plant Signaling & Behavior

INTERNATIONAL SYMPOSIUM ON

PLANT SIGNALING & BEHAVIOR 2014

ABSTRACTS



DEPARTMENT OF BOTANY
UNIVERSITY OF DELHI
DELHI 110007, INDIA

plant Signaling & Behavior



Contents

1. Welcome 1
2. Programme 2-18
3. Abstracts 19-156
    a) Oral presentations 19-67
    b) Poster presentations 68-156

ORGANIZING SECRETARY

Professor Satish C. Bhatla
Head, Department of BotanyUniversity of Delhi, Delhi – 110007, India

ORGANIZING COMMITTEE

INTERNATIONAL

František Baluška
University of Bonn, Germany

Liz Van Volkengurgh
university of Washington, USA

Stefano Mancuso
University of Florence, Itlay

Mary Jane Beilby
University of New South Wales, Australia

Susan J. Murch
University of British Columbia Canada

Jinxing Lin
Beijing Forestry University, China

Tomonori Kawano
The University of Kitakyushu, Japan

NATIONAL

Professor A. K. Bhatnagar
Department of Botany, Delhi University

Professor S. B. Babbar
Department of Botany, Delhi University

Professor A. K. Pandey
Department of Botany, Delhi University

Professor Amar Kumar
Department of Botany, Delhi University

Professor Suman Lakhanpaul
Department of Botany, Delhi University

Dr. Rupam Kapoor
Department of Botany, Delhi University

Dr. Rajesh Tandon
Department of Botany, Delhi University

Dr. Renu Deswal
Department of Botany, Delhi University

Dr. Arun Jagannath
Department of Botany, Delhi University

Professor J. P. Khurana
Department of Plant Molecular Biology, Delhi University

Professor N. Tuteja
International Centre for Genetic Engineering and Biotechnology, Delhi

Professor R. Srinivasan
National Research Centre on Plant Biotechnology, Delhi

Welcome

During the past year a number of exciting developments have occurred for the Society of Plant Signaling and Behavior. In July 2013 participants from Europe, Asia, Australia, South and North America attended the sixth meeting of the Society in Vancouver, BC Canada, the first time our group has met in North America. Preceding the scientific meeting, a strategic session was held to identify ways to increase the impact of our research. Steps are being taken to strengthen the financial sustainability of the Society, including establishing tax-exempt status in the US and creating an Advisory Board to develop broader funding mechanisms. The journal Plant Signaling and Behavior is growing exponentially in impact and continuing to expand its format. A number of journalists attended the Vancouver meeting leading to publicity in Canada and the USA. One article, published in the New Yorker Magazine (Dec 16-23 2013) by Michael Pollan, did an exceptionally good job of drawing into focus the challenges our group has faced bringing forward ideas of plant behavior, communication, and cognition. While the basic biology of plants is represented by numerous scientific societies worldwide, the unique focus of our group is to discover integrative properties of plants previously unrecognized, including information storage, behavioral responses, and communication. Success will rely on precision in developing hypotheses, experimental protocols, and the language used in descriptions of results and interpretations. Progress will rely on willingness to move beyond known paradigms. Inspiration flows from interdisciplinarity, not only among scientific disciplines but also by folding insights from non-scientific fields such as literature, religion, and philosophy into a strong scientific base.

This March 2014 we are meeting in Delhi, India, our first time in South Asia. Novel features of this meeting include encouragement to contributors in Poster Sessions through Best Poster Awards. Since there will be two Poster Sessions, each Session will have separate prizes for three best posters to be judged by a Committee. It is further proposed to publish all abstracts and selected research papers from oral presentations in a special issue of Plant Signaling and Behavior published by Landes Bioscience.

We hope that the PSB2014 meeting will be successful in furtherance of the Society's objectives for the benefit of scientific community.

Elizabeth VanVolkenburgh
President
Society for Plant Signaling and Behavior

FrontisekBaluska
Editor-in-Chief
Plant Signaling and Behavior

Satish C. Bhatla
Organising Secretary
International Symposium on Plant Signaling and Behavior 2014

PSB 2014 – Oral Sessions

March 7, 2014

Session Number	Abstract Number	Time	Speaker	Title
Conference Opening : **Chair:** Elizabeth Van Volkenburg				
		9.30 a.m.	Elizabeth Van Volkenburg	Welcome & Opening Ceremony
CONTRIBUTIONS OF SIR J. C. BOSE TO PLANT ELECTROPHYSIOLOGY				
I	Plenary Lecture (L1)	9.45 a.m.	Minorsky, P.V.	Jagadish Chandra Bose and the Western response to Indian science
	L 2	10.20 a.m.	Virginia Shepherd	J.C. Bose and the roots of Plant Neurobiology
Tea break: 10.45 a.m. – 11.45 a.m.				
ELECTROPHYSIOLOGICAL RESPONSES				
II	Plenary Lecture (L1)	11.45 a.m.	L. A. Katicheva	Participation of passive ionic flows in the variation potential generation in wheat leaves
	L 2	12.05 p.m.	R. F. Oliveira	Action potentials in abscisic acid-deficient tomato mutant generated spontaneously and evoked by electrical stimulation
PLANT RESPONSES TO ENVIRONMENT - BIOTIC				
III	L 1	12.25 p.m.	Amar Kumar	Characterization of transcriptome and miRNAome of tomato roots infected with parasitic root-knot nematode (*Meloidogyne incognita*) by high-throughput sequencing
	L 2	12.45 p.m.	Mariya Khodakovskaya & Mohamed H. Lahiani	Effect of carbon nanotubes on plant cell stress signaling
	Lunch: 1.05 p.m. – 2.00 p.m.			

III	L 3	2.00 p.m.	R. Oelmuller	Beneficial and pathogenic fungi in the rhizosphere: How do roots distinguish?
	L 4	2.20 p.m.	Sharmila Chattopadhyay	The intricate position of GSH, a dynamic signalling molecule, in plant defence signalling network
	L 5	2.40 p.m.	Shubhra Chakraborty	Regulatory networks involved in plant-pathogen interaction: An omics approach
PHOTOSENSORY MECHANISMS				
IV	L 1	3.00 p.m.	A. G. Volkov	Phytosensors and phytoactuators
	L 2	3.20 p.m.	B. C. Tripathy	Involvement of phytochrome A and calmodulin-dependent kinases in suppression of photomorphogenesis in rice seedling grown in red light with their shoot bottom exposed
	L 3	3.40 p.m.	J. P. Khurana	Functional analysis of the blue light sensing cryptochrome 2 from *Brassica* and rice
	L 4	4.00 p.m.	K. Yokawa	Volatile ether induces ROS/calcium spikes and light escape-like tropism of roots
Tea break &Poster Session: 4.20 p.m. onward				

March 8, 2014

Chair:				
Session Number	**Abstract Number**	**Time**	**Speaker**	**Title**
SIGNALING IN PLANT DEVELOPMENT AND REPRODUCTION				
V	L 1	9.00 a.m.	C. Karounos	Environmental phosphorus mediates glucosinolates in mustard plants
	L 2	9.20 a.m.	F. Baluska	Update on the Darwin's 'Root Brain' – Oscillatory Command Centre
	L 3	9.40 a.m.	H. Paves	Role of *Arabidopsis thaliana* myosins in gravotropism of stems
	L 4	10.00 a.m.	P. Khurana	Functional Genomics of Thermal Tolerance in Wheat
	L 5	10.20 a.m.	Rujin Chen	Compound leaf development in legume
	L 6	10.40 a.m.	R. K. kar	Reactive oxygen species (ROS)-mediated growth and gravisensitivity of embryonic axes during germination of *Vigna radiata* seeds
	Tea break: 11.00 a.m. – 11.30 a.m.			
	L 7	11.30 a.m.	S. Yalovsky	Regulation of cell polarity and pattern formation in plants
PLANT HORMONES AND SIGNALING				
VI	L 1	11.50 a.m.	A. S. Raghavendra	Guard Cell Alkalization Precedes the Increase in Reactive Oxygen Species or Nitric Oxide during Stomatal Closure by Plant Hormones as well as Microbial Elicitors
	L 2	12.10 p.m.	Cho H.T.	Spatio-temporal dynamics of subcellular localizations of PIN-FORMED auxin
	L 3	12.30 p.m.	Bharti, N.	Photomodulation of strigolactone accumulation and physiological effect of associated biomolecules during sunflower seedlings growth

	Lunch: 12.50 p.m. – 1.50 p.m.			
VI	L 4	1.50 p.m.	Lee R.D.	Pro-rich receptor-like kinase RHS10 may transduce cell wall signals to inhibit root hair tip growth in angiosperms
	L 5	2.10 p.m.	Geetika Sirhindi	Understanding the role of jasmonic acid signalling molecules in nickel tolerance mechanism during seedling growth in *Glycine max* L.
	L 6	2.30 p.m.	M. J. Beilby	The role of Melatonin and Serotonin in circadian rhythm of Characeae *Chara australis*
	L 7	2.50 p.m.	A. Jelinkova	Mapping the trafficking pathways of PIN1 with engineered BFA-resistant ARF-GEFs in *Nicotiana tabacum*
	L 8	3.10 p.m.	González-Fontes A.	Boron deprivation inhibits root cell elongation via an ethylene/auxin/ROS-dependent
	L 9	3.30 p.m.	D. A. Chamovitz	The glucosinolate breakdown product Indole-3-Carbinol acts as an auxin antagonist in roots of *Arabidopsis thaliana*
	Tea break: 3.50 p.m. – 5.00 p.m.			
	MECHANISMS OF PLANT COMMUNICATION			
VII	L 1	5.00 p.m.	J. D. Blande	Plant communication by volatiles: mechanisms and environmental interference
	L 2	5.20 p.m.	Renee M. Borges	The volatilome of a nursery for seeds and wasps: why do plant signals change?
	L 3	5.40 p.m.	Stefano Mancuso	Plant bioacustic – a new frontier in plant communication

VII	L 4	6.00 p.m.	A. Novoplansky	Say it with flowers: flowering acceleration by root communication
	Poster Session			

March 9, 2014

FIELD TRIP

March 10, 2014

Chair:

Session Number	Abstract Number	Time	Speaker	Title
PLANT RESPONSES TO ENVIRONMENT - ABIOTIC				
VIII	L 1	9.00 a.m.	Patrick J. Hussey	Interactions of the plant cytoskeleton with membranes
	L 2	9.25 a.m.	C. Pandolfi	Salt stress priming in Zea mais
	L 3	9.45 a.m.	G. K. Pandey	Negative regulation of ABA signaling and positive regulation of abiotic stresses by a rice protein phosphatase 2C
	L 4	10.05 a.m.	K. J. Appenroth	Increase of starch accumulation in duckweed (Lemnaceae) under abiotic stress
	L 5	10.25 a.m.	I. Karsai	Phenotypic Plasticity and Fluctuating Asymmetry of Leaf Morphology of Three *Quercus* (Oak) Species in Response to Environmental Factors
PLANT SIGNALING AND BIOTECHNOLOGY				
IX	L 1	10.45 a.m.	Anita Grover	Genetic engineering of *Brassica juncea* for fungus resistance using signaling protein gene NPR1
	L 2	11.05 a.m.	K. P. Dorota	Annexins a new player in plant stress adaptation to the adverse environment
Tea Break: 11.25 a.m. – 12.00 p.m.				
	L 3	12.00p.m.	N. Tuteja	RNA splicing factor promotes crop improvement under drought and salinity stress
REDOX SIGNALING				
X	Plenary Lecture (L 1)	12.20 p.m.	Sampa Das	Reactive Oxygen Species (ROS): The key player in signaling events of Chickpea (*Cicer arietinum* L.)-*Fusarium oxysporum* f. sp. *ciceris* race 1 (Foc1) interplay.
X	L 2	12.40p.m.	T. Kagenishi	Extracellular ATP Inhibits Endocytic Vesicle Recycling and Gravitropism of Arabidopsis Roots via NADPH Oxidase-Mediated ROS Signaling
	L 3	1.00 p.m.	Tomonori Kawano	Protection of plant cells and green algae from oxidative copper toxicity by catalytically active metal-binding DNA oligomers
Lunch: 1.20 p.m. – 2.20 p.m.				
PLANT SIGNALING MOLECULES				
XI	L 1	2.20 p.m.	H.G. Edelmann	The root cap orchestrates maize seedling development via root- and shoot-affecting, ethylene-dependent signals
	L 2	2.40 p.m.	Wei Siao	Arabidopsis Synaptotagmin-Like Proteins (SYTLs) and Synaptic Vesicle Protein 2-Like (SVL) in endocyticvesicle recycling pathways of root apex cells
	L 3	3.00 p.m.	Weiming Shi	Shoot-supplied ammonium triggers two distinct responsive signaling pathways in Arabidopsis
	L 4	3.20 p.m.	Zhi Qi	Glutamate Receptor-Like Channel3.3 Is Involved in Mediating Glutathione-Triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in Arabidopsis
	L 5	3.40 p.m.	Paco Calvo	Probabilistic predictive processing vs. ecological psychology models of plant intelligence
Poster Session: 3.40 p.m. – 4.40 p.m.				
Closing Session followed by High Tea: 4.40 p.m.				

PSB 2014 – Poster Sessions

March 7-8 (up to Lunch), 2014

Poster Session A			
Session Number	Abstract Number	Poster	Title
I. CONTRIBUTIONS OF SIR J. C. BOSE TO PLANT ELECTROPHYSIOLOGY			
I	**No Posters**		
II. ELECTROPHYSIOLOGY RESPONSES			
II	P2.1	Vladimir S.	A participation of changes in cytoplasmic and apoplastic pH in variation potential-induced photosynthetic response
III. PLANT RESPONSES TO ENVIRONMENT - BIOTIC			
	P3.1	Bhatia V.	Transgenic Arabidopsis overexpressing E-β-farnesene synthase in cytosol and plastids confers repellence to green peach aphids
	P3.2	Choudhary P.	Development and analysis of expressed sequence tags induced by toxin, isolated from *Fusarium oxysporum* in chickpea
III	P3.3	Das D.R.	Role of cyclops in an Aeschynomeneae legume *Arachis hypogaea*
	P3.4	Jain P.	Expression profiling of NILs carrying rice blast resistance gene *Pi9* to understand molecular basis of host-pathogen interactions
	P3.5	Kaur P.	Identification and characterization of miRNAome of tomato roots infected with root knot nematode (*Meloidogyne incognita*)
	P3.6	Kumar S.	Putative roles of selected OY-M phytoplasma effector proteins in plant hosts: A bioinformatic approach
	P3.7	Jain P.K.	Host mediated RNAi of a parasitic gene for eliciting root knot nematode resistance
	P3.8	Masahito N.	Involvement of phospholipase C and D in plant immune responses against *Ralstonia solanacearum*
	P3.9	Mazumdar-Leighton S.	Towards developing Rubisco as a physiologically-relevant substrate for measuring herbivory
	P3.10	Nagar R.	NGS based small RNA transcriptome profiling of sheath blight resistance and susceptible rice lines
	P3.11	Purwar S.	Signaling through cAMP controls pathogenesis in Karnal bunt fungus (*Tilletia indica*) in wheat (*Triticum aestivum*)
III	P3.12	Singh Vijayata	*Flowering Locus D*, a putative histone demethylase of *Arabidopsis thaliana*, functions as a modulator of systemic acquired resistance
	P3.13	Saha C.	Endophytes isolated from *Typha angustifolia* alter nitrogen use efficiency in Rice
	P3.14	Shukla N.	Transcriptome profiling of roots of susceptible and resistant tomato cultivars at various stages of infection with root knot nematode (*Meloidogyne incognita*)
	P3.15	Thakur B.	Sheath blight: Efficacy of resistance inducers
	P3.16	Sarkar M.	*Arachis* and *Oryza* in an intercropped field are colonized by nod+ and nod divergent Bradyrhizobial populations respectively
IV. PHOTOSENSORY MECHANISMS			
	P4.1	Burman N.	Functional characterization of HY5 homolog in rice

IV	P4.2	Mishra Sushma	Over-expression of *indica* rice cryptochrome 2 gene induces partial photomorphogenesis in dark, semi-dwarfism and early flowering
V. SIGNALING IN PLANT DEVELOPMENT AND REPRODUCTION			
	P5.1	Bamel K.	Acetylcholine suppresses shoot formation and callusing in leaf explants of *in vitro* raised seedlings of tomato, *Lycopersicon esculentum* Miller var. Pusa Ruby
	P5.2	Borah P.	OsFBK, a rice F-box protein, is a component of the Ubiquitin-26S proteasome system (UPS) and involved in anther and root development
	P5.3	Dwivedi A.	*In silico* analysis indicates Receptor Like Kinase and Kinase Interacting Protein 1 genes isolated from *Cenchrus ciliaris* interact during apomictic female gametophyte development
V	P5.4	Dwivedi V.	Analysis of root system architecture in Chickpea
	P5.5	Kalra C.	Ameliorating effect of nitric oxide on *in vitro* caulogenesis of *Linum usitatissimum* oniron-deficient culture medium possibly by its internal mobilization
	P5.6	Khurana A.	ROS is also involved in *in vitro* flowering of *Lemna aequinoctialis* under non-inductive conditions
	P5.7	Kumar R.	Isolation and molecular characterization of heterotrimeric G-protein family from *Brassica nigra*
	P5.8	Kumar V.	Exploring the role of histone deacetylases in epigenetic regulation of fiber development in *Gossypium hirsutum* L.
	P5.9	Mishra B.S.	Unknown function protein having Serine Rich Repeat is involved in glucose and auxin dependent root growth and development
	P5.10	Sharma B.	Structural and biochemical changes accompanying attainment of stigma receptivity and pollen-stigma interaction in sunflower
	P5.11	Singh G.	YABBY and MYB transcription factors genes as master regulators for plants developmental process- a review
V	P5.12	Singh M.	Glucose control of root growth direction in *Arabidopsis thaliana*
	P5.13	Singh S.P.	Cellular characterisation of *Nicotiana* tapetum developmental stages and differential gene expression analysis to reveal the PCD signalling in their degeneration
	P5.14	Thomas S.	Tomato root tips regulate lateral root formation during early seedling development
VI. PLANT HORMONES AND SIGNALING			
	P6.1	Gayatri G.	Signaling components involved during stomatal closure by three microbial elicitors, compared with the effects of abscisic acid
	P6.2	Gupta A.	Brassinosteroid and glucose signaling interaction during early seeding growth and development in *Arabidopsis thaliana*
VI	P6.3	Kaur H.	28-Homobrassinolide in plant signaling and behavior
	P6.4	Kushwah S.	The interaction between glucose and cytokinin signal transduction pathway in *Arabidopsis thaliana*
	P6.5	Maitra-Majee S.	Drought induced 19 protein: A putative interacting partner of Aux/IAA13 in rice
			Understanding the role of phosphatidic

VI	P6.6	Pandit Shatakshi	acid-sphingosine kinase1 interaction during ABA signaling
	P6.7	Sharma P.	Narrating the role of JA signaling molecule in alleviation of copper toxicity in *Cajanuscajan* (L.) Millsp
	P6.8	Singh A.	Functional characterisation of wheat *Brassinosteroid independent-1* (*TaBRI* 1) overexpressed in *Arabidopsis thaliana*
	P6.9	Yadav S.	Auxin-induced nitric oxide accumulation and tyrosine nitration of proteins during adventitious root development in sunflower

March 9, 2014

FIELD TRIP

March 8 (Post Lunch) – 10, 2014

Poster Session B

Session Number	Abstract Number	Speaker	Title
VII. MECHANISMS OF PLANT COMMUNICATION			
VII	P7.1	Sharma R.	Cholinergic system: A novel approach to understand allelopathy
VIII. PLANT RESPONSES TO ENVIRONMENT - ABIOTIC			
VIII	P8.1	Agarwal M.	Landscape of open chromatin during abiotic stress in *Arabidopsis*
	P8.2	Agrawal V.	Salt and heavy metal stress modulated biochemical and physiological changes and their amelioration through glutathione in Chickpea
	P8.3	Bazihizina N.	Root acclimation enhance zinc tolerance in tobacco plants
	P8.4	Bhatt D.	Identification and characterization of stress inducible promoters from *Oryza sativa*
	P8.5	Chandna R.	Characterization of glutamate receptor in *Arabidopsis thaliana*
	P8.6	Fatma M.	Nitric oxide interacts with sulfur assimilation and alleviates decrease in photosynthesis and growth of salt treated mustard by enhancing antioxidant metabolism
	P8.7	Grover M.	Heat stress signalling in plants as a context sensitive language acceptance problem
	P8.8	Jogawat A.	The root endophytic fungus *Piriformospora indica* improves growth of rice seedlings during high salt stress
	P8.9	Khalid H.	*In vitro* culture approach to study the effect of salt stress on *Camelina sativa* L. cv Calena: an emerging biofuel crop

	P8.10	Khan M.I.R.	Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency and antioxidant metabolism
	P8.11	Marik A.	Study reveals *Brassica juncea* natural resistance-associated macrophage Protein 4.1 (BjNRAMP4.1) as an interactor of G-protein coupled receptor like protein associated signalling
	P8.12	Manuka R.	Identification of abiotic stress responsive with no lysine kinase in rice
	P8.13	Mukherjee S.	Analysis of salt-stress induced modulation of ouabain-sensitive ATPase activity in sunflower seedling roots using a novel fluorescence imaging approach
VIII	P8.14	Chaturvedi A.K.	Physiological approaches for mitigating high temperature stress effects in rice
	P8.15	Pandey B.K.	Comparative transcriptome analysis of signaling regulators in traditional and modern indica rice genotypes under phosphorus deficiency
	P8.16	Pandey S.	*In silico* analysis of riceascorbate peroxidase gene expression reveals its role in stress
	P8.17	Pandey V.	Elucidating the role of *Trichoderma harzianum* on drought-specific expression of genes in different genotypes of rice (*Oryza sativa* L.)
	P8.18	Per T.S.	Variation in phytocystatin activity in mustard seeds is correlative with efficient antioxidant metabolism and protection of cadmium-inhibited photosynthesis
	P8.19	Roy Chowdhuri S.	"Lipidomics"- an approach to assess the plasticity of the membrane for ecological adaptation in eastern himalayan mosses
	P8.20	Sehrawat A.	Proteome and S-nitrosoproteome analysis suggest a cross talk between nitric oxide and cold stress signaling
	P8.21	Seth C.S.	Synthesis of phytochelatins and antioxidants in response to lead accumulation in Indian mustard (*Brassica juncea* L.)
VIII	P8.22	Sharma E.	Comparative analysis of drought stress responsive genes in contrasting rice cultivars
	P8.23	Singh A.	Transcriptional expression analysis of potato tuberization in response to heat stress
	P8.24	Syeed S.	Protective role of exogenously applied proline on photosynthetic inhibition by salt stress through changes in proline and antioxidant in mungbean cultivars

IX. PLANT SIGNALING AND BIOTECHNOLOGY

	P9.1	Arora K.	Functional complementation of rice blast resistance gene *Pi54* using RNA interference approach
	P9.2	Baluska F.	Serine rich peptide domain regulates the activity of AtMTM1 towards ATX1
	P9.3	Gupta N.C.	Molecular characterization and expression analysis of a wound-responsive promoter cloned from *Arabidopsis thaliana*
IX	P9.4	Kaul T.	Glyphosate and sulphonyl urea tolerant "Swarna" rice for sustainable weed management, high water-use efficiency and enhanced yield
	P9.5	Kumar R.	A GH3 gene, *SlGH3.2*, is required in the regulation of various auxin-mediated responses during tomato (*Solanum lycopersicum*) development
	P9.6	Mandal S.	Localization of hydrogen peroxide in

			glandular trichomes and its effect on artemisinin production in *Artemisia annua* colonized by arbuscular mycorrhizal fungi *Glomus intraradices*
IX	P9.7	Reddy S.	Pyramiding genes of "ascorbate-glutathione pathway" for conferring multiple abiotic stress tolerance in rice
	P9.8	Sardar A.	Understanding the role of a putative protein kinase from *Arabidopsis* in plant defense response
	P9.9	Singh H.R.	Synergistic effect of potato class I chitinase and mung bean defensin gene on defense signaling against blister blight in transgenic tea
	P9.10	Srivastava V.K.	P-protein forisome from *Pisum sativum* functions in sealing of wounds and confers mode of recovery from biotic stress
X. REDOX SIGNALING			
X	P10.1	Agurla S.	Effect of salicylic acid and its esters on stomatal closure and increase in reactive oxygen speices, nitric oxide in *Pisum sativum* guard cells
	P10.2	Jain P.	Enzymatic scavenging of reactive oxygen species accompanying sodium chloride-induced stress in sunflower seedlings
	P10.3	Singh S.	Understanding of calcium ion regulation and ROS-interacting enzymes in *Anabaena* sp. PCC 7120 under varied calcium chloride levels
	P10.4	Thakur A.	Correlation between accumulation of reactive oxygen species, their enzymatic scavenging mechanisms, Ca^{+2} and modulation of protein kinase C activity during seed development in sunflower
XI. PLANT SIGNALING MOLECULES			
	P11.1	Arya G.C.	Isolation and functional characterization of heterotrimeric G-proteins from *Brassica rapa*
	P11.2	Augustine R.	Glucosinolates in plant fitness
	P11.3	Baluska F.	Potential Involvements of Arabidopsis Glutamate-Receptor-Like Receptors in Endocytosis
XI	P11.4	Bhardwaj D.	*Pisum sativum* G-protein beta subunit interacts with small pathogenesis-related cysteine rich protein to regulate stomatal functions
	P11.5	Kilaru A.	Elucidation of *N*-acylethanolamine pathway and its physiological role in *Physcomitrella patens*
	P11.6	Mathur V.	Molecular interaction studies of *Brassica* annexin with methyl jasmonate
	P11.7	Mitra S.	Fatty acid derived oxylipin signalling molecules in some eastern himalayan mosses analysed by headspace sample enrichment probe-gas chromatography mass spectrometry (HS-SEP-GC-MS)

ORAL PRESENTATIONS



Jagadis Chandra Bose and the Western response to Indian science

Minorsky, P.V.
Department of Natural Sciences, Mercy College, Dobbs Ferry NY 10522 USA
email: pminorsky@mercy.edu

More than 75 years after his death, Jagadis Chandra Bose (1858-1937) remains a controversial figure. Amongst his adherents, Bose is variously celebrated as a polymath genius, an inventor, a national hero, and more recently, the father of the plant neurobiology movement. In marked opposition, Bose's detractors in academia, by accusing Bose of virtually every academic malfeasance, including technical incompetence, poor exposition, vitalism and mysticism, effectively marginalized Bose from the history of plant biology in the West. Bose was a genius but he was not perfect; two of his hypotheses in particular, one related to the unity of life and non-life, and the other to the ascent of sap in plants, were based on naïve inductive arguments. Because of these erroneous theoretical constructs, Bose's interpretation of his own experiments were often misguided, although his experimental findings, at least those that have been re-examined, have almost entirely been confirmed. The various charges of scientific incompetence and dishonesty raised against Bose by Western scientists are examined and found to be without merit. It is argued that the vitriolic rejection of Bose's botanical contributions by Western plant physiologists in the first third of the 20th century cannot be understood solely in terms of scientific dialectics, and that the racism, classism and orientalism rampant in the West during this era played a huge role in Bose's rejection by the West. Evidence is presented that, to a man (and one woman), Bose's critics were eugenicists (USA and Germany) or aristocrats (Great Britain), who had in common the shared assumption of inherent self-superiority. However, given that the modern plant neurobiology movement, which was founded in a less overtly racist era, has encountered similar but less strident resistance indicates that there are deeper philosophical differences between plant neurobiology and the reductionist orthodoxy.



