

**Abstracts & Souvenir**

**National Symposium on  
Germplasm to Genes: Harnessing  
Biotechnology for Food Security and Health**

**August 9-11, 2015**



**Society for Plant Biochemistry and Biotechnology**  
ICAR-National Research Centre on Plant Biotechnology  
LBS Centre, Pusa Campus, New Delhi 110 012



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***Compiled and Edited by:***

PK Mandal  
C Viswanathan  
KV Bhat  
Shelly Praveen  
Akshay Talukdar  
Monika Dalal  
BL Patil  
Gopala Krishnan S  
SV Amitha CR Mithra  
Amolkumar U Solanke  
Veda Krishnan

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# National Symposium on Germplasm to Genes: Harnessing Biotechnology for Food Security and Health

**August 9-11, 2015**

**New Delhi**

## PROGRAMME

09-08-2015	
11:00 AM – 3:00 PM	Registration at ICAR-NRC on Plant Biotechnology, IARI, New Delhi
4:00 - 4:30 PM	Inauguration
4:30 - 5:30 PM	Special Lecture: Dr. Raghavendra Gadagkar, President INSA & Professor IISc, Bengaluru
5:30 - 6:15 PM	High Tea
6:15 - 7:30 PM	Felicitatation of Prof. VL Chopra on his 80 <sup>th</sup> Birthday
8:00 PM	Dinner

10-08-2015	
<b>Technical Session I:</b> <b>Genes, Promoters and Transgenics (Coordinator: Dr Shelly Praveen)</b> <i>(Dr. Deepak Pental: Chair, Dr. HS Dhaliwal: Co-chair, Dr. BL Patil: Rapporteur)</i>	
09:00 - 09:30 AM	<b>Prof. K Veluthambi</b> (MKU, Madurai) <b>Topic:</b> Gene targeting in rice by positive/negative selection: Knock-out of the <i>OsMADS1</i> gene
09.30 - 09.50 AM	<b>Dr MV Rajam</b> (Delhi University, South Campus) <b>Topic:</b> Host induced RNA interference for resistance against <i>Fusarium</i> wilt in tomato
09:50 - 10:10 AM	<b>Dr. PK Jain</b> (ICAR-NRCPB, New Delhi) <b>Topic:</b> <i>Arabidopsis</i> promoters for directing need-based gene expression in plants
10:10 - 10:30 AM	<b>Dr. Karabi Datta</b> (Calcutta University) <b>Topic:</b> Plant genetic resources and genes used in enhancing crop improvement
10:30 - 10:45 AM	<b>Presentation: Ms Pratikshya Borah</b> (Delhi University, South Campus) <b>Topic:</b> OsFBK1, a rice F-box protein, is a component of the UPS and is involved in anther and root development
10:45 - 11:00 AM	Discussion
11:00 - 11:30 AM	Tea Break



<b>Technical Session II:</b> <b>Genomics and Bioinformatics (Coordinator: Dr KV Bhat)</b> (Dr. CR Bhatia: Chair, Dr. PK Gupta: Co-chair, Dr. K Gaikwad: Rapporteur)	
11:30 - 12:00 Noon	<b>Dr. Akhilesh Tyagi</b> (NIPGR, New Delhi) <b>Topic:</b> Plant genomes for forward and reverse genetics
12:00 - 12:20 PM	<b>Prof. NK Singh</b> (ICAR-NRCPB, New Delhi) <b>Topic:</b> Development of genomic resources for pigeonpea, jute and mango: plants uniquely important for India
12:20 - 12:40 PM	<b>Dr. V Siva Reddy</b> (ICGEB, New Delhi) <b>Topic:</b> Genomics of cotton fibre development and genome response under biotic and abiotic stress
12.40 - 01:00 PM	<b>Dr. DT Singh</b> (CloudSeq, Singapore) <b>Topic:</b> Rice genomics in the Cloud
01.00 - 01.15 PM	<b>Presentation : Mr PK Jayaswal</b> (ICAR-NRCPB, New Delhi) <b>Topic:</b> Eukaryotic tree of life based on 98 rice genes conserved in 49 other model organisms
01:15 - 01:30 PM	Discussion
01:30 - 02:30 PM	Lunch Break

<b>Technical Session III :</b> <b>Plant Development and Stress Biology (Coordinator: Dr Jasdeep Padaria)</b> (Dr. RB Singh: Chair, Dr. KC Bansal: Co-chair, Dr Monika Dalal: Rapporteur)	
2:30 - 3:00 PM	<b>Prof. JP Khurana</b> (Delhi University, South Campus) <b>Topic:</b> Characterization of blue light sensing cryptochrome 2 from <i>Brassica</i> and rice
3:00 - 3:20 PM	<b>Dr. Utpal Nath</b> (IISc, Bangalore) <b>Topic:</b> Genetic regulation of leaf size and biomass in <i>Arabidopsis thaliana</i>
3:20 - 3:40 PM	<b>Dr. Alok Krishna Sinha</b> (NIPGR, New Delhi) <b>Topic:</b> A blue light mediated MKK3-MPK6-MYC2 module regulating <i>Arabidopsis</i> seedling development
3:40 - 4:00 PM	<b>Dr. RC Bhattacharya</b> (ICAR-NRCPB, New Delhi) <b>Topic:</b> Devising strategies for aphid resistance in Indian mustard
4:00 - 4:15 PM	<b>Dr. Y Sreenivasulu</b> (IHBT, Palampur) <b>Topic:</b> Isolation and analysis of novel seed development mutants in <i>Arabidopsis</i>
4:15 - 4:30 PM	Discussion
4:30 - 5:00 PM	Tea



<b>Technical Session IV:</b> <b>Emerging Technologies (Coordinator: Dr. Debasis Chattopadhyay)</b> (Dr. EA Siddiq: Chair, Dr. PA Kumar: Co-chair, Dr. D Pattanayak: Rapporteur)	
5:00 - 6:15 PM	<b>Panel discussion</b> Dr. KK Narayanan (Metahelix, Bengaluru), Dr. M Ravi (IISER, Trivandrum), Dr. S Ganesh Ram (TNAU, Coimbatore), Dr. A Mohanty (El DuPont India. Hyderabad), Dr. Paras Yadav (ILS)
6:15 - 7:30 PM	<b>Poster session 1</b> <b>Chair:</b> Dr. Sanjay Kapoor (Delhi University, South Campus) <b>Co-Chair:</b> Dr. Manoj Prasad (NIPGR, New Delhi)
7:30 - 8:00 PM	GB Meeting of Society for Plant Biochemistry and Biotechnology <b>Chair:</b> Dr. SL Mehta, President, SPBB <b>Co-Chairs:</b> Dr. ML Lodha & Dr. Srinivasan
8:00 PM	Dinner

<b>11-08-2015</b>	
<b>Technical Session V:</b> <b>Genetic Resources, Allele Mining and Marker Assisted Breeding</b> (Coordinator: Dr Gurinder Randhawa) (Dr. JS Sandhu: Chair, Dr. KV Prabhu: Co-chair, Dr. Swarup Parida: Rapporteur)	
09:00 - 09:30 AM	<b>Dr. AK Singh</b> (ICAR-IARI, New Delhi) <b>Topic:</b> Molecular breeding for improvement of biotic and abiotic stress resistance in Basmati rice
09:30 - 09:50 AM	<b>Dr. Akshay Pradhan</b> (Delhi University, South Campus) <b>Topic:</b> Marker-assisted breeding in <i>Brassica juncea</i> – a reality check
09:50 - 10:10 AM	<b>Dr. Kuldeep Singh</b> (PAU, Ludhiana) <b>Topic:</b> Alien genetic resources in rice: their characterization and utilization for rice improvement
10:10 - 10:30 AM	<b>Dr. HD Upadhyay</b> (ICRISAT, Hyderabad) <b>Topic:</b> Mining allelic variations associated with agronomic and nutritional traits using mini core and genome-wide association mapping in large germplasm collection
10:30 - 10:45 AM	<b>Presentation: Dr. Rajkumar Zunjare</b> (ICAR-IARI, New Delhi) <b>Topic:</b> Marker-aided pyramiding of $\beta$ -carotene hydroxylase ( <i>crtRB1</i> ) and lycopene- $\epsilon$ -cyclase ( <i>lycE</i> ) genes for provitamin-A enrichment in quality protein maize (QPM) inbreds
10:45 - 11:00 AM	Discussion
11:00 - 11:30 AM	Tea Break



<b>Technical Session VI:</b> <b>Biochemistry, Proteomics and Metabolomics (Coordinator: Dr. Archana Sachdev)</b> (Dr. ML Lodha: Chair, Dr. HS Nainawate: Co-chair, Dr Ranjeet Ranjan: Rapporteur)	
11:30 - 12:00 Noon	<b>Dr. RP Sharma</b> (University of Hyderabad, Hyderabad) <b>Topic:</b> Reverse genetic modulation of ethylene biosynthesis as tool to regulate fruit ripening in tomato
12:00 - 12:20 PM	<b>Dr. Shubra Chakraborty</b> (NIPGR, New Delhi) <b>Topic:</b> Search for pathogenicity vs immunity determinates in crop plants: a proteomic approach towards translational research
12:20 - 12:40 PM	<b>Dr. RS Sangwan</b> (CIAB, Mohali) <b>Topic:</b> Specialized secondary metabolism in <i>Withania somnifera</i> (Ashwagandha): New calls for revisions in phytochemical biosynthesis concepts
12:40 - 01:00 PM	<b>Dr. AM Kayastha</b> (BHU, Varanasi) <b>Topic:</b> Plant $\beta$ -galactosidase immobilization on functionalized nano materials and their applications
01:00 - 01:15 PM	<b>Presentation: Dr. SRCK Rajendran</b> (GBPUA&T, Pantnagar) <b>Topic:</b> Synthesis and characterization of Finger millet protein stabilized nanoemulsion system for the delivery of hydrophobic bioactives
01:15 - 01:30 PM	Discussion
01:30 - 02:30 PM	Lunch

<b>Technical Session VII:</b> <b>Biosafety, IPR, Biotechnology Education and Training (Coordinator: Dr PK Mandal)</b> (Dr. KR Koundal: Chair, Dr. Paramjit Khurana: Co-chair, Dr. S Archak: Rapporteur)	
2:30 - 3:45 PM	<b>Panel Discussion</b> Dr. SR Rao (DBT, Govt. of India), Dr. S Raina (Nath Seeds, Aurangabad), Dr. Anil Kumar (GBPUA&T, Pantnagar), Dr. PC Sharma (Indraprastha University, Delhi), Dr. M Lakshmikumar (M/s Lakshmikumar & Sridharan Attorneys, New Delhi), Dr. Pratibha Brahmi (ICAR-NBPGR, New Delhi) Dr. Gurinder Randhawa (ICAR-NBPGR, New Delhi)
3:45 - 4:30 PM	<b>Poster Session II :</b> <b>Chair :</b> Dr. C Viswanathan (ICAR-IARI, New Delhi) <b>Co-chair:</b> Dr. Ashwani Pareek (JNU, New Delhi)
4:30 - 5:30 PM	Concluding session and prize distribution <b>Chair:</b> Dr. SL Mehta; <b>Co-chair:</b> Dr. R Srinivasan

# Society for Plant Biochemistry and Biotechnology

*TR Sharma and ML Lodha*

The Society for Plant Biochemistry and Biotechnology (SPBB) was established in May, 1991 with its Headquarters at Division of Biochemistry, Indian Agricultural Research Institute, New Delhi, and registered under the Societies Registration Act of XXI of 1860 on 15th November, 1991. The aims and objectives for which the society was established are as under:

- i. To advance Plant Biochemistry, Molecular Biology, Molecular Genetics and Biotechnology research in India.
- ii. To provide forum to Plant Biochemists, Molecular Biologists, Molecular Geneticists and Biotechnologists for exchange of scientific knowledge.
- iii. To foster high standards in teaching and research in the areas of Plant Biochemistry, Molecular Biology, Molecular Genetics and Biotechnology.
- iv. To collect, collate and disseminate information on various aspects of Plant Biochemical, Molecular Biology, Molecular Genetics and Biotechnological research.
- v. To work in association and cooperation with other National Societies/ Associations having similar objectives.
- vi. To publish a journal covering Plant Biochemistry, Molecular Biology, Molecular Genetics and Biotechnology research.
- vii. To do and perform all other acts, matters and things which may assist in, conduce to or be necessary for the fulfillment of the objectives and aims of the Society.

The Society has 777 life Members (upto July, 2015) including Ph.D. scholars, 02 Student/ordinary members and 5 Institutional Members. It has 24 foreign members from 12 different countries. The members are from the disciplines of Biochemistry, Biotechnology and Molecular Biology, Plant Physiology, Genetics, Microbiology, Molecular Genetics, and Mycology and Plant Pathology.

The Society had the honour of being led by distinguished scientists/academicians as Presidents : Dr. S. Ramachandran, then the Secretary, DBT (1992-1994); Dr. R.S. Paroda, then Secretary, DARE & DG, ICAR (1995-2000); Prof. V.L. Chopra, then Secretary, DARE & DG, ICAR (2000-2005); Dr. S.L. Mehta, former DDG (Education), ICAR and Vice Chancellor, MPUAT, Udaipur (2006 – till date).

As its main activity, the Society is publishing the 'Journal of Plant Biochemistry and Biotechnology' (JPBB) since January 1992. Initially, the Journal was published biannually, but since January 2013, four issues per year are being published due to heavy pressure of a large number of MS received from India and abroad. The latest Thompson Reuter 'Impact Factor' 2014 of the Journal is 1.09. It has been our endeavour to timely publish the Journal while maintaining its standard and constantly improving its quality. The members of the Society receive the Journal online, free of cost. Based on the quality/impact factor of the Journal, the Society has been graded and put in category 'A' by the Indian Council of Agricultural Research.

The Journal publishes review articles, research papers, short communications and commentaries in the areas of plant biochemistry, plant molecular biology, microbial and molecular genetics, DNA finger printing, micropropagation, and plant biotechnology including plant genetic engineering, new molecular tools and techniques, genomics and bioinformatics.

The Journal is being Abstracted/Indexed in : Science Citation Index Expanded (SciSearch), Journal Citation Reports/Science Edition, SCOPUS, Chemical Abstracts Service (CAS), Google Scholar, CSA, CAB International, Biological Abstracts, BIOSIS, CAB Abstracts, Elsevier Biobase, Food Science and Technology Abstracts, Global Health, Indian Science Abstracts, OCLC, SCImago, Summon by ProQuest (<http://www.springer.com/life+sciences/journal/13562>).



Earlier, the Journal received a rare distinction of being covered in Institute of Scientific Information (ISI)'s Electronic Library Project. From Volume 20 - Issue 1 (Jan - June, 2011), the Journal is being co-published by the Society and Springer (India) Private Limited.

Another main activity of the Society has been to organize Symposia/Seminars on topics of current National interest. Since its inception, the Society has organized the following National Symposia/Seminars and International Conference:

- i. The first National Symposium on the "Role of Plant Biochemistry and Biotechnology in Improving Crop Productivity" at the Indian Agricultural Research Institute, New Delhi from November 18-19, 1991.
- ii. The second National Symposium on the "Developments in Plant Molecular Biology & Genetic Engineering" at the Tamil Nadu Agricultural University, Coimbatore from December 29-31, 1993.
- iii. The third National Symposium on the "Role of Plant Biotechnology in Improving Agriculture-challenges and opportunities, and Physiological & Biochemical Basis of Crop Yield" at the University of Rajasthan, Jaipur from March 23-25, 1995. This Symposium was co-sponsored by the Society for Plant Physiology & Biochemistry.
- iv. The fourth National Symposium on "Current Trends in Plant Biochemistry and Biotechnology" was organized at CCS Haryana Agricultural University, Hisar from February 23-25, 1996.
- v. The fifth National Symposium on the "Role of Plant Biochemistry and Biotechnology in Improving Crop Productivity" was organized at ICAR Research Complex for NEH Region, Barapani, Meghalaya from March 18-20, 1997.
- vi. The sixth National Symposium on the "Role of Plant Biochemistry and Biotechnology in Improving Crop Productivity" was held at University of Agricultural Sciences, Bangalore from March 4-6, 1999.
- vii. The seventh National Symposium on "Biotechnology for Sustainability in Agriculture" was held at GB Pant University of Agriculture and Technology, Pantnagar from April 27-29, 2000.
- viii. The eight National Symposium on the "Relevance of Plant Biochemistry and Biotechnology-Modern Trends" was held at The American College, Madurai (TN) from March 1-3, 2001.
- ix. The ninth National Symposium on the "New Opportunities and Challenges for Improving Crop Productivity through Biotechnology" was organized at the Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar from February 13-15, 2002.
- x. ICAR sponsored Workshop on "Harnessing the Benefits of Biotechnology" was organized in collaboration with the NRC on Plant Biotechnology, Pusa Campus from March 27-29, 2008.
- xi. The first "International Conference on Plant Biotechnology for Food Security : New Frontiers" was organized by the Society and the National Research Centre on Plant Biotechnology, in association with the Indian Agricultural Research Institute, New Delhi from Feb 21-24, 2012.
- xii. The tenth National Conference on "Science of Omics for Agricultural Productivity: Future Perspectives" was organized at GBPUAT during 4-6 March, 2014.

The Society started 'Dr. N.B. Das Memorial Lecture' in 1993 in the memory of renowned Biochemist late Prof. N.B. Das, the first Head of the Division of Biochemistry, Indian Agricultural Research Institute, New Delhi. The first Memorial Lecture was delivered by Prof. M.S. Naik, then the Head, Division of Biochemistry, Indian Agricultural Research Institute, New Delhi in December, 1993 at the time of second National Symposium. Dr. P.V. Sane, then the Director, National Botanical Research Institute, Lucknow delivered the second Memorial lecture in February, 1996 at the time of the fourth National Symposium.

## About the Symposium

With the global population projected to touch 9.6 billion by 2050 and the climate change threatening agriculture, the challenge of meeting the demand for food and feed is daunting. The 'National Food Security Act 2013' of India puts extra onus on us for accelerating food production to meet the goal of making food available to the poor and needy. Thus emphasis on agricultural research, and development and adoption of farm technologies is imperative to enable sustained increase in farm productivity. Genetic improvement of plants, animals and other organisms including microbes that are part of the broad agricultural production system is expected to play a key role in this regard. The dizzying pace of discoveries in biology in general and molecular biology in particular and the imaginative innovations they are spawning hold great promise to face the above challenges. For instance, the discovery and refinement of the genome editing technologies in the past few years have opened opportunity for plant improvement which until recently was in the realm of imagination. Further, these technologies are likely to radically change our views and treatment of genetically modified crops. Similarly, the explosive growth of genomics data unleashed by the high-throughput sequencing technologies is challenging our ability to handle, comprehend and utilize the new information. It is critical, therefore, that we should quickly adapt and profit from these emerging technologies, and find the best solutions to our pressing problems of health, hunger, malnutrition and environmental degradation etc. India being one of the mega centres of plant diversity is well endowed with a rich diversity of flora and fauna. Whereas germplasm resources are being collected and conserved, their utilization has lagged behind. In this regard, modern biotechnological tools and genomic information could be applied for rapid and efficient characterization of germplasm. Further, biotechnology could be used to quickly put together the best combination of alleles into new varieties. Therefore, the present National Symposium 'Germplasm to Genes: Harnessing Biotechnology for Food Security and Health' is proposed to review the latest developments in Molecular Biology and Biotechnology and to deliberate on strategies and action plans including policy interventions and investments, to keep pace with the latest developments. This will not only enable us to participate with the global community in the discovery and innovation but also make us technology competent and self-reliant. The past experience shows that in agriculture foreign technology cannot be directly supplanted and indigenization is most critical for success. Hence, the programmes are planned under the following Technical Sessions to cover various aspects.

### Technical Sessions

1. Genes, Promoters and Transgenics
2. Genomics and Bioinformatics
3. Plant Development and Stress Biology
4. Emerging Technologies
5. Genetic Resources, Allele Mining and Marker Assisted Breeding
6. Biochemistry, Proteomics and Metabolomics
7. Biosafety, IPR, Biotechnology, Education and Training





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# Technical Session I: Genes, Promoters and Transgenic Plants

*Invited Lecture*

## Gene Targeting in Rice by Positive/Negative Selection: Knock-Out of the *OsMADS1* Gene

*P Kannan<sup>1</sup>, Bharat B Majhi<sup>1</sup>, Debjani Basu<sup>1</sup>, Grace L Chongloi<sup>2</sup>, Reena V Kartha<sup>2</sup>,  
Usha Vijayraghavan<sup>2</sup> and K Veluthambi<sup>1,\*</sup>*

<sup>1</sup>Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai-625021; <sup>2</sup>Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012 (\*Email: kveluthambi@rediffmail.com)

Gene targeting (GT) via homologous recombination is a powerful tool in functional genomics. We attempted targeted disruption of the *OsMADS1* gene by *Agrobacterium*-mediated transformation involving the 'positive/negative selection' strategy. Rice *OsMADS1* is a flower-specific gene, which controls identity of floret meristem and its determinate development. In the GT vector, the positive selectable marker hygromycin phosphotransferase gene (*hpt*) is flanked by a 4.28-kb 5' sequence of *OsMADS1* and a 4-kb 3' sequence of *OsMADS1*. The cytotoxic diphtheria toxin-A subunit gene (*DT-A*), a negative selectable marker, is placed on both ends of the T-DNA to eliminate the plants with ectopic (random) T-DNA integrations. Rice transformation of 2,643 scutellum-derived calli with the GT vector yielded two independent *hph*-resistant transgenic plants. Southern blot results confirmed that the *hph* gene was integrated as a single copy in the *OsMADS1* locus and no ectopic T-DNA integration had occurred. The T<sub>0</sub> plants, upon selfing and forwarding to the T<sub>1</sub> generation, displayed 3:1 segregation of hygromycin-resistant (Hyg<sup>R</sup>) and -sensitive (Hyg<sup>S</sup>) plants. The homozygous, knock-out T<sub>1</sub> plants were identified by Southern blotting using the 3' part of the *OsMADS1* gene as the probe. The GT frequency in our experiment was 0.1 %. In the *OsMADS1*-targeted homozygous plants, lemma and palea were open and elongated, lodicules were converted into lemma/palea-like structures, anthers were deformed and their numbers were reduced. Carpels were either absent or were replaced by flower-like structures. Thus, *OsMADS1* knock-out has been successfully achieved by gene targeting.

*Invited Lecture*

## Host Induced RNA interference for Resistance against *Fusarium* Wilt in Tomato

*MV Rajam*

Department of Genetics, University of Delhi South Campus, Benito Juarez Road,  
New Delhi 110021 (\*E-mail: rajam.mv@gmail.com)

Crop yield and quality is significantly affected by pathogens, and agrochemicals have been traditionally used for crop protection against pathogens. An alternative method to use of agrochemicals and plant breeding for disease control is transgenics. Transgenic approaches, including RNA interference (RNAi) have gained relevance for crop protection. Here, we have been exploiting host induced RNAi (HI-RNAi) technology for engineering tomato for resistance against fungal infections. We examined the potential of RNAi for the control of *Fusarium* wilt in tomato by targeting a key polyamine biosynthesis gene, ornithine decarboxylase (ODC) of the pathogen. The target fungal ODC gene fragment was cloned in



a hairpin RNA construct and generated transgenic tomato plants. These transgenic lines expressed the intended small interfering RNAs (siRNAs) and exhibited moderate to high resistance when challenged with the spores of *F. oxysporum*. These results confirm that the ODC gene is vital for fungal growth and is a suitable target for disease control through RNAi and also implicate the transfer of dsRNA/siRNAs from host plant cells to the fungal cells. To further validate the uptake of plant-derived siRNAs by fungal cells in a visual manner, silencing of the GFP transgene was observed in *F. oxysporum* GFP-transformants upon infecting the dsGFP expressing tomato transgenic plants. These results suggest that HI-RNAi can complement the existing transgenic strategies for disease control.

*Invited Lecture*

### **Arabidopsis Promoters for Directing Need-Based Gene Expression in Plants**

*Pradeep K Jain\*, SR Bhat and R Srinivasan*

ICAR-NRC on Plant Biotechnology, Pusa Campus, New Delhi (\*Email: jainpmb@gmail.com)

Gene expression is regulated, both qualitatively and quantitatively, by promoter sequences. Skillful use of genetic engineering techniques depends upon our ability to make an organism produce a gene product at the required expression level, in specified cells or tissues at appropriate time. This can only be achieved by properly fusing appropriate promoter elements with the gene. Arabidopsis with its so many advantages is an ideal source for mining of different plant promoters. Promoter trapping is the random insertion of a promoterless reporter gene in the genome followed by screening for reporter gene expression in tissue/condition-specific manner. This technique can be used for isolation of different types of promoter. More than a decade back we developed promoter trap lines which have been very useful for screening and identification of different promoters. We have isolated and characterized several tissue specific promoters, namely anther-, trichome-, root-, lateral organ junction-specific and wound-responsive. The promoter region of peroxidase gene, fatty acyl-CoA reductase gene and ethylene response factor gene was involved in anther, wound and trichome-specific expression, respectively. In plants, promoter trapping is the most convenient method to tag cryptic promoters and several cryptic promoters, including our root-specific promoter have been identified. Recently we have isolated and characterized nematode-responsive root-specific (NRRS) promoters which show preferential expression upon nematode infection, exclusively in the root in one case and in the galls in other. All these promoters have several applications and their appropriate utilization towards need based expression will be immensely useful.

*Invited Lecture*

### **Plant Genetic Resources and Genes Used in Enhancing Crop Improvement**

*Karabi Datta*

Plant Molecular Biology and Biotechnology Laboratory, Botany Department, University of Calcutta, Kolkata 700019 (\*Email: krbdatta@yahoo.com)

Germplasm available in the gene bank and grown in different agro-ecosystems or maintained by the farmers as traditional varieties or landraces provide the sources of most of the genes encoding the traits which are essential for crop improvement. In continuation of our work in sheath blight since 1995, we



have expanded this work in cloning a number of genes (e.g. *oxo*, *npr1*, etc.) and green tissue specific promoters (e.g. PD540, PEPC etc.) and introduced those genes driven by different promoters in rice. Enhanced sheath blight resistance in rice has been established with delayed vertical movement of the pathogen, *Rhizoctonia solani* with reduced infection resulting significantly less yield loss. Pyramiding PR and defense related genes also have been introduced in rice showing further enhanced sheath blight resistance. Pod borer causes significant yield loss in Chickpea. Considering the value and importance of transgenics, we have cloned the pod specific promoter (Pmsg) from soybean and introduced the fusion cry 1Ab/Ac genes driven by Pmsg and enhanced significant plant protection against the pest in Chickpea. Jute grows in dry season and faces considerable yield loss due to drought and biotic stress. First time, we have developed reproducible transformation system in jute and introduced a number of genes. Malnutrition is a severe problem in India with estimated 42% children and women are suffering from anemia and Vitamin-A deficiency. In continuation of our Golden Rice research, we have cloned and introduced a number of genes along with RNAi approach in rice to enhance iron, provitaminA and reduced phytate with a possibility of higher bioavailability of iron. It is also possible to enhance the drought and salinity tolerance in rice by introducing transcription factor genes (DREB) driven by stress inducible promoter rd29.

*Oral presentation*

### **OsFBK1, a Rice F-Box Protein, is a Component of the UPS and is Involved in Anther and Root Development**

*Pratikshya Borah<sup>1,2\*</sup>, Shane W Rydquist and Jitendra P Khurana<sup>1,2</sup>*

<sup>1</sup>Interdisciplinary Centre for Plant Genomics, University of Delhi South Campus, New Delhi;

<sup>2</sup>Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi

(\*Email: pratikshya.borah@gmail.com)

The Ubiquitin-26S proteasome system (UPS) is a large group of proteins, one of such groups is known as the SCF complex (Skp1-Cullin-Rbx1-F-box) that is involved in substrate identification and ubiquitination. The substrate adaptor, F-box, in one of the largest group of proteins found in plants. The present research deals with a rice F-box protein, OsFBK1, that has a Kelch domain at its C-terminal, a unique combination in plants. Y2H showed that OsFBK1 is a part of a unique SCF complex. Expression analysis of this gene reveals its up-regulation in reproductive stages, roots and in response to auxin and ABA. Rice transgenics over-expressing OsFBK1 and its RNAi plants clearly revealed the role of this gene in anther and root development. Electron and confocal microscopy of the transgenic anthers demonstrated differences in their morphology and lignification. The putative interacting partners of OsFBK1 were identified from Y2H libraries of anther and root tissue and their interactions were validated by Bimolecular Fluorescence Complementation and Co-immunoprecipitation assays. Thus, it could be hypothesized that OsFBK1 might be involved in lignification processes in anthers, and in controlling proliferation of roots.



## **S101: Monitoring Adventitious Presence of Transgenes in the Okra and Brinjal Germplasm being Conserved in the National Gene Bank**

*Rakesh Kumar Bairwa<sup>1</sup>, Monika Singh<sup>2</sup>, Rajesh K Bhoge<sup>2</sup>, Chitra Devi<sup>3</sup>  
and Gurinderjit Randhawa<sup>2\*</sup>*

<sup>1</sup>ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi 110 012; <sup>2</sup>Division of Genomic Resources; <sup>3</sup>Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012 (\*E-mail: gjr@nbpgr.ernet.in)

In India, several genetically modified (GM) crops/events including Bt brinjal and Bt okra have been under field trials. While harnessing their benefits, it is also imperative to study their impact on biodiversity. India is one of the 12 Mega-Diversity Centres and one of the Vavilovian Centre of Origins of crops like brinjal, okra. There are delicate pockets where wild and weedy relatives of these crops are being conserved on- farm and as ex situ collections in the National Gene Bank, hence unintentional introgression of transgenes in brinjal and okra needs to be monitored. National Gene Bank at National Bureau of Plant Genetic Resources, New Delhi has conserved 4,338 and 3,414 accessions of brinjal and okra respectively, including their wild relatives. Monitoring of adventitious presence of transgenes in conserved ex situ collections, comprising 50 accessions of okra and brinjal each was undertaken using Polymerase Chain Reaction (PCR)/Real-time PCR assays targeting CaMV 35S promoter, nos terminator along with endogenous gene. Based on preliminary studies, adventitious presence of transgenes has not been detected in any of the tested sample. To ensure GM-free conservation of germplasm in genebanks, this approach would be efficient and reliable.

## **S102: Spatio-Temporal Variation of Cry Protein in Transgenic Pigeonpea (*Cajanus cajan* L.) Lines**

*Alok Das\*, Alok Shukla, Arvind Kumar Singh and NP Singh*

ICAR-Indian Institute of Pulses Research, Kanpur 208 024, Uttar Pradesh  
(\*Email: adas@icar.org.in)

Pigeonpea (*Cajanus cajan* L. Millisp.) is an important grain legume and a major source of dietary protein for human consumption. Bt-pigeonpea presents a potential alternative for insect resistance management in pigeonpea. Presence of Cry protein in different plant tissues is preferred since pod borer larvae feed on these organs at different larval stages. Quantitative ELISA (DAS) was performed using different plant tissues and at different developmental stages to observe the expression variability. Expression was higher (18.39-39.07 ng/mg of TSP) in leaves pre-flowering (92 DAS) as compared to post flowering (160 DAS) (16.00 - 18.09 ng/mg of TSP). In pod wall the expression was higher (12.34 -13.67 ng/mg of TSP) in comparison to immature seeds (9.59 -10.1 ng/mg of TSP) and flower (10.25-13.17ng/mg of TSP). Variations in expression of Cry protein and the mechanism involved need to be fully understood, so as to plan rational insect resistance management strategy.



### **S103: Role of miRNAs in Fibre Quality and Development in Allotetraploid *G. hirsutum* and *G. barbadense***

*KV Padmalatha*<sup>1</sup>, *H Suresh*<sup>2</sup>, *IS Katageri*<sup>2</sup> and *M Udayakumar*<sup>1</sup>

<sup>1</sup>Department of Crop Physiology, University of Agricultural Sciences (UAS), Bangalore; <sup>2</sup>Dharwad Farm, UAS, Dharwad (\*Email: kv.padmalatha@gmail.com)

Cotton is one of the most important economic crops providing excellent natural fibre. *G. hirsutum* which represents over 95% of the annual cotton crop, is characterized by high yields while *G. barbadense* offers superior fibre quality properties like length, fineness, and strength. Recent studies indicated that cotton miRNAs play important roles in modulating fibre development. In this study, to elucidate role of miRNAs in fibre quality and development, miRNA profiles were analysed during cotton fibre initiation (0 dpa) and elongation (10 dpa) stages in *G. hirsutum* and *G. barbadense* through high throughput small RNA sequencing. In total, 697 known miRNAs belonging to 38 families and 183 novel miRNAs were identified. About 129 known and 67 novel miRNAs were significantly differentially expressed ( $p \leq 0.05$  and  $\log_2$  Fold Change  $\geq 1$ ) in *G. barbadense* as compared to *G. hirsutum*. miRNA targets were predicted and functional analysis of targets has been done. A total of 620 genes were potentially targeted by the identified miRNAs, whose functions are involved in a various biological processes including fibre development, metabolism and signal transduction. The identification of miRNAs and their targets broadens our understanding of the complicated regulatory mechanism of miRNAs in cotton fibre quality and development.

### **S104: Event-Specific Detection in GM Cotton and Maize using Loop-Mediated Isothermal Amplification Assays**

*Gurinderjit Randhawa*<sup>\*</sup>, *Monika Singh*, *Rajesh K Bhoge* and *Rashmi Chhabra*

Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012 (\*E-mail: gjr@nbpgr.ernet.in)

Loop-mediated Isothermal Amplification (LAMP) is an isothermal nucleic acid amplification technique, in which amplification and detection of target gene is completed in a single step by incubating the reaction mix at constant temperature. A rapid and sensitive technology based on LAMP has been applied for event-specific detection of genetically modified (GM) cotton and maize for the first time in India. Visual and real-time LAMP assays have been developed for two major commercialized Bt cotton events (MON531 and MON15985) and six imported GM maize events. Efficiency in terms of sensitivity, applicability and cost-effectiveness of LAMP assays was compared with conventional Polymerase Chain Reaction (PCR)/real-time PCR assays. Real-time LAMP assays are faster and more user-friendly than PCR; and visual LAMP assays are most cost-efficient. Due to portability of real-time isothermal system, the developed real-time LAMP assays, coupled with a fast DNA extraction method, may facilitate on-site identification of specific GM events.



## **S105: An Efficient Agrobacterium- Mediated Genetic Transformation Protocol for *Cenchrus ciliaris* L. using Shoot Apices**

Shashi\* and Vishnu Bhat

Department of Botany, University of Delhi, New Delhi 110 007

(\*Email: shasbot.du@gmail.com)

A rapid and an efficient Agrobacterium- mediated transformation system has been developed for the first time in *Cenchrus ciliaris* genotype IG-3108. *Cenchrus ciliaris* is one of the tropical range grasses known for its perenniality and high palatability. Due to its apomictic mode of reproduction, improvement of this polymorphic grass was restricted to in vitro selection based genetic manipulation approaches. To improve the nutritive value and introduce genes for sexuality, an optimized protocol for transforming this grass was very essential. Shoot apical meristems from sterile seedlings of buffel grass were found to be suitable for Agrobacterium-mediated transformation. While it was possible to transform embryogenic calli successfully, the transgenic calli did not regenerate. Hence, transformation variables such as density of bacterial cells, duration of infection, co-cultivation period, acetosyringone concentration and vacuum infiltration for achieving high transformation frequency using shoot apex explants were optimized. The shoot apex explants were co-cultured with EHA 105 harboring the binary vector pCAMBIA 1301. Maximum transformation frequency (1.42%) was obtained by infection with Agrobacterium suspension of OD<sub>600</sub> = 1.0 for 30 minutes, under a negative pressure of 0.5 × 10<sup>5</sup> Pa and co-cultivated for 3 days on MS medium containing 3mg/L TDZ and 400 μM acetosyringone. Hygromycin-resistant plants were rooted and transplanted into plastic pots. PCR assay and southern blot analysis confirmed the transgenic status of plants and the stable integration of the target gene into the genome of regenerated plants.

## **S106: Effect of Exogenous Amino Acids in Agrobacterium Mediated Genetic Transformation of Chickpea (*Cicer arietinum* L.)**

Alok Shukla, Mohd Jamal Ansari, Alok Das\* and NP Singh

ICAR-Indian Institute of Pulses Research, Kanpur 208 024, Uttar Pradesh

(\*Email: adas@icar.org.in)

Chickpea is one of the most important source of dietary protein to a large section of world population in arid and semiarid areas. *In vitro* regeneration of healthy chickpea shoots from explants after Agrobacterium mediated genetic transformation is an important step for realization of increased shoot frequency. Here we report the effect of exogenous amino acids (L-Cysteine, L-Arginine & L-Glutamine) on axillary meristem explants of chickpea cv. DCP 92-3 after co-cultivation with *Agrobacterium tumefaciens*. We employed previously standardized regeneration system of chickpea that utilizes a cytokinin, benzyl amino purine for induction of multiple shoots (10.4 ± 1.66) from AMEs. Co-cultivated explants were subjected to different amino acids concentration to calculate regeneration response percentage, modified MS medium with L-Cysteine, L-Glutamine and L-Arginine synergistically helped to recover higher regeneration response percent (79%). After four cycles of selection, shoots with amino acid treatment have twice the frequency as compared to control. This modified medium can be effectively utilized for higher transformed frequency in chickpea.



## **S107: Structural Diversity of miRNA393a Gene and its Implication for Salinity Adaptation in Rice**

*Tapan Kumar Mondal\* and Showkat Ahmad Ganie*

ICAR-National Bureau of Plant Genetic Resources, Pusa campus, New Delhi 110012  
(\*Email: mondaltk@rediffmail.com)

MiRNAs are non-coding small RNA, evolutionary conserved regulatory elements for gene expression. We investigated extent of sequence variation of one salt responsive miRNA, Os-393a gene among the salt tolerant and susceptible rice cultivars. We found that there is no variation in mature sequence, its cleavage site of the target gene (TIR1, LOC\_Os05g05800), promoter as well as precursor miRNA sequence. But there are variations in the primary miRNA sequences, more among the susceptible genotypes than the tolerant genotypes. Further, salt stress altered the expression pattern of miR393a-TIR1 module in a time dependent manner in the roots and shoots of salt tolerant and susceptible genotypes. Promoter methylation of this regulatory module was also altered at different time points under salt stress. Together, differential expression were complemented by the differential promoter methylation, variation of primary miRNA genes and cis-element abundance of the promoter region of miR393a-TIR1 module. Additionally, the variations of primary miRNA genes were utilized to develop genome-wide miRNA-based genetic markers which are potential for genotyping application.

## **S108: Development of Effectual Plant Regeneration Protocol for Development of Transgenics in Sandalwood (*Santalum album* Linn.)**

*MK Tripathi<sup>1,4\*</sup>, D Bele<sup>1</sup>, G Tiwari<sup>2</sup>, RP Patel<sup>3</sup> and SS Tomar<sup>4</sup>*

<sup>1</sup>Horticultural Biotechnology Laboratory; <sup>2</sup>Department of Medicinal and Aromatic Plants; <sup>3</sup>Department of Plant Pathology, KNK-College of Horticulture, Mandsaur; <sup>4</sup>Zonal Agricultural Research Station, Morena; Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh  
(\*E-mail: drmanojtripathi64@gmail.com)

Santalwood (*Santalum album* L.) is highly valued for its fragrant sandal oil used in perfumes and cosmetics. Conventional breeding of sandalwood can be an expensive and difficult task because of their long generation time, sexual incompatibility and heterozygous nature. In vitro regeneration techniques can be used to clone superior lines, conservation of germplasm and for Agrobacterium-mediated gene transfer techniques. During present investigation, induction of morphogenic callus cultures and plantlet regeneration in different explants of sandalwood was attempted. The aim was to identify suitable culture media and explant(s) with higher regeneration potential for development of transgenic plants tolerant to various biotic and abiotic stresses. For all explants cultures, culture media MS2D (MS+2.0mg/l-12,4-D+30gl-1 sucrose) proved well for callus initiation. Culture media MS.5D.5B (MS+0.5 mg/l-1 2,4-D+0.5 mg/l-1 BA+30 gl-1 sucrose) induced direct somatic embryogenesis and average number of somatic embryo per explant in higher frequencies, while, nutrient media MSD.5B (MS+1.0mg/l-12,4-D+0.5mg/l-1BA+30gl-1sucrose) enhanced the frequency of indirect somatic embryogenesis. Inoculation media MS2N.5Td (MS+2.0mg/l-1NAA+0.5mg/l-1TDZ+30gl-1sucrose) promoted direct organogenesis and plantlet regeneration and medium MSN.5Td (MS+1.0mg/l-1NAA+0.5mg/l-1TDZ+30gl-1sucrose) supported occurrence of indirect organogenesis. Regeneration medium MS2TdGA (MS+2.0mg/l-1TDZ+1.0mg/l-1GA3+15gl-1sucrose) regenerated plantlets in higher frequencies from indirect somatic embryos, while plantlet regeneration via indirect organogenesis was attained in higher frequencies on regeneration medium MSTd.5GA.5N(MS+1.0mg/l-1TDZ+0.5mg/l-1GA3+0.5mg/l-1NAA+15gl-1sucrose) for most of the explants cultures. For regeneration ability, maximum numbers of shoots were recovered from mature embryo culture followed by hypocotyl and mature cotyledon cultures. The explant with higher regeneration potential can be used for commercial plant production, germplasm conservation and development of transgenics.

### **S109: Molecular Characterization of Oleosin Storage Protein Gene from *Jatropha curcas* using Candidate Gene Based Approach**

Vimala Devi S<sup>1</sup>, Akriti Verma<sup>2</sup> and Vishal Singh<sup>1</sup>

<sup>1</sup>Tree Improvement Lab, ICAR-Central Agroforestry Research Institute, Gwalior Road, Jhansi 284003;

<sup>2</sup>Vellore Institute of Technology, Vellore, Tamil Nadu (\*Email: vimal123@gmail.com)

*Jatropha curcas* is an important renewable source for alternative biofuels. Tree improvement for high yield and oil content is essential to make this a commercially viable. An effort was made to use the candidate genes from *Arabidopsis thaliana* for retrieving information on oleosin storage protein gene from *Jatropha curcas*. Search for molecular information on oleosin gene lead to identification of a 541 bp gene (At1g48990) coding for oleosin family protein. It is involved in lipid storage and located in monolayer-surrounded lipid storage body, integral to membrane. It contains InterPro domains: Oleosin (InterPro: IPR000136). In silico analysis using this gene sequence in the whole genome of *Jatropha curcas* ([www.kazusa.or.jp/jatropha/](http://www.kazusa.or.jp/jatropha/)) produced a sequence with significant alignment viz., ID of Jcr4S06252. FGENESH and Pfam analysis predicted 3 genes for the *Jatropha* sequence Jcr4S06252, and the domain characterization revealed the function of second gene coding for oleosin like protein. The primers designed for this coding region of *Jatropha* were used to PCR amplify the gene from *Jatropha curcas*, which resulted in the amplicon size of ~ 1500-1600 bp. This was further validated in the germplasm accessions of the *Jatropha* maintained at the ICAR-CAFRI research farm, revealing allelic variation in the germplasm for this trait. This data in combination with phenotypic data can be used to screen and characterize germplasm for oil content.

### **S110: Development of NPR1 Overexpressing *Brassica juncea* Transgenic Plants for Improved Resistance to Alternaria Blight**

Sajad Ali, Chamil Nayankantha, Sandya Rawat, Zahoor Ahamad Mir and Anita Grover\*

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012  
(\*Email: anitagrover@hotmail.com)

Alternaria leaf blight caused by *Alternaria brassicae* is a major problem in *Brassica juncea* cultivation. Systemic acquired resistance (SAR) is a long-lasting plant defense response that confers broad-spectrum resistance to viral, bacterial, and fungal pathogens and induces expression of pathogenesis-related (PR) genes. NPR1 (non-expressor of PR genes) is a positive key regulator of salicylic acid mediated systemic acquired resistance and activator of many PR genes and plays role in SA/JA signalling crosstalk. Here we report the isolation and characterisation of NPR1 gene from *Brassica juncea*. BjNPR1 gene was isolated from the BjcDNA library using Arabidopsis probe. The BjNPR1 cDNA is 1130 bp, with a 1782 bp open reading frame. The transgenic lines overexpressing NPR1 gene (driven by 35S promoter) acquire significant levels of resistance to *Alternaria brassicae* than wild type Bj plants. Based on preliminary observation there was delay in lesion appearance, size and spread of infection in BjNPR1 transgenic plants as compared to wild plants where lesions appeared second day of post infection. The size of lesions in transgenic lines were 4 to 6 mm on 9th day of post infection as compared to wild lines which shows 11 to 15 mm. This is a preliminary report and has to be studied further in details.



## **S111: Four Rice Seed-Specific NAC Transcription Factors Exhibit Variations in Properties**

*Pinky Agarwal\* and Iny Elizebeth Mathew*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi

(\*Email: pinky.agarwal@nipgr.ac.in)

NACs are a class of plant-specific transcription factors (TFs) and are known to play important regulatory roles, from development to stress conditions. Four seed-specific NAC encoding genes of rice, namely, ONAC020, ONAC026, ONAC023 and ONAC025 express at high levels, during five seed development stages, in five genotypes with a range of seed weights, as shown by QPCR. The levels and patterns of expression are different amongst the genotypes, which can be attributed to their promoter sequences only to a certain extent. However, promoter-reporter transgenic plants show that the promoters are responsible for seed-specific expression. Transcript fusion occurs between ONAC020 and ONAC026. All the genes not only have varying levels of transactivation ability but are repressors as well. The transcript fusion forms also show differences in these properties. Out of the four, only ONAC026 is nuclear localized. BiFC experiments show that it interacts with all forms of ONAC020 and ONAC023 to mobilize them to the nucleus. Summarily, though the four NAC TFs are seed-specific, our model indicates a balance amongst their properties to regulate rice seed development.

## **S112: Mutation in Coproporphyrinogen III oxidase Gene Impairs Male and Female Gametophyte Development in *Arabidopsis thaliana***

*Pritu Pratibha<sup>1,2</sup>, Ramamurthy Srinivasan<sup>3</sup>, Shripad Ramchandra Bhat<sup>3</sup> and Yelam Sreenivasulu<sup>1,2\*</sup>*

<sup>1</sup>CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, H.P.; <sup>2</sup>Academy of Scientific and Innovative Research, Palampur, Kangra, H.P.; <sup>3</sup>ICAR-National Research Centre on Plant Biotechnology, IARI, New Delhi 110012 (\*Email: sreenivasulu@ihbt.res.in)

Heme biosynthesis represents one of the most essential metabolic pathways in living organisms. Coproporphyrinogen III oxidase (CPPO) enzyme catalyzes the conversion of coproporphyrinogen into protoporphyrinogen in the heme biosynthetic pathway. Here we report that a mutation in the *Arabidopsis thaliana* CPPO gene causes short siliques with significant seed sterility. The *Atcpo* mutant displays defects in male gametophyte development including nonviable pollen, unfused polar nuclei in the female gametophytes. Improper differentiation of the central cell subsequently led to defects in the formation of the endosperm. As a result, post-fertilization embryo development was also arrested at the globular stage. The results suggest that the highly accumulated ROS are responsible for the impairment in male and female gametophyte development. Further, the mutant phenotype was completely rescued by the overexpression cDNA of *AtCPPO*. The present results contribute towards enhancing our understanding of the complex network of cellular processes related to tetrapyrrole metabolism in plants especially in reproductive development.



### **S113: A Novel Indel in the Promoter Region of *Lax1* Gene in Rice (*Oryza sativa* L.)**

Sheel Yadav, DP Wankhede, Sonika Singh, Amit Kumar Singh, Sundeep Kumar and Rakesh Singh\*

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012

(\*Email: rsingh@nbgpr.ernet.in)

Rice (*Oryza sativa* L.) is a cereal crop which serves as the major staple food for nearly half of the global population. Grain yield in rice is a polygenic trait and has been targeted for improvement through various molecular breeding approaches. A large number of genes and QTLs controlling this trait have been reported in various studies. One such gene, *lax 1* which is reported to be a bHLH (basic helix-loop-helix) transcription factor gene is shown to control grain number (panicle size) in rice. The promoter region of this gene was partially sequenced to identify any sequence variations. A 10 bp Indel was identified at 205 bps upstream from the transcription start site in the promoter region of the *lax 1* gene. A sequence specific primer was designed flanking the Indel to achieve desired amplification of this genomic region. The amplification profile showed the presence of a 10 bps unique deletion in Basmati rice genotypes and corresponding addition in non Basmati rice genotypes. The development of such a sequence specific primer might help identification and differentiation of Basmati rice varieties from the non Basmati ones and can be used to check/detect adulteration of Basmati rice grains/seeds with non Basmati adulterants. Further investigation of the genomic region containing the Indel might lead to its functional characterization in terms of alteration of a transcription factor binding site.

### **S114: Development and Demonstration of a Modified Binary Vector, Pore-R2::GFP, for Testing Bidirectional Promoter Activity**

Ritesh Kumar Raipuria<sup>#</sup>, Vajinder Kumar<sup>#</sup>, Sunil Kumar Singh, Seema Dargan and Shripad Ramachandra Bhat

ICAR-National Research Centre on Plant Biotechnology, New Delhi 110012

(\*Email: raipuriaritesh@gmail.com) <sup>#</sup>Authors contributed equally

Promoters are mostly believed to drive gene expression in one direction. However, in silico studies, transcriptome studies and T-DNA promoter trap studies suggest that a significant proportion of promoters might be bidirectional. In particular, head-to-head oriented genes with short (<200 bp) intergenic regions are likely to exhibit bidirectional promoter activity. Currently available vectors for testing promoter activity like pBI101 and pORE-R2, have MCS upstream to the reporter gene. Hence, testing bidirectional promoter activity requires separate cloning and plant transformation. To avoid this double exercise and to simultaneously test bidirectional promoter activity, we developed a base vector named as pORE-R2::GFP, having sGFP and uidA genes in head-to-head orientation separated by MCS. To test the efficacy of this vector, we cloned six deletion fragments from the upstream region of At3g53420 (Aquaporin) gene, which is known to be constitutively expressed in all organs at various developmental stages. The 1772 bp (-1419/+353) fragment behaved as a unidirectional promoter. However, the deletion fragment 1070 bp (-811/+259) showed both GUS and GFP expression. Successive deletions to 827 bp (-474/+353) or 733 bp (-474/+259) displayed dramatic reduction in GUS expression but high GFP expression in seedlings, root and leaf tissue. Our results clearly show the utility of the new vector for quick identification of bidirectional promoters and also reveal that promoter deletion fragments could uncover novel regulatory sequences.



