-Arabidopsis-Phenomics-Study-in-T-DNA-Insertion-Mutant-Population-3514.*
3. Gupta NC, Samkumar A & Rao M. 2016. Sclerotinia stem rot disease and its management in *Brassica spp*. *Agriculture*, *http://www.biotecharticles.com/Agriculture-Article/Sclerotinia-Stem-Rot-Disease-and-its-Management-in-Brassica-Sp-3515.*
4. Reddy SVR, Sharma RR, Barthakur S & Srivastav M. 2015. Rapid- and- efficient RNA isolation protocol for recalcitrant tissues of mango. *ICAR News* **21(4)**: 1-2.

20 DISTINGUISHED VISITORS

16-12-2015	Prof. Oron Shagrir, Vice Rector and Prof. Shmuel Wolf, Dean, Hebrew University

21 IMPORTANT COMMITTEES

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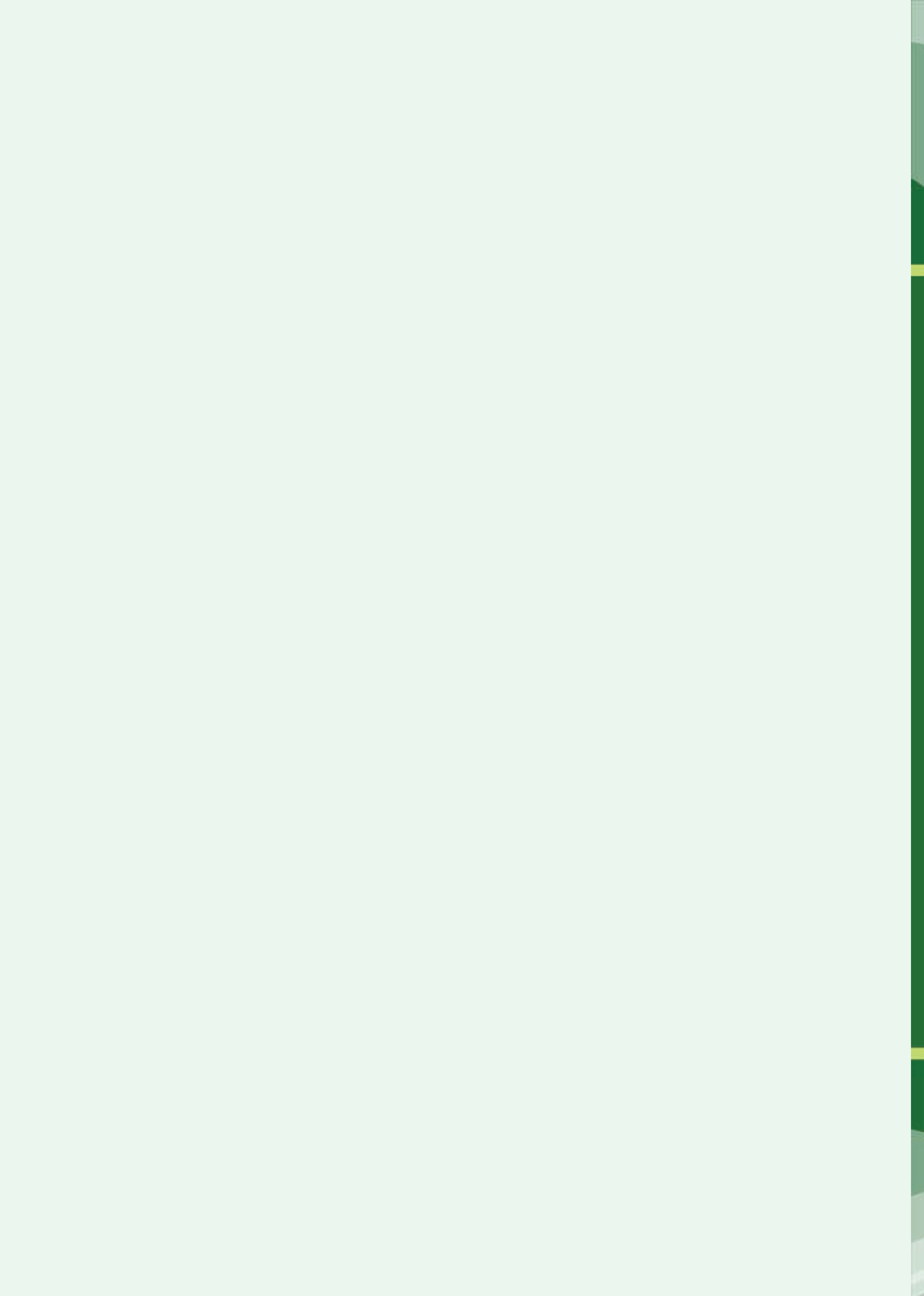