J.C. Bose and the roots of plant neurobiology

Virginia Shepherd
UWSCollege, University of Western Sydney,
& Department of Biophysics, School of Physics, The University of NSW
Sydney, Australia. vas@phys.unsw.edu.au

In 1900, Sir J. C. Bose began an insightful, controversial and prodigious body of research into plant signalling and behaviour. This research would last until his death in 1937, at which time Bose was much beleaguered in the West. An experimenter and inventor of extraordinary ingenuity, Bose was at the forefront of physics in 1900. He had discovered millimetre waves, explicated their properties, envisaged and invented the first semiconducting diode detectors for radio waves. Lauded by distinguished contemporaries including Lord Rayleigh and J.J. Thompson, Bose began his contentious plant research by inventing unique, delicate instruments for simultaneous recording, in real time, of plant movements and bioelectric potentials. He documented signals, including action potentials, coupled to behaviour in plants making touch-induced movements (including *Mimosa pudica*), in plants making spontaneous movements (including the Indian telegraph plant), and in 'ordinary', slow-moving plants. Bose concluded that all plants actively respond to their environments and co-ordinate their responses through electrical signalling.All plants are sensate, responding to the world through a fundamental motif involving coupled oscillations in electric potential, turgor pressure, contractility, and growth. Plants have a nervous system, an electromechanical pulse, a form of intelligence, and can access their past states in a form of learning. Together with his panexperientialist philosophy, these conclusions were deeply at odds with emerging mechanistic materialist concepts of plants as simple insensate automata. At Charles Darwin's behest, Sir John Burdon-Sanderson had measured changes in electric potentials during trap closure in Venus' Flytrap, and professional enmity developed between Bose, Burdon-Sanderson, and another prominent physiologist, Auguste Waller. Recently, a concept that plants are 'neuronal, knowledge-accumulating systems' has re-emerged in the mainstream, with attendant research that aims to understand how plants perceive and process experiences, coordinating responses to the world via integrated informationnetworks including molecular, chemical, and electrical levels of signalling.



Participation of passive ionic flows in the variation potential generation in wheat leaves

L.A.Katicheva, A.V.Bushueva, R.V.Batrakov, V.S.Sukhov, V.A.Vodeneev
Department of Biophysics, N.I. Lobachevsky State University of Nizhny Novgorod,
603950 Gagarin St., 23, Nizhny Novgorod, Russia
email: katicheva_la@mail.ru

Higher plants show two types of electric reactions in accordance with the nature of the stimulation: an action potential (AP) and a variation potential (VP). Though little is known about the mechanisms of induction of functional changes by AP or VP, it is considered that ionic concentration shifts in cells at electrical reactions development play the main role in such transformation. Unlike AP, ionic nature of the VP remains unclear. It is considered that VP's depolarization is based on transient inactivation of the proton pump at the plasma membrane. At that participation of passive ionic flows in VP generation is also possible. The aim of the present study is to distinguish the involvement of passive ionic flows – Ca^{2+}, Cl^- and K^+ - in the generation of VPs. For the recognition of the passive ionic flows at VPs resistance was registered simultaneously with electrical activity. Changes of the VP parameters under inhibitors addition served as indicators of participation of calcium, chlorine or potassium channels in the VP generation. It has been shown that cell input resistance decreases during the VP generation, which indicates ionic channels activation. At the same time VP amplitude decreased under calcium ions chelator EGTA addition to the medium. When anion channels were blocked by ethacrinic acid addition the VP had poor depolarization speed and amplitude. Potassium channel blocker tetraethylammonium added to the medium led to suppression of the VP repolarization phase. It has been also shown that addition of proton pump inhibitor leads to the membrane potential depolarization and to the VP generation inhibition. The results suggest that the VP may be formed not only by proton pump transient suppression but also by appearing of passive ionic fluxes through the membrane.

The work is partly supported by the Russian Foundation for Basic Research (project 13-04-97152).



Action potentials in abscisic acid-deficient tomato mutant generated spontaneously and evoked by electrical stimulation

Macedo F.C. O[1]., Dziubinska H[2]., Trebacz K[2]. and Oliveira R. F[1].
[1] Departament of Biological Science, University of São Paulo, Piracicaba -13418 900 – Brazil. [2] Department of Biophysics, Marie Curie-Sklodowska University, PL-20-033 Lublin, Poland.
email: rfo@usp.br

Action potentials generated spontaneously (SAPs) and evoked by electrical stimulation (APs) in tomato plants (*Solanum lycopersicum* L.) cv. Micro – Tom ABA-deficient mutants (*sitiens* - MT*sit*) and its wild type (MTwt) were characterized by continuous monitoring of electrical activity for 66 h and by application of an electric current supplied extracellularly through Ag/AgCl electrodes, respectively. The tomato plants grew in a room, where the temperature was kept at 22 – 25°C, 40 – 60% humidity, and a photoperiod of 14 h light (white fluorescent lights of 120 µmol m^{-2} s^{-1} PAR at the plant level). The MT*sit* were grown in a covered glass box where the humidity was approximately 70-90%. The experiments were carried out in a Faraday cage. Four measuring electrodes (0.2 mm diameter silver wire) were inserted along the stem or petiole in different arrangements. The reference electrode was inserted into the stem or placed on the ground. The electrodes were connected to a data acquisition system with 4 channels which was connected to a computer using the software "RealView". MT*sit* generated SAPs which spreaded along the stem, including petioles with na amplitude of 66 mV, duration ½ of 33 s and velocity of 6.5 cm min^{-1}. Amplitude and velocity were 47% and 38% higher in MT*sit* than in MTwt, respectively. The largest number of SAPs was registered in the early morning in both genotypes. MT*sit* was less responsive to electrical stimuli. The excitation threshold and the refractory period were greater than MTwt. After stimulation of 8V/4s, an AP was generated in the MTwt and propagated with na amplitude of 21.2 mV and speed of 5.6 cm min^{-1}. Stimuli of lower intensity did not trigger AP in these plants. In MT*sit* an AP was measured after stimulation of 9V/9s and spreaded with an amplitude of 38 mV and speed of 11.5 cm min^{-1}.



Characterization of transcriptome and miRNAome of tomato roots infected with parasitic root-knot nematode (*Meloidogyne incognita*) by high-throughput sequencing

Kumar A. [1],Kaur P.[1], Shukla N.[1], Chauhan R.[2], Joshi G.[1], Chenni V.[3], Heisnam D.[1], Katiyar-Agrawal S.[3], Agrawal M.[1], Goel S. [1], Jagannath J.[1] and Shankar R.[2]
[1]Botany and [3]Plant Molecular Biology Departments, Delhi University, Delhi 110007, India; [2]Computational Biology & Bioinformatics, CSIR-IHBT, Palampur 176061, HP
email: akumar23j@gmail.com

One of the most economically important plant parasitic nematode (PPN) groups is root-knot nematode (RKN, *Meloidogyne* species) because it has a wide-host range. RKN species are obligate biotrophs and it takes between 3-6 weeks to complete their life cycle in the host roots. Roots are invaded by infective juveniles (J2) that migrate within the root to a site where they initiate a feeding site with highly modified host cells. Feeding cells formation is activated by nematode secretions, which causes dramatic morphological and physiological changes in root cells and converts them into nutrient sink.We have taken genome-wide approaches to investigate genes from both tomato and parasitic nematode that are involved in various stages of plant disease development by transcriptome and miRNAome analyses. Fifteen separate libraries for transcriptome and small RNAs comprising both susceptible and resistant tomato cultivars were prepared from various stages of disease development and sequenced using Illumina Hiseq technology. With respect to transcriptome, differential expression analysis revealed that a set of tomato genes were up and down-regulated during various stages of disease development (259 differentially expressed genes; DEGs) as well as in susceptible and resistance responses (68 DEGs). In the miRNAome, out of 337 miRNAs identified, 47 were conserved (belonging to 19 families) and 223 were novel with star sequence and 67 were without star sequence. Digital expression profiling indicated up-regulation of 107 miRNAs in susceptible and 96 miRNAs in resistant cultivars. While down-regulation of 16 miRNAs in susceptible and 13 miRNAs in resistant cultivar was observed. Moreover, 54 miRNAs were upregulated in both susceptible and resistant cultivars, whereas only 4 were down-regulated in both cultivars.We show that a set of genes and miRNAs from tomato are up- and down-regulated at various stages of disease development and these are likely to play an important role in determining the outcome of plant-nematode interaction.



Effect of Carbon Nanotubes on Plant Cell Stress Signaling

Mohamed H. Lahiani[1] and Mariya Khodakovskaya[1]
[1]Department of Applied Science, University of Arkansas at Little Rock, Little Rock, AR, 72204, USA
email: mvkhodakovsk@ualr.edu

Carbon Based Nanomaterials (CBM) are gaining a lot of attention due to their potential application in different fields including electronics, medicine, agriculture and horticulture. It was described recently that carbon nanotubes were able to penetrate roots/seed coats and regulate growth of different plant species. The effects of carbon nanotubes on plant transcriptome and overall plant signaling are poorly understood. We performed analysis of total gene expression (Affymetrix platform) in tomato seeds and plants exposed to carbon nanomaterials. Total transcriptome profiling revealed that carbon nanomaterials can affect tomato genes involved in abiotic/biotic stress responses, signal transduction, cellular responses, transport and plant/water relations. It was found that the number of differently regulated genes in tomato plants exposed to carbon nanotubes were associated with response of plants on biotic stress including pathogen attacks. Data of microarray analysis were confirmed by real-time PCR and contributed to the understanding of mechanism of activation of seed germination and plant growth for seeds/plants treated with carbon nanotubes.



plant Signaling & Behavior

International Symposium on Plant Signaling and Behavior 2014

Beneficial and pathogenic fungi in the rhizosphere: How do roots distinguish?

Ralf Oelmüller
Institute of Plant Physiology, Friedrich-Schiller-University, Dornburger Str. 159, D-07743 Jena

A huge number of beneficial and pathogenic fungi release biomolecules into the rhizosphere which initiate signaling events in the roots leading to mutualistic or pathogenic plant/fungus interactions. The combination and concentration of the individual biomolecules in the rhizosphere is critical for the plant´s decision to invest in either growth or defense. These biomolecules activate receptors in the roots and induce cytoplasmic calcium elevation in a phosphorylation-dependent manner. Furthermore, they stimulate phospholipid signaling and AGC kinase activities which coordinate the balanced response between growth/development and defense, by cross-talking to the Ca^{2+} signals. We use transgenic Arabidopsis and *Nicotiana attenuata* plants expressing the calcium sensor aequorin to isolate and identify biomolecules from the exudates of the beneficial root-colonizing fungi *Piriformospora indica* and *Mortierellahyalina*, and from the pathogenic fungi *Alternaria brassicae* and *Verticillium dahliae*. The exudate componentsinduce a rapid (< 90 s) and transient increase in cytoplasmic calcium levels in the roots. We isolated Arabidopsis non-allelic EMS mutants which do not respond to these biomolecules. Their initial characterization has demonstrated that the calcium response is necessary for the proper plant response to the fungi, and that some mutants are impaired in abscisic acid (ABA) signaling. I will give a progress report on signaling events which allow roots to distinguish between beneficial and pathogenic fungi in the rhizosphere.



The intricate position of GSH, a dynamic signalling molecule, in plant defence signalling network

Sharmila Chattopadhyay
Plant Biology Lab., CSIR-Indian Institute of Chemical Biology, Kolkata-700 032, India

Glutathione (GSH) is a nearly ubiquitous, most abundant non-protein tripeptide thiol compound found in both prokaryotes and eukaryotes. A broad range of functions have been proposed for GSH *in planta*, like redox control, detoxification of heavy metals and electrophilic xenobiotics, etc. The role of GSH in plant defence has also been known in addition to its substantial role in stress tolerance and antioxidant signalling. However, how GSH interact with other established signalling molecules in rather complicated defence signalling network is yet to be known. To this end we tried to understand the interaction of GSH with salicylic acid (SA), jasmonic acid, ethylene and abscisic acid to combat stress. For that a transgenic tobacco line viz. *NtGB*, exhibiting enhanced GSH content as confirmed by HPLC analysis, has been developed. Southern Blot and Western blot analysis further confirmed the integration and protein expression pattern of GOI in *NtGB* lines at T_2 generation. Transcriptomic and proteomic profiling of *NtGB* line identified the genes and proteins altered at enhanced GSH condition and probably regulated by GSH as well. Several SA related genes viz *PR*1, *GLS, MAPKK, NPR*1, etc. and proteins viz. HSP 70, ADC, NBS-LRR, CA, PR10 etc. were identified. QRT-PCR analysis further confirmed the expression level of SA-related genes in *NtGB* line. Interestingly, in addition to SA, other major phytohormones have also been noted to be interactive with GSH. Stress tolerance potential of *NtGB* line displayed significant capacity against salt & draught stress and *Pseudomonas syringae* infection in comparison to wild type.Together, comprehensive transcriptomic and proteomic investigation on *NtGB* line demonstrating the interplay of GSH with established signalling molecules will be discussed.



Regulatory networks involved in plant-pathogen interaction: An omics approach

Subhra Chakraborty
National Institute of Plant Genome Research, JNU Campus, Aruna Asaf Ali Marg, New Delhi-110067, India.
email: schakraborty@nipgr.ac.in, subhrac@hotmail.com

The ultimate phenome of eukaryotic organism is highly sophisticated and is modulated by cellular cross-talks of diverse pathways. Cellular cross-talk is mediated by many genes and proteins in multicellular organisms including plants, and understanding their language may uncover various signaling networks that control and regulate a multitude of biological and physiological processes such as immunity. Patho-stress due to fungi is one of the most crucial environmental constraints on plant growth and development and thus crop productivity. Vascular wilt caused by *Fusarium* is ubiquitous evolutionary and effects crop plants across families. To determine the cellular circuitry that operates in plant immunity during plant-fungal interaction, we have developed and analyzed comparative organ-specific transcriptome and sub-cellular proteome in food crop, chickpea. Towards this, we have ESTablished chickpea unigene dataset (*Ca*Unigene) comprising 2013 genes of immune response pathway, two-third of which represent new chickpea genes hitherto undiscovered. The transcriptomic analyses revealed a large set of non-canonical genes besides many unexpected candidates with known biochemical functions, which have never been associated with immune response. The detailed analysis of the *Ca*EST datasets and global gene expression by microarray revealed diverse and overlapping organ-specific immune-responsive transcript signatures in legumes. Further, the extracellular matrix (ECM) is a dynamic structure that serves as repository for important signaling components and acts as a frontline defense. To better understand the regulatory networks and metabolic pathways involved in plant-pathogen interaction, we developed comparative patho-stress responsive ECM proteome of chickpea. The differentially displayed proteomes revealed more than thousands protein spots. The LC-ESI-MS/MS analyses led to the identification of couple of hundred proteins, presumably associated with cell differentiation, which regulate diverse functions like carbohydrate metabolism, cell wall restructuring, cell defense and rescue, and cell signaling. Network modeling based on our datasets illustrates immune responsive gene regulatory functional modules, wherein translational regulation was found to be one of the major determinants of species-specific adaptation for cell survival. This study may provide new insight into the underlying mechanisms of immunity in plant.



ytosensors and phytoactuators

Volkov A. G. and Markin V. S.
Department of Chemistry, Oakwood University, Huntsville, AL 35896;
Department of Neurology, University of Texas Southwestern Medical Center at Dallas, TX 75390-8813
email: agvolkov@yahoo.com

Plants continuously sense a wide variety of perturbations and produce various responses known as tropisms in plants. It is essential for all plants to have survival sensory mechanisms and actuators responsible for a specific plant response process. Plants are ideal adaptive structures with smart sensing capabilities based on different types of tropisms, such as chemiotropism, geotropism, heliotropism, hydrotropism, magnetotropism, phototropism, thermotropism, electrotropism, thigmotropism, and host tropism.Plants can sense mechanical, electrical and electromagnetic stimuli, gravity, temperature, direction of light, insect attack, chemicals and pollutants, pathogens, water balance, etc. Here we show how plants sense different environmental stresses and stimuli and how phytoactuators response to them. Plants generate various types of intracellular and intercellular electrical signals in response to these environmental changes. This field has both theoretical and practical significance because these phytosensors and phytoactuators employ new principles of stimuli reception and signal transduction and play a very important role in the life of plants.



Involvement of phytochrome A and calmodulin-dependent kinases in suppression of photomorphogenesis in rice seedlings grown in red light with their shoot bottom exposed

Baishnab C Tripathy, Ansuman Roy and Varsha Gupta
School of Life Sciences, Jawaharlal Nehru University, New Delhi - 110067, India
email: bctripathy@mail.jnu.ac.in

Rice seedlings grown on germination paper in red-light (400 µmoles photons m^{-2} s^{-1}) for 48 h having their shoot-bottom exposed had suppressed photomorphogenesis and were deficient in chlorophyll. Seedlings grown under identical light regime having their shoot-bottom covered in vermiculite or in aluminum foil were green and accumulated chlorophyll. Etiolated seedlings having their shoot-bottom exposed when grown in 4 min Red/Far-red cycles for 48 h accumulated Chl demonstrating the reversal of suppression of photomorphogenesis by far-red implicating the involvement of phytochrome. Immunoblot analysis showed the persistence of photo-labile Phytochrome A protein for 48 h in seedlings grown in red-light (400 □mol photons m-2 s-1) with their shoot bottom exposed, suggesting its involvement in suppression of photomorphogenesis in red light. This was further corroborated in *PhyA* mutant (*phyA*) studies that showed *phyA* seedlings grown in red-light having their shoot bottom exposed were green and accumulated chlorophyll. Results demonstrate that red-light-induced suppression of photomorphogenesis, perceived in the shoot bottom, is a red-HIR response of PhyA. Treatment of cytokinin analog benzyladenine to cytokinin-deficient red-light-grown R/V--seedlings resulted in up-regulation of gene expression of both *G*□ and *G*□̃ To probe further, modulators of signal transduction pathway i.e., AlF_3 (G-protein activator) or $LaCl_3$, (Ca^{2+} channel blocker) or NaF (nonspecific phosphatase inhibitor) or calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-nafthalene-sulfonamide were added along with Hoagland solution to the roots of 4-d-old etiolated seedlings, grown on germination paper and transferred to red light. AlF_3, $LaCl_3$, NaF failed to elicit any photomorphogenic response. However, calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-nafthalene-sulfonamide significantly reversed the red-light-induced suppression of photomorphogenesis. Phosphorylation of proteins assayed in the absence or presence of trifluoperazine revealed respective upregulation or down-regulation of phosphorylation of several plastidic proteins in R/V- -seedlings. These results suggest that signal transduction of red-light perceived by shoot bottom to suppress photomorphogenesis is mediated by calmodulin-dependent-protein kinases.



Functional analysis of the blue light sensing cryptochrome 2 from *Brassica* and rice

Khurana J.P.
Interdisciplinary Centre for Plant Genomics & Department of Plant Molecular Biology,
University of Delhi South Campus, New Delhi -110021, India
email: khuranaj@genomeindia.org

Photomorphogenically active light is principally absorbed by 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. Most of our understanding on how these sensory photoreceptors function has emerged through molecular genetic analyses of *Arabidopsis* mutants, the yeast two-hybrid analysis and whole genome microarray profiling. However, despite the fact that light has a profound effect on plant growth and productivity and manipulating these sensory receptors can have far reaching implications, relatively little work has been done on crop plants. In the past nearly 15 years, our group has made concerted efforts to understand the role of blue light sensing 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* being an allotetraploid harbors 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. CRY2 from rice also exhibits essentially similar characteristics as in *Brassica*. However, its over-expression in both rice and *Arabidopsis* causes constitutive photomorphogenesis (both in light and dark) and early flowering under long day conditions. Microarray analysis has revealed that quite a few of the light-regulated genes are in fact turned-on even when the CRY2 over-expressing transgenic seedlings are grown in dark. These observations are unique since *Arabidopsis* CRY2 over-expression does not confer such a phenotype. 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.



plant Signaling & Behavior

International Symposium on Plant Signaling and Behavior 2014

Volatile ether induces ROS/calcium spikes and light escape-like tropism of roots

Ken Yokawa, Saskia Gall, Tomoko Kagenishi, Matthias Weiland and František Baluška
IZMB, University of Bonn, Kirschallee 1, 53115 Bonn, Germany
email: yokawa@uni-bonn.de

Stressed plants emit volatile infochemicals such as ethylene and methyl jasmonate which are used not only for cell-cell communication but also for long-distance plant-plant signaling, affecting plant ecosystem. Although many studies reported that roots emit diverse volatiles into the rhizosphere, little is known how these volatiles affect growth, tropic movements and physiological responses of roots. Here, the treatment of gas-phase ether in the darkness induced the light-escape behavior of maize roots. Interestingly, the number of root hair was increased in dark-grown and ether-exposed Arabidopsis roots even in the hair-less *rhd2-4* mutant, which lacks the NADPH oxidase necessary for root hair formation. Generation of reactive oxygen species and Ca^{2+} signals were observed after the exposure of Arabidopsis and maize root cells to ether. Finally, ether is also inducing escape tropism of maize roots. Our data indicate that stress-induced ether released from roots might be relevant in soil as an alarm rhizosphere signal, inducing escape and avoidance tropisms.



Environmental phosphorus mediates glucosinolates in mustard plants

Karounos C., Miller D., Smith J.J. and McLetchie N.
Department of Biology, University of Kentucky, Lexington, Kentucky. U.S.A
email:ckaroun@gmail.com

Phosphorus deficient soils cover 30% of the world's usable land. We investigated a possible role of the secondary metabolite glucosinolate, produced by *Brassica rapa* (field mustard) in phosphorus poor soils. Glucosinolates are biomedically relevant as they lower cancer rates in humans and are ecologically important as they ward off competitors via allelopathy. Our experiments assayed root responses of both *B. rapa* and a natural competitor, *Lolium perenne* (ryegrass). By measuring root growth, shoot growth and number of lateral root formations, we determined shoot and root behavior and architecture in response to glucosinolates. We hypothesized that glucosinolates inhibit root growth, shoot growth, and lateral formation, in *L. perenne* but not in *B. rapa*. Past research using *B. rapa* in low phosphorus conditions indicate that glucosinolate biosynthetic processes are co-expressed with phosphorous use efficiency, suggesting that *B. rapa* up-regulates glucosinolate production despite nutrient stress and greater cost under nutrient deficiency. Phenethyl isothiocyanate is the most common glucosinolate produced by *B. rapa*. We applied phenethyl isothiocyanate to *L. perenne* competing with a *B. rapa* plant and found that it inhibited lateral root branching, root growth and shoot growth. In *B. rapa*, phenethyl isothiocyanate did not significantly inhibit shoot growth or lateral branching but slightly inhibited root growth. Previous research suggests a self-inhibitory effect of glucosinolates, however the low degree of inhibition and the lack of inhibition of shoot and lateral roots suggest *B. rapa* showed partial resistance. In *L. perenne*, roots were completely stunted suggesting necrosis or apoptosis. We believe our findings present evidence linking ecological strategy to both RNA transcript and metabolite levels and we propose an ecological explanation to the up-regulation of glucosinolate biosynthetic processes in low phosphorus conditions. Future work will identify the effects of glucosinolates on the root cap, meristem, transition zone and elongation zone in *L. perenne*.



Update on the Darwin's 'Root Brain' – Oscillatory Command Centre

František Baluška and Stefano Mancuso
IZMB, University of Bonn, Kirschallee 1, 53115 Bonn, Germany
LINV – DiSPAA, Department of Agri-Food and Environmental Science, University of Florence, Sesto Fiorentino, Italy
email: baluska@uni-bonn.de

The plant root apex acts as the most prominent plant sensory organ; sensing diverse biotic and abiotic parameters. However, the motoric responses to these sensory stimuli are accomplished in the sub-apical rapid cell elongation region. This spatial discrepancy has been solved when we have discovered and characterized the transition zone. Cells of this zone are very active in the cytoskeletal rearrangements, endocytosis/endocytic vesicle recycling, and in electric activities. The oscillatory nature of the transition zone suggests that it acts as some kind of command center. Our recent data reveal that Glutamate-like Receptors (GLRs) and plant synaptotagmins control synaptic activities in the transition zone cells of Arabidopsis roots. In accordance with the early proposal of Charles and Francis Darwin, cells of this root zone receive sensory information fromthe root cap and instruct the motoric responses of cells in the elongation zone. Here we discuss the most recent data and discoveries supporting the Darwin's 'Root Brain' theory.