## **S115: Expression Level Variations of the Gibberellin Signalling Gene GAS4, in Bold-Seeded and Small-Seeded Varieties of *Brassica juncea* (Indian Mustard) during Seed Development**

Bharat Joshi<sup>1,2</sup>, Manu Agarwal<sup>1</sup>, Amar Kumar<sup>1</sup>, Shailendra Goel<sup>1</sup> and Arun Jagannath<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Delhi, Delhi 110007; <sup>2</sup>Swami Shradhanand College, University of Delhi, Alipur, Delhi 110036 (\*Email: jagannatharun@yahoo.co.in)

*Brassica juncea* (Indian mustard) is the second most important oilseed crop of the Indian subcontinent after groundnut. Earlier studies on genetic diversity of *B. juncea* had identified two distinct gene pools – the bold-seeded Indian gene pool and the small-seeded East European gene pool. The plant hormone, gibberellin/gibberellic acid, is known to influence a variety of plant growth and developmental processes including seed size. The expression of the gibberellin signalling gene, GIBBERELIC ACID-STIMULATED ARABIDOPSIS4 (GASA4) increases in response to gibberellins. In the present study, using intron-spanning primers, we isolated four paralogs of the GASA4 gene from the bold-seeded Indian variety, Varuna and three paralogs from the small-seeded east European variety, Heera. Expression variations among the GASA4 paralogs was analyzed in two consecutive growing seasons by harvesting developing siliques from two bold-seeded varieties (Varuna and Pusa Bold), and two small-seeded varieties (Heera and EH2). Paralog-specific primers were designed to identify specific sequence/s that express during seed development. Of the four paralogs identified for GASA4 gene, only one was found to express during seed development. Analysis of expression levels by qRT-PCR indicated that transcript levels of GASA4 in the small-seeded varieties increased during initial stages followed by a rapid decline. However, in bold-seeded varieties, expression levels of this gene were significantly higher even in later stages indicating a more active GA signalling mechanism. Our observations indicate that the GASA4 gene might play an important role in determination of seed size in *B. juncea*.

## **S116: Loss of Function in Nucleotide Sugar Transporter (AtNSTP) Gene Causes Defects in Pollen Development and Embryo sac Progression in *Arabidopsis thaliana***

Rimpy Diman<sup>1,2</sup>, Ramamurthy Srinivasan<sup>3</sup>, Shripad Ramchandra Bhat<sup>3</sup> and Yelam Sreenivasulu<sup>1,2,\*</sup>

<sup>1</sup>Biotechnology Division; <sup>2</sup>Academy of Scientific and Innovative Research (AcSIR), CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh; <sup>3</sup>ICAR-National Research Centre on Plant Biotechnology, IARI, New Delhi (\*Email: Sreenivasulu@ihbt.res.in)

Nucleotide sugar transporters (NSTPs) are transmembrane proteins which are localized in Golgi/ER membrane and transports nucleotide sugars from cytosol to its lumen. Transported nucleotide sugars are act as donor in post transcriptional modification of proteins by donating sugar moiety in glycosylation processes. The *Arabidopsis thaliana* genome contains more than 50 different nucleotide sugar transporter genes. Here, we report that mutation in nucleotide sugar transporter (AtNSTP) gene causes impaired gametophyte development including collapsed, nonviable pollen grain and an arrest of female gametophyte at single nucleate stage. Crossing results confirms that both gametophytes are defective but male gametophyte is more contributes towards sterility. Complementation analysis completely regains fertility with completely filled elongated siliques with normal gametophyte development. Therefore, we conclude that mutation in AtNSTP gene affects transport of such nucleotide sugars which are involve in posttranscriptional modification of such protein which is possibly play crucial role in gametophyte development in *Arabidopsis thaliana*.



### **S117: Cry2Aa-based Resistance towards Pod Borer (*Helicoverpa armigera*) in Transgenic Pigeonpea**

N Murali Mohan\*, R Maniraj, KY Srinivasa Rao, Shweta Singh, Komal Jhala, T Arulprakash, K Karthik, D Pattanayak and Rohini Sreevathsa

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi  
(\*Email: mohandurga101@gmail.com)

Pigeonpea (*Cajanus cajan* [L.] Millspaugh) is an important pulse crop predominantly cultivated in tropical and subtropical regions of the world. Globally, pigeon pea is cultivated nearly on 4.64 Mha, with an annual production of 3.43 million tons and a mean productivity of 780 kg/ha. Pod borer (*Helicoverpa armigera*) is the one of the important insect pests, and has turned out to be a serious production constraint in pigeonpea. In the present study, we have developed transgenic pigeon pea plants through apical meristem-targeted in planta transformation using *Agrobacterium* strain EHA105 harbouring pBinAR: CaMV35S: Cry2Aa: OcsT construct. Putative T<sub>0</sub> seeds were selected through kanamycin treatment and obtained T<sub>1</sub> plants were independently screened for the presence of Cry2Aa transgene through PCR analysis. PCR positive T<sub>1</sub> transformants were tested for their efficacy against *Helicoverpa armigera* larvae in pigeon pea by detached leaf bioassay. T<sub>1</sub> pigeon pea lines positive for leaf bioassay were further carried for T<sub>2</sub> generation. Southern analysis of the T<sub>2</sub> transgenic pigeon pea lines showed stable integration of transgenes. The high expressing transgenic lines with superior bioefficacy against *Helicoverpa armigera* are being advanced further.

### **S118: A Chimeric Bt Insecticidal Protein Cry1AcF Confers Resistance to *Helicoverpa armigera* in Transgenic Pigeonpea**

R Maniraj\*, N Murali Mohan, KY Srinivasa Rao, Shweta Singh, Komal Jhala, T Arulprakash, K Karthik, D Pattanayak and Rohini Sreevathsa

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi  
(\*Email: rmani607@gmail.com)

Pigeonpea is an important food crop in India, it's a good source of protein food. Pod borer (*Helicoverpa armigera*) is an important insect pest which causes huge production losses in pigeon pea. Cry proteins are used as one of the means to develop insect-resistant transgenic plants, however development of resistance in pest population is another challenge. Along with various other strategies, used to delay resistance in the insects, use of pyramided or chimeric genes is one of them. In this direction, chimeric gene, Cry1AcF was developed. It was observed that toxicity towards *Helicoverpa armigera* increased in combination of Cry1Ac and Cry1F. We have developed transgenic pigeonpea plants through in planta transformation using *Agrobacterium* strain EHA105 harbouring pBinAR: CaMV35S: Cry1AcF: OcsT construct. Putative transformants from the T<sub>0</sub> seeds were selected on kanamycin and T<sub>1</sub> plants were obtained. Promising transformants were selected based on molecular and bioefficacy analysis. Stability in integration and inheritance of the transgenes is being analysed in the advanced generations.



## **S119: Chickpea Protection against *Helicoverpa* Pest: From Bt Toxins to Plant Mediated RNAi**

Surender Khatodia\* and SM Paul Khurana

Amity Institute of Biotechnology, Amity University Haryana, Manesar 122413, Haryana  
(\*Email: skhatodia@ggn.amity.edu)

Chickpea is world's second most widely grown annual legume crop. *Helicoverpa armigera*, the pod borer is a major constraint to global chickpea production. Genetic improvement of chickpea for insect resistance by traditional methods has been hampered by narrow genetic diversity in the elite gene pool. Genetic transformation of chickpea using various versions of single Bt genes as well as pyramids also been carried out. However, with intensive cultivation of Bt crops, pest resistance to transgenic plants is increasing in *H. armigera* even in transgenic plants expressing two different types of Bt toxins. The sustainability of Bt transgenic crops is already threatened by the accelerated emergence of insect resistance in *Helicoverpa* and Bt chickpeas have yet to be commercialized. Plant mediated RNAi is an attractive approach for crop protection is to knock down the crucial physiology-related genes of insect pests, which is paving the way for a new generation of insect-resistant transgenic crops. Common siRNAs for the target genes of *H. armigera*, designed in silico could be used to study the lethal effect of down-regulating crucial target genes in chickpea. This study focuses on the future prospects of using plant mediated RNAi for *H. armigera* resistance in chickpea. The plant mediated RNAi approach holds great promise for future development but further studies will be required to optimize RNAi-based strategies for chickpea protection against *H. armigera* using integrated pest management strategies.

## **S120: Characterization of Resistance in Back-Crossed Rice Plants of Variety ASD-16, BPT-5204, CR1009, Khitish and Shatabdi Containing Transgene Against Rice Tungro Bacilliform Virus**

Gaurav Kumar<sup>1</sup>, S Robin<sup>2</sup>, R Rabindran<sup>3</sup>, J Tarafdar<sup>4</sup> and I Dasgupta<sup>1</sup>

<sup>1</sup>Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021; <sup>2</sup>Department of Rice, <sup>3</sup>Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu; <sup>4</sup>Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia 741 252 (\*Email: gauravkumar.bhu@gmail.com)

Rice tungro disease of rice is the most damaging viral diseases of rice prevalent in south and Southeast Asia. It is a complex disease resulting from infection by two viruses: Rice Tungro Bacilliform Virus (RTBV), and Rice Tungro Spherical Virus (RTSV); the green leafhopper (GLH) *Nephotettix virescens* being the natural vector. RTBV can also be inoculated through an agrobacterium mediated inoculation procedure known as agroinoculation. Previously, a transgenic rice line of the variety Pusa Basmati-1 was developed, having dsRNA construct against RTBV, which triggered RTBV resistance by using the conserved RNA-interference pathways of the plant. The transgenic Pusa Basmati-1 was subsequently used for back-crossing to transfer the resistance to the popular rice varieties, ASD-16, BPT-5204, CR-1009, Khitish and Shatabdi. The present work includes the resistance assay of these backcross lines. The procedure includes the molecular detection as well as biochemical confirmation of the transgene as well as the phenotypic comparison and determination of the virus levels by quantitative PCR. The backcrossed transgenic plants were inoculated with RTBV and RTSV using both GLH and agroinoculation and analysed for degrees of stunting at successive time-points post inoculation compared with their respective parents and viral inoculum. The results identified lines resistant to RTBV.

## **S121: Genetic Transformation of Rice with Chitinase gene, LOC\_Os11g47510 for Sheath Blight Resistance**

*Richa<sup>1,3</sup>, Ila Mukul Tiwari<sup>1</sup>, R. Nagar<sup>1</sup>, Vinay Sharma<sup>3</sup>, J Botella<sup>2</sup> and TR Sharma<sup>1\*</sup>*

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>2</sup>The University of Queensland, St Lucia Campus Brisbane, Queensland, Australia; <sup>3</sup>Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali 304 022

(\*Email: trsharma@nrcpb.org)

Sheath blight disease (SB), caused by the fungus *Rhizoctonia solani* Kühn, is one of the most devastating diseases of rice (*Oryza sativa* L.), causing extensive yield losses. The objective of this study was to develop a plant transformation gene cassette containing chitinase gene, LOC\_Os11g47510 and its transformation in susceptible lines for developing rice lines resistant to *R. solani*. To achieve this, mature scutellum calli of rice were used as explants for genetic transformation using Biolistic approach. After 16h of bombardment, the calli were transferred to the selection media containing hygromycin. After three rounds of selection, survived putative transformed calli were transferred to regeneration medium which was supplemented with cytokinin (BAP & kinetin) and Auxin (NAA) along with hygromycin as a selection agent and exposed to light. Out of 678 bombarded calli, 20 calli were regenerated into plants. Putative transformants were evaluated and molecular analysis of putative transformants showed the presence of 970 bp PCR product specific to region between *gfp* and chitinase gene and a total of 2.9% stable transformation efficiency was achieved. Results on the expression of this gene will be included in this presentation.

## **S122: Molecular Characterization of Flowering Repressors TFL1/CEN-like Genes in Banana**

*AK Chaurasia<sup>1</sup>, HB Patil<sup>1</sup>, VR Subramaniam<sup>1</sup>, Bal Krishna<sup>1</sup>, AP Sane<sup>2</sup> and PV Sane<sup>1</sup>*

<sup>1</sup>Plant Molecular Biology Lab, Jain R&D lab, Agri Park, Jain Hills, Shirsol Road, Jalgaon 425001 Maharashtra; <sup>2</sup>Plant Gene Expression Lab, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001 (\*Email: molbio@jains.com)

TERMINAL FLOWER1 (TFL1) or CENTRORADIALIS (CEN)-like genes are highly conserved in plant species and are involved in regulating the flowering time. These function as repressors of flowering as they delay vegetative to reproductive phase transition and maintain meristem indeterminacy. These genes encoding a small protein of about 175 amino acids belong to the phosphatidyl ethanolamine-binding protein (PEBP) superfamily. In this study, seven TFL1/CEN-like genes were isolated from banana (AAA cultivar Grand Nain) and named as MUSA CENTRORADIALIS1 (MCN1) to MCN7. For all these genes, functionally reported critical amino acids (His88 and Asp140) in rice, maize and Arabidopsis were found conserved besides almost 75% similarity in their amino acid sequence. Relative expressions of MCN1-3, MCN5 and MCN7 were significantly higher during vegetative stage than flowering stage when the expression of florigen equivalent MaFT genes goes up. Over expression of MCN1, MCN2 and MCN3 but not MCN4 and MCN6 in mutant background of Arabidopsis (*tfl1-14*) fully complemented the function of TFL1. Our results suggest that TFL1/CEN-like genes are evolutionarily conserved and are involved in the maintenance of vegetative meristem identity in banana.



## S123: Comparative Genomics Based In Silico Assessment of Dof Gene Family of *Medicago truncatula*, Chickpea and Pigeonpea

Dinesh Yadav\*, Neha Malviya, Jeya Nasim and Sujata Sharma

Department of Biotechnology, D.D.U Gorakhpur University, Gorakhpur 273 009, U.P.

(\* Email:dinesh\_yad@rediffmail.com)

Dof (DNA binding with One Finger) is a plant specific transcription factor (TF) family recognized by the presence of unique and conserved 50-52 amino acid long Dof domain with four cysteine residues. In silico whole genome analysis of *Medicago* (470Mbp), chickpea (740 Mbp) and pigeonpea (833Mbp) revealed 37, 37 and 38 Dof genes respectively. In *Medicago*, they were distributed on all the 8 chromosomes with a maximum of 7 Dof genes on chromosomes 2 and 3. Similarly, in chickpea they were distributed among the 8 chromosomes with a maximum of 9 Dof genes on chromosome 7. In pigeonpea, the 38 Dof genes were distributed among 8 out of 11 chromosomes with a maximum of 11 on chromosome 11 while chromosomes 2, 5 and 9 lacked Dof genes. The prevalence of intronless or single introns containing Dof genes was uniformly observed among these legumes. The comprehensive phylogenetic analyses of Dof genes of these legumes along with rice and *Arabidopsis* revealed several orthologs and paralogs. The structural and functional insights into Dof proteins of these legumes have been assessed based on in silico predicted 3D structures and Cis regulatory elements analyses. The bioinformatics based characterization of Dof TF of these legumes could provide a direction for molecular cloning and expression profiling.

## S124: Identification of a Vital Development Gene, *glp-1*, and Its Utilization in RNAi Mediated Silencing for Conferring Resistance Against Root Knot Nematode in *Arabidopsis*

Deshika Kohli<sup>1,2</sup>, Anil Kumar<sup>1</sup>, Navneeta Bharadvaja<sup>2</sup>, K Subramaniam<sup>3</sup>, A Sirohi<sup>4</sup>,  
R Srinivasan<sup>1</sup> and PK Jain<sup>1</sup>

<sup>1</sup>ICAR-NRC on Plant Biotechnology, Pusa Campus, New Delhi; <sup>2</sup>Department of Biotechnology, Delhi Technological University, Delhi; <sup>3</sup>Department of Biological Science and Bioengineering, Indian Institute of Technology, Chennai; <sup>4</sup>Division of Nematology, ICAR-IARI, New Delhi  
(\*Email: deshika15@gmail.com)

Plant Parasitic Nematodes (PPNs) are among the most harmful biotic pests affecting severely the crop production throughout the world. Among them *Meloidogyne incognita*, a root knot nematode (RKN) is the most damaging species owing to its wide host range. In recent years, RNA interference has emerged as a new biotechnological tool to control the establishment of these harmful nematodes. Moreover, RNAi has also contributed in increasing our understanding of plant pathogen interaction through targeting of nematode parasitic genes. In the present study we have identified *glp-1* gene involved in the development of *Meloidogyne incognita*, from ESTs and genomic sequences using computational biology approaches. qRT-PCR analysis indicated the involvement of this gene in early development of *M. incognita*. *Arabidopsis* RNAi transgenic lines (expressing dsRNA construct of *glp-1* gene) showed reduction in *M. incognita* infection (40-45%) in terms of number of galls and females. Further, molecular analysis revealed reduced expression levels in nematode females isolated from RNAi lines as compared to control plants. Host-mediated RNAi silencing of this gene may be studied and employed in crops for better management of RKN and also for better understanding of plant-pathogen interaction.



## **S125: Promoter Analysis of *GA2ox1*, a Gibberellin Catabolism Gene, Reveals Its Role in Blue Light-Mediated Photomorphogenesis in Arabidopsis**

*Sushma Mishra and Jitendra P Khurana*

Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi 110021 (\*Email: sushmamishra87@gmail.com)

The young light-grown seedlings characteristically display short hypocotyl, open apical hook with expanded cotyledons, and plastid development in dicot systems like Arabidopsis. This photomorphogenic development occurs by the co-ordination of light signals perceived by photoreceptors and endogenous cues like phytohormones. One such example is the interaction between cryptochromes (CRYs), the blue light-sensing photoreceptors, and the phytohormone gibberellin (GA). Prompted by the observations made in our laboratory on *Brassica* CRY1-ox transgenics, which showed reduced plant height accompanied by altered transcript levels of GA metabolism genes, it was decided to work out the intricacies associated with this cross-talk in Arabidopsis. The real-time PCR analyses confirmed that blue light stimulates the expression of *AtGA2ox1*, a gene encoding GA-catabolism enzyme. Moreover, Arabidopsis transgenics containing *GA2ox1* promoter-reporter (GUS) construct showed higher promoter activity in blue light grown seedlings in comparison to the dark grown ones; it is likely that higher activity of GA catabolism enzyme leads to low bioactive GA levels and hence shorter plant height. The trans-acting factors interacting with *GA2ox1* promoter were identified by yeast-one-hybrid assay, and the interaction confirmed by electrophoretic mobility shift assay. Work is in progress to elucidate the precise role of these factors in light regulated expression of *GA2ox1*.

## **S126: Functional Characterization of a Central Effector of Light Signaling, AtHY5, Ortholog in Rice**

*Naini Burman, Akanksha Bhatnagar and Jitender P Khurana*

Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi (\*Email: nainiburman21@gmail.com)

To perceive light, plants have developed an extensive array of signal transduction network, which integrates with almost all the important pathways. This pathway is highly hierarchical with photoreceptors like phytochromes and cryptochromes being at the top. They are followed by early signaling factors like HFR1 and central integrators like COP1. Then lies the downstream effectors like HY5. Most of the components of light signaling pathway have been characterized in Arabidopsis, a model dicot plant. In rice, *OsZIP48*, one of the HY5 ortholog, was functionally characterized using transgenic approach. Over-expression transgenics of *OsZIP48* caused dwarf phenotype and accumulation of more chlorophyll content. There was a high degree of floret sterility, which severely affected the grain yield. RNAi transgenics of *OsZIP48* segregated into two types of seedlings in T<sub>2</sub> generation; one which resembled wild type and vector control in morphology and the other which showed lethal phenotype. Microarray analysis revealed that a number of hormonal pathways were affected in these transgenics. These data obtained by analysis of rice transgenics for *OsZIP48* provide evidence that rice ortholog of HY5, i.e. *OsZIP48*, performs overlapping functions with HY5 as well as some monocot specific roles in regulating plant development.



## **S127: Embryo Targeted In planta Transformation in Elite Indian Cultivars of Wheat for Developing Thermotolerant Transgenics**

Uttara Chaturvedi, Arul Prakash and Jasdeep Chatrath Padaria\*

ICAR-National Research Centre on Plant Biotechnology, New Delhi 110012

(\*Email: jasdeep\_kaur64@yahoo.co.in)

Ascorbate Peroxidase (APX) is a central component of the glutathione-ascorbate cycle which detoxifies reactive molecules accumulated in the cell during abiotic stress conditions. APX plays a significant role in providing tolerance in plants to various abiotic stresses. Wheat is one of the most important cereal crops of the world and high temperature stress especially at grain filling stage is causing immense yield loss. The present study aimed at developing thermotolerant wheat using the transgenic approach for which an efficient embryo targeted In planta transformation protocol was standardized. pAPX gene (Genbank:JX126968) from thermotolerant Indian wheat cv Raj3765 was used as gene of interest to be transferred to thermo susceptible elite Indian wheat cultivar HD2967. Abrasion strategy was adopted where dry mature seeds were infected by gentle abrasion of the embryonal end on a sand paper, then co-cultivated with *Agrobacterium* inoculum with vacuum pump at a pressure of 80 kPa for initially 15 minutes and allowed to grow till maturity in soilrite medium. T<sub>1</sub> seeds were collected and screened for putative transgenics by germinating seeds under hygromycin selection (50 mg/L). The putative transgenics were confirmed by gene specific PCR and southern hybridization analysis. The method proved to be competent with the transformation efficiency of 24% as per PCR analysis. Leaf disc and peroxidase enzyme assay after heat stress treatment in transgenic plants confirmed expression of the transgene.

## **S128: Allele Mining for Phosphorus Starvation Tolerance Gene (*Pstol 1*) in *O. rufipogon*, a Wild Species of Rice**

Shivali Thakur, Palvi Malik, Kuldeep Singh, Kumari Neelam\*

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab

(\*Email: kneelam@pau.edu)

Phosphorus deficiency of soil is considered as a major constraint to the production of rice. Rice needs phosphorus to survive and thrive. It is a key element in plant metabolism, root growth, maturity and yield. Phosphorus Starvation Tolerance 1 (*PSTOL1*) gene from traditional rice variety Kasalath (Indian origin) has been cloned. Our aim is to identify the novel alleles of *PSTOL1* gene in *O. rufipogon* accessions and further its transfer to the elite cultivars. Primers were designed to amplify functional region of *PSTOL 1* gene. Nearly 200 accessions of *O. rufipogon* were surveyed using gene specific primers. Those accessions with amplification were sequenced along with Vandana, a cultivar having *PSTOL 1* gene. The elite rice cultivars PR114, PAU 201, PR122, PR121 and PB3 did not had amplification indicating absence of *PSTOL 1* gene. Sequencing results indicated presence of three novel haplotypes for *PSTOL 1* gene. As none of the modern cultivars has phosphorus starvation tolerance gene in them, transferring this important gene for phosphorus uptake will have major impact on reducing cost incurred in phosphorus fertilizers and also having good yield under phosphorus deficiency.

**S129: Sequence Analysis of *Dro 1* Gene at Exon 4 in Wild Species of *Oryza***

Kumari Neelam, Gurpreet K Sahi, Preeti Sharma, Kishor Kumar, Amanpreet Kaur,  
Gaurav Madan and Kuldeep Singh\*

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab  
(\*Email: kuldeep35@pau.edu)

Drought is an important abiotic factor limiting rice yield worldwide. In present study, 1600 accessions of wild species (AA, CC and CCDD) were screened to assess genetic variation for drought tolerance under field conditions. Severe water stress was imposed at vegetative stage by withholding water initially for 25 days and then extended further. Tolerance was based on extent of leaf rolling and drought score (leaf drying). Results showed that 35 accessions (28 of *O. rufipogon*, 4 of *O. longistaminata*, and 1 each of *O. nivara*, *O. officinalis* and *O. latifolia*) showed the resistant and moderately resistant reaction at 25 days of drought stress. The primers were designed targeting 1-bp deletion within exon 4 of DEEPER ROOTING 1 (*DRO 1*) gene, responsible for enhancing rice yield under drought in Kinandang Patong. The most tolerant, thirteen accessions of *O. rufipogon*, *O. officinalis* and *O. latifolia* were sequenced. Nine accessions of *O. rufipogon* along with *O. officinalis* and Nagina 22 had shown 1bp insertion of nucleotide A in exon 4 of *Dro 1* gene as reported in Kinandang Patong, a deep rooting and drought tolerant cultivar. Four of the *O. rufipogon* accessions formed different haplotype indicating presence of novel allele for *Dro1* gene. The process of transferring drought tolerance from *O. rufipogon* to elite rice cultivars is in progress.

**S130: Genetic Transformation of Safflower (*Carthamus tinctorius* L.) and *Arabidopsis* for Increased Oil Content**

Ch Anil Kumar and V Dinesh Kumar\*

Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500 030  
(\*Email: dineshkumarv@yahoo.com)

Increasing the oil content through transgenic approach has been reported by over expressing single genes involved in TAG formation and it has been opined that expression of multiple genes might actually lead to better increased oil content. We have attempted to assess the effect of simultaneous expression of glycerol-3-phosphate acyltransferase (*GPAT9*) and diacyl glycerol acyl transferase (*DAGAT1*) genes in increasing the oil content in safflower after validating the constructs in *Arabidopsis*. Plant Transformation vectors carrying the genes (*GPAT9* and *DAGAT1*), either singly or together were developed and mobilized into *Agrobacterium tumefaciens* strain LBA4404 and GV3101 to facilitate plant transformation. *Arabidopsis* was transformed with these constructs independently and the transgenic plants were identified and advanced to the T2 generation. Initial oil content analysis with these lines has shown elevated levels of oil content. Putative safflower transgenic shoots have been obtained with the double gene cassette. These shoots have been grafted onto control root stocks, out of 50 grafted shoots only 6 survived in the green house but none of them had set seeds. Alternatively, in planta transformation was carried out with double gene construct and concoction of single gene constructs independently, and analysis of the progeny obtained is in progress.



### **S131: Overexpression of *AtICE1* Gene Improves Abiotic Stress Tolerance in Transgenic Rice**

*Rakesh Kumar Verma, Santosh Kumar VV, Shashank Kumar Yadav and Viswanathan Chinnusamy\**

Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: viswanathan@iari.res.in)

Abiotic stresses for example drought, soil salinity and freezing, greatly affect plant productivity and quality. Many genes are involved in the process of adaptation to adverse environmental conditions. *ICE1* (Inducer of CBF Expression 1), an upstream transcription factor that regulates the transcription of CBF genes and DREB1/CBF (Dehydration Responsive Element Binding/C-repeat Binding Factor) transcription factor can bind to the DRE/CRT cis-element and activates the expression of downstream stress-responsive genes. Arabidopsis *AtICE1* gene was isolated by RT-PCR and cloned in a modified pCAMBIA1300 under stress inducible *AtRD29A* promoter and transformed into indica rice variety Pusa Sugandh 2 by the Agrobacterium-mediated transformation. Cold, drought and salt stress tolerance of transgenic events were compared with the non-transgenic plants in seedling, vegetative and flowering stages. Leaf width and length significantly decreased in transgenic plants. Transgenic plants exhibited enhanced tolerance to these stresses in terms of RWC, chlorophyll and membrane stability. Stomatal density was observed higher while stomatal size was smaller in transgenic plants as compared to non-transgenics. The results suggest that the Arabidopsis *ICE1* is functional in rice and the over-expression of *ICE1* improves the tolerance of rice to abiotic stresses.

### **S132: Functional Characterization of ABA Receptors in Rice**

*Santosh Kumar VV, Shashank K Yadav, Sanya Shrivastava, Rakesh K Verma and Viswanathan Chinnusamy\**

Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi

(\*Email: viswanathan@iari.res.in)

Abcisic Acid (ABA) plays crucial roles in development and stress responses of plants. Bioinformatic analyses led to the identification of 12 PYR/PYL homologs ABA receptor (ABAR) from rice. However, the specific function of members of ABARs in developmental and stress responses needs to be understood. Expression analysis of ABARs in different tissues and stress conditions revealed differential expression patterns suggesting non-redundant roles of ABARs in development and stress responses. Transgenic rice lines expressing OsABAR6 under *AtRD29A* promoter showed hypersensitivity to ABA in germination assay. This suggests that ABAR6 conferred enhanced ABA perception and thus function as ABA receptor. The expression levels of ABA pathway genes are significantly higher in rice transgenics as compared with non-transgenic plants under drought stress. Analysis of drought tolerance of RD29A:ABAR6 transgenic rice lines in pot culture under greenhouse conditions revealed that RD29A:ABAR6 transgenic rice lines are more tolerant than non-transgenic lines in terms of maintenance of RWC, chlorophyll content, survival and recovery growth. Results from this study suggest that ABARs are potential candidate genes for improving drought tolerance of rice.

## Technical Session II: Genomics and Bioinformatics

*Invited Lecture*

### **Plant Genomes for Forward and Reverse Genetics**

*Akhilesh K Tyagi*

National Institute of Plant Genome Research, New Delhi 110067 (\*Email: akhilesh@genomeindia.org)

Plants are pillars of world-wide food security. Improvement of their yield and its protection are necessary to mitigate hunger of millions of people. Decoding genome sequences is expected to complement efforts being made to improve food production. We have sequenced genomes of rice, tomato and chickpea thereby covering three major groups of plants responsible for our food security i.e. cereals, vegetables and pulses. In the post-genomic era, significant progress has been made in transcriptomics and gene discovery by way of forward and reverse genetic approaches. Efforts are on to fill the gap between the genome and the phenotype. This may lead to regular practice of genomics-assisted molecular breeding. One of the primary aims of ongoing investigations in our group is to understand gene function, phylogeny and regulatory networks. Several general conclusions of great relevance emerging from these studies include: i) New genes and taxon-specific clades can be identified by using molecular model based comparative genomics, ii) Signatures of co-evolution in interacting proteins are discernible, iii) Duplicated genes have undergone neo-/sub-/pseudo-functionalization, iv) Highly significant number of genes are differentially expressed in spatial/temporal manner and in response to stress, and v) such analysis provides useful input for selecting target genes/promoters for functional genomics. As we integrate molecular biology to genetic enhancement activity and overcome practical impediments in taking the knowledge from lab to land, we hope to reap the benefit of genomics research for plant improvement.

*Invited Lecture*

### **Development of Genomic Resources for Pigeonpea, Jute and Mango: Plants Uniquely Important for India**

*Nagendra K Singh*

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012  
(\*Email: nksingh4@gmail.com)

India started its genomics journey with sequencing of rice genome, was a key partner in tomato genome and is presently involved in wheat genome. These are crops of global significance. There are also plants uniquely important to India with large production volume and economic stakes. Genomic resources for these are priority as it will pay rich dividend in productivity enhancement through genetic improvement. We started work on pigeonpea, jute and mango through national partnerships with funding support from ICAR. After developing comprehensive transcriptome sequence assemblies we created high density genetic map and the first draft of the genome of pigeonpea of variety 'Asha' in 2011. A large number of genic SSR and SNP markers were mined and QTLs for semi-dwarf stature, early maturity and determinacy were mapped, which are now being used in pigeonpea breeding. India is the largest producer Jute, a tropical fibre crop for packaging and industrial usage. We have assembled a draft



genome popular jute variety 'JRO524', developed high density genetic map of 503 SNP markers and identified QTLs for fibre quality in collaboration with CRIJAF. We assembled the first draft of mango genome for variety 'Amrapali' (estimated size 450 Mbp) using overlapping pair-end MiSeq reads. Large number of SSR and SNP have been mined by resequencing 96 popular mango varieties of India. These genomic resources will speed up varietal improvement in pigeonpea, jute and mango for enhancement of productivity, quality and stress tolerance.

*Invited Lecture*

### **Genomics of Cotton Fibre Development and Genome Response Under Biotic and Abiotic Stress**

Saravanan Kumar<sup>1</sup>, Krishan Kumar<sup>1</sup>, Deepak P Patil<sup>1</sup>, Gurusamy Dhandapani<sup>2</sup>, Mogilicherla Kanakachari<sup>2</sup>, Mullapudi LV Phanindra<sup>2</sup>, Kethireddy Venkata Padmalatha<sup>2</sup>, AH Prakash<sup>4</sup>, Hiremath Vamadevaiah<sup>3</sup>, Ishwarappa S Katageri<sup>3</sup>, Sadhu Leelavathi<sup>1</sup>, Polumetla Ananda Kumar<sup>2,5</sup> and Vanga Siva Reddy<sup>1\*</sup>

<sup>1</sup>Plant Transformation Group, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067; <sup>2</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi; <sup>3</sup>Agricultural Research Station, University of Agricultural Sciences, Dharwad; <sup>4</sup>ICAR-Central Institute for Cotton Research, Regional station, Coimbatore, Tamil Nadu; <sup>5</sup>Present address, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500040 (\*Email: vsreddy@icgeb.res.in)

Cotton is commercially important fibre crop and is the main source of natural fibre used in textile industry. Among the four cultivated species of cotton, *G. hirsutum*, an allotetraploid with AADD genome occupy more than 90% of cultivated area in the world. Cotton fibres are single-celled seed trichomes developed from the epidermal layer of the ovule, can grow up to 30-40 mm in length. Development of fibre cell can be divided into four distinct, but overlapping stages such as initiation, elongation, secondary cell wall (SCW) synthesis and maturation. The fibre cell initiation usually occurs just before the anthesis and mature into fiber in about 60-75 days. Fiber development is closely associated with massive amounts of cellulose synthesis (> 90%) and its deposition in the cell. During the maturation stage fibre cells undergo dehydration and produce mature cotton lint. Genome wide high throughput transcriptomic and proteomic approaches were adopted to unravel the molecular events leading to the development of cotton fiber. Also these approaches were used to understand the influence of abiotic and biotic stress on the development of cotton fibers. A comparative transcriptome and proteome analysis was carried out to understand the genes/pathways involved at various stages of fibre cell initiation and differentiation using a fuzzless-lintless mutant and its wild type progenitor. Also we have identified the role of glycoproteome in the development of cotton fiber and its strength. Results from these studies will be presented and discussed.

## Rice Genomics in the Cloud

Dadabhai T Singh

CloudSeq Pte Ltd, Singapore (\*Email: dt@cloudseq.sg)

Rice genomics has progressed rapidly since the publication of the first draft of the genome sequence by IRGSP in 2005. Over the past decade the NGS technology has matured, stabilized and moved to higher glories with long reads. As a result, sequencing has become a “commodity” with global surge in the demand for NGS service providers. Sequence data generation has become routine for hundreds of genomics labs around the world. However, the major bottleneck and/or challenge now are the efficient data management and data analysis in a cost effective way. Several institutes and big companies have spent millions of dollars in setting up huge data centres to manage and analyze critical genomics data locally. It becomes a very tiring and tedious task to maintain these data centres, especially with volatile human resources. Cloud Computing has revolutionized the way BIG data is stored, managed and analyzed today. Initial apprehensions of data security in the Cloud have been largely mitigated by the strict compliance to international security standards such as MTCS, ISO 27017 etc. by several Cloud service providers. Several prestigious institutes around the world have already migrated their voluminous data to the Cloud. The ability to access, manage and analyze the sequence data from anywhere in the world through secure web, opens a tremendous opportunity to young and brilliant scientists in Rice Genomics to achieve their best without the constraints of NGS infrastructure and infostructure. We endeavour to deliver that at CloudSeq.

### Oral Presentation

## Eukaryotic Tree of Life Based on 98 Rice Genes Conserved in 49 other Model Organisms

Pawan Kumar Jayaswal<sup>1</sup>, Asheesh Shanker<sup>2</sup>, Tilak Raj Sharma<sup>1</sup>, Nagendra Kumar Singh<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012;

<sup>2</sup>Banasthali University, Rajasthan (\*Email: nksingh@nrcpb.org)

Rapid developments in the NGS technologies incorporated large datasets in the public domain. Comparative analysis of genomes illuminates aspects of mutational changes and evolution of life on planet earth. Here we compared the genome sequences of 49 model organisms, including 17 plants, 10 vertebrates, 13 invertebrates and 9 fungi representing different taxa of life. Analysis based 66,338 predicted CDS and 44,235 EST unigenes of rice revealed that 36,002 of the predicted genes were expressed in rice while other genes did not show any homology with rice EST database. Among all *Zea mays* showed the highest number of genes matching with rice followed by *Hordeum vulgare* and *Triticum aestivum*. Potato blight causing fungus shared the highest number of 1266 rice gene homologs among nine model fungi. Comparing 36002 rice unigenes with 21 model animal species found that 840 genes were commonly expressed in all these animals. *Bos torus* shared the highest number of 1076 genes while *Dictyostelium discoideum* shared the lowest number of 487 genes with rice. Comparative analysis of all the 49 model organisms revealed 98 genes that were commonly expressed in all these species. A eukaryotic tree of life based on these conserved genes was developed using Bayesian theory with GTR+G+I (General Time Reversible, Gamma distribution and evolutionary invariable sites) model. We estimated that *T. aestivum* and *H. vulgare* diverged from a common ancestor about 10 million years ago, while *Glycine max* and *Cajanus cajan* diverged about 33.65 million years ago.



## S201: Computational Identification of MicroRNA and Their Targets in *Zea mays* L.

Kirandeep Kaur\*, Naveen Duhan, Inderjit Singh Yadav, Yogesh Vikal

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141 004

(\*Email: k.k.flora20@gmail.com)

Maize (*Zea mays* L.) is the third most important cereal crop in Asia. It is vulnerable to large number of pathogens and diseases. MicroRNAs (~ 22 nt) play critical role in various biological processes of resistance, development and stress responses, still little is known about the miRNA responsive to a particular disease. Advancement in the computational approaches has wide open the ways for prediction of miRNAs in plants having role in plant defense. In the present study 2,019,524 ESTs sequences were fetched from dbEST and were processed with seqclean for automated trimming and validation of sequence. A total of 17,55,524 sequences passes the strict filtering criteria, which were further masked for repeat elements and clustering was performed to create a unigene dataset. The Unigene dataset was used for finding the putative miRNAs by performing a local BLAST with the matured and validated miRNAs retrieved from miRBase. Stem loop structure of potential matched segments was generated. Finally, a total 30 miRNAs were selected on the basis of MFE value. These were found to hybridize with some important genes such as respiratory burst oxidase, histidine kinase 2, lipoxigenase 1, HSP70 etc. These miRNA will be further validated for their role in plant defense.

## S202: Comparative Transcriptome Profiling of Two *Brassica juncea* Cultivars with Contrasting Nitrogen Use Efficiency under Various Nitrate Treatments

Parul Goel<sup>1,2</sup>, Rohit Chauhan<sup>1</sup>, Mohit Kumar Swarnkar<sup>1</sup>, Vandna Chawla<sup>1</sup>, Ravi Shankar<sup>1</sup>  
and Anil Kumar Singh<sup>1,2, \*</sup>

<sup>1</sup>Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176 061, H.P.; <sup>2</sup>Academy of Scientific and Innovative Research, New Delhi (\*E-mail: anil@ihbt.res.in)

Nitrogen (N) is one of the major essential mineral elements and often a primary limiting factor for plant growth and yield in agriculture. Rapeseed crop has relatively high demand of N because N content in its seeds and plant tissues is greater than in most other grain crops. However, no study has been performed to understand the global N-response of *Brassica* species. In the present work, RNA-seq analysis of two *B. juncea* cultivars namely, Pusa Bold (with high NUE) and Pusa Jaikisan (with low NUE) was performed under various nitrate treatments and time points. In total 42 RNA-seq libraries were sequenced using Illumina GAIIX and 446,580,418 paired end reads were generated. Quality filtered reads were used for de novo assembly that resulted in 46,556 transcripts with N50 value 1,393bp. Transcripts were functionally annotated using BLASTx, GO, EC and KEGG programmes. In this study, we identified contigs encoding for 9 nitrate transporters, 3 ammonium transporters, 2 nitrate reductase, 1 nitrite reductase, 6 GS/GOGAT, 2 glutamate dehydrogenase and 4 asparagine synthetase and most of them were found to be induced within 2h of nitrate treatment in Pusa Bold, while in Pusa Jaikisan their induction was found to be at later time points. In addition, several kinases and transcription factors were also identified that may act downstream to nitrate signalling. Our study will provide a catalogue of nitrate responsive genes and some other novel genes that may have role in nitrate regulatory network in *B. juncea*.



### **S203: Identification of Genomic Regions for Delayed Senescence Related Traits in a WL711/C306 RIL Population in Wheat**

Neeta Dwivedi<sup>1</sup>, Kalpana Singh<sup>1,\*</sup>, Vimal Kumar Semwal<sup>1</sup>, Beerpal Singh<sup>1</sup>, Jitesh Kumar<sup>1</sup>,  
Nagendra Kumar Singh<sup>2</sup>, Renu-Khanna Chopra<sup>1</sup> and Ravinder Kaur

<sup>1</sup>Plant Physiology Laboratory, Water Technology Centre, ICAR-Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup> ICAR-National Research Centre on Plant Biotechnology, New Delhi 110012 (\*Email: kalpanaschauhan@yahoo.co.in)

Drought, the most prominent threat to agricultural production worldwide, accelerate leaf senescence, leading to decrease in canopy size, loss in photosynthesis and reduced yields. A population of 206 recombinant inbred lines (RILs) derived from WL711/C306 was phenotyped for delayed senescence related traits such as green flag leaf area at anthesis (A), A+25 and A+30 days after anthesis, senescence score, SPAD chlorophyll value under water deficit stress (WDS) and irrigated (IRR) environments. WL711 showed higher GFLA than C306 at A and A+25 DAA and C306 showed higher GFLA than WL711 at A+30 DAA under WDS. The RIL population showed considerable variation, normal distribution and transgressive segregation for GFLA at all stages under both water regimes. The genetic linkage map of WL711/C306 RIL population was constructed comprising of 346 markers. The total map distance was 4526.8cM with an averaged interval of 12.9cM between adjacent markers. Major QTLs for GFLA at A+25 DAA and GFLA at A+30 DAA were detected on chromosome 3BS and 4DS under WDS respectively. Major QTLs for senescence score were identified on chromosomes 2DS, 3BS, 3DL, 4AL and 6DL. After validation, markers associated with QTLs may be used by wheat breeders in the marker assisted breeding for delayed senescence related traits in wheat.

### **S204: Transcriptional Properties of ONAC024 in Rice Seed**

Iny Elizebeth Mathew\* and Pinky Agarwal

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India  
(\*Email: iny.elizebeth@nipgr.ac.in)

NACs constitute one of the largest families of plant-specific transcription factors (TFs). In our previous microarray analysis, we found that *ONAC024* is a seed-preferential NAC gene. This gene shows high expression in rice seed developmental stages, even in five different genotypes varying in seed weights. Specific expression of *ONAC024* in the seeds can be credited to its 2 Kb promoter region as evidenced by the promoter-GUS analysis of transgenic plants. We also obtained three different forms (A, B and C) of the transcript due to short direct repeat (SDR) mediated alternative splicing. Presumably, forms B and C act as competitors to the main form, as they have arisen due to change in the C-terminal domain only. By themselves, these proteins are localized in the cytoplasm. Only the most abundant form A is localized to the nucleus following its interaction with another NAC TF, *ONAC023*, as shown by BiFC experiments. Further, the different forms also show varying levels of transactivation ability. Over expression of this protein *in planta* points to the necessity of maintaining the temporal expression of *ONAC024* at an optimal level, as these plants show a slower growth rate with weaker and thinner aerial parts. The presentation will focus primarily on these properties in the functioning of *ONAC024* as a TF, in rice seed development.



## **S205: *In Silico* Functional Analysis and Molecular Modeling of Inositol Pentakisphosphate 2 Kinase (IPK1), a Potential Low Phytate Trait Gene from *Glycine max***

Nabaneeta Basak, Veda Krishnan, Vanita Pandey, Mansi Punjabi, Alkesh Hada, Ashish Marathe, Monica Jolly and Archana Sachdev\*

Division of Biochemistry, Indian Agricultural Research Institute, Pusa, New Delhi 110012  
(\*Email: arcs\_bio@yahoo.com)

Inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPK1) [EC: 2.7.1.158], the bottleneck enzyme in the phytic acid pathway optimizes the metabolic flux of phytate generation in soybean. Given its distinct role in the metabolism of highly phosphorylated inositols, in the present study, 1371 bp nucleotide sequence representing the full length cDNA of *Gmipk1* was cloned and sequenced. The differential spatial expression profiling of *Gmipk1* revealed it to be seed specific and hence a right candidate for developing the low phytate trait. Biocomputational tools were employed to predict the protein's properties, conserved domains, and secondary structures. Using *in silico* physicochemical approach a three dimensional (3D) GmIPK1 protein model (PMD id - PM0079931) was developed. Cleft analysis followed by simulations performed with docking software using ADP as the most likely ligand indicated Gly19, Asn22, Val38, Ser148 and His155 to be the preferred catalytic nodes. This study provides some fundamental knowledge on the distinct mechanistic steps performed by the key residues to elucidate the structure-function relationship of GmIPK1, as an initiative towards engineering "low phytate soybean".

## **S206: Comparative Analysis of Anther Transcriptomes of CMS (*Moricandia arvensis*) *Brassica juncea* Male Sterile and Fertility Restorer Lines**

Deepak Singh Bisht\*, Ajay Mahato, Vajinder Kumar, Viswanathan Satheesh and Shripad R Bhat

ICAR-National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110012 (\*Email: deebisht@gmail.com)

Cytoplasmic male sterility (CMS) and fertility restoration system involves cross talk between the nuclear and mitochondrial (mt) genomes. We have identified the mt-gene imparting male sterility and mapped the fertility restorer gene to the A9 chromosome of *B. juncea*. The *Moricandia arvensis* based CMS system is being used to breed hybrid varieties. The present study was undertaken to identify the candidate fertility restorer gene through comparative transcriptome analysis. We obtained 99.3 million and 121.0 million raw reads from CMS line and restorer line, respectively, using Illumina HiSeq 2000 platform. The reads were assembled into 126988 unigenes with an average length of 1768 bp. BLAST analysis retrieved annotation for only 81624 unigenes. A total of 67552 unigenes were assigned 512751 GO terms and 7967 unigenes had COG classifications, distributed in 25 categories. RPKM analysis revealed 1852 transcripts as differentially expressed between the two samples with log<sub>2</sub> (fold change) >2. Twenty-six DEGs were picked at random and tested by qRT-PCR, and 23 of them showed differential expression between male sterile and male fertile anther, consistent with the gene expression differences estimated based on transcriptome analysis. The results provide a comprehensive framework for understanding the anther development and the mechanism of CMS in *B. juncea*.



## S207: Utility of the Internal Transcribed Spacer Region (ITS2) of nrDNA in Elucidating the Species Relationships in Genus *Cucumis*

Nidhi Shubhanand<sup>1,2,3</sup>, Madhoolika Agrawal<sup>2</sup> and KV Bhat<sup>3</sup>

<sup>1</sup>Division of Genomic Resources, National Bureau of Plant Genetic Resources, New Delhi 110 012; <sup>2</sup>Department of Botany, Banaras Hindu University, Varanasi 221 005; <sup>3</sup>Department of Botany, MKR Government Degree College, Ghaziabad 201003 (\*Email: nidhi.shubhanand@gmail.com)

The genus *Cucumis* L. (Cucurbitaceae) includes two important vegetables of commerce, cucumbers and melons. Tracing the species relationships of cucumbers and melons is important for introduction of disease resistant genes from wild relatives and development of new varieties and thus promoting the beneficial utilization of genetic resources. Despite the presence of rich diversity for the genus *Cucumis* in India, the crop-wild relative complex has not been studied exhaustively for establishing their identity and developing a system for classification, conservation and management. Present study was planned to analyse the phylogenetic informativeness of the Internal Transcribed Spacer region 2 (ITS2) of nuclear ribosomal DNA for species relationship studies of *Cucumis* species collected from India. The analysis included commercially important species such as melon (*C. melo* L.) and cucumber (*C. sativus*) along with their wild relatives. The phylogenetic analysis was performed based upon the genetic distance matrix using the neighbor-joining (NJ) method. Cucumber along with *Cucumis hystrix*, *Cucumella*, *Mukia* and *Dicaelospermum* formed a well distinguished clade. *Cucumis setosus* was found to be close to *Cucumella silentvalleyii*. All the cultivated and wild melo species formed the second clade. *Cucumis prophetarum* and *C. dipsaceus* were branched as a separate group showing their African origin. Sequence alignment showed the presence of at least eleven significant regions in ITS2 with insertions/deletions. Internal transcribed spacer region 2 showed twenty five single nucleotide polymorphic sites. These regions were clearly defining the differences in various *Cucumis* species collected from India.

## S208: Application of PERL Scripts in Accelerating Genome Data Analysis

Hukam Chand Rawal\*, Kanti Kiran, Himanshu Dubey, Rajdeep Jaswal and TR Sharma

ICAR-National Research Centre on Plant Biotechnology, New Delhi  
(\*Email: hukam.rawal@gmail.com)

Genome data analysis is one of the important tasks of many biologists. It includes sequencing of genomic DNA and various types of genome analysis using bioinformatics tools. PERL scripting is a best companion for biological data analysis since the emergence of bioinformatics. Although many blogs and forums provides in-built PERL scripts, while dealing with genome level analysis, we need many in-house PERL scripts at one or other steps as per our needs and available data. At genome-level analysis we need to parse the large outputs from different tools, we need to speed up the annotation job as with a genome of even 60 Mb size, we may predict more than 10,000 genes and analysis of those genes will take extra time and efforts without scripts and programming. We analyzed more than 20 genomes of *Puccinia* species and developed many in-house PERL scripts. These scripts can automatically search and assign the best function to a gene from the multiple significant hits of BLAST output to accelerate the functional annotation process of thousands of genes and parsing the large outputs from software for useful data (eg. FASTA sequences from FGGENESH or repeats from MapRep) in one go. In-house PERL scripts which helped us to accelerate our genome analysis program will be explain in this paper for the larger interest of the community.



## **S209: Genetic and Genome-Wide Transcriptome Analysis of an EMS Induced Mutant with Short Root, Short Grain Showing Enhanced Drought Tolerance in Rice**

*Lima JM<sup>1,2</sup>, Nath M<sup>1,3</sup>, Bahadur K<sup>1</sup>, Ramkumar M<sup>1</sup>, Pattnaik S<sup>1</sup>, Sharma RP<sup>1</sup>, Mohapatra T<sup>1,4</sup>, SV Amitha CR Mithra<sup>1,\*</sup> and Singh NK<sup>1</sup>*

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, New Delhi; <sup>2</sup>North Orissa University, Baripada, Odisha; <sup>3</sup>International Centre for Genetic Engineering and Biotechnology, New Delhi; <sup>4</sup>Central Rice Research Institute, Cuttack (\*Email: amithamithra.nrcpb@gmail.com)

Drought is one of the complex traits that severely affect plant growth and yield. Mutagenesis is an important research tool that provides valuable resources to understand functional genomics. From the National repository of rice EMS mutants at NRCPB, New Delhi, 500 mutants of the upland rice variety Nagina22 at M<sub>6</sub> generation were screened at vegetative and reproductive stage in field structure and PVC pipes under soil moisture deficit conditions. From this a gain of function mutant for drought stress performing better in terms of various physiological drought measures under moisture deficit conditions was identified. The mutant showed identical pattern in SSR genotyping at 72 selected loci to wild type (WT). However it had altered morphology showing reduced plant height, panicle length, seed size and root length as compared to the WT. Scanning Electron Microscopy (SEM) studies revealed higher number of partially opened and completely closed stomata in the mutant than the WT under moisture stress condition. Gene expression analysis using microarray revealed a total of 65 differentially expressed genes (DEGs) in normal condition and 745 DEGs in stress conditions of mutant as compared to the WT. F<sub>2</sub> populations derived from the cross with the WT revealed that the mutant phenotype is governed by two genes with inhibitory gene action, consistently showing 13:3 wildtype: mutant plants. Efforts are underway to map and identify the causal genes governing moisture deficit stress tolerant phenotype in the mutant. Further, differentially expressed genes identified may reveal the mechanisms responsible for moisture stress tolerance in rice.