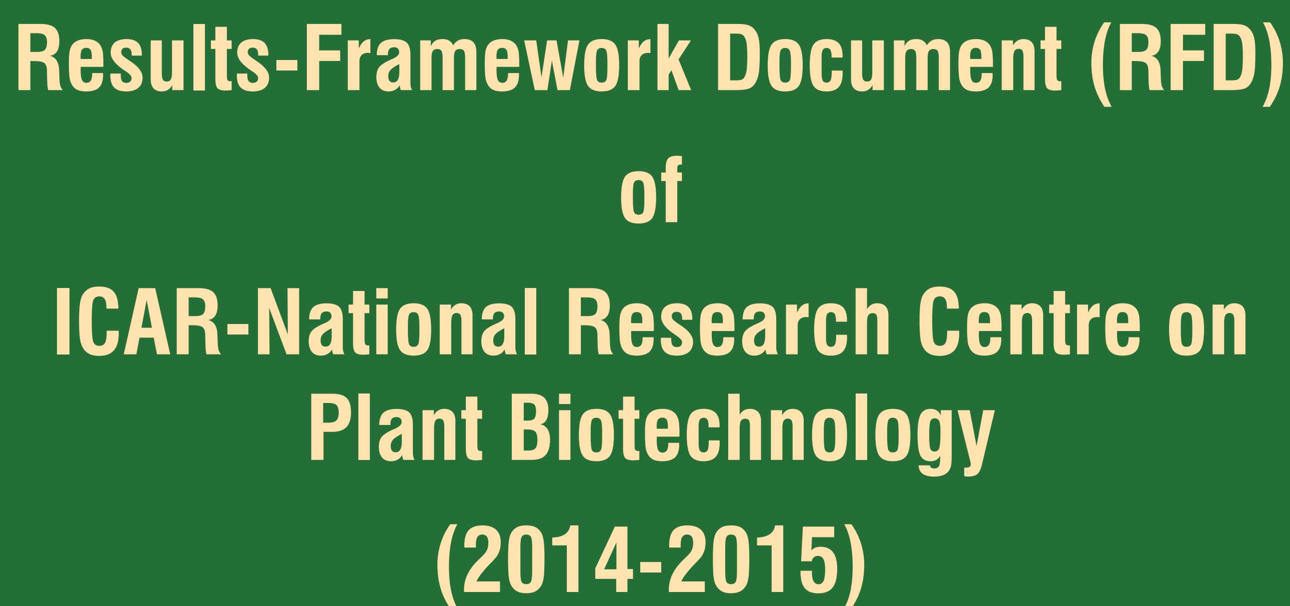
Results-Framework Document (RFD)
of
ICAR-National Research Centre on
Plant Biotechnology
(2014-2015)

22 ICAR–NRCPB 2014–15

Section 1:

Vision, Mission, Objectives and Function

Vision:

Contributing to sustainable food, nutritional, ecological and livelihood security of the country through development and deployment of tools and techniques of modern plant biotechnology

Mission:

Enhancing and sustaining crop productivity and quality by generating and harnessing the genomic, bioinformatic and trained human resources in harmony with ecology and environment

Objectives:

1. Strengthening frontier molecular biology research for enhancing agricultural production and productivity
2. Capacity building in plant biotechnology

Functions:

- To undertake plant molecular biology research for understanding molecular mechanisms underlying basic biological processes
- To devise tools and techniques of biotechnology for crop improvement
- To apply the knowledge of genomics and bioinformatics for advancing agricultural production
- To serve as a national lead centre for plant molecular biology and biotechnology research and create trained manpower in the area of plant biotechnology.

Section 2: *Inter se* priorities among Key objectives, Success Indicators and Targets