Role of *Arabidopsis thaliana* myosins in gravotropism of stems

Talts K., Ojangu, E.-L., Ilau B., Dolja V.*, Paves H.
Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 12618, Estonia; *Department of Botany and Plant Pathology, Oregon State University
email: heiti.paves@ttu.ee

The positioning and dynamics of vesicles and organelles, and thus the growth of plant cells, is mediated by the acto-myosin system. In *Arabidopsis* there are 13 class XI myosins which mediate vesicle and organelle transport in different cell types, and 4 class VIII myosins with unknown function. Plant stems respond to gravitropic stimulation with a rapid, local and reversible change in cell growth rate. Involvement of myosins in gravitropic response of *Arabidopsis* stem was examined using a simple time lapse assay on T-DNA single and multiple mutant lines. Gravitropic response of all 17 different single myosin mutant lines was undistinguishable from behavior of wild type *Arabidopsis* stems after gravitropic stimulation. Decrease of amplitude and speed of stem upwards bending became evident in triple, quadruple, and quintuple mutants of class XI myosins. We have shown before that the class XI myosins (XI-K, XI-1 and XI-2) have partially redundant roles in the growth of shoot epidermis. Results of the gravitropism study suggest that these myosins have overlaping functions also in gravitropic behavior of the stems. Stem gravitropic response of quadruple mutant of class VIII myosins was identical to that of wild type plants, indicating that *Arabidopsis* stems are able to bend upwards without participation of class VIII myosins.



Functional Genomics of Thermal Tolerance in Wheat

Paramjit Khurana
Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi-110021, India.
email: param@genomeindia.org

Climate change and abiotic stress affects agriculture and crop production adversely. Wheat is a temperate crop and prone to various abiotic stresses therefore mapping and characterizing ESTs offers a manageable approach to understand the complex architecture and functioning of the wheat transcriptome.Tolerance to heat stress is a complex phenomenon and controlled by multiple genes, hence a program on functional genomics of heat stresshas been initiated to understand the transcriptional profile of the sensitive and tolerant cultivars of wheat under heat stress by subtractive hybridization, both at the vegetative and reproductive stages. Heat tolerance related gene transcripts were identified based on their putative functions and validated by cDNA macroarray and northern/RT-PCR analysis, with the aim to unravel the complexity associated with heat stress response in wheat. HSP encoding genes are found to be under the control of Heat Shock Factors (HSF), transcription factors that regulate the expression of HSP genes. One such gene, a chloroplastic small heat shock protein (sHSP26) was found essential for tolerance to heat stress. Transgenic *Arabidopsis* plants over-expressing wheat HSP26 were more tolerant to high temperature induced damage to the photosynthetic machinery, and the antisense transgenic plants were found to be severely sensitive to heat stress.Similarly, a newly identified heat shock factor (HSF) possesses the characteristic domains of class A type plant HSFs and shows high similarity to rice OsHsfA2d. The transcription factor activity of TaHsfA2d was confirmed through transactivation assay in yeast. Transgenic *Arabidopsis* plants overexpressing TaHsfA2d not only possess higher tolerance towards high temperatures but also showed considerable tolerance to salinity stress. They also showed higher yield and biomass under constant heat stress conditions. Analysis of putative target genes through quantitative RT-PCR showed higher and constitutive expression of several abiotic stress responsive genes in transgenic *Arabidopsis* plants over-expressing TaHsfA2d. TaHsfA2d can also functionally compliment the T-DNA insertion mutants of AtHsfA2, although partially. These observations suggest that TaHsfA2d may be useful in molecular breeding of crop plants, especially wheat, to improve yield under abiotic stress conditions.



Compound leaf development in legume

Rujin Chen, Jianling Peng, Liangfa Ge, Yuan Wang, Yuhui Chen
Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, Oklahoma 73401, USA
email: rchen@noble.org

As the primary organs of photosynthesis and transpiration, plant leaves play important roles in growth, survival and production of plants. In nature, diverse leaf forms exist, reflecting the origin and early diversification of land plants. However, the underlying mechanisms are largely not clear. Previous studies have shown that the class I knotted-like homeodomain transcription factor proteins (KNOXIs) are required for the development and maintenance of the shoot apical meristem (SAM). Development of lateral organs from the periphery of SAM requires downregulation of KNOXI proteins in the incipient sites of lateral organ primordia. In simple leafed species such as *Arabidopsis thaliana*, *KNOXI* downregulation is permanent. On the other hand, in compound leafed species such as tomato (*Solanum lycopersicum*) and *Cardamine hirsuta*, *KNOXI* genes are initially downregulated and subsequently reactivated in leaf primordia. Extensive expression and genetic studies confirm that KNOXI proteins are required for compound leaf development in tomato, *C. hirsuta* and many other eudicot species. Surprisingly, in pea (*Pisum sativum*), *Medicago* and others, which belong to the so-called Inverted Repeat Lacking Clade (IRLC) of legumes (Fabaceae), *KNOXI* genes are not expressed in leaf primordia and therefore not likely associated with compound leaf development. Instead, previous studies have shown that *FLORICAULA/LEAFY* orthologs, *UNIFOLIATA* (*UNI*) and *SINGLE LEAFLET1* (*SGL1*) plays a key role in compound leaf development in pea and *Medicago*, respectively. Other important regulators of compound leaf development, including *FUSED COMPOUND LEAF1*, *PALMATE-LIKE PENTAFOLIATA1*, *MtPINFORMED10*, *ELONGATED PETIOLULE1* and *MtPHANTASTICA*, have been recently identified from *M. truncatula*, providing some new insights on the regulatory mechanisms that underlie compound leaf development in the legume.



Reactive oxygen species (ROS)-mediated growth and gravisensitivity of embryonic axes during germination of *Vigna radiata* seeds

Singh, K.L., Mukherjee, A. and Kar, R.K.
Department of Botany, Visva-Bharati University, Santiniketan 731235, West Bengal, India
email: rupkumar.kar@visva-bharati.ac.in

Seed germination starts with imbibition and culminated with the onset of growth of the embryonic axes. This earliest spell of growth is initiated under subtle regulation after breaking the rest of quiescent or dormant seed. We have tried to characterize early axis growth and understand its regulation during germination of *Vigna radiata* seeds. Early phase of growth is mainly due to cell elongation in the undifferentiated axis leading to radicle emergence. However, the first spell of growth is gravisensitive and gravity-induced curvature occurred in the upper part of axis which is distinct from elongation zone of radicle. Cell elongation, either in the curved region or elongation zone of axis, is correlated with accumulation of superoxide (as revealed by NBT staining) supporting the positive role of reactive oxygen species in plant growth and development including seed germination. Auxin plays a role only in bending growth of axis, since TIBA and NPA, known auxin transport inhibitors, could inhibit such gravity-induced bending but were ineffective for germination. NAD(P)H oxidase located in plasma membrane is mainly ascribed for apoplastic generation of superoxide that may be further metabolized to other ROS molecules (H_2O_2 and OH.) that may participate in growth processes. Auxin mediates its effect on gravisensitive growth through ROS as both TIBA and NPA treatment precludes $O_2^{\cdot-}$ accumulation in the corresponding zone. Cytosolic calcium level (Ca_{cyt}) that can act as a signal may be involved in bending response and Ca_{cyt} comes from apoplast not from endosomal stores as $LaCl_3$ and EGTA but not LiCl could inhibit bending response as well as $O_2^{\cdot-}$ accumulation (as shown by NBT staining). In-gel assay for NAD(P)H oxidase showed two different sets of bands against NADPH and NADH separately. A possibility of involvement of two different NAD(P)H oxidases in auxin-mediated bending growth and radicle tip growth is being explored.



Regulation of cell polarity and pattern formation in plants

Shaul Yalovsky, Ora Hazak, Daria Bloch, Hasana Sternberg
Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978 Israel
email: shauly@tauex.tau.ac.il

Cell polarity is a basic entity of leaving organisms and involves spatial organization of the cytoskeleton and cellular membranes. In plants, a unique mechanism for the directional transport and asymmetric distribution of the phyto-hormone auxin links cell polarity and pattern formation. In eukaryotes, Rho superfamily small G proteins orchestrate cytoskeleton organization, membrane dynamics and have been implicated to play central roles in the regulation of cell polarity. Work carried out in our laboratory over the last fifteen years has demonstrated the involvement of ROPs, the plant-specific Rho family proteins, in regulation of cell polarity and auxin dependent patterning. In my talk I will present our current understanding of ROP function and present some new unpublished data on ROP signal transduction and its interaction with other signaling cascades in the cell.



plant Signaling & Behavior

International Symposium on Plant Signaling and Behavior 2014

Guard cell alkalization precedes the increase in reactive oxygen species or Nitric Oxide during stomatal closure by Plant Hormones as well as Microbial Elicitors

Agepati S. Raghavendra, Gunja Gayatri, Srinivas Agurla and Mallikarjuna Rao Puli
Department of Plant Sciences, School of Life Sciences,
University of Hyderabad, Hyderabad 500 046, India
email: asrsl@uohyd.ernet.in

Stomatal closure is an effective adaptation to restrict H_2O loss as well as the entry of pathogens into leaves. Guard cell signal transduction leading to stomatal closure is a complex phenomenon, involving signaling components, such as cytosolic pH, reactive oxygen species (ROS), nitric oxide (NO), free Ca^{2+} and phospholipids, besides ion-channels. We have been examining the components of signal transduction during stomatal closure induced by plant hormones, abscisic acid (ABA) or methyl jasmonate (MJ) and fungal elicitors (chitosan). Use of fluorescent dyes illustrated an increase in not only ROS or NO but also the cytoplasmic pH in guard cells. Butyrate (a weak acid) reduced the cytosolic pH/ROS/NO production and prevented stomatal closure by ABA. Methylamine (a weak base) enhanced the cytosolic alkalization and aggravated stomatal closure by ABA. The kinetics of changes in cytosolic pH, ROS or NO suggested that the sequence of events in guard cells in response to ABA or MJ or chitosan is the rise in pH, followed by that of ROS and then NO. Both ROS and NO are known to elevate cytosolic Ca^{2+}, which in turn can promote the efflux of K^+, Cl^-, decrease guard cell turgor, and cause stomatal closure. Besides the direct influence of pH on ROS or NO levels, it is possible that these components exert interactive effects. The effects of ROS or NO can be enhanced at alkaline pH. The interrelationships and interactions of cytosolic pH, with NO, ROS and free Ca^{2+} need further detailed examination. We propose that besides ROS and NO, the cytosolic pH is an important secondary messenger in guard cells and even otherplant tissues. Stomatal guard cells are excellent model systems to evaluate the role of cytosolic pH in modulating the events of signal transduction.



Spatio-temporal dynamics of subcellular localizations of PIN-FORMED auxin transporters

Ganguly A., Park M., Kesawat M.S., Lee R.D., and Cho H.-T.
Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea
email: htcho@snu.ac.kr

Different members of PIN-FORMEDs (PINs) contribute for inter- and intracellular auxin transport depending on their distinctive subcellular localizations. PINs with a long hydrophilic loop (HL) (PIN1~4 and 7, 'long PINs') localize predominantly to the plasma membrane (PM) whereas short PINs (PIN5 and 8) were shown to localize to internal compartments. However, the subcellular localization of the short PINs has been observed in limited cell types, and the role of the HL in PIN trafficking has yet to be deciphered in more detail. In this study, we tested whether long PIN's HL can provide its original phosphorylation and trafficking nature to the short PIN by adopting transplantation of the HL. The results showed that the transplanted long PIN-HL was sufficient for phosphorylation and PM-trafficking of the chimeric short PIN but failed to provide the short PIN with the long PIN's polarity. On the other hand, unlike previous observations, PIN5 (a short PIN) showed evident PM-localization in diverse cell types where PIN5 is originally or ectopically expressed, or even polar PM-localization in certain cell type. Furthermore, in the root epidermal tissue, the subcellular localization of PIN5 switched from the PM to internal compartments according to the developmental stage. Our results suggest that the long PIN-HL is partially modular for the trafficking behavior of PINs and that the intracellular trafficking of PIN is plastic depending on cell type and developmental process.



plant Signaling&Behavior

International Symposium on Plant Signaling and Behavior 2014

Photomodulation of strigolactone accumulation and physiological effect of associated biomolecules during sunflower seedling growth

Bharti N., Tripathi S. and Bhatla S.C.
Laboratory of Plant Physiology and Biochemistry, Department of Botany, University of Delhi, Delhi-110007, India
email: bharti.niharika@gmail.com; sm.trip@gmail.com; bhatlasc@gmail.com

Strigolactones (SLs) were originally identified as germination stimulants for root parasitic plants and are now established as plant hormones which control various developmental and physiological responses. Plants produce a mixture of SLs and the amount and type of SLs differ according to species and age. To date, more than 20 SLs have been characterized and more are being discovered. Most of the SLs characterized so far have been obtained from root exudates. SLs are classified as terpenoid lactones and their biosynthesis is facilitated by carotenoid cleavage dioxygenase 7 (CCD7) and carotenoid cleavage dioxygenase 8 (CCD8) which cleave 9-cis-β-carotene and 9-cis-β-apo-10′-carotenal, respectively. Present findings highlight the effect of light on SL accumulation in sunflower seedling roots and cotyledons. CCD activity and SL content have been estimated in roots, cotyledons and leaves of sunflower seedlings in the absence and presence of light. Light-grown seedlings consistently exhibited higher CCD activity than dark-grown ones in all tissue systems investigated. The enzyme activity was also more in roots as compared to cotyledons. The enzyme activity in roots decreased with age. SL was not detected in cotyledons and roots of 2-d-old light and dark-grown seedlings. SL accumulation was always more (approx 5 times) in roots of light- grown seedlings. Maximum SL content was observed in 4-d-old roots of light-grown seedlings. SLs were also detected in first pair of leaves of the sunflower seedlings. More than one type of SLs are likely to be present in leaves and root tissue. Present work further investigates the probable cross-talk events occuring between strigolactone, ethylene and auxin. This has been achieved by pharmacological observations obtained from lateral root initiation and hypocotyl elongation in 6-d-old seedling roots subjected to various treatments. CLSM localization of PIN1 elucidates the effect of several treatments on auxin gradient in the elongation zone of primary roots obtained from 2-d-old seedling.



Pro-rich receptor-like kinase RHS10 may transduce cell wall signals to inhibit root hair tip growth in angiosperms

Lee R.D., Lee Y.S., Lin C., Park M., and Cho H.-T.*
Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea
*email: htcho@snu.ac.kr

Root hairs are tip-growing tubes that emerge from the root trichoblast cell. Arabidopsis root hairs have been a model system for studying the mechanisms of cell morphogenesis. In plants there are hundreds of receptor-like protein kinases (RLKs) which are involved in diverse development processes. So far, few RLKs have been implicated in root hair morphogenesis. The RHS10 (AT1G70460) RLK has a Pro-rich N-terminal region and is described as a member of the PERK (Pro-rich extensin-like receptor kinase) family. As PERKs have extensin-like motifs, the extracellular domain of RLKs might mediate cell wall-related signals. We characterized the RHS10 function in the root hair system by analyzing the extensin domain, functional orthologous relationship, ROS (reactive oxygen species)-mediated signaling, and the root hair growth rate. The RHS10 loss-of-function mutant grew longer root hairs than wild type, whereas its overexpression greatly inhibited root hair growth. The serial deletion analysis of the RHS10 extensin domain, using the root hair assay system, showed that a minimum number of Pro residues are required for RHS10 to inhibit root hair growth. Root hair-specific overexpression of rice and poplar RHS10 orthologs in Arabidopsis considerably inhibited root hair growth as seen with AtRHS10 overexpression, indicating that the RHS10 function in root hair development has been conserved in angiosperms. ROS has been known to be necessary for root hair tip growth. Consistently with its hair-inhibitory function, RHS10 overexpression reduced ROS accumulation in the root. Root hair growth rates of the *rhs10* mutant were considerably higher in the late stage than the wild type plant, suggesting that RHS10 probably modulates the saturation point of root hair growth. The next challenge for the molecular mechanism of the RHS10 RLK in root hair growth will be to identify the probable ligand and the downstream targets.



plant Signaling&Behavior

International Symposium on Plant Signaling and Behavior 2014

Understanding the role of jasmonic acid signalling molecules in nickel tolerance mechanism during seedling growth in *Glycine max* L.

Geetika Sirhindi*, Mudaser Mir Ahmed and Poonam Sharma
Department of Botany, Punjabi University, Patiala – 147 002, Pb., India
email: geetika@pbi.ac.in

Soyabean (*Glycine max.* L.) seedlings subjected to heavy metal stress exhibit a decrease in extension growth of 15 day old seedlings. Exogenous application of lower concentrations of jasmonic acid (JA; 1 µM and 1 nM) showed an integrative role with 2 mM nickel (Ni^{+2}) ions as stress signals to *G. Max* L. seedlings. Presence of JA deteriorates the growth of seedlings in absence of any stress signals. However, presence of stress signals in the environment helped in revealing the protective potential of JA but in very dose dependent manner. Presence of Ni in environment imposed detrimental effects on metabolic mechaniary of the seedlings as chlorophyll content decreased to its lowest level in such environment. Supplementation of different concentrations of JA helped in modulating and protecting the photosynthetic metabolic components of seedlings from harmful effects of Ni^{+2} ions. JA performed its protective role by reallocating the sugars and photosynthetic pigments along with upregulating the antioxidant enzymes and accumulation of osmolytes in the cell. The foremost enzymes showed upregulation in their activity by JA were peroxidises and ascorbate peroxidise (APOX) in particular followed by superoxidase dismutase (SOD). In present findings it can be concluded that JA is an oxylipin signal molecule integrate with Ni^{+2} and control the downstream stress response by triggering and regulating the biochemical reactions responsible for enhancing the antioxidant activities in *G. max* L. 15 day old seedlings but in very dose manner.



The role of Melatonin and Serotonin in circadian rhythm of Characeae *Chara australis*

Mary J Beilby[1], Christina Turi[2] and Susan Murch[2]
[1] School of Physics, Biophysics, The University of NSW, Sydney, NSW 2052, Australia
[2] University of British Columbia, Okanagan Campus, 3333 University Way, Kelowna, British Columbia V1V 1V7, Canada

Melatonin (MEL) acts as circadian rhythm indicator and antioxidant in animal systems. Serotonin (SER), an animal neurotransmitter, forms part of the same metabolic pathway. Recent experiments suggest that MEL performs similar functions in plants. MEL and SER are closely related by structure to growth hormone IAA. Our experiments are performed at single cell level in giant-celled Characeae, sister plants to ancestors of land plants. Characeaeproduce endogenous MEL and SER in similar amounts to leaf and shoot portions of higher plants. They respond to exogenous MEL by an increase in quantum yield of photosystem II. To explore the role of MEL in circadian rhythm, the apex cells of plants acclimated to 12/12 hours light/dark regime were harvested every hour for 24 hours and analyzed for MEL, SER and IAA. MEL and SER exhibited distinct concentration peaks 4 hours after light-on. IAA concentration increased at the about the same time and continued to rise for another 3 hours. There was also a sharp peak in MEL concentration shortly after light-off at 6 pm. Plants pre-treated in continuous darkness for 3 days exhibited similar peaks in MEL and SER concentration with IAA following closely. Plants pre-treated in continuous light for 3 days, showed diminished concentration changes of MEL and SER, while IAA concentration oscillated with shorter period. The MEL concentration peak in the dark was not observed in continuous light or dark. The persistence of MEL concentration peak under dark treatment suggests its involvement in circadian mechanism, which can be analyzed further in future experiments.



Mapping the trafficking pathways of PIN1 with engineered BFA-resistant ARF-GEFs in *Nicotiana tabacum*

Jelínková A., Müller K., Pařezová M. and Petrášek J.
Institute of Experimental Botany AS CR, 165 02 Prague 6, Czech Republic
email: jelinkovaa@ueb.cas.cz

The processes of vesicle trafficking play an important role in the targeting of PIN-FORMED1 (PIN1) auxin efflux carriers to the plasma membrane (PM). They were mostly characterized in *Arabidopsis thaliana*. The goal of this *in vivo* study is to characterize the subcellular trafficking pathways of *Arabidopsis thaliana* PIN1 in BY-2 tobacco cells, good model for auxin transport assays. Combined treatments with brefeldin A (BFA) and endocytosis inhibitors were used and their impact on the uptake of endocytic tracer FM4-64 and PIN1-GFP distribution followed microscopically. Inhibitors of cytoskeletal dynamics were used to determine the cytoskeletal requirements of these processes. Our results showed that BFA, potent inhibitor of adenosine ribosylation factor (ARF)-guanine exchange factors (GEF)-dependent anterograde membrane trafficking in *Arabidopsis thaliana*, inhibits endocytosis in tobacco BY-2cells. These results are supported by the fact that all *Nicotiana tabacum* ARF-GEFs contain in their Sec7 domain Met at the position that has been shown previously to confer sensitivity of these GEFs to BFA. The absence of BFA-resistant ARF-GEFs in tobacco makes BY-2 cells favourable model for studying ARF-GEF endomembrane trafficking in plants. By inserting point mutation into the Sec-7 domain of individual tobacco ARF-GEFs it is possible to generate BY-2 cell lines carrying pathway-specific BFA resistance after their inducible overexpression. We show here that point mutation in the Sec-7 domain of the close homolog of *Nicotiana tabacum* BFA-GNOM-LIKE protein 1 (NtGNL1) confer pathway-specific BFA resistance in *Ntgnl1*^{M683L} lines. The endocytosis of FM4-64 and PIN1-GFP was increased rapidly upon inducible overexpression of *Ntgnl1*^{M683L}allele in contrast to control *NtGNL1* allele. These observations uncovered preferential role of this NtGNL1 homolog in the endocytosis of PM proteins, including PIN1. In addition, they demonstrate the potential of this tobacco BY-2 system for selective mapping of ARF-GEF-regulated endomembrane trafficking pathways.

Supported by the Grant Agency of the Czech Republic, project GPP305/11/P797.



Boron deprivation inhibits root cell elongation via an ethylene/auxin/ROS-dependent pathway

Martín-Rejano E. M., Camacho-Cristóbal J. J., Herrera-Rodríguez M. B., Navarro-Gochicoa M. T., Rexach J. and González-Fontes A.
Departamento de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide, E-41013 Sevilla, Spain
email: agonfon@upo.es

Modifications in root architecture can be essential to take up limiting nutrients in soils. In *Arabidopsis thaliana* seedlings, it has been described that boron deficiency significantly decreased primary root elongation when compared to control treatment. The aim of this work was to study in detail the temporal responses of arabidopsis roots to low boron supply. For this, several arabidopsis reporter lines and mutants with impaired auxin and ethylene signaling were used. Seedlings were grown in 10 µM boron for 5 days and then transferred to a boron deficient medium (0 µM) or control medium (10 µM) during 4-5 h. Root cell elongation were measured using the length of the first epidermal cell with a visible root hair protuberance (LEH), a parameter that reflects rapid effects on elongation much more sensitively than macroscopic root length measurements. Quantitative RT-PCR of the expression levels of several aminocyclopropane-1-carboxylic acid synthase (ACC synthase, ACS) gene isoforms and histochemically analyses by using pACS::GUS lines, suggest an enhancement in ACC and/or ethylene synthesis in arabidopsis roots under boron deficiency. LEH of the ethylene-insensitive mutant *ein2-1* was less sensitive to boron deprivation than that of wild-type plants. All these results show that the rapid reduction of cell elongation triggered by boron deficiency is mediated, in part, by an ethylene-dependent pathway. Known the interaction between ethylene and auxin, experiments were conducted to analyze the possible involvement of auxin in the rapid inhibition of root cell elongation elicited by boron deprivation. Using the auxin reported lines and two arabidopsis mutants affected in basipetal auxin transport, we concluded the involvement of this basipetal transport in the inhibition of root cell elongation under B deficiency, which was supported by using PEO-IAA, a synthetic antagonist of the auxin receptor function. In addition, staining experiments performed with detectors of ROS suggest that boron deprivation could cause an oxidative damage and cell death in the root elongation zone.

Research supported by Ministerio de Economía y Competitividad (BFU2012-37445) and Junta de Andalucía (BIO-266 and P09-CVI-4721), Spain.



The glucosinolate breakdown product Indole-3-Carbinol acts as an auxin antagonist in roots of *Arabidopsis thaliana*

Ella Katz[1], Sophie Nissani[1], Marcelo Ehrlich[2], Melkamu Woldemariam[3], Georg Jander[3], and Daniel A. Chamovitz[1]
[1]Department of Molecular Biology and Ecology of Plants, and [2]Department of Cell Research and Immunology, Tel Aviv University, Tel Aviv, ISRAEL; [3]Boyce Thompson Institute for Plant Research, Ithaca, NY, USA
email: dannyc@tauex.tau.ac.il

Indole-3-Carbinol (I3C) is a phytochemical produced endogenously in cruciferous vegetables as one of the autolysis product of glucosinolates. I3C is synthesized upon tissue rapture, and in leaves this acts to deter herbivore insects. In mammals, I3C has anti-carcinogenic properties. However little is known about the direct effects of I3C in general, and in plants specifically. To explore the mechanism by which I3C influences plants, we are using Arabidopsis roots as a model system. Exogenously supplied I3C inhibited root-elongation within 30 minutes of application. The I3C taken up by the roots was not metabolized into other compounds indicating a direct effect of I3C. This inhibition was accompanied by changes in the expression of ~300 genes, including many auxin-induced genes. Accordingly, we observed reduced auxin signaling in the root apical meristem, suggesting that I3C acts as an auxin antagonist. This hypothesis was supported by experiments examining the responses to simultaneous application of both auxin and I3C. Thus our results suggest that I3C is a novel phytohormone that modulates auxin signaling.