## **S210: Evidences for Functional Adaptation of Cyanobacterial Genomes Towards Different Ecological Niches**

*Ratna Prabha, DP Singh\*, Ashutosh Kumar Rai and Arun K Sharma*

ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Maunath Bhanjan 275103 (\*Email: dpsfarm@rediffmail.com)

Cyanobacteria were subjected to a variety of niche-specific competitive forces in the environment that result in unique characteristics of each genome. We analyzed complete genome sequence of 84 cyanobacteria with respect to functional composition of their genomes and the part of genome contributing to particular cellular processes. The results reflect both cellular and ecological strategies associated with adaptation to environmental conditions. The size of cyanobacterial genomes varied from 1.44 Mb to 9.06 Mb. It was observed that cyanobacteria exhibiting marine habitats tend to have lower genome size as compared with those residing in other habitats. Functional characterization of cyanobacterial genomes was done using the Clusters of Orthologous Groups (COG) and entire genes of each genome were subjected to COG assignment. Only 400 COGs (15%) were common in all the 84 cyanobacteria while rest represent functions of pan-genome for the organisms in which 6.68% functions are strain-specific i.e. owned only by individual single member. It was observed that across all

cyanobacteria, genes with metabolic functions gained maximum share. Next most abundant functional category in most of the cyanobacteria was that of poorly categorized genes. Clustering on the basis of functional categories divided all the cyanobacteria in two groups, (i) cyanobacteria with diverse habitats (ii) aquatic cyanobacteria. In general, habitats seem to influence the functional profile. While marine cyanobacteria preferred genes for 'Information Storage and Processing' over 'Cellular Processes and Signalling', later one is preferred by cyanobacteria from other habitats. It has already been suggested that bacterial genomes contain specific functional gene inventories which is in concurrence with their survival in the particular ecological niche. This study facilitates a better understanding of the functional profile of cyanobacteria inhabiting diverse ecological niches and help to identify shifts in their functional profile towards ecological adaptation.

### **S211: Genome Size Estimation of Guggul [*Commiphora wightii* (Arnott) Bhandari]: A Study by Fluorescence Activated Cell Sorting Analysis**

Ashok Kumar Bishoyi<sup>1</sup>, Aarti Kavane<sup>1</sup>, Geetha KA<sup>1</sup> and SR Bhat<sup>2</sup>

<sup>1</sup>ICAR- Directorate of Medicinal and aromatic Plants Research, Boriavi, Anand, Gujarat; <sup>2</sup>ICAR- National Research Centre on Plant Biotechnology, New Delhi (\*Email: ashokbiotech4@gmail.com)

Guggul [*Commiphora wightii* (Arnott) Bhandari] is a rare, endangered and threatened medicinal plant which has not received much scientific attention. Guggul reproduces via adventive embryony, a form of sporophytic apomixis. We have initiated transcriptomics investigation of adventive embryony in guggul. Genome size is a fundamental parameter and knowledge of genome size is the first step while planning high throughput genome/transcriptome sequencing. We have employed Fluorescence Activated Cell Sorting (FACS), a convenient, rapid and extensively used method for estimating genome size in guggul. Propidium iodide stained nuclei isolated from different tissues (fruit walls, ovules, leaves, petals) were tested for the FACS analysis and well expanded leaf tissue was used for the determination of genome size. Relative fluorescence intensity of the stained nuclei was analysed by using laser beam of PA II Ploidy analyser (Partec, Germany). Tomato (*Lycopersicon esculentum*) and Isabgol (*Plantago ovata*) were used as reference standards for the analysis. The fluorescence peaks of guggul overlapped with tomato implying that genome size of guggul is comparable to tomato. The results were confirmed by the second reference standard isabgol. The study showed that approximate genome size of guggul is  $1.123 \pm 0.06$  pg or  $(1.098 \pm 0.058$  Gbp). This information is being used to plan genomic survey, sequencing and transcriptomic analysis of guggul.



## S212: Reconstitution of Pigeonpea Sterility Mosaic Virus Genome Sequence by Next Generation Sequencing of Small RNAs using Ion Proton Technology

Basavaprabhu L Patil

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi  
(\*Email: blpatil2046@gmail.com)

Pigeonpea sterility mosaic virus (PPSMV), a species of the genus Emaravirus, is the causal agent of Sterility mosaic disease (SMD) of pigeonpea. For a long time the genome sequence of PPSMV was not known and very recently the genome sequence of PPSMV from Patancheru (Telangana) has been published. In a virus-infected plant, small interfering RNAs (siRNAs) corresponding to the viral genome form a large proportion of the small RNA population. 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. For the first time here we report the NGS of small RNA population from virus infected pigeonpea plants collected from Gulbarga (Karnataka) and New Delhi, 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 significantly reconstituted. The data revealed that the virus infected pigeonpea sample from Gulbarga was infected by PPSMV, while the sample from Delhi was infected by a geminivirus, Mungbean yellow mosaic India virus (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 (21nt, 22nt & 24nt) across the viral genomes was studied. 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.

## S213: Genome Wide Identification of Target Mimics (eTMs) for miRNAs in Wheat (*Triticum aestivum*)

Tinku Gautam<sup>1</sup>\*, Anuj Kumar<sup>2</sup>, HS Balyan<sup>1</sup> and PK Gupta<sup>1</sup>

<sup>1</sup>Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Ch Charan Singh University, Meerut 250 004, UP; <sup>2</sup>Bioinformatics and Documentation Laboratory Camp Office, Uttarakhnad Council for Biotechnology, Silk Park, Prem Nagar, Dehradun 248007  
(\*Email: tinkugoutam@gmail.com)

The role of miRNAs in the regulation of gene expression in plants is known for more than a decade now. A novel mechanism called “target mimicry” was discovered in 2007, providing another layer of regulation for modulating miRNA activities. Target mimicry involves sequestering of miRNAs by newly discovered endogenous target mimics (eTMs). Very few studies are available in plants for the identification of endogenous TMs, and no study on TMs has so far been undertaken in wheat. In the present study, in silico genome-wide search was made for putative endogenous eTMs for 116 miRNAs (miRbase 21) in wheat. For these 116 miRNAs, 381 TM sites for 83 miRNAs were predicted. In these TMs, miRNA binding sites were well conserved, but sequences outside the binding sites varied a lot. The density of TM sites was found to be much higher within the CDS region (208 mimic sites), relative to 3'UTR (105 mimic sites), 5'UTR (55 mimic sites), 5'UTR+CDS (3 mimic sites) and ncRNA regions (2 mimic sites). A precise manipulation of an endogenous RNA regulatory network affords an opportunity to achieve a desired outcome of enhanced plant development or response to environmental stresses.



## **S214: Comparative Genome Analysis Reveals Species-Specific Genes Involved in the Different Life Styles of Fungi**

Meenakshi Sharma, BN Devanna, NK Singh and TR Sharma

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012  
(\*Email: trsharma@nrcpb.org)

Fungal pathogens cause severe devastation to crops worldwide and their different types of host infections make them fall into various groups such as, biotrophs, hemi-biotrophs, necrotrophs and saprotrophs. Each group has a battery of conserved as well as species-specific genes which define their respective lifestyles. We report here comparative genomic analysis of species-specific genes, belonging to four different pathogens like *Puccinia triticina*, *Magnaporthe oryzae*, *Sclerotinia sclerotiorum* and *Neurospora crassa* representing different modes of parasitism and infection strategies. Our study revealed that all these fungal genomes exhibit tremendous diversity. Species-specific secretory proteins belong to different classes of proteins linked to pathogenicity and effectors. Analysis of species-specific pathogenicity related proteins, revealed the presence of maximum number of transporters and binding proteins in biotrophic fungi, few in hemibiotrophs, but completely absent in the necrotrophs and saprotrophs. The species-specific genes reported might be contributing to the divergence and different lifestyles of respective fungi. The network analysis revealed the presence of certain hub genes, lack of which could hamper the whole biosynthetic pathway of each fungal species. This study provides insight on specific genes that are found to be associated with their nutritional strategy, host specificity and pathogenicity factors.

## **S215: Genome-wide Analysis of Novel Unknown Expressed Protein Genes for Drought Tolerance in Rice**

Ankit Singh<sup>1</sup>, Karikalan J<sup>1</sup>, Apoorva Bhatnagar<sup>1</sup>, Viswanathan Chinnusamy<sup>2</sup>, SV Amitha CR Mithra<sup>1</sup> and Pranab Kumar Mandal<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa campus, New Delhi 110012;

<sup>2</sup>Department of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012  
(\*Email: pranabkumarmandal@gmail.com)

Drought is one of the most limiting factors for stable crop production worldwide, which adversely affects rice productivity in many parts of the world and is expected to aggravate further due to global climate changes. Identification and functional characterization of stress responsive genes is an important step towards understanding and improving drought tolerance of rice. Around 111 Drought Tolerant QTLs (DTLs) which have 3.0 or more LOD score from different mapping populations, derived from genotypes such as Vandana, Azucena, Bala, IRAT109, IR62266-42-6-2, IRGC 105491 were selected to identify and validate novel drought tolerant genes. These 111 DTLs contain a total of 21,594 genes of which 5636 (26%) are expressed protein genes of unknown function. Genome-wide transcriptome meta-analysis of expressed protein genes in contrasting genotypes (N22, IR64, IRAT109 and Zhenshan97) showed 44 genes to have more than 2 fold differential expression under water stress. Of these, 21 genes are from yield related DTLs. To validate the expression of these 21 expressed protein genes, we have initiated expression study with four drought tolerant indica genotypes namely Dagardeshi, Nagina22, Vandana, Sahbhagidhan, and two drought sensitive genotypes IR64 and Swarna in vegetative as well as reproductive stages under well watered and water stress conditions. Gene03-51470 seems to be a promising candidate for further analysis since it shows consistent differential expression across both the stages in tolerant and sensitive genotypes under drought. However, other genes showed clear contrasting expression only at the vegetative stage.



## **S216: Microbial Diversity Analysis of Hot Water Spring of Tattapani, Himachal Pradesh, Through Metagenomic Approach**

*Mahesh Mahajan, Dushyant P Singh, Etika Goyal, Amit K Singh, Kanika Kumar\**

ICAR-National Research Centre on Plant Biotechnology, IARI, Pusa Campus, New Delhi

(\*Email: kumarkanika@rediffmail.com)

Thermal water springs harbour a large diversity of microorganisms but their microbial community diversity has not been fully studied as yet. Only 0.1%-1% of the total microorganisms from environment can be cultured. Metagenomics bypasses the need for isolation or cultivation of microorganisms, as it deals with direct isolation of nucleic acids from environmental samples. In present study metagenomic profile of microbial community inhabiting hot water spring at Tattapani in Himachal Pradesh, situated at an altitude of 656m above sea level, was analysed. The physical parameters of water were measured as pH 7.5, temperature ~63°C, TDS 4800 mg/L. Universal 16S rRNA gene specific primer pair U789F-U1053R was used to amplify partial 16S rRNA gene. In-silico analysis of the sequences showed presence of both archaea and bacteria in hot water spring. Bacterial phyla included chloroflexi, bacteroidetes, firmicutes, proteobacteria, actinobacteria, thermodesulfobacteria, caldiserica, aquificae, deinococcusthermus, and many unclassified bacteria. Phylum archaea included euryarcheota, crenaeota along with many unclassified archaea. To the best of our knowledge results obtained using metagenomic approach demonstrated for the first time the presence of both bacteria and archaea in the water of Tattapani hot spring.

## Technical Session III: Plant Development and Stress Biology

*Invited Lecture*

### **Characterization of Blue Light Sensing Cryptochrome 2 from Brassica and Rice**

*Jitendra P Khurana\*, Pooja Sharma, Sushma Mishra and Dibyendu Kumar*

Interdisciplinary Centre for Plant Genomics & Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021 (\*Email: khuranaj@genomeindia.org)

Light regulates plant development through the combinatorial interaction of red/far-red sensing phytochromes and blue/UV-A receptors, cryptochromes and phototropins. Recently, a few other blue light and UV-B sensing photoreceptors have also been identified. In the past nearly 15 years, our group has made concerted efforts to understand the role of cryptochromes (CRYs) in crop plants like Brassica and rice. We have characterized *CRY1* and *CRY2* genes from Brassica napus, an oilseed crop, and *CRY2* from rice. *Brassica napus* harbours two copies of *CRY2* and they differ in their expression profile. One of them, *BnCRY2a*, has been characterized in detail; it represents a light labile species. When over-expressed in *B. juncea*, it causes shortening of the hypocotyl, akin to *CRY1*, but stimulates leaf expansion and promotes precocious flowering in transgenics. The effect of *CRY1* and *CRY2* in Brassica transgenics is fluence dependent. The *OsCRY2* over-expression in both rice and Arabidopsis causes constitutive photomorphogenesis and early flowering in Arabidopsis; some of the light-regulated genes are up-regulated even in dark. Besides posing some interesting basic questions, these data provide evidence that both *CRY2* genes can be used as a potential tool for altering plant height and flowering time in crop plants.

*Invited Lecture*

### **A Blue Light Mediated MKK3-MPK6-MYC2 Module Regulating Arabidopsis Seedling Development**

*Alok Krishna Sinha*

National Institute of Plant Genome Research, New Delhi 110067 (Email: alok@nipgr.ac.in)

Mitogen activated protein kinase (MAPK/MPK) cascade are known to regulated several stress responses and developmental processes in eukaryotes. They usually consist of three tier components consisting of MAPK, MAPK kinase (MAPKK/MAP2K/MKK) and MAPKK kinase (MAPKKK/MAP3K/MKKK) connected to each other with an event of phosphorylation. Light signal transduction pathways have extensively been studied in plants and are known to control several developmental processes. However, till date a direct connection of MAPK and light signaling pathways is not reported. Here, we present a MAPK module, consisting of MKK3-MPK6 that is activated by blue light in a MYC2 dependent manner. MYC2 is a transcription factor that negatively regulates blue light signaling during Arabidopsis seedling development. MPK6 physically interacts and phosphorylates MYC2, and is phosphorylated by MKK3. Furthermore, MYC2 binds to MPK6 promoter and regulates its expression in a feedback regulatory mechanism in blue light signaling. The mutational and physiological studies illustrate the function of MKK3-MPK6-MYC2 module in Arabidopsis seedling development and provide a new mechanistic view of photomorphogenesis.



## Genetic Regulation of Leaf Size and Biomass in *Arabidopsis thaliana*

Krishna Reddy Challa and Utpal Nath\*

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012

(\*Email: utpal@mcbl.iisc.ernet.in)

Plant growth is driven by cell proliferation and expansion, processes that together control final organ size. Perturbation in either expected to affect organ growth, shape and size. Leaves of model plant *Arabidopsis thaliana* grow predominantly by cell division at early stage, while the later growth is contributed solely by cell expansion. A large number of genes affecting leaf growth have been isolated and their mutants have been studied. An important class of homologous genes that suppresses leaf growth codes for TCP transcription factors. It has been demonstrated that loss-of-function mutations in five miR319-targeted TCP genes result in prolonged proliferation whereas their gain-of-function causes precocious organ maturity. However, the direct targets of these TCP transcription factors that promote cell differentiation have not been identified, mainly due to high degree of redundancy in TCP function. To address the cellular function of TCP genes, we have generated a chemically-inducible form of TCP4 protein that is resistant to miR319 – called here as rTCP4-GR – and expressed under its endogenous promoter in the jaw-D background, a transgenic line where several TCP transcripts are reduced due to over-expression of miR319. Thus, a strong TCP4 function can be induced in this line with much reduced genetic redundancy. By using a combination of phenotypic characterization, transcriptome profiling and genetic interaction studies on rTCP4-GR plants, we show that TCP4 represses organ growth by committing the proliferating cells to differentiation by directly promoting brassinosteroid-dependent auxin response. Our studies provide a molecular basis of TCP function and organ size control.

Invited Lecture

## Devising Strategies for Aphid Resistance in Indian Mustard

RC Bhattacharya

ICAR-National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute Campus, New Delhi 110012

Aphids, (Hemiptera: Aphididae) have coevolved with their host plants and emerged as one of the most economically important insect pest of crop Brassica including Indian mustard (*B. juncea*). Their atypical feeding mechanism and parthenogenetic reproduction made them intractable to control below economic threshold level of damage to the crops. Breeding efforts for developing aphid resistance in mustard has been stalled due to unavailability of accessible resistance source. Study based on defense related unigenes indicated that aphids in the process of colonization on the mustard plants escape the endogenous host-defense response. The missing perception of aphid-associated molecular cues by the host plant renders the basal host-defense attenuated against aphids. Interestingly though, elicitation of the endogenous defense by application of exogenous chemical agent potentially reduced the population growth of aphids. One of our strategies for developing aphid resistance in mustard aims to develop transgenic plant types which can deliver specific dsRNA or siRNAs and mediate gene-silencing in the aphids feeding on such plants. In this endeavour, the feasibility of delivering host generated dsRNA into the aphids ingesting on the transgenic plants and consequent attenuation of the aphid-transcript have been demonstrated. Several metabolically important genes of mustard aphid as potential targets for RNAi and their siRNA binding domains have been identified. For adequate expression different phloem specific promoters have been analysed for their potency in driving Phloem-specific gene-expression in mustard. One of the advantages of using dsRNA as insecticidal gene is less likelihood of resistance evolution in the insect population.

## Isolation and Analysis of Novel Seed Development Mutants in Arabidopsis

Y Sreenivasulu<sup>1</sup>, Sunil Kumar Singh<sup>1</sup>, Pritu Pratibha<sup>1</sup>, Rimpdy Diman<sup>1</sup>, Isha Sharma<sup>1</sup>,  
Vajinder Kumar<sup>2</sup>, R Srinivasan<sup>2</sup> and SR Bhat<sup>2</sup>

<sup>1</sup>CSIR-IHBT, Palampur (HP), <sup>2</sup>ICAR-NRC on Plant Biotechnology, New Delhi

Seeds are the important food resource for the living organisms including humans. Seed development starts with the specification of micro- and mega-spore mother cells (MMCs) and completes with the subsequent gametophyte development, fertilization, embryo development and maturation. These processes are regulated by the expression of a number of genes and their interactions. What these programs are and how are they integrated into unique regulatory networks to form a mature seed within the plant genome remains a major unanswered question. Identification and understanding the function of novel genes involved in seed development should permit us to plan novel approaches to breed and engineer seeds with new agronomic traits. With this idea, we developed T-DNA insertion mutant lines of *Arabidopsis* and screened for the mutant lines showing defects in seed development. A number of novel genes such as Tumor Necrosis Factor Receptor (TNF-R) Associated Factor (TRAF) like gene, Coprophorphyrinogen oxidase (CPPO), Nucleotide sugar transporter (NST) and Pectinmethylesterase inhibitor (PMEI) genes were identified, which are having significant role in male and female gametophyte development and seed formation. It was found that *TRAF* gene plays a key role in the ubiquitin proteasome system (UPS) mediated hormone signaling of male and female gametophyte development and early seed development. ROS mediated regulation of male and female gametophyte development was documented for the CPPO gene.

### S301: Role of Catalase in Mitigating the Effect of Heat Stress in Wheat (*Triticum aestivum* L.)

Shweta Singh<sup>1</sup>, Ranjeet R Kumar<sup>1</sup>, Suneha Goswami<sup>1</sup>, Khushboo Singh<sup>1</sup>, Sadhana<sup>1</sup>, Sushil K Sharma<sup>1</sup>  
Himanshu Pathak<sup>2</sup>, Raj D Rai<sup>1</sup>

<sup>1</sup>Division of Biochemistry, Indian Agricultural Research Institute, Pusa, New Delhi, 110012;

<sup>2</sup>CESCRA, Indian Agricultural Research Institute, Pusa, New Delhi 110012

Ambient temperature has increased since the beginning of the century and are predicted to continue rising under climate change. Such increases in temperature can cause heat stress; a severe threat to wheat production in many countries, particularly during reproductive and grain-filling phases. Heat stress reduces plant photosynthetic capacity and causes oxidative damage to enzymes, membranes, DNA etc. The present experiment was carried out to study the effect of delayed sowing on oxidative injury and catalase activity in 18 wheat genotypes (*Triticum aestivum* L.) under pot culture conditions. It was observed that heat stress caused due to delayed sowing significantly increased hydrogen peroxide accumulation in different wheat genotypes (*Triticum aestivum* L.). Varieties with increased catalase activity (HD2643, HD2967, HD2733, HD2985, and HD2864) showed lower hydrogen peroxide accumulation, whereas varieties with decreased catalase activity (HD3043, HD3090, HD2189, HD2329) showed higher hydrogen peroxide accumulation. An inverse relationship was established between the hydrogen peroxide accumulation and catalase activity in wheat (*Triticum aestivum* L.) under the heat stress. The amount of hydrogen peroxide accumulated and catalase activity seems to be closely associated with the tolerance/susceptibility of a genotype to heat stress.



### **S302: Role of Abscisic Acid in Regulating the Expression of EcMYB Gene for Drought Tolerance in Finger Millet**

*Sarita Kumari, Shipra Sharma, Pushpa Lohani and Anil Kumar*

Department of Molecular Biology & Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar

Finger millet is mostly cultivated in arid and semiarid regions of the world and is reported to be tolerant to biotic and abiotic stresses. EcMYB gene was isolated and cloned from finger millet in our lab. The gene expression gets induced in drought tolerant genotype of *Eleusine coracana* (PRM-6107) at the onset of drought. The present study was performed to investigate the role of Abscisic acid (ABA) in regulating the expression of this gene in tolerant genotype (PRM-6107) and sensitive genotype (PES-400) under drought stress. In two separate experiments, 20 days old seedlings were subjected to 15% PEG (6000 MW) and ABA (25 $\mu$ M, 50 $\mu$ M, 100 $\mu$ M) treatments respectively and expression analysis of EcMYB and NCED gene (one of the gene of biosynthetic pathway of ABA) was carried out. Our results showed that EcMYB gene expressed in both the genotypes in response to ABA whereas there was no expression of NCED gene. On exposure to drought, EcMYB gene as well as NCED gene expressed only in tolerant genotype (PRM-6107). Co expression of these two genes indicates that EcMYB gene is expressed only when optimum amount of ABA accumulates in the plant. From our study it could be concluded that expression of EcMYB gene occurs following ABA dependent signaling pathway.

### **S303: RNA Sequence Analysis to Understand Response to GA<sub>3</sub> Application in Grape (*Vitis vinifera* L.) cv. Thompson Seedless**

*Smita Maske-Ghule\*, Anuradha Upadhyay, J. Satisha*

ICAR- National Research Centre for Grapes, Manjri Farm P.O., Solapur Road, Pune 412307, Maharashtra (\*Email: biosmitas@gmail.com)

In table grape production, exogenous gibberellic acid (GA<sub>3</sub>) is applied at different stages of bunch development to achieve desirable bunch shape and berry size. RNA sequence based transcriptome analysis was used to understand the mechanism of gibberellin action at rachis, flower cluster and berry stage in table grape variety Thompson Seedless. At rachis and flower cluster stage 123 and 264 genes were found to be significantly differentially expressed within 6h of GA application. The number of DEG reduced considerably at 24h. However, at berry stage major changes occurred even at 24h and number of DEGs at 6 and 24h were 174 and 191 respectively. We performed a clustering analysis of genes regulated by GA<sub>3</sub> and various expression profiles that showed stage specific and common gene cluster. The gene ERF5, GRF10, peroxidase 64 was expressed commonly in both flower cluster and berry stage. Functional categorization and enrichment analysis revealed that several transcripts involved in abiotic and biotic stimuli, sucrose and hexose metabolism, hormone and secondary metabolism were enriched in response to application of GA<sub>3</sub>. Selected genes were validated by Real time PCR. This information will help in identifying the gene and signaling pathways associated with berry characteristics.

### **S304: Whole Transcriptome Analysis to Identify Genes Associated with Salinity Stress in Thompson Seedless Grapevine (*Vitis vinifera* L.)**

Tulshi Gaonkar\*, Anuradha Upadhyay, Manisha B Shinde, AK Upadhyay, J Satisha

ICAR-National Research Centre for Grape, Manjari Farm post, Solapur Road, Pune 412307, Maharashtra (\*Email: tulsi0812@gmail.com)

Salt stress is one of the environmental perturbations that negatively affect grapevine growth and yield. Identification of potential stress related genes is of great importance. RNA sequence based transcriptome analysis was used to study salinity stress response in grape variety Thompson Seedless. A total of 370 genes were differentially expressed in response to salt stress. The number of DEG at 6h, 24h and 7days of stress were 181, 55 and 213 respectively. Pathway analysis revealed that differentially expressed genes were mainly involved in primary and secondary metabolism, regulation of transcription, hormone response, development and protein degradation. Functional categorization and Fisher's Exact test showed that at all the three time points GO term for nucleic acid binding transcription factor activity were over represented. A group of 52 transcription factors mainly belonging to WRKY, EREB, MYB, NAC, BHLH families were upregulated. Among hormone related genes, enzymes 9-cis epoxy dioxygenase, allene oxide cyclase and SAM dependent carboxyl methyltransferase involved in the biosynthesis of Abscisic acid, Jasmonic acid and Salicylic acid respectively were significantly affected by salinity stress. Several stress related genes like LEA, dehydrin, expansin, laccase and lipoxygenase were upregulated as early as six hours of stress. This information will be useful for the identification of key genes and their subsequent use for the management of salinity stress in grape.

### **S305: A Universal Stress Protein, AtUSP18 Acts as Negative Regulator of Ethylene Mediated Abiotic Stress Tolerance in *Arabidopsis thaliana***

Monika Bhuria<sup>1,2</sup>, Mohit Kumar Swarnkar<sup>1</sup> and Anil Kumar Singh<sup>1,2\*</sup>

<sup>1</sup>Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176 061 H.P.; <sup>2</sup>Academy of Scientific and Innovative research, New Delhi (\*Email: anil@ihbt.res.in)

Role of universal stress proteins (USP) in abiotic stress tolerance is well established in bacteria, but their function in plants is not yet known. An *A. thaliana* protein, AtUSP18 that was found to be central interactor among eight other USPs was functionally characterized. Expression of AtUSP18 was shown to be induced under salt, cold, oxidative and heat stress. A T-DNA insertion mutant line from ABRC was found to have reduced expression of AtUSP18 with T-DNA insertion found to be present in its promoter region. Arabidopsis overexpressing AtUSP18 and functionally complemented Atusp mutant lines showed delayed germination and inhibited green cotyledon formation under high salinity and osmotic stress as compared to wild-type (WT) and Atusp mutant line. The ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) was found to recover the hypersensitive response of overexpression lines to high salinity. Addition of ACC with its synthesis inhibitor aminoethoxyvinylglycine (AVG) was also found to recover the hypersensitive response. However, addition of ACC with its action inhibitor, silver nitrate was not able to recover the hypersensitivity of transgenic plants. These results indicate that AtUSP18 might act as a negative regulator of ethylene-mediated salt and osmotic stress tolerance during early growth of Arabidopsis seedlings.



### **S306: Mutational Analysis for Improving Grain Quality and Stress Responses in Rice**

*Bishun Deo Prasad<sup>1</sup>, Pankaj Kumar<sup>1</sup> and Wasim Siddiqui<sup>2</sup>*

<sup>1</sup>Department of Plant Molecular Biology & Genetic Engineering; <sup>2</sup>Department of Post Harvest Technology; Bihar Agricultural University, Sabour, Bhagalpur, Bihar (\*Email: dev.bishnu@gmail.com)

Rice (*Oryza sativa* L.) is a major staple cereal crop of Asia ensuring food security to millions of people. The realized yield potential in rice is mainly achieved through genetically stable high yielding varieties. Continuous breeding for specific trait predominated by few varieties leads to narrow genetic base making the rice ecosystem highly vulnerable to different environmental stress. The possibility of increasing the genetic variability of rice varieties might be useful for breaking yield plateau, developing stress tolerance, and improving grain quality in rice. In present investigation, two rice varieties of rice namely Rajendra Mahsuri-1 and Rajendra Kasturi were irradiated with different doses of gamma rays. The LD50 for both the genotypes were found to be 350Gy. A large number of M2 generation mutants were screened for different agronomical, nutritive and abiotic stress tolerance parameters. Different mutants group like early maturing, reduced plant height, increase in length and number of panicle, Increase in length/breadth (LB) ratio of mature grain, change in grain colour were identified. The mutants were also screened on 15% PEG for drought tolerance and 150mM NaCl in hydroponically grown seedlings. These different mutants can be either directly used for varietal improvement or used in breeding programme for rice improvement.

### **S307: Effect of Gamma Radiation on Seed Germination, Survival Percentage, Plant Height, Tiller Production, and Pathogen Reaction in Mega Rice Variety of Bihar**

*Pankaj Kumar<sup>1</sup>, Bishun Deo Prasad<sup>1</sup>, Abhijeet Ghatak<sup>2</sup>, Awadhesh Kumar<sup>3</sup>, Anupam Adarsh<sup>4</sup>*

<sup>1</sup>Department of Plant Molecular Biology & Genetic Engineering; <sup>2</sup>Department of Plant Pathology; <sup>3</sup>Department of Plant Breeding & Genetics; <sup>4</sup>Department of Horticulture (Vegetable & Floriculture), Bihar Agricultural University, Sabour, Bhagalpur, Bihar (\*Email: dev.bishnu@gmail.com)

The use of gamma radiation for inducing variation in Rice (*Oryza sativa* L.) is well established. Various attempts have been made by researchers to determine the most effective mutagenic treatment for the induction of desirable traits in rice. In the present investigation, effect of different doses of gamma radiation on seed germination, survival percentage, plant height, tiller production, insect and pest resistance of Rajendra Mahsuri-1, a mega variety of Bihar, was analysed. The Lethal Dose 50 (LD50) was determined on the basis of germination of gamma irradiated seeds of Rajendra Mahsuri-1 and found to be 350Gy. Parameters like no. of chlorophyll mutants and early vigour were observed at early seedlings stage whereas, plant height, panicle length, early maturing, grain quality, insect and pest resistance were analysed at different stages on the M<sub>2</sub> generation. The result indicated that increasing dosage of gamma radiation from 300 to 500 Gy reduced field survival on the M<sub>1</sub> generation. Although no concrete pattern were observed between different doses of gamma radiation on observed phenotypes but the significant reduction in plant height, increase in panicle length, effective tillers and rice gain size on the M<sub>2</sub> generation clearly indicates the usefulness of this investigation in architectural modification of Rajendra Mahsuri-1.

### **S308: Identification of Genes Involved in Arsenic (As) Response by Deep Transcriptome Profiling of a Hyper-Tolerant Fern Species, *Adiantum Capillus-veneris***

Khushboo Gupta<sup>1</sup>, Vishal Sharma<sup>1,2</sup>, Mohit Kumar Swarnkar<sup>1</sup>, Alka Kumari<sup>3</sup> & Anil Kumar Singh<sup>1,2\*</sup>

<sup>1</sup>Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176 061, H.P.; <sup>2</sup>Academy of Scientific and Innovative Research, New Delhi; <sup>3</sup>Biodiversity Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, H.P.

(\*Email: anil@ihbt.res.in)

The fern species *Adiantum capillus-veneris* is known as hypertolerant to heavy metal, Arsenic (As). In the present study, *A. capillus-veneris* plants were treated with different concentration of arsenic viz. 150  $\mu$ M, 300  $\mu$ M and 450  $\mu$ M and the samples were collected after 3d and 7d of treatments. Using illumina platform, a total of 156,790,210 of high quality (HQ) reads were generated. These HQ reads were subjected to de novo assembly resulting into 38,365 contigs with average length of 979 bp and N50 of 1438 bp. A total of 22,331 (58.20%) transcripts were annotated using gene ontology. Out of the total transcripts 12,822 were involved in 258 KEGG pathways suggesting presence of genes encoding plant hormone signal transduction, oxidative phosphorylation and transporters. A total of 8,965 (23.36%) transcripts were identified as transcription factors, out of which MYB, ERF, bHLH, bZIP, C2H2 and NAC families were found to be highly represented. A total of 6,639 sequences containing 9,059 SSRs were identified among which, di repeats (70.10%) were most abundant followed by tri (10.30 %) and tetra (0.45 %) type of repeat motif. Differential expression analysis has identified several transcripts, which are differentially expressed in response to As treatment.

### **S309: DNA-Methylation Profiling During Dormancy and Fruit Set in Apple under High and Low Chill Conditions using Methylation Sensitive Amplified Polymorphism**

Gulshan Kumar<sup>1,2</sup>, Usha Kumari Rattan<sup>1</sup> and Anil Kumar Singh<sup>1,2\*</sup>

<sup>1</sup>Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176 061, H.P.; <sup>2</sup>Academy of Scientific and Innovative Research, New Delhi

(\*Email: anil@ihbt.res.in)

Apple is an important cash crop for most of the temperate regions of the world. During winters, low temperature plays a crucial role in phenological events of apple tree. Exposure to sufficient chilling makes the dormant buds ready to resume active growth in response to higher temperature during spring. Epigenetic regulation through cytosine methylation has remarkable effect on various developmental and adaptive processes by regulating gene expression. In present study, methylation sensitive amplified polymorphism was used to analyze the changes in cytosine methylation pattern during dormancy and fruit set under high and low chill conditions. Under low chill conditions, differential banding pattern shows increase in methylation events by 6%, while demethylation events were decreased by 2.8% as compared to high chill conditions. Accordingly, transcriptome analysis also shows higher expression of DNA methyltransferases such as CMT and DRM, and S-adenosyl methionine (SAM) synthase (involved in biosynthesis of SAM, a cofactor for methyltransferase) under low chill conditions. The polymorphic bands were found to be associated with GO terms like oxygen binding, sequence-specific DNA binding transcription factor activity and pyrimidine nucleotide-sugar transmembrane transporter activity. These observations indicate that DNA methylation is regulated by chilling acquisition and plays crucial role in setting and release of dormancy in apple.



### **S310: Natural Variation in Nitrate Starvation Induced Root System Architecture in Wheat Genotypes**

*Manju Rani, PK Mandal and Subodh K Sinha\**

ICAR-National Research Centre on Plant Biotechnology, New Delhi (\*Email: subsinha@gmail.com)

Roots play major role in determining overall growth and developments of plants as they explore underground strata for water and nutrients. In addition to it, roots have significant impact on ability to adapt different environmental constraints such as nutrient availability. They exhibit different morphological responses to different nitrogen level indicating its developmental plasticity in relation to nitrogen availability. In order to understand the genetic diversity in root morphology among different wheat genotypes in response to nitrate starvation, seven root system architecture parameters such as Total Root Size (TRS), Main Root Size (MRP), Branch Angle (BA), Lateral Root Size (LRS), 1st Order Lateral Root (LR) Number, 2nd Order Lateral Root Number and LR density/MR, were studied at seedling stage in seventeen wheat genotypes grown in solution culture as well as solid neutral (in terms of plant nutrients) media. We observed nitrate starvation induces overall root system architecture in order to provide nitrate to plants for its normal growth and developments. However, the ability of such modulation is genotype depended. Further the solid neutral media, in contrast to solution culture, induced much pronounced effect on overall architecture indicating their nitrate foraging ability in natural soil condition. In general, total root size and lateral root numbers increased greatly under nitrate limitation condition.

### **S311: Potential Biochemical and Molecular Indicators of Salinity Tolerance in Contrasting Pigeonpea (*Cajanus cajan* L.) Genotypes to Salt Stress Imposition**

*Vijay Kapale, Monika Awana, OP Yadav, Suresh Kumar, RD Rai and Archana Singh\**

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110012  
(\*Email: sarchana19@gmail.com)

Pigeonpea (*Cajanus cajan* (L.) Millsp), commonly known as 'Arhar' or 'Tuhr' is one of the major grain legume (pulse) crops. It is tolerant to drought and high temperature but it is having very limited tolerance to salinity. Based on morphological and biochemical expression of antioxidant potential (AP), lipid peroxidation (LP) and total phenolic contents (TPC), the most salt-susceptible (ICP1071) and the salt-tolerant (ICP7) genotypes were selected out of 160 genotypes. LP and TPC estimations were found more in shoot than that in the root, but AP was found to be comparable. Partial CDS for hybrid proline-rich protein (HYPR, 218 bp), cold, drought salt regulatory protein (CDS, 207 bp) and cyclophilin protein (CYP, 323 bp) were cloned from ICP7 genotype and sequences were submitted to NCBI with the accession Nos. KP996197, KP996198 and KR262820, respectively. Salt stress imposition caused 29% increase in global-methylation level in root of the salt-susceptible genotype, while it caused 58.2% decrease in global-methylation in root of the salt-tolerant genotype. Quantitative methylation and expression analyses of these genes are in progress and the results will be presented. These findings will help in understanding the important epigenetic, molecular and biochemical mechanisms underlying the salinity tolerance in pigeon pea.



### **S312: Terminal Heat Stress Induced WRKY Transcription Factor (TaWRKY10) From Bread Wheat: Regulation and Characterization**

*Sushma Khomdram and Sharmistha Barthakur\**

ICAR-National Research Center on Plant Biotechnology, Pusa campus, New Delhi 110012

(\*Email: sbthakur@yahoo.com)

The changing climate leading to terminal heat stress in wheat has become an important issue which will have a significant influence on the metabolic processes, and thus on its yield and grain quality. To isolate and functionally characterize novel genes regulating adverse terminal stress effects, we have constructed SSH libraries from grain filling stages and cloned and characterized novel genes modulated differentially under terminal stress. One candidate gene belongs to the WRKY group of transcription factors involved in various developmental processes including stress tolerance. Comprehensive transcript profiling of WRKY10 was carried out in two diverse wheat cultivars, WR544 and HD2967. Differential expression was observed in both the cultivars with higher induction in WR544, a late sown cultivar. Highest induction was observed in early boots and post anthesis spikelets. A 650bp full CDS was cloned, sequenced, and in silico and phylogenetic analyses was carried out. Further validation of TaWRKY10 was done in E.coli by cloning in pET28a. Heterologous expression also showed tolerance to abiotic stresses in E.coli and transient expression was confirmed in Nicotiana. The results of the above will be presented.

### **S313: Effect of Pollination on Apomictic and Sexual Embryo Development in *Commiphora wightii*- A Data Deficient Indian Medicinal Plant**

*Aarti Kavane\*, Ashok Kumar Bishoyi and KA Geetha*

ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat

(\*Email: aartikawane@gmail.com)

Apomixis is an asexual mode of reproduction, where seeds are produced without fertilization. The degree of apomixis and polyembryony vary both within and between species. Apomixis with adventive polyembryony has been reported in *Commiphora wightii*. In the present investigation, effect of pollen grains on the apomictic embryo development was studied in the selected accessions of Guggul (DMAPRCW 02, DMAPRCW 22 and DMAPRCW 37) by monitoring embryo and endosperm development by histological methods and flow cytometry, respectively. Controlled pollinations were done in one set of flowers which were bagged and another set of flowers were pollinated with viable pollen grains and a third set of flowers were allowed natural pollinations. The study revealed that pollinations increased fruit setting (parthenocarpy) and number of apomictic embryo initials in the studied accessions. The flow cytometric analysis of developing ovules of the pollinated flowers showed both 2x:4x and 2x:3x ploidy levels of endosperm. The study reveals that apomixis is facultative and triple fusion endosperm developments also occur based on the availability of pollen grains in the studied accessions.



### S314: Development of Integrated Linkage Map for Powdery Mildew Resistance Locus *er1* in Pea

Harsha Kumara Hegde<sup>G1</sup>, Viveka Katoch<sup>2</sup>, Rajeev Rathour<sup>1</sup> and Rakesh Kumar Kapila<sup>1\*</sup>

<sup>1</sup>Department of Agricultural Biotechnology, CSK HPKV Palampur, 176 062, H.P.; <sup>2</sup>Department of Vegetable Science & Floriculture, CSK HPKV Palampur 176 062, H.P.

(\*Email: rkkapila@gmail.com)

Powdery mildew (*Erysiphe pisi* D.C.) of pea is an important disease which causes considerable losses. Three genes, two recessive, *er1* and *er2*, and one dominant *Er3* are reported to govern powdery mildew resistance in pea. Among these genes *er1* offers complete resistance in comparison to the resistance of *er2*. Therefore, the present investigation was undertaken to know the genetics of resistance to powdery mildew in the genotype 'JI1559' using 600 F<sub>2</sub> plants derived from a cross between EC387115 (susceptible) and presumptive *er1* donor 'JI1559'. For linkage analysis, 22 previously reported *er1* linked and other markers known to map close to *er* locus were surveyed. Ten markers that were polymorphic, were used for genotyping of 135 F<sub>2</sub> resistant plants for recessive-class linkage analysis. The segregation of F<sub>2</sub> population fitted well in 3S:1R Mendelian ratio indicating monogenic recessive inheritance. Linkage analysis further led to construction of an integrated linkage map of *er1* locus comprising 9 markers with a total coverage length of 25.8 cM. SSR markers, A5, AA467, AD59 and AD159 mapped near to *er1* locus at map distances of 2.7, 3.1, 3.8 and 3.8 cM, respectively. The genic marker *er1-1/AsuHPI-B*, recently developed for a loss of function mutation in *PSMLO1* gene of the same genotype co-segregated with *er1* locus in the present study validating the marker as well as the allele *er1-1* of the genotype, 'JI1559'. In addition to this genic marker (*er1-1/AsuHPI-B*), the other closely linked markers (A5, AA467, AD59 and AD159) will provide multiple/alternate options to the plant breeders for tracking the *er1-1* gene/allele in gene pyramiding and other marker assisted breeding programmes in pea.

### S315: Contrasting Wheat Genotypes Exhibit Changes in Global/Site-specific DNA Methylation and Expression of HKT Genes on Salt Stress Imposition

Beena AS, Monika Awana, Archana Singh, RD Rai and Suresh Kumar\*

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110012

(\*Email: sureshkumar3\_in@yahoo.co.uk)

Epigenetic mechanisms have been shown to modulate gene expression in plants under environmental stresses. Though effect of salt stress on cytosine methylation has been studied in crop plants, its functional consequences are still unexplored. Our studies on changes in cytosine methylation in contrasting wheat genotypes on salt stress imposition revealed that basic methylation level in shoot of both the genotypes was higher (5.4-6.6%) compared to that in the root (5.4-6.6%), and salt stress caused further increase (by 10%) in methylation in tolerant genotype. Quantitative analyses of cytosine methylation revealed that HKT2;3 gene in tolerant genotype was hypermethylated (88%) in both root and shoot compared to the methylation status (74%) of the gene in susceptible genotype. Salt stress was found to increase cytosine methylation even in susceptible genotypes (from 74% to 79%), and the increase was more prominent in root than that in the shoot. Most of the cytosine in CG context was methylated in both the genotypes, but a significant variation in methylation level was observed in CHH context. Expression analysis of HKT2;3 gene indicated that hyper-methylation of the gene in shoot of salt-tolerant genotype down-regulated its expression, while its hyper-methylation caused up-regulated expression in root of the salt-tolerant genotype. Thus, understanding epigenetic regulation of gene expression in different tissues under varying environmental conditions may be helpful in managing salt stress tolerance in crop plant. Further details shall be presented.



### **S316: TDZ Enhances Direct Shoot Regeneration from BAP Induced Embryogenic Calli in Lentil (*Lens culinaris* Medik.)**

*Abhilasha Sinha<sup>1</sup>, Nishi Kumari<sup>1</sup>, Bishun Deo Prasad<sup>2</sup>, Ravi Shankar Singh<sup>1</sup> & Awadhesh Pal<sup>1,\*</sup>*

<sup>1</sup>Department of Plant Breeding and Genetics; <sup>2</sup>Department of Plant Molecular Biology & Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur 813210, Bihar  
(\*Email: awapal@gmail.com)

Lentil (*Lens culinaris* Medik.) is a rabi pulse, well fortified with protein, iron, zinc etc. but contributes only 7% to the total national pulse production. With a productivity of 600 kg/ha, India lags far behind the nations like Canada (1971 kg/ha) and Turkey (1483 kg/ha) and hence is the biggest importer of lentil. Efficient shoot regeneration procedure is a pre-requisite for in vitro mutagenesis, somatic embryogenesis, somatic hybridization and transgenic development for leading to rapid release of new genotypes for the improvement of lentil. The current study was performed using lentil var. HUL57, where, Murashige and Skoog (MS) medium containing 3 mg/L Benzyl amino purine (BAP) induced maximum callusing from embryogenic axis. These callus when subjected to MS containing 1 mg/L Thidiazuron (TDZ) and 1 mg/L kinetin produced highest number of shoots per calli (2.1), while that with 2 mg/L TDZ alone was at par (1.9) after 25 days of inoculation. Nodular callus was observed at basal ends of the shoots in agar solidified MS media supplemented with different concentration of various auxins. Such shoots when transferred to autoclaved coco-peat supplemented with ¼ MS with 3 mg/L IBA, resulted in 2-3 roots per shoot of 1-1.5 cm length within 15 days of inoculation. The rooted plantlets were successfully hardened and transferred to pots containing 2:1 soil and sand.

### **S317: Sequencing and Characterization of Stress Associated Transcription Factors for Abiotic Stress Tolerance in Sugarcane**

*Manimekalai R<sup>1\*</sup>, Arun meena<sup>1</sup>, Selvi A<sup>1</sup>, Gomathi R<sup>2</sup>*

<sup>1</sup>Sugarcane Breeding Institute, Crop Improvement Division, Biotechnology, Coimbatore 641007, Tamil Nadu, India; <sup>2</sup>Sugarcane Breeding Institute, Crop Production Division, Plant Physiology, Coimbatore 641007, Tamil Nadu, India (\*Email: rmanimekalaiicar@gmail.com)

Plants experience the oxidative stress when there is imbalance between the production and removal of Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>). During the extreme situation of abiotic factors, plants undergo oxidative stress. Many genes and transcription factors have been implicated in oxidative stress response. In the present study, the NAC transcription factor gene was sequenced and characterized in sugarcane and wild species. Primers have been synthesized based on the sorghum genome sequence. Ninety days old potted plants were given oxidative stress treatment using 100 ppm H<sub>2</sub>O<sub>2</sub> solution. cDNA was amplified using stress associated NAC gene primers and obtained a partial gene of 215 bp in sugarcane. Full length (2.0 kb) genome fragment was amplified from *Erianthus* sp, which showed 91% nucleotide identity with sorghum stress associated NAC gene. A total of 85 NAC genes of sugarcane were analysed in comparison with rice and sorghum NAC genes. Identified orthologues between sugarcane, rice and sorghum NAC proteins. The rice NAC proteins OsNAC30, OsNAC125, OsNAC18, OsNAC4, OsNAC126 that are reported to be stress responsive genes are orthologues to ScNAC36, ScNAC74, ScNAC82, ScNAC44 and ScNAC77 respectively. Thirty four orthologs were identified in the phylogenetic tree of sorghum and sugarcane NAC proteins. The full length genes of stress associated NAC transcription factors are being sequenced for further characterization.



### **S318: Molecular Characterization of Virulent *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) Isolates and their Responsive Susceptibility Genes in Rice**

Ningombam Rabichandra Meitei and Rhitu Rai\*

ICAR-National Research Centre on Plant Biotechnology, IARI, LBS Building, Pusa Campus, New Delhi (\*Email: rhitunrcpb@yahoo.com)

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes the bacterial leaf blight (BLB) of rice by injecting the transcription activator like effectors (*TALe*) into host nucleus and upregulating host susceptibility genes. The host target specificity of *TALe* genes is determined by number and order of near identical, 33-35 amino acid repeats present in the central domain of each *TALe* gene. This work aimed at genotyping Indian *Xoo* isolates for their *TALe* gene content and identifying the host susceptibility gene induced by these isolates. Comparison of the *TALe* profiles revealed considerable degree of diversity amongst isolates in terms of *TALe* gene size and number. The candidate host susceptibility genes studied for induction in response to infection were rice sucrose transporters, *SWEET11-15*. The induction of *SWEET11* by three out of four *Xoo* isolates, reiterated the prevalence of cognate *TALe* *pthXo1* even in Indian isolates which fails to find its target in the recessive allele of *SWEET11* i.e. *xa13*, hence culminating in resistance. However, one of the isolates compatible with *xa13* facilitated its proliferation in host by binding to another *SWEET* gene target i.e. *SWEET14*. These results strongly support the importance of *TAL* effectors and its targets to play major determinative role in BLB disease.

### **S319: Gene Stacking: A Most Promising Approach for Developing Durable Resistance in Rice against *Magnaporthe oryzae***

M Kumari\*, AK Rai, PK Singh, BN Devanna, K Arora, J Singh, R Kapoor and TR Sharma

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012  
(\*Email: mandeep.dharwal@gmail.com)

Rice is the most dominant staple food throughout the world. Rice blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most menacing biotic stresses that severely affects the food security across the world. Due to highly variable nature of *M. oryzae* it may break the resistance in varieties having single resistance gene. Gene stacking is an emerging way to combine two or more resistance genes together to get more durable resistance. In the present study, we have developed a transgenic rice line by stacking two alleles of blast resistance gene Pi54 and Pi54rh together in a single vector (pBluescript) under the control of native and constitutive promoter CaMV35S using hygromycin as a selectable marker. Nearly one thousand calli from susceptible rice line Taipei-309 were transformed via biolistic method. Putative transformants expressing the two genes were analysed using PCR and Southern blot analysis. Further RT-PCR and phenotyping study are in progress. Since both the alleles, Pi54 and Pi54rh confer resistance against different strains of *M. oryzae* individually; the generated rice line in the present study may show broad-spectrum resistance against rice blast. Further the same strategy can be used to stack multiple disease resistance genes together in a single gene cassette to develop more durable disease resistant varieties in a single step.



### **S320: RNA-Seq Analysis of Developing Ovules from Apomictic and Sexual Ovules of *Commiphora wightii* (Guggul) to Identify Genes Expressed during Adventive Embryony**

Vajinder Kumar<sup>1\*</sup>, Ashok Kumar Bishoyi<sup>2</sup>, Vishwanathan Satheesh<sup>1</sup>, Gaurav Sharma<sup>1</sup>, Aarti Kawane<sup>2</sup>, K. A. Geetha<sup>2</sup>, Kishor Gaikwad, Ramamurthy Srinivasan, Shripad Ramachandra Bhat<sup>1</sup>

<sup>1</sup>ICAR-National Research Center on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>2</sup>ICAR-Directorate of Medicinal and Aromatic Plants, Boriavi, Anand 387310, Gujarat

(\*Email: vgarg\_23@yahoo.com)

Adventive embryony is a sporophytic apomixis found mostly in tree species. While several species displaying gametophytic apomixis are being intensively investigated to identify genes governing apomixis, adventive embryony has received little attention. We have carried out detailed transcriptome analysis of pre- and post-anthesis stage ovules of guggul, a perennial tree species reproducing through adventive embryony. High quality RNA isolation protocol was optimized to obtain contaminant-free RNA (RIN value >8.0). Fragmented cDNA (~500 bp) was sequenced on ROCHE-454 platform. A total 53232 and 73068 raw reads were obtained in apomictic and sexual samples. Quality trimming resulted in 36359 and 45107 reads with an average length of 450 bp. These reads were assembled in iAssembler using default parameters and a total of 16529 and 20670 contigs were generated. Annotation and overlap analysis suggests presence of 2320 common and 17408 unique transcripts in the two samples. Both dynamic transcriptional changes due to rapid growth of ovule tissue from early (0-3 DPA) to late post-anthesis stages (30-35 DPA) and genotypic differences contribute to diversity of transcripts. This fits with our expectation of finding more unique genes compared to the common genes in two different samples. Further deep transcriptome sequencing and differential gene expression studies and pathway analysis are being pursued to identify key genes associated with the adventive embryony.