S. No.	Objectives	Wt	Actions	Success Indicators	Unit	Wt	Targets/ Criteria Value				
							Excellent	V. Good	Good	Fair	Poor
							100	90	80%	70%	60%
1.	Strengthening frontier molecular biology research for enhancing agricultural production and productivity	58	Generation of genomic resources base for gene discovery and crop genetic enhancement	Generation of genomic resources	Mb	10	540	450	360	270	180
				Generation of EST sequences	Mb	10	840	700	560	420	280
			Identification and isolation of useful genes and promoters for the development of transgenics	Cloning and characterization of genes	Number	10	15	12	9	6	3
				Designing of constructs for transgenic development	Number	10	10	8	6	4	2
				Mapping/tagging of agronomically important QTLs/genes	Number	8	8	7	6	5	4
			Maintenance and up gradation of GM crop & plant genome database at nrcpb.org	Volume of data added as genomic and EST resources	Number (lakhs)	10	25	20	15	10	5
2	Capacity building in plant biotechnology	22	Human resource development	M.Sc. degree awarded	Number	8	6	5	4	3	2
				Ph.D. degree awarded	Number	8	4	3	2	1	0
				Long term trainees (training on plant biotechnology research at NRCPB)	Number	6	42	35	28	21	14
*	Publication/ Documentation	5	Publication of the research articles in the journals having the NAAS rating of 6.0 and above	Research articles published	No.	3	38	35	32	29	26
			Timely publication of the Institute Annual Report (2013-2014)	Annual Report published	Date	2	30.06.2014	02.07.2014	04.07.2014	07.07.2014	09.07.2014

S. No.	Objectives	Wt	Actions	Success Indicators	Unit	Wt	Targets/ Criteria Value				
							Excellent	V. Good	Good	Fair	Poor
							100	90	80%	70%	60%
	Fiscal resource management	2	Utilization of released plan fund	Plan fund utilized	%	2	98	96	94	92	90
	Efficient Functioning of the RFD System	3	Timely submission of Draft RFD for 2014-2015 for Approval	On-time submission	Date	2	May 15, 2014	May 16, 2014	May 19, 2014	May 20, 2014	May 21, 2014
			Timely submission of Results for 2013-2014	On-time submission	Date	1	May 1 2014	May 2 2014	May 5 2014	May 6 2014	May 7 2014
	Enhanced Transparency / Improved Service delivery of Ministry/Department	3	Rating from Independent Audit of implementation of Citizens' / Clients' Charter (CCC)	Degree of implementation of commitments in CCC	%	2	100	95	90	85	80
			Independent Audit of implementation of Grievance Redress Management (GRM) system	Degree of success in implementing GRM	%	1	100	95	90	85	80
	Administrative Reforms	7	Update organizational strategy to align with revised priorities	Date	Date	2	Nov.1 2014	Nov.2 2014	Nov.3 2014	Nov.4 2014	Nov.5 2014
			Implementation of agreed milestones of approved Mitigating Strategies for Reduction of potential risk of corruption (MSC)	% of implementation	%	1	100	90	80	70	60
			Implementation of agreed milestones for ISO 9001	% of implementation	%	2	100	95	90	85	80
			Implementation of milestones of approved Innovation Action Plans (IAPs)	% of implementation	%	2	100	90	80	70	60

Section 3: Trend Values of the Success Indicators

S. No.	Objective	Action	Success Indicator	Unit	Actual Value for FY 12-13	Actual Value for FY 13-14	Target Value for FY 14-15	Projected Value for FY 15-16	Projected Value for FY 16-17
1.	Strengthening frontier molecular biology research for enhancing agricultural production and productivity	Generation of genomic resources base for gene discovery and crop genetic enhancement	Generation of genomic resources	Mb	200	200	450	500	500
			Generation of EST sequences	Mb	620	600	700	750	750
		Identification and isolation of useful genes and promoters for the development of transgenics	Cloning and characterization of genes	Number	5	12	12	15	15
			Designing of constructs for transgenic development	Number	5	5	8	8	8
			Mapping/tagging of agronomically important QTLs /genes	Number	6	8	7	8	8
		Maintenance and up gradation of GM crop & plant genome database at nrcpb.org	Volume of data added as genomic and EST resources	Number (millions)	18	40	20	25	25
2	Capacity building in plant biotechnology	Human resource development	M.Sc. degree awarded	Number	5	4	5	4	4
			Ph.D. degree awarded	Number	2	3	3	3	4
			Long term trainees (training on plant biotechnology research at NRCPB)	Number	47	38	35	37	38
*	Publication/ Documentation	Publication of the research articles in the journals having the NAAS rating of 6.0 and above	Research articles published	No.	35	35	35	36	36
		Timely publication of the Institute Annual Report (2013-2014)	Annual Report published	Date	-	-	02.07.2014	-	-
	Fiscal resource management	Utilization of released plan fund	Plan fund utilized	%	100	100	96	96	96
	Efficient Functioning of the RFD System	Timely submission of Draft RFD for 2014-2015 for Approval	On-time submission	Date	-	-	May 16, 2014	-	-
		Timely submission of Results for 2013-2014	On-time submission	Date	-	-	May 2 2014	-	-