Plant communication by volatiles: mechanisms and environmental interference

James D. Blande

Department of Environmental Science, University of Eastern Finland, Kuopio 70211, Finland

email: james.blande@uef.fi

It is well established that plants emit a plethora of volatile chemicals that reflect their physiological and phenological condition. These chemicals are available for interpretation to a range of cohabiting organisms including herbivorous and predatory arthropods and neighbouring plants. During the last thirty years, substantial evidence has been accumulated to support the broad hypothesis that plants exposed to volatile signals from their damaged-neighbours have enhanced defences. However, the mechanisms underlying the plant responses have been difficult to disentangle. In our recent studies we have observed both active and passive roles for volatiles compounds transferred from damaged to undamaged plants. The active process involves the receiving plant detecting and responding to the volatile signal, which often results in the receiving plant being primed to respond to attack with a more vigorous than usual defence response. The passive process simply involves the volatile compounds emitted by damaged plants adsorbing to the surfaces of undamaged neighbours and altering the receiver plant's interactions with its antagonists. These two processes can co-occur and may have either similar or opposite effects on a plant's defences. One of the most controversial issues related to plant-plant interactions mediated by volatiles is the ecological significance of the process. Most studies conducted under field conditions have only found effective interactions over short distances. In our research we have shown that atmospheric pollutants, such as ground-level ozone, can reduce the distance over which volatile-mediated plant-plant interactions occur. The most likely reason is the degradation of the volatiles before they provide an effective stimulus to the receiver plant. In this talk I will present our recent work on plant-plant interactions with a focus on the co-occurrence of active and passive processes. I will also give an overview of how atmospheric pollutants can interfere with the process with ozone as an example.



plant Signaling&Behavior

International Symposium on Plant Signaling and Behavior 2014

The volatilome of a nursery for seeds and wasps: why do plant signals change?

Renee M. Borges

Centre for Ecological Sciences, Indian Institute of Science

email: renee@ces.iisc.ernet.in

Plants produce volatile organic compounds (VOCs) in a variety of contexts that include response to abiotic and biotic stresses, attraction of pollinators and parasitoids and repulsion of herbivores. Some of these VOCs may also exhibit diel variation in emission. In the nursery pollination system of *Ficus racemosa*, we examined variation in VOCs released by fig syconia throughout syconium development and between day and night. Syconia are globular enclosed inflorescences which serve as developing nurseries for pollinating and parasitic fig wasps. Syconia are attacked by gallers early in their development, serviced by pollinators in mid phase, and are attractive to parasitoids in response to the development of gallers at later stages. VOC bouquets of the different development phases of the syconium were distinctive, as were their day and night VOC profiles. Diel patterns of release of volatiles could not be correlated with their predicted volatility as determined by Henry's law constants at ambient temperatures. Therefore factors other than Henry's law constant such as stomatal conductance or VOC synthesis must explain diel variation in VOC emission. Using an adaptation of Weighted Gene Co-Expression Network Analysis (WGCNA) that is used in proteomics, we found that the volatilome had seven distinct modules of co-emitted VOCs that could be interpreted on the basis of syconium ecology. Some modules were characterised by the response of fig syconia to early galling by parasitic wasps and consisted largely of green leaf volatiles (GLVs). Other modules, that could be characterized by a combination of syconia response to oviposition and tissue feeding by larvae of herbivorous galler pollinators as well as of parasitized wasps, consisted largely of herbivore-induced plant volatiles (HIPVs). Only understanding the biology of this nursery can help in making sense of the volatilome produced by the syconia at different stages and diel phases of their development. Future studies will target the expression of key genes of the volatilome.



Plant bioacustic – a new frontier in plant communication

Stefano Mancuso[1], Frantisek Baluska[2], Ana Rodrigo Moreno[1]
[1] LINV – DiSPAA, Department of Agri-Food and Environmental Science, University of Florence, Sesto Fiorentino, Italy
[2] IZMB, University of Bonn, Kirschallee 1, 53115 Bonn, Germany
email: stefano.mancuso@unifi.it

Vibrations travelling through air and substrate are fundamental in animal communication. In plant, germination and growth, as well as defense, can be influenced by sounds. However, the physiological and ecological significance of these many responses to sound in plants is still unknown. Recently, we demonstrated that young corn roots grow toward the source of continuous tone around 200-300 Hz; the same range of frequencies emitted by growing roots and by many natural phenomena as, for example, the running water or the insect feeding.
We discuss here the most recent data supporting the role of acoustic vibration as a new and important long distance signaling mechanism in plants.



Say it with flowers: flowering acceleration by root communication

Falik, O., Hoffmann, I. and Novoplansky, A.
Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus 84990, Israel
email: anovopla@bgu.ac.il

The timing of reproduction is a critical determinant of fitness, especially in organisms inhabiting seasonal environments. Increasing evidence suggests that inter-plant communication plays important roles in plant functioning. Here, we tested the hypothesis that flowering coordination can involve communication between neighboring plants. We show that soil leachates from *Brassica rapa* plants growing under long-day conditions or treated with GA accelerated flowering and decreased allocation to vegetative organs in target plants growing under non-inductive short-day conditions. The results suggest that besides endogenous signaling and external abiotic cues, flowering timing may involve inter-plant communication, mediated by root exudates. Flowering communication (FC) is expected to be common where synchronized flowering predictively increases fitness via e.g. increased pollinator attraction and outcrossing probability, decreased probability of seed predation, or improved seed dispersal; however, the degree and prevalence of FC are hypothesized to depend on multiple evolutionary and ecological factors. The study of flowering communication is expected to illuminate neglected aspects of plant reproductive interactions and to provide novel opportunities for controlling the timing of plant reproduction in agricultural settings.



Interactions of the plant cytoskeleton with membranes

Patrick J. Hussey
School of Biological and Biomedical Sciences, University of Durham, South Road, Durham. DH1 3LE UK
email: p.j.hussey@durham.ac.uk

Complex animals use a wide variety of adaptor proteins to produce specialized sites of interaction between actin and membranes. Plants do not have these protein families, yet actin-membrane interactions within plant cells are critical for the positioning of subcellular compartments, for coordinating intercellular communication, and for membrane deformation. Novel factors are therefore likely to provide interfaces at actin-membrane contacts in plants, but their identity has remained obscure. Here the plant-specific Networked (NET) superfamily of actin-binding proteins is discussed. Members of this family localize to the actin cytoskeleton and specify different membrane compartments. The founding member of the NET superfamily, NET1A, is anchored at the plasma membrane and predominates at cell junctions, the plasmodesmata. NET1A binds directly to actin filaments via a novel actin-binding domain that defines a superfamily of thirteen Arabidopsis proteins divided into four distinct phylogenetic clades. Members of other clades identify interactions at the tonoplast, nuclear membrane, and pollen tube plasma membrane, emphasizing the role of this superfamily in mediating actin-membrane interactions. This and further work on specific members of the NET superfamily will be discussed.



Salt stress priming in *Zea mays*

Pandolfi C.
Department of Agrifood and Environmental Science - University of Florence
email: camilla.pandolfi@unifi.it

Salt stress in plants is one of the main causes limiting agricultural productivity in the world's irrigated land. A way to tackle the problem is to try to enhance plant tolerance to salt stress by understanding basic natural mechanisms that naturally occur in plants under changing environmental conditions. As such, acclimation to external environmental changes occurs in plants thanks to internal adjustments within tissues and cells, enabling cell metabolism to proceed under these somewhat altered conditions (Demmig-Adams et al., 2008). The physiological mechanisms beyond this acquired resistance to salinity remain largely a matter of conjecture. In present contribution three specific questions on the salt-acclimation process will be answered: (1) which of the two major components – specific ion toxicity or osmotic stress – has a bigger role in plant acclimation to salinity, (2) whether the acclimation affects take place in plant root or shoot tissues, and (3) which are the major ionic mechanisms that are triggered by salt acclimation.



Negative regulation of ABA signaling and positive regulation of abiotic stresses by a rice protein phosphatase 2C

Singh A., Bagri J., Pandey G.K.
Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India
email: gkpandey@south.du.ac.in

Arabidopsis PP2C belonging to clade A have been extensively worked out and known to negatively regulate ABA signaling. However, rice (*Oryza sativa*) orthologs of *Arabidopsis* clade A PP2C are scarcely characterized functionally. We have identified a clade A PP2C from rice, which is highly inducible under ABA, salt and drought stresses and localized predominantly in the nucleus. Sequence analysis suggest that *ArabidopsisHighly ABA inducible* 3 (*HAI3*) is the closest ortholog of this OsPP2C. Genetic analysis revealed that *Arabidopsis* plants overexpressing rice PP2C are highly insensitive to ABA and tolerant to high salt and mannitol stresses during seed germination, root growth and overall seedling growth. At adult plant stage in *Arabidopsis* and rice, OsPP2C overexpression leads to high tolerance to salt, mannitol and drought stresses with far better physiological parameters such as water loss, fresh weight, chlorophyll content and photosynthetic potential (Fv/Fm) than wild type plants. Expression profile of various stress marker genes in OsPP2C overexpressing plants revealed interplay of ABA dependent and independent pathway for abiotic stress tolerance. For the first time, this study has identified a potential rice PP2C, which regulate ABA signaling negatively and abiotic stresses positively. Transgenic rice plants overexpressing this gene might provide an answer to the problem of low crop yield and productivity during adverse environmental conditions.



Increase of starch accumulation in duckweed (Lemnaceae) under abiotic stress

K. Sowjanya Sree[1], Klaus-J. Appenroth[2]*
[1] Amity University, Noida, UP, India
[2] University of Jena, Plant Physiology, Jena, Germany
email: klaus.appenroth@uni-jena.de

Abiotic stresses often result in suppressed photosynthesis and growth of plants. We addressed the question whether growth is delayed because of inhibited photosynthesis. Using several duckweed species and creating stress to these plants by (1) application of heavy metals, (2) application of salt (NaCl), and (3) lack of defined nutrients, we showed that this is not the case. Instead, photosynthesis was inhibited to a lesser degree than growth. This became evident by detecting the accumulation of starch under these stress conditions. (1) Cobalt ions and other heavy metals (Cd^{2+}, Ni^{2+}, Tl^{+}, Hg^{2+}, Ag^{+}) induced the accumulation of starch at concentrations when growth was almost completely suppressed (80 %). In *Lemna minor*, application of 100 µM Co^{2+} resulted in the accumulation of 40 % starch per dry weight after 4 days. (2) Application of NaCl at concentrations of 50 mM or higher also resulted in accumulation of starch but the highest values (species-dependent between 10 and 45 % starch) could be observed only after 7 days. (3) Depletion of phosphate, nitrate or sulphate in the growth media had similar effects to salt addition. In the absence of phosphate starch can accumulated to approximately 50% of dry mass in several species (*Spirodela intermedia, Landoltia punctata, Lemna aequinoctialis*) after 14 days. We suggest the following common molecular mechanism that underlies these observations: Stress factors commonly suppress growth more effectively than photosynthesis. The resulting surplus of carbohydrates that is not being used for growth is therefore stored as starch. This hypothesis has biotechnological relevance since this may be applied toward increasing starch accumulation in duckweed and thus could be used to optimize bioethanol production from this aquatic crop.



Phenotypic Plasticity and Fluctuating Asymmetry of Leaf Morphology of Three *Quercus* (Oak) Species in Response to Environmental Factors

Karsai I. and Kusi J.
Department of Biological Sciences, East Tennessee State University,
Johnson City, TN, 37614 USA
email: karsai@etsu.edu

Modification of leaf morphology is associated with phenotypic plasticity and fluctuating asymmetry (FA) in plants. Our hypothesis is that by modification of leaf shape the trees are able to adapt to varying climatic conditions. Variation in leaf morphology of *Quercus* (oaks) has been studied extensively but the level and sources of plasticity and FA variation remained elusive. We investigated the sources of variation in *Q. alba* (white oak), *Q. palustris* (pin oak) and *Q. velutina* (black oak). Leaves were sampled from four different branch positions of each species and their digital images were processed for 15 quantitative trait measurements and also 7 indices were calculated from the data. Nested ANOVA and General Linear Model (GLM) was used to determine the sources of variation and relationship of leaf morphology with leaf position. Tree ID, interval between the leaf base and the largest secondary vein, interval between the largest secondary vein and the apex, length of the largest secondary vein, and maximal blade width of the left half of the leaf were the main sources of leaf shape variations in each species. Leaf position explained variation in FA and leaf lobing only in *Q. alba*. In each species FA was low in leaves that were exposed to the sun indicating the connection of developmental stability and environmental stress (heat and light exposure in this case). All the species exhibited plasticity in response to light exposure but it was most enhanced in *Q. palustris*. Fluctuating asymmetry was greater in the inner (shade) leaves which support our hypothesis that shade leaves would exhibit greater FA because they experience less severe environmental conditions. In summary the study showed that the trees react and adapt to the small scale environmental disparities by modifying their organ size and shape.



Genetic engineering of *Brassica juncea* for fungus resistance using signaling protein gene NPR1

Anita Grover, Sajad Ali, Sandhya Rawat and Eduardo Blumwald
National Research Center on Plant biotechnology, Pusa Campus, New Delhi-110012
email: anitagrover@hotmail.com

Fungal infection is one of the major factors for yield loss in many important crop plants like cereals, pulses, vegetables and oilseed plants. But unfortunately in situations where resistant germplasm is not available in sexually compatible relatives, genetic engineering remains the only option to develop fungus-resistant plants.All over the world, biotechnology-based fungal disease resistance programmes can be basically classified into three categories, namely: Production of transgenic plants over-expressing antifungal molecules like proteins and toxins, Generation of hypersensitive response through R genes and Manipulation of upstream regulatory genes: One of the effective strategies for broad spectrum plant disease resistance has been to exploit SAR pathway. Activation of the regulatory genes which encode signal proteins and transcriptional factors is a prerequisite for subsequent activation of an array of downstream antifungal genes. Hence, manipulation of regulatory genes will induce a wide spectrum of defense genes in one go instead of individual antifungal genes. Potential of regulatory genes for developing fungi- and bacteria-resistant transgenic plants has been demonstrated in *Arabidopsis* using *NPR1, PAD4 and RIN4* and might in future be the the effective way of developing durable and broad spectrum resistance in plants against pathogens.We have developed defense-induced cDNA libraries in *Brassica juncea* and isolated and sequenced cDNA clones of about 15 antifungal genes, chiinase, glucanase, thionin, defensin to name a few. Signalling genes including positive (NPR-1) and negative regulator (RIN-4) genes of defense responses have also been isolated and characterized. *Brassica juncea* transgenic plants with NPR1 gene have been developed Their molecular analysis and their *in vitro* and *in vivo* response to *Alternaria* infections was done and will be discussed. We have isolated three pathogen-inducible promoters by making Genome Walker library of *Brassica juncea*. These are of chitinase, glucanase and PR1. Out of these glucanase and PR1 promoter were validated in Arabidopsis transgenics. Their inducibility by developmental stage and by biotic and abiotic stresses will be discussed.



Annexins a new player in plant stress adaptation to the adverse environment

Dorota Konopka-Postupolska, Michał Szalonek, Wojciech Rymaszewski, Radomira Vankova, Peter Dobrev and Jacek Hennig
Institute of Biochemistry and Biophysics PAS, Pawinskiego 5A, 02-106 Warsaw, Poland
email: konopka@ibb.waw.pl

Sessile organisms like plants, are inevitably subjected to unfavorable environmental changes. Thus, they have to develop various tolerance strategies enabling them to continue growth and development despite of non-optimal conditions. Recognizing such mechanisms is one of the most important aims of modern biology with crucial importance for agriculture. In our previous research we found that the tolerance to adverse environmental conditions of model plant Arabidopsis is correlated with the level of expression of the calcium- and phospholipids binding proteins, annexins. Analysis of available complete plant genomes revealed that in land plant annexins are a monophyletic clade of homologs; in a genome of any single species there are several genes coding for individual annexins but they are not expressed in the same tissue and/or on the same level. Despite abundance of annenxins' and ubiquity, their primarily physiological role has not been elucidated yet. However, the basic biochemical characteristic of this family indicates they can modulate membranes-related processes, and especially protect the structure and function under stress conditions. To further assess the problem of annexin function in stress response we employed transgenic approach. Potato plants over-expressing endogenous potato annexin 1 (AnnSt1_OE) were generated and their tolerance to the drought was analyzed. We found that annexin AnnSt1 improved the functioning of photosynthetic membranes during water deficit. Transgenic plants for longer time were able to maintain the proper chloroplast functioning (biosynthetic pathways) and support effective photosynthesis than parental cv. Sante line. It was accompanied with modulation of hormonal homeostasis and resulted in delaying of drought-induced leaf senescence. Taken together all these data strongly suggest that annexin-mediated tolerance goes far beyond local membrane protection but also involved modulation of intracellular signaling pathways, probably the ones that begin with the release of the structural membrane components.



RNA splicing factor promotes crop imovement under drought and salinity stress

Narendra Tuteja
International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi - 110067, India
email: narendra@icgeb.res.in

The moisture availability is very important for plant growth and yield. Soil moisture stress trends to withhold water from roots and is affected by abiotic stresses including drought and other abiotic stresses, which cause significant water deficits. These stresses also cause adverse effects on the growth and productivity of crops, which resulted in significant economic losses worldwide. Minimizing these losses is a major area of concern for all countries. Therefore, it is desirable to develop multi-stress tolerant varieties. Since stresses affect the cellular gene expression machinery, therefore, molecules involved in nucleic acid metabolism including helicases are likely to be the target. Most helicases are members of DEAD-box protein superfamily and play essential roles in basic cellular processes such as DNA replication, repair, recombination, transcription, ribosome biogenesis and translation initiation. Earlier, we have shown the first direct evidence of a role of PDH45, PDH47 and SUV3 helicases in salinity stress tolerance in plant. Here a novel role of a plant p68 RNA helicase in drought and salinity stress tolerance has been described. The p68 (Ddx5) is one of the prototypic members of the DEAD-box helicase family. In animal systems the p68 is reported to be involved in RNA splicing, RNA-induced silencing and in transcription. However, very little is known in plant system and role of plant p68 in stress tolerance has not been reported so far. Here, we report the followings: a) cloning of a *p68* gene by screening of pea cDNA library using *p68* DEAH-box protein from *Arabidopsis thaliana* as a probe, b) the pea p68 protein shows self-interaction, c) *p68* transcript is upregulated in pea plant in response to salinity, dehydration and cold stress, d) *p68* overexpression driven by constitutive cauliflower mosaic virus-35S promoter in tobacco transgenic plants confers salinity and drought tolerance by improving the growth, photosynthesis and antioxidant machinery, e) T_1 transgenic plants were able to grow to maturity and set normal viable seeds under continuous stress, without yield penalty. These findings provide first direct evidence for the novel function of p68 helicase in drought and salinity stress tolerance without yield loss. The results also suggest that RNA helicase can be exploited as candidate gene for engineering abiotic stress tolerance in crop plants of economic importance.



Reactive Oxygen Species (ROS): The key player in signaling events of Chickpea (*Cicer arietinum* L.)-*Fusarium oxysporum* f. sp. *ciceris* race 1 (Foc1) interplay.

Anirban Bhar, Moniya Chatterjee, Sumanti Gupta, Sampa Das
Div. of Plant Biology, Bose Institute, P 1/12 C.I.T Scheme VII M, Kolkata- 700054
email: sampa@mail.jcbose.ac.in

Early pathogenic entry is sensed by generation of reactive oxygen species (ROS) in plant followed by oxidative burst that lead to altered redox state at site of infection. These ROS play pivotal roles in pathogen perception, recognition and downstream defense signaling. But, how these redox alarms converge and coordinate *in planta* into a defensive network is still illusive. Present investigation demonstrates the role of *Fusarium oxysporum* f.sp *ciceris* Race 1 (Foc1) induced redox responsive transcripts in regulating downstream defense signaling in chickpea. Foc1 induced redox signals were found to trigger intracellular transporters which activated some transcription factors. These redox regulators, cellular transporters and transcription factors were found to coordinate into a well orchestrated defensive network with sugars acting as internal energy modulators. Similar to transcriptomic data proteomic study also indicate ROS as initial trigger for downstream defense signaling. Respiratory burst oxidase (RBOH), Cationic peroxidase (OCP), Vacuolar sorting receptor (VSR), Polyol transporter (PLT), Sucrose synthase (SUS), Serine threonine kinase and Zinc finger protein appeared as key molecular candidates controlling important hubs of the defense network. Foc 1 infected root proteome revealed that Pathogenesis related protein 1 (PR1), Thaumatin like protein, β glucanase etc are the important defense modulators in this host pathogen interplay. Many of these defense proteins are also regulated by cellular redox state directly or indirectly. Further investigation and characterization of these key molecular candidates are required to unravel the exact mechanism of action of these proteins to impart effective resistance against wilt causing pathogen.



plant Signaling&Behavior

International Symposium on Plant Signaling and Behavior 2014

Extracellular ATP inhibits endocytic vesicle recycling and gravitropism of Arabidopsis roots via NADPH Oxidase-mediated ROS signaling

Tomoko Kagenishi, Ken Yokawa and František Baluška
Institute of Cellular and Molecular Botany, University of Bonn
Kirschallee 1 D-53115 Bonn Germany
Email: baluska@uni-bonn.de

Extracellular ATP (eATP) is known as a signal molecule both in mammal and plant cells. In mammals, eATP study has accelerated since that the purinoceptor was cloned from brain. By contrast, plant eATP receptor was revealed only recently. Nevertheless, studies demonstrate that eATP is involved in regulating plant growth and adaptation to their environment. For example, exposure to eATP was shown to inhibit root gravitropism and growth in Arabidopsis. Here, we have analyzed the expression site of Arabidopsis eATP receptor gene At5G60300 by microarray database of the Genenvestigator and it has high expression in roots. Moreover, it was shown that a root apex shows high activity of eATP release induced by touch stress. eATP may play important roles for environmental sensing and adaptation. However, the processes behind the eATP signaling are largely unknown. The aim of our present study is to investigate the mechanisms of eATP signaling in root apex. In the result of our study, endocytic vesicle recycling in root cells was modified by eATP. Here, we report that the eATP-induced inhibition of root gravitropism and endocytic vesicle recycling require ROS generation by NADPH oxidase AtRBOHC.



Protection of plant cells and green algae from oxidative copper toxicity by catalytically active metal-binding DNA oligomers

Tomonori Kawano,[1,2,3] Junichiro Iwase,[1,2] Hiroka Furukawa,[1,2] François Bouteau,[2,4,5]& Stefano Mancuso[3,6]

1 Graduate School of Environmental Engineering, The University of Kitakyushu, Kitakyushu, Japan

2 University of Florence LINV Kitakyushu Research Center (LINV@Kitakyushu), Kitakyushu, Japan

3 Université Paris Diderot, Sorbonne Paris Cité, Paris 7 Interdisciplinary Energy Research Institute (PIERI), Paris, France

4 Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain (FRE 3597), Paris, France

5 Institut de Biologie des Plantes, Bât 630, 91405 Orsay, France

6 LINV- DiSPAA, Department of Agri-Food and Environmental Science, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino (FI), Italy

email: kawanotom@kitakyu-u.ac.jp

Impact of copper ions on the oxidative and calcium signal transductions leading to cell death in plant cells have been documented. Copper induces a series of biological and chemical reactions in plant cells including the oxidative burst reflecting the production of reactive oxygen species and stimulation of calcium channel opening allowing a transient increase in cytosolic calcium concentrations. These early events completed within few min after the contact with copper are known to trigger the development of cell death. Here, effects of the DNA fragments with copper-binding motifs as novel of plant cell-protecting agents were assessed using the cell suspension culture of transgenic tobacco (cell line BY-2) expressing the aequorin gene. Addition of GC-rich double-stranded DNA fragments, prior to the addition of copper ions, effectively blocked both the copper-induced calcium influx and cell death. In addition, the DNA-Cu complex examined was shown to possess the superoxide-scavenging catalytic activity, suggesting that DNA-mediated protection of the cells from copper toxicity is due to removal of superoxide. Lastly, the cell protecting action of CG-rich DNA oligomer was also examined in green algal models such as Cu-stressed green paramecia.



plant Signaling&Behavior

International Symposium on Plant Signaling and Behavior 2014

The root cap orchestrates maize seedling development via root- and shoot-affecting, ethylene-dependent signals

Hans G. Edelmann and Achim Hahn

Institute for Biology and its Didactics, University of Cologne, - 50931 Germany

email: h.edelmann@uni-koeln.de

Seedlings of maize are characterized by a pronounced root growth, thereby assuring anchorage in the soil and the supply with water and nutrients, and also enabling an appropriate shoot elongation growth. Besides various other compounds affecting root elongation growth, the plant hormone ethylene inhibits growth in a concentration-dependent manner. However, exogenously applied ethylene is no longer capable of inhibiting elongation growth when the root cap has been surgically removed prior to hormone treatment. Reconstitution of the root cap positively correlates with the developing capacity of the root to respond to ethylene again. In contrast, the removal of the root cap does not per se affect growth inhibition controlled by auxin (IAA) and cytokinin (kinetin). Furthermore, semi-quantitative RT-PCR results support earlier findings that the maize root cap is a site of high gene expression activity with respect to sensing and responding to hormones such as ethylene. As detected employing GC-measurements the root cap is not only relevant for the processing of exogenously supplied ethylene, but strongly affects endogenous ethylene synthesis/emission in the shoot with appropriate impacts on growth of both root as well as elongation growth of the shoot.