### **S321: Polyamines: Unexplored Elixir Conferring Tolerance in Rice against Arsenic Induced Toxicity**

Atrayee Sengupta, Jayita Saha, Mayukh Chakrabarty, Ushasi Bhaumik, Kamala Gupta\* and Bhaskar Gupta\*

Department of Biological Sciences, Presidency University, 86/1 College Street, Kolkata 73

(\*Email: bhaskar.dbs@presiuniv.ac.in; kamala.dbs@presiuniv.ac.in)

The slogan "Rice is life" used by the Food and Agricultural Organization (FAO), well designates rice as one of the most important staple food consumed worldwide. But often commercially available rice serves as a potential sink for inorganic arsenic, a known human carcinogen. Ubiquitous presence of arsenic in the ground water, whose origin are both natural and anthropogenic, worsen the situation even more. The scenario is much grave in the South-East Asian countries, where these contaminated ground water are used for irrigation and drinking purposes. On other hand polyamines, including the diamine putrescine (Put2+), triamine spermidine (Spd3+) and tetramine spermine (Spm4+) are naturally occurring, low molecular weight, straight chain, aliphatic amines regulating plant growth and developmental processes. Several reports emphasise the roles of polyamines in alleviating abiotic stress. Taking cue from all the previous study depicting role of polyamines in abiotic stress, we are trying to scrutinize the role of spermidine, one well known higher polyamine, in arsenic toxicity. In this report we have performed several biochemical, physiological and transcriptomic analyses to investigate the effect of exogenous spermidine on arsenate induced oxidative stress responses in locally grown rice cultivars. Our results indicate that exogenous spermidine differentially influenced enzymatic antioxidative system during arsenic toxicity.



### **S322: Improved Drought Resistance in a Wheat Stay Green CMS line is Associated with Enhanced Antioxidant Competence Compared to its Fertile Line under Water Deficit Stress**

*Renu Khanna-Chopra\* and Vimal Kumar Semwal*

Stress Physiology Lab, Water Technology Centre, Indian Agricultural Research Institute, New Delhi 110012 (\*Email: [renu.chopra3@gmail.com](mailto:renu.chopra3@gmail.com))

Reproductive sink regulates monocarpic senescence in wheat as desinking delays senescence. Wheat fertile (HW 2041) and its isonuclear male sterile line (CMS) were subjected to post-anthesis water deficit stress (WDS) to understand the association between sink strength, oxidative stress, antioxidant defense and drought resistance. CMS plants maintained better water relations and exhibited delayed onset and progression of flag leaf senescence in terms of green leaf area, chlorophyll and protein content than fertile plants. Stay green character in CMS plants under WDS was associated with less ROS generation, lower damage to membranes and better antioxidant defense both in terms of antioxidant enzyme activities and metabolite content compared to fertile plants. CMS plants also exhibited delay in expression of senescence associated genes i.e. WRKY53, serine and cysteine protease (WSP and WCP2), glutamine synthetase (GSI and GSII) and catalase under WDS compared to fertile plants. CMS line exhibited better antioxidant defense in chloroplasts than its fertile line. In conclusion stay green trait contributed to improved drought resistance (DR) in wheat. Reproductive sink regulated drought response as CMS wheat plants exhibited stay green character and improved DR coupled with enhanced antioxidant competence than its fertile line under WDS.

### **S323: Direct Shoot Organogenesis from Petiole Attached with Leaf of Okra (*Abelmoschus esculentus* L.)**

*Mariya S Zaman\*, Akarsh Parihar, BR Patel, N Subhash, Ghanshyam Patil, Dipanki D Patel and RS Fougat*

Centre of Excellence on Biotechnology, Department of Agricultural Biotechnology, Anand Agricultural University, Anand 388110, Gujarat

An efficient and reproducible procedure is developed for direct shoot regeneration using petiole attached with leaf explants of okra (*Abelmoschus esculentus* L.). The new shoots mainly emerged from the distal end and lateral side of the petiole, whereas in leaf explants, callus was initiated from the basal part, wounded tissue and proximal end of the petiole connected to leaf. The regeneration medium that induced the highest number of shoots in the leaf – petiole explants was Murashige and Skoog (MS) medium supplemented with 1 mg/l N6-benzyladenine (BA) and 0.1 mg/l  $\alpha$ -naphthalene acetic acid (NAA). The frequency of shoot regeneration was greatly influenced by the type of explant, the carbon source, the orientation of the explant, and the basal medium used in the regeneration medium. Explants produced shoot buds and adventitious shoots within two weeks. Plantlets were rooted on MS alone or MS containing different concentrations of NAA. The addition of low concentration of  $\alpha$ -naphthalene acetic acid to the medium was most effective in inducing root formation. The regenerated plantlets will be acclimatized in the greenhouse and successfully transferred to the field. The timely application of fungicide such as bavistin may be used to promote the survival rate effectively.

### **S324: Differential Gene Expression of N-Metabolizing Enzymes in Diverse Wheat Genotypes under N-Starvation**

Gayatri, Shruti Sinha, Subodh Kumar Sinha, Monika Dalal, Amita Choudhary and PK Mandal\*

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012

Nitrogen use efficiency (NUE) is imperative for sustainable agriculture, and is more important in case of prime cereal like wheat. However, the lack of information on molecular basis of NUE is major limitation towards improvement of NUE in crop plants. In the process of NUE, understanding how nitrogen metabolizing genes respond to N starvation is also essential. Therefore, in this study, we tried to elucidate the expression patterns of five nitrogen metabolizing genes (TaNR; TaNiR; TaGS; TaGOGAT and TaGDH) among 24 diverse wheat genotypes at seedling stage under N-stress in hydroponics condition. Expression of the five genes was quantified using qPCR in the shoot tissues. Genotypic variation was prominent for all the genes and did not follow any specific trend under N-stress, except for NR and NiR genes expression, where all the genotypes showed down regulation. GS and GOGAT expression were also down regulated except in six (C-306, RAJ-1482, K-7903, PBW-550, WL-410 and WH-542) and eight ((NIPHAD-4, HUW-206, KENPHAD-39, HD-2888 HS-1097-17, K-7410, NP-12) genotypes respectively. However, GDH expression was up regulated except in 10 (N-846, KALYANSONA, NIPHAD-4, K-68, HS-1097-17, PBW-175, HS-365, K-7410, WH-542, WL-410) genotypes. This study provides an outlook on expression of major nitrogen metabolizing enzymes under N-stress, and elucidates some of the gene interactions may play a role in the nitrogen-use-efficiency.

### **S325: Genetic Characterization of Novel Source of Resistance against *Fusarium* Wilt of Muskmelon and Validation of Fom-2 Gene**

Harshawardhan Choudhary\*, BB Beesanakoppa, TK Behera, Sri Dhar and V Shanmugam

Division of Vegetable Science, Indian Agricultural Research Institute, Pusa, New Delhi 12

(\*Email: harshahit2001@yahoo.co.in)

*Fusarium* wilt is a devastating disease of muskmelon and novel source of resistance in Indian snapmelon (*Cucumis melo* var. *momordica*) line has been identified after field screening. A genotype of snapmelon DSM-11-6 has been made homozygous by selfing for 6 generations from a population collected from Punjab. The fungus (*Fusarium oxysporum* f.sp. *melonis*) was isolated from diseased plant from IARI and DSM-11-6 showed resistance under artificial inoculation. Highly susceptible lines Hara Madhu & Kashi Madhu were crossed with resistant line DSM-11-6 as pollen parent. The inheritance of the resistance gene was found to be governed by single dominant gene in both the crosses. Sixty seven lines of melon germplasm were screened for resistance to *Fusarium* and the same set of lines were used for genetic characterization with 12 SSR/STS markers from Fom-2 region of genetic map and 2 functional SCAR markers Fom2- R408 and Fom2- S342 developed from LRR domain of Fom-2 gene for resistant and susceptible alleles respectively. Eight lines showed resistance and 59 were susceptible. Out of 8 resistant lines, 6 lines were showing presence of both susceptible and resistant allele of Fom-2 and only two lines MR-1 and DSM-11-6 were having Fom-2 R allele in homozygous condition and no recombinants was observed. The distinctness of DSM-11-6 from MR-1 was established on the basis of SSR138 which has been earlier reported to have no recombinants between SSR 138 and Fom-2. The identification of new source of Fom-2 gene will help in its introgression to muskmelon cultivars by MAS.



## S326: High Temperature and Salt Stress Induced MicroRNA Profiles in Indian Rice Varieties

Neeti Sanan-Mishra

Plant Molecular Biology Group, International Center for Genetic Engineering and Biotechnology, New Delhi

microRNAs are established as key modulators of plant biology. They act as genetic buffers in plant gene regulation providing protection against various abiotic and biotic stress conditions. Next generation sequencing technologies are high throughput approaches that can be utilized to study the global expression patterns of miRNAs under various stress physiologies. Thus, we adopted the approach of comparative miRNA profiling across different tissues in rice varieties. This revealed the profiles of several known and novel miRNAs. The expression patterns of selected stress deregulated miRNAs were experimentally validated. The targets of these miRNAs were also predicted and validated. Time kinetics studies helped to capture the narrow windows of miRNA target correlations indicating their role in tissues development. This data was compiled and developed as a web server, ARMOUR, which is now being used to select miRNAs for functional analysis. The studies would lead to novel outcomes that can be utilized to prepare “smart plants”, which will be an enduring step to fight against abiotic stresses mediated decline in crop yields in rice.

## S327: Coevolutionary Dynamics of Rice-*Magnaporthe oryzae* Host-Pathogen System

PK Singh<sup>1,3</sup>, S Thakur<sup>1</sup>, S Ray<sup>1</sup>, P Jain<sup>1</sup>, S Kumar<sup>1</sup>, R Rathour<sup>2</sup>, V Sharma<sup>3</sup> and TR Sharma<sup>1</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, New Delhi 110012; <sup>2</sup>Department of Agricultural Biotechnology, CSK HPKV, Palampur, H.P. 176062; <sup>3</sup>Department of Bioscience and Biotechnology, Banasthali University, Tonk, Rajasthan 304 022

Considering the co-evolutionary relationship between avirulence (Avr) gene of *Magnaporthe oryzae* and resistance (R) gene of its host rice plant in the host-pathogen interaction, there are two predominant hypotheses arms race and trench warfare implemented for this relationship. Arms race suggests that both R and Avr genes are under diversified selection whereas trench warfare predicts that either R or Avr is under balanced selection, and the other is under diversified selection. Allele mining is the best way to deal with variants of a gene existed in the population and to determine the evolutionary selecting forces imposed on the variants. Using the approach in this coevolutionary study, we analyzed three R-Avr gene sets, Pita-AvrPita, Pizt-AvrPizt and Pi54-AvrPi54. Results of neutrality tests and sequence analyses reveal that AvrPita, Pizt and Pi54 are diversified and highly variable at their respective loci, whereas, Pita, AvrPizt and AvrPi54 are highly conserved and balanced in the nature. These results conclude that trench warfare hypothesis is fully applicable for rice-*M. oryzae* system in the Indian field condition. However, more study on this aspect would give us a clear insight about the evolutionary dynamics of host and pathogen. Understanding the specific host-pathogen interaction is crucial in improving agriculture and food production.



### **S328: Polyamines: a Prima Facie Role in Orchestrating Ion Homeostasis during Salinity Stress in Rice**

*Mayukh Chakrabarty, Atrayee Sengupta, Jayita Saha, Ushasi Bhaumik, Bhaskar Gupta\* and Kamala Gupta\**

Department of Biological Sciences, Presidency University, 86/1 College Street, Kolkata 700073

(\*Email: bhaskar.dbs@presiuniv.ac.in; kamala.dbs@presiuniv.ac.in)

Polycationic molecules in the form of polyamines which possess multi-dimensional roles in plant systems are an important area of research. Although the modulations of polyamine level during stress in plants have been reported by many research groups but still a number of questions remain unanswered. Our group has made an attempt to understand whether polyamines have important regulatory role in maintaining the ion homeostasis- sodium and potassium balance. The potassium and sodium transporters along with a number of other key transporters help plants to tide over the stress by maintaining ion homeostasis. We felt that it would be interesting to investigate the probable role of polyamines in synchronizing these transporters to avoid excess accumulation of toxic ions in plants during stress. At the same time it was observed that a number of cationic channels show varied expression maintaining an appropriate ion balance. Our study has focused on a number of important transporter families in rice plants, including HAK, NhKT, HKT, AKT and their expression levels in presence and absence of exogenously applied polyamines during salt stress. The concentration of sodium and potassium ions during stress along with the inter-relationship between their accumulation and the presence of polyamines has been elucidated in our work.

### **S329: Application of Plant Proteomics for Crop Improvement in Agriculture Research**

*Dinesh Kumar Thakur<sup>1</sup>\*, Akanksha Sharma<sup>1</sup> and Shiv Chandrakar<sup>2</sup>*

<sup>1</sup>Indira Gandhi Krishi vishwavidyalaya, Raipur CG 492012; <sup>2</sup>N.M. College of Agriculture, Navsari Gujarat (\*Email: dinesh.igkv@gmail.com)

Proteomics has become a useful tool and provide a new approach to the identification of numerous proteins that play crucial roles in plant growth and development and to carry out a functional analysis of the genome and its relationship with the environment. Seeds are one of the most important factors in crop production, as seed viability is related to crop yields. Stress is a key limiting factor that affects the growth, leading to delayed seed germination, development and reducing the quality of the seeds. Salinity and cold are other inhibitory factors in crop plant growth and production and together with drought cause osmotic stress in plant. Proteins associated with the primary function of an organ are specifically accumulated in that organ/tissue or organelle. The need for organ-specific proteomic analyses to identify proteins that are commonly accumulated in organs under a wide range of abiotic stresses. The knowledge of these key proteins that play crucial roles in the proper growth and development of a crop plant are critical to propel the biotechnological improvement of crop plants. These proteins maintain cellular homeostasis under a given environment by controlling physiological and biochemical pathways. Genomics and proteomics are the two major wheels; proteomics is a powerful tool to provide a path toward increasing the efficiency of indirect selection for inherited traits. In crop plants a comprehensive functional genomics is also performed for analyzing the functions of the plant genes and proteins, which can eventually be placed into the pipeline for crop improvement programs.



### **S330: Delineating the Role of Heat Shock Factor- and Heat Shock Protein-Encoding Genes in Stress Tolerance of Foxtail millet**

Roshan Kumar Singh, Jananee Jaishankar, Mehanathan Muthamilarasan and Manoj Prasad\*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, JNU Campus, New Delhi

(\*Email: manoj\_prasad@nipgr.ac.in)

Heat and drought are the immediate outcomes of climate change, which severely affect crop productivity. Of the several molecular mechanisms reported to combat these stresses, role of heat shock factors (HSF) and heat shock proteins (HSP) are imperative. Therefore, the study on identification and characterization of HSFs and HSPs in foxtail millet (*Setaria italica*), a C4 model crop cultivated predominantly in arid and semi-arid regions has been performed. The study identified 22 HSF (SiHSF) and 113 HSP-encoding genes (SiHSP), which were subjected to further analyses including gene structure prediction and chromosomal location, duplication and divergence studies, phylogenetic classification, promoter analysis, in silico expression profiling using RNA-seq data of tissues and stress library, and comparative physical mapping. Tissue-specific expression profiling of candidate SiHSF and SiHSP genes at different time points of heat, drought and salinity stress in two contrasting foxtail millet cultivars revealed differential expression patterns and comparatively higher expression of SiHSP-27 in tolerant cultivar. Over-expression of SiHSP-27 in bacteria and yeast systems conferred tolerance to heat stress in respective host systems. Development of SiHSP-27 over-expressing rice lines is in progress. Taken together, the results indicate that SiHSP-27 could be a potential candidate for enhancing stress tolerance through transgene-based approaches.

### **S331: Dehydration-responsive microRNAs: Identification and Characterization in Naturally Stress Tolerant Model Crop Foxtail millet (*Setaria italica*)**

Amita Yadav, Yusuf Khan and Manoj Prasad\*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, JNU Campus, New Delhi

(\*Email: manoj\_prasad@nipgr.ac.in)

The miRNA-mediated gene regulatory networks responsive to dehydration stress in foxtail millet (*Setaria italica*), known for its abiotic stress tolerance, remain largely unexplored. To dissect dehydration-responsive miRNAs systematically, 4 small RNA libraries were constructed from control and dehydration-treated seedlings of 2 cultivars of foxtail millet. Using Illumina technology, 55 known miRNAs (representing 23 miRNA families) and 136 novel miRNAs (representing 47 families) were identified in foxtail millet and their secondary structures were predicted. Among the identified miRNAs, 18 known miRNAs and 33 novel miRNAs were identified to be differentially expressed during dehydration stress. In tolerant cv. IC403579, 35 dehydration responsive miRNAs (13 known & 22 novels) have been observed and majority of these (32) miRNAs was up-regulated. In sensitive cv. IC480117, 32 miRNAs (8 known & 24 novels) were differentially expressed and most of them (22 miRNAs) were shown to be down-regulated after stress treatment. These results suggest that the opposite expression behavior of foxtail millet miRNAs after dehydration stress might play more important roles in providing the contrasting characteristics to these cultivars. Predicted targets of these identified miRNAs were found to encode various DNA binding proteins, transcription factors or important functional enzymes, indicating their involvement in wide range of regulatory functions and some specific biological processes. Further, expression patterns of ten novel dehydration-responsive miRNAs and some of their targets were validated by quantitative real-time PCR. Overall, this study provides novel insights onto post-transcriptional regulation by miRNAs in drought response and/or tolerance in millets.

### **S332: Proline Metabolism in Rice Genotypes as Reliable Mechanism for Stress Tolerance**

Ashu Singh\* and RS Sengar

Tissue culture Lab, College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut 250110 (\*Email: ashubiot25@gmail.com)

Abiotic stress is a major constraint to rice production in water-limited environments. Deciphering the cellular responses of plants exposed to different abiotic stresses is very important. There are many cellular mechanisms by which organism cope up the effects of environmental stresses. Plants have adapted to respond to these stresses at the molecular and cellular levels as well as at the physiological and biochemical levels, thus enabling them to survive. This triggers expression of various stress related genes and they in turn produce compounds that play role in stress management of a plant system. In this review we focus mainly on gene products which are, low molecular weight osmoprotectants and osmolytes such as proline, glycine betaine, and sugar alcohols are synthesized and accumulated and their synthesis and degradation have been studied well. The accumulation of osmoprotectants such as proline is an important process for the adaptation to adverse environmental conditions. The increase of osmoprotectants is achieved either by altering metabolism or by its transport and in some case both. Generally, proline protects plants from stress through different processes, including by the adjustment of cellular water, detoxification of ROS, protection of membrane integrity, and stabilization of enzymes and proteins. Thus it is important to analyze the proline regulon/biosynthesis in rice to understand the molecular mechanisms of stress tolerance and to produce monocots with higher stress tolerance by gene transfer.

### **S333: High Frequency of Plant Regeneration through Repetitive Somatic Embryogenesis in Guggul**

Aarti Kavane<sup>1\*</sup>, Ashok Kumar Bishoyi<sup>1</sup>, Geetha KA<sup>1</sup> and SR Bhat<sup>2</sup>

<sup>1</sup>ICAR-Directorate of Medicinal and aromatic Plants Research, Boriavi, Anand, Gujarat; <sup>2</sup>ICAR-National Research Centre on Plant Biotechnology, New Delhi (\*Email: aartikawane@gmail.com)

Guggul [*Commiphora wightii* (Arnott) Bhandari], an important Indian medicinal plant, reproduces via adventive embryony and shows autonomous endosperm formation. We have initiated molecular investigation of apomixis in guggul using genomics approaches. To test the candidate genes governing adventive embryony, we have simultaneously initiated optimisation of tissue culture regeneration and transformation protocols for guggul. Murashige & Skoog and Woody Plant media supplemented with different levels and combinations of auxin (2,4-D, Picloram, 2-iP, Dicamba, IAA and NAA) and cytokinins (Kinetin, BAP, Zeatin and TDZ) were tested for inducing callus from different explants such as leaf, immature ovule, fruit wall, seedling etc. All explants responded to callus induction in MS medium supplemented with 2, 4-D and Kinetin, but organogenesis was not obtained in any of the growth regulator combinations tested. However, when ovule generated calli were transferred to the MS medium with Kinetin, BAP and NAA, somatic embryos were obtained after 9-10 weeks of subculture. Primary somatic embryos upon transfer to fresh medium continued to give rise to secondary embryos, both on the induction medium and also in medium with lower concentrations of cytokinins. Direct somatic embryogenesis was also observed from the epicotyl regions of in-vitro raised seedlings, when cultured on embryogenesis-induction medium. This is the first report of repetitive somatic embryogenesis in guggul, and lays foundation for plant transformation of this species.



### **S334: Introgression of Drought, Submergence and Salinity Governing QTLs into mega Varieties of Rice through Marker-Assisted Backcross Breeding – a multi-Institutional Network Initiative**

Renu Singh<sup>1</sup>, Yashi Singh<sup>1</sup>, Neera Yadav<sup>1</sup>, Nisha Singh<sup>1</sup>, PC Sharma<sup>2</sup>, SL Krishnamurthy<sup>2</sup>, SK Sharma<sup>2</sup>, JL Dwivedi<sup>3</sup>, AK Singh<sup>3</sup>, PK Singh<sup>4</sup>, Nilanjay<sup>5</sup>, NK Singh<sup>5</sup>, Rajesh Kumar<sup>5</sup>, SK Chetia<sup>6</sup>, T Ahmad<sup>6</sup>, M. Rai<sup>7</sup>, P Perraju<sup>8</sup>, Anita Pande<sup>9</sup>, DN Singh<sup>9</sup>, NP Mandal<sup>10</sup>, JN Reddy<sup>10</sup>, ON Singh<sup>10</sup>, JL Katara<sup>10</sup>, B Marandi<sup>10</sup>, P Swain<sup>10</sup>, RK Sarkar<sup>10</sup>, DP Singh<sup>10</sup>, T Mohapatra<sup>10</sup>, Suchit Xalaxo<sup>11</sup>, S Verulkar<sup>11</sup>, G Padmawathi<sup>12</sup>, T Ram<sup>12</sup>, KSN Prasad<sup>13</sup>, K Kondayya<sup>13</sup>, PV Ramana Rao<sup>13</sup>, M Girija Rani<sup>13</sup>, T Anuradha<sup>13</sup>, Y Suraynarayana<sup>13</sup>, RM Kathiresan<sup>14</sup>, K Paramasivam<sup>15</sup>, S Nadarajan<sup>15</sup>, S Thirumeni<sup>15</sup>, M Nagarajan<sup>16</sup>, AK Singh<sup>16</sup>, Arvind Kumar<sup>17</sup>, E Septiningsih<sup>17</sup>, US Singh<sup>17</sup>, AM Ismail<sup>17</sup>, D Mackill<sup>17</sup> and Nagendra K Singh<sup>1\*</sup>

<sup>1</sup>National Research Centre on Plant Biotechnology, New Delhi; <sup>2</sup>Central Soil Salinity Research Institute, Karnal (Haryana); <sup>3</sup>Acharya Narendra Dev University of Agriculture and Technology, Faizabad (UP); <sup>4</sup>Banaras Hindu University, Varanasi (UP); <sup>5</sup>Rajendra Agricultural University, Samastipur (Bihar); <sup>6</sup>Assam Agricultural University, Jorhat (Assam); <sup>7</sup>Central Agricultural University, Umiam (Meghalaya); <sup>8</sup>JawaharLal Nehru Krishi Vishwavidyalaya, Rewa (MP); <sup>9</sup>Birsa Agricultural University, Ranchi (Jharkhand); <sup>10</sup>Central Rice Research Institute, Cuttack (Odisha); <sup>11</sup>Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhatisgarh); <sup>12</sup>Directorate of Rice Research, Hyderabad; <sup>13</sup>Acharya N.G. Ranga Agricultural University, Maruteru (AP); <sup>14</sup>Annamalai University, Annamalai Nagar (TN); <sup>15</sup>PAJANCOA, Karikal (Puduchery); <sup>16</sup>Indian Agricultural Research Institute, New Delhi; <sup>17</sup>International Rice Research Institute, Los Banos, Philippines (\* E-mail: nksingh4@gmail.com)

Rice crop faces severe production losses due to various abiotic stresses, namely, drought, submergence and salinity. In this multi-institutional, IRRI-India collaborative project, seven major drought governing QTLs (*qDTY1.1*, *qDTY2.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2*, *qDTY9.1* and *qDTY12.1*) have been introgressed into Swarna- Sub1, Samba Mahsuri- Sub1 and IR 64- Sub1. Similarly, submergence tolerance gene 'Sub1' has been introgressed into ten mega varieties of northern and southern regions of India namely, Pooja, Pratikshya, Sarjoo 52, HUR 105, Ranjit, Bahadur, Rajendra Mahsuri, MTU 1075, ADT 39 and ADT 46 whereas salinity tolerance QTL '*SaltoI*' has been incorporated into seven popular rice varieties namely, Gayatri, CR 1009, MTU 1010, ADT 45, Sarjoo 52, Pusa 44 and PR 114. The transfer of the QTL/gene of interest has been done using marker- assisted backcross breeding approach (MAB) wherein at each generation the presence of QTL was ensured using peak marker or the gene based functional marker followed by recombinant selection to ensure minimal linkage drag on both the sides of the QTL. At each stage, rigorous phenotypic selection was carried out for the target trait as well as agronomic traits. At BC<sub>3</sub>F<sub>3/5</sub> generation, the best advanced lines from each combination are being screened for recipient parent genome (RPG) recovery using an in-house developed high density 50K SNP chip. A very high RPG recovery of >90% has been observed in few of the advanced lines. The improved varieties will help in enhancing the rice production in adverse ecologies of our country.



### **S335: Introgression of Superoxide Dismutase from Salt Tolerant Cell Lines of *Arachis hypogaea* Confers Multiple Stress Tolerance**

*Neelam Prabha Negi\* and Neera Bhalla Sarin*

School of Life Sciences, Jawaharlal Nehru University, New Delhi (\*Email: sapphirenegi@yahoo.com)

Antioxidant enzymes play an important role in conferring abiotic stress tolerance. Several enzymes act jointly to maintain redox status homeostasis. ROS-scavenging systems play an important role in cell functioning because ROS are extremely cytotoxic. Superoxide dismutase is the first enzyme involved in the detoxification of ROS and converts superoxide ( $O_2^{\cdot-}$ ) radicals to hydrogen peroxide, which is further converted to non-toxic water and monodehydroascorbate by the APX enzyme utilizing ascorbate as electron donor. A functional role of *AhCuZnSOD* in alleviation of abiotic stress was further exploited through its overexpression in the model plant tobacco and subsequently used for the transformation of a highly important oilseed crop, *Brassica juncea* (Indian mustard). Compared with the wild type plants, transgenic plants survived under longer period of water deficiency and salinity stress and displayed improved recovery after rehydration. The enhanced levels of antioxidant enzymes in the transgenic plants correlated with higher relative water content, improved photosynthetic efficiency, less electrolyte damage, elevated accumulation of compatible osmolytes, less malondialdehyde as well as  $H_2O_2$  accumulation and  $O_2^{\cdot-}$  accumulation under stress conditions as compared to untransformed wild-type controls. The overall results demonstrate the profound effect of *AhCuZnSOD* in bestowing multiple abiotic stress tolerance at cellular and whole plant level via ROS detoxification.

### **S336: Deciphering the Role of ADP Glucose Pyrophosphorylase for Thermotolerance in Wheat**

*Ravi Mehndiratta\* and Santosh Dhillon*

Department of Molecular Biology, Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar 125004 (\*Email: ravi\_hau@hotmail.com)

Wheat (*Triticum aestivum* L. em. Thell) is one of the most important cereal crops of the world. High temperature in the month of February-March is not congenial for grain development, thus leading to reduced yield. Starch, accounting for 65–75% of wheat kernel weight, is the major factor severely affected by natural stresses during filling periods. ADP-glucose pyrophosphorylase (AGPase) catalyzes the conversion of Glc-1-P and ATP to pyrophosphate and ADPGlc, and is then thought to be the rate-limiting enzyme of starch synthesis. There are also some indications that the enzyme AGPase, the initiator of starch biosynthesis, directly affects wheat productivity by synthesizing starch that constitutes 70% of the wheat grain. The present study is being done to investigate the high temperature sensitivity of AGPase by comparing the expression of the enzyme using qPCR in a pair of contrasting wheat cultivars for response to high temperature. Heat stress would be provided naturally by late sowing of wheat in the month of December and January. CTD, grain filling duration and other morpho-physiological parameters would be recorded. Putative cDNAs would be sequenced and further bioinformatics tools would be applied to understand the role of AGPase during grain filling stage in wheat.



### S337: Biochemical Changes in Wheat (*Triticum aestivum* L) Inoculated with Arbuscular Mycorrhiza (AM) Fungi under Water Stress

Babita Rani\*, S Madan and KD Sharma

Department of Biochemistry, Crop Physiology lab, CCS Haryana Agricultural University, Hisar 125004, Haryana (\*Email: babitachahalkharb@gmail.com)

The effect of Arbuscular Mycorrhiza (AM) fungi on biochemical changes in drought tolerant (WH 1025) and drought susceptible (WH 1105) varieties of wheat was investigated in pot culture under well watered and water stress conditions. The seeds were treated with AM fungi before sowing and water stress was created by withholding irrigation at heading stage. WH 1105 suffered greater damage to cellular membrane due to high level of reactive oxygen species (ROS) as indicated by hydrogen peroxide ( $H_2O_2$ ), superoxide radical ( $O_2^{\cdot-}$ ) and malondialdehyde (MDA) content under stress. However, when treated with AM fungi, the level of ROS enzymes viz. superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) was found to be higher in WH 1105, but still lower as compared to AM treated samples from WH 1025. Basal level of ROS enzymes was found to be higher in tolerant variety. Though activity of ROS enzymes increased in both the varieties under stress, it was higher in WH 1025 than WH 1105. Results also showed that under water stress, mycorrhizal inoculation significantly decreased the  $H_2O_2$ , superoxide radical and MDA content by enhanced activities of SOD, APX and GR both in tolerant and susceptible varieties. Thus the study suggested that mycorrhizal symbiosis can play a vital role in sustaining under drought conditions in host plants.

### S338: Molecular Characterization of bZIP Transcription Factor from Finger Millet (*Eleusine coracana* L.) under Abiotic Stresses

Ch Ramakrishna, Sonam, Kanika, JC Padaria, Amolkumar U Solanke\* and TR Sharma

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi.

(\*E-mail: Amol.Solanke@icar.gov.in)

Transcription factors play an important regulatory role in almost all aspects of plant life, including growth, development, and responses to abiotic and biotic stresses. The bZIP transcription factor family is one of the largest families in higher plants and is involved in plant growth, development, and environmental responses. Here, we isolated and characterized a novel bZIP transcription factor from finger millet (*Eleusine coracana* L.) commonly known as Ragi. Relative expression analysis of *EcbZIP* gene showed up regulation in different developmental stages and stress conditions. *EcbZIP* ORF is 1722 bp long and sequencing analysis revealed two homologs of this gene differing at 17 amino acids. *EcbZIP* gene encodes a putative protein of 573 amino acids with a predicted molecular weight of 61 kDa and isoelectric point (pI) of 5.49. *EcbZIP* protein is predicted to contain two major domains i.e. BRLZ-Basic region luciferase zipper domain and transmembrane domain. To determine its role, transgenic tobacco plants over expressing *EcbZIP* were generated. Stable integration and over expression of *EcbZIP* in transgenic tobacco plants was proved by Southern blot analysis and quantitative real time PCR. The transgenic lines when subjected to 10% PEG, 250mM NaCl, 400mM Mannitol and 2.5mM DTT stress, showed better germination, growth with increased biomass and chlorophyll content as compared to wild type plants. These results indicate that *EcbZIP* transcription factor is an important constituent in transcriptional reprogramming involved in diverse stress responses.



### **S339: Paclobutrazol Application Induces Expression of Functional *Flowering Locus T (FT)* in Mango**

Vyavhare Sayali N, Chaudhary Rakesh S, Bal Krishna, Subramaniam VR and Sane PV

Molecular Biology Lab, Jain R&D, Jain Hills, Jain Irrigation Systems Ltd, Jalgaon 425001, Maharashtra  
(Email: balkrishna.yadav@jains.com)

Alternate bearing is a common problem in many commercially important mango varieties including the popular Alphonso and Dashehari. Besides management practices to overcome alternate bearing, growth regulators such as Paclobutrazol (PBZ) are used to induce flowering in alternate bearers. In this study, we have investigated the expression of flowering inducing gene *Flowering Locus T (FT)* and the flowering suppressing gene *Terminal Flower (TFL)* in response to PBZ and gibberellic acid (GA) application in Alphonso (an alternate bearer) and Ratna (a regular bearer). While PBZ application induced 100 % flowering, GA application resulted in no flowering in both the varieties. Gene expression analysis showed that *MaFT1* but not *MaFT2* expression was high in PBZ treated plants of both the varieties while GA application suppressed *MaFT1* expression. The expression of *MaTFL* that acts as a suppressor of flowering was however induced by GA. These data strongly suggest that PBZ induces flowering through the expression of functional *FT* responsible for flowering whereas GA blocks functional *FT* expression but induces expression *TFL* (an *FT* antagonist) and blocks flowering. *MaFT1* and not *MaFT2* is the florigen equivalent in mango while *TFL* is its antagonist.

### **S340: PG-eIF4a, Encoding a Eukaryotic Translation Initiation Factor 4A in *Pennisetum glaucum*, Functions in Salinity and Drought Stress Tolerance in Tobacco and Rice (*Oryza sativa* L. cv. Swarna)**

Bhattacharjee B and Tyagi JP

Centre for Biotechnology, ICAR Research Complex for NEH Region, Barapani, Meghalaya  
(Email: bijoyabhattacharjee@yahoo.com)

Abiotic stress severely limits plant growth and development as well as crop yield. Analysis of genes whose expression is induced under stress condition is important to understand the mechanism of abiotic stress tolerance and possibly to use for breeding stress tolerant plants. Abiotic stresses severely affect the cellular gene expression machinery; therefore, it is possible that the molecules involved in nucleic acid metabolism including Eukaryotic Translation Initiation Factor 4A (eIF-4A) are likely to be the targets. The full length cDNA clone encoding *eIF4A* isolated from *Pennisetum glaucum* (PG-*eIF4A*) was PCR amplified and over-expressed in tobacco and rice through *Agrobacterium*-mediated transformation. A total of 105 independent transgenic lines of Swarna were assessed for salinity and drought stress tolerance at germination, seedling & vegetative stage and reproductive stage of growth. This study provides insights into the mechanism of PG-*eIF4A* mediated stress tolerance by controlling the generation of stress induced reactive oxygen species (ROS) and also by protecting the photosynthetic machinery through a strengthened antioxidant system. It is evident from the study that *eIF-4A* may provide stress tolerance by efficient protein synthesis. This finding will help researchers to identify new genes/QTL for abiotic stress tolerance and utilize in plant breeding programs.



### S341: Reactive Oxygen Species Generation and Antioxidative Metabolism in Rice Genotypes Differing in Grain Iron Concentration

Ritu Saini<sup>1</sup> and Sunita Jain<sup>2</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, <sup>2</sup>Department of Molecular Biology, Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar 125004

Iron (Fe<sup>2+</sup>) is a vital trace element in plants and component of many important oxido-reductases. Both iron deficiency and high levels of iron affect plant growth and productivity. In the present investigation, reactive oxygen species (ROS) generation, malondialdehyde (MDA) content and enzymes and metabolites of antioxidant pathway were studied in root and shoot tissues of six rice genotypes (Govind, Super Basmati, HKR120, Pusa1121, HBC19 and Palman) differing in grain iron concentration (35-400 µg/g). Plants were grown in pots and irrigated with Yoshida solution containing different iron concentrations (0, 0.1mM, 0.5mM EDTA-Fe (II)). 0 and 0.5 mM EDTA-Fe (II) treatments resulted in higher ROS (O<sub>2</sub><sup>·-</sup> and H<sub>2</sub>O<sub>2</sub>), MDA and antioxidative metabolites (ascorbate and glutathione) in both the tissues. Plants grown at 0 mM EDTA-Fe (II) had enhanced activity of superoxide dismutase (SOD) and glutathione reductase (GR) in both root and shoot tissues, whereas the activities of catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) declined as compared to plants treated with 0.1mM EDTA-Fe (II). Activities of APX, POX and GR increased and those of CAT and SOD decreased at 0.5mM EDTA-Fe (II). The results suggested that iron deficiency as well as excess iron in the soil causes oxidative stress in growing rice plants.

### S342: Physiological and Molecular Basis of Water-Deficit Stress Tolerance in F<sub>1</sub> Hybrids and Their Parental Lines

Sasmita Pattnaik<sup>1,2</sup>, Kapil K Tiwari<sup>1</sup>, Chandra Prakash<sup>1</sup>, Vinod Kumar<sup>1</sup>, Maninder Sandhu<sup>1</sup>, SV Amitha CR Mithra<sup>1</sup>, Jogeswar Panigrahi<sup>2</sup>, Niranjana Behera<sup>2</sup>, Singh NK<sup>1</sup> and Trilochan Mohapatra<sup>3</sup>

<sup>1</sup>National Research Centre on Plant Biotechnology, New Delhi; <sup>2</sup>School of Life sciences Sambalpur University, Burla; <sup>3</sup>Central Rice Research Institute, Cuttack. (\*E-mail: sasmita.iari@gmail.com; amithamithra.nrpcb@gmail.com)

Drought is one of the major challenges to rice productivity. Tolerance to drought is a complex quantitative trait and to address this, understanding of physiological and molecular basis of tolerance mechanism is vital. In many crops such as rice, F<sub>1</sub> hybrids are long known to be better at tolerating drought stress than their parental lines. The present investigation was aimed at understanding the physiological and molecular basis of water-deficit stress tolerance in 20 F<sub>1</sub> hybrids and their parental lines. To understand the genetic relationship of the parental lines and their bearing on drought tolerance of hybrids, they were genotyped using 60 genome-wide SSR markers. PIC values and genetic distance estimates revealed that certain parents were genetically nearly identical (Rasi and Samleswari) whereas some were quite unrelated (CSR30 and Keshav). For physiological characterization, plants were grown in pots in completely randomized design with three replicates each under control and drought stress. Data on various morphological characters and physiological attributes (relative water content, chlorophyll content and cell membrane stability) were recorded. The highest degree of drought tolerance was observed for five F<sub>1</sub> Hybrids. To further study the drought tolerance among F<sub>1</sub> hybrids at molecular level, 10 reported miRNAs genes and their targets were subjected to expression analysis. This study is expected to throw light on the basis of drought tolerance in F<sub>1</sub> hybrids as against their parents.



### **S343: Cloning and Characterization of Novel Heat-Responsive Transcription Factor Gene (*HSF-2*) in Wheat (*Triticum aestivum* L.) under Heat Stress**

Khushboo Singh<sup>1</sup>, Ranjeet R Kumar<sup>1</sup>, Suneha Goswami<sup>1</sup>, Sushil K Sharma<sup>1</sup>, Kavita Dubey<sup>1</sup>, Sweta Singh<sup>1</sup>, Sadhana Maruya<sup>1</sup>, Himanshu Pathak<sup>2</sup> and Raj D Rai<sup>1</sup>

<sup>1</sup>Division of Biochemistry, Indian Agricultural Research Institute, New Delhi; <sup>2</sup>CESCRA, Indian Agricultural Research Institute, New Delhi

Wheat, an important food grain crop in the world is adversely affected by high temperature. HSFs play central role in regulating the expression of stress-associated genes (SAGs) under heat stress which in turn modulates the thermotolerance of plants. Here, we cloned and sequenced a novel heat-responsive transcription factor (*HSF-2*) gene from wheat cv. HD2967. The 1.5kb long gene showed 97% similarity with transcription factor reported from *Triticum aestivum* (accession no JQ771755) and 90% with *Hordeum vulgare* (accession noAK373036) in BLASTn homology search. HSF2 has open reading frame of 374 amino acids with 5' UTR region of 213 bp and 3'UTR region of 163 bp (NCBI GenBank submission: accession no KP063542). Conserved domain search showed the presence of HSF\_DNA-binding domain, winged helix DNA-binding domain and transcriptional regulator. The cloned transcription factor gene was further characterized for its location on wheat chromosomes using Ensembl Plants. The gene was observed to be localized on 3B chromosome. We observed 23 phosphorylation sites in the amino acid sequence of the cloned gene (serine - 15 sites, threonine - 6 sites and tyrosine - 2 sites). There is a need to further exploit this gene using tools of genetic engineering in order to develop heat-tolerant wheat.

### **S344: Spatial and Temporal Expression of *AP2/ERF-N22* during Water Deficit Stress in Rice (*Oryza sativa* subsp. *Indica*)**

Vaibhav Kumar, Nanditha Krishnan, Kishwar Ali and Aruna Tyagi\*

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110012  
(\*Email: at\_bio@iari.res.in)

Rice is the staple food for two-third of the world's population with a production of 496.4 mt (2014-FAO estimate). Abiotic stresses like drought and salinity are the most limiting factors for stable crop production and result in significant yield losses. To cope with abiotic stress the plant expresses stress responsive genes which in turn are regulated by transcription factors. *AP2/ERF* family of plant specific transcription factors play important roles in plant growth, development and in expression of various stress responsive genes. The present study was carried out to analyze the expression pattern of stress responsive *AP2/ERF-N22* for different stress levels and developmental stages of rice. Differential patterns of expression of *AP2/ERF-N22* were observed for different treatments i.e. control drought, drought + ABA and ABA at different stress levels. Among all developmental stages, vegetative stage showed higher expression as compared to anthesis stage. Among treatments, drought induced the expression of *AP2/ERF* at both vegetative and anthesis stages while ABA treatment in combination with drought increased the *AP2/ERF* expression only during mild stress but under severe stress it decreased at both vegetative and anthesis stages. ABA alone induced *AP2/ERF* expression at vegetative stage only.



### **S345: Isolation and Characterization of Rhizobacteria from Rice Grown in Jharkhand Vis-A-Vis Evaluation of Their Plant Growth Promoting Activity**

*SK Lal<sup>1</sup>, KU Tribhuvan<sup>1</sup>, NK Sinha<sup>1</sup>, K Thamilarasi<sup>2</sup>, SR Meena<sup>1</sup> and Kanchan Kumari<sup>3</sup>*

*<sup>1</sup>ICAR- Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand; <sup>2</sup>ICAR- Indian Institute of Natural Resins and Gums, Ranchi, Jharkhand; <sup>3</sup>University of Delhi, Delhi  
(\*Email: shambhumku@gmail.com)*

Agro climatic region of Jharkhand in India is suitable for rice production and there exists a rich biodiversity of rice cultivars. However, in terms of nutrient availability, 90% of area is medium to low in available nitrogen; about 66% area is low in available phosphorus content and nearly 70% area has low to medium potassium content. To address the problems of poor nutrient availability, beneficial microbes such as plant growth promoting bacteria (PGPR) may be used. PGPR increases nutrient uptake from soils, reducing the need of fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils. In this regard, rhizobacterial strains from local landraces and varieties of rice crop grown in different regions of Jharkhand will be isolated, identified and their plant growth promoting activity will be analysed. On the basis of isolated PGPR performance at in vitro and in vivo condition, the efficient strains will be selected and used as biofertilizer, phytostimulator, biopesticide or biocontrol agent and thus reducing the burden of chemical fertilizer, improving soil health and increasing the production potential of crop.

### **S346: Effect of Chitosan Treatments on Nutritional Quality and Shelf Life of Guava (*Psidium guajava*) Fruits during Storage**

*Shilpa Chawla\*, Veena Jain and Ajay Pal*

Department of Biochemistry, CCS Haryana Agricultural University, Hisar 125004, Haryana  
(\*Email: sunsshine2009@gmail.com)

Current investigation was carried out to study the effect of chitosan on nutritional and keeping quality of the guava fruit variety 'Hisar Surkha'. The fruit treated with various concentrations of chitosan viz. 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% for five minutes were air dried and stored at room temperature. Samples were analyzed at three day interval until complete decay for loss in weight, firmness, total soluble solids (TSS), titratable acidity, ascorbic acid content, total antioxidant activity, total and reducing sugars. Treatment with 1.5% chitosan showed the most promising effects on quality of fruit as is evident from delaying the decrease of ascorbic acid, sugars and titratable acidity as compared to control and other treatments. Further analysis showed that fruits treated with 1.5% chitosan exhibited slower rate of physiological loss in weight and decreasing firmness up to 15th day as compared to control and other treated fruits. Also total antioxidant activity was the highest in 1.5% chitosan treated fruits. The results thus indicate that treatment with 1.5% chitosan resulted in enhancing the nutritional quality and shelf life of guava fruit.

### **S347: Effect of Calcium Chloride on Carbohydrate and Cell Wall Components and Cell Wall Degrading Enzymes in Ber Fruits during Storage**

Veena Jain, Shilpa Chawla, Poonam Choudhary and Sunita Jain

Department of Biochemistry, CCS Haryana Agricultural University, Hisar 125004, Haryana  
(Email: sunsshine2009@gmail.com)

The ber (*Ziziphus mauritiana* Lamk.) fruits of two varieties Umran (shelf life 8 to 9 days) and Kaithali (shelf life 4 to 5 days) harvested at physiological maturity were treated with two concentrations (1 and 2 %) of calcium chloride for five minutes. Samples were taken at two day interval until complete decay and analyzed for various cell wall degrading enzymes and its components and carbohydrate components. Pretreatment of fruits with  $\text{CaCl}_2$  led to slight increase in total sugar content in both the varieties. It resulted in decline of reducing sugar content in Umran only. However, non-reducing sugar increased up to 4<sup>th</sup> day and then decreased linearly in Umran. Conversely, in Kaithali, non-reducing sugar decreased up to 6<sup>th</sup> day and then increased. Pretreatments of ber fruits resulted in slowing down the cell wall degradation process during storage. This is evident from the decrease in activities of cell wall degrading enzymes viz. pectin methyl esterase, polygalacturonase and cellulase thereby retaining more cell wall components (cellulose, hemi-cellulose and pectin content) as compared to control during storage in both the varieties.

### **S348: Bulbing and Flowering Related Genes of Onion**

VS Dalvi<sup>1</sup>, YA Patil<sup>1</sup>, Bal Krishna<sup>1</sup>, VR Subramaniam<sup>1</sup>, AP Sane<sup>2</sup> and PV Sane<sup>1</sup>

<sup>1</sup>Plant Molecular Biology Lab, Jain R&D lab, Agri Park, Jain Hills, Shirsoli Road, Jalgaon 425001, Maharashtra; <sup>2</sup>Plant Gene Expression Lab, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001

Flowering and bulbing are the most important phenomena in the life cycle of onion and both are driven by the same gene family, the *Flowering locus T (FT)*. *FT* induces bulbing or flowering in response to growing environment, especially day length and temperature in onions. Along with *FT*, *TERMINAL FLOWER 1 (TFL1)*, *Constans (CO)* and *Flowering locus C (FLC) / Vernalization (VRN)* like genes are also involved in flowering. In a short day onion variety (JISL 5) having no strict requirement of vernalization for flowering, there are at least 11 *FT* and one *TFL1* like gene. Sequence analysis revealed that six out of 11 *FT* like genes exhibit conserved position of Tyrosine, Glycine and Tryptophan in B segment indicating these to be taking part in flowering and bulb induction. Based on the expression patterns, *AcFT6* appears to be involved in bulbing, and *AcFT1* and *AcFT2* in bulb development while *AcFT3* and *AcFT5* are key flower inducers. Other *FT* like genes might have some other functions as shown by differential expression patterns. *AcTFL1* expression was found to be the highest in harvested bulbs; however, it went down as the bulbs sprouted and initiated reproductive phase.



### **S349: The bZIP Transcription Factor, *OsZIP62*, in Rice is a Functional Ortholog of Flowering Locus D (*FD*) in Arabidopsis**

Amarjot Kaur<sup>1,\*</sup>, Aashima Nijhawan<sup>1</sup>, Mahesh Yadav<sup>1</sup> and Jitendra P Khurana<sup>1,2</sup>

<sup>1</sup>Department of Plant Molecular Biology; <sup>2</sup>Interdisciplinary Centre for Plant Genomics, University of Delhi South Campus, New Delhi (\*Email: just\_amar1988@yahoo.co.in)

The bZIP proteins form a major class of transcription factors involved in regulating seed germination, floral induction, stress signaling, hormone signalling and many other aspects of plant development. They are highly conserved and exclusively present in eukaryotes, and are characterized by the presence of a conserved bZIP domain containing the basic region involved in binding to specific regions of DNA for modulating transcription. Apart from this, they also contain a leucine zipper region known to be involved in homo- and hetero-dimerization. Our lab identified a few *bZIPs* that may be involved in regulating flowering in rice employing whole genome transcriptome analysis. Among these, *OsZIP62* showed some promising results as it was able to functionally complement *fd* mutant of Arabidopsis. To further strengthen this hypothesis, yeast one-to-one interaction of *OsZIP62* with OsFTLs was performed and a few of them did show interaction. Also, yeast-three-hybrid assay involving some 14-3-3 proteins, which serve as chaperones, showed similar results. RNAi transgenics for *OsZIP62* have been generated in rice and showed a slight delay in flowering time in comparison to WT. These data thus provide evidence that *OsZIP62* is a putative ortholog of Arabidopsis *FD* and may be involved in regulating transition to flowering.

### **S350: Identification of Novel Alleles of *HKT1;5* and *HKT2;3* Metal Ion Transporter Genes Associated with Salt Tolerance in Indian Wild Rice Accessions**

Shefali Mishra, Balwant Singh, Vandna Rai and Nagendra Kumar Singh

ICAR-National Research Centre on Plant Biotechnology, IARI, Pusa Campus, New Delhi 110012

Soil salinity is a major factor limiting crop productivity in 6.73 Mha area in India. Rice (*Oryza sativa* L.) is one of the most important food crops worldwide; hence a major challenge is to find effective genetic sources of tolerance for adaptation to the salt affected areas. High affinity potassium transporter (*HKT*) gene family is functionally divided into two sub families based on the amino acid sequence in the first pore loop. *HKT* subfamily I has five sodium specific transporters with serine residue while *HKT* subfamily II has four members which are either Na<sup>+</sup>/ K<sup>+</sup> co-transporters or Na<sup>+</sup>/ K<sup>+</sup> antiporters with glycine residue in the first pore loop at position 88. *HKT* gene family members are potential candidates for allele mining as they are promising source of tolerance to salinity. In this study we examined allelic variability and phylogeny of two members (*HKT 1;5* and *HKT 2;3*) of *HKT* gene family in a collection of wild rice accessions. A wide range of variability was observed among examined genes, some of which have the potential in rice crop improvement programme.



### **S351: Antioxidant Enzymes Confer Tolerance in Rice (*Oryza Sativa*) Cultivars Exposed to NaCl**

*Pragya Mishra<sup>1,2</sup>, Shikha Sinha<sup>1,2</sup>, Vagish Mishra<sup>2</sup>, Nagendra Kumar Singh<sup>2</sup> and Vandna Rai<sup>2</sup>*

<sup>1</sup>Banasthali Vidyapith, Jaipur; <sup>2</sup>National Research Centre on Plant Biotechnology, Pusa campus, New Delhi 110012 (Email: pragya.bioinfo@gmail.com)

Salt stress is a major abiotic stress that negatively affects crop growth and productivity. Different plants respond to stress in different ways by altering their metabolism and activating various defense mechanisms. To evaluate the genotypic variation for NaCl stress in terms of biochemical response, rice seedling of seven varieties namely CSR13, CSR36, CSR27, IR64, IR20, MI48 and PB1 differing in salt tolerance were grown in Hoagland's medium for 15 days. These were exposed to NaCl (150 mM) for 24h. CSR36 and CSR27 showed minimum reduction in plant growth whereas, MI48 and PB1 showed higher growth reduction. Higher levels of superoxide radicals and hydrogen peroxide were observed in MI48 and PB1 as compared to CSR27 and CSR36 cause oxidative stress. In contrast to this, higher activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and non enzymatic antioxidants such as proline and ascorbate was observed in CSR27 and CSR 36. However, in MI48 and PB1 comparatively less increase was observed. From the results it appears that reactive oxygen species generated due to stress and scavenged by antioxidant enzymes is important for conferring tolerance.