S. No.	Objective	Action	Success Indicator	Unit	Actual Value for FY 12-13	Actual Value for FY 13-14	Target Value for FY 14-15	Projected Value for FY 15-16	Projected Value for FY 16-17
	Enhanced Transparency / Improved Service delivery of Ministry/ Department	Rating from Independent Audit of implementation of Citizens' / Clients' Charter (CCC)	Degree of implementation of commitments in CCC	%	-	-	95	-	-
		Independent Audit of implementation of Grievance Redress Management (GRM) system	Degree of success in implementing GRM	%	-	-	95	-	-
	Administrative Reforms	Update organizational strategy to align with revised priorities	Date	Date	-	-	Nov.2 2014	-	-
		Implementation of agreed milestones of approved Mitigating Strategies for Reduction of potential risk of corruption (MSC)	% of implementation	%	-	-	90	-	-
		Implementation of agreed milestones for ISO 9001	% of implementation	%	-	-	95	-	-
		Implementation of milestones of approved Innovation Action Plans (IAPs)	% of implementation	%	-	-	90	-	-

Section 4 (a): Acronyms

S.No	Acronym	Description
1	EST	Expressed Sequence Tags
2	Mb	Mega base pair
3	QTL	Quantitative Trait Loci
4	GM	Genetically Modified
5	NRCPB	National Research Centre on Plant Biotechnology
6	M. Sc.	Master of Science
7	Ph. D.	Doctorate in Philosophy
8	ASRB	Agricultural Scientist Recruitment Board
9	CSIR	Council for Scientific and Industrial Research
10	SAUs	State Agricultural Universities
11	NARS	National Agricultural Research System

Section 4 (b): Description and definition of success indicators and proposed measurement methodology

Sl. No.	Success Indicator	Description	Definition	Measurement	General Comments
1	Generation of genomic resources	Genome sequencing projects are the basis for many aspects of molecular biology and precise crop breeding or enhancement such as gene discovery, isolation and marker development	Total no. of bases sequenced in different crops genome	Mb	-
2	Generation of EST sequences	This is also sequence information related or specific to functional aspects of gene	Total no. of bases sequenced in different ESTs of different crops and microbes under stress	Mb	-
3	Cloning and characterization of genes	This is an important step in unraveling the role of individual genes	Total no. of genes cloned and characterized	Number	-
4	Designing of constructs for transgenic development	This is an important step in transgenic development which are required for both functional validation of genes and crop improvement	How many number of transgenics have been developed	Number	-
5	Mapping/tagging of agronomically important QTLs / genes	This is an important step for dissecting agronomically and nutritionally important traits which have a complex inheritance pattern	Total no. of QTLs and genes, which are mapped in different mandate crops	Number	-
6	Volume of data added as genomic and EST resources	This is major resource repository of all the sequence related information available across the world	How much volume (number of entries) of data is incorporated in the database	Number (millions)	-
7	M. Sc. degree awarded	Pertains to human resource development which is one of the major mandate of the organization	No. of students awarded M. Sc. degree during the year	Number	-

Sl. No.	Success Indicator	Description	Definition	Measurement	General Comments
8	Ph.D. degree awarded	Pertains to human resource development which is one of the major mandate of the organization	No. of students awarded Ph.D. degree during the year	Number	-
9	Long term trainees (training on plant biotechnology research at NRCPB)	Pertains to human resource development which is one of the major mandate of the organization; generates revenue	No. of long term trainees trained for dissertation work during the academic year	Number	-

Section 5: Specific performance requirement from other department that is critical for delivering agreed results

Location Type	State	Organization Type	Organization Name	Relevant Success Indicator	What is your requirement from this organization	Justification for this requirement	Please quantify your requirement from this Organization	What happens if your requirement is not met?
Urban	Delhi	Funding Agency	Department of Biotechnology	Generation of genomic resources	Project funding	Project oriented additional funding	10 %	No major impact
Urban	Delhi	Funding Agency	Department of Science and Technology	Generation of EST sequences	Project funding	Project oriented additional funding	3 %	No major impact

Section 6: Outcome/Impact of activities of Department/Ministry

Sl. No.	Outcome/ Impact	Jointly responsible for influencing this outcome / impact with the following organization(s)/ department(s)/ministry(ies)	Success Indicators	Unit	2012-13	2013-14	2014-15	2015-16	2016-17
1	Per cent students employed	ASRB/ Public funded Scientific Res. Institutes and Universities like CSIR, Central Universities, SAUs etc./ Biotechnology companies	M.Sc. degree awarded	Per cent	100	100	NA	NA	NA
			Ph.D. degree awarded	Per cent	100	100	NA	NA	NA
2	Knowledge enhancement after imparting training	NA	Long term trainees (training on plant biotechnology research at NRCPB)	Per cent	Data not available	48.78	NA	NA	NA