Arabidopsis Synaptotagmin-Like Proteins (SYTLs) and Synaptic Vesicle Protein 2-Like (SVL) in endocytic vesicle recycling pathways of root apex cells

Wei Siao and Baluška František
Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1
D-53115 Bonn, Germany

To gain more understanding on the signal transmission in plants, this report focuses on the roles of Arabidopsis Synaptotagmin-Like Proteins (SYTLs) and Synaptic Vesicle Protein 2-Like (SVL) in the endocyticvesicle recycling pathway, and the physiological functions of these proteins concerning with the stressresponses and the signal transmission in the roots. We first demonstrate that cells of the *syt1* and *syt3* mutants have smaller BFA compartments in the transition zone of roots, indicating that the endocytic pathway is modulated by SYT1 and SYT3. We show here that SYT1 and SYT2 protein are co-localizing with SVL in the plasma membrane and endosomes, but have no physical interaction with each other, which is different from that in the animal synaptic vesicles. To investigate the functions of SYTLs on plant endocytosis, the protein-protein interactome network study on SYTLs, Adaptor Protein 2 (AP2), Clathrin Light Chain (CLC) and Stonin is now in process. We have also performed the phenotypic screenings of SYTLs mutants and SVL mutants under salt conditions, heavy metal treatments and UV light exposures. All this give us new insights into the sensory functions of SYTLs and SVL in Arabidopsis roots. Furthermore, we study small signal molecules such as plant hormones, NAD and amino acids that could be transmitted by the recycling endocytic vesicles which are regulated by SYTLs and SVL. We are also analyzing possible roles of the SYTLs- and SVL-mediated endocytic vesicle recycling/secretion together with the electrical signalstraveling through the plant bodies by using voltage-sensitive fluorescent protein (VSFP) transgenic Arabidopsis seedlings.



plant Signaling & Behavior

International Symposium on Plant Signaling and Behavior 2014

Shoot-supplied ammonium triggers two distinct responsive signaling pathways in Arabidopsis

Baohai Li Guangjie Li and Weiming Shi
State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, China
email: wmshi@issas.ac.cn

Ammonium (NH_4^+) toxicity is not only a common phenomenon in cell biology, but also a significant ecological and agricultural issue. Particularly, with increasing atmospheric deposition, plants need to deal with unprecedented NH_4^+ stress from aboveground source. However, the signaling pathway in response to shoot-supplied ammonium (SSA) remains unclear in plants. Recently, we found that under SSA condition the responsive signaling pathway clearly includes both local and systemic components in *Arabidopsis thaliana*. Firstly, SSA-induced leaf chlorosis, as a shoot-localized behavior, is mediated by AMOS1/EGY1-dependent plastid retrograde signalling and integrated with absisic acid (ABA) pathway. Secondly, SSA-mediated reduction of lateral root formation is a typical systemic signaling process that involves local ethylene and auxin production in shoots, and also long-distance auxin transport and auxin response in roots, but independent of ABA pathway.



Glutamate Receptor-Like Channel3.3 Is Involved in Mediating Glutathione-Triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in Arabidopsis

Feng Li, Jing Wang, Chunli Ma, Yongxiu Zhao, Yingchun Wang, Agula Hasi, &Zhi Qi
Inner Mongolia University, Hohhot, P.R.China
email: qizhi@imu.edu.cn

The tripeptide reduced glutathione (GSH; g-glutamate [Glu]-cysteine [Cys]-glycine) is a major endogenous antioxidant in bothanimal and plant cells. It also functions as a neurotransmitter mediating communication among neurons in the central nervous system of animals through modulating specific ionotropic Glu receptors (GLRs) in the membrane. Little is known about suchsignaling roles in plant cells. Here, we report that transient rises in cytosolic calcium triggered by exogenous GSH in Arabidopsis (*Arabidopsis thaliana*) leaves were sensitive to GLR antagonists and abolished in loss-of-function *atglr3.3* mutants. Like the GSH biosynthesis-defective mutant PHYTOALEXIN DEFICIENT2, *atglr3.3* showed enhanced susceptibility to the bacterial pathogen Pseudomonas syringae pv tomato DC3000. Pathogen-induced defense marker gene expression was also decreased in *atglr3.3* mutants. Twenty-seven percent of genesthat were rapidly responsive to GSH treatment of seedlings were defense genes, most of whichwere dependent on functional AtGLR3.3, while GSH suppressed pathogen propagation through the AtGLR3.3-dependent pathway. Eight previously identified putative AtGLR3.3 ligands, GSH, oxidized glutathione, alanine, asparagine, Cys, Glu, glycine, and serine, all elicited the AtGLR3.3-dependent cytosolic calcium transients, but only GSH and Cys induced the defense response, with the Gluinduced AtGLR3.3-dependent transcription response being much less apparent than that triggered by GSH. Together, these observations suggest that AtGLR3.3 is required for several signaling effects mediated by extracellular GSH, even though these effects may not be causally related.



Probabilistic predictive processing vs. ecological psychology models of plant intelligence

Calvo P.
Department of Philosophy, University of Murcia, Murcia – 30100. Spain
email: fjcalvo@um.es

The exploitation of abstract knowledge in one form or another seems to be the *sine qua non* of the capacity of mammals to generalize beyond the observed data. Most likely, other animals are equally able to induce underlying regularities in the environmental data pool, even if they that lack the neurocomputational resources of a cerebral cortex. Do plants generalize too? A positive answer to this question presupposes that a sound general model of animal cognition is forthcoming. Although there is no scientific consensus as to how best to model animal cognition, 'probabilistic predictive processing' has been gaining momentum in recent years. In this talk, I shall consider the possibility that plants are likewise best modeled by a predictive processing model under Bayesian rules of inference. However, as I shall try to show, a more comprehensive picture of both animal and plant intelligence maybe obtained, were we to recast predictive processing under the ecological principles of emergence and self-organization.



POSTER PRESENTATIONS



P2.1

A participation of changes in cytoplasmic and apoplastic pH in variation potential-induced photosynthetic response

Sukhov V., Sherstneva O., Surova L., Katicheva L. and Vodeneev V.
Department of Biophysics, N.I. Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Gagarin Avenue, 23, 603950, Russia
email: vssuh@mail.com

Variation potential (VP) is wounding-induced electrical signal which causes numerous functional responses in higher plants. Photosynthesis is one of targets of this electrical signal. It is known that VP can induce short-term inactivation of photosynthetic processes. Lowering of photosynthesis dark stage activity, possibly, initiates this inactivation; however, mechanism of VP influence on this stage is still discussed. The aim of our studywas analysis of participation of pH changes in apoplast and cytoplasm in development of VP-induced photosynthetic response in pea plants. It has been shown that burning-induced VP decreased CO_2 assimilation rate and photosystem I and II quantum yields as well as increased non-photochemical quenching of fluorescence in pea. Further, experiments with pH-sensitive fluorescent dyes showed that VP generation was accompanied with pH increase in apoplast and pH increase in cytoplasm which reflected proton influx into cell. Imitation of this influx using protonophore CCCP induced photosynthetic response which was similar with VP-induced response. Experiments with isolated chloroplasts showed that decrease of external pH, which imitated cytoplasmic pH decrease at VP generation, induced growth of the non-photochemical quenching of fluorescence and lowering of photosystem I and II quantum yields. Thus proton influx and changes in cytoplasmic and apoplastic pH are probable mechanism of VP-induced photosynthesis inactivation. Influence of the H^+ influx and pH changes on photosynthesis can be connected with lowering of CO_2 flow into cell, pH dependent decrease of ferredoxin:NADPH oxidoreductase activity, lumen acidification and appropriate induction of non-photochemical quenching of fluorescence, etc. This work was supported by the Russian Foundation for Basic Research, project no. 14-04-01899 A.



P3.1

Transgenic Arabidopsis overexpressing E-β-farnesene synthase in cytosol and plastids confers repellence to green peach aphids

Bhatia V.[1,3], Maisnam J.[2], Uniyal P.L.[3], Bhattacharya R.C.[1]
[1]National Research Center on Plant Biotechnology, PUSA Campus, New Delhi-110012, India
[2]Division of Agricultural Chemicals, Indian Agricultural Research Institute, PUSA Campus, New Delhi- 110012. India
[3]Department of Botany, University of Delhi, Delhi- 110007. India
email: bhattacharyarc@rediffmail.com

Aphids, hemipteran insect pests have infochemical communication, an interaction among organisms based on chemicals. Infochemicals mainly comprise of pheromones and allelochemicals. Pheromones can be sex pheromones (mating signal) or alarm pheromones (warning signal) and are produced for intraspecific interaction while allelochemicals are emitted for interspecific interaction. E-β-farnesene (E-β-f) is a well identified alarm pheromone in aphids. In order to mimic the alarm response produced by aphids in danger, the transgenic mediated continuous emission of E-β-f has been speculated as a potential strategy to develop direct or indirect defence in plants against aphids. However, the success of terpenoid engineering in plants is limited by a few factors including the availability of both the substrate pool and the incorporated branch point enzyme in the same sub-cellular compartment, the specific activity of endogenous enzymes if any sharing the same substrate and the costs of engineering in terms of effects on general growth and physiology of the plant.Farnesyl diphosphate (FDP) is the common substrate for many downstream key end products including E-β-f. The availability of FDP in different sub-cellular compartments may be limiting for E-β-f synthesis. The generation of FDP in cytosol through mevalonic acid (MVA) pathway is known but the reports on the availability of plastidic pool of FDP are rarely available. In the present study an E-β-f synthase gene of *Citrus reticulata* was expressed in transgenic Arabidopsis for cytosolic and plastidic localization of the enzyme. A comparative assessment was carried out for the generation of E-β-f harnessing the available FDP pool in both the compartments. Assay for potential induced repellence mediated by E-β-f demonstrated that transgenic Arabidopsis had lower aphid infestation in comparison to control plants. The present study highlighted the potency of E-β-f in generating novel aphid repellence.



P3.2

Development and analysis of expressed sequence tags induced by toxin, isolated from *Fusarium oxysporum* in chickpea

Choudhary P.[1], Khanna S.M.[1], Lakhera P.C.[2], Jain P.K.[1] and Srinivasan R.[1]
[1]National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi-110012. India
[2]Department of Biotechnology, Hemwati Nandan Bahuguna Garhwal University, Srinagar, Pauri Garhwal-246174, Uttarakhand. India
e-mail: srinivasan53@gmail.com

Fusarium wilt caused by fungal pathogen *Fusarium oxysporum* f. sp. *ciceris* is one of the most destructive diseases of chickpea (*Cicer arietinum* L.) in the Indian subcontinent and the Mediterranean basin causing yield losses from 10 - 15% which may escalate to complete loss under favourable conditions. In this study, the filtrates obtained from the cultures of *F. oxysporum* were found to cause wilting in chickpea and the compounds responsible for toxicity were isolated and identified as 3-O-methyl-8-O-methyl fusarubin, 8-O-methyl fusarubin, fusarubin, bikaverin and fumonicin.Three SSH libraries were constructed from the shoot samples of WR315 (wilt resistant) and BG256 (wilt susceptible) genotypes in which wilt symptoms were induced by toxin. A total of 2000 clones were generated of which 1728 ESTs were sequenced giving 1611 high quality ESTs which assembled into 1210 unigenes. BLASTN analysis of unigene dataset revealed highest identity with *Medicagotruncatula* followed by *Pisum sativum*, *Glycine max*, *Phaseolusvulgaris*, *Oryza sativa*, *Lotusjaponicus*, *Arabidopsisthaliana* and *Populusalba*. BLASTX analysis of 1210 unigenes showed 803 total hits of which majority of top matches were from proteins of legume species. Functional annotation of unigenes by Blast2GO resulted in gene ontology functional classification terms for 553 unigenes of which 453 were functionally annotated and 100 were mapped but not annotated. ESTs representing known stress-responsive proteins were present in abundance, indicating their high expression in fusarium wilt stressed seedlings. The expression profile of a few unigenes was validated using semi-quantitative RT-PCR, northern blotting and real time PCR. These high quality wilt responsive ESTs can be helpful for systematic analysis of stress responsive gene signatures that may help in identifying genes involved in wilt tolerance in chickpea.



P3.3

Role of cyclops in an Aeschynomeneae legume *Arachis hypogaea*

Das D.R. and Das Gupta M.
Department of Biochemistry,University of Calcutta.35, Ballygunge Circular Roa, Kolkata-700019. West Bengal, India
email: maitrayee_d@hotmail.com

Cyclops or IPD3 is a member of common symbiotic pathway that is required for both nodulation and mycorrhization. It consists of 11 exons. It encodes a protein with a C-terminal coiled-coil motif and two nuclear localization signals that direct the protein to the nucleus, colocalizing with CCaMK, a Ca2+/calmodulin-dependent protein kinase essential for decoding calcium signatures in Sym Pathway. Functionally Cyclops is associated with intracellular colonisation and infection of the symbionts. Our objective was to describe the role of Cyclops in an aeschynomeneae legume *Arachis hypogaea*, where rhizobial invasion bypass the infection-thread (IT). Instead, the invasion occurs through epidermal cracks and the rhizobia are endocytosed directly by a cortical cell and thereby trigger nodule development.*Arachis hypogaea* Cyclops (*Ah*Cyclops) was cloned from both genomic DNA and root cDNA (Accession No. JQ839282).Ahcyclops consists 10 exons as opposed to 11 exon of Model legumes.While cloning the gene we came across a variant of exon five that has two deletions leading to a premature stop codon. Real time data shows an inverse expression pattern of the variant with the normal cyclops following infection with rhizobia. Down regulation of the gene using RNA interference (RNAi) of *Ah*cyclops considerably increased the number of nodules which is consistent with the coupling of bacterial internalization and nodule development in aeschynomeneae legumes. The RNAi nodules were properly developed with proper dissemination of symbionts. However, these nodules showed improper development of the symbiosome. The role of Cyclops in an aeschynomeneae legume would be discussed in the backdrop of our knowledge of its role in IT legumes.



P3.4

Expression profiling of NILs carrying rice blast resistance gene *Pi9* to understand molecular basis of host-pathogen interactions

Jain P.[1,3], Singh P. K.[1,3], Singh A. K.[2], Gopala Krishnan S.[2],Sharma V.[3] and Sharma T. R.[1]
[1]National Research Centre on Plant Biotechnology, Pusa Campus,New Delhi-110012. India.
[2]Indian Agricultural Research Institute, New Delhi-110012, India.
[3]Department of Bioscience & Biotechnology, Banasthali University,Tonk, Rajasthan-304 022, India[3].
email: trsharma@nrcpb.org

Annual rice production worldwide suffers loss of 10-30% due to rice blast. Rice blast is caused by hemibiotrophic fungal pathogen *Magnaporthae oryzae*. In this study, resistant near isogenic line (NIL) carrying *Pi9* rice blast resistance gene in background of Pusa Basmati-1 was used to investigate molecular basis of Pi9 mediated resistance response. The NIL exhibited broad spectrum resistance to several *M. oryzae* strains collected from different regions of India. Expression profiling of resistant NILs and susceptible control (Pusa Basmati-1) rice line, with and without pathogen inoculation was done. The leaf samples were collected after 24 hours of pathogen inoculation for RNA isolation. High quality RNA samples were used for preparing cDNA libraries for transcriptome profiling using RNA Sequencing (Illumina platform). We obtained 1254 and 781 significant differentially expressed genes after multiple correction (FC $\geq$2 & FC$\leq$ 2) in incompatible and compatible interactions, respectively. The significant genes (211) were found after multiple correction (FC $\geq$2 & FC$\leq$ 2) as common genes in both compatible and incompatible interactions. The functional analysis of these genes showed that genes involved in hormone signalling (Jasmonic Acid, Ethylene, ABA and Auxins) and secondary metabolism were upregulated. This study will help to find genes involved in early response to *M. oryzae* and to reveal the sophisticated transcriptional reprogramming during interaction between Rice-*M. oryzae* pathosystem.



P3.5

Identification and characterization of miRNAome of tomato roots infected with root knot nematode (*Meloidogyne incognita*)

Kaur P.[1], Cheeni V.[2], Joshi G.[1], Heisnam D.[1], Katiyar-Agarwal S.[2], Agarwal M.[1], Jagannath A.[1], Goel S.[1], Kumar A.[1]

[1]Department of Botany, University of Delhi, Delhi- 110007, India.

[2]Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi-110021, India.

email: kaurpritam.pritam@gmail.com; cvjaykumarreddy@gmail.com

Small RNAs are noncoding, endogenous 20-24 nucleotide long molecules that regulate the expression of genes by degrading mRNA or by repressing translation. They are known to play important roles in regulating plant development, signal transduction, abiotic and biotic stress responses. We are interested in investigating the role of miRNAs both from tomato and root knot nematode (RKN) during susceptible and resistance responses following RKN infection of tomato roots. RKNs are obligate, sedentary endoparasites that complete their lifecycle in the host roots. Parasitic second juvenile stage of nematode (J2) invades plant near root tip, migrates towards the vascular bundle and triggers xylem parenchyma cells to develop into feeding cells called giant cells. Nematode secretions cause formation and development of feeding cells in roots by altering plant gene expression patterns. Deep sequencing was performed on 15 small RNA libraries prepared from roots of susceptible and resistant tomato cultivars at different stages of disease development. In total, 517 million reads were obtained and 47% reads belonged to putative small RNA population. A total of 1774 tomato miRNAs were predicted by bioinformatics analysis. Forty seven conserved miRNAs (belonging to 19 families), 223 novel miRNAs with star sequence and 67 novel miRNAs without star sequence were identified. The digital expression profiling indicated that 203 miRNAs were upregulated in which 107 miRNAs were from susceptible and 96 miRNAs were from resistant cultivars. On the other hand, 29 miRNAs were down-regulated, out of which 16 and 13 miRNAs belong to susceptible and resistant cultivars, respectively. While 54 miRNAs were up-regulated in both susceptible and resistant cultivars, only 4 miRNAs were down-regulated. Also, 10 miRNAs were differentially regulated in both susceptible and resistant culivars. Additionally, our analysis identified both conserved and novel miRNAs from the root-knot nematode, which were found to be up- and down-regulated during disease development.



P3.6

Putative roles of selected OY-M phytoplasma effector proteins in plant hosts: A bioinformatics approach

Kumar S., Singh V. and Lakhanpaul S.

Department of Botany, University of Delhi, Delhi – 110007. India.

email: sach_inom@yahoo.co.in

Over 1000 plant taxa are known to be affected by phytoplasma which bring an array of deformations in the hosts. Phytoplasma, the plant pathogenic mollicutes are fastidious microorganisms rendering the research efforts to understand them rather difficult in the past decades inspite of their huge economic importance. The niche of phytoplasmas is limited to phloem in plants, yet they have an ability to exercise the adverse effect on the distant organs. Therefore, the identification and characterization of effector molecules is an important research area. Onion Yellow (OY) phytoplasma, one of the most widely spread group has been reported in large number of taxa from India including from some important crop plants. In order to identify the effector molecules of OY phytoplasma, screening was performed on all the proteins available in the phytoplasma proteomic database for the strain M using SignalP, TMHMM, PSORT and Predict NLS. Out of 749 proteins, 65 were found to contain a N-terminal signal peptide while comparison of the predicted signal peptidase site and the transmembrane helices present, resulted in only 33 proteins to be putatively secretory. Structure prediction of five of these secretory proteins using iTASSER and their functional prediction using Profunc was carried out. These proteins appear to be interacting with the host genomic machinery either directly or via intermediate ligands resulting in differential expression of the selected genes involved in plant growth and development. The putative mechanism leading to malformations in the host taxa caused by phytoplasma infestations is hereby proposed.



P3.7

Host mediated RNAi of a parasitic gene for eliciting root knot nematode resistance

Kumar A.[1], Verma G.[1], Dwivedi A.[1], Abdin M.Z.[2], Sirohi A.[3], Subramaniam K.[4], Srinivasan R.[1] and Jain P.K.[1]

[1]NRC on Plant Biotechnology, Indian Agricultural Research Institute (Pusa) Campus, New Delhi 110012, India

[2]Center for Transgenic Plant Development, Deptt. of Biotechnology, Faculty of Science, Jamia Hamdard University, New Delhi 110062. India

[3]Department of Nematology, Indian Agricultural Research Institute, New Delhi 110012. India

[4]Department of Biological Science and Bioengineering, Indian Institute of Technology, Kanpur 208016. India

email: jainpmb@gmail.com

Meloidogyne incognita, root knot nematode is an economically important plant parasitic nematode which infects a large number of crop plants and leads to enormous losses to the tune of hundreds of billion dollars worldwide. *M. incognita* infects the root cells of the host plant, and transforms them into multinucleate, enlarged cells, which act as their feeding cells. Traditionally, control of nematodes has been through the application of resistant cultivars, nematicides, and integrated pest management strategies. However, all traditional technologies have several drawbacks and none of them offers an effective strategy to impart nematode resistance. Of late, RNA interference (RNAi) has shown promising results and can become an ideal method for imparting nematode resistance in plants. In our study, we have identified *msp25,* a gene from *M. incognita* that codes for a parasitism protein and is expressed in the dorsal oesophageal gland cells. A dsRNA hairpin construct was prepared for this gene in pBC binary vector and transformed into *Arabidopsis thaliana* using floral dip method. The T-3 RNAi lines were infected with *M. incognita* and the nematodes extracted from the roots of these plants showed 45 to 55% reduced (number of knots, females and egg masses) infection compared to the wild type. The *msp25* gene expression was also reduced in the nematode females isolated from the RNAi lines as compared to control plants. Thus, host-delivered RNA interference of *msp25* gene significantly reduced the root knot nematode population. The identification of such essential parasitic genes and their silencing using HD-RNAi offers an effective strategy to control nematode infection in important plants.



P3.8

Involvement of phospholipase C and D in plant immune responses against *Ralstonia solanacearum*

Masahito N. and Akinori K.
Faculty of Agriculture, Kochi University, Nankoku 200, Japan
email: s11dre01@s.kochi-u.ac.jp

Phosphatidic acid (PA) is a key molecule involved in cell membrane biosynthesis and intracellular signal transduction in plants. We previously identified a novel disease susceptibility factor DS1 (disease suppression 1) having a PA phosphatase activity in Nicotiana benthamiana. Silencing of DS1 induced high PA levels and disease resistance in N. benthamiana inoculated with the phytopathogen Ralstonia solanacearum. This phenotype of DS1-silenced plants was compromised by treatment with PA inhibitors, suggesting that PA might play a crucial role in plant immune responses against R. solanacearum. To understand the molecular mechanisms underlying disease resistance regulated by PA and its synthesis pathway, here we focused on the two types of enzymes that generate PA, phospholipase C (PLC) and D (PLD). Firstly, we isolated NbPLC2 and NbPLDα1 genes from N. benthamiana leaves, and analyzed the expression pattern of these genes in the plants inoculated with various R. solanacearum strains. Significant induction of NbPLC2 was observed in N. benthamiana during the infection with virulent or avirulent strains. In contrast to NbPLC2 expression, NbPLDα1 was induced in the plants inoculated with the avirulent strain, but not with the virulent strain. To elucidate the role of NbPLC2 and NbPLDα1 on plant immune responses, we created gene knockdown plants for NbPLC2 or NbPLDα1 using virus-induced gene silencing. Silencing of NbPLC2 or NbPLDα1 reduced expression of defense-related genes in response to R. solanacearum infection. Consistent with the expression level, the growth of R. solanacearum was enhanced in NbPLC2- or NbPLDα1-silenced plants compared with that of control plants. These data indicate that both NbPLC2 and NbPLDα1 are required for the appropriate induction of plant immune responses against R. solanacearum.



P3.9

Towards developing Rubisco as a physiologically-relevant substrate for measuring herbivory

U Bhardwaj[1], A Bhardwaj[2], R Kumar[1], S Leelavathi[2], V SivaReddy[2] and S Mazumdar-Leighton[1]

[1] Department of Botany, University of Delhi, Delhi- 110007;
[2]Plant transformation Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India
email: smazumdar@botany.du.ac.in

Plants respond to herbivory by production of protease inhibitors. Production of defense proteins like plant protease inhibitors is energetically expensive for plants and involves JA-mediated signaling pathways and dedicated cellular responses. During a defense mode, production of Rubisco in herbivore-attacked plants is typically down-regulated, potentially altering plant quality. Ingestion of plant protease inhibitors elicits complex, physiological responses in lepidopteran larvae that may enable adaptation to host plant choice. Understanding Rubisco digestion in the presence of ingested plant protease inhibitors is of potential significance for studying host plant utilization by pests and beneficial Lepidoptera in the tropics. We have cloned and expressed in *E. coli*, conserved domains of the Large Subunit (LS) of Rubisco (RbcL) from various species of host plants. Cognate and non-cognate recombinant RBCL were tested as protein substrates for digestive serine proteases of two agricultural pests and one indigenous species of silkworm in the presence of plant protease inhibitors using *in vitro* protease assays, and zymography. Results obtained suggest that unfolding and proteolysis of Rubisco in the insect gut is unaffected by the presence of plant protease inhibitors, due to the production of inhibitor-insensitive larval proteases.



P3.10

NGS based small RNA transcriptome profiling of sheath blight resistance and susceptible rice lines.

Nagar R., Kumari A. and Sharma T. R.
National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi-110012. India
email: trsharma@nrcpb.org

Sheath blight (*Rhizoctonia solani*) is the second most destructive fungal disease of the rice followed by the Blast. Increasing evidence is emerging to support the notion that host endogenous small RNAs represent a fundamental layer of post-transcriptional gene regulation to fine-tune gene expression in host adaptive responses to pathogen infection. Thus, understanding regulatory mechanisms controlling plant defence responses against pathogens in which small RNA genes participate and the target genes and the traits they regulate is of great importance for basic research in plant biology as well as for the development of novel strategies for engineering disease-resistant plants. To elucidate the likely association of small RNA in sheath blight resistance response, we have been carried out expression profiling of small RNA in the rice lines using deep sequencing (Illumina HiSeq). Analysis of NGS sequence data reveals that out of the total miRNA present in Sanger database release 20, a set of miRNA was found to be differentially expressed in sheath blight resistance and susceptible rice lines. A total of 18 and 35 miRNA were found to be differently expressed in susceptible and resistant rice line at 24 hours post inoculation respectively. Target of these miRNAs were predicted and pathway analysis of these targets was also done. The predicted targets of these miRNA mostly include transcription factor, cell wall synthesis enzymes, protein kinases and DNA binding proteins. Functional validation and further characterization of these miRNAs would be helpful to understand the molecular insight of *Rhizoctonia solani* regulated miRNA network in plants.