### **S352: Global Expression Profiling of Rice miRNAs at Flowering Stage under Drought Stress**

*Chandra Prakash<sup>1</sup>, Sasmita Pattnaik<sup>1</sup>, Ramkumar MK<sup>1</sup>, Viswanathan C<sup>2</sup>, Mohapatra T<sup>3</sup>, SV Amitha Mithra CR<sup>1</sup> and Singh NK<sup>1</sup>*

<sup>1</sup>ICAR-National Research Center on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>2</sup>Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi 110012; <sup>3</sup>ICAR-Central Rice Research Institute, Cuttack, Odisha 753006 (Email: cpsingh9@gmail.com)

MicroRNAs (miRNAs) are small RNAs that control gene expression through silencing of target mRNAs. They play a major role in plants developmental processes and stress tolerance. Present investigation was undertaken to study the effect of drought at flowering stage in Nagina-22, IR-64 and extreme bulks of recombinant inbred lines (RILs) for spikelet fertility under drought, in terms of expression of different miRNAs present in rice. A novel miRNA expression array, consisting of 522 miRNAs from miRbase and 102 candidate miRNAs earlier identified in our laboratory under drought stress from Nagina22, was designed and used for the present study. Plants were grown under two water regimes, drought and well irrigated conditions and leaf sampling was done at flowering stage. 100ng of total RNA was used for miRNA microarray experiment as per manufacturer (Agilent) protocol and data analysis was done using GeneSpring GX v12.5. Out of 624 miRNAs, 53, 62, 47 and 17 were found to be differentially expressed in Nagina-22, IR-64, bulks of highly fertile and highly sterile RILs respectively under drought stress as compared to well irrigated conditions. Integration of findings from this study with their target genes and QTL data will aid in identification and use of robust candidate genes for rice improvement under drought.



### S353: Evaluation of *Aegilops Tauschii* and *Aegilops Speltooides* for Acquired Thermo Tolerance: Implications in Wheat Breeding Programmes

Suboot Hairat and Paramjit Khurana

Department of Plant Molecular Biology, University of Delhi, South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi 110 021

The current temperature increase induced by global warming have led to the prediction of severe and frequent heat waves, which could have dramatic ecological and social impacts. The aim of this study was to examine the effect of two separate episodes of heat stress, mimicking heat wave conditions on the physiology of two *Aegilops* species: *A. tauschii* and *A. speltooides* and its potential adaptive mechanism. A significantly lesser inhibition of photosystem II efficiency and net photosynthetic rate (Pn) was observed in *A. tauschii* as compared to *A. speltooides*. Measurement of the minimum fluorescence (Fo) versus temperature curves revealed a higher inflection temperature of Fo for *A. tauschii* than *A. speltooides*, reflecting greater thermo stability of the photosynthetic apparatus. Light energy participating in photochemistry revealed that *A. speltooides* showed increased steady state fluorescence and a lower absorbed light allocated to photochemistry (\*PSII) relative to *A. tauschii*. Significantly higher free radicals scavenging ability was shown by *A. tauschii* as compared to *A. speltooides*. These results suggest that *A. tauschii* under severe high temperature stress showed faster recovery and a better thermo stability of its photosynthetic apparatus and thus makes it a better candidate for alien species introgression in wheat breeding programmes for imparting thermotolerance to wheat.

### S354: Use of Host Delivered RNA Interference for Increasing Tolerance to Sheath Blight Pathogen (*Rhizoctonia solani*) in Transgenic Rice

Ila Tiwari<sup>1</sup>, Arun Jesuraj<sup>2</sup>, Richa Kamboj<sup>1</sup>, Ramawatar Nagar<sup>1</sup>, Amolkumar U Solanke<sup>1</sup>, J Bottella<sup>2</sup> and TR Sharma<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>2</sup>The University of Queensland, St Lucia Campus Brisbane, Queensland, Australia.

(\*Email: trsharma@nrpcb.org)

*Rhizoctonia solani*, a causal agent of sheath blight disease in rice is a devastating plant pathogen and no rice cultivar showed complete resistance to this fungus. The effectiveness of Host Delivered RNAi (HD-RNAi) in the sheath blight fungus *Rhizoctonia* was tested by targeting predicted pathogenicity gene *RPMK* (*map kinase gene*). For ShB assay of transgenic lines fungal mycelia plug along with a scleroatia as inoculum was used. Parameters like lesion length and Highest Relative Lesion Height (HRLH%) were taken into consideration for assessing infection under pot culture experiments. Detached leaf assay showed lesion expansion proceeded aggressively with time intervals in non-transgenic as compared to transgenic lines. Transgenic plants showed phenotypes with delayed and less lesion development in the transgenic rice lines (21mm) as compared to non-transformed control (73mm). Highest relative lesion height percentage (HRLH%) was 3.04% in transgenic line as compared to 9.89% in control. RNAi based transgenics are advantageous as they do not produce any functional foreign proteins and target organisms in a sequence-specific manner. This is the first ever report that has revealed the utility of HD-RNAi to control *R. Solani* and the strategy can also be extended to other crops and pathogens.

### **S355: Isolation and Characterization of Heavy Metal Tolerant Bacteria from Coal Mining Environment vis-a-vis Evaluation of Their Heavy Metal Mobilizing and Plant Growth Promoting Activity**

SK Lal<sup>1\*</sup>, Sudhir Kumar<sup>2</sup> and Kanchan Kumari<sup>3</sup>

<sup>1</sup>ICAR- Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand; <sup>2</sup>ICAR-RC for NEH Region Manipur Centre, Imphal; <sup>3</sup>University of Delhi, New Delhi  
(\*Email: shambhumku@gmail.com)

Heavy metal contamination is a major global concern due to its toxicity and deteriorating effects on human health and environment. Heavy metals are generally classified into three categories: toxic metal (As, Cd, Co, Cr, Cu, Hg, Pb, Sn, Zn), precious metal (Ag, Au, Pd, Pt, Ru) and radionuclides (Am, Ra, Th, U). These metals can't be degraded biologically and chemically and are difficult to eliminate from environment. Hg and Cd metal ions show toxicity even at concentration of 0.001-0.1 mg/L. The elevated level of heavy metal concentration in soil affects the microbial community and plant growth which leads to substantial changes in ecological dynamics of rhizospheric niche. Certain group of bacteria which are thriving well in polluted site have the ability to withstand and detoxify toxic metal. In this study, bacteria inhabiting nearby coal mines and polluted environment will be identified and thereafter potential for heavy metal resistance, heavy metal mobilization activity, bioremediation, microbial assisted phytoremediation and plant growth promoting activity will be analyzed both *in vitro* and *in vivo*. On the basis of performance of isolated heavy metal tolerant bacteria both *in vitro* and *in vivo* condition, the efficient bacterial strains will be selected for reclamation of heavy metal polluted and degraded soil.

### **S356: Plant Growth Regulators and Its Effect on Seed Set and Seed Yield of *Flemingia semialata***

NK Sinha<sup>1\*</sup>, J Ghosh<sup>2</sup>, Md Monobrullah<sup>2</sup>, VD Lohot<sup>2</sup> and Nirmal Kumar<sup>1</sup>

<sup>1</sup>ICAR- Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand 843 010; <sup>2</sup>ICAR-Indian Institute of Natural Resins and Gums, Ranchi, Jharkhand 843 010  
(\*Email: nksinha.cazri@gmail.com)

*Flemingia semialata* is one of the most important leguminous species for intensive lac production. Seed yield of this species is quite low because of poor seed set and high rate of seed shattering. The present study was carried out to study the effect of different levels of plant growth regulators on morpho-physiological and biochemical characters and their relationship on yield attributing traits that helps in improving seed set and shattering. Plant Growth Regulators (PGRs) at different levels were sprayed at pre flowering and anthesis stage. Among the ten treatments, T6 (NAA 30 ppm) recorded highest shoot length (102.8), number of branches (6.33), and percent green leaves per plant (10) whereas number of raceme/plant (5.0), floret number/ raceme (127.67), number of pods / raceme (91.44) was recorded highest in T3 (Thiourea 1000 ppm). The application of PGRs increased the chlorophyll content and caretenoid. Lowering the water saturation deficit and increasing the relative water content and leaf porosity due to the application of PGRs resulted higher seed set (49.1-76.7 %) than that of control (37.3 %). Thus the present study revealed that the application of PGRs improved the seed set and seed yield may be because of high relative water content and leaf porosity, and low water saturation deficit.



### **S357: Temporal and Spatial Expression of Putative Heat Shock Protein 70 Protect the Cells from Heat Injury in Wheat (*Triticum aestivum*)**

Suneha Goswami<sup>1</sup>, Ranjeet R Kumar<sup>1</sup>, Pooja Verma<sup>1</sup>, Kavita Dubey, Khushboo Singh<sup>1</sup>, Jyoti P Singh<sup>1</sup>, Mahesh Kumar<sup>1</sup>, Sushil K Sharma<sup>1</sup>, Gyanendra P Singh<sup>2</sup>, Himanshu Pathak<sup>3</sup> and Raj D Rai<sup>1</sup>

<sup>1</sup>Division of Biochemistry, <sup>2</sup>Division of Genetics, <sup>3</sup>Center for Environment Science and Climate Resilient Agriculture, Indian Agricultural Research Institute, New Delhi 110012

Heat stress adversely affects growth, development and yield of winter wheat (*Triticum aestivum*). Plants have, however, evolved mechanisms to adapt to such conditions mainly by the expression of stress-associated chaperones, the heat shock proteins (HSPs), for modulating the tolerance level. Here, we report cloning of cytosolic putative HSP70 of 1678 bp from a thermotolerant cultivar (C306) of wheat. BLASTn search showed maximum homology with predicted HSP70 protein reported from *Hordeum vulgare*. *In silico* characterisation showed presence of nucleotide-binding domain of the sugar kinase/HSP70/actin superfamily in the sequence. Putative HSP70 showed temporal and spatial variations in the expression under the heat stress (HS). We observed abundance of HSP70 protein, H<sub>2</sub>O<sub>2</sub>, proline, and guaiacol peroxidase activity during the seed-hardening stage under HS; however accumulation was more in the thermotolerant C306 than in thermosusceptible HD2329 cultivar. The expression of HSP70 showed negative correlation with CMS and positive with TAC under HS; changes were less pronounced in C306 than in HD2329 at all the stages of growth studied. HSP70 seems to play diverse roles associated with the thermotolerance, and partially protect the wheat from the terminal HS. Being the important member of family of the HSPs, HSP70 needs to be studied in detail, to be used for developing climate-smart wheat crop, through genetic engineering and breeding approaches.

### **S358: De Novo Assembly for the Identification of Novel and Conserved Heat-Responsive microRNAs in Wheat (*Triticum Aestivum* L.) at Pollination Stage**

Ranjeet Ranjan Kumar<sup>1</sup>, Suneha Goswami<sup>1</sup>, Sushil Kumar Sharma<sup>1</sup>, Khushboo Singh<sup>1</sup>, Sadhna Maruya<sup>1</sup>, Sweta Singh<sup>1</sup>, Yugal K Kalra<sup>2</sup>, Gyanendra Pratap Singh<sup>2</sup>, Himanshu Pathak<sup>3</sup> and Raj Deo Rai<sup>1</sup>

<sup>1</sup>Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012; <sup>3</sup>CESCR, Indian Agricultural Research Institute, New Delhi 110012

MicroRNAs (miRNAs) are small endogenous RNAs of ~22 nucleotides that play a regulatory role by negatively affecting the expression of genes at the post-transcriptional level. Information of miRNAs on some important crops like soybean, Arabidopsis, rice etc. are available, but no such study on heat-responsive novel miRNA has been reported in wheat (*Triticum aestivum* L.). In the present investigation, wheat cultivar HD2985 was used in small RNA library construction and Illumina HiSeq 2000 was used to perform high throughput sequencing of the library after cluster generation; 110,896,604 and 87,743,861 reads were generated in the control (22°C) and heat shock treated (42°C for 2 h) samples, respectively. Forty four mature miRNA were found in *T. aestivum* from miRBase v19. In the expression profiling, we could observe 30, 14 and 9 mature miRNA in the control and 25, 10, 11 mature miRNA in the heat-treated samples based on the reference genome of *Oryza sativa*, *Zea mays* and *Sorghum bicolor*. We could deduce 187, 308, 138 and 70 target genes in *T. aestivum* (tae), *O. sativa* (osa), *Z. mays* (zma) and *S. bicolor* (sbi) to be regulated by 19, 37, 8 and 13 identified miRNAs. MiR analyzer based search showed the presence of 16, 2 (*osa*), 8, 9 (*zma*) and 1 (*sbi*) novel miRNAs in the control and treated samples. Target prediction and pathway analysis revealed their involvement in stress tolerance; signal transduction etc. Novel miRNAs are new additions to miRNA database of wheat.

### **S359: RNAi in Aphids: A Potential Way for Developing Aphid-Resistance in Indian Mustard**

Raghavendra Aminedi<sup>1</sup>, Deepa Bhatt<sup>1</sup>, Varsha Jain<sup>1</sup>, Deepa Dhatwalia<sup>1</sup>, Raj K Bhatnagar<sup>2</sup> and Ramcharan Bhattacharya<sup>1</sup>

<sup>1</sup>National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute Campus, New Delhi 110 012; <sup>2</sup> International Centre for Genetic Engineering and Biotechnology, New Delhi 110 012 (\*Email: rcb@nrcpb.org; rcbhattacharya1@gmail.com)

*Brassica juncea* (Indian mustard) is a chief oil yielding crop and is severely damaged by aphids (*Hemiptera: Aphidoidea*). Due to lack of resistance source within the crossable germplasms, attaining aphid resistance through conventional breeding has been difficult in *B. juncea*. Recently, RNAi mediated gene silencing is being talked as a potential way to develop insect resistance in crops. In such strategy gene silencing of essential aphid-specific genes mediated through host delivered dsRNA has been hypothesized. The feasibility of the proposed mechanism has been proven in model plant *Arabidopsis*. However, to attain applicable resistance identification of potential target gene(s) in aphid is pivotal. In our continuous effort for identifying potential target aphid genes two potential domains MpDE4A and MpDE8A have been identified through bioinformatics intelligence. dsRNA targeted to these two domains when supplemented in artificial diets can potentially disrupt survivability of aphids. Interestingly, the qRT-PCR data on gene-expression revealed significant down-regulation of the target genes as the cause of aphid-mortality. In addition genes associated with aphid-parthenogenesis are also being assayed for their amenability to RNAi mediated silencing and consequent effect on aphid reproduction. The results pertaining to the efficacy of the different target aphid-genes in effecting aphid-mortality through RNAi will be presented and discussed.

### **S360: In Vitro Selection in Ashwagandha (*Withania somnifera* (L.) Dunal.) against Leaf Blight Disease**

RP Patel, A Jhankare, MK Tripathi, Gyanendra Tiwari, GN Pandey and BK Patidar

RVSKVV, K.N.K. College of Horticulture, Mandsaur (M.P.)

Asgandh/Ashwagandha (Winter Cherry) is one of the most important medicinal crops commercially cultivated in India. Ashwagandha [*Withania somnifera* (L.) Dunal.] belongs to family Solanaceae. It is commercially cultivated in western Madhya Pradesh (Mandsaur, Neemuch) and eastern Rajasthan. It is cultivated in 10000-15000 hectare area to produce 2500-3500 tonnes with average productivity (6-7 q/ha.) Disease tolerant/resistant Ashwagandha cv JA-20 and MSW-100 cell line were selected against leaf blight disease caused by *Alternaria alternata*. For this purpose callus and cell suspension culture derive from mature embryos and hypocotyls explants were exposed to purified toxic culture filtrate produced by fungus supplemented with MS culture medium. Two selection methods were used: a continuous method in which four cycle of selection were performed on toxic medium whereas during discontinuous method, a pause was given after the second and third cycle of selection using non-toxic medium. Approximately 310 calli of JA-20 and 340 of MWS-100 obtained from mature embryo and hypocotyls explants culture and 675 of JA 20 and 725 of MSW-100 cell clumps/embryoids acquired from cell suspension cultures were exposed to media with effective concentration (10.0 ml/l) of phytotoxin for selection. The discontinuous method proved to be superior as it allowed the calli to regain their regeneration capability. Continuous exposure with toxic culture filtrate resulted up to 77% mortality. A total 2 lines of JA-20 and one of MWS-100 were documented resistant/tolerant amongst an array of putative resistant/ tolerant lines during S<sub>1</sub> generation.



### **S361: Heterologous Expression of Manganese Superoxide dismutase (Mn - SOD) from Wheat (*Triticum aestivum* L.) under the Heat Stress**

Kavita Dubey<sup>1</sup>, Suneha Goswami<sup>1</sup>, Khushboo Singh<sup>1</sup>, Shweta Singh<sup>1</sup>, Sadhana Maruya<sup>1</sup>, Gyanendra K Rai<sup>2</sup>, Yugal K Kala<sup>3</sup>, Gyanendra P Singh<sup>3</sup>, Sushil . Sharma<sup>1</sup>, Ranjeet R Kumar<sup>1</sup>, Himanshu Pathak<sup>4</sup>, Raj D Rai<sup>1</sup>

<sup>1</sup>Division of Biochemistry, ICAR- Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu; <sup>3</sup>Division of Genetics, ICAR- Indian Agricultural Research Institute, New Delhi 110012; <sup>4</sup>CESCRA, ICAR-Indian Agricultural Research Institute, New Delhi 110012

Superoxide dismutase (SOD), the metalloenzyme, provides first line of defence against abiotic stress in all plants. Oxidative damage occurring under abiotic stress is basically counteracted by SOD. In this study, we cloned a full length gene of 696 bp encoding manganese SOD (*Mn-SOD*) from *Triticum aestivum*. BLASTn homology showed maximum similarities with SOD gene reported from *Hordeum vulgare* and *Triticum aestivum*. Southern blot analysis showed the presence of three copies of SOD gene in wheat. Conserved domain search showed the presence of manganese iron binding domain in the amino acid sequence. Quantitative Real-Time PCR showed significant increase in the relative expression of *MnSOD* under heat stress. Abundance of transcripts was observed in HDR77, as compared to PBW343 under HS. Relative expression was observed maximum (1.9- fold) in HDR77, as compared to 1.5- fold in PBW343 during grain-filling stage in response to HS. The gene was mobilised in pMalc5x vector and transformed using BL21 strain of *E.coli*. The gene was expressed under the influence of 1.5 mM IPTG and the fusion protein of size 68kDa was separated through column chromatography. The fusion protein was digested using Factor Xa and a protein of size 25.5 kDa corresponding to Mn-SOD was purified using ion-exchange chromatography. Findings suggest that *Mn-SOD* regulates the various heat stress-associated genes through balancing the accumulation of H<sub>2</sub>O<sub>2</sub> inside the cells and in turn modulates the thermotolerance level of wheat under the heat stress.

### **S362: Development of SSH Library from *Pennisetum glaucum* for Identification of Drought Stress Responsive Genes**

Rajendra Prasad Meena, Radha Yadav and Jasdeep Chatrath Padaria

National Research Centre on Plant Biotechnology, IARI, New Delhi 110012

(Email: jasdeep\_kaur64@yahoo.co.in)

The rapidly changing global climate is leading to enhanced environmental stress conditions which are causing paramount adverse impacts on agriculture and food security. Drought is one of the main environmental constraints to agricultural productivity worldwide. Many plants have evolved multiple, interconnected strategies that enable them to survive drought stress. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the most widely grown millet, which has an efficient adaptive mechanism to cope with drought and other environmental stresses. Physiological and biochemical parameters like Relative Water Content, Membrane Stability Injury, chlorophyll content and lipid peroxidation confirmed the maximum drought stress after 10 days, 15 days and 20 days of water withdrawal in *P. glaucum* at flowering stage. *P. glaucum* plants at flowering stage were subjected to drought stress through water withdrawal for 10 days, 15 days and 20 days and drought responsive suppressive subtractive hybridization (SSH) libraries were constructed using leaf samples. A total of 1551 ESTs were obtained which were assembled in 54 contigs and 235 singletons. BlastX (NCBI data base) analysis of obtained ESTs showed homology to drought responsive gene like *delta-1-pyrroline-5-carboxylate synthase*, *cell wall-associated hydrolase*, *cbl-interacting serine threonine-protein kinase 1*, *Glutathione peroxidase* and *Calmodulin-binding transcription activator*. The drought responsive ESTs libraries generated in *P. glaucum* will thus be a good source of studying genes and involved in mechanism of abiotic stress tolerance.

### S363: Identification of Blast Resistance Genes in Kalanamak Rice Landrace Using Allele Specific STS Markers

Neeraj Pal<sup>1</sup>, Aparna Gupta<sup>1</sup>, Anita Kumari<sup>1</sup>, AK Gaur<sup>1</sup>, ID Pandey<sup>2</sup>, Shrawan Kumar<sup>3</sup>,  
TR Sharma<sup>3</sup> and Anil Kumar<sup>1</sup>

<sup>1</sup>Department of Molecular Biology & Genetic Engineering, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand; <sup>2</sup>Department of Genetics & Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand; <sup>3</sup>National Research Centre on Plant Biotechnology, ICAR- Indian Agricultural Research Institute, New Delhi 110 012

Rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases of rice crop production in different parts of the world. The disease causes considerably large grain yield losses in almost all major ecosystems where it is grown. To develop cultivars having resistance against different races of *M. oryzae*, availability of molecular markers along with marker-assisted selection strategies are essential. In this study, presence of seven major blast resistance genes (*Pi54*, *Pi9*, *Pita*, *Pi1*, *Pi2*, *Pi5* and *Pib*) were determined in 65 landraces of Kalanamak rice using gene based STS markers namely YL155/YL87, C1454, 195R-1/195F-1, CPI54 which showed close-set linkage to four major rice blast resistance (R) genes, *Pita*, *Pi5*, *Pi9*, *Pi54* respectively. Three other microsatellite marker namely RM208, RM224, AP-5659-5 were found to co-segregate with the *Pib*, *Pi1*, *Pi2* genes respectively. Among the 65 accessions, 59 accessions of Kalanamak rice was found to possess one or more blast resistance gene. Out of 59 landrace, one was positive for *Pi9*, 2 for *Pi54*, 5 for *Pi5*, 6 for *Pi1*, 25 for *Pita*, 24 for *Pi2* and 42 for *Pib*. One accession, 3216-N (Kalanamak-50) showed positive bands associated with five major rice blast resistance genes indicating broad spectrum blast resistance against different races of *M. oryzae*. The information on blast resistance in present study will further aid plant breeders in choosing appropriate breeding strategy for blast resistance and improvement of Kalanamak rice.

### S364: Isolation and Characterization of a Hypovirulence Associated Mycovirus from *Fusarium* species

Saurabh Kulshrestha<sup>1</sup>, Mohit Sharma<sup>1</sup>, and Kirti Singh<sup>1</sup>

<sup>1</sup>Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh (\*Email: saurabh\_kul2000@yahoo.co.in)

*Fusarium*, a genus of filamentous fungi, is having a broad host range including rice, horticultural crops, ornamentals and in almost all agricultural commodities. Utilizing a biological control method is a tactful choice. In the field, a number of strains within a species of fungi present, some of them are virulent whereas others are hypovirulent. The hypovirulent isolates are either genetically hypo virulent or are infected by mycoviruses. From phytopathological perspective, both of them are interesting as they could be developed as biocontrol agents. In present investigation, an attempt was made to isolate hypovirulence associated dsRNA mycoviruses from *Fusarium* spp. *Fusarium* was isolated using baiting method from soil and was characterized by amplifying the internal transcribed region (ITS) and its sequencing. Cultures which showed slow and abnormal growth phenotype were suspected to be infected by mycovirus. When inoculated on apple and pear this isolate does not produced symptoms. After curing, cultures exhibited normal morphology, pigmentation and normal growth rate. Presence of mycoviral dsRNA was first confirmed by isolation of dsRNA elements using CF-11 chromatography followed by RT-PCR amplification with primer pairs designed from the coat protein sequences of *Fusarium graminearum* mycovirus. *Fusarium* strain cured for mycoviruses was found to be more pathogenic as compared to uncured strain. This confirms the association of mycovirus with hypovirulence in *Fusarium* spp.



### S365: Siderophore Monooxygenase a Potent Drug Target for Bacterial Diseases

Saroja Narsing Rao<sup>1\*</sup> and GS Jagannatha Rao<sup>2</sup>

<sup>1</sup>Dept of Biotechnology, Dayananda Sagar College of Engg, Bengaluru 78; <sup>2</sup>UNT Health Science Centre, Fort Worth, TX-76107, USA (\*Email: Saroja95@yahoo.com)

Iron is an essential nutrient and is typically present at too low concentrations to support proliferation of microbial pathogens in mammalian hosts. To overcome this iron deficiency during infection many invasive human pathogens such as *Mycobacterium tuberculosis*, *Aspergillus fumigates*, *Shewanella putrefaciens* produce siderophores to scavenge ferric iron from the host for the bacteria to proliferate. Biosynthesis of these siderophores involves the action of novel flavin –containing N-hydroxylating monooxygenases (NMO). Disruption of these monooxygenases in *Burkholderia cepacia* and *A. fumigates* results in loss of effective persistence and colonization and has been reported to be absolutely essential for virulence. We are targeting the enzymes that hydroxylate siderophores to form a hydroxamate moiety, which is essential for iron binding and the lack of homologous enzymes in humans. These enzymes are an attractive drug target for the treatment of tuberculosis and other bacterial diseases. Putrescine monooxygenase (*pubA*) is a flavin –dependent monooxygenase that catalyzes the NAD(P)H- and oxygen –dependent hydroxylation of the putrescine to give N-hydroxyputrescine involved in the biosynthetic pathway of the siderophore putrebactin is targeted. Cloning of *pubA* gene of *Shewanella putrefaciens* was done. The recombinant vector construct transformed into *E. coli* BL21 cells for full length expression. IPTG induced protein expression of recombinant entry proteins fused with 6-His and V5 epitope tags at their N termini and purification by IMAC is in progress.

### S366: Phosphorylation Based Regulation of Submergence Tolerance Gene *Sub1A1* by MPK3 Module in Imparting Submergence Tolerance to Rice

Pallavi Singh and Alok Krishna Sinha\*

National Institute of Plant Genome Research, New Delhi

The sudden inundation of rice fields lead to decrease in oxygen and soil pH, which in turn leads to deprivation of nutrients. It becomes imperative to understand how rice plant perceives and transduces these varying signals and produce responses to the stress in question. *SUBMERGENCE 1* (*SUB1*), a QTL imparts submergence tolerance to wild rice landraces. Among indica accessions of rice with *SUB1A*, those with *SUB1A-1* allele are typically submergence tolerant, while those with *SUB1A-2* allele are typically submergence intolerant. These two alleles encode proteins that only differ at a single amino acid, Ser-186 in *SUB1A-1* and Pro-186 in *SUB1A-2*, a putative mitogen activated protein kinase (MAPK) phosphorylation site in *SUB1A-1*. Thus, *SUB1A* is designated as the master regulator, orchestrating a plethora of response during submergence stress tolerance. The connection between MAPK signalling cascade and submergence tolerance is currently unknown. In the present study, we report that MPK3 is activated by submergence in a *SUB1A*-dependent manner. MPK3 physically interacts with and phosphorylates *SUB1A-1* in an allele specific manner. Furthermore, *SUB1A1* binds to the MPK3 promoter and regulates its expression in a feedback regulatory mechanism during submergence stress signalling. We present molecular and physiological evidence for the cardinal role of the MPK3-*SUB1A-1* module in acclimation of rice seedling to adverse effects of submergence. Overall, the results provide a more detailed and holistic understanding of submergence tolerance in *Oryza sativa* that will inform the deployment of this trait in varietal development.

### **S367: Understanding the Basis of Systemic Resistance Induced by *Trichoderma* in Castor Bean**

V Dinesh Kumar\*, RD Prasad, RBhuvanewari, KB Durga Bhavani and Velu Mani Selvaraj

Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500 030

(\*Email: dineshkumarv@yahoo.com)

Elicitors released by *Trichoderma* upon colonizing the root system is known to bring about induced systemic resistance (ISR) in plants through altered transcriptome and proteome of the host plants enabling them to fight diseases. Seedling blight disease, caused by *Phytophthora parasitica* var *nicotiana*, and vascular wilt disease, caused by *Fusarium oxysporum fsp ricini* (F. o. r), are among the major diseases of castor that result in yield reduction up to 30 to 77%. Efficacy of *Trichoderma* in inducing systemic resistance against these two diseases was studied using different strains of *Trichoderma*. Colonization of castor roots by *Trichoderma* induced resistance against both the diseases. When compared with untreated seedlings *Phytophthora* disease severity was reduced by 85.7% in *Trichoderma* (Th4d strain)-treated seedlings and similar results were obtained with respect to *Fusarium* disease. ISR by *Trichoderma* was confirmed by studying the expression pattern of a few signature genes known to be up-regulated during ISR. To further understand the differentially expressed genes during ISR, we have adopted RNA sequencing technique in a time series experiment and the results have shown global changes in the gene expression pattern. ISR is being further characterized by comparing proteome and metabolomes in *Trichoderma* treated and untreated castor seedlings.

### **S368: Stress Inducible Expression of *AtNHX1* Confers Salinity and Oxidative Stress Tolerance in Transgenic Mungbean**

Sanjeev Kumar, Debeepasad Sahoo, Sagarika Mishra, and Lingaraj Sahoo\*

Department of Bioscience and Bioengineering, Indian Institute of Technology Guwahati,

Guwahati 781039 (\*E-mail: ls@iitg.ernet.in)

Salinity is one of the major abiotic stresses affecting mungbean (*Vigna radiata* L.) productivity. To generate mungbean plants that can adapt to saline soil, *AtNHX1*, encoding an Arabidopsis vacuolar antiporter gene driven by stress inducible *rd29A* promoter was over expressed in a salt sensitive cultivar (K-851) by *Agrobacterium* mediated transformation and the putative transformed plants were selected using herbicide, phosphinothricin (PPT). Presence and integration of the transgenes (*Bar* and *AtNHX1*) were confirmed in T1 and T2 transgenic lines by Polymerase Chain Reaction (PCR) and Southern hybridization. Semi-quantitative RT-PCR and quantitative real time PCR (qRT-PCR) revealed higher expression of *AtNHX1* and *Bar* in T2 transgenic lines than wild-type plants (WT). The inducible expression of *AtNHX1* conferred enhanced salt tolerance to transgenic mungbean. These T2 transgenic lines grew well in the presence of 200 mM NaCl while wild type exhibited chlorosis and died within 3 weeks. These transgenic lines retained more chlorophyll, accumulated more proline and ascorbate, revealed triggered activity of glutathione reductase, catalase, peroxidase and superoxide dismutase, and higher level of relative water content and lower level of lipid peroxidation, hydrogen peroxide and oxygen radical production. Moreover, these transgenic plants accumulated higher Na<sup>+</sup> content in shoot and roots. In addition, the transgenic plants also exhibited considerable tolerance to oxidative damage induced by methyl viologen (MV).



### **S369: 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**

Manisha Negi<sup>†</sup>, Raghavendrarao Sanagala<sup>†</sup>, Vandna Rai and Ajay Jain\*

ICAR-National Research Centre on Plant Biotechnology, Lal Bahadur Shastri Building, Pusa Campus, New Delhi 110012 (\*E-mail: [ajay1762jain@gmail.com](mailto:ajay1762jain@gmail.com)). <sup>†</sup>Both the authors contributed equally

Pi-deprived plants trigger an array of species-specific and spatiotemporally regulated responses. Further, there is an antagonistic cross talk between Pi and iron (Fe). Root system plays a pivotal role in Pi acquisition and its subsequent mobilization to aerial parts of plant. However, the effects of Fe availability on Pi deficiency-mediated morphophysiological and molecular responses of root system of rice (*Oryza sativa*) are far from being elucidated. Here, in this study rice seedlings were germinated in Petri plates lined with moist filter paper for 4 days at 28°C. Extensive variations were observed in radical length. To minimize the intrinsic variability, seedlings in the range of 2-3 cm were selected for the treatment. Element-contamination free aerated hydroponic system was developed for detailed temporal (2 d and 4 d) analysis of the effects of Pi and/or Fe deficiency treatments on 10 different root traits of rice seedlings. After 4 d treatment, Fe availability exerted variable and significant effects on Pi deficiency-mediated developmental responses of some of these root traits. Further, ICP-MS analysis revealed noticeable influence on ionic profiles of macro-(P, K, S and Mg) and micro-(Fe, Zn, Mn and Cu) elements during growth under different nutrient regime. Analysis of the roots revealed differential effects of Fe availability on Pi deficiency-mediated relative expression levels of genes involved in Pi homeostasis. Together these results revealed variable effects of Fe availability on Pi deficiency-mediated morphophysiological and molecular responses of rice.

### **S370: Transformation of elite Indian bread wheat with DREB gene from finger millet (*Eleusine coracana*)**

Tanu Tiwari, Zahoor Ahmad Mir, Priyanka Monga, Amolkumar U. Solanke, Jasdeep Chatrath Padaria\*

National Research Centre on Plant Biotechnology, New Delhi-110012  
(\*Email: [jasdeep\\_kaur64@yahoo.co.in](mailto:jasdeep_kaur64@yahoo.co.in))

Environmental stresses hamper plant growth and productivity. Some plants are to adapt to environmental stresses due to presence of a number of abiotic stress responsive genes/transcriptional factors. *DREB* (Dehydration Responsive Element Binding) identified in a heat responsive cDNA library of *Eleusine coracana* was isolated, cloned in pBI121 and mobilized in *Agrobacterium tumefaciens* strain EHA105. Transformation of Indian elite wheat cultivars (HD2894 & HD2967) with *EcDREB* gene using *Agrobacterium* mediated transformation *Inplanta* method (shoot tip infection) was carried out for developing thermotolerant transgenic wheat. A total 175 two day old seedlings were transformed and grown to maturity (T0 plants) in phytotron. A T1 seeds (367) were obtained which were screened by kanamycin selection (80mg/L) for 8 hours with mild shaking (150 rpm) at two day old seedlings stage for presence of transgene. Transformation efficiency of 6.85% was obtained as 12 seedlings survived by kanamycin selection. The putative transgenics were confirmed by PCR analysis and further validation for stable *EcDREB* gene integration and expression is under progress.

## Technical Session IV: Emerging Technologies

### S401: DNA Barcoding: Utility in Plant Species Delineation and Limitations

Mahesh C Yadav

Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources,  
New Delhi (Email: mcyadav@yahoo.com)

DNA barcoding is an emerging genomic tool for rapid and accurate species identification and phylogenomics studies in both animals and plants. Unlike in animals where mitochondrial locus *cox1* have been found to be useful for DNA barcoding, for plants, two-locus DNA barcode system of chloroplast genes namely *matK* and *rbcl* have been recommended as a standard barcode by consortium on barcode of Life (CBOL). We amplified and sequenced partial *rbcl* gene and *trnH-psbA* spacer region of chloroplast genome from 100 rice accessions belonging to 16 different species of genus *Oryza*. Ten SNPs were identified, which could distinguish different genome species. The wild species of AA-genome namely *O. nivara*, *O. rufipogon*, *O. barthii*, *O. longistaminata*, *O. meridionalis* and *O. glumaepatula* grouped in the same clade in the phylogenetic tree and could not be differentiated from each other using *rbcl* sequences alone. Nevertheless, these species were separated from other genome species of rice. A polymorphism in mono-nucleotide repeats region in *trnH-psbA* spacer was observed along with Indels at three sites. A 6-bp InDel clearly distinguished *O. brachyantha* from other *Oryza* species. The utility and limitations of *rbcl*, ITS region, *trnH-psbA* and *matK* loci for DNA barcoding in rice will be discussed.

### S402: Phytofabricated Nanoparticles and Their Role in Human Health

Abhijeet Singh\*, Jitendra Mittal and Madan Mohan Sharma

Department of Biosciences, Manipal University, Jaipur, Rajasthan  
(\*Email: abhijeet.singh@jaipur.manipal.edu)

In the present scenario, there are growing concerns over the potential impacts of bioengineered nanoparticles in the health sector. However, our understanding of how bioengineered nanoparticles may affect organisms within natural ecosystems lags far behind our rapidly increasing ability to engineer novel nanoparticles. To date, research on the biological impacts of bioengineered nanoparticles has primarily consisted of controlled lab studies of model organisms with single species in culture media. Here we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles. Silver nanoparticles were successfully synthesized from 3mM  $\text{AgNO}_3$  via a green synthesis process using leaf extract as reducing as well as capping agent. Nanoparticles were characterized with the help of UV-vis absorption spectroscopy, X-ray diffraction and TEM analysis which revealed the size of nanoparticles of 35 nm size. Further the nanoparticles synthesized by green route are found highly toxic against pathogenic bacteria and plant pathogenic fungi viz. *Escherichia coli*, *Pseudomonas syringae* and *Sclerotinia sclerotiorum*. The most important outcome of this work will be the development of value-added products and protection of human health from pathogens viz., bacteria, virus, fungi etc.



### **S403: Use of Biomimetic Nanoparticles in Human Health**

*Madan Mohan Sharma\**, *Yash Mishra* and *Abhijeet Singh*

Department of Biosciences, Manipal University, Jaipur, Rajasthan

(\*Email: [madanmohan.sharma@jaipur.manipal.edu](mailto:madanmohan.sharma@jaipur.manipal.edu))

Bio-nanotechnology is the advance area of research in modern science. Nanoparticles exhibit improved physical and chemical properties. Silver (Ag) nanoparticles possess strong antimicrobial effects, making them suitable for use in medical devices such as catheters, wound dressings, skin donation, prostheses, contraceptives etc. Hence, in the present study, we described a cost effective and environment friendly technique for production of silver nanoparticles from 3 mM AgNO<sub>3</sub> solution through fungal extract. This extract functions as reducing agent as well as capping agent. Synthesized nanoparticles were characterized using UV-Vis absorption spectroscopy, SEM and TEM. Further these myco-synthesized nanoparticles were found to be highly toxic against different bacterial and pathogenic fungal species viz. *Escherichia coli*, *Pseudomonas syringae*, *Sclerotinia sclerotiorum* and *Aspergillus flavus*. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications make this method potentially exciting for the large-scale synthesis. Toxicity studies of silver nanoparticles on human system opens new vistas for a new range of antimicrobial agents. Development of value-added products, bacterial and fungal control agents from fungus will be the most important outcome of this work.

## Technical Session V: Genetic Resources, Allele Mining and Marker Assisted Breeding

Invited Lecture

### Molecular Breeding for Improvement of Biotic and Abiotic Stress Resistance in Basmati Rice

Singh AK<sup>1\*</sup>, Gopala Krishnan S<sup>1</sup>, Bhowmick PK<sup>1</sup>, Haritha B<sup>1</sup>, Prabhu KV<sup>1</sup>, Singh NK<sup>2</sup>, Sharma TR<sup>2</sup>, Nagarajan M<sup>1</sup>, Vinod KK<sup>1</sup>, Singh UD<sup>3</sup>, Prakash G<sup>3</sup>, Mondal KK<sup>3</sup>, Bashyal BM<sup>3</sup>, Chander S<sup>4</sup>, Ellur RK<sup>1</sup>, Khanna A<sup>1</sup>, Ashtosh Yadav<sup>1</sup>, Fiyaz Ahmad<sup>1</sup> and Vivek Kumar Singh<sup>1</sup>

<sup>1</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012;

<sup>2</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa campus, New Delhi 110012;

<sup>3</sup>Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110012;

<sup>4</sup>Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: aks\_gene@yahoo.com)

Basmati rice is popular across the globe due to its unique grain and cooking quality characteristics. Traditional varieties of Basmati rice suffered from lodging owing to tall height, yielded less than 2.5 tons/ha and had long duration. Genetic improvement of Basmati rice at ICAR-IARI has led to development of a number of high yielding Basmati rice varieties with significant improvement yield and in per day productivity, which has helped the annual national earning of foreign exchange to the tune of ~ US \$ 5 billion. However, Basmati rice were highly susceptible to various biotic stresses such as bacterial blight (BB), blast, sheath blight (ShB) and brown plant hopper (BPH) and abiotic stress like salinity. Several MAS derived varieties such as Improved Pusa Basmati 1 and Pusa 1592 with resistance to BB; Pusa 6 (Pusa1612) and Pusa Basmati 1609 with blast resistance have been released. Further, an array of isogenic lines carrying different genes in Basmati background have been developed for combating multiple biotic stresses such as BB (*xa13*, *Xa21*, *Xa33*, *Xa38*), blast (*Pi54*, *Piz5*, *Pi9*, *Pi1*, *Pita*, *Pib*, *Pi5*), sheath blight (qSBR11-1), BPH (*Bph 18*, *Bph20* and *Bph 21*), Bakanae, and abiotic salt tolerance (Saltol). Genomic resources complimented by suitable genetic resources have helped in mapping genes, discovering novel QTLs for yield, grain and cooking quality traits. Genetic enhancement of rice in tandem with modern molecular tools has made noteworthy contribution in not only improving the productivity of Basmati rice but also significant improvement in quality.

Invited Lecture

### Marker-Assisted Breeding in *Brassica juncea*: a Reality Check

AK Pradhan

Department of Genetics, University of Delhi, South Campus, New Delhi 110021

(Email: pradhancgmcp@gmail.com)

*Brassica juncea* is a natural allotetraploid (AABB) between *B. rapa* (AA) and *B. nigra* (BB) and is a major oilseed crop of India. There are two distinct and genetically diverse gene pools in *B. juncea*, the east European and the Indian gene pools. To broaden the genetic diversity in the Indian gene pool lines, it is imperative to utilize the east European gene pool of mustard as this gene pool contains several desirable traits pertaining to oil and meal quality, disease resistance and yield components. The marker-based backcross breeding (trait/targeted breeding) would be the suitable method to mobilize the desired trait



from the east European lines to Indian cultivars. However, hybrids between these two gene pool lines are heterotic for yield. The concept has been successfully exploited in developing some productive hybrids namely, DMH-1, DMH-4 and DMH-11. Our experience on marker-assisted backcross breeding only through foreground selection in *B. Juncea* for the introgression of desired traits from the east European gene pool line to the Indian gene pool lines is not very encouraging because of retention of large fragment of donor genome around the gene of interest leading to linkage drag in near-isogenic lines.

*Invited Lecture*

### **Alien Genetic Resources in Rice: Their Characterization and Utilization for Rice Improvement**

*Kuldeep Singh<sup>1\*</sup>, Kumari Neelam<sup>1</sup>, Amanpreet Kaur<sup>1</sup>, Amandeep Kaur<sup>2</sup>, Dharminder Bhatia<sup>2</sup>, JS Lore<sup>2</sup>, Preetinder Singh<sup>2</sup>, NK Dhillon<sup>3</sup>, Gurpreet K Sahi<sup>1</sup>, Kishore Sinha<sup>1</sup>, Sarbjeeet Kaur<sup>1</sup>, Shiwali Thakur<sup>1</sup> and M Sheshu<sup>4</sup>*

<sup>1</sup>School of Agricultural Biotechnology, <sup>2</sup>Dept. Plant Breeding, Genetics and Biotechnology, <sup>3</sup>Dept. Plant Pathology Punjab Agricultural University, Ludhiana 141 004; <sup>4</sup>ICAR-Indian Institute of Rice Research, Hyderabad (\*Email: kuldeep35@pau.edu)

Rice serves as a source of calories for more than half of the world's population. Contrary to the requisite growth rate of >1 percent it has fallen down. Thus, there is a need to increase the yield per unit area through development of new varieties. Here we present the potential for utilization of wild species of rice for improving its productivity and sustainability. Hybrids have been successfully produced between cultivated rice and wild species in the genus *Oryza* through a series of alien introgression lines, monosomic alien addition lines (MAALs), backcross inbred lines (BILs) and chromosome segmental substitution lines (CSSLs). At PAU Ludhiana we are maintaining more than 1200 accession of wild species of rice and characterized these for a series of traits. We have generated about 3000 introgression lines (ILs). 326 ILs were evaluated for blast resistance and novel sources of leaf and neck blast resistance have been identified from *O. glumaepatula* and *O. glaberrima*. More than 1500 accession were screened against 10 pathotypes of *Xoo*. More than 1000 accession were screened against brown plant hopper (BPH) and many were confirmed to be resistant to BPH over two years of screening. We identified five lines derived from crosses with *O. glaberrima* against root knot nematode. A varying number of wild species accessions (20-100 accessions) were used for mining of alleles at Phospholipase D (PLD), PSTOL1, and DRO1 by sequencing whole gene including the promoter regions.

*Invited Lecture*

### **Mining Allelic Variations Associated with Agronomic and Nutritional Traits Using Mini Core and Genome-Wide Association Mapping in Large Germplasm Collection**

*Hari D Upadhyaya*

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Telangana (Email: H.Upadhyaya@cgiar.org)

Plant genetic resources are important for future genetic progress and an insurance against unforeseen threats to production. ICRISAT genebank conserves over 123,000 accessions of its mandate crops (chickpea, pigeonpea, groundnut, sorghum, and pearl millet) and six small millets from 144 countries.



Representative core (10% of entire collection) and mini core (10% of core or 1% of entire collection) collections have been developed as resource to discover new sources of variations for enhanced use of germplasm in crop breeding. To date, 257 sets of mini core collection have been distributed to users in 35 countries. Using these resources, trait-specific genetically diverse germplasm for resistance to abiotic and biotic stress and for agronomic and nutritional traits have been identified, and made available to researchers globally. Genome-wide association mapping in chickpea, groundnut and sorghum unraveled many significant marker-trait associations, with many of these markers co-mapped on the same linkage groups previously reported as harboring QTL or candidate genes associated with stress tolerance and/or phenological variations associated with productivity traits. Further, germplasm and breeding lines with unique traits are being sequenced. This will facilitate to associate sequence differences with agronomically beneficial traits. Increased use of agrobiodiversity together with modern genomics tools is crucial to coping with new challenges to agricultural production.

### Oral Presentation

## Marker Aided Pyramiding of $\beta$ -carotene hydroxylase (*crtRB1*) and lycopene e-cyclase (*lcyE*) Genes for Provitamin-A Enrichment in Quality Protein Maize (QPM) Inbreds

Rajkumar Zunjare\*, Firoz Hossain, Vignesh Muthusamy, Anchal Baveja, Nepolean Thirunavukkarasu, Supradip Saha and HS Gupta

ICAR-Indian Agricultural Research Institute, New Delhi (\*Email: raj\_gpb@yahoo.com)

Yellow maize exhibits tremendous natural variation for carotenoids, but concentration of provitamin-A is quite low (<2  $\mu\text{g/g}$ ) compared to target level (15  $\mu\text{g/g}$ ). In the present study, four popular QPM hybrids (HQPM-1, HQPM-4, HQPM-5 and HQPM-7) of the country were targeted for enrichment of provitamin-A by introgressing the favourable alleles of *crtRB1* and *lcyE* genes using marker-assisted selection (MAS). HP704-22 and HP704-23 (CIMMYT-HarvestPlus germplasm) were used as donors, while four elite QPM parents viz. HKI161, HKI163, HKI193-1 and HKI193-2 were the recipients. The recurrent parents possess low provitamin-A (<2.5  $\mu\text{g/g}$ ), and donors were rich in provitamin-A (>20  $\mu\text{g/g}$ ). Foreground selection for opaque2 was undertaken using SSRs ( $\phi$ 057), while InDel markers were used for *crtRB1* and *lcyE*. Strong segregation distortion for the *crtRB1* has been observed.  $BC_1F_1$ ,  $BC_2F_1$  and  $BC_2F_2$  populations were genotyped for *opaque2* and *crtRB1/lcyE* alleles. Introgressed versions of the selected QPM inbreds having favourable alleles of *opaque2*, *crtRB1* and *lcyE* possess provitamin-A as high as >20  $\mu\text{g/g}$ . High recovery of recurrent parent genome among selected backcross progenies were observed. Improved inbreds resemble their respective original inbreds for plant, ear and grain characteristics. These MAS-derived inbreds will play important role for nutritional security of the country.



## **S501: Chemical Evaluation of *Mucuna* species Germplasm for L-Dopa: an Anti-Parkinson's Drug Yielding Plant from India**

Archana P Raina<sup>1\*</sup> and RC Mishra<sup>2</sup>

<sup>1</sup>Germplasm Evaluation Division, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012; <sup>2</sup>ICAR-National Bureau of Plant Genetic Resources, Regional Station Cuttack 753006, Odisha (\*Email: aprraina@yahoo.co.in)

*Mucuna* species are important medicinal plants of India due to the presence of bioactive compound L-dopa (L-3,4-dihydroxy phenylalanine) in seeds, which serves as potential drug for the treatment of Parkinson's disease and acts as an intermediate precursor of the neurotransmitter dopamine. The present study was undertaken with the objective of phytochemical evaluation of five species of *Mucuna* (*M. pruriens* var. *pruriens*, *M. monosperma*, *M. nigricans*, *M. gigantea*, *M. pruriens* var. *nivea*) collected from wild habitats of Odisha state of India for active compound L-dopa by High Performance Thin Layer Chromatography (HPTLC). Among the five *Mucuna* species, L-dopa content was highest in *M. pruriens* var. *pruriens* germplasm varying between 5.34-7.60 % on dry weight basis. Other species of *Mucuna* having high L-dopa content in seeds were *M. gigantea* (6.76%), *M. nigricans* (6.16%) and *M. monosperma* (4.61%); while *M. pruriens* var. *nivea* showed least L-dopa content of 1.22%. Promising accessions with high L-dopa content in *M. pruriens* var. *pruriens* were IC599330, IC599336, IC599342, IC599290 and IC599350. These superior accessions along with other rare species of *M. monosperma*, *M. nigricans* and *M. gigantea* can be exploited for large scale cultivation for their pharmacological important constituent L-dopa.

## **S502: Genetic Studies in Cassava (*Manihot esculenta* Crantz.) Germplasm and Isolation of Mutants with Altered Starch for Augmenting Industrial Value**

S. Kanagarasu, V Thiruvengadam, S Ganesh Ram and A John Joel\*

Department of Plant Genetic Resources, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 3 (\*Email: jnjoel@gmail.com)

The present investigation was carried out with the 97 cassava germplasm lines for morphological traits, physicochemical, biochemical and pasting properties for identification of elite germplasm lines. There was high variability in the germplasm lines for the traits analysed. Tuber yield, plant height and commercial roots possessed high values of GCV, heritability and genetic advance. Four landraces viz., Tall chilly kallan, Tall pachai konntai, Tall adukku muttan and Kottaram 2 having tuber yield of more than 5.80 kg and amylose content of less than 16 per cent with acceptable yield and biochemical traits were identified as elite genotypes. Attempts made to isolate low amylose mutants using a popular cassava hybrid H 226 through induced mutagenesis resulted in the generation of TML 070 (14.2 per cent), TML 370 (14.8 per cent) and TML 069 (14.9 per cent) as compared with the control H 226 (28.5 per cent). SEM revealed that the starch granules from low amylose mutant TML 370 possessed larger granule size and less granular distribution. Molecular characterization of TML 370 revealed two SNPs with functional effect on GBSS I gene. Based on the loss in restriction sites, CAPS markers were developed to aid the MAS programs in cassava.

### **S503: Microsatellite Analysis and Their Segregation Pattern in Eucalyptus Interspecific Hybrids**

*M. Sumathi\* and R Yasodha*

Division of Plant Biotechnology, Institute of Forest Genetics and Tree Breeding, Coimbatore 641 002, Tamil Nadu (\*Email: gmsumathibt@gmail.com)

Simple sequence repeats (SSRs) are dispersed ubiquitously within the genome and have been proven useful as markers in plant genetic analysis because of their hyper variability, co-dominance and reproducibility. Similar to agricultural crops, economically important forest trees like Eucalyptus, having world's largest area under industrial plantations, has been targeted for marker assisted selection. In this study, efforts were made to investigate the segregation pattern in the interspecific hybrids of *Eucalyptus tereticornis* X *Eucalyptus grandis* and *Eucalyptus tereticornis* X *Eucalyptus camaldulensis* using genomic and EST-SSRs. After analyzing 320 SSRs in 100 F<sub>1</sub> individuals of each cross, it was found that 110 loci were polymorphic and useful for further analysis. The markers were segregating in F<sub>1</sub> progenies as fully informative markers, with 1:1:1:1 and 1:2:1 ratio. Maternally and paternally informative markers were segregating in 1:1 ratio with about 5% of SSR loci showing segregation distortion in *Eucalyptus tereticornis* X *Eucalyptus grandis* cross. Compared to genomic SSRs, EST-SSRs showed high level of polymorphism and markers were fully informative. The fully informative segregation pattern (1:1:1:1 and 1:2:1) were efficient in detection of hybrid purity. The information generated in this study would be used in generation of genetic map followed by QTL analysis in Eucalyptus for adventitious rooting traits and wood property traits.

### **S504: Identification of Microsatellite Marker Suitable for Marker Assisted Selection of Downy Mildew Resistance in Table Grape Variety Thompson Seedless**

*Pushpa M Deore\*, Roshni R. Samarth, Anuradha Upadhyay and Indu S Sawant*

ICAR-National Research Centre for Grapes, Manjari Farm Post, Solapur Road, Pune 412307, Maharashtra (\*Email:push.deore@gmail.com)

Downy mildew disease is one of the major risk factors for viticulture. Marker assisted breeding was initiated to introgress the downy mildew resistance into Thompson Seedless, a popular susceptible table grape variety in India. The grape genome sequence corresponding to RPV3, a major QTL for downy mildew resistance was analyzed and primers were designed for 10 microsatellite regions. These primers were screened for polymorphism using several susceptible and resistant genotypes and 2 markers (VVDM2 and VVDM5) showed polymorphism between resistant and susceptible parents. These polymorphic markers and earlier reported markers (VMC7E2, UDV305, UDV737) linked to QTL RPV3 were used to analyze 51 F<sub>1</sub> progenies of cross between Carolina Black Rose (resistant parent, source of RPV3 locus) and Thompson Seedless. These F<sub>1</sub> progenies were also screened in vitro and in field for their response to downy mildew pathogen and rated according to the UPOV scale (1-9). The co-segregation data on the molecular marker and disease resistance in 51 F<sub>1</sub> were analyzed by means of ANOVA single marker analysis. Significant association (P<0.01) of VVDM5\_153 allele was observed with downy mildew resistance with R<sup>2</sup> value of 0.1125. This marker was validated for its use in marker assisted selection for downy mildew resistance.



## **S505: Molecular Marker Assisted Development of Blast Resistance Rice Varieties for Northern Hill Zone of Chhattisgarh**

*Jitendra Kumar Tiwari<sup>1\*</sup> and Ashish Kumar Tiwari<sup>2</sup>*

<sup>1</sup>Department of Genetics and Plant Breeding, RMD, College of Agriculture and Research Station, Ambikapur, IGKV (C.G.); <sup>2</sup>Department of Genetics and Plant Breeding, IGKV, Raipur (C.G.)

(\*Email: tiwarijk5@gmail.com)

Blast disease of rice caused by the fungus *Magnaporthe grisea* (Hebert) Barq, is one of the most destructive disease of the crop causing severe yield loss. In Northern Hill Zone (NHZ) of Chhattisgarh, most of the rice varieties are susceptible to blast disease pathogen. Identification and incorporation of new blast resistance genes into modern rice cultivars are important breeding strategies to control the damage caused by new races of blast. Certain accessions of NHZ of Chhattisgarh rice germplasm possess virulent blast resistance gene. Being adapted to the locations where these germplasm originated, and having coevolved with the local population of the blast fungus, use of these germplasm could have a competitive edge over other exotic resistance donors currently being used in the breeding programs. The germplasm accessions will be screened for the presence of major blast resistance (R) genes and one susceptible gene using a set of SSR markers. PCR based allele specific SSR markers will be obtained for identification of blast resistance genes in the selected germplasm.

## **S506: Applications of Single Nucleotide Polymorphism (SNP) in Varietal Improvement of African Rice (*Oryza glaberrima* Steud.)**

*Abubakar Mohammad Gumi\* and Tapan Kumar Mondal*

Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi (\*Email: muhammadag@yahoo.co.uk)

African rice (*O. glaberrima*) consists of over 1,500 cultivated accessions known for their low yield but tolerant to various biotic and abiotic stresses. Several contrasting genotypes for many abiotic stresses such as drought, salinity etc. exists among the African rice gene pool. The techniques of SNP detection in plants is comparatively new and ever since, protocols have been designed and constantly modified to enable researchers to discover SNPs both experimentally as well as through *in silico* analysis. For majority of crop species, sequence availability is still a limiting factor for *in silico* SNP discovery, though consortium efforts have generated large amount of sequence data from some important model crops such as maize, Arabidopsis, wheat, rice, barley and brassicas. Therefore, application of SNP technology to identify important gene loci/QTLs of desired phenotypes will help in varietal improvement of the African rice germplasm through introgression of genes from other domesticated and wild relatives of rice.



## S507: Resynthesis of *B. juncea*: Enriching Diversity for Mustard Improvement

Mahesh Rao<sup>1\*</sup>, Megha Kaushik<sup>2</sup>, NC Gupta<sup>1</sup>, Rohit Chamola<sup>1</sup> and SR Bhat<sup>1</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa, New Delhi 110012; <sup>2</sup>Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan 303007 (\*Email: amritkushinagar@gmail.com)

Indian mustard *B. juncea* ( $2n=4x=36$ ) is an amphidiploid evolved in nature by chromosome doubling of hybrids between progenitor diploid species, *B. rapa* ( $2n=20$ ) and *B. nigra* ( $2n=16$ ). Low genetic base in the germplasm pool is limiting the scope of further improvement and exclusively retarding the potentiality of hybrid breeding. Resynthesis of *B. juncea* utilizing diverse germplasm lines of the parental species could lead to enhancement of valuable diversity in the gene pool. About 41 different crosses were made in 2012-13 between twelve *B. rapa* and six *B. nigra* lines. Embryo rescue produced 305 hybrid embryos that yielded 85 plants from 15 cross combinations. Use of SSR primers revealed only 14 plants as true hybrid from seven different crosses. In the following year, 0.2% colchicine treatment at the apical bud was given and pollen fertility was simultaneously tested before and after the treatment. Ten individual plants from six cross combinations were found fertile and harvested separately. In next generation (2014-15) plants raised from individual seeds showed variable level of fertility. Bud pollination facilitated fertilization and self seed set in 13 plants from five cross combination. So far, five new resynthesized *B. juncea* lines have been recovered from different *B. rapa* and *B. nigra* cross combinations which will be further characterized and used in mustard breeding program.

## S508: Marker Assisted Identification of Potential Restorers and Assessment of Their Restoration Potential in Rice Germplasm

Kushwaha Amar Kant<sup>1</sup>, Singh Gagandeep<sup>1</sup>, Nagarajan M<sup>2</sup>, Bhowmick PK<sup>1</sup>, SV Amitha CR Mithra<sup>3</sup>, Singh AK<sup>1</sup> and Gopala Krishnan S<sup>1,\*</sup>

<sup>1</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India; <sup>2</sup>Rice Breeding and Genetics Research Centre, ICAR-IARI, Aduthurai, Tamil Nadu, <sup>3</sup>ICAR-National Research Centre on Plant Biotechnology, ICAR-Indian Agricultural Research Institute, New Delhi (\*Email: gopal\_icar@yahoo.co.in)

Productivity of rice needs to be enhanced significantly in order to meet increasing demand. Hybrid rice technology is one of the potential mean to achieve this goal. The rice hybrids currently under cultivation in India have narrow genetic base limiting the extent of realized heterosis. Therefore, an attempt was made to identify prospective restorers and maintainers from a set of 59 diverse rice genotypes. Screening for the presence of restorer genes was carried out using gene linked SSR markers viz., RM 3873 and RM 6100 as well as candidate gene based markers viz., DRRM-RF3-5, DRRM-RF3-10 and DRCG-RF4-14 for Rf3 and Rf4, respectively. Test crosses were performed with the 4 different CMS lines using 59 genotypes as pollen parent. The restoration/maintenance ability of the genotypes were assessed based on pollen and spikelet fertility of the test cross hybrids. Based on marker alleles and pollen fertility, the efficacy of the markers in identifying potential restorers/maintainers was estimated. It was observed that candidate gene based markers performed better in identification of prospective restorers/maintainers as compared to gene linked markers. The identified restorers and maintainers can be utilized in diversification of parental lines in hybrid rice breeding.



### **S509: Genetic Diversity for Yield Traits in Chickpea (*Cicer arietinum* L.) under Rainfed Rice Based Cropping System**

*PL Johnson\*, MK Singh, RN Sharma and HC Nanda*

Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur 492 012, Chhattisgarh (\*Email: pyare\_johnson@yahoo.co.in)

Chickpea is the prime pulse crop of India. In Chhattisgarh, it is cultivated in around 0.261 million hectare, with an average productivity of 1002 kg/ ha. Here mostly those varieties survive which have adaptability under rice based cropping system. In the present investigation 112 genotypes (85 Germplasm accession, 12 improved varieties and 15 advance breeding lines) of chickpea were grown under rice based cropping system. Augmented design was used to conduct the experiment. Genotypes were sown in six blocks each block 20 genotypes, with row-to-row spacing of 30 cm and plat to plant 10 cm. Cluster analysis was carried out for these genotypes were grouped into Five and cluster clusters. The intra and inter cluster distances were computed for all the clusters. The highest inter cluster distance was observed clusters IV and VII (6.296) followed by I and VII (5.867), IV and V (5.681), I and IV (5.658). The highest intra-cluster distance was observed for cluster V (2.328). Hence, genotypes belonging to this cluster viz. ICC 251834, ICC 275466, ICC 269530, Indira Chana-1, ICC 269563, RG 2011-01, RG 2011-03 and JG 11. Therefore, the results indicated that the genotypes belonging to clusters possessing high inter cluster distance between them may be used in the future hybridization programmes for the development of high yielding chickpea genotypes.

### **S510: Survey of Parental Polymorphism between Katarni and Three Elite Rice Genotypes through Microsatellite and Gene Based Molecular Markers and Their Utilization in Validating the F<sub>1</sub> Population**

*Kumari Neha, Smriti, Nitu Kumari, Tirthartha Chattopadhyay, Satyendra, SP Singh, Shashikant, PK Singh and Mankesh Kumar\**

Department of Plant Breeding and Genetics, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813210 (\*Email: mankesh2008@gmail.com)

Katarni rice is one of the famous fine aromatic landraces of rice in Bihar. It is renowned for its special aroma, unique grain and cooking qualities. The Katarni landrace is sensitive to photoperiod and commonly suffers from the problem of lodging due to its very tall stature (150-170 cm plant height). In the present study, with an objective to introgress dwarfism and photoperiod insensitivity traits in this landrace, crossing was made between Katarni (as recipient parent) and 3 dwarf and photoperiod insensitive elite lines namely Rajendra Sweta, IR64 and BPT5204 (as donor parent). The survey of parental polymorphism was done through 115 Simple Sequence Repeat (SSR) markers, located throughout the 12 rice chromosomes, including those SSR markers linked to aroma, dwarfing and heading date genes in rice. Among the SSR markers used in the present study, 72 were found to reveal parental polymorphism. Polymorphism of functional markers for *sd-1* gene (responsible for semi dwarfing trait) and *badh2* gene (responsible for the accumulation of aromatic compound 2-acetyl pyrroline in rice grains) was also observed between the test genotypes of the present study. Furthermore, the polymorphic molecular markers were used to validate the F<sub>1</sub> population derived from the crossing programme.

### **S511: Molecular and Morphological Characterization Reveals High Level of Genetic Variation in Wild-Relatives of Rice**

Shailesh Tiwari<sup>1</sup>, Mahesh C Yadav<sup>1\*</sup>, Nilmani Dikshit<sup>2</sup>, Dipti R Pani<sup>3</sup>, M Latha<sup>4</sup> and Vijay K Yadav<sup>5</sup>

<sup>1</sup>Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi; <sup>2</sup>ICAR-NBPGR Regional Station, Akola, Maharashtra; <sup>3</sup>ICAR-NBPGR Regional Station, Cuttack, Odisha; <sup>4</sup>ICAR-NBPGR Regional Station, Thrissur, Kerala; <sup>5</sup>Department of Genetics and Plant Breeding, CSA University of Agriculture and Technology, Kanpur  
(\*Email: mcyadav@yahoo.com)

Analysis of variability and genetic structure of wild-relatives of cultivated rice is important for the conservation, management and gainful utilization of these valuable genetic resources in crop improvement programmes. To better understand the relationship among the wild rice accessions, we analysed 48 accessions belonging to two species of wild rice *Oryza nivara* and *O. rufipogon* including two cultivated varieties of *O. sativa* collected from six different states of India. These accessions were characterized using 28 morphological descriptors including 15 qualitative and 13 quantitative traits, displaying a wide range variation for plant height, panicles colour, shape and size, peduncle length, kernel size and colour. These accessions were also genotyped for 25 microsatellite loci located on eight out of twelve chromosome of the rice genome. A total of 312 alleles were detected with an average value of 12.48 alleles per microsatellite locus and high PIC value with mean 0.779. Genetic similarities between 48 accessions of wild rice were estimated using Dice similarities coefficient, which ranged from 0.031 to 0.865 with an average of 0.0169. The dendrograms constructed based on Dice coefficient of similarity grouped the accessions into two major clusters. Two accessions collected from Kerala were highly divergent from the rest of the 46 accessions of *Oryza* species.

### **S512: Molecular Analysis of Genetic Variation and Differentiation in a Select Set of Terminal-Heat Tolerant and Susceptible Genotypes of Bread Wheat Using Microsatellite Markers**

Anil Patidar<sup>1</sup>, Mahesh C Yadav<sup>1\*</sup>, Shailesh Tiwari<sup>1</sup> and Tejpal Pal Singh<sup>2</sup>

<sup>1</sup>Division of Genomic Resources, <sup>2</sup>Division of Germplasm Evaluation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi (\*Email: mcyadav@yahoo.com)

Wheat is a staple food crop in India. High temperatures during grain-filling period of bread wheat (*Triticum aestivum* L.) adversely affect the plant growth, yield and grain quality in many regions of world. We have molecularly analysed a set of bread wheat genotypes comprising of terminal-heat tolerant and susceptible accessions using polymorphic microsatellite markers located on chromosome 7B and 7D of wheat genome. The 22 primer-pairs amplified a total of 95 alleles with an average of 4.3 alleles per locus. The PIC value ranged from -0.176 to 0.843. The average frequency of unique alleles was 0.23, whereas the frequency of rare alleles was 0.45. These unique/rare alleles could be used for DNA fingerprinting and differentiation of individual/group of genotypes from the rest. A large number of genotypes (220) exhibited 2- alleles per genotype, while 34 genotypes amplified 3-alleles per genotype. The Dice coefficients of genetic similarity varied from 0.250 to 0.893 with an average of 0.511. UPGMA dendrogram grouped all the genotypes into two major clusters, which clearly distinguished all the genotypes of bread wheat. A moderate level of molecular variation was observed between genotypes of bread wheat.



### S513: Assessment of Genetic Variability and Divergence Analysis in Cowpea (*Vigna unguiculata* (L.) Walp)

Kiran Tigga<sup>1\*</sup>, Er Rahul Sahu<sup>2</sup>, Dinesh Kumar Thakur<sup>1</sup> and RL Pandey<sup>1</sup>

<sup>1</sup>RMD College of Agriculture & Research Station, IGKV, Ajirma, Ambikapur (C.G.); <sup>2</sup>Krishi Vigyan Kendra, IGKV, Kumhrawand Jagdalpur, Bastar (C.G.) (\*Email: kiranascoraipur@gmail.com)

In the present study, genetic variability and divergence analysis of 22 genotypes of cowpea was carried out. The magnitude of genotypic and phenotypic variation was high for seed yield per plot, seed yield per plant, swelling index, swelling capacity, plant population per plot, days to maturity, plant height, seed volume, hydration index, days to 50% flowering, hydration capacity and 100 seed weight. High heritability coupled with high genetic advance was observed for days to maturity, seed volume, swelling capacity, swelling index and seed yield per plant. Divergence analysis revealed that considerable amount of genetic divergence was present in the material under study. Based on Mahalanobis's  $D^2$  statistic, genotypes were grouped into four non-overlapping clusters. Intercrossing of genotypes from different clusters showing superior mean performance may help in obtaining higher yields. Genotypes belonging to cluster I may produce better heterosis and segregants with the genotypes of cluster III. The present investigation revealed that selection for the characters such as seed yield per plant, biological yield, number of capsules per plant, days to maturity and number of primary branches per plant is important to evolve high yielding genotypes of cowpea. Development of dual type cowpea can also be taken up as one of the breeding objective.

### S514: PGRdup: an R Package to Aid in Identification of Probable Duplicates from Plant Genetic Resources Collections

J Aravind<sup>1\*</sup>, J Radhamani<sup>1</sup>, Kalyani Srinivasan<sup>1</sup>, B Ananda Subhash<sup>2</sup> and RK Tyagi<sup>1</sup>

<sup>1</sup>Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi;

<sup>2</sup>Centre for Development of Advanced Computing, Thiruvananthapuram, Kerala

(\*Email: aravindj@nbgr.ernet.in)

Duplicate accessions are the bane of any crop genebank. Long term conservation of orthodox seeds being a costly affair, such duplicates use up valuable resources without adding to the value of the collection. Moreover, this also adds to the cost of characterization and evaluation of germplasm to facilitate their end use. FAO estimates that 70-75% duplication exists both within and between collections among the ~7.4 million accessions held in more than 1,750 genebanks worldwide. 'PGRdup' is an R package developed to facilitate the search for probable duplicate accessions in germplasm collections using passport databases. Primarily this package implements a workflow designed to fetch sets of germplasm accessions with similar passport data particularly in fields associated with accession names within or across passport databases. It offers a suite of functions for data pre-processing, creation of a searchable Keyword in Context (KWIC) index of keywords associated with accession records and the identification of probable duplicate sets by fuzzy, phonetic and semantic matching of keywords. It also has functions to enable the user to review, modify and validate the probable duplicate sets retrieved. Even though the occurrence of similar passport data does not imply 100% genetic identity of accessions, this package can be used for prioritization of accessions for resource intensive activities in the collection.

### **S515: Distribution and Remobilization of Iron and Zinc in Various Tissues of Wheat-*Aegilops* Substitution Lines of Group 2 and 7 Chromosomes**

*Prachi Sharma, Imran, Pritesh Vyas and HS Dhaliwal\**

Eternal University, Sirmour, Himachal Pradesh (\*Email: hsdhaliwal07@gmail.com)

The QTL controlling high grain iron and zinc have been mapped on chromosomes 2 and 7 of *Aegilops* species. In the present study, the iron and zinc uptake and distribution was investigated in various parts of wheat-*Aegilops* substitution lines of group 2 and 7 chromosomes at preanthesis, milking and maturity growth stages. Lower leaves, flag leaves and straw of the whole plant were collected for the analysis of iron and zinc using Atomic absorption spectroscopy. The study reveals that the higher amount of iron and zinc content was observed in the parts of substitution lines and *Aegilops* species as compared to the cultivars. The highest concentration of iron and zinc was observed in the lower leaves and flag leaves of the milking stage. The tissue with the lowest micronutrient concentration was straw in all the stages. Micronutrient uptake begins in preanthesis, until milking stage maximum iron and zinc is accumulated in the lower leaves and flag leaves, and reduces at the maturity stage. In the substitution lines the lower leaves and flag leaves show 4-5 fold reduction in the micronutrient concentration whereas very low (1.5 fold) remobilization is observed in the cultivars at the time of grain filling.

### **S516: Development of Intron Flanking EST-PCR Markers for Characterization of Precise Transfer of Genes for High Grain Micronutrients from Non-Progenitor *Aegilops* species to Wheat**

*Sheikh Imran<sup>1</sup>, Sharma Prachi<sup>1</sup>, Verma Shailender Kumar<sup>2</sup>, Kumar Satish<sup>2</sup>, Malik Sachin<sup>3</sup>, Kumar Sundip<sup>3</sup>, Vyas Pritesh<sup>1</sup>, Chugh Vishal<sup>1</sup> and Dhaliwal HS<sup>1\*</sup>*

<sup>1</sup>Eternal University, Sirmour, Himachal Pradesh, India; <sup>2</sup>Indian Institute of Technology Roorkee, Roorkee, Uttarakhand; <sup>3</sup>GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (\*Email: hsdhaliwal07@gmail.com)

Bread wheat (*Triticum aestivum* L.) is the staple food for over 35% of global population. Its biofortification will help in combating wide spread deficiency of micronutrients. The wheat-*Aegilops* substitution lines with high grain Fe and Zn have been developed and utilized for fine transfers of useful genes using induced homoeologous pairing and radiation hybridization. The anchored, transferable and polymorphic wheat SSR markers used earlier for identification of alien *Aegilops* chromosomes are not effective for tagging fine transfers. More than forty genes such as *IDS*, *NAAT*, *NAS*, *Nramp*, *YSL*, *ZIP*, *YSI* and *IRT* are involved in Fe and Zn homeostasis. Compared with exons, the introns are much more variable and suitable for DNA marker development. In the present study polymorphic intragenic introns of the genes have been targeted for the PCR amplification. For intron flanking EST-PCR marker development, full mRNA sequences of the genes were retrieved from the NCBI database, and on BLASTn against the wheat genome in Ensembl Plants got the genomic sequences revealing the exonic and intronic sequences of the genes. These polymorphic primers amplifying intragenic intronic regions of the genes are being used for characterization and validation of precise transfers with least linkage drag.



### **S517: Marker Assisted Breeding of Recombinant 1RS.1BL for Improvement of Bread Making Quality and QTL for Useful Root Traits of Wheat**

*Kaur Rajdeep, Sharma Prachi, Sheikh Imran, Singh Jasmeet, Vyas Pritesh and Dhaliwal HS\**

Eternal University, Sirmour, Himachal Pradesh (\*Email: hsdhaliwal07@gmail.com)

The 1RS.1BL translocation has been extensively used as a source of genes on 1RS (*Lr26*, *Yr9*, *Sr31* and *Pm8*) for multiple disease resistance and enhanced yield in cultivars. However, cultivars with the 1RS.1BL translocation have sticky dough due to the presence of *Sec-1* on 1RS and absence of *Glu-B3/Gli-1* of 1BS. Many QTLs for useful root traits have also been mapped on 1RS. A highly recombinant 1RS lacking *Sec-1* but with useful root QTL and *Glu-B3* and *Gli-1* of 1BS are available which are being used for marker assisted breeding of wheat cultivars for higher yield and bread making quality. In the present study PCR based markers were used for characterization of cultivars and 1RS recombinants for the absence of *Sec-1* and presence of *Glu-B3/Gli-1*. Marker SCM-9 was used to identify rye translocation, SECA2 for *Sec-1* and O11B3 for *Glu-B3* in various cultivars and recombinants. SDS-PAGE was also used to confirm the presence of secalins. The SDS-microsedimentation test of various 1RS recombinants and parental lines showed that 1RS.1BL with *Sec-1* had lowest MST value while addition of *Glu-B3/Gli-1* with negligible improvement. The work on contribution of root QTL in 1RS recombinants is in process.

### **S518: Influence of Environment on Andrographolide Content in *Andrographis paniculata* (Burn. F.) Wall. Ex. Nees.**

*Anjali Sharma\*, Narendra Gajbhiye, Ashok Kumar Bishoyi and Geetha KA*

ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat  
(\*Email: anjuksharma31@gmail.com)

Medicinal and aromatic plant improvement is entirely different from the other agricultural crops, since quality in terms of secondary metabolites production, which is not apparently dependant on morphological characters and also highly influenced by the environment is very important. A study on influence of environment on andrographolide accumulation in Kalmegh (*Andrographis paniculata* (Burn. f.) Wall. ex. Nees.) was carried out in three agro-climatic zones of India viz., Anand, Faizabad and Coimbatore. Thirteen genotypes of Kalmegh were used for the study. Plants were harvested at 130 days after transplanting (DAT) and andrographolide content was estimated in the herbage by HPLC method. The study showed significant influence of environment in andrographolide accumulation in the species. Maximum andrographolide accumulation was obtained under Anand condition, wherein andrographolide content varied from 1.65% to 2.48% and the content significantly reduced in Tamil Nadu conditions (0.89% to 1.66%). Genotypes also behaved differently under different environmental conditions. Under Anand condition AAP1 showed better andrographolide content (2.48%). However, in Tamil Nadu conditions genotype DMAPR AP 42 showed higher andrographolide content (1.66%). At Faizabad, DMAPR AP 62 has the highest andrographolide (2.17%) content. Hence the study revealed importance of environment for the production of higher active principle in medicinal plants.



### **S519: Genomic Resources in Foxtail Millet (*Setaria italica*) for Crop Improvement**

Garima Pandey, Mehanathan Muthamilarasan, Chandra Bhan Yadav, Kajal Kumari, Sarika Gupta, Bonthala Venkata Suresh, Yusuf Khan, Swarup K Parida, Debasis Chattopadhyay and Manoj Prasad\*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, JNU Campus, New Delhi  
(\*Email: manoj\_prasad@nipgr.ac.in)

Foxtail millet (*Setaria italica* L.) is an experimental model crop for studying functional genomics of millets and bioenergy grasses such as Napier grass, Switch grass, Pearl millet etc. So, it is imperative to generate adequate genomic resources in terms of molecular markers for its genetic improvement. Considering the importance of molecular markers in molecular breeding, a variety of DNA-based markers have been explored and developed. An attempt was made to explore various regions of foxtail genome representing microsatellite repeats, transposable elements, ESTs, miRNA and introns to develop molecular markers. As a result 15,573 genomic SSRs, 20,278 TE-based markers, 447 eSSRs, 176 miRNA-based markers, and 4049 ILP markers were successfully developed. Further validation of these markers on different accessions of foxtail millet, other millets and some non millets was done to assess their polymorphic potential and cross genera transferability. Results demonstrated that these markers will be useful in germplasm characterization, transferability, construction of genetic linkage map for gene/quantitative trait loci discovery, phylogenetics and comparative mapping studies for crop improvement of millets, non millets and bioenergy grass species.

### **S520: Development of *Diplotaxis erucoides* Specific Markers for Marker Assisted Introgression of Alien Genes into *Brassica juncea***

Naresh Vasupalli\*, Viswanathan Satheesh and SR Bhat

ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012  
(\*Email:naribiotech@gmail.com)

*Diplotaxis erucoides* is a wild species which is highly resistant to Alternaria leaf spot. To introgress genes for Alternaria resistance, we developed an advanced back cross population by hybridizing the synthetic amphidiploid (*D. erucoides* x *B. rapa*) with *B. juncea*. Molecular markers designed based on available sequence data of Brassicaceae gave very low polymorphism between *B. juncea* and *D. erucoides*. Therefore, we undertook a genome survey sequencing of *D. erucoides* using Ion-torrent platform. We generated 60 million single end reads with an average read length of 130 bp. Reference assembly was done in CLCbio7.5.1 using available Brassica database (*B. rapa*, *B. oleracea* chromosomes, mitochondria and chloroplast genome) as a reference to eliminate the redundant sequences. The filtered sequences (15 million reads) generated 4000 contigs (>1 kb) by de novo assembly. The unique contigs showing no significant similarity in blast search against nucleotide data base in NCBI were used for STS primer development. More than 80% primers out of sixty, gave polymorphic amplification pattern when tested by PCR (present in *D. erucoides* and amphidiploids but absent in *B. juncea*). These primers are now being used to test the advanced backcross population for alien gene introgression and to tag the Alternaria resistance gene(s).



## **S521: Molecular Mapping of Gynoecious Trait (*F* Locus) Utilizing Indian Monoecious Line and Exotic Gynoecious Line of Cucumber (*Cucumis sativus*)**

TK Behera\*, H Choudhary, AD Munshi, R Amarnath and Rajni

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi

(\*Email: tusar@rediffmail.com)

The gynoecious sex form has extensively been exploited in heterosis breeding for enhancing productivity of cucumber worldwide. Indian cucumber cultivars are basically monoecious in nature and associated with crown fruit inhibition whereas exotic gynoecious lines are not stable at high temperature and their fruit quality does not match with that of indigenous cucumber. The introgression of gynoecious trait into Indian cucumber varieties requires molecular mapping of gynoecious trait. The  $F_2$  population of the cross G 421 (exotic gynoecious) x Pusa Uday (Indian monoecious) was used to study of the inheritance and molecular mapping of gynoecious trait (*F*). Out of 233 SSR markers screened for parental polymorphism, five primers pairs were found polymorphic. Another functional dominant SCAR marker CsACS1G specific to the *F* locus was also used for mapping. This SCAR marker amplifying the promoter sequence of the gene CsACS1G was amplified in FF and Ff plants but not in monoecious (ff) plants, thus clearly distinguishing gynoecious and monoecious plants in the  $F_2$  population. All these six markers were used for mapping the  $F_2$  population consisting of 209 individuals. Linkage analysis revealed that two markers, namely SSR-13251 and SSR-15516 were located on the same side of the gene of interest at a distance of 1.5 and 4.5 cM, respectively with the *F* locus (SCAR marker) positioned at the top of chromosome 6. These three molecular markers can be effectively utilized for marker assisted introgression of gynoecious trait in cucumber.

## **S522: Genetic Mapping of Gynoecious Trait in Cross Between Gynoecious and Parthenocarpic Line (PPC-2) and Indian Cucumber Variety Pusa Uday**

GS Jat\*, AD Munshi, TK Behera, H Choudhary, C Bharadwaj, P Das and R Amarnath

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi

(\*Email: harshahit2001@yahoo.co.in)

Parthenocarpic gynoecious lines of cucumber have dark green coloured fruits with shorter shelf life which is not preferred by Indian consumers. Study of inheritance and molecular mapping of gynoecious trait is important for transfer of this trait to Indian cucumber lines which have better fruit quality but are monoecious in nature. For this, a  $F_2$  mapping population was developed from the cross PPC-2 (gynoecious and parthenocarpic) X Pusa Uday (monoecious). The  $F_2$  population consisting of 214 individuals segregated as 156 gynoecious plants and 59 monoecious plants suggesting monogenic inheritance of the gynoecious trait (*F*). Out of 312 SSR markers screened for parental polymorphism, seventeen were polymorphic which were used for mapping the *F* locus. The linkage studies identified four co-dominant markers, namely SSR-13251, SSR15955, SSR 21886 and SSR 7248 to be in the same linkage group 6. SSR 13251 was found to be tightly linked to the *F* locus at 1.0 cM and is suitable for MAS of gynoecious trait. The SSR- 15516 marker which was found to be associated with *F* gene at 4.5 cM in another mapping population did not show polymorphism in this population, demonstrating the importance of validation of molecular markers in specific populations.

### **S523: Marker Assisted Introgression of *crtRB1*-Favourable Allele into QPM Version of an Elite Maize Inbred-HKI1128 for Enrichment of Kernel $\beta$ -Carotene**

Rajat Goswami<sup>1\*</sup>, Firoz Hossain<sup>1</sup>, Suphiya Khan<sup>2</sup>, Vignesh Muthusamy<sup>1</sup>, Aanchal Baveja<sup>1</sup>, Rajkumar Zunjare, Konsam Sarika, Nepolean Thirunavukkarasu<sup>1</sup>, Supradip Saha<sup>1</sup> and HS Gupta<sup>1</sup>

<sup>1</sup>ICAR-Indian Agricultural Research Institute, New Delhi; <sup>2</sup>Banasthali Vidyapith, Jaipur, Rajasthan  
(\*Email: rajatgoswami13@gmail.com)

Yellow maize possesses abundant genetic variation for kernel carotenoids, but traditional maize varieties are low in  $\beta$ -carotene (<2  $\mu\text{g/g}$ ). HKI1128, an elite parental inbred with good general combining ability and agronomic performance, was earlier improved for protein quality through marker-assisted selection (MAS) of opaque2 allele. In the present study, a QPM version of HKI1128 with high lysine and tryptophan was targeted for improvement of  $\beta$ -carotene using introgression of  $\beta$ -carotene hydroxylase (*crtRB1*) that enhances  $\beta$ -carotene by 8-10 folds.  $F_1$ s generated between QPM version of HKI1128 and donor germplasm (HP704-22: CIMMYT-HarvestPlus) were genotyped for hybridity, and true heterozygotes were backcrossed to recurrent parent. Individual plants with opaque2 allele in homozygous condition and *crtRB1* in heterozygous state were successfully selected in  $BC_1F_1$ . Segregating progenies of  $BC_2F_1$  and  $BC_2F_2$  were genotyped for *crtRB1* allele. Segregation of *crtRB1* gene deviated from expected ratio of 1:1 in backcross generations. Genome-wide SSRs were used for background selection and the progenies with high recovery of recurrent parent genome were selected. MAS-derived inbreds possess high degree of phenotypic similarity to original inbred for plant, ear and grain characteristics. Improved version of HKI1128 provides ample scope to develop maize hybrids with enhanced lysine, tryptophan and  $\beta$ -carotene.

### **S524: Interspecific Hybridization for YVMV Resistance through Protoplast Fusion in Okra**

Mariya S Zaman\*, Akarsh Parihar, BR Patel, N Subhash, Ghanshyam Patil,  
Dipanki D Patel and RS Fougat

Centre of Excellence on Biotechnology, Department of Agricultural Biotechnology, Anand Agricultural University, Anand 388110, Gujarat (\*Email: mariyazaman232@gmail.com)

Yellow Vein Mosaic Virus (YVMV) of Okra (*Abelmoschus esculentus*) transmitted by whitefly (*Bemisia tabaci*) is the most serious disease of okra. Several wild species of okra show high degree of YVMV resistance and *A. moschatus* subsp. *tuberosus* reported to be one of them, but it is not cross compatible with cultivated species through hand pollination. Therefore, to introgress resistant genes from wild species to cultivated ones, especially, where the problem of cross incompatibility exists, intervention of *in vitro* techniques like somatic hybridization/embryo rescue is required. Thus, an attempt for protoplast fusion has been made to isolate, characterize, fuse and select hybrid cells, followed by their *in vitro* regeneration into whole plants. By enzymatic method, viable protoplasts were isolated from four cultivated (GO2, GAO5, Pusa Sawani, and Parbhani Kranti) and one wild (*A. moschatus* sub sp. *tuberosus*) species. Successive fusions were made between all four cultivars with one wild species using PEG (Polyethylene Glycol) as fusogen. The development of callus from the fused protoplast was obtained through NAA and BA growth regulator supplements which will be further subjected for development of plantlet through induction of shooting and rooting.



## S525: Transfer of HMW Glutenin Subunits from *Aegilops kotschy* to Wheat through Pollen Irradiation Hybridization

Singh Jasmeet<sup>1</sup>, Sheikh Imran<sup>1</sup>, Sharma Prachi<sup>1</sup>, Kaur Rajdeep<sup>1</sup>, Vyas Pritesh<sup>1</sup>, Kumar Sundip<sup>2</sup> and Dhaliwal HS<sup>1\*</sup>

<sup>1</sup>Eternal University, Sirmour, Himachal Pradesh; <sup>2</sup>GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (\*Email: hsdhaliwal07@gmail.com)

Bread wheat (*Triticum aestivum* L.) is the major staple cereal for most of the world population which is being consumed as bread, biscuits and chapattis. Variation in high molecular weight (HMW) glutenin protein composition is associated with dough elasticity contributing to 47-60% of the bread making quality of wheat flour. HMW Glutenin subunits are controlled by Glu-A1, Glu-B1 and Glu-D1 loci present on the long arms of the wheat chromosomes 1A, 1B and 1D respectively. Each of the Glu locus encodes for x and y glutenin subunits by two tightly linked loci Glu-1x and Glu-1y. Some of the *Aegilops* species possess higher molecular weight x and y glutenin subunits than bread wheat cultivars. In the present study, a wheat-*Aegilops* substitution line 1U/1S of *Aegilops kotschy* was used for fine transfer of HMWG subunits of 1U/1S to wheat through pollen irradiation hybridization. The transfer of HMWG subunits was characterized by SDS-PAGE. Micro SDS-sedimentation (MST) test results revealed that the derivatives with addition of HMWG subunits of 1U/1S and substitution of 1B/1D had double MST values as compared to the wheat cultivar PBW343. GISH is being done to confirm the precise transfer. Analysis of micronutrients and total protein content is in progress.

## S526: Marker Assisted Pyramiding of *Opaque2* and *Opaque16* Alleles for the Enhancement of Lysine and Tryptophan in Maize Endosperm

Konsam Sarika\*, Firoz Hossain, Vignesh Muthusamy, Rajkumar Zunjare, Aanchal Baveja, Nepolean Thirunavukkarasu, Rajat Goswami and HS Gupta

ICAR-Indian Agricultural Research Institute, New Delhi (\*Email: konsams@gmail.com)

Quality protein maize by virtue of recessive opaque2 (o2) allele possesses nearly two-fold lysine and tryptophan compared to normal maize. A recessive opaque16 (o16) allele in combination with o2 increases lysine by as high as 60%, over o2o2 genotype. In the present study, three parental inbred lines (HKI161, HKI193-1, and HKI193-2) of two hybrids, HQPM-4 and HQPM-7 were targeted for the improvement by pyramiding o2 and o16 genes. The F<sub>1</sub>s, generated from CML161 (o2o2) and CML193 (o2o2) with QCL3024 (Chinese germplasm) possessing o16 gene were used as donor parents. F<sub>1</sub>s were further backcrossed to recurrent parent HKI161, HKI193-1 and HKI193-2. Desirable plants with o2o2 and O16o16 in BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub> were successfully selected using foreground selection for SSRs associated with o2 (umc1066) and o16 (umc1149 and umc1141). Background selection among the desirable progenies was carried out using ~90 SSRs. Based on background selection and morphological characteristics for plant and ear types, desirable segregants were further advanced. Progenies (o2o2) having endosperm opaqueness akin to respective recurrent parents, were selected through light-box test. Improved versions of HKI161, HKI193-1, and HKI193-2 with o2o2/o16o16 would help in developing nutritionally improved QPM hybrids.



### **S527: Improvement through Biotechnology in Soybean (*Glycine max* (L.) Merrill)**

*Dinesh Kumar Thakur\*, Santosh Kumar Sinha, Kiran Tigga and Tulesh Kumar Gendley*

Department of Genetics & Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raj Mohini Devi  
College of Agriculture & Research Station, Ajirma, Ambikapur (Chhattisgarh) 497001  
(\*Email: dineshthakur.in@gmail.com)

Conventional breeding strategies have been very successful in improving soybean productivity and quality. Today, the molecular based plant breeding techniques are assuming an increasingly more important role in genetic improvement of soybean germplasm. Several emerging technologies, such as molecular characterization and genetic transformation, are already being used extensively for the purpose of plant improvement. Transfer of desirable gene across the sexual barrier using DNA marker technology and application of marker assisted selection for identification of QTL for yield and yield contributing traits may open up avenues for further soybean improvement programme. Other emerging sciences, including genomics and proteomics, are also starting to impact plant improvement. Tools provided by biotechnology will not only replace classical breeding methods, but rather will help provide new discoveries and contribute to improved nutritional value and yield enhancement through greater resistance to disease, herbicides and abiotic factors. In soybeans, biotechnology has and will continue to play a valuable role in public and private sector for soybean breeding programs. So, conventional breeding strategies have priority, and in combination with biotechnology (molecular technologies) have provided the possibility of broadening genetic variability of cultivated soybean and of creating new economically important genotype that is better adapted to new market, production and environment demands.

### **S528: Estimation of Withanolide-A in Roots of Diverse Genotypes of Ashwagandha (*Withania somnifera*)**

*Surya Chauhan, Arunabh Joshi, G Rajamani and Devendra Jain\**

Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur 313 001, Rajasthan  
(\*Email: devroshan@gmail.com)

*Withania somnifera* (Family: Solanaceae) is commonly known as Ashwagandha and is used in traditional system of medicine since long time. Historically, the plant has been used as an aphrodisiac, liver tonic, anti-inflammatory bronchitis, asthma, ulcers, emaciation, insomnia and senile dementia. Clinical trials and animal research support the use of Ashwagandha for alleviation of anxiety, cognitive and neurological disorders and inflammation. Ashwagandha roots are used in the preparation of many Ayurvedic medicines. Many compounds have been reported from the root part which mainly consists of Withanolides, steroidal lactones. In present study, the roots of 25 diverse Ashwagandha genotypes were collected and morphological parameters such as root length, root diameter and dry root yield were recorded. Total alkaloid, crude fiber and starch content were also estimated from the dried roots of Ashwagandha genotypes. Methanol soluble root extract of the plant was subjected to TLC and HPLC analysis. Thin layer chromatography (TLC) showed the presence of withanolide-A in dried roots. Quantification through reverse-phase HPLC revealed varied concentrations of withanolide-A in different germplasm grown under field conditions. Accumulation of withanolide-A was reported highest in the genotype UWS-59, which makes this genotype superior for medicinal use.



## **S529: Genomic Resource Creation for Genetic Diversity Characterization and Implementation of Marker Assisted Selection in *Stevia rebaudiana* Bertoni**

*Aparna Shree Raina, Navgeet Paul and Ram Kumar Sharma\**

Biotechnology Division, CSIR - Institute of Himalayan Bioresource Technology, Post Box 6, Palampur 176061, Himachal Pradesh (\*Email: rksharma.ihbt@yahoo.com)

*Stevia rebaudiana* Bertoni is a therapeutically important crop species which although native to Paraguay is recently gaining hold in the international market. It is widely known for its zero calorie sweetener property and also known for its antioxidant activity, anti-inflammatory and immunomodulatory activity, a blood glucose-lowering effect and renal protective properties. Despite its multifarious importance, a comprehensive genomic resource is yet unavailable. Introduction of various Next Generation Sequencing (NGS) platforms have greatly facilitated global transcriptome sequencing for identification of key pathway gene(s) and genome wide markers in non model organisms. In the current study, genome wide SNP and SSR markers were ascertained by special transcriptome sequencing of five diverse genotypes of *S. rebaudiana*, besides identification of important gene(s) of steviol glycoside pathway. In total, 206162421 high quality reads were generated which were assembled into 48248 contigs (42.03 Mb) with an average length of 873 bp. Using this data, 102688 high quality SNPs and 1215 microsatellite markers were ascertained. Annotations with KEGG, GO and EC assigned known function to more than 50% of the markers. Conclusively, functionally relevant marker resource created here can futuristically be used for diversity characterization and selection of superior genotypes for mass cultivation besides genome wide association studies in this crop.

## **S530: Genetic diversity analysis and identification of species specific marker in *Elaeis oleifera* – the American oil palm**

*Mandal PK<sup>1,2\*</sup>, Jayanthi M<sup>1,3</sup>, Laha S<sup>1</sup>, Sujatha G<sup>1</sup>, and Pillai RSN<sup>1</sup>*

<sup>1</sup>ICAR-Indian Institute of Oil Palm Research, Pedavegi, West Godavari District 534450; <sup>2</sup>Present Address: ICAR-NRC on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>3</sup>Present address: Division of Nematology, ICAR-IARI, Pusa Campus, New Delhi 110012 (\*Email: pranabkumarmandal@gmail.com)

*Elaeis oleifera*, commonly known as the American oil palm, is preferred for its oil quality but is not cultivated on a large scale due to its low yield. However, it is used in breeding programmes to transfer oil quality traits to *Elaeis guineensis*, the widely cultivated species. At the IIOPR regional centre at Palode, Kerala, collection of 23 palms belonging to *E. oleifera* are available which were subjected to genetic diversity studies using 54 random and 19 microsatellite (SSR) primers. SSR primers were designed from the reported microsatellite sequences in NCBI. A total of 276 loci with an average of 5.11 band per primer were obtained for random primers. We obtained 37 alleles for 19 SSRs. In total, 313 bands were obtained for the analysis of genetic diversity. Random primers formed two major clusters, one with 18 palms and other with two palms. Three pairs of palms showed maximum similarity (0.895). Palm No. 16 was genetically distant from most of the palms, with least similarity value of 0.699 (Eo-01). This study could be used for *E. oleifera* improvement breeding programme involving the genetically diverse palms. We also found some species specific markers (2 random and 3 SSRs), which could be used in the identification of the two species and interspecific hybrids at seedling stage.



### **S531: Shoot Tip Grafting (STG), a Bio-Safe Technique in Commercial Production of Disease Free Planting Material and in Establishment of Healthy Diverse Exotic Citrus Germplasm Repository: Present Status**

Vijayakumari N

ICAR-National Research Centre for Citrus, Nagpur (Email: vasu91098@yahoo.co.in)

Citrus orchards suffer from severe losses due to virus and virus like diseases, particularly tristeza and greening bacterium which reduce the average life span of citrus orchards to less than 10 years in tropical countries. A key measure for preventing such losses is the elimination of the pathogens in propagative bud wood and producing certified elite and healthy planting stock. In vitro shoot tip grafting (STG) has been proved effective in elimination of virus and virus like diseases and in establishment of healthy citrus orchards worldwide. The National Research Centre for Citrus embarked in the complete technical knowhow, for the large scale production and release of certified disease free planting material through STG in *Citrus reticulata* cv. Nagpur mandarin to meet the enormous demand of quality planting stock. Through successful transfer of STG technology from lab to land so far 3.15 lakh of certified quality bud grafts of Nagpur mandarin were produced and distributed to citrus growers/nursery which facilitated, establishment of more than 800 hectares of healthy citrus orchards in farmer fields of central India. Through the STG based Citrus quarantine program, 21 exotic citrus cultivars introduced from U.S.A., France, and Australia, and maintained in the citrus repository, were cleaned as post entry quarantine check. Out of 21 exotic cultivars, 16 micro grafted cultivars were tested and 14 exotic cultivars were declared free from major graft transmissible pathogens and recommended for field evaluation.

### **S532: Development of Microsatellite Marker Resources for Genetic Characterization of Economically Important *Dendrocalamus latiflorus***

Abhishek Bhandawat, Gagandeep Singh and Ram Kumar Sharma\*

Biotechnology Division, CSIR - Institute of Himalayan Bioresource Technology, Post Box 6, Palampur 176061, Himachal Pradesh (\*Email: rksharma.ihbt@yahoo.com)

Bamboo belongs to family Poaceae, and is an essential bioresource with high economic and ecological importance. It comprises of nearly 1200 species worldwide. India is a major biodiversity hotspot for bamboos with about 130 species representing 18 genera. *Dendrocalamus latiflorus* is a clumping bamboo with great importance in Asia. Juvenile shoots have remarkable taste, rich in nutrients, fiber, phytosterols and phenolics. They possess anti-oxidant, anti-microbial, anti-ageing properties which imparts them diverse medicinal properties. Despite the huge economic importance, *D. latiflorus* has poor genomic, especially, molecular marker resources. We utilized public transcriptome and expressed sequence databases for massive development of genic microsatellite (SSR) markers in *D. latiflorus*. In total, 12,118 primer pairs were predicted, of which, 85 were successfully validated for genetic characterization and establishing phylogenetic relationship in selected bamboo species. Additionally, 2,189 functionally relevant SSR markers, with presence of SSRs track in protein coding genes, which might have implications on various physiological and developmental processes, were identified. In conclusion, SSR marker resource developed in this study can be efficiently utilized in genetic diversity studies, conservation and management, and selection of genotypes with better nutritional and therapeutic potential.



### S533: Mapping of Panicle Blast Resistance Genes in Rice

Amolkumar U. Solanke<sup>1\*</sup>, Vishesh Kumar<sup>1</sup>, Vivek K Singh<sup>1</sup>, R Rathour<sup>2</sup> and TR Sharma<sup>1</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi; <sup>2</sup>CSK HP Agricultural University, Palampur 176062 (\*Email: Amol.Solanke@icar.gov.in)

Rice blast caused by *Magnaporthe oryzae* is the most devastating disease affecting rice crop. Panicle/neck blast is a severe form of this disease as it causes direct yield loss. Here we report mapping and cloning of genes associated with panicle blast resistance from rice using a RIL population generated from Tetep and HP2216. An inbred line namely, RIL4, from this population was found to be tolerant to both leaf and panicle blast. We genotyped RIL4 along with its parents using in-house designed 50K SNP array. On the basis of distribution of SNPs, contribution of each parent in RIL4 was identified for each chromosome. This line was further crossed with HP2216 to get insight into panicle blast resistant genes. Phenotypic analysis of F<sub>2</sub> population showed 15:1 ratio for leaf as well as panicle blast resistance at Palampur, HP, suggesting duplicate gene action. To identify genes involved in panicle blast resistance, two F<sub>2</sub> derived F<sub>3</sub> progenies with 92 plants each were chosen for genotyping. Pita and Pi54 gene specific primers were used for genotyping in order to identify their role in imparting tolerance to panicle blast. This analysis will help to identify known/new gene(s) associated with panicle blast tolerance in rice.

### S534: Development and Use of N and P Use Efficiency (N/PUE) Gene Specific SSR and ILP Markers in Wheat (*Triticum aestivum*)

Anuj Kumar<sup>\*</sup>, Supriya Kumari, Gautam Saripalli, Tinku Goutam, HS Balyan and PK Gupta

Department of Genetics & Plant Breeding, Ch. Charan Singh University, Meerut 250004  
(\*Email: anujbioinfo91@gmail.com)

It would be desirable to develop wheat varieties that give high yield with low fertilizer (N and P) inputs in wheat (*T. aestivum* L.) which is a major staple food crop, so as to avoid environmental pollution and to reduce cost of production. For this purpose, we developed 17 SSRs and 31 ILP markers using sequences of 10 NUE and 6 PUE related wheat genes/orthologs (identified by us). These markers are perfect markers for use in marker assisted selection (MAS) and are genome specific for each of the three sub-genomes (A, B and D). SSR primers for the six NUE related wheat orthologs (TaNRT2.4, TaNRT2.6, TaAMT1-1, TaGdh, TaGS1;1, and TaANR1) were synthesized and used for polymorphism survey among 21 wheat genotypes, which are the parents of different mapping populations. Only one functional SSR marker for the gene TaANR1 showed polymorphism between the two parental genotypes (C306 and HUW468) of one RIL mapping population. This is being utilized by us for mapping QTLs for NUE related traits in wheat. The detailed results will be presented and discussed.