P3.11

Signaling through cAMP Controls pathogenesis in Karnal bunt fungus (*Tilletia indica*) in wheat (*Triticum aestivum*)

Shalini Purwar[1], Shanthy Sundaram[1] and Anil Kumar[2]
[1]Center for Biotechnology, University of Allahabad, Allahabad, Uttar Pradesh, India;
[2]G B Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India
email: purwarshalini@gmail.com

The molecular signals required for establishing and maintaining the infectious dikaryons would have to be identified by investigating the role of c-AMP in fungal pathogenesis. Tilletia indica is fungal pathogen that infects the spike of wheat during development stages. Mating is an essential step in the life cycle of *T.indica*. Obtain direct evidence for the role of cAMP in a signaling cascade controlling mating and virulence in *T. indica*, the gene encoding the regulatory subunit of the cAMP-dependent protein kinase (rpkA) was cloned, and sequenced. The cloned amplification products were used to identify genomic clones and its expression cheek by RT PCR. Two isolates, KB3 and KB9, in which expression rpkA high, were unable to induce mating between two MS culture, and were nonpathogenic on even in susceptible wheat genotype. These results were confirmed among 3 different isolates were studied by inoculating cAMP treated and without treated monoteliosporic cultures on host genotypes comprising of susceptible and resistant wheat genotype against *T.indica*, at physiological growth stage of spike, Z-49. After maturity, coefficient of infection (CI) was calculated based on the percentage of infection. It is concluded that, *T.indica*, the basidiomycete bunt pathogen of wheat, exhibit dimorphism in which mating between haploid budding cells establishes a pathogenic filamentous cell type, and cAMP signaling cascade controlling mating and virulence in *T. indica*.



P3.12

Flowering Locus D, a putative histone demethylase of *Arabidopsis thaliana*, functions as a modulator of systemic acquired resistance

Vijayata Singh, Zeeshan Zahoor Banday, Deepjyoti Singh, Shweta Roy and Ashis K. Nandi
School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India.
email:vijayata.cb@gmail.com

Localized pathogen infections in plants lead to development of systemic acquired resistance (SAR) that protects the plant from subsequent infections, and provide long term immune memory. The infected tissue release vascular mobile signals that get distributed throughout the plant to generate infection memory. Even though, several mobile signals are known, genetic regulation of infection memory development and retention are not well known. Through screening of EMS mutagenized *Arabidopsis* population, we identified a novel mutant *rsi1* (reduced systemic induction 1) that modulates SAR. The *rsi1* mutant displays compromised SAR activation without any defect in local defense. Positional cloning of this mutant revels that the mutant has a lesion in *FLOWERING LOCUS D* (FLD) locus. *FLD* is a well known flowering regulator that epigenetically modulates flowering phenotype in *FLC* dependent manner. Our results suggest that *FLD* is required for systemic accumulation of SA and upregulation of several pathogenesis related genes. Petiole exudates swapping experiments reveals that FLD function is necessary for perception of mobile signals in distal tissues; it has no defect in generation of mobile signals. *FLD* encodes a plant homolog of mammalian *LSD1* and is involved in H3K4 demethylation. In general, Histone3 lysine4 (H3K4) demethylation indicates an activation mark of genes. On the basis of chromatin immune-precipitation (ChIP) analysis we found that FLD mediated H3K4 demethylation of *PR1*, *PDF1.2WRKY29* and *WRKY6* is important for immune memory formation. Priming of these defense related genes were also inhibited in *rsi1* mutant. Further we supported our results by using Lysine specific demethylase1 inhibitor PCPA upon SAR activation and memory formation. These all results suggest that, FLD mediated histone demethylation is important for immune memory development in plants.



P3.13

Endophytes isolated from *Typha angustifolia* alter nitrogen use efficiency in Rice

Chinmay Saha[1], Gairik Mukherjee[1], Dipayan Bose[2]& Anindita Seal [1]
[1]Dr. B.C. Guha Centre for Genetic Engineering & Department of Biotechnology, University of Calcutta, Kolkata-700019, India. [2]Department of Botany (Appl. Microbiology), Banaras Hindu University, Varanasi- 221005, India.
email: aninditaseal@gmail.com

Typha angustifolia is a wetland macrophyte that grows naturally in uranium (U) mine associated, nutrient-limited, metal-polluted wetlands. A group of microbes were found to endophytically colonize the plant. The endophytes showed several properties reported for plant growth promotion and were positive for the marker gene of nitrogen fixation *nifH*. Inoculation of soil with the bacterial consortium showed significant growth promoting effect on *Typha* as well as rice, as observed by considerable increase in the biomass of the plants. Nitrogen use efficiency was also found to increase in microbes-treated rice. Real time PCR was used to check the expression of marker genes involved in nitrogen uptake and assimilation like Ammonium Transporter (AMT1.1), Glutamine Synthatase (GS1.1) and Nitrate Transporter (NRT2.3a). The results indicated that the Endophytic consortium upregulated NRT and GS genes significantly in roots and GS and AMT genes in shoots of rice plants. Our results suggest that *Typha* endophytes alter nitrogen metabolism and nitrogen use-efficiency in rice.



P3.14

Transcriptome profiling of roots of susceptible and resistant tomato cultivars at various stages of infection with root knot nematode (*Meloidogyne incognita*)

Shukla N.[1*], Chauhan R.[2*], Kaur P.[1], Joshi G.[1], Katiyar-Agarwal S.[3], Agarwal M.[1], Jagannath A.[1], Goel S[1]., Shankar R.[2] and Kumar A.[1]
[1] Department of Botany University of Delhi, Delhi 110007, India. [3]Department of Plant Molecular Biology University of Delhi, South Campus, New Delhi 110021, India; [2]Computational Biology & Bioinformatics, CSIR-IHBT, Palampur 176061, Himachal Pradesh, India
email: nehashukla04@gmail.com

Root-knot (*Meloidogyne* spp.) nematodes are sedentary phytonematodes that develop an intimate and sustained obligate parasitic relationship with their host plant roots. Infective second stage juveniles (J2) invade the host roots and select few xylem parenchymal cells to form specialized feeding cells called giant cells to act as nutrient sinks. Giant cells formation is activated by nematode secretions, which manipulate plant gene expression leading to formation of hypertrophied and multinucleate giant cells. Our objective is to study transcriptome profile of tomato roots during nematode infection at different stages of infection as well as during susceptible and resistance responses using high throughput sequencing. More than one billion good quality paired-end reads were generated and utilized for reference-based assembly. Almost 93,234 transcripts with an average length of 2,150 bp were assembled on plant genome, which were represented by a group of 40,924 unigenes. Of these, 85,125 transcripts were annotated while 8,109 were found to be novel. All the assembled transcripts were used to derive the associated KEGG, EC and GO annotations. Overall 254 KEGG pathways were identified and plant-pathogen interaction pathway was found to be preeminent (6.5%) in the whole transcriptome.A total of 1,589 different enzyme classes were identified where serine/threonine protein kinase was predominant (18%). GO analysis revealed that metabolic process was prominent (21.6%) among the primary biological processes. Plant transcription factor-based studies categorized 26,450 transcripts in 83 transcription factor families. Differential expression analysis revealed that a set of genes were up- and down-regulated during various stages of disease development as well as in susceptible and resistance responses. In susceptible response, 259 differentially expressed genes (DEGs) were found among different infected time points whereas 230 genes showed significant differential expression between uninfected and infected roots. In susceptible and resistance responses, 68 DEGs were found during initial stages of disease development.



P3.15

Sheath blight: Efficacy of resistance inducers

Bhavna Thakur, Alok Shukla, J. Kumar[1] and Gurdeep Bains
Department of Plant Physiology, G.B. Pant University of Agricultural and Technology, Pantnagar, Uttarakhand-26314 India;
[1]Department of Plant Pathology, G.B.Pant University of Agriculture and Technology, Pantnagar, Uttarakhand-263145 India.
email: bhavna.thkr@gmail.com

Rice sheath blight caused by the pathogen *Rhizoctonia solani* is one of the major diseases in rice (*Oryza sativa* L.). Plant responses to attack by pathogen are complex, and involve the induction of expression of a large array of defense related genes encoding diverse proteins. The present investigation was conducted to evaluate the effects of individual and combined application of biological resistance inducers *viz. Trichoderma harzianum* (TH38), *Pseudomonas fluorescens* (PF28) and chemical resistance inducer salicylic acid (SA) against sheath blight disease of rice *(Oryza sativa* L. cv. Pusa Basmati1 and Kalanamak 3119). The experiment was conducted in completely randomized design (CRD) with eleven treatments and three replications. Application of resistance inducers was done in the form of seed treatment and three subsequent sprays at different growth stages of rice *viz.* early vegetative, reproductive and maturity prior to pathogen challenge. Disease index was significantly reduced in the treatments TH38+PF28, PF28+SA and SA as compared to inoculated control which recorded highest disease index. The expression of chitinase gene was higher in the treatments TH38+PF28+SA and TH38+SA while a higher level of expression of lipoxygenase (LOX) gene was observed in the treatments TH38, SA, TH38+SA and PF28+SA. The expression of phenylalanine ammonia-lyase (PAL) gene was significantly higher in PF28+SA. Alterations in phenolic content and malondialdehyde (MDA) was also recorded in the treated plants during the course of study. A combination of biocontrol agents and chemical resistance inducers can provide promising integrated alternatives in suppression of diseases. Thus, activating induced systemic resistance (ISR) and systemic acquired resistance (SAR) simultaneously can increase the efficacy of disease control.



P3.16

Arachis and *Oryza* in an intercropped field are colonized by nod+ and nod divergent Bradyrhizobial populations respectively

Guha S., Sarkar M., Dasgupta M
Department of Biochemistry, University of Calcutta.35, Ballygunge Circular Road, Kolkata-700019, West Bengal, India
email: maitrayee_d@hotmail.com

To develop root nodule symbiosis, rhizobial invasion in Aeschynomene legumes bypass the intracellular infection-threads. Instead, invasion occurs through epidermal cracks, which is also the entrance for the endophytic colonizers in plants. Unlike the endophytes which remain intercellularly colonised, the rhizobia are endocytosed directly by a cortical cell for intracellular symbiosis. In several Aeschynomene legumes, this process is demonstrated to be nod factor independent. The phenomenon was first brought into notice when the sequenced genomes of the photosynthetic *Bradyrhizobium* strains, ORS278 and BTAi1 were found to be devoid of nod genes. These strains naturally associate with *Oryza* but couldalso nodulate *Aeschynomene legumes.* Our objective was to check the nod genes of Bradyrhizobial colonizers in an Aeschynomene legume *Arachis* and compare and contrast them with those that were collected from *Oryza* grown in the same field. For this, we worked out the genetic diversity of culturable bradyrhizobia strains collected from the nodule interior of *Arachis hypogaea* cultivated in a field at 23°24"N, 88°30"E. A total of 19 *Arachis* and 18 *Oryza* isolates were subjected to MLSA using 16S *rRNA*, *glnII, dnaK*. These phylogenetic analyses indicated that the population of *Bradyrhizobia* isolated from *Oryza* formed a separate cluster with little overlap with the *Bradyrhizobial* population isolated from *Arachis* indicating population segregation between *Arachis* and *Oryza*, in spite of having comparable modes of colonization. Nod locus of *Arachis* colonizers but not that of *Oryza* colonizers could be PCR amplified by divergent primers under identical conditions. This indicated that the nod locus in *Oryza* colonizers was either absent or too divergent from that in *Arachis* colonizers. This study indicated that *Arachis* though an Aeschynomene legume probably undertakes nod dependent interactions for RNS but direct involvement of nod factors or Nod Factor Receptors (NFRs) is yet to be understood.



To bee or not to bee? Defensive animal mimicry by plants

Simcha Lev-Yadun,
Department of Biology & Environment, Faculty of Natural Sciences, University of Haifa - Oranim, Tivon 36006, Israel
email: levyadun@research.haifa.ac.il

While defensive plant mimicry by animals is a very well-known phenomenon, defensive animal mimicry by plants has received much less attention. Concerning plant biology, only animal mimicry for pollination received significant (although not sufficient) attention. The suggested types of defensive animal mimicry by plants belong to two general types: direct animal mimicry, and mimicry of cues for animal action. Direct animal mimicry includes bee, caterpillar, ant, butterfly eggs, butterflies, aphids and beetle. Indirect animal mimicry includes tunnelling, chewing damage, carrion and dung odors and bird dropping. More types are expected to exist. Some cases (butterfly eggs, tunnelling) were tested experimentally and found to operate. The current proposed defensive animal mimicry types can be classified as Batesian mimicry, masquerade, a mixture of Batesian mimicry & Masquerade and probably the related phenomenon of, animal perceptual exploitation. How plants look in the U.V. and the olfactory aspect should be studied as was done in pollination biology. Overlap with other defensive and physiological functions should be considered. Too little attention was given to defensive animal mimicry by plants and certainly there are scientific treasures waiting there to be found and explored.



P4.1

Functional characterization of HY5 homolog in rice

Naini Burman, Akanksha Bhatnagar and Jitendra P. Khurana
Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi- 110021
email: nainiburman21@gmail.com

Light is an important environmental signal, which is perceived by plants to adapt to ambient conditions. To perceive this signal, plants have evolved four major classes of photoreceptors - phytochromes, cryptochromes, phototropins and UVR8. These photoreceptors perceive the light signal and pass on this signal to master regulators, which in turn bring about changes in downstream components, leading to changes in gene expression. One of these master regulators is HY5, also known as *LONG HYPOCOTYL 5*,which plays an important role in photomorphogenesis. HY5 is a bZIP transcription factors which has a leucine zipper domain and basic residues for DNA binding. In dark, HY5 interacts with COP1/SPA1 and is constitutively degraded by COP9 signalosome complex. However, as soon as the cell perceives light, phytochromes (activated by red/far-red light) move into the nucleus where it inhibits COP1 activity by excluding it from the nucleus and targeting it to the cytoplasm. Likewise, blue light activated CRY1 disturbs COP1/SPA1 interaction and prevents degradation of HY5. As a result, HY5 is able to activate the downstream light-responsive genes involved in photomorphogenesis. Most of the work on HY5 and its interacting partners to regulate photomorphogenesis has largely emerged through work on *Arabidopsis*. In our endeavour to characterize HY5 from monocots, we have identified its orthologs in rice based on presence of COP1-binding and bZIP domains. The work presented here deals with the functional characterisation of one of the HY5-homolog in rice. The overexpression and RNAi transgenics of this gene reveal that this it is involved in controlling plant height and lateral root development. The overexpression transgenics show close to 30% reduction in height of last and second last internodes and also close to 50% sterility. The RNAi lines show an increase in plant height and more lateral root development as compared to wild type. The complementation analysis of *AtHY5* mutants with this rice gene shows that it could be a functional equivalent of *Arabidopsis* HY5.



P4.2

Over-expression of *indica* rice cryptochrome 2 gene induces partial photomorphogenesis in dark, semi-dwarfism and early flowering

Pooja Sharma[1,2], Sushma Mishra[1], Dibyendu Kumar[1,3] and Jitendra P. Khurana[1]
[1]Interdisciplinary Centre for Plant Genomics & Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India. [3]Waksman Institute of [2]Plant Biology Major, Iowa State University, Ames, IA 50011, USA. Microbiology, Rutgers University, New Jersey 08854, USA.
email: sushmamishra87gmail.com

Cryptochromes, the blue/UV-A light photoreceptors in plants, have been best studied in *Arabidopsis thaliana*, which possesses three members: CRY1, CRY2 and CRY3. Among these, CRY2 primarily regulates photoperiodic flowering, apart from its minor role in blue light-mediated inhibition of hypocotyl elongation. Besides *Arabidopsis*, CRY2 has been functionally characterized from pea, *japonica* rice, sorghum, tomato and apple. Since the *indica* and *japonica* rice differ markedly in their morphology, cooking quality and the environmental conditions under which they are cultivated, we decided to functionally characterize CRY2 from *indica* rice. The *CRY2*cDNA from *indica* rice was isolated and sequenced previously in our laboratory; it is 1956 bp long and codes for a protein of *ca*73.8 kDa. *OsiCRY2* cDNA sequence shows 8 SNPs vis-à-vis *japonica CRY2* cDNA. Although *OsiCRY2* transcript levels are not affected by light but its protein is degraded rapidly when exposed to blue light. For functional characterization, the *OsiCRY2* gene was over-expressed in both rice and *Arabidopsis* and stable, homozygous lines raised for phenotypic analyses. The transgenic lines exhibited partial photomorphogenesis even in complete darkness, faster emergence of leaves and increased seed weight per 100 seeds. Similarly, the dark-grown *Arabidopsis* seedlings over-expressing *OsiCRY2* developed short hypocotyl and partially open apical hook. The rice transgenics were semi-dwarf throughout the life cycle but flowered at the same time as the wild-type due to the photoperiod-insensitivity of the cultivar used for transformation. The *Arabidopsis* transgenics, on the other hand, were found to be early flowering by 10-14 days under long day conditions. These photomorphogenic effects observed in transgenics of both rice and *Arabidopsis* are unique and impart new knowledge to our understanding of the developmental roles cryptochrome 2 plays in diverse plant species.



P5.1

Acetylcholine suppresses shoot formation and callusing in leaf explants of *in vitro* raised seedlings of tomato, *Lycopersicon esculentum*Miller var. Pusa Ruby

Bamel K.[1,2], Gupta R[1]. And Gupta S. C.[1]
[1]Department of Botany, University of Delhi, Delhi – 110007, India
[2]Department of Botany, Shivaji College, University of Delhi, Delhi – 110027, India
email: kbamel@yahoo.in

We have shown earlier that exogenous application of animal neurotransmitter acetylcholine (ACh) induces rooting and promotes secondary root formation in leaf explants of tomato (*Lycopersicon esculentum* Miller var. Pusa Ruby). Inhibition of enzymatic breakdown of ACh by neostigmine simulates the action of ACh. Now, we present experimental evidence to show that ACh causes decrease in shoot formation in leaf explants of tomato (*Lycopersicon esculentum* Miller var. Pusa Ruby) when cultured on shoot regeneration medium. The optimum response was obtained at 10^{-4} M ACh-enriched medium. ACh also causes decrease in percentage of cultures forming callus and reduces the callus mass. Inhibitors of ACh hydrolysis, neostigmine and physostigmine, also suppresses callogenesis and caulogenesis whereas choline and acetate, the breakdown products of ACh do not alter the morphogenic response induced on the shoot regeneration medium. Neostigmine showed optimal reduction in shoot formation at 10^{-5}M. The explants cultured on neostigmine augmented medium showed decline in the activity of ACh hydrolyzing enzyme acetylcholinesterase. ACh and neostigmine added together showed marked reduction in callus mass. These results strongly support the role of ACh as a natural regulator of morphogenesis in tomato plants.



P4.2

OsFBK, a rice F-box protein, is a component of the Ubiquitin-26S proteasome system (UPS) and involved in anther and root development

Pratikshya Borah, Shane W. Rydquist and Jitendra P. Khurana
Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology
University of Delhi South Campus, New Delhi 110021, India
email: pratikshya.borah@gmail.com

Regulated proteolysis of proteins takes place in response to distinct environmental and developmental cues so that abundance of crucial cellular regulators could be controlled. This is particularly important for plants that require a high degree of plasticity in their developmental processes owing to their sessile nature. Proteins poised for proteolysis are, in majority, ubiquitinated and are turned-over by the 26S proteasome with the concomitant release of ubiquitin for reuse. Central to the Ubiquitin-26S proteasome system (UPS) are a large group of proteins, known as the SCF complex (Skp1-Cullin-Rbx1-F-box) that are involved in substrate identification and ubiquitination. The substrate adaptor, F-box protein, is one of the largest group of proteins found in plants. The sheer number of these proteins in rice (971) and *A. thaliana* (897) as compared to humans (69) clearly indicates the importance of this family in plants. The present research deals with a rice F-box protein, OsFBK, that has a Kelch domain at its C-terminal, a unique combination in plants. Analysis by Y2H showed that this protein is a part of an SCF complex. Expression analysis of this gene revealed its up-regulation in the reproductive stages, roots and during dehydration stress. Transgenics over-expressing this gene and its corresponding RNAi lines in rice clearly reveal the role of this gene in anther and root development. The putative interacting partners were identified from Y2H libraries of panicle, anther and root and their interactions were validated by Bimolecular Fluorescence Complementation and Co-immunoprecipitation assays. Thus, from these results it could be inferred that OsFBK might be involved in lignification process in anthers. Also, its role in early panicle stages is being studied as it has been found to be interacting with a MADS-5box transcription factor. The transgenic and the protein studies would thus be instrumental in understanding the precise role of OsFBK in rice development.



P5.3

In silico analysis indicates Receptor Like Kinase and Kinase Interacting Protein 1 genes isolated from *Cenchrus ciliaris* interact during apomictic female gametophyte development

Anuj Dwivedi and Vishnu Bhat
Plant Developmental Biology, Department of Botany, University of Delhi, Delhi-110 007
email: bhatv64@rediffmail.com

Receptor like kinases (RLK) were initially classified into three classes such as S-domain, Leu-rich repeat and the epidermal growth factor like class based on their extracellular domains. They are architecturally related to tyrosine and serine/threonine receptor kinases and are known to play diverse physiological roles, including control of shoot and floral meristem size in Arabidopsis, mediation of brassinosteroid signal transduction pathway by BRI1 and control of the stigma's ability to recognize and reject self pollen in self-incompatibility interactions. In Petunia, another protein namely Kinase interacting protein 1 (KIP 1) was reported as interacting very strongly with Petunia receptor like kinase. In order to understand the roles of these two genes, a partial RLK and a full length KIP 1 gene had been isolated earlier from apomictic *Cenchrus ciliaris.* To determine whether RLK and KIP 1 are specifically expressed in the embryo sac of apomictic *Cenchrus ciliaris,* in-situ hybridization was carriedout on the ovaries of both apomictic and sexually reproducing flowers. While RLK specifically expressed in the ovules of apomictic flowers, KIP 1 expression was limited to ovules at mature embryo sac stage in both apomictic and sexual flowers. But, the KIP 1 gene was differentially expressed between ovules at megaspore mother cell and mature embryo sac stage indicating its possible role in the formation of mature embryo sac and fertilization. In order to understand their possible interaction during embryo sac development, *in silico* analysis of these two genes was carried out using I-TASSAR, Combfunc and CSSB softwares. The structure and binding sites of both the proteins alongwith their molecular and cellular function have been deciphered. To further validate the *in silico* results, the partial peptides are being produced in heterologous organism such as *E.coli* after cloning the gene fragment in the expression vector.



P5.4

Analysis of root system architecture in Chickpea

Dwivedi V., Parida S.K. and Chattopadhyay D.
National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, India
email: dwivedivikas1989@gmail.com

Chickpea (*Cicer arietinum*) is the third most consumed legume in the world. It is a major source of protein, therefore, a very important crop in developing countries. Annual crop yield is very low due to several biotic and abiotic factors. The advancement of modern biotechnological approaches has now potential to dissect with complex traits for genetic enhancement of chickpea. Development of root biomass and its architecture helps the plant to access water more efficiently from soil. The dynamic nature of root is controlled by external factors such as environmental signals, water and nutrients availability, salinity and some other factors. The manipulation of root architecture could minimize the negative impact of a number of abiotic factors and can improve yield. Root System Architecture (RSA) is vaiable between two species and also within the species. RSA is a highly plastic and dynamic trait.Many methods are used for study of root system. The development and architecture of roots hold potential for the use and manipulation of root characteristics to both increase yield and optimize agricultural land use. To understand the variation in chickpea root system architecture in field conditions, 42 different varieties of chickpeawere studied. Phenotypic root system architecture (RSA) was analyzed using different tools to identify genotypes with contrasting root traits. We aim to obtain chickpea cultivars with improved root architecture by combining genomics and molecular genetics approaches.



P5.5

Ameliorating effect of nitric oxide on *in vitro* caulogenesis of *Linum usitatissimum* on iron-deficient culture medium possibly by its internal mobilization

Charu Kalra and Shashi B. Babbar
Department of Botany, University of Delhi, Delhi 110007, India
Department of Botany, Deen Dayal Upadhyaya College, Delhi, India
email: charu149@yahoo.com

Iron, which is generally provided in the culture media along with a chelate, plays an important regulatory role in *in vitro* morphogenesis. A series of experiments were conducted to study role of iron in caulogenesis from the hypocotyls explants of *Linum usitatissimum* cultured *in vitro* and the influence of SNP (sodium nitroprusside) – a NO (nitric oxide) donor on its absorption and internal mobilization. The absence of iron and chelate in the medium drastically inhibited the caulogenic response. Transfer experiments which involved transfer of the explants from Basal medium (BM) to the same deficient in both iron and chelate (DBM) or vice versa after different time intervals, revealed that iron is obligatorily required for caulogenesis during initial 4-6 days of culture. The explants cultured on BM for initial six days and then transferred to DBM had caulogenic response equivalent to that of the explants cultured continuously on BM. While, the explants deprived of iron and chelate for only initial six days behaved as if cultured continuously on DBM. However, the explants cultured on DBM augmented with SNP (5 μM) exhibited caulogenic response equivalent that of those cultured on BM. The observed mitigating effect of SNP was due to NO was confirmed as NO scavengers, (2-(4-caroxyphenyl)-4, 4, 5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) and methylene blue, provided individually along with SNP abrogated the mitigating effect the latter on morphogenesis. The comparison of iron contents of variously treated explants revealed (i) a significant higher content of iron in the explants cultured on BM than those incubated on DBM and (2) SNP provided alone in BM or DBM or along with either of the NO scavengers did not affect the iron content. As the restoration of caulogenic response on DBM by NO was not accompanied with any increase in endogenous levels of iron in the explants the observed mitigating effect of NO could possibly be because of internal mobilization of iron to the sites of differentiation.