### **S535: Validation of Molecular Markers Linked to Glucosinolate Genes/QTLs in a RIL Population of *Brassica juncea***

Pushpa HD, D K Yadava\*, Sujata Vasudev, Naveen Singh, Navinder Saini and KV Prabhu

ICAR-Indian Agricultural Research Institute, New Delhi (\*Email: dkygenet@gmail.com)

Complex inheritance of glucosinolates restricts breeders to breed for low glucosinolates genotypes in Brassicas. During past two decades, many studies have reported molecular markers linked to genes/QTLs for low glucosinolates in Brassicas, however, there is limited information on validation and use of these markers. Objective of this study was to confirm previously reported QTLs for glucosinolate trait in a recombinant inbred line (RIL) population of a cross between Pusa Mustard-21 (low erucic acid) and EC-597325 (double low) genotypes of *B. juncea*. To utilize the available information through MAS, six sets of already reported markers closely linked to low glucosinolates QTLs, spread across 'A' genome (A2, A3 and A9) were employed. Four of these markers revealed polymorphism between high and low glucosinolate parents i.e. GER 1 amplified 650bp and of 950bp, GER 5 amplified 310 bp and 350bp, At5gAJ67 amplified 500bp and 450bp and Myb28 amplified 900bp and 920bp fragments in EC597325 and Pusa Mustard-21, respectively. These four markers were utilised in RIL population consisting of 624 plants. Marker and trait (glucosinolate content) association was tested for goodness of fit using  $\chi^2$  test. GER1 and GER5 had higher phenotypic variance ( $R^2$  value) compared to the others. This study demonstrates the strength of MAS for low glucosinolate trait.

### **S536: Introgression of Diverse Traits to Improve Drought Adaption in Rice by Marker Assisted Multi-Parent Backcross Breeding Strategy**

Prathibha MD<sup>1</sup>, Mohan Kumar MV<sup>1</sup>, Mallikarjun NM<sup>2</sup>, Raju BR<sup>1</sup>, Sumanth Kumar K<sup>1</sup>, Rajanna MP<sup>3</sup>, Udayakumar M<sup>1</sup> and Sheshshayee MS<sup>1</sup>

<sup>1</sup>Department of Crop Physiology, UAS, GKVK, Bangalore 560065; <sup>2</sup>Department of Genetics and Plant breeding, UAS, GKVK, Bangalore 560065; <sup>3</sup>ZARS, VC Farm, Mandya 571401

(\*Email: msshesh1@uasbanglore.edu.in)

Saving water and improving yield potentials are the most important challenges of rice improvement programs. Global opinion towards improving productivity under water limited conditions strongly favours a trait based strategy over selection for absolute yield under stress. In this context, mining water associated with roots, efficient use of water and Cellular Level Tolerance (CLT) have greatest relevance. In an earlier investigation, a panel of germplasm was characterised for molecular and phenotypic diversity leading to the discovery of markers by Association Mapping and trait donor genotypes (AC-39020 for roots and CLT, IET-16348 for WUE). Marker Assisted Backcross Breeding (MABB) was initiated to introgress traits into IR-64. Foreground selection was done with 20 SSR markers associated with specific traits and 120 genome wide SSRs for background selection. A total of 10,000 DCBC<sub>3</sub>F<sub>2</sub> lines have been generated and a set of 1500 lines were screened under aerobic field conditions. The best lines (250) were advanced to DCBC<sub>3</sub>F<sub>3</sub> and phenotyped for root traits and  $\Delta 13C$ . This study significantly contributed for the development of trait introgressed lines that can be used for commercial cultivation. Several QTL-NILs that have great potential in academic understanding of QTL interactions have been developed.



### **S537: Development of PCR Based Marker for Detection of Stripe Rust of Wheat Caused by *Puccinia striiformis tritici***

Sangeeta Gupta, Sapna Sharma, Deepika Kulshrestha, Bishn M Bashyal, VK Singh and Rashmi Aggarwal\*

Division of Plant Pathology, ICAR-IARI, New Delhi (\*Email: teenaiari1@gmail.com)

A quick and reliable PCR-based diagnostic assay has been developed to detect *Puccinia striiformis* f. sp. *tritici*, the causal organism of stripe rust, the most important disease in wheat. Three set of primers designed for  $\beta$ -tubulin, 6-squalene monooxygenase and 8- ketopantoate reductase genes using whole genome of *P. striiformis* amplified 239, 1518 and 358 bp long fragments respectively in pathotypes (78S84 and 46S119). A fragment of 1518 bp unique to *P. striiformis* races distinguished it clearly from other *Puccinia* spp. races and other fungal isolates. Since the detection limit of marker in conventional PCR assay is 10pg, qPCR based assay was developed to enhance the sensitivity and utility of marker. This assay amplified a product of 302 bp and could detect as little as 1fg of DNA template and can diagnose disease before symptom expression. The PCR-assisted identification of *P. striiformis* may be performed as soon as there is sufficient fungal material in the infected leaves (2 days after inoculation) without the need to wait for the formation of urediospores (12–15 days). The universality of the marker was tested by detecting the presence of the pathogen on wheat leaves at different time intervals and also from wheat leaves collected randomly from wheat fields. This method provides a rapid and reliable tool for efficient detection and monitoring of *P. striiformis* and helps in identifying and quantifying the level of resistance in wheat germplasm.

### **S538: Evolutionary Significance, Genetic Structure and Linkage Disequilibrium Pattern in a Diverse Set of Rice Germplasm**

Vinod Kumar<sup>1</sup>, Kapil K Tiwari<sup>1</sup>, Swarup K Parida<sup>1</sup>, Anshuman Singh<sup>1</sup>, Sourabh Jain<sup>1</sup>, Nagendra K Singh<sup>1</sup>, Trilochan Mohapatra<sup>1,2\*</sup> and SV Amitha CR Mithra<sup>1</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, ICAR, New Delhi-110012, India; <sup>2</sup>ICAR-Central Rice Research Institute, Cuttack, Odisha 753006 (\*Email:tmnrpcb@gmail.com)

Phylogenetic analysis, population structure and linkage disequilibrium (LD) studies are predominantly used to decipher evolutionary relationships between individuals and genome-wide association studies. Here we present the genetic architecture of rice as deduced from a set of 1476 rice accessions which included cultivated Asian rice (*Oryza sativa*) as well some of the wild accessions of *O. rufipogon* and *O. nivara* species. The array used for genotyping had 5246 SNPs and the samples were processed in Illumina Infinium platform (Illumina Inc., San Diego, CA). The Infinium data were loaded in GenomeStudio and clusters were refined with GenTrain (>0.7) and GenCall score (>0.3). After excluding monomorphic and SNPs with minor allele frequency < 0.02, 4,668 (~89%) high-quality SNPs were identified with 91% genotyping frequency. Neighbor-joining tree was constructed through TASSEL while principal component analysis plot was created through SNPRelate where both programs classified all the accessions into four distinct groups according to their geographical locations, gene-pool variability and cultivated and wild nature. Average LD even in 100kb genomic region observed was very low (0.28) which might be due to higher number of accessions from different species and reduced chances of high LD haplotype blocks.

### S539: Genetic Diversity in Pearl Millet Inbred Lines Using SSR Markers

Sonali Sangwan<sup>1\*</sup>, Shikha Yashveer<sup>1</sup> and Ramesh Kumar<sup>2</sup>

<sup>1</sup>Department of Molecular Biology, Biotechnology and Bioinformatics; <sup>2</sup>Department of Genetics and Plant Breeding, CCSHAU, Hisar 125004, Haryana (\*Email: sonalisangwan03@gmail.com)

Pearl millet (*Pennisetum glaucum*) is a cereal cultivated in drought prone semi-arid regions of Africa and the Indian subcontinent for food, forage and fodder. It is a principal source of energy, protein, vitamins and minerals for millions of poor people in the regions where it is cultivated. Pearl millet productivity can be increased by growing varieties/hybrids with improved characters for which new allele combinations need to be discovered. All this generate the need for genetic diversity study. PCR based markers like SSRs have been adjudged as more reliable for such studies. A set of thirty SSR markers were used to study diversity in thirty-six pearl millet genotypes. DNA from leaf tissue was isolated using CTAB method. PCR followed by PAGE revealed a good polymorphism among these genotypes. Mean allele per locus and PIC obtained was 10.5 and 0.8, respectively. Similarity coefficient ranged from 0.73 to 0.88 using NTSYS-pc and UPGMA algorithm divided genotypes into nine clusters where four genotypes namely HMS 30B, HTP 92/80, HMS 37B and IPS 98-2 did not fall into any cluster.

### S540: Marker Trait Association and Identification of Putative Candidate Genes for Red Rot Resistance in Sugarcane

Nandita Banerjee, Ram JiLal, Sanjeev Kumar and RK Singh\*

ICAR-Indian Institute of Sugarcane Research, Lucknow 226002 (\*Email: ikrps@yahoo.com)

Red rot caused by *Colletotrichum falcatum* is a serious disease of sugarcane that persistently generates new pathogenic strains, causing the breakdown of resistance in newly released varieties. Hence in order to aid the breeding process, marker trait associations (MTAs) was investigated for resistance to two races of red rot (Cf08 and Cf09), on a panel of 119 sugarcane genotypes using 732 SSR markers. Of the two models used for MTA, general linear model (GLM) identified one marker, (IISR\_198\_170) with Cf08, and five (IISR\_46b\_170, IISR\_148\_200; SCB10\_410, ESTA69\_400, IISR\_137\_240) with the race Cf09 whereas mixed linear model (MLM) was able to reduce the false positives and identify only two MTAs for Cf09 (IISR\_46b\_170, IISR\_137\_240), that were able to explain 14.5%, and 11.7% of the total trait variation, respectively. The EST sequence of IISR\_46b mapped on chromosome 2 when BLASTed on the sorghum genome and the 100 kb region in the vicinity of this marker harboured four candidate genes coding for cyclic nucleotide-gated ion channel, cytochrome P450, lachrymatory-factor synthase and concovalinA-like lectin family proteins that have been reported to have important roles in plant-pathogen interactions. These genes may be putative 'positional candidate genes' for red rot resistance in sugarcane.



## S541: Linkage Mapping and Marker Assisted Selection for Mineral Rich (Iron and Zinc) Rice in Segregating Rice Lines Derived from PAU201 × Palman 579

Naveen Kumar\*, RK Jain and Vijay K Chowdhury

Department of Molecular Biology, Biotechnology and Bioinformatics, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana (\*Email: nimbhal@gmail.com)

Molecular markers provide novel tools for linkage mapping of QTLs of target traits and can greatly enhance the efficacy of breeding programs. To understand the genetic basis and improve mineral (iron and zinc) concentration in rice, F<sub>3</sub>, F<sub>4</sub>, BC<sub>1</sub>F<sub>2</sub> and BC<sub>1</sub>F<sub>3</sub> populations were derived from the cross between high-yielding (PAU201) and iron-rich (Palman 579) indica rice varieties. Large variation for various physio-morphological traits and mineral content was observed in all the populations. Iron and zinc content varied from 0.9- 149.9 and 0-143.1 µg/g respectively. Transgressive segregation for grain iron content was noticed in F<sub>3</sub> population with one of the plants having exceptionally higher iron (746.8µg/g) content. Phenotypic correlation analysis showed positive correlation between grain iron and zinc content in BC<sub>1</sub>F<sub>2</sub> population but not in others. DNA fingerprint of 33 F<sub>4</sub> plants developed using 61 polymorphic SSR markers distributed on the entire genome of rice showed scattering of F<sub>4</sub> population between the two parental genotypes; the population was inclined towards Palman 579. SSR data was used to identify QTLs for grain mineral content and various agronomical traits. A total of 128 alleles were identified in 33 PAU201 × Palman 579 F<sub>4</sub> plants in addition to three new recombinant alleles. Composite interval mapping revealed six QTLs for mineral content (five for Fe and one for Zn) in rice grains on chromosome 5, 6, 7 and 9 and sixteen QTLs for various agronomical traits.

## S542: Genetic Variation for Grain Mineral Contents (Iron And Zinc) in PAU201 × Palman579 F<sub>5</sub> Population in Rice (*Oryza sativa* L.)

Amit Pippal\*, Naveen Kumar, Rajinder K Jain and Virendra K Sikka

Department of Molecular Biology Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar 125004 (\*Email: amitpippal@gmail.com)

Rice (*Oryza sativa* L.), is the staple diet of more than half of the world population. Micronutrient malnutrition, particularly Fe and Zn deficiency affect over three billion peoples worldwide, mostly in developing countries. Micronutrient enrichment of plants/crops with high bioavailable micronutrient content using conventional breeding and genetic engineering approaches is being used to improve the nutritional quality of major crops. In the present study, variability for iron and zinc content in dehusked rice grains of a collection of 288 F<sub>5</sub> rice plants of cross PAU201 × Palman579 rice genotypes was assessed. F<sub>5</sub> population displayed large variation for various physio-morphological traits including plant height, effective number of tillers per plant, panicle length and grain yield per plant. Dehusked rice grain samples harvested from the F<sub>5</sub> population also demonstrated huge variation in iron content (4.7 – 312.1 µg/g, PAU201 – 44.6 ± 0.77 µg/g; Palman579 – 335.0 ± 1.93 µg/g) and zinc content (2.2 – 78.9 µg/g, PAU201 – 19.6 ± 1.29 µg/g; Palman579 – 15.2 ± 1.19 µg/g). However, because of the critical nutritional status of human population, there is an urgent need for development of such rice varieties that would be more nutritious with improved iron and zinc content.



### **5543: Marker-Assisted Selection of Promising Aerobic × Basmati Segregating Lines under Water-Limited Conditions in Rice (*Oryza sativa*)**

Rahul Kumar Meena\* and RK Jain

Department of Molecular Biology, Biotechnology and Bioinformatics, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana (\*Email: rahulbt24@gmail.com)

Water scarcity is a major threat for irrigated rice cultivation. To tackle the problem of water scarcity, research has been directed towards the development of “aerobic rice” varieties that combine the drought-resistant characteristics of upland varieties with the high-yielding traits of lowland varieties. Aerobic rice requires 50% or less of the amount of water used by lowland rice. In the present study, experiments were conducted to evaluate segregating F<sub>3</sub> populations of crosses derived from Pusa1121 (Basmati) × MAS26 (aerobic), MASARB25 (aerobic) × IB370 (Basmati) and MAS25 (aerobic) × IB370 (Basmati) for various physio-morphological and/or root traits and allelic diversity for BADH2 (aroma) gene and microsatellite markers linked to the traits promoting aerobic adaptation. The F<sub>3</sub> population(s) of all the crosses showed enormous variation for plant height, panicle length, effective number of tillers per plant, root length, root thickness, fresh and dry root weight, 1000 grain weight, grain length/breadth ratio and yield per plant. A number of promising F<sub>3</sub> plants have been selected, which had higher grain yield, root length and biomass greater than the parents and Basmati specific allele at BADH2 locus (either in homozygous or heterozygous condition) for further progeny analysis.

### **5544: Improving the Number of Grains per Panicle in Mega Rice Variety Samba Mahsuri by Incorporating a Dominant QTL qGN4-1**

Vijay Kumar Singh<sup>1</sup>, Anshul Sharma<sup>1</sup>, M Nagarajan<sup>2</sup>, Ashok Kumar Singh<sup>2</sup>, Brahma Deo Singh<sup>3</sup> and Nagendra kumar Singh<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012; <sup>2</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi 110012; <sup>3</sup>School of Biotechnology, Banaras Hindu University, Varanasi 221005  
(\*Email: nksingh@nrcpb.org)

Rice is the most important food crop of the world feeding more than half of the human population. Large number of well-filled grains per panicle is an important yield contributing trait in rice. Identification of a major QTL for grain number per panicle on chromosome 4 (qGN4-1) from our laboratory has provided the opportunity to apply marker assisted backcross breeding to develop rice cultivars with high grain number. Here we report, transfer of qGN4-1 into Samba Mahsuri, a fine grained mega variety of South and East India, using marker-assisted backcross breeding. Donors for qGN4-1 were RILs derived from Pusa1266/Pusa Basmati-1, the cross used for the identification of this QTL. The SSR markers NKSSR 04-19, RM3276, HvSSR 04-49 and RM2441 were used as peak and recombinant markers for foreground selection and minimizing linkage drag. Data on BC<sub>2</sub>F<sub>4</sub> plants showed an increase of more than 100 grains per panicle. Phenotypic characteristics such as plant height, flag leaf length and width, panicle length, primary and secondary branches in the panicle showed significant increase, whereas fewer tillers with sturdy stem were found showing typical new plant type. The study was further expanded to develop a series of near isogenic lines with qGN4-1 in twelve mega varieties of rice to validate the stability of this QTL across genetic backgrounds.



### S545: Exploration, Characterization, Evaluation and Genetic Structure of Indian Wild Rice

Balwant Singh<sup>1</sup>, Shefali Mishra<sup>1</sup>, Nisha Singh<sup>1</sup>, Kabita Panda<sup>1</sup>, Bikram Pratap Singh<sup>1</sup>, Ashok Kumar Singh<sup>2</sup>, Vandana Rai<sup>1</sup> and Nagendra Kumar Singh<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012;

<sup>2</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi 110012

(\*Email: nksingh@nrcpb.org)

Modern high yielding rice varieties carry very small fraction of natural genetic variation present in the easily crossable gene pool due to breeding and domestication bottlenecks. To explore the untapped genetic potential of Indian wild rice expeditions were made around diversity hot spots in different eco-geographical regions of India. A set of 418 accessions were collected and studied at morphological and molecular levels using 46 morphological traits describing DUS characteristics and genome specific pSINE1 markers. Genetic diversity was evaluated using HvSSR markers. The mean observed and expected heterozygosity varied from 0.129 to 0.156 and from 0.617 to 0.645, respectively. Majority of genetic variations (68%) resided among individuals while species were separated by only 11% of the genetic variation, as revealed by markers. Population structure was analysed by a panel of 48 genome wide unlinked SNP markers revealing three populations in the Indian wild rice. Genetic structure and migrants per generations' analysis found small to moderate level of hybridization between the populations. The collection is being phenotyped for different biotic and abiotic stresses and promising accessions have been identified for drought, flooding and salinity tolerance which will be used to generate introgressed lines for rice variety improvement.

### S546: Development and Validation of EST- SSR Markers of Isabgol (*Plantago ovata* Forsk) Using Next-Generation Sequencing Approach

Vinay Kumar<sup>1\*</sup>, YM Shukla<sup>2</sup>, RS Fougat<sup>1</sup> and CG Joshi<sup>3</sup>

<sup>1</sup>Department of Agricultural Biotechnology, Anand Agricultural University, Anand (Gujarat);

<sup>2</sup>Department of Biochemistry, Anand Agricultural University, Anand (Gujarat); <sup>3</sup>Department of Animal Biotechnology, Anand Agricultural University, Anand (Gujarat)

(\*Email: vinaybt\_04@rediffmail.com)

Isabgol (*Plantago ovata* Forsk), is a crucial medicinal and industrial crop cultivated in India, generating appreciable foreign exchange. Seed husk of *P. ovata* is known for its pharmaceutical value and is used in medicinal formulations, food and beverages. However there are hardly any genomic resources in isabgol which hinders its improvement. In the present study, transcriptome sequencing of two genotypes of isabgol, EC-124345 (resistant to downy mildew) and Niharika (susceptible to DM) using 454- pyrosequencing chemistry was carried out for development of EST-SSR markers. Denovo assembly of transcriptome produced a total of 8101 and 9745 unigenes from EC 124345 and Niharika genotypes from which 1,440 and 1,824 potential simple sequence repeats were identified, respectively. Trinucleotide repeats were the most abundant (76.7%) followed by di, hexa and pentanucleotide repeats. GAA was the most frequent trinucleotide repeat followed by TGG, TTC and CAG. Thirty-four markers were randomly selected for validation in six genotypes of Isabgol and six allied species of *Plantago*, of which, 28 were successfully amplified and showed expected amplicon size. Eighty-five per cent SSR markers showed cross species amplification. Four SSR markers out of 28 produced polymorphism within *P. ovata* suggesting less variability within the species. The EST-SSR loci identified from the mining of the isabgol transcriptome, could be useful in identification of gene based or linked markers for improvement of isabgol by molecular breeding.

### **S547: Physical Mapping and Sample Sequencing of Chromosome 2AS Specific BACs in Wheat Using SNaPshot Technology**

RS Tomar<sup>1</sup>, Vinod Kumar<sup>1</sup>, Ajay Mahato<sup>1</sup>, Sandeep Kaushik<sup>1</sup>, Bharat Yadav<sup>2</sup>, Naveen Sharma<sup>3</sup>, Chanderkant Chaudhary<sup>3</sup>, IS Yadav<sup>2</sup>, Preeti Sharma<sup>2</sup>, OP Gupta<sup>2</sup>, SV Amitha Mithra<sup>1</sup>, Kishore Gaikwad<sup>1</sup>, TR Sharma<sup>1</sup>, Paramjit Khurana<sup>3</sup>, JP Khurana<sup>3</sup>, Hana Simkova<sup>4</sup>, Jaroslav Dolezel<sup>4</sup>, Jane Rogers<sup>5</sup>, BS Gill<sup>6</sup>, Kuldeep Singh<sup>2</sup> and NK Singh<sup>1</sup>

<sup>1</sup>ICAR- National Research Centre on Plant Biotechnology, LBS Building, IARI campus, New Delhi; <sup>2</sup>Punjab Agricultural University, School of Agricultural Biotechnology, Ludhiana, Punjab; <sup>3</sup>Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi; <sup>4</sup>Institute of Experimental Botany AS CR, Rozvojova, Prague, Czech Republic; <sup>5</sup>Eversole Associates, 18 High Street, Little Eversden, CB23 1HE, Cambridge, UK; <sup>6</sup>Wheat Genetics Resource Center, Kansas State University, Manhattan, USA (\*Email: nksingh@nrcpb.org)

India is a partner to International Wheat Genome Sequencing Consortium with the responsibility of physical mapping and sample sequencing of chromosome 2A. For creating a minimum tiling path for 2AS, 55,648 BACs specific to 2AS were fingerprinted using SNaPshot technique. BAC DNAs were isolated, digested with restriction endonucleases, labelled, washed and subjected to capillary electrophoresis for generating High Information Content Fingerprints (HICF) using DNA Analyzer (ABI 3730xl). High quality fingerprints were processed through GeneMapper 4.1, FPB and finally through Genoprofiler software. FPC (finger print contig) assembly was constructed using LTC software which included 38,692 clones into the assembly of which 29,853 were assembled into 372 contigs with minimum number of clone  $\geq 5$ . In addition, we performed two separate genome assemblies of shotgun sequence reads of chromosome 2AS from sequencing data generated using 454 and Illumina chemistries. Final hybrid contig assembly using CAP3 software produced a total of 544,189 contigs with ~390 Mbp of sequence data with N50 value of 1017bp. A total of 19,382 genes were predicted using FGENESH with an average gene length of 505bp. A total of 46,168 SSRs were mined from this draft genome assembly. A RIL mapping population of C306/WL711 is being used for genetic mapping of SSR and genic SNPs to enrich the genetic map of 2A which will be used to anchor the physical map.

### **S548: Morphological and Agronomical Characterization of Common Wheat Landrace (*Triticum aestivum* L.) Collected from Different Regions of India**

Sonia Goel<sup>1</sup>, H Kumaraswamy<sup>1</sup>, Sapna Grewal<sup>1</sup>, Kalpana Singh<sup>2</sup>, Ranjit Singh<sup>1</sup> and Nagendra Kumar Singh<sup>1\*</sup>

<sup>1</sup>ICAR-National Research Centre for Plant Biotechnology, Pusa campus, New Delhi 110012; <sup>2</sup>Water Technology Centre, ICAR-Indian Agricultural Research Institute, New Delhi 110012 (\*Email: nksingh@nrcpb.org)

Wheat landraces are potentially a useful germplasm resource of genetic variability for traits such as stress tolerance and grain quality characteristics. For thousands of years, wheat landraces have evolved under the influence of natural and artificial selection as performed by many generations of farmers. In the recent years the importance of genetic resources has been increasingly recognized. The objective of this study was to assess the morphological and agronomic characteristics of original germplasm of



common wheat (*Triticum aestivum* L.) maintained in ex situ collection in ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Total of 200 accessions of wheat were planted under field condition and their agro-morphological characters such as plant shape (at tillering), leaf-flag attitude (at the beginning of heading), spike attitude (at full ripeness), spike awnedness, spike color, plant height, ear length and 1000 grain weight were recorded. Principal components analysis (PCA) was carried with plant height, ear length and 1000 grain weight. This approach helped in identification of top ranking wheat genotypes with good grain characteristics which could be conserved and utilized in winter bread wheat breeding programs.

### **S549: Identification of SNPs from Expressed Sequence Tags and Their Effect on Structural and Functional Aspects of Proteins in Pigeonpea**

DP Wankhede\*, J Aravind, Satya P Mishra and Sundeeep Kumar

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110112

(\*Email: dpwankhede@nbpgr.ernet.in)

ESTs are partial sequences of gene transcripts which represents the expressed portion of the genome. Identifying ESTs have often been a first step towards elucidation of complex regulatory network underlying varied biological processes and thus played major role in identifying array of genes involved in biological processes and stress response. ESTs deposited in ESTdb of NCBI constitute a wealth of valuable information on transcripts from different tissues/developmental stage/stress conditions/strains/genotypes. We have used Pigeonpea (*Cajanus cajan*) EST resources to harness sequence variations in the form of Single Nucleotide Polymorphism (SNP) with the aim to study effect of SNP on proteins. To achieve this, coding sequences (CDS) were identified from the genome of Pigeonpea (1,91,705 contigs) using Augustus gene prediction software. ESTs from Pigeonpea (25,576) were downloaded from ESTdb, NCBI. Using CDS sequences as query and ESTs as reference, total 23,317 raw SNPs were identified using MUMmer program. To eliminate erroneous SNPs, stringent filters were applied. After removing possible erroneous and doubtful SNPs, a total of 474 high quality SNPs were selected for identification of non-synonymous SNPs (nsSNP). Sequence carrying nsSNPs included several transcription factors known to be involved in generating responses for biotic and abiotic stresses, genes involved in growth and development, signaling proteins etc. Effects of identified nsSNPs on protein structure, stability, active sites and possible protein-protein interactions are also studied. The study provides a greater insight into biotic and abiotic stress mechanism as well as in growth and development of this important pulse crop.





## **S550: Plant Tissue Culture: An Approach for in vitro Germplasm Conservation**

*Susmita Shukla\* and Taramla Raman*

Amity Institute of Biotechnology, Amity University, Noida, U.P.

(\*Email: shuklasusmita@yahoo.co.in)

Conservation of germplasm is an essential for ensuring and maintaining plant species from extinction by providing the genetic material and resources for breeding, preservation and research. This genetic material can be in the form of seeds or tissues. The exploitation, heavy depreciation and clearing of forest land have limited availability of germplasm and their in situ conservation almost impossible. Ex situ conservation also has many limitations such as availability of land and other natural factors like pest infestation making it undesirable for germplasm conservation. Employing in vitro techniques for germplasm conservation is a more suitable approach and has advantages over in situ and ex situ techniques. It is carried out under aseptic controlled environment which generates disease free stock, high rate of multiplication, minimal storage space requirement, no genetic erosion as genetic fidelity is maintained and lesser labor cost. The most important factor for considering in vitro conservation is the requirement of very less starting material irrespective of their natural environment. The pool of stored material can be revived and used to generate into whole organisms. *Stereospermum suaveolens* and *Stereospermum personatum* are important tree species which are being heavily exploited for their medicinal properties. This leads to uprooting and cutting of the trees resulting in decline of these tree species. These species have been successfully propagated by plant tissue culture technique and successful acclimatization was accomplished.

## **S551: Genetic Variability for Inductive Root Traits under Osmotic Stress in Progenitors of Bread Wheat (*T. aestivum* L.)**

*Pramod Awkale and Monika Dalal\**

ICAR-National Research Centre on Plant Biotechnology, New Delhi

(\*Email: monikadalal@hotmail.com)

Root system is an important drought avoidance mechanism in plants. Maintenance of root growth during water deficit (inductive root growth) can significantly contribute to yield stability by enhancing the ability of plant to extract water and nutrients from deeper layers of soil. The bread wheat (*T. aestivum* L.) is evolved from the natural crossing and polyploidation of three diploid progenitor species namely *T. urartu* (A genome), *Aegilops tauschii* (D genome) and *Ae. speltooides* (S genome, closest to B genome). These diploid wild species are potential resources for various agronomic traits including abiotic stress tolerance. However, these species have not been explored for inductive root traits. Therefore to characterize this untapped genetic resource, seedlings of nine accessions of diploid wheat (three accessions each representing A, B and D genomes) were phenotyped for inductive root growth under two levels of osmotic stress viz. -0.5 (S1) and -1.48 bars (S2) for 10 days. Root traits such as primary and total root length, root diameter and surface area were recorded. Significant genetic variability was observed for root traits among the accessions with different genomes. The accessions with AA genome showed higher root length followed by DD and BB genomes under control as well as osmotic stress conditions.



## S552: Population Structure Analysis of Diverse Germplasm of Finger Millet (*Eleusine coracana*) for Their Utilization and Improvement With Respect to Nutritional Quality Using Genotype by Sequencing (GBS)

Divya Sharma<sup>1</sup>, Apoorv Tiwari<sup>1</sup>, Salej Sood<sup>2</sup>, NK Singh<sup>3</sup>, JP Jaiswal<sup>3</sup> and Anil Kumar<sup>1</sup>

<sup>1</sup>Department of Molecular Biology & Genetic Engineering, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand; <sup>2</sup>ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand; <sup>3</sup>Department of Genetics & Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand

Finger millet is an important food crop grown mainly by subsistence farmers in arid and semi-arid regions of the world. The existence of variation with respect to seed calcium and protein content certainly makes it a target crop of study with an aim to frame biofortification strategies based on breeding approaches by the development of molecular markers, marker-trait studies and eventually selection of superior genotypes with respect to nutritional quality and accumulation of nutrients in finger millet. With an aim to identify molecular markers linked to calcium and protein content, attempts have been made to genotype and characterize a diverse collection of 128 genotypes from India, Nepal, Africa and Germany through Genotype By Sequencing (GBS). It generated a data of 33 GB which resulted in a genome wide set of >23000 Single Nucleotide Polymorphisms (SNPs) segregating across the entire collection and several thousand SNPs segregating amongst different accessions which were used to show pattern of population structure in the finger millet gene pool. The phylogenetic study of all genotypes of finger millet clearly shows that there are three clusters of accessions. In all the three clusters there was a mixture of genotypes from South Asia and East Africa. Among the Indian genotypes in cluster 1, most of the genotypes were from North India. In cluster 2 and 3, there was an equal proportion of genotypes from North India and South India. Overall this data will be a useful resource for breeders to improve nutritional quality.

## S553: Diversity Analysis of Maintainers and Restorers in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) Using SSR Markers

Subhash Chandra<sup>1</sup>, SP Singh<sup>1\*</sup>, Anju M Singh<sup>1</sup>, C Tara Satyavathi<sup>1</sup>, Mukesh Sankar S<sup>1</sup>, Neelu Jain<sup>1</sup>, KV Prabhu<sup>1</sup> and TR Sharma<sup>2</sup>

<sup>1</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>ICAR-National Research Centre on Plant Biotechnology, New Delhi 110012  
(\*Email: sumerpalsingh@yahoo.com)

Analysis of molecular diversity was carried out among 32 genotypes of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Materials comprised of 29 restorers and 3 maintainers. Polymorphism data using 70 SSR primers was generated in these genotypes. A total of 42 primers showed polymorphism and amplified 106 alleles. The number of alleles per SSR marker per locus varied from 2 to 5. Polymorphic information content (PIC) values ranged from 0.117 to 0.841 per locus with an average PIC of 0.348. Dendrogram based on Jaccard's similarity coefficients were generated based on an average linkage algorithm (UPGMA) using molecular data. Genotypes were grouped into four clusters based on molecular data. Clustering patterns based on Jaccard's similarity efficiently discriminated the genotypes and correlated well with their pedigree and origin. The highest similarity index (0.74) was observed between WGI 58 and WGI 148 and the lowest similarity index (0.27) was observed between 841 B and PMMI 269. SSR marker screening also revealed heterozygosity in some of the inbreds. Observed heterozygosity ranged from 0 to 0.56 indicating the greater efforts needed in maintenance breeding of highly cross pollinated crops like pearl millet.



### **S554: Validation of Molecular Markers for Genes Controlling Quality Traits in Bread Wheat (*Triticum aestivum* L.)**

Anjali Rai\*, Poornima Sharma, Sumit Kumar, Swati Chaudhary, Arvind K Ahlawat, and Anju M Singh

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: anjalirules22@gmail.com)

In the present study, molecular markers for genes determining different quality traits and rust resistance genes were validated using a set of genotypes that included Indian wheat varieties, landraces and exotic cultures. Molecular markers for glutenin genes were screened and compared with the SDS-PAGE while those for other genes were confirmed with technological tests for quality. The allele-specific PCR (AS-PCR) primers for the alleles *Glu-A1a*, *Glu-A1b* and *Glu-B1a*, *Glu-A1c*, *Glu-A1u* and *Glu-B1i*, *Glu-D1a* and *Glu-D1d* at *Glu-1* loci and the AS-PCR primers for all the *GluA3* and *Glu-B3* alleles could be appropriately amplified and their corresponding subunits could be identified on the SDS-PAGE gels. The AS-PCR primers for alleles of kernel texture genes (PinD1) could also distinguish between the hard (ab or ba) and the soft (aa) grained genotypes which produced corresponding grain hardness index scores. One of the three markers (RIS) produced amplicon in all the varieties possessing the 1BL/1RS translocation and not in varieties devoid of the translocation. Among the other two primers, from B genome of wheat, while amplification occurred in DNA of all varieties devoid of translocation, a differential result was produced in varieties confirming 1BL/1RS translocation with RIS marker. The possible reasons for differential response of these primers and the bearing of the size of the segment translocated from *Secale cereale* on these results is discussed.

### **S555: Comparison of Indian and Australian Wheat Germplasm for Quality Traits Using Predictive Tests and Molecular Markers**

Arvind K Ahlawat\*, Anjali Rai, Poornima Sharma, Swati Chaudhary, GP Singh and Anju M Singh

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: ahlawatarvind@yahoo.com)

Millers and bakers prefer high milling recovery and baking quality in wheat. The quality of the flour of Indian wheat varieties is not very suitable for producing either bread or biscuits. Since, Australia is a leading exporter of wheat and its wheat flour is known and traded for product-specific qualities, a comparison was made for various predictive quality tests and for the presence of alleles of genes for different quality traits between Indian wheat germplasm including released and popular varieties and the Australian cultivars imported under an Indo-Australian collaborative project. It was found that the Australian varieties grown in the same environment had lower grain- and test- weight as compared to Indian wheat lines. The Australian varieties could be distinguished into two distinct groups based on hardness index and molecular markers as hard or soft while all Indian wheat lines were hard grained. The hard grained lines from Australia also possessed all quality traits required for superior bread making quality and the soft grained lines possessed traits required for biscuit quality. The Indian wheat lines had intermediate sedimentation values, weak but less extensible gluten and low to intermediate protein content in the flour thus categorized as all purpose flour. The alleles coding for HMW-GS and LMW-GS as judged with SDS-PAGE and molecular markers were more randomly distributed in Indian lines while they were of particular type in case of Australian varieties.



## S556: Mapping and Identification of QTLs for Grain Protein Content in a Biparental Bread Wheat Cross

Swati Chaudhary<sup>1</sup>, Arvind K Ahlawat<sup>1</sup>, K Gopalreddy<sup>1,2</sup>, J Jaiswal<sup>3</sup> and Anju M Singh<sup>1\*</sup>

<sup>1</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana 132001; <sup>3</sup>G B Pant University of Ag. and Technology, Pant Nagar, Uttarakhand 263145 (\*Email: anju\_mahendru@yahoo.co.in)

Wheat is a staple source of energy and protein for the world population. Though the protein of wheat is limiting in two essential amino acids, its more consumption fulfil an extent for the reduced content of these amino acids. Moreover, high protein is very important for processing quality especially for bread making. In the present study, grain protein content was determined in a RIL mapping population consisting of 288 lines grown over three locations viz., Delhi, Pant Nagar and Bihar for two consecutive crop seasons (2011-12 and 12-13) and in Delhi in 2013-14. The RILs showed a wide range of grain protein content from 9% to 20% and transgressive segregants were identified. 714 SSR markers were screened of which 189 markers were found polymorphic between the two parents (WH542 and *T. dicoccum*/*T. tauschii*/BCN) of the mapping population. The RILs were screened with polymorphic markers of which 136 segregated in Mendelian fashion. A framework map was created and QTLs for protein content were identified. Further markers screening is being attempted and will help in saturating the map and identifying more closely linked markers to protein QTLs.

## S557: Marker-assisted Breeding for Developing Wheat Variety to Resistance for Bipolaris Leaf Spot (*Bipolaris sorokiniana*)

Sanjay Singh<sup>1\*</sup>, SV Amitha CR Mithra<sup>1</sup>, GP Singh<sup>2</sup>, PK Singh<sup>2</sup>, VK Singh<sup>2</sup>, AK Singh<sup>3</sup> and JC Padaria<sup>1</sup>

<sup>1</sup>Nation Research Centre on Plant biotechnology, Pusa Campus, New Delhi; <sup>2</sup>Indian Agricultural Research Institute, Pusa, New Delhi; <sup>3</sup>National Bureau of Plant Genetics Resource, Pusa Campus, New Delhi (\*Email: sanjay\_singh777@yahoo.com)

Bipolaris leaf spot caused by *Bipolaris sorokiniana* can cause significant yield losses and reduce grain quality of wheat (*Triticum aestivum* L. em. Thell). This is most damaging disease in the warm and humid wheat growing regions of the world such as Eastern India, South East Asia, Latin America, China and Africa. The disease becomes severe during the grain filling stage and causes significant grain yield loss and grain quality deterioration in susceptible varieties. The average loss caused by *B. sorokiniana* in South Asia is reported to be around 17%. The current Project aims at Marker-assisted introgression of genes/QTLs of Bipolaris leaf spot in the exotic donor Chiyra 3 and SW89-5422 and Shangai. Three popular wheat varieties like HD2967, HD2733 and PBW343 which are highly adapted but susceptible to leaf spot and three tolerant varieties namely Chiyra3, SW89-5422 and Shangai are selected for development of tolerant varieties against the leaf spot. Crosses between three susceptible and tolerant varieties were generated and harvested F1 seed of all six crosses. Seven SSR markers linked to QTLs associated with spot blast resistance of wheat have been tested in a set of known spot blast susceptible and resistance wheat genotypes. SSR marker Xgwm148 and Xgwm111 linked to QTL on chromosome 2B generated band of expected size in the resistant genotypes and will be used in marker assisted breeding of spot blotch resistance high yielding wheat varieties.



## **S558: Characterization of Synthetic Amphiploids of Interspecific Hybrid Derived from *O. punctata* and *O. sativa* Cv. PR122**

*Kishor Kumar, Gurpreet Singh, Kumari Neelam and Kuldeep Singh\**

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab

(\*Email: kuldeep35@pau.edu)

*Oryza punctata* (BB,  $2n=24$ ) is a wild species of rice having many useful agronomic traits. An interspecific hybrid (AB,  $2n=24$ ) was produced by crossing *O. punctata* and *O. sativa* cv. PR122 (AA,  $2n=24$ ). This hybrid was completely sterile. To restore the fertility, F1 hybrids were treated with colchicine. Chromosome doubling significantly improves the fertility of synthetic amphiploids (AABB,  $2n=48$ ) by regular pairing of chromosome in meiosis confirmed by analysis of pollen fertility. Synthetic amphiploids were characterized by cytological and flow cytometric tools. Meiotic studies revealed presence of 24 bivalents in synthetic amphiploids. The diploid F1 hybrid showed 28871.37 mean fluorescent value of tissue homogenate with 3.70% CV whereas in synthetic amphiploid, the mean fluorescent value observed was 62843.37 with CV 2.88%. These amphiploids will be further backcross with PR122 to develop a series of addition and substitution lines and can be utilized in breeding program for enhancing genetic diversity among cultivated rice with incorporation of several quality traits and disease resistance genes.

## **S559: Identification of Candidate Gene(s) for the QTL Controlling Spikelet Number per Panicle Transferred from *Oryza longistaminata* to *O. sativa***

*Amanpreet Kaur, Kumari Neelam, Priti Sharma, Inderjit S Yadav and Kuldeep Singh\**

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab

(\*Email: kuldeep35@pau.edu)

Rice is a source of calories for more than half of the world population and varieties with higher yield potential and greater stability are required to feed the ever-expanding population. Wild germplasm of rice constitutes a reservoir of agronomically important genes which can be exploited to develop high yielding cultivars. This study utilized  $BC_3F_6$  progenies derived from a cross between *O. sativa* cv PR114 and *O. longistaminata* acc IRGC 104301B to fine map a major QTL controlling spikelet number per panicle to a 167.1 kb region bracketed by SSR markers RM13743 and RM13750. Twenty three genes predicted by Rice Genome annotation project (RGAP) database (<http://rice.plantbiology.msu.edu/>) were identified in the QTL region. Among these, three genes viz. LOC\_Os02g44860, LOC\_Os02g44990 and LOC\_Os02g45010 were selected for sequencing on the basis of their functional annotation. Sequence comparison of these genes showed nucleotide variations between the parental lines. A six base pair deletion was observed in the 5'UTR region in PR114 for LOC\_Os02g45010. Functional variations were observed for LOC\_Os02g44990 between the parents. Thus, these two genes are identified as putative candidate genes for the spikelet number QTL.



### **S560: Marker Assisted Transfer of Spikelet Number QTL qSPP2.2 from *Oryza longistaminata* Derived Introgression Line to Punjab Basmati 3**

Sadhan Debnath, Amanpreet Kaur, Kumari Neelam, Dharminder Bhatia and Kuldeep Singh\*

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab

(\*Email: kuldeep35@pau.edu)

Basmati, the unique aromatic quality rice is a nature's gift to Indian sub-continent. Punjab Basmati 3 (PB3) an improved version of Basmati 386 is a photoperiod sensitive, semi-dwarf lodging tolerant variety which is about 105 cm tall. It is the first Basmati variety to be resistant to bacterial blight and also resistant to all the ten presently prevalent pathotypes of bacterial blight in Punjab. It possesses extra-long slender and translucent grains with strong aroma, good cooking and eating quality characteristics. Though 1000 grain weight of PB3 (approx. 28 gm) is comparatively more than Indica varieties, it is low yielding (avg. 16 Q/ acre) which is primarily due to less number of grains (70 to 80) per panicle. The present study, therefore, is planned to precisely transfer the spikelet number QTL qSPP2.2 from *Oryza longistaminata* derived introgression line into PB3. More than 1000 BC1F1 plants were planted which will be analysed with the polymorphic SSR markers linked to QTL qSPP2.2. The plants positive for the target markers will be analysed with DNA markers linked to aroma, waxy locus, bacterial blight resistance genes *xa13* and *Xa21* for background selection. About 400 F<sub>2</sub> plants were planted as two replications on which data will be recorded for all the yield component traits including plant height (cm), days to flowering, numbers of tillers per plant, number of spikelets per panicle, filled grains per panicle, grain length (mm), grain width (mm), L/B ratio etc.

### **S561: Molecular Mapping and Simultaneous Transfer of Bacterial Blight Resistance Gene(s) from *Oryza rufipogon* to Cultivated Rice**

Kaur Sarabjeet<sup>1</sup>, Kumari Neelam<sup>1</sup>, Lore Jagjeet<sup>2</sup> and Kuldeep Singh<sup>1\*</sup>

<sup>1</sup>School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab;

<sup>2</sup>Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab

(\*Email: kuldeep35@pau.edu)

Rice is a source of calories for more than half of the world population and varieties with higher yield potential and stability are required to feed the ever-expanding population. The biotic stresses like pathogens, insects, pests etc limit the worldwide production of rice. Among these, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is responsible for 30-50% yield losses. There are ten pathotypes of *Xoo* prevalent in Punjab and is continuously evolving. This emphasizes the need to have novel source of resistance against *Xoo* pathotype. The present study focuses on mapping and transfer of novel Bacterial blight resistance gene/(s) from *Oryza rufipogon* acc. IRGC93216 to cultivated rice PR114. F<sub>2</sub> population derived from the cross between *Oryza rufipogon* and PR114 was used as mapping population. F<sub>2</sub> plants were inoculated with the *Xoo* pathotype tar 950 and data was recorded. The total of 180 SSR markers from the Universal Core Genetic map of rice were used to carry out parental polymorphism and 90 markers were found polymorphic. These are being applied to the 300 F<sub>2</sub> plants. The process of genetic mapping of bacterial blight gene is continued and simultaneous backcrossing is being done to transfer the disease to the cultivated rice.

## S562: Towards Physical Mapping of an Adult Plant Stripe Rust Resistance Gene from *Triticum monococcum*

Bawa Preeni<sup>1</sup>, Yadav Bharat<sup>1</sup>, Sharma Priti<sup>2</sup>, Inderjeet Singh Yadav<sup>1</sup>, Chhuneja Parveen<sup>1</sup>, Kaur Satinder<sup>1</sup> and Singh Kuldeep<sup>1\*</sup>

<sup>1</sup>School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab;

<sup>2</sup>Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab (\*Email: kuldeep35@pau.edu)

Stripe rust (*Puccinia striiformis* f. sp. *tritici*) is one of the major devastating disease which causes large reduction in wheat yield. Wild species are the most important reservoir for many agronomic traits. *Triticum monococcum* (Tm) and *Triticum boeoticum* (Tb) are diploid species having A genome. At PAU, a BAC library is available for physical mapping of 2AL chromosome (IWGSC, 2010). Tm and Tb are having Adult Plant Stripe rust resistance. A QTL for stripe rust is mapped previously at PAU, on chromosome 2A at 3.6 cM interval between Xwmc407 and Xwmc170. Phenotyping of Tb/Tm RIL population for stripe rust resistance against 46S119 and 78S84 was done. NBS-LRR related genes were identified in 2AL using Next Generation Sequencing data. Functional annotation of the domains was done to identify unique domains. Sequences containing unique NBS-LRR domains were BLAST with BAC end sequences data of 2AL. Linear Topological Contig #849 containing maximum number of domains was selected and four BAC clones were identified. After sequencing of these BACs, primers from SSRs were designed which are being applied on RIL population to identify the physical distance of the stripe rust resistance gene on 2AL, the gene can further be cloned and be used in Marker Assisted Selection.

## S563: Identification of High Sucrose Saccharum Genotypes Using Sequence Tagged Microsatellite Markers

Amrinder Singh Brar<sup>1\*</sup>, Surinder K Sandhu<sup>2</sup> and D Bhatia<sup>2</sup>

<sup>1</sup>School of Agricultural Biotechnology, Punjab Agricultural University; <sup>2</sup>Department of Plant Breeding and Genetics, Punjab Agricultural University (\*Email: amrinderbrar-coasab@pau.edu)

Sugarcane (*Saccharum* spp. complex) is an important agricultural cash crop in India. Owing to the bottlenecks like polyploidy, high heterozygosity, long duration, slow rate of multiplication, narrow genetic pool and above all, non-flowering in sub-tropics, this makes sugarcane improvement through conventional breeding an arduous task. Since sugarcane is valued both for cane and sugar yield, the rigorous screening for high sucrose content is an integral part of a breeding process. Molecular marker that can differentiate sugarcane genotypes for sucrose content can certainly fasten the breeding process in sugarcane. A germplasm assemblage of 48 sugarcane genotypes comprising interspecific introgression lines advanced breeding lines, commercial varieties, *S. spontaneum* and *S. barberi* clones were field evaluated for sucrose content at regular intervals. Genotypes were screened using 30 STMS primers including six STMS markers previously found to be annotated with sucrose content. Out of six, primer NSK 45 amplified a fragment of about 180bp length, reported for association with high sucrose content, was validated in high sucrose genotypes viz., CoC 671, CoJ 88 and CoPb 08212. Similarly a primer NSK 46 was amplified in SES 594, ISH-29 and ISH-108, having low sucrose content.



### **S564: Allelic Diversity for Salt Stress Responsive Candidate Genes among Indian Rice Landraces**

*Deepika Singh<sup>1</sup>, Balwant Singh<sup>1</sup>, Shefali Mishra<sup>1</sup>, Ashok Kumar Singh<sup>2</sup>, Tilak Raj Sharma<sup>1</sup> and Nagendra Kumar Singh<sup>1\*</sup>*

<sup>1</sup>NRC on Plant Biotechnology, IARI, New Delhi 11 0012; <sup>2</sup>Division of Genetics, IARI, New Delhi 11 0012 (\*Email: nksingh4@gmail.com)

Salt stress adversely affects growth and productivity of crops. Rice, one of the most important food crops has contributed to Green Revolution with the development of semi-dwarf high yielding varieties but most of these are rated salt-sensitive. Landraces of rice are known to possess greater genetic variation for the trait and hence are being exploited for enhancing salt tolerance in the high yielding varieties. We analyzed, allelic diversity in 13 candidate genes for salt tolerance by targeted re-sequencing in sixty-nine diverse land races and varieties of rice. Multiple sequence alignment revealed total 43 SNPs. Nucleotide diversity ranged from 0.00007 to 0.01430. Number of haplotypes varied from zero to nine whereas haplotype diversity ranged from 0 to 0.350. The *SOS1* and *SKC1* genes revealed the highest allelic variability among the tested genotypes. This haplotype variability may be used for trait-association analysis to identify useful functional markers for enhancing salt stress tolerance, through molecular breeding.

### **S565: Evaluation of Elite Rice Accessions for Genetic Variability and Identification of Associated Markers for Iron and Zinc Content Under Aerobic Condition**

*Rakesh Kumar Prajapat<sup>1\*</sup>, Namrata Pawar<sup>1</sup>, Deepak Pawar<sup>2</sup>, HE Shashidhar<sup>1</sup> and HV Vijayakumar Swamy<sup>1</sup>*

<sup>1</sup>Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, Karnataka; <sup>2</sup>NRCPB, IARI, New Delhi (\*E-mail:rpk123000@gmail.com)

More than half of the world's population, especially women and children in the developing countries, suffer from micronutrient deficiency or 'hidden hunger' resulting from the consumption of meagre amount of bioavailable vitamins and minerals containing diet. Rice being the major staple food crop of the world, increasing nutrient contents of rice will enhance nutrition uptake of global population. The objective of the present study was to evaluate genetic variability parameters, and discern correlation for Iron (Fe) and Zinc (Zn) content in grains of RPHP rice accessions grown in Kharif-2012 under aerobic condition. The study showed that grain yield per plant, culm biomass and iron content showed higher genotypic as well as phenotypic co-efficient of variation. Significant positive correlation was observed for plant height with biomass of culm ( $r = 0.29^*$ ), grain yield per plant ( $r = 0.41^{**}$ ) and Fe content ( $r = 0.42^{**}$ ). Number of tiller showed positive correlation with biomass of culm ( $r = 0.48^{**}$ ) and grain yield per plant ( $0.49^{**}$ ). Single-marker analysis using 19 SSR markers showed that RM1089 and RM144 were associated with grain Fe content and Zn content respectively. The genetic information generated and molecular markers identified from this study can be used for future biofortification programme of rice.



## 556: Genome Wide Analysis of *Cajanus cajan* and Discovery of RGAs against *Fusarium* Wilt Disease

Soma S Marla\*, S Gahoi, SP Mishra, Rajesh Kumar, Neeta Singh and DP Wankhede

Division of Genomic Resources, ICAR-NBPGR, New Delhi (\*Email: soma.marla@nbpgr.ernet.in)

*Fusarium* wilt is a major disease hampering efforts by legume breeders to improve Pigeonpea productivity. NGS analysis of nearly 13 mln raw sequence reads of popular variety 'Asha' yielded 1,91,705 contigs. Among the predicted coding gene sequences from contigs, 104 disease resistance analogues containing known disease resistance domains such as ARC-NBS-LRR, Transmembrane, kinases were chosen for further study. Comparative Genome studies of *Glycine max*, *Medicago truncatulum*, field bean and Pigeonpea revealed presence and synteny of most of these disease resistance analogues. Primer sets were designed and putative RGAs were PCR amplified from genomic DNA from 31 land races and varieties of pigeonpea (NBPGR, New Delhi). The study will contribute to ongoing field disease evaluation and germplasm characterization studies at National Bureau of Plant Genetic Resources, India.

## S567: Physical Mapping of QTLs for Salt Tolerance by Bulk Segregant Analysis of Recombinant Inbred Line Using a 50K SNP Chip in Rice (*Oryza sativa* L.)

Sushma Tiwari<sup>1</sup>, Krishnamurthy SL<sup>2</sup>, Vinod Kumar<sup>2</sup>, Balwant Singh<sup>1</sup>, Amitha Mithra SV<sup>1</sup>, Vandna Rai<sup>1</sup>, Ashok K Singh<sup>3</sup>, Nagendra K Singh<sup>1\*</sup>

<sup>1</sup>National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi; <sup>2</sup>Central Soil Salinity Research Institute, Karnal; <sup>3</sup>Division of Genetics, Indian Agricultural Research Institute, New Delhi (\*Email: sushma2540@gmail.com)

Agronomically important traits of crop plants are usually quantitatively inherited because of their control by multiple genes across loci. Genotyping of entire mapping population using large number of polymorphic marker is a prerequisite for genetic mapping of these quantitative trait loci (QTLs). Bulk segregant analysis (BSA), though rapid method to identify markers or genomic regions linked to a trait, has been applied mainly for simply inherited traits. In the present study, we describe a method for rapid identification of physical position of QTLs by BSA approach in a recombinant inbred lines population of 'CSR11/MI48' segregating for reproductive stage salt tolerance using 50K rice SNP chip. Phenotyping for salt tolerance was done in controlled microplots under moderate (pH 9.5) and high sodicity (pH 9.9) conditions at CSSRI Karnal. Thirty extreme tolerant and sensitive RILs were identified on the basis of their stress susceptibility index for grain yield from three year data. DNA from each of the extreme RILs was pooled in equimolar concentration to prepare tolerant and sensitive bulks. SNP genotyping of the parents and bulks using the 50K SNP chip revealed 6083 polymorphic SNPs and 139 QTLs, showing homogeneity of SNP alleles in the RIL bulks. A much larger number of QTLs were identified with this method because of the high density of the SNP assay and multiple rounds of recombination in the RILs, most of which were also narrowed down to less than 500 kb.

## Technical Session VI: Biochemistry, Proteomics and Metabolomics

*Invited Lecture*

### Search for Pathogenicity Vs Immunity Determinates in Crop Plants: A Proteomic Approach towards Translational Research

*Subhra Chakraborty\**

National Institute of Plant Genome Research, Jawaharlal Nehru University Campus,  
Aruna Asaf Ali Marg, New Delhi 110067 (\*E-mail:subhrac@hotmail.com)

Agricultural productivity and nutritional quality are the two key issues to the sustainable food production worldwide. Patho-stress is a major impediment during post-harvest storage and plant productivity. To investigate the cellular pathways in non-host vs host specific resistance in plant, we conducted a system study during fungal pathogenicity. Transcriptomic analysis revealed diverse and overlapping organ-specific immune-responsive transcript signatures in plants. Comparative patho-stress responsive proteome and phosphoproteome analyses led to the identification of couple of hundred proteins, phosphopeptides, and their site of modifications; presumably associated with cell differentiation, which regulate diverse functions like carbohydrate metabolism, cell wall restructuring, innate defense, redox homeostasis, protein folding and degradation. Network analysis identified major protein hubs pointing towards the onset and context of disease signalling, metabolic pathway activations and specific adaptation for cell survival. Furthermore, CabHLH1, a member of basic helix loop helix family protein was found to be the major determinant of innate immunity. These findings will not only impact plant biology, but in near future would be useful for identifying biomarkers, expedite the functional determination of the immunity-related proteins and their prioritisation as potential molecular targets for better adaptation.

*Invited Lecture*

### Specialized Secondary Metabolism in *Withania somnifera* (Ashwagandha): New Calls for Revisions in Phytochemical Biosynthesis Concepts

*Rajender Singh Sangwan\**

Center of Innovative and Applied Bioprocessing, C-127, Phase-8, Industrial Area, S.A.S. Nagar,  
Mohali 160071, Punjab (\*Email: sangwan@ciab.res.in ; sangwan.lab@gmail.com)

Plants are known to produce a myriad of chemicals that primarily do not appear essential for growth, development, survival and procreation. A long and continuing trail of chemical investigations on these phytochemicals has led to recognition of more than 200,000 individual structures that still represent only a small fraction of their naturally occurring number as only about 15% of the more than 250,000 plants have been chemically screened. These phytochemicals, belonging to diverse chemical classes such as terpenoids, alkaloids, flavonoids etc., are synthesized through specialized biochemical pathways typically referred to as secondary metabolism or more precisely as specialized metabolism. Last few decades of research on secondary metabolism have led to early concepts about their biosynthesis in a class-specific manner. Our extensive research on metabolomics, biochemistry and functional genomics of secondary metabolism of Ashwagandha (*Withania somnifera*), under New Millennium Indian Technology Leadership Initiative (NMITLI), have led to close to 50 lead publications on its phytochemical biology. Probably today this represents the best studied Indian medicinal plants for its science. These investigations cover withanolides, tropine and withanamides representing terpenoids, tropane alkaloids and indolyl classes of molecules. Results of some of these studies have implications exceptional to the then concepts. These include relative significance of MVA/MEP pathways, de novo biosynthesis across tissues and special indolyl functions in fruit.

## Plant $\beta$ -Galactosidase Immobilization on Functionalized Nano Materials and their Applications

Arvind M Kayastha

School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi 221005

$\beta$ -galactosidase enzyme was isolated from the seeds of pea (PsBGAL) and chickpea (CpGAL), with 910- and 1840-fold purification and final specific activity of 77.33 and 220  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ , respectively. Studies revealed that PsBGAL is a monomeric enzyme of Mr 55 kDa; whereas CpGAL is a heterodimeric enzyme of Mr 85 kDa, and resolves into two subunits of 48 and 38 kDa on SDS-PAGE. These enzymes were covalently immobilized with suitable nano-material using Response Surface Methodology (RSM). A lactose sensing nano-probe was developed by immobilizing PsBGAL onto gold nanoparticles, which showed 140.81% immobilization and significant enhancement in catalytic efficiency compared to soluble enzyme. Similarly, CpGAL was covalently attached to functionalized graphene nano-sheets with 84.2% immobilization efficiency. Immobilized enzymes were characterized using SEM, TEM and FTIR. Immobilized  $\beta$ -galactosidase could hydrolyse milk lactose at comparatively broad range of temperature and pH. The enzyme kinetics also showed excellent reusability and storage stability, under dry/wet conditions. This forms the first report on exploitation of  $\beta$ -galactosidase from a plant source for lactose hydrolysis and its use for quality check of lactose hydrolyzed milk and hidden lactose mainly in dairy products.

Oral Presentation

## Synthesis and Characterization of Finger Millet Protein Stabilized Nanoemulsion System for the Delivery of Hydrophobic Bioactives

Subin Raj Cheri Kunnumal Rajendran, Dinesh Pandey, Manoj Singh, Mamta Metwal and Anil Kumar\*

Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263145  
(\*E-mail:ak\_gupta2k@rediffmail.com)

Bioactives such as omega-fatty acids, phytosterols, vitamins, carotenoids are all known to reduce the risk associated with a number of chronic diseases. However, these compounds are faced with problems such as low bioavailability, bioactivity, biostability, solubility, limited retention time and poor uptake or absorption into the human body from natural sources such as fruits and vegetables. These constraints also limit their use in functional foods and nutraceuticals. In this regard, we have prepared a nanodelivery vehicle using finger millet protein as the encapsulating matrix over oil in water nanoemulsion of sesame oil containing dissolved  $\beta$ -carotene which acts as a model compound. During in vitro digestion using simulated gastric and intestinal fluids, sustained release of  $\beta$ -carotene was observed from the nano delivery vehicle. The delivery system was shown to have high encapsulation and loading efficiencies with particle size in the range of 100-1000nm as characterized by electron microscopy and Zetasizer. Such products are beneficial to consumers and farmers alike, providing a better alternative to pharmaceuticals and value added farm produce. This delivery oriented food derived nanoparticle system has applications as an oral agent for the controlled release and enhanced absorption of lipophilic bioactives and thus great potential in nutrient security.



## **S601: Transcription Factor Mediated Regulatory Surveillance of Therapeutic Metabolite Biosynthesis in *Swertia chirayita*, an Endangered Medicinal Herb of North-Western Himalayas**

*Jibesh Kumar Padhan\* and Rajinder Singh Chauhan*

Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat 173234, Himachal Pradesh (\*E-mail: jkpadhan@gmail.com)

*Swertia chirayita* (Gentianaceae), an endangered medicinal herb, is a reservoir of many pharmacologically important molecules exhibiting a diverse range of therapeutic value. These molecules belong to various classes of secondary metabolites such as xanthenes and their derivatives, flavonoids, terpenoids and secoiridoid glycosides. Molecular resources related to the biosynthesis of these therapeutic metabolites and the regulatory components associated thereof are very sparse, thereby limiting genetic improvement strategies. The current study focused on identification of Transcription Factors (TFs)/Transcription Regulators (TRs) through computational mining of transcriptomes of *S. chirayita* grown in two different environmental conditions. All transcripts obtained from field-grown (SCFG) and tissue-cultured (SCTC) plant transcriptomes were annotated to proteins (according to possible open reading frames) and searched through InterProScan. PlantTFcat identified a total of 4,100 and 3,210 transcripts genes belonging to 108 TF/TR/CR families in SCFG and SCTC transcriptomes, respectively. SCFG transcripts encoding TFs predicted to be involved in secondary metabolites biosynthesis were 872, out of which 108 were unique to SCFG only. Similarly, predicted TFs in SCTC were 614, out of which 28 transcripts were found to be unique to SCTC. Understanding of regulatory mechanisms of these TFs/TRs could be useful in devising genetic improvement strategies in order to enhance secondary metabolite production in *S. chirayita*.

## **S602: Isolation of novel acid soil-tolerant isolates of *Rhizobium* from Pigeonpea and proteomic characterization by utilizing MALDI-TOF / TOF and Peptide Mass Fingerprinting approach**

*Himanshu Dubey\**

Proteomics Laboratory, College of Biotechnology, Birsa Agricultural University (B. A. U.), Kanke, Ranchi-834006 (Jharkhand); All India Network Project on Soil Biodiversity-Biofertilizers (AINP-BNF), ICAR, New Delhi (\*Email: dubeybiotech99@live.com)

Biological processes account for approximately 60% of the biosphere's fixed nitrogen. As concerns mount to the growing input of reactive nitrogen into environment, as part of a "nitrogen cascade", an increased need to understanding "Biological Nitrogen Fixation (BNF)" has become of paramount importance. *Rhizobium* spp. survival in soil is influenced by a combination of many variable parameters, with soil-acidity being prominent ones. Using 16S rRNA Ribotyping analysis we have identified novel strains of *Rhizobium* (GenBank Accession Numbers KF309195, KF309203 and KF309204) from pigeonpea, which are tolerant to acidic soil pH regimes. "Two-Dimensional Gel Electrophoresis (2-DE)", followed by MALDI-TOF-TOF (Peptide-Mass Fingerprinting) was performed to characterize several important "Unique" protein differences amongst acid tolerant / acid in-tolerant *Rhizobium* isolates. Analysis of 14 "Unique" protein spots identified the genes implicated in the acid-soil tolerance. One of the important protein was identified as "Chain A, Structure of Periplasmic Binding Protein" (Accession Number: gi88192851, Molecular weight: 33,300Da and PI: 7.80). The periplasmic binding proteins serve as chemo-receptors, recognition constituents of transport systems, and initiators of signal transduction pathways. The existence of such unique proteins in the acid-tolerant isolates of *Rhizobium* is believed to explain the molecular basis of the factors responsible for imparting selective acid tolerance.



### **S603: Modified HPLC Method for Seed Phytate Analysis and Accumulation Profiles in Indian Soybean Genotypes**

Vanita Pandey<sup>1</sup>, Ashish Marathe<sup>1</sup>, Veda Krishnan<sup>1</sup>, Nabaneeta Basak<sup>1</sup>, Mansi Panjabi<sup>1</sup>, Alkesh Hada<sup>1</sup>, Monica Jolly<sup>1</sup>, S K Lal<sup>2</sup> and Archana Sachdev<sup>1\*</sup>

<sup>1</sup>Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: arcs\_bio@yahoo.com)

Identifying or designing low phytic acid soybean demands an improved understanding of its dynamics and a need for a reliable and accurate phytate quantification method. For the extraction of phytic acid, a modified method utilizing sonication of sample in 0.78 M HCl, followed by varied durations of mechanical agitation and sample quantification using high performance liquid chromatography (HPLC) was optimized. The modified method was found to be accurate, precise and reproducible and required relatively lesser time for sample preparation. The objective of this work was to evaluate the phytic acid content and study its accumulation profile in both black and yellow genotypes. Phytic acid was found to accumulate in a linear and continuous manner during the initial developmental stages of the seeds of both yellow (DS 9814) and black (MM 64) genotypes evaluated and reached steady levels on maturity. The observed phytic acid content in the 20 genotypes screened ranged, from 2.38-4.72 g/100g soybean flour and among them black soybean genotypes showed the maximum variability ( $P < 0.0001$ ). The black soybean genotypes JS 76205 (2.38 g/100g), Kalitur (2.50 g/100g) and UPSL 652 (2.54 g/100g), inherently rich in the antioxidant - anthocyanin content, reported the lowest phytic acid levels and hence can be utilized as nutraceutical genotypes with nutritional as well as environmental benefits.

### **S604: Genetic Determinants of Resistant Starch Characteristics in Foxtail Millet**

Annvi Dhaka, Mehanathan Muthamilarasan and Manoj Prasad\*

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, JNU Campus, New Delhi

(\*Email: manoj\_prasad@nipgr.ac.in)

Starchy foods are potential candidates for reducing glycaemic and insulinemic responses. Among different dietary starches, resistant starch (RS) has been reported to reduce blood glucose and insulin levels, lowers cholesterol and improves gastrointestinal health. Adequate levels of RS in diet assists in overcoming obesity-related conditions including cardio-vascular disease and Type-2-diabetes, and also consumption of RS reduces the risk of colorectal cancer. Noteworthy, foxtail millet (*Setaria italica*) is rich in RS and high fiber ( $\beta$ -glucans) in its whole and partly-milled grains, and a positive relationship between RS and amylose content has been established in several studies. In view of this, an attempt has been made to identify and characterize the genes involved in amylose biosynthesis. Key gene families involved in starch biosynthesis pathway namely adenosine 5' diphosphate-glucose pyrophosphorylase, starch synthase, starch branching enzyme, starch debranching enzyme, starch phosphorylase and starch disproportionating enzyme have been identified and characterized using computational approaches. Expression profiling of candidate genes at various time points of seed development in different cultivars provided insights to delineate their role in high RS content. The study provides informative clues and will significantly improve the understanding on molecular genetics and genomics of high RS content in foxtail millet.



## **S605: Reverse Genetic Modulation of Ethylene Biosynthesis as Tool to Regulate Fruit Ripening in Tomato**

*Kapil Sharma, Soni Gupta, Yellamaraju Sreelakshmi and Rameshwar Sharma\**

Repository of Tomato Genomics Resources, School of Life Sciences, University of Hyderabad

(\*Email: rameshwar.sharma@gmail.com)

Tomato fruit ripening involves coordination of several regulatory steps transforming the green, unpalatable fruit to a fleshy aromatic red fruit, rich with carotenoids and other phytonutrients. The analysis of naturally occurring tomato mutants impaired in ripening such as rin and cnr revealed a genetic hierarchy determining initiation of ripening, as fruits of these mutants failed to undergo characteristic changes in color and aroma. Downstream of genetic regulation of ripening by transcription factors, the climacteric rise of ethylene also plays an important role and mutants defective in perception of ethylene such as Nr fail to ripen normally even after exposure to exogenous ethylene. The inhibition of ethylene emission by antisense suppression of genes involved in ethylene biosynthesis such as ACS2 or ACO1 inhibit ripening. At present there are no naturally occurring mutants comprised in ethylene biosynthesis in tomato. It is believed that ACS2 is one of principle gene regulating climacteric rise of ethylene in tomato. A collection of tomato accessions obtained from different sources was analyzed for the frequency of naturally occurring SNPs in ACS-2 gene and few of the lines bearing SNPs in ACS-2 gene were phenotyped. Using reverse genetic screening of EMS-mutagenized M2 population of tomato by TILLING led to the identification of few mutations in ACS-2 gene. One of the mutants bearing a missense mutation displayed a gain of function phenotype. The mutant plants showed elevated production of ethylene in fruits during transition to red ripe stage. The gain of function phenotype was associated with increased level of the lycopene in red ripe fruits. The mutant fruits also showed accelerated ripening and on vine senescence. The ACS-2 mutant might be useful for developing early maturing variety with high carotenoid content in fruits.

## **S606: Differential Expression of Enzymes Involved in Organic Acid Metabolism in Relation to Nitrogen Use Efficiency in Wheat**

*Shruti Sinha, Gayatri, Monika Dalal, SK Sinha and PK Mandal\**

National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012

(\*Email: pranabkumarmandal@gmail.com)

Improvement of Nitrogen use efficiency (NUE) is imperative to reduce cost of cultivation and environmental pollution. Plants require glycolytic and TCA cycle enzymes for the supply of C-skeleton to incorporate inorganic NH<sub>4</sub><sup>+</sup> ion and form amino acids. It is important to study these enzymes for better understanding of N-metabolism which may help in selecting NU efficient genotypes. In the present study, eight wheat genotypes with varying degree of NUE were grown under N-stress along with control. Measurement of chlorophyll content and in vitro assay of enzymes namely, Pyruvate Kinase (PK), Citrate Synthase (CS) and Isocitrate Dehydrogenase (ICDH) was carried out. Gene expression of the three enzymes was studied using gene-specific primers in the leaf tissues of 15 days old seedlings. Chlorophyll content was lower in all the genotypes under stress, with highest reduction in Kalyansona and NP- 846. Interestingly, these two genotypes also exhibited higher enzyme activity. Genotypic variation, both in enzyme activity and gene expression was evident from the study. However, there was no correlation of gene expression with enzyme activity which might be because of other isoforms of these enzymes present but not separately analyzed. The other reason might be post-transcriptional and post-translational modification/ regulation of these proteins.



## **S607: A Modified Protocol for Sequential Extraction of Gliadins, Glutenins and Other Proteins from Wheat Seeds**

*RK Gupta<sup>1</sup>, Monika Dalal<sup>1</sup>, SK. Sinha<sup>1</sup>, G Thomas<sup>2</sup> PR Choudhury<sup>3</sup>, AK Ahlawat<sup>4</sup>,  
A Mahendru Singh<sup>4</sup> and PK Mandal<sup>1,\*</sup>*

<sup>1</sup>ICAR-NRC on Plant Biotechnology, Pusa, New Delhi 110012; <sup>2</sup>Dept. of MCE, SHIATS, Allahabad 211007; <sup>3</sup>ICAR-Head Quarter Krishi Bhawan, Shashtri Bhawan, New Delhi; <sup>4</sup>Div. of Genetics, ICAR-IARI, Pusa, New Delhi 12 (Email: pranabkumarmandal@gmail.com)

The introduction of gluten-containing grains occurred about 10,000 years ago. Celiac Disease is a malabsorptive disorder which occurs in more than 1% individuals upon ingestion of gluten. Gluten is the main structural protein of wheat with equivalent immunogenic proteins found in other cereals viz., rye and barley. The immunogenic protein fractions of gluten are gliadins (monomeric) and glutenins (aggregated) proteins. Though, gluten can be extracted by removing starch and albumins/globulins from wheat flour, the extraction of celiac responsible gluten protein fractions require suitable and reliable protocols. Moreover the gluten fractions should be devoid of detergents/regents harmful for the T-cells which are used in identifying the fractions based on their elicitation. In the present study, the sequential extraction of different fractions from seed sample was standardized. After removing albumins (water) and globulins (0.5M NaCl), gliadins were extracted in 50% propanol-1. The precipitate was treated with 50% propanol-1; 0.08 M Tris-HCl; 1% DDT at 450C temperature to extract glutenins. All these protein fractions were analyzed by SDS-PAGE and no nonspecific background bands were observed. We are now standardizing extraction of each of the gliadin and glutenin fractions without using any reducing agents known to be lethal for the T-cell cultures.

## **S608: Proline Biosynthesis, Degradation and Accumulation in Rice Genotypes under Aerobic Environment**

*Anjali Dahiya<sup>1</sup>, Sunita Jain<sup>2</sup> and Veena Jain<sup>1</sup>*

<sup>1</sup>Department of Chemistry and Biochemistry; <sup>2</sup>Department of Molecular Biology, Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar 125004  
(\*Email:sunitajain60@gmail.com)

Proline as an osmoprotectant plays a major role in counteracting the effects of abiotic stresses in plants. In the present investigation, aerobic (MAS25, MAS26) and low-land (HKR47, PAU201, HBC19 and Pusa1121) indica rice genotypes were grown under submerged and aerobic conditions in pots. Proline content and activities of proline metabolizing enzymes pyrroline-5-carboxylate synthetase (P5CS), pyrroline-5-carboxylate reductase (P5CR) and proline oxidase were observed in root and shoot tissues at vegetative stage. High basal level of proline content and proline synthesizing enzymes, P5CS and P5CR, were observed in aerobic rice genotypes under both aerobic and submerged conditions in both the tissues as compared to lowland rice genotypes. However, proline oxidase, the proline degrading enzyme had low activity in these genotypes. Proline content and P5CS and P5CR activities increased in all the rice varieties under aerobic conditions in both the tissues, however, the percent enhancement was more pronounced in lowland varieties as compared to aerobic varieties. In contrast, activity of proline oxidase decreased under aerobic condition. High level of proline content (~ 1.5 fold), P5CS and P5CR activities in aerobic rice may be one of the reasons for its better performance under aerobic conditions in comparison to lowland varieties.



## **S609: Comparative Analysis of ADP-glucose Pyrophosphorylase (AGPase) Large Subunit (LS) in Some Monocot and Dicot Plants**

*Saripalli Gautam<sup>1</sup>, Ritu Batra<sup>2</sup>, HS Balyan<sup>1,2</sup>, PK Gupta<sup>1,2</sup> and Kulvinder S Gill<sup>3</sup>*

<sup>1</sup>Molecular Biology laboratory, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut 250004; <sup>2</sup>Bioinformatics Infrastructure Facility, Department of Genetics and Plant Breeding, Charan Singh University, Meerut 250004; <sup>3</sup>Department of Crops and Soil Sciences, Washington State University, Pullman, USA (\*Email: saripalligautam86@rediffmail.com)

AGPase is a rate limiting enzyme involved in starch biosynthesis and thus has a significant role in crop productivity. Comparative analysis of AGPase LS at nucleotide and protein levels may lead to identification of structural features, which are conserved and functionally important. Using as reference the well characterized *Sh2* gene of maize encoding AGPase LS, true orthologues were identified for six monocots (maize, wheat, rice, sorghum, barley and Brachypodium) and three dicots (chickpea, Arabidopsis and potato). The results suggested conserved nature of AGPase LS across species. The identity based on cDNAs was 69-89% in monocots and 73-75% in dicots. Similarly, the identity based on amino acid sequences was 69-89% in monocots and 73-75% in dicots. The average size of orthologues from monocots was 6.2 kb and from dicots, it was 4.2 kb. This was largely due to differences in the size of UTRs and the introns. The orthologues contained two major conserved domains (AGPase\_PP and LbH\_GIP\_AT\_C). Indels of 2 to 7 amino acid residues specifically at N-terminal region indicated that this region may be crucial for regulation of starch biosynthesis. Phylogenetic analysis revealed separate clusters for monocots and dicots.

## **S610: Towards Understanding of the Heterotetrameric Structure of ADP-Glucose Pyrophosphorylase (AGPase) Enzyme in Wheat**

*Saripalli Gautam<sup>1</sup>, Ritu Batra<sup>2</sup>, HS Balyan<sup>1,2</sup>, PK Gupta<sup>1,2</sup> and Kulvinder S Gill<sup>3</sup>*

<sup>1</sup>Molecular Biology laboratory, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut 250004; <sup>2</sup>Bioinformatics Infrastructure Facility, Department of Genetics and Plant Breeding, Charan Singh University, Meerut 250004; <sup>3</sup>Department of Crops and Soil Sciences, Washington State University, Pullman, USA (\*Email: ritubiotech24@gmail.com)

The crystal structure of the native heterotetrameric AGPase enzyme is not available for any plant species, except that for the small subunit (SS) of potato AGPase. Therefore, only limited information is available for the structure-function relationship of this important enzyme. In the present study, information about primary sequence and secondary structural analysis was compiled for both subunits of wheat AGPase. The 3D structure of AGPase subunits was also determined through homology modelling using I-TASSER, SWISS-MODEL and Phyre2 utility packages. Each of the three models so generated were verified through SAVES, PDBsum and SWISS-MODEL. Based on their respective quality parameters, the 3D model generated by SWISS-MODEL was found to be the best. The Ramachandran plot analysis using PROCHECK/RAMPAGE of the best 3D model of both subunits showed that more than 90% of the residues are in the most favoured region; the corresponding figures for other quality parameters were as follows: VERIFY3D (97 and 99.32%), DFire energy (-581.76 and -2654.7), QMEAN6 (0.695 and 0.715) and Z-Score value (-0.859 and -0.415). The crucial amino acid residues that will be identified after performing LS-SS interaction studies may be targeted in future wet lab efforts for site directed mutagenesis which may lead to development of thermostable AGPase variants in wheat.

## S611: Compositional and Hydrolytic Enzymatic Changes in Guava (*Psidium guajava* L.) fruit during ripening

Reena Devi\* and Veena Jain

Department of Biochemistry, CCS Haryana Agricultural University, Hisar 125004

(\*Email: reena.8jan@gmail.com)

Fruits of two cultivars of guava viz. Hisar Surkha (shelf life 4-5 days) and L-49 (shelf life 7-8 days) harvested at five different stages of maturity, viz. immature green (IG), mature green (MG), turning (T), ripe (R) and over-ripe (OR) were analysed for various changes in cell wall components and their hydrolyzing enzymes. NDF, ADF, hemicellulose, cellulose, pectin and silica content reached their maximum at mature green stage followed by a decline up to OR stage in both the cultivars. However, lignin was maximum value at T stage and then decreased till OR stage. L-49, the firm cultivar showed higher content of cell wall components than the soft variety, Hisar Surkha at all stages of ripening. The activity of polymethylesterase increased upto T stage followed by a decrease in both the cultivars. The polygalacturonase (PG) and cellulase activity increased drastically throughout ripening. PG and cellulase activities were very low initially (1.55 and 2.33 units; 1.25 and 1.9 units in L-49 and Hisar Surkha for PG and cellulase, respectively) and increased later. The two varieties exhibited significant differences in their activities, which was higher in Hisar Surkha throughout ripening.

## S612: MADS Box Transcription Factor *APETALA1* Regulates Growth of Perennial Trees in Response to Photoperiod

Abdul Azeez<sup>1,2\*</sup> and Rishikesh P Bhalerao<sup>2</sup>

<sup>1</sup>Plant Molecular Biology Lab, Jain Irrigation Systems Ltd, Jalgaon, Maharashtra; <sup>2</sup>Umea Plant Science Center, Department of Forest Genetics and Plant Physiology, SLU, 90187 Umea, Sweden

(\*Email: drazeez.abdul@jains.com)

Plants, being sessile, have developed highly complex pathways to sense the change in environment and modulate the pattern of their growth. Trees of temperate and boreal forest where temperature goes below -40°C during winter stop their growth well ahead of winter. Most of these trees anticipate winter by sensing the reduction in day length and when day length falls below a critical value they stop making new leaves and form bud like structure to protect shoot apical meristem (SAM). We identified a tree ortholog of Arabidopsis floral meristem identity gene *APETALA1* (*API*) which Like-*API* (*LAP1*) plays a central role in short day mediated growth cessation in perennial tree hybrid aspen (*Populus tremula* x *P. tremuloides*). *LAP1* acts downstream of *CONSTANTS/FT* (*CO/FT*) module and directly regulates the *AINTEGUMENTA-like* (*AIL1*) transcription factor which is a positive regulator of core cell cycle genes. Therefore downregulation of *LAP1* after exposure to short days results in growth cessation. Thus our data demonstrate a novel role for tree ortholog of *API* in photoperiod mediated growth control in perennial trees. Our finding can explain how an evolutionarily conserved signaling molecule *LAP1* regulates two extremely diverse developmental processes such as flowering and perennial growth using the same photoperiodic signal.



### S613: Molecular and Biochemical Characterization of Root Nodule Bacteria Isolated from *Flemingia macrophylla* (Willd.) Merr.

Kishor U Tribhuvan<sup>1\*</sup>, K Thamilarasi<sup>2</sup>, VD Lohot<sup>2</sup> and KK Sharma<sup>2</sup>

<sup>1</sup>Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand; <sup>2</sup>Indian Institute of Natural Resins and Gums, Ranchi, Jharkhand (\*Email: kish.tribhuvan@gmail.com)

*Flemingia macrophylla* is an important lac host plant which is able to support almost all known lac insect species. Towards developing biofertilizer for *F. macrophylla*, nodule bacteria were isolated from the root nodules of *F. macrophylla* using YEMA medium. A total of 52 bacterial isolates were isolated from nodules of *F. macrophylla*. The bacterial isolates were identified based on 16S rDNA sequence analysis. Isolates mainly represented the genera *Bacillus*, *Rhizobium*, *Pseudomonas*, *Lycobactor* and *Ensifer*. The bacterial isolates were also tested in vitro for plant growth promoting properties including Indole Acetic Acid (IAA) production, potassium solubilization, zinc solubilization, siderophore production, urea utilization, catalase production and nitrate reduction. On the basis of biochemical tests performed in vitro, twelve efficient bacterial strains were selected. The consortia of the selected efficient strains along with *Rhizobium* consortia, *Pseudomonas* consortia and *Bacillus* consortia were inoculated to the soil to validate the effect of bacterial isolates on growth and root nodule formation ability in *F. macrophylla*. It was found that *Rhizobium* inoculated plants showed two fold increase in biomass as compared to control. The study shows that *Rhizobium* bacterial isolates are efficient biofertilizer for the production of healthy and quick growing seedlings at nursery stage.

### S614: Molecular and Metabolomic Characterization of Ageratum Enation Virus Associated with Leaf Curl Disease of *Amaranthus hypochondriacus*

Ashish Srivastava<sup>1,2</sup>, S Kumar<sup>2</sup>, SK Raj<sup>2</sup>, OP Sidhu<sup>3</sup> and I Dasgupta<sup>1</sup>

<sup>1</sup>Department of Plant Molecular Biology, University of Delhi South Campus, Delhi 110021; <sup>2</sup>Plant Molecular Virology Lab, CSIR-National Botanical Research Institute (NBRI), Lucknow 226001;

<sup>3</sup>Phytochemistry Lab, CSIR-National Botanical Research Institute (NBRI), Lucknow 226001 (\*Email: ash12biotech@gmail.com)

*Amaranthus hypochondriacus* is an important subsidiary food crops in the tropics and subtropics. Severe attack of leaf curl disease with a significant incidence (10-12%) was observed on intensively cultivated plants of amaranth at breeding plots of the CSIR-NBRI, Lucknow, India during December 2010. The full-length begomovirus genome was amplified by RCA, and linearized by digestion with BamH1. The product obtained was cloned, sequenced, and deposited in GenBank (JF682242). Based on highest nucleotide sequence identities and closest phylogenetic relationship the begomovirus under study was identified as ageratum enation virus (AEV). *A. hypochondriacus* infected with AEV was investigated for identifying alterations in the anatomical structures, sap translocation and metabolomic variations using light microscopy, NMR spectroscopy and GC-MS, respectively. Combination of GC-MS and NMR spectroscopy identified 68 polar and non-polar metabolites that were present in different levels in healthy and virus-infected *A. hypochondriacus*. Contrast of T<sub>1</sub> and T<sub>2</sub> weighted MR images showed significant differences in the spatial distribution of water, lipids and macromolecules indicating alterations in the cortical region and disruption of vascular bundles in AEV infected stem tissues. Microscopic examination of AEV infected stem revealed severe hyperplasia with a considerable reduction in size of stem cells. Higher accumulation of TCA cycle intermediates in AEV infected plants indicated enhanced rate of respiratory metabolism. The results demonstrated potential of MRI, NMR spectroscopy and GC-MS for studying anatomical and metabolic variations in virus-infected plants.

### **S615: Cloning and Functional Validation of Cation Chloride Cotransporter Gene from Rice cv. CSR 27**

Shikha Sinha<sup>1,2</sup>, Pragma Mishra<sup>1</sup>, Vandna Rai<sup>1</sup> and Nagendra Kumar Singh<sup>1</sup>

<sup>1</sup>ICAR-National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110012; <sup>2</sup>Banasthali Vidyapith, Jaipur (\*Email:nksingh@nrcpb.org)

There are two genes coding for cation chloride cotransporters (CCCs) in rice. CCC mediates coordinated symport of K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup>, and participates in long distance transport of Cl<sup>-</sup> in plants. Earlier study in our lab has demonstrated that a CCC gene was co-located in the QTL interval qKSH-1.1 for K<sup>+</sup> ion concentrations in shoot. In the present investigation, full length CCC gene with its native promoter was PCR amplified from the salinity stress tolerant cultivar CSR 27, cloned in plant transformation vector pCAMBIA1305.1 and sequenced. The construct was later transformed into *A. tumefaciens* strain EHA105. The mature scutellum calli of rice variety ‘‘Tapei 309’’ were used as explant for genetic transformation by *Agrobacterium* method. The putatively transformed calli that survived three cycles of selection of 10 days each on selection medium containing hygromycin were transferred to regeneration medium. Out of 1531 calli transformed, 33 independent calli were regenerated into plants. Molecular analysis of the transformants showed the presence of PCR product of 3387bp specific to the CCC gene and 500bp specific to hygromycin gene. Now efforts are underway to check the efficacy of this overexpression construct on salt tolerance.

### **S616: Evaluation of Antioxidant Enzyme Activity and Lipid Peroxidation Product in Presence of Jasmonic Acid during *Alternaria brassicae* Infection in Arabidopsis**

Snigdha Tiwari, Aditi Bist, Manu Gaur, Dinesh Pandey and Anil Kumar\*

Department of Molecular Biology and Genetic Engineering, College of Basic Sciences & Humanities, GBPUA&T, Pantnagar 263145 (\*Email:ak\_gupta@rediffmail.com)

Amongst the oilseed crops, rapeseed and mustard (*Brassica* spp.) play a central role in agricultural economy of the world. These crops are affected by a number of diseases which limits the productivity of crop. The productivity of rapeseed and mustard is seriously hampered by the disease *Alternaria* blight by a hemibiotrophic *Alternaria brassicae*. Jasmonic acid (JA) acts as a defense signal against this necrotrophic pathogen by triggering Induced Systemic Resistance. In case of necrotrophic pathogen, ROS accumulation and induction of cell death occurs which leads to pathogen colonization, progression and symptom development. Therefore the aim of the present study determine whether effect of JA on disease resistance in two ecotypes (WS and Columbia) of *Arabidopsis thaliana* inoculated with pathogens and treated with or without exogenous application of jasmonic acid by examining the antioxidative enzymes activities of APX, CAT, GPx and lipid peroxidation product i.e. MDA during *A. brassicae* infection. It was found that methyl jasmonate treatment was found to be effective in decreasing the disease incidence by lowering the disease index in both susceptible (WS) and tolerant ecotype (Col). Along with this it also enhances the antioxidant activities of enzymes like Ascorbate peroxidase, Catalase, Guaiacol Peroxidase and decreases MDA level which is the product of lipid peroxidation. In addition, JA treatment reduces the expression of metacaspase I. Together these data indicate that JA mediates the defense response against the disease by reducing the level of reactive oxygen species and cell death enzymes.



## **S617: Identification and Characterization of Genes Involved in Starch Biosynthesis Using Developing Spike Transcriptome Data of Finger Millet (*Eleusine coracana*): Structural and Functional Expression Analysis**

*Rajhans Tyagi<sup>1,3</sup>, Garima Saxena<sup>2</sup>, Sanjay Gupta<sup>3</sup>, Vijay Kumar Garg<sup>1</sup>, Sanjeev Agarwal<sup>4</sup> and Anil Kumar<sup>1,\*</sup>*

<sup>1</sup>Molecular Biology and Genetic Engineering, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand; <sup>2</sup>Department of Bioinformatics, Banasthali Vidhyapith, Banasthali, Rajasthan; <sup>3</sup>Department of Biotechnology, SBS Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun; <sup>4</sup>Department of Biochemistry, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (\*Email:ak\_gupta2k@rediffmail.com)

Finger millet is considered to be a boon for diabetes patients and obese people. Starch is the major storage form of carbohydrate in millets, consists of amylopectin, a branched-chain polymer of glucose, and amylose, a straight-chain polymer, which determine the quality of starch either resistant or soluble in nature. Attempts were made in the present study to generate the first large scale developing spikes transcriptome sequencing data of two genotypes differing in their calcium content: GP1- low (LC) and GP45- high (HC) using Illumina platform and subsequently reads were assembled with Trinity assembler. A total of 14,405 contigs in high grain calcium and 14,403 contigs were found in low grain calcium. These contigs were then retrieved on the basis of blast search against the 25 rice starch biosynthetic genes and further assembled. Four genes ADP glucose pyrophosphorylase, starch synthase, starch branching enzyme and starch debranching enzyme, were identified which were significantly taking part in the biosynthesis pathway of starch. Starch debranching enzyme pullulanase was showing the higher expression in HC in comparison to LC. Higher expression of debranching enzyme in HC genotype indicates that HC genotype might be more resistant than LC genotype. On the basis of annotation, characterization and analysis, four potential candidate genes were abundantly expressed of starch biosynthetic pathway in finger millet which may be further validated through biochemical and molecular analysis.



### **S618: Is High Grain Calcium Resulted Due to Oxalate Accumulation in Finger Millet (*Eleusine coracana*)? Bioinformatics Approaches for Characterization of Metabolic Networks of Oxalic Acid Biosynthesis**

Naved Akbar<sup>1</sup>, Apoorv Tiwari<sup>1</sup>, Rajesh Kumar Pathak<sup>1</sup>, BR Singh<sup>1</sup>, KP Singh<sup>2</sup> and Anil Kumar\*

<sup>1</sup>Department of Molecular Biology & Genetic Engineering, College of Basic Sciences & Humanities, G B Pant University of Agriculture & Technology, Pant Nagar; <sup>2</sup>Department of Biotechnology, Shri Venkateshwara University, Gajraula, U.P. (\*Email:ak\_gupta2k@rediffmail.com)

Finger millet (*Eleusine Coracana*) is rich in calcium content. However, grain calcium accumulation is similarly dependent on accumulation of phytate, oxalate and pectate. The genes involved in oxalic acid biosynthetic pathway in finger millet are remains unknown. Transcriptome data analyses of finger millet developing spikes of two genotypes (GP-1 & GP-45) differing in grain calcium content were used for understanding the role of metabolic networks of oxalic acid biosynthesis in relation to calcium accumulation. Several oxalic acid biosynthetic pathways have been proposed in other plants. It seems that glyoxalate play a key role in the formation of oxalic acid. In order to investigate the metabolic machinery of oxalic acid biosynthesis, seven major genes (SGAT, GGAT, ICL, GLO, MHAR, APO and OXO) were identified through in silico analysis from rice taking as a reference. After identification of major genes de novo assembly of all contigs involved in oxalic acid biosynthesis were performed. Expression analysis on the basis of 'Fragments Per Kilobase of Exon Per Million Fragments Mapped' (FPKM) values show all genes were highly expressed in GP-1 genotype in comparison to GP-45. The higher expression of oxalic acid pathway genes in GP-1 genotype indicates the possibility of more oxalate formation, however high grain calcium genotype GP-45 showed lower expression thus suggests that high accumulation of calcium is not related with oxalate accumulation and may be more bio-available. From this it can state that grain calcium accumulation in finger millet is not much related to oxalic acid accumulation.

### **S619: In vitro Manipulation of nifLA Operon in *Azotobacter chroococcum* for Efficient Nitrogen Fixation**

Gothandapani S<sup>1</sup>, HK Das<sup>1</sup>, Sekar S<sup>2</sup> and JC Padaria<sup>1\*</sup>

<sup>1</sup>Biotechnology and Climate Change, ICAR-National Research Centre on Plant Biotechnology, IARI Campus, New Delhi 110012; <sup>2</sup>Department of Industrial Biotechnology, School of Biotechnology, Bharathidasan University, Trichy, Tamil Nadu 620024 (\*Email: jasdeep\_kaur64@yahoo.co.in)

Modern agriculture has become highly dependent on chemical fertilisers but use of chemical fertilizers is environmentally and economically inhibitory. Biological nitrogen fixing microbes are limited in meeting the nitrogen requirement of agricultural crops due to negative regulation of microbial nitrogen fixing cascade in the presence of nitrogen fertilizer. The study involved genetic engineering of free-living nitrogen fixing microbe, *Azotobacter chroococcum* so that it can fix nitrogen constitutively even when used along with synthetic fertilizers like urea. Based on data sequences available, a *nif A* gene promoter sequence was designed and synthesised. The efficiency of this promoter was evaluated by cloning it in a promoterless plasmid construct having *lacZ* as reporter gene. The developed construct (pDJG101) was transformed in *E. coli* and the promoter strength was evaluated by quantitative and qualitative assays. Intensity of blue colour and ONPG assay confirmed the expression of gene under the synthetic *nif A* promoter. Using Tn5: Interposon mutagenesis, the *nif L* gene of *A. chroococcum* was partially deleted and continuous subculturing was done to ensure partial deletion of *nif L* gene in all the chromosomes of *A. chroococcum*. PCR analysis and southern hybridization confirmed the same. In vitro manipulation was carried out to transfer the *nifA* synthetic promoter in *A. chroococcum* having deletion in *nifL* upstream of *nifA* gene in correct orientation. Transformed strains would be analysed further.



## **S620: Allelic Combinations of Genes for Gluten Strength and Extensibility in Indian Wheat Varieties**

Anju M Singh\*, Anjali Rai, Poornima Sharma, Arvind K Ahlawat, Swati Chaudhary and Sumit K Singh

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012

(\*Email: anju\_mahendru@yahoo.co.in)

The market for bakery industry in India is valued at Rs. 69 billion. The two major bakery products, i.e. breads and biscuits hold about 82% of the market share. However, millers and bakers are not satisfied with the quality of the wheat flour of Indian varieties required for either bread or biscuit making. Knowledge of genetic constitution of germplasm for quality related genes/QTLs is important before taking up quality breeding. In the present study, a database of 180 Indian wheat germplasm including released varieties has been created using the molecular markers for glutenin genes. A good number of germplasm possessed either HMWGS or LMWGS desirable for bread or for biscuit quality. However, the number of varieties with combination of alleles of HMW- and LMW-GS genes desirable for good bread or biscuit quality was negligible. In addition, varieties having 1BL/1RS translocation were also identified. It was found that the 1BL/1RS translocation affected the dough mixing quality and produced weaker gluten. The gluten strength in varieties possessing 1BL/1RS translocation was compensated to some extent by HMW/LMWGS alleles that otherwise produced stronger gluten. The database generated in the present study will be useful in making designed crosses to develop further germplasm suited for a particular end-product.

## **S621: Biochemical Analysis of Color Variation Amongst Legumes Including Naurangi of Uttarakhand by Determination of Polyphenols, Isoflavonoids and Anti-Oxidant Activities**

Anita Kumari, Pallavi Shah, AK Gaur and Anil Kumar\*

Department of Molecular Biology and Genetic Engineering, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (\*Email: ak\_gupta2k@rediffmail.com)

Pulses belong to the family of leguminosae. They are an important source of macronutrients (such as proteins, carbohydrates, and dietary fiber), micronutrients, vitamins, carotenoids, phenolic compounds, antioxidant, antimutagenic and antiaging activities. Antioxidant based drugs are used for prevention and treatment of complex diseases like cancer, have attracted a great deal of research interest in natural antioxidants. Therefore, in this study, methanolic crude extract of eight pulses (black soyabean, white soyabean, pale yellow naurangi, brown naurangi, mix naurangi, black naurangi, yellow-green naurangi and sea-green naurangi) of Uttarakhand region were analyzed for total phenolics content (TPC), total flavonoid content (TFC) and antioxidant properties. TPC of pulses were determined using a spectrophotometric technique, based on Folin-Ciocalteu reagent and calculated as Gallic acid equivalents per gram of extract. Aluminium chloride colorimetric method was used for flavonoids determination and free radical-scavenging activities of the extracts were measured as decolorizing activity by trapping of unpaired electron of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Highest antioxidant activity as correlated with % of DPPH scavenging capacity was observed in yellow-green naurangi (70.40±2.27) followed by mix naurangi (62.33±3.92) and sea-green naurangi (60.48±0.81). The highest TPC were shown by Pale-yellow naurangi (5.36±0.03 mgGAE/g) followed by brown naurangi (4.97±0.03 mgGAE/g) and TFC by brown naurangi (33.8±0.4 mgCE/g) followed by black soyabean (32.6±0.4 mgCE/g). Out of 8 pulses, yellow green naurangi showed the best results in all the parameters and can be used as main staple pulse having better nutritional and health benefits.

## **Technical Session VII: Biosafety, IPR, Biotechnology Education and Training**

### **S701: Protection of Genetic Resources by Intellectual Property Right Management**

*Maneesha SR<sup>1\*</sup>, Gograj Singh Jat<sup>2</sup> and Dikshant Gautham<sup>3</sup>*

<sup>1</sup>ICAR-CCARI, Goa, <sup>2</sup>IARI, New Delhi and <sup>3</sup>ICAR-NBPGR, New Delhi  
(\*Email: sr.maneesha@gmail.com)

Genetic resources, the potential hereditary materials of any organism belonging to a particular region should be conserved by various means for several years for the advantage of the breeders and the farmers. Unauthorized exploitation of these genetic resources can be prevented by intellectual property rights management to ensure the sovereignty of a particular nation. The conventional morphological descriptors fail to discriminate the genetic resources accurately because of the environmental effect and the gene interactions. DNA profiling or DNA fingerprinting is a valuable technique for the recognition of a meticulous genetic resource, which can be executed by various hybridization based markers (e.g. RFLP, VNTR), PCR based markers (e.g. RAPD, SSR, ISSR) or markers involving the both techniques (e.g. AFLP) and Sequence generated informations (e.g. SNP). DNA fingerprint has not been accepted as the proof of uniqueness of genetic resources by the Union for Protection of New Varieties (UPOV), but the release of new varieties with DNA fingerprint information will certainly assist the breeders to reinforce the declare for the new variety. Patenting of the whole genome sequence information by IPR is an expensive, laborious and time consuming process. Hence sequences of short gene fragments with substantial genotypic distinctiveness can be protected by IPR for the safety of genetic resources worldwide.



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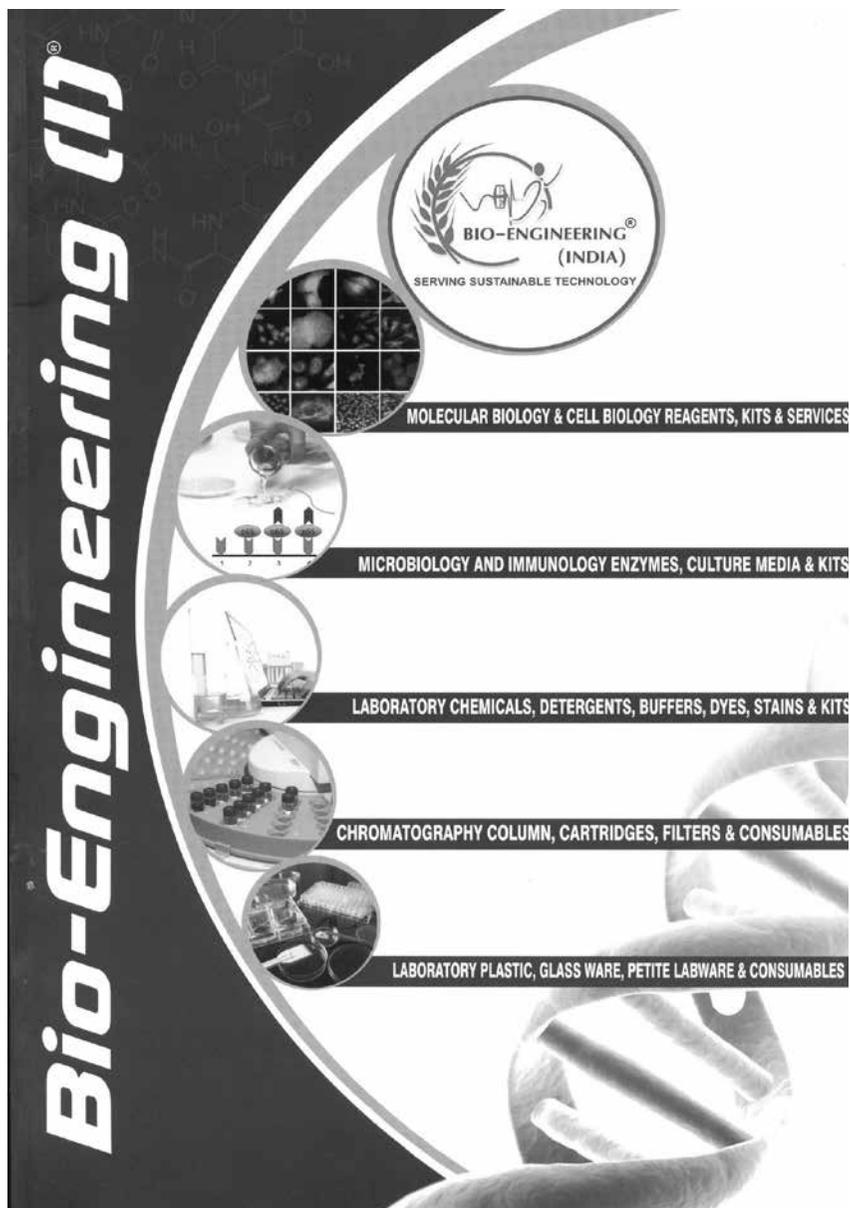
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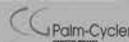
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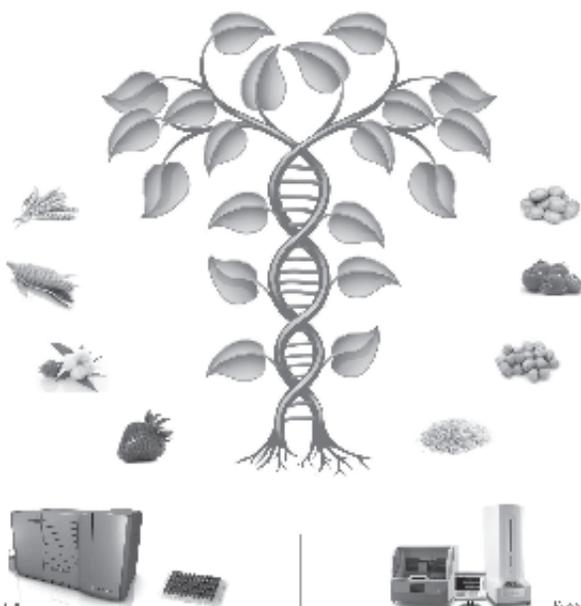
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