P5.6

ROS is also involved in *in vitro* flowering of *Lemnaaequinoctialis* under non-inductive conditions

Ashima Khurana and Shashi B. Babbar
Botany Department, Zakir Husain Delhi College, Delhi, 110002, India
Department of Botany, University of Delhi, Delhi, 110007, India
email: babbars@rediffmail.com

Nitric oxide (NO) has been shown to be involved in a plethora of developmental processes in plants, including seed germination, seed dormancy, rhizogenesis, caulogenesis, hypocotyl elongation, leaf expansion, mesocotyl extension, stomatal movement, xylem differentiation and fruit senescence besides playing important role in biotic and abiotic resistance. An earlier investigation revealed the repressive effect of NO in flowering of *Arabidopsisthaliana*. Contrary to this, NO donors, used at lower concentrations than used in earlier study, induced flowering in the two strains of *Lemna aequinoctialis* (a duckweed), that too, under non-inductive photoperiodic conditions. NO scavengers substantially negated this floral inductive effect of NO donors. As SNP, one of the NO donors used in the study, also releases CN^- besides NO, effect of cyanide donors was also studies. These also induced flowering in both the strains under non-inductive conditions as was reported earlier too. Through a series of experiments, NO was also indicated to be partly involved in SA-induced flowering in the two strains of *Lemna*. As the observations indicated partial role played by NO, the involvement of some other component(s) was explored. Since the NO scavengers were able to negate NO and cyanide-induced flowering only partially, the possibility of involvement of reactive oxygen species (ROS) in the observed flower induction by NO and CN^- was investigated. This was considered all the more relevant because cyanide is well known to enhance production of ROS by the inhibition of COX. The possibility of involvement of ROS was checked by the using specific ROS scavenger DMTU (Dimethyl thiourea) provided along with cPTIO in the medium already containing $K_3[Fe(CN)_6]$. DMTU was found to reduce the percentage of flowering below the level brought down by cPTIO alone, indicating involvement of ROS in the observed flower induction. Floral inductive effect of ROS was further validated, as ROS generator, methyl viologen (MV) induced flowering in both the strains. The levels of lipid peroxidation, as evidenced by malondialdehyde (MDA) content, in the plants subjected to different treatments also compared. Among all the treatments, lipid peroxidation was found to be maximum in the plants subjected to SNP followed by $K_3[Fe(CN)_6]$ and MV. In all the treatments, except that involving SA, the levels of lipid peroxidation was directly proportional to the percentages of flowering recorded previously. The complexity of cross-talk involved among signal molecules, NO, ROS, SA and CN^- in this important developmental transition, ushering in the reproductive phase in the life cycle of these plants, is well reflected by these results.



P5.7

Isolation and molecular characterization of heterotrimeric G-protein family from *Brassica nigra*

Kumar R and Bisht N.C.
National Institute of Plant Genome Research, New Delhi-110067. India
email: kumarroshan08@gmail.com

Heterotrimeric G-proteins, comprising of α, β and γ subunits, are one of the major signalling transducers of the cell, controlling a wide range of biological processes across phyla. These signalling molecules are well characterized in model plant *Arabidopsis* and rice. Although, in recent years the G-protein inventories are possible from few plant species, however roles played by G-proteins in regulating various growth and developmental traits from any of the crop species are lacking. Crops belonging to genus *Brassica* are cultivated globally as a source of vegetable, oilseed, condiment and fodder. *Brassica nigra* (BB), a major condiment crop, is a diploid *Brassica* species, and one of the progenitor species of globally cultivated allotetraploid species *B. juncea* (AABB) and *B. carinata* (BBCC). In this study, we have isolated one Gα, three Gβ and four Gγ coding sequences from *B.nigra*. Transcript expression profiling of these genes in various tissue types revealed differential temporal and spatial expression pattern, thereby suggesting transcriptional sub-functionalization of multiple G-protein genes. All the eight G-protein genes are expressed during seed maturation and germination, wherein during seed germination the expression of *BniB.Gα1* transcript was found to be highest among all the G-protein genes. In response to various abiotic and biotic stress conditions, most of the G-protein genes were differentially regulated and in general their transcript accumulation increased at later time point of treatments. Interestingly, the *B. nigra* G-protein subunits showed interaction specificity among them, when tested using both membrane based split ubiquitin system and yeast two hybrid screens, thereby suggesting that differential functional combinations of heterodimer and heterotrimer may exist in *B. nigra*. This study is a first step towards our understanding on the roles played by G-protein signalling components in controlling various growth and development processes in polyploid *Brassica* species.



P5.8

Exploring the role of histone deacetylases in epigenetic regulation of fiber development in *Gossypium hirsutum* L.

Kumar V.,Rai K. M., Singh S. K., Yadav V. K., Singh S. P. and Sawant S. V.
CSIR-National Botanical Research Institute, Lucknow – 226001, India
email: rajvirendraraj@gmail.com

The epigenetic regulation of gene expression is controlled by the DNA methylation, post translational modifications of histones, small RNAs and histone variants. The epigenetic regulation control many fundamental biological processes in plants such as flowering, seed germination, root hair development and their response to pathogens. The histone acetylation is reversibly controlled by Histone acetyltransferases (HATs) and Histone deacetylases (HDACs) by either adding or removing acetyl moiety from various lysine residues of H3 or H4 respectively, thus controlling the gene expression. In plants, three families of HDACs, i.e. RpD3/HDA1, SIR2 and HD2 (plants specific) have been identified. *Gossypium hirsutum* (AD genome) is an allotetraploid species and evolved by polyploidization events between diploid *G. raimondii* (D-genome) and *G. arboreum* (A-genome). Cotton fiber is a single cell trichome that originates from epidermal cells of ovule at or just before anthesis. It undergoes through four overlapping developmental stages i.e. initiation, elongation, secondary cell wall synthesis, and maturation to eventually giving lint fiber. In the present study, we performed genome-wide *in-silico* analysis to identify 17 HDAC orthologs in *G. raimondii, G. arboreum and G. hirsutum* on the basis of homology search with the previously known HDACs in the *Arabidopsis thaliana*. We also identify the homeologs (A and D-genome specific) of these HDACs in *G. hirsutum* and analyze their genomic organization. We found that Conserve domains (CD) are present in RpD3 and SIR2 families but not in the HD2 family. Cellular localization prediction reveals that HDACs mostly localize in the nucleus. Phylogenetic analysis reveals HDACs are evolutionary conserved among the eukaryotes. Tandem and segmental duplication pattern suggests the expansion of HDAC family during the evolution. The temporal differential expression analysis of HDAC alleles shows the contribution of A- and D-genome specific HDACs in the fiber development. Overall, our study provides a further understanding of HDACs evolution and their molecular role in cotton fiber development.



P5.9

Unknown function protein having Serine Rich Repeat is involved in glucose and auxin dependent root growth and development

Mishra B.S. and Laxmi A.
National Institute of Plant Genome Research, New Delhi - 110 067, India
email: ashverya_laxmi@nipgr.ac.in

About 20% to 40% of all eukaryotic genomes sequence have genes which encode for proteins of unknown function. In *Arabidopsis* about 13% of genes encode for proteins having a completely unknown function and more than 30% of the genes are poorly characterized. Proteins with unknown function can be further classified as proteins with obscure features that lack currently defined motifs or domains. They also include proteins with defined features which contain at least one previously defined domain or motif. Transcriptome and proteome profiling experiments suggest that in plants many of these proteins play important roles during different biological processes. Studying the functions of these uncharacterized genes remains one of the main challenges in modern biology. Glucose (Glc)-auxin microarray, data analysis suggested that major groups of genes regulated by auxin and Glc encoded for proteins whose molecular and biological functions were unknown. Detailed functional characterization of a gene of unknown function has been carried out. This gene is 6.8 fold up regulated by auxin and 3.5 fold down regulated by Glc. The cDNA of this gene is 1026 bp in length and encodes for a protein of 341amino acids which is found to be enriched with serine rich repeats. The sequence of this protein does not match with any known proteins whose function has been characterized, and does not have any known conserved domain. Overexpression and down-regulation of this gene affect *Arabidopsis* seedling root growth and development. This protein is localized in nucleus in diffused and as speckles-like bodies and interacts with an exon junction complex (EJC) protein Y14. However, the biological significance of this interaction is yet to be explored.



P5.10

Structural and biochemical changes accompanying attainment of stigma receptivity and pollen-stigma interaction in sunflower

Sharma B.* and Bhatla S.C.
Department of Botany, University of Delhi, Delhi-110007, India
Department of Botany, MM (PG) College, Modinagar, Uttar Pradesh
email: basudhashama@gmail.com; bhatlasc@gmail.com

Stigma development in sunflower is accompanied with structural and biochemical changes which marks the onset of signaling events associated with successful pollen-stigma interaction. Among the structural changes, a modification in the morphology of receptive stigma and accumulation of elaioplasts and endoplasmic reticulum in the basal region of the papillae make stigma adaptive for receiving pollen grains. Hydration and germination of pollen subsequently leads to the directed growth of pollen tubes towards the ovary for successful fertilization. Extracellular lipidic secretions between the papillae and in the transmitting tissue appear to play a critical role in stigma receptivity. Several biochemical changes are associated with attainment of stigma receptivity. Stigma-specific marker enzymes (esterases and peroxidases), which also constitute the components of stigma surface proteins, exhibit new isoforms during attainment of receptivity, indicating their critical role in pollen-stigma interaction. A gradual increase in signaling molecules, such as calcium, reactive oxygen species (ROS) and nitric oxide (NO), and the activity of ROS scavenging enzymes (superoxide dismutase and peroxidase) indicates their modulation by external and internal stimuli. Internal stimuli (derived from mature embryo sac) and external stimuli (pollination) also exert significant effects on the initial stages of pollen-stigma interaction. A flexibility in sporophytic self-incompatibility has been reported among the members of Asteraceae. Compatible pollen grains germinate and penetrate stigma surface, and enter the nutrient-rich transmitting tissue, whereas incompatible pollen grains are rejected on the stigma surface or pollen tubes fail to penetrate the stigmatic tissue and grow parallel to the papillae. The present work, thus, highlights structural and functional relationship of stigma development and pollen-stigma interaction.



P5.11

YABBY and MYB transcription factors genes as master regulators for plants developmental process- a review

Govinda, Singh S.,and Rohilla J.
Department of Biochemistry,Maharashi Dayanand University,Rohtak, Rohtak –124501. India
email: govind22053@gmail.com

Regulation of gene expression at the level of transcription controls many crucial biochemical and physiological processes in all plants.Transcription factors play importent role in controlling and regulating these processes at gene level. The mode of action of TFs is to recognize and bind to a segment of DNA in the promoter and/or enhancer region . TFs may be a zink finger,bLBH or Homeobox. YABBY TFs and MYB TFs gene families are large families and involved in various cellular processes like differentiation, development,metabolism,abiotic and biotic stresses,defenceand leaf tissues patterning. MYB proteins comprise a large family of plant transcription factors, members of which perform a variety of functions in plants.The MYB proteins such as PhMYB3,PhMYB,An2ZmMYBC1 and ZmMYBP1 controls the anthocyanin synthesis whileAtMYB011,AtMYB017 and AtMYB023 help in trichome initiation,branching, formation and root hairs patterning. The maize has R2R3-MYB genes as the most studied. A total of157 genes encoding R2R3-MYB Proteins has been Identified in the Maize genome which performs different biochemical developmental processes. YABBY genes are transcription factors with zinc finger and high mobility group-related domains. The YAB genes FIL and YAB3 are expressed on the abaxial side of emerging lateral primordia and may be involved in abaxial cell-type specification.YABBY genes are presents in Oryza sativa subsp. indica, Oryza sativa subsp. japonica,Sorghum bicolor,Zea mays,Arabidopsis lyrata,Carica papaya,Populus trichocarpa and Vitis vinifera.Tobbacco has ten and Arabidopsis six YABBY genes which maintains different functional processes.The current review will provide necessary information in understanding the regulatory processes and to develops the plans for crop improvement.



P5.12

Glucose control of root growth direction in *Arabidopsis thaliana*

Manjul Singh, Aditi Gupta, and Ashverya Laxmi
National Institute of Plant Genome Research, New Delhi – 110067, India
email: manjul@nipgr.ac.in

The ability for plant organs to guide their growth at a specified angle from the gravity vector (gravitropism) ensures that the shoot is positioned to maximize its light-harvesting capabilities, and the roots are positioned downward so as to maximize the uptake of water and nutrients. The ability to re-adjust their growth direction by sensing any deflection relative to the direction of gravity involves gravity perception, signal transduction followed by differential growth. Directional growth of roots is a complex process that is modulated by various environmental signals. Here, we show that presence of glucose (Glc) in the medium could also extensively modulate seedling root growth direction. Glc modulation of root growth direction is dramatically enhanced by simultaneous brassinosteroid (BR) application. Glc could enhance BR receptor BRASSINOSTEROID INSENSITIVE1 (BRI1) endocytosis from plasma membrane to early endosomes. Glc induced root deviation is highly enhanced in PP2A defective mutant, *roots curl in naphthyl phthalamic acid 1-1 (rcn1-1)* suggesting the role of phosphatase in Glc induced root-growth deviation. RCN1, therefore, could act as a link between glucose and BR signaling pathway. Polar auxin transport (PAT) works further downstream to BR in controlling Glc induced root deviation response. Glc also affects other root directional responses such as root waving and coiling leading to altered root architecture. High light intensity could also mimic the Glc induced changes in root architecture which were highly reduced in Glc signaling mutants. Thus, under natural environmental conditions changing light flux in the environment may lead to enhanced Glc production/response and is a way to manipulate root architecture for optimized development via integrating several extrinsic and intrinsic signaling cues.



P5.13

Cellular characterisation of *Nicotiana*tapetum developmental stages and differential gene expression analysis to reveals the PCD signalling in their degeneration

Singh S.P., Goel R., Yadav V.K., KumarV., Iqbal Z and Sawant S.V.
CSIR- National Botanical Research Institute, Lucknow-226001, India
email: spsinghbiome@gmail.com

Tapetum is the innermost wall layer of microsporangium. It providing enzymes, wall materials and nutrients required for pollen development. It undergoes PCD like degeneration which is crucial for pollen fertility. Developmental stage specific detailed cellular characterisation and the signalling cascade that results in their apoptotic like degeneration is needed to be done. The anatomical and Transmission Electron Microscopy (TEM) of *Nicotiana tabacum* petiteHevana anther revels that tapetum is short lived, originates in -5 stage of anther development and completely degenerated up to S_6. The demarcated tapetal initial at S_{-5} which further differentiates at S_{-3} and distinct tapetum with several trans-golgi networks (TGN) with originating vacuoles were seen at S_{-1}. Tapetal cell swell and empty vacuoles get enlarged and filled vacuoles related to secretary pathway showing abundance at S_1. Multinucleate tapetal cell, filled with various secondary metabolites with originating orbicules (ubisch bodies) towards pollen sac in S_2 and S_3. Tapetal cell degenerate leaving various vesicles like structure, nucleus and cytoplasm collapse releasing cell remnants in the pollen sac in S_4 while degeneration was completed in S_5 and S_6. To understand the reprogramming in S_3 to S_4 transition that leads to apoptotic like tapetal degeneration, we performed 454 pyro-sequencing of S_3 and S_4 anther. 2354 genes were differentially expressed between S_3 and S_4. Further analysis reveals that ROS generation leads to the oxidative stress in the tapetal cell, vacuolar processing enzymes (VPEs) and other protease like cystein protease activate the downstream death signalling. We find some unique genes expressed during the death signalling in the tapetal cell which adds to the present knowledge of tapetal degeneration mechanism.



P5.14

Tomato root tips regulate Lateral Root Formation during early seedling development

Sherinmol Thomas, Yellamaraju Sreelakshmi and Rameshwar Sharma
Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad- 500046. India
email:sherintn@gmail.com

Lateral roots are derived from the primary roots to optimize primary function of root to acquire water and minerals. Lateral root formation (LRF) is also a major determinant of root architecture development. LRF is profoundly affected by environmental cues. However, the mechanism by which the environment regulates lateral root formation is not fully deciphered. In this study we demonstrate that in tomato lateral root initiation can be induced by excising the root tip. Our result suggests that root tip exerts some level of regulatory effect on lateral root formation. We also examined the effect of light and plant hormone ethylene on LRF after excision of root tips. It is known that light influences the formation of the plant hormone auxin in germinating seedlings and auxin transported from the developing leaves to the root is required for LRF during early seedling development. Inhibition of Polar Auxin Transport in seedlings normally results in no LRF but induced LRF upon excision of root tips. Using specific light wavelengths we found that WL stimulated LRF, and multiple photoreceptor control the LRF stimulation in tomato. Using an ethylene precursor we showed that LRF as a result of root tip excision is not related to ethylene.



P6.1

Signaling components involved during stomatal closure by three microbial elicitors, compared with the effects of abscisic acid

Gunja Gayatri[1], Mallikarjuna Rao Puli[1], Srinivas Agurla[1], Appa Rao Podile[1], Kazuyuki Kuchitsu[2] and Agepati S. Raghavendra[1]
[1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, [2]Department of Applied Biological Science, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510 Japan
email: gayatri7257@gmail.com

Apart from the regulation of photosynthesis and water status of the leaves, stomata also act as main access points for the entry of the pathogens. Stomatal guard cells respond to various external stimuli through signaling components, e.g. ROS, NO, G-proteins, calcium and protein kinases/protein phosphatases. Stomatal closure is one of the plant innate immune response to restrict the entry of the pathogen. Stomatal closure is often mediated by microbial elicitors, produced from either microbes or within the plant tissue. We studied the effect of three microbial elicitors namely, flg22 (a 22 amino acid peptide from eubacterial flagellin), harpin (from *Pseudomonas syringae* pv. *syringae*) and cryptogein (from *Phytophthora cryptogea*) on signaling components during stomatal closure in guard cells of *Arabidopsis thaliana*. We observed the changes in the pattern of signaling components involved during stomatal closure by elicitors and compared with the pattern by abscisic acid (ABA, a well-known phytohormone). The three elicitors (Flg22, cryptogein and harpin) induced stomatal closure, in a concentration dependent manner. The elicitors raised markedly the levels of ROS and NO in guard cells. Time course monitoring of ROS and NO indicated that ROS acted upstream of the NO during stomatal signaling by these elicitors, a situation similar to ABA. Impaired effect of the elicitors on stomatal closure and restricted increase in ROS levels in guard cells of *atrbohD/F* mutant confirmed that ROS was essential during stomatal signaling. Decreased levels of NO in *atrbohD/F* mutant guard cells suggested that the ROS action was upstream of the NO. Incomplete reversal of stomatal closure in *nia*1 and *nia*2 mutant plants, indicating that NO was important but not essential for elicitor-induced closure. A tentative scheme of signal transduction events during stomatal closure induced by microbial elicitors would be described.



P6.2

Brassinosteroid and glucose signaling interaction during early seedling growth and development in *Arabidopsis thaliana*

Aditi Gupta, Manjul Singh and Ashverya Laxmi
National Institute of Plant Genome Research, New Delhi – 110067, India
email: aditi0225@gmail.com

Brassinosteroids (BRs) are important class of phytohormones which govern many processes in plants such as, cell proliferation and elongation, seed germination, hypocotyl elongation, photomorphogenesis, stomatal development and senescence. Sugars/Glucose (Glc) can also act as signals to control physiology, metabolism, cell cycle, development and gene expression in plants. Both Glc and BRs are fundamental to plants and regulate similar processes. There are few evidences for BR and Glc signaling interactions in plants but its molecular basis is not explored. Here, we studied the independent and combined effects of BR and Glc on some of the very important physiological aspects such as; hypocotyl and root development in dark and light grown Arabidopsis seedlings respectively. Exogenous BR application leads to randomizationin etiolated-seedling growth; a novel response which we designate as BR-reset of hypocotyl gravitropism. BR-reset of hypocotyl gravitropism involves both BR-receptor and -signaling elements. Glc antagonizes the BR-reset of hypocotyl gravitropism involving both HXK -dependent, and -independent Glc-signaling pathway components. Cytokinin signaling antagonizes this BR reset of gravity sensing and/or tropism by affecting ethylene biosynthesis/signaling. Glc seems to act independently of cytokinin and ethylene signaling pathways and may antagonize the response via affecting BR-regulated gene-expression quantitatively and spatially, by altering protein degradation, and by antagonizing BR-induced changes in microtubule organization and cell patterning associated with hypocotyl agravitropism. Modulation of auxin homeostasis lies further downstream to BR-Glc regulation of hypocotyl directional growth. Glc and BR also interact via auxin mediated mechanisms to regulate hypocotyl elongation in etiolated seedlings and primary root elongation, lateral root production and root hair production in light grown seedlings. In conclusion, our studies have provided important insights about the interdependence between Glc and BR signal transduction pathways in regulating early seedling growth and development in Arabidopsis.



P6.3

28-Homobrassinolide in Plant Signaling and Behavior

Kaur H[1]., Sirhindi G[1]*., Bhardwaj R[2]., Sharma P[1]. and Mir. M.[1]
[1]Department of Botany, Punjabi University, Patiala-147 002. Punjab, India
[2]Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India
email: geetikasir123@gmail.com

ROS production is an integrated cellular process occurring in plants upshot signaling for various metabolic processes in plants. H_2O_2 and NO are two important signaling molecules which facilitate normal growth and development for better survival against various stresses as a whole. Molecular and physio-biochemical studies have convincingly shown their essential roles in plant growth and development under changing environmental conditions. Role of phytohormones has been explored in modulating concentration and/or production of ROS and RNS at cellular level to regulate normal growth of plants. However, the details and mechanism by which ROS or RNS concentration regulated in plants by exogenous application of various phytohormones are not well understood. The present study was aimed to explore 28-HBL potential for regulated production of ROS and RNS by modulating the antioxidants of cell under normal and high salinity conditions. Production and accumulation of ROS and RNS was enhanced by exogenous application of different concentration of 28-homoBL, which directly enhanced the activity of various antioxidants responsible for regulating ROS and RNS concentrations. It was revealed from the present investigation that 28-homoBL enhanced the accumulation of free amino acids such as proline and glycine betaine which act as osmoprotectant under normal and salinity stress. These osmoregulators reduced the lipid peroxidation thus protect the plasma membrane from disintegration. Thus it has been suggested that 28-homoBL a plant steroid hormone, which has a potential for understanding the complex regulated network of signaling molecules responsible for well mannered behavior of plants under changing environmental conditions.



P6.4

The interaction between glucose and cytokinin signal transduction pathway in *Arabidopsis thaliana*

Kushwah S. and Laxmi A.
National Institute of Plant Genome Research, Aruna Asaf Ali Road, New Delhi-110067, India
email: ashverya_laxmi@nipgr.ac.in

Sugar and Cytokinins (CKs) are fundamental to plants and regulate a number of similar processes. We hypothesize that there may be an extensive overlap between CK and GLC signaling pathways. In this study, whole genome transcript profiling along with physiological analysis has been performed to find out the interdependence/overlap between GLC and CK signal transduction pathways in Arabidopsis seedlings. GLC could transcriptionally affect 76% of CK-regulated genes at whole genome level, 89% of which are agonistically regulated. GLC may also affect CK-regulated gene expression via non-transcriptional pathways. GLC can regulate several genes involved in CK metabolism and signaling. The role of GLC and CK was studied in regulating some of the important physiological parameters such as hypocotyl length, root length, root directional responses, chlorophyll level and anthocyanin content. Physiologically, both GLC and CK could regulate hypocotyl length in dark. GLC and CK signaling may integrate at the level of Type A ARRs in controlling hypocotyl length. Both GLC and CK signaling can not alter hypocotyl length in dark in auxin signaling mutants *AUXIN RESPONSE2*/INDOLE-3-ACETIC ACID7 (*AXR2*/*IAA7*) and *AXR3*/*IAA17* suggesting that they may involve auxin signaling component as a nodal point. Here, we demonstrate that there is a huge overlap between CK- and GLC-regulated gene expression and physiological responses.



P6.5

Drought induced 19 protein: A putative interacting partner of Aux/IAA13 in rice

Maitra-Majee S., Buttar B.S. and Khurana J.P.
Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi - 110021, India
email: susmita1977@gmail.com

Auxin is one of the best studied phytohormone and plays many diverse roles in regulating plant development. In recent years, auxin receptors have been identified and they represent components of E3 ligase involved in ubiquitination and 26S proteasome mediated degradation. Interestingly, one of the target protein class is encoded by auxin inducible *Aux/IAA* genes. The Aux/IAA protein harbours four conserved domains; domain I has a transcriptional repressor function, domain II forms the degron and domain III and IV are involved in homo- or hetero-dimerization. *Aux/IAA* gene products serve as negative regulators of auxin action, thus degradation of Aux/IAA is a prerequisite for auxin signal transduction to commense. Recently, TIR1,one of the auxin receptors, has been identified to stimulate degradation of Aux/IAA. In our study, we tried to look for other interacting partners of Aux/IAA with the aim to identify its possible targets that are associated with auxin response pathway. In rice, *Aux/IAA* gene family is comprised of 31 members. We have chosen OsIAA13 to look for its interacting partners since ealrier studies from our laboratory had shown that this gene expresses at a high level in the young seedling coleoptiles on auxin application. Screening of coleoptile library using Y2H approach resulted in identification of Di19 protein as one of its interacting partners. The interaction has been verified by *invitro* pull-down assay and *in vivo* BiFC and FRET analysis. Di19 protein is drought induced protein and has zinc finger motif that is suggestive of it being involved in DNA-protein or protein-protein interactions. Although, in *Arabidopsis*, recent studies have given a clue of it being involved in DNA-protein interaction, but its function remains to be studied in rice. Thus, our finding that Di19 interacts with Aux/IAA may provide clue towards a probable role of OsIAA13 in auxin-mediated plant development.



P6.6

Understanding the role of Phosphatidic acid-Sphingosine kinase1 interaction during ABA signaling

Shatakshi Pandit and Girish Mishra
Department of Botany, University of Delhi, New Delhi-110007, India
email: girishmishra@yahoo.com

Abscisic acid (ABA) mediates plant response to environmental stresses. During drought, ABA levels in plant increases, which promotes the closing of opened stomata and inhibits the opening of closed stomata. Several signaling proteins have been implicated in mediating the ABA effects in signaling networks perhaps linked through G proteins, protein phosphatases, kinases and phospholipases. Involvement of protein-protein interaction and lipid signaling molecules (Phosphatidic acid, PIP2, S1P) has been reported in past several years. Sphingosine Kinase (SK1), a cytosolic protein, phosphorylates sphingosine to generate sphingosine-1-phosphate (S1P) which is recruited to the plasma membrane in response to abiotic and biotic stresses. Phosphatidic acid (PA) is a central lipid signaling molecule that links different components in the ABA signaling network in guard cells. PA interacts with SK1 and ABA response in guard cells is diminished in PLDα1 and SK T-DNA mutants of Arabidopsis. Interaction between SK1-PA may be critical in ABA signaling and it is imperative to decipher its role. To determine the region of SK1 and PA interaction, C terminal and N terminal deletion mutants of SK1 were prepared and recombinant protein expression of all deletion mutants of SK1 were carried out. Lipid overlay assay was performed to identify the PA binding region on SK1which was mapped between 1-216 amino acids. In silico 3D structure prediction has revealed hydrophobic pocket on SK1, which can act as the site of PA docking. Deletion of this hydrophobic region from SK1 impedes PA binding.



P6.7

Narrating the role of JA signaling molecule in alleviation of Copper toxicity in *Cajanuscajan* (L.) Millsp.

Poonam Sharma, Geetika Sirhindi, Harpreet Kaur and Mudaser Ahmad Mir
Department of Botany, Punjabi University, Patiala-147002, Punjab, India
email: geetikasir123@gmail.com

Jasmonic acid (JA) is a well characterized signaling molecule in plant defense responses involved in regulating various stress signaling pathways of abiotic and biotic stresses. In the present study the effect of JA seed priming treatment on 12th day of growth of *Cajanus cajan* (L.) Millsp. seedlings under toxic concentration of copper (Cu) has been studied. This study is first time report of JA as defensive molecule for *C. cajan* under Cu toxicity.14% increase in rate of seed germination on 3rd day of sowing in JA primed seeds in presence of toxic concentration of Cu showed that application of JA may trigger the enzymes of cell surface which is involved in germination of seeds. However this triggering of germination promoting enzymes is dose dependent, as 1μM JA inhibits germination upto 20% under same Cu toxicity. Exogenous application of JA inhibits the overall growth of *C. cajan* seedlings and the effect was more pronounced in Cu treated seedlings thus confirming the theme that JA are stress signaling molecules. The results showed that presence of 1μM JA in medium triggered Cu uptake by plants which may be due to the activation and/or synthesis of Cu uptake associated transporters. However, lower concentrations of JA (1nM and 1pM) decreased Cu uptake by seedlings in presence of 5mM Cu in growing medium. These results suggested that JA may regulate gene expression stimulated transporter proteins for Cu at higher concentration. All concentrations of JA work in concert with Cu to ameliorate activities of SOD, CAT and GPOX which are responsible for mitigation of free radicals into H_2O_2 and ultimately to H_2O and O_2 in the cell. These observations suggest that JA may be an important signaling molecule in plants to sense Cu stress and regulating various metabolic processes in response to stress.



P6.6

Understanding the role of Phosphatidic acid-Sphingosine kinase1 interaction during ABA signaling

Shatakshi Pandit and Girish Mishra
Department of Botany, University of Delhi, New Delhi-110007, India
email: girishmishra@yahoo.com

Abscisic acid (ABA) mediates plant response to environmental stresses. During drought, ABA levels in plant increases, which promotes the closing of opened stomata and inhibits the opening of closed stomata. Several signaling proteins have been implicated in mediating the ABA effects in signaling networks perhaps linked through G proteins, protein phosphatases, kinases and phospholipases. Involvement of protein-protein interaction and lipid signaling molecules (Phosphatidic acid, PIP2, S1P) has been reported in past several years. Sphingosine Kinase (SK1), a cytosolic protein, phosphorylates sphingosine to generate sphingosine-1-phosphate (S1P) which is recruited to the plasma membrane in response to abiotic and biotic stresses. Phosphatidic acid (PA) is a central lipid signaling molecule that links different components in the ABA signaling network in guard cells. PA interacts with SK1 and ABA response in guard cells is diminished in PLDα1 and SK T-DNA mutants of Arabidopsis. Interaction between SK1-PA may be critical in ABA signaling and it is imperative to decipher its role. To determine the region of SK1 and PA interaction, C terminal and N terminal deletion mutants of SK1 were prepared and recombinant protein expression of all deletion mutants of SK1 were carried out. Lipid overlay assay was performed to identify the PA binding region on SK1which was mapped between 1-216 amino acids. In silico 3D structure prediction has revealed hydrophobic pocket on SK1, which can act as the site of PA docking. Deletion of this hydrophobic region from SK1 impedes PA binding.



P6.7

Narrating the role of JA signaling molecule in alleviation of Copper toxicity in *Cajanuscajan* (L.) Millsp.

Poonam Sharma, Geetika Sirhindi, Harpreet Kaur and Mudaser Ahmad Mir
Department of Botany, Punjabi University, Patiala-147002, Punjab, India
email: geetikasir123@gmail.com

Jasmonic acid (JA) is a well characterized signaling molecule in plant defense responses involved in regulating various stress signaling pathways of abiotic and biotic stresses. In the present study the effect of JA seed priming treatment on 12th day of growth of *Cajanus cajan* (L.) Millsp. seedlings under toxic concentration of copper (Cu) has been studied. This study is first time report of JA as defensive molecule for *C. cajan* under Cu toxicity.14% increase in rate of seed germination on 3rd day of sowing in JA primed seeds in presence of toxic concentration of Cu showed that application of JA may trigger the enzymes of cell surface which is involved in germination of seeds. However this triggering of germination promoting enzymes is dose dependent, as 1μM JA inhibits germination upto 20% under same Cu toxicity. Exogenous application of JA inhibits the overall growth of *C. cajan* seedlings and the effect was more pronounced in Cu treated seedlings thus confirming the theme that JA are stress signaling molecules. The results showed that presence of 1μM JA in medium triggered Cu uptake by plants which may be due to the activation and/or synthesis of Cu uptake associated transporters. However, lower concentrations of JA (1nM and 1pM) decreased Cu uptake by seedlings in presence of 5mM Cu in growing medium. These results suggested that JA may regulate gene expression stimulated transporter proteins for Cu at higher concentration. All concentrations of JA work in concert with Cu to ameliorate activities of SOD, CAT and GPOX which are responsible for mitigation of free radicals into H_2O_2 and ultimately to H_2O and O_2 in the cell. These observations suggest that JA may be an important signaling molecule in plants to sense Cu stress and regulating various metabolic processes in response to stress.



P6.8

Functional characterisation of wheat *Brassinosteroid independent-1* (*TaBRI* 1) overexpressed in *Arabidopsis thaliana*

Akanksha Singhand Paramjit Khurana
Department of Plant Molecular Biology, University of Delhi South Campus
Benito Juarez Road, New Delhi-110021, India

Brassinosteroids are hormones that are important for plant growth, development and immune responses. They are sensed by the transmembrane receptor kinase *Brassinosteroid Insensitive*-1 *(BRI1)* when they bind to its extracellular Leu-rich repeat (LRR) domain. Binding of brassinosteroid to *BRI*1is known to induce conformational changes that create a docking platform in BRI1 for co-receptor binding. The TaBRI1 protein consists of a putative signal peptide for delivery to the plasma membrane, followed by nine leucine rich repeats (LRR) domain, followed by a transmembrane domain and a C-terminal kinase domain. The sub-cellular localization of TaBRI1 indicates that it is localized in plasma membrane.The overexpression transgenic lines *TaBRI*1 were raised in Arabidopsis by floral dip method. These lines showed faster germination, early flowering and seedling establishment as compared to the wild-type. *TaBRI*1 overexpressing transgenic plants showed enhanced primary root growth under control condition as compared to the wild type plant. However when treated with epibrassinosteroid (Epi-BL), inhibition of primary root growth in a dose- dependent manner was observed. These results indicate that overexpression of *TaBRI*1 renders the roots of plants more sensitive to brassinosteroid induced root growth inhibition. Analysis of transgenic lines under various abiotic stress condition is under way.



.P6.9

Auxin-induced nitric oxide accumulation and tyrosine nitration of proteins during adventitious root development in sunflower

Yadav S. and Bhatla S.C.
Department of Botany, University of Delhi, Delhi-110007, India
e-mail: bhatlasc@gmail.com

Indole-3-acetic acid (IAA) induced adventitious rooting (AR) in sunflower (*Helianthus annuus* L.). hypocotyls highlights a clear demarcation of nitric oxide (NO)-dependent and NO-independent roles of auxin in this developmental process. Evidence for NO accumulation in the interfascicular cells 2days after IAA treatment highlights the involvement of NO specifically during root initiation. Treatment of hypocotyl explants with 1-napthylphthalamic acid (NPA), an inhibitor of polar auxin transport in plant cells, leads to complete abolition of NO-associated fluorescence. Using NO specific probe (MNIP-Cu), rapid NO accumulation as a response to IAA treatment has been observed in the protoplasts from the hypocotyls of sunflower seedlings. Incubation of protoplasts in presence of NPA and PTIO (NO scavenger) leads to significant reduction in NO accumulation, indicating that NO signals represent an early signaling event during auxin-induced response. A surge in NO production has also been demonstrated in whole hypocotyl explants showing adventitious root development. Evidence of tyrosine nitration of cytosolic proteins as a consequence of NO accumulation has been provided by western blot analysis and immunolocalization in the sections of AR producing hypocotyl segments. Most abundant anti-nitrotyrosine labeling is evident in proteins ranging from 25–80 kDa. Tyrosine nitration of a particular protein (25 kDa) is completely absent in presence of NPA. Immunofluorescent localization experiments have revealed that non-inductive treatments (such as PTIO) for AR develpoment from hypocotyl segments coincide with symplastic and apoplastic localization of tyrosine nitrated proteins in the xylem elements, in contrast with negligible (and mainly apoplastic) nitration of proteins in the interfascicular cells and phloem elements. Tyrosine nitrated proteins are abundant around the nuclei in the actively dividing cells of the root primordium. Thus, NO-modulated rapid response to IAA treatment through differential distribution of tyrosine nitrated proteins is evident as an inherent aspect of the AR development.



P7.1

Cholinergic System: A Novel Approach to Understand Allelopathy

Rashmi Sharma and Rajendra Gupta
Acharya Narendra Dev College, University of Delhi, Delhi 110019, India
email: yrs1810@gmail.com

Components of the cholinergic system: acetylcholine, acetylcholinesterase, choline and acetylcholine receptors, are the principal agents of nerve transmission in animals. Plants though nerveless, have long been known to possess most of the components of the cholinergic system. Plants have a repertoire of allelochemicals which help them to dominate their habitat by affecting the growth of other plants in their vicinity. Allelochemicals belong to highly diverse categories of chemical but the mechanism of action of most of them is unknown. A hitherto overlooked fact is that many of these allelochemicals are cholinergic chemicals. They affect the cholinergic system by either inhibiting acetylcholine synthesis or its hydrolysis or by blocking the acetylcholine receptor or by simply disrupting the Na^+ Channel. It was found in the present study that methanolic extract of 45 invasive weeds including *Lantana camara*, *Ageratum conyzoides*, *Argemone mexicana*, *Rununculus scleratus*, *Prosopis juliflora* and *Cyperus rotundus* have very high levels of anticholinesterases. Anticholinesterases block the enzyme acetylcholinesterase resulting in increase in the levels of acetylcholine in the system. Thus, an allelopathy-acetylcholine nexus sounds highly plausible. This is an interesting observation and further work in this direction may lead to exciting discoveries about the mechanism of action of allelochemicals.



P8.1

Landscape of Open Chromatin During Abiotic Stress in *Arabidopsis*

Vivek Kumar Raxwal[1], Sourav Ghosh[2], Shailendra Goel[1], Arun Jagannath[1], Amar Kumar[1], Vinod Scaria*[,3] and Manu Agarwal*[,1]
[1] Department of Botany, University of Delhi, Delhi-110007, India
[2] Genomics and Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India
[3] G N Ramachandran Knowledge Centre for Genome Informatics, CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India
email: agarwalm71@gmail.com, vinods@igib.in

Plants perceive abiotic stresses through a complicated chain of events that include changes in membrane structure, generation of Ca^{+2} signatures, activation of specific kinases and phosphatases leading to activation of transcription factors that either induce or repress transcription of genes. Recent advances in technologies have resulted in unprecedented discovery of both cis- and trans-regulatory elements involved in induction and repression of gene expression. However a genome wide perspective of cis-regulation in *A. thaliana* under abiotic stresses is lacking. In an attempt to identify genome-wide cis-regulatory elements permissive for gene activity, we isolated and sequenced the open chromatin regions of *Arabidopsis* seedlings subjected to various abiotic stress conditions. Two cross-validating techniques, FAIRE-Seq and DNase-Seq, were employed to define the chromatin regions that opened up under sustained stressful conditions. A map of open regions in stress relative to control conditions was generated leading to identification of O_c-O_s (i.e., chromatin regions open in control as well as stress), C_c-O_s (i.e., chromatin regions closed in control while open in stress) and O_c-C_s (i.e., chromatin regions open in control while closed in stress) loci. It is well known that components of different abiotic stress pathways interact and therefore genes that exhibited similar chromatin state in more than one stress were catalogued. The highlight of this analysis was identification of 134 genes in C_c-O_s configuration under all the stress conditions imposed in the current study. Further, to correlate the chromatin state with gene expression changes in response to stress imposition we carried out mRNA-Seq of *Arabidopsis* transcriptome under same conditions. Superimposition of gene expression data on the different chromatin states revealed novel components involved in stress signaling pathways.



P8.2

Salt and heavy metal stress modulated biochemical and physiological changes and their amelioration through glutathione in Chickpea

Veena Agrawal, Dinesh Kumar and Vinay Shankar
Department of Botany, University of Delhi, Delhi – 110 007, India
email: drveena_du@yahoo.co.in

Nodal explants from 28-day-old *in vitro* grown seedlings of chickpea were exposed to 0, 1, 10, 50, 100 and 200 mg/l NaCl with or without 10 mg/l glutathione for 14 days. Changes in the expression level of antioxidant enzymes; lipid peroxidation; relative permeability; nutrients concentration; photosynthetic pigments, protein and proline content were investigated. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) activities were found to be increased under salt as well as glutathione supplemented medium. Malondialdehyde (MDA) concentration increased by 3-fold at 200 mg/l NaCl, while ion leakage increased by 37% over control. A significant decrease in the concentration of photosynthetic pigments (chlorophylls a, b, total chlorophyll and carotenoid) suggested aberration in chloroplast functioning under salt stress. Concentration of Nitrogen, Phosphorus, Potassium, Calcium, Carbon, Magnesium and Sulphur showed an initial increase up to 10mg/l NaCl while at higher NaCl level there was significant decline in their concentrations. Five folds increase in proline concentration occurred in the nodal explants over control when exposed to 200 mg/l of NaCl. Three isoforms of SOD, one of CAT and four of GPX were expressed during native PAGE analysis. The SDS-PAGE of the stressed nodal explants revealed the over expression of several polypeptide bands related to NaCl stress. Similar trend has been observed during Pb and Cd stress. Work pertaining to antioxidant enzymes; lipid peroxidation; relative permeability; nutrients concentration; photosynthetic pigments, protein and proline will be presented.



P8.3

Root acclimation enhance zinc tolerance in tobacco plants

Nadia Bazihizina[1], Cosimo Taiti[1], Lucia Marti[1], Ana Rodrigo-Moreno[1], Christiana Giordano[2], Stefania Caparrotta[1], Massimo Gori[1], Elisa Azzarello[1], Stefano Mancuso[1]
[1] LINV - Department of Agrifood Production and Environmental Sciences – University of Florence, Viale delle Idee 30, 50019 Sesto F.no, Florence, Italy
[2] Centro di Microscopie Elettroniche " Laura Bonzi" (Ce.M.E.), ICCOM, CNR, Via Madonna del Piano, 50019 Sesto F.no, Florence, Italy
email: nadia.bazihizina@unifi.it

Heavy metal tolerance can be induced in plants following pre-treatment with low and non-toxic metal concentrations but results are still controversial. Tobacco plants were exposed to increasing Zn^{2+} concentrations (250 and/or 500 µM $ZnSO_4$) with or without a 1-week acclimation period with 30 µM $ZnSO_4$. Elevate Zn^{2+} resulted toxic for plants, with a marked decline in plant growth, meanwhile pre-treatment with 30 µM $ZnSO_4$ improved it. In the short-term (within 24 h after Zn^{2+} addition), the acclimation process was associated with a superior ability to maintain a negative root membrane potential and a lower cytoplasmic Zn^{2+} concentration in root cortical cells. The different response at the plasma membrane level between acclimated and non-acclimated plants was associated with a 2-fold increase in the expression of the tobacco orthologue of the *Arabidopsis thaliana* vacuolar Zn transporter in acclimated roots. The acclimation process activated a set of physiological adjustments in the short-term that enabled plants to withstand toxic Zn^{2+} concentrations at the root-level; this appeared to be depended on improved plasma membrane functionality, probably associated with enhanced Zn^{2+} compartmentalization in vacuoles.



P8.4

Identification and characterization of stress inducible promoters from *Oryza sativa*

Bhatt D., Sharma M., Ghosh P., Shaikh N., Bandyopadhyay A., Char B., Zehr U. and Bhattacharya A.
Maharashtra Hybrid Seed Company Ltd. (MAHYCO) Jalna Maharashtra, India
email: anjanabha.bhattacharya@mahyco.com

Abiotic stresses like drought, heat and salinity are major constraints limiting the overall crop productivity. Plant acclimation to these stresses is often correlated with increased expression of stress-induced genes. This regulated gene expression is often associated with the presence of various cis regulatory elements (CREs) located upstream of the gene. We mined genome wide expression databases for identification of highly up regulated genes under stressed conditions. Further characterization of regulatory elements present in their upstream region may be utilized for developing inducible gene expression system. Rice, a major staple crop and an important monocot model system, was utilized for identification of stress inducible promoters. We utilized public domain databases like PLEXdb and RiceXpro for mining of up regulated genes under various abiotic stress conditions. A total number of 231 differentially regulated probe sets were identified, which represent 197 non redundant genes, from different sets of experiments those related to abiotic stress. Out of which 63 were occurring at least twice in different experiments. Upstream 1.5 kb sequence was identified using RAP-DB (Rice Annotation Project – Database) that does not include coding sequence of neighboring gene. Analysis for the presence of regulatory elements and motifs was performed using suitable databases viz. PlantCARE and PLACE. Based on our findings promising promoters were selected and cloned in a binary vector. Constructs were then mobilized to *Agrobacterium* and transient infiltration assays were performed using tobacco leaves which lead to the identification of several putative promoters. All putative promoters were transformed in rice for further characterization. The methods presented here can be applied for the analysis of stress inducible promoters that direct the synthesis of genes controlling respective biological processes.



P8.5

Characterization of glutamate receptor in *Arabidopsis thaliana*

Chandna R. and Ahmad A
Molecular Ecology Laboratory, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi-110062, India
email: rubychandna@gmail.com

Ionotropic glutamate receptors (iGluRs) function as glutamate-activated ion channels that mediate rapid excitatory neurotransmission in the brain of human beings. The homologues of the iGluRs have also been found in higher plants. It has been reported that large family of 20 glutamate receptor–like genes (GLRs) are present in *A. thaliana* and that has been divided into three sub-groups based on sequence similarity. Function role of these receptors in plants is not known so far. Indirect evidence for the participation of glutamate (glu) receptors in Glu-mediated calcium signalling has been reported. We conducted real-time PCR analysis to investigate the expression of AtGLR_2 during different stress conditions. Real time-PCR study showed that AtGLR_2 gene isoforms expressed differentially during stress conditions. AtGLR2.3 and AtGLR2.5 were found to be maximally expressed among other AtGLR isoforms. The expression of these two genes was observed to be up-regulated during heavy metal stress in comparison to other stresses. For in vivo monitoring of the expression of these glutamate receptors, we used FRET approach by using variants of green fluorescent proteins (GFP), cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP).



P8.6

Nitric oxide interacts with sulfur assimilation and alleviates decrease in photosynthesis and growth of salt treated mustard by enhancing antioxidant metabolism

Fatma M. and Khan N.A.
Plant Physiology and Biochemistry Division, Department of Botany, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India
email: meharfatma30@gmail.com

The effects of salt stress on growth parameters, photosynthetic attributes and some key enzymes of the oxidative defense system were studied in the leaves of mustard (*Brassica juncea* L.) grown with/without 100 mM NaCl and treated sulfur (S; 200 mg S Kg^{-1} soil) or nitric oxide (NO; 100 mM). The results showed that salt stress resulted in a decrease in the plant characteristics studied, but the exogenous application of NO together with S enhanced photosynthesis and growth. Application of NO with S counteracted the adverse effect of NaCl stress on oxidative stress by increasing S-assimilation resulting in the increase in glutathione (GSH) production, protecting activity of ribulose 1,5-bisphosphate carboxylase (Rubisco) and net photosynthesis. The activity of antioxidant enzymes increased with exogenous application of NO and S under salt stress. The results suggest that NO signaling in S-assimilation reduces harmful effect of salt stress and improves growth, photosynthesis by enhancing antioxidant metabolism in mustard.



P8.7

Heat stress signalling in plants as a context sensitive language acceptance problem

Monendra Grover
Centre for Agricultural Bioinformatics, Indian Agricultural Statistics Research Institute, Pusa Campus, New Delhi -110 012
email: monendra_grover@yahoo.com

In heat stress signalling in plants, in addition to the listing of the individual components, it is essential to understand how individual components dynamically interact during the biosynthesis process. In this context the concept of computational complexity is applied to heat stress signalling in plants. It is shown that heat stress signalling system accepts context sensitive languages and models of computation of this system are universal. It can be envisaged that context sensitive process of heat stress signaling is composed of several simpler processes that in turn accept context sensitive languages. Heat stress signaling can be defined into several automata. The combination and communication of these processes results in greater computational power of the heat stress signaling. An important role is played by modularity in biological systems. In our case protocols involving communication between the several automata in heat stress signaling serve an important function.



P8.8

The root endophytic fungus *Piriformospora indica* improves growth of rice seedlings during high salt stress

Abhimanyu Jogawat[1], Madhunita Bakshi[2], Manoj Kumar[1], Meenakshi Dua[2], Narendra Tuteja[4], Ralf Oelmüller[5] and Atul K. Johri[1]

[1]School of Life Sciences, [2]School of Environmental Sciences, Jawaharlal Nehru University, New Delhi – 110067, India. [3]Amity Institute of Microbial Technology, Amity University, Noida, 201303, UP, India. [4]International Centre for Genetic Engineering and Biotechnology, New Delhi - 110067, India. [5]Friedrich-Schiller-University; Jena; Jena, Germany

email: abmanu.jnu@gmail.com

The root endophytic *Piriformospora indica* symbiotic relationship has been described to intensify biotic as well as abiotic stress tolerance of its host plants. We analysed the beneficial effect of *P. indica* colonization on rice seedlings during high salt stress conditions (upto 300 mM NaCl). The growth parameters of rice seedlings such as root and shoot lengths or fresh and dry weights were found to be improved in *P. indica*-inoculated rice seedlings as compared with non-inoculated control seedlings, irrespective of whether they are exposed to salt stress or not. However, salt-stressed seedlings performed much better in the presence of the fungus compared with non-inoculated control seedlings. The photosynthetic pigment content [chlorophyll (Chl) a, Chl b, and carotenoids] was significantly higher in *P. indica*-inoculated rice seedlings under high salt stress conditions as compared with salt-treated non-inoculated rice seedlings, in which these pigments were found to be decreased. Proline accumulation was also observed during *P. indica* colonization, which may help the inoculated plants to become salt tolerant. Taken together, *P. indica* rescues growth diminution of rice seedlings under salt stress.



P8.9

In vitro culture approach to study the effect of salt stress on *Camelina sativa* L. cv Calena: an emerging biofuel crop

Haya Khalid, Maya Kumari*, and M Nasim

Defence Institute of Bio-Energy Research (DIBER), Goraparao, Haldwani, Nainital - 263 139 Uttarakhand, India

email: madhulogin@gmail.com

Camelina *Camelina sativa* L. Crantz. (Brassicaceae) is native to Central Asia and the Mediterranean. It is gaining rapid importance as an alternative biofuel crop with 30–40% seed oil. Considering its importance, the present study was designed with the aim to find the effect ofdifferent concentrations (0, 25, 50, 75, 100, 125, 150, 175, 200 mM) of sodium chloride (NaCl) on growth, physiological and biochemical parameters of *in vitro* (MS medium) grown Camelina plants. Observations were recordedon growth parameters viz. rate of germination, cotyledon opening, true leaf emergence, seedling height, plant water content and biomass. Effect of salt stress was also characterized at physiological and biochemical level by recording electrical conductivity (EC), relative water content (RWC), photosynthetic pigments, malondialdehyde content (MDA) and guiacol peroxidise (GPX). There was significant decline in all the growth parameters with increasing NaCl concentration compared to control. Plants survived salt concentration till 200mM with significant effect on growth, physiological and biochemical parameters. The rate of germination, opening of cotyledon by and true leaf emergence declined by 30.9%, 17.63% and 28.78% respectively in 200 mM salt treatment with respect to control. Biomass also significantly decreased with increase in concentration to 200 mM. The plant height, RWC and plant water content decreased by 85.5%, 10.8%, and 9.85% respectively in stressed plants with respect to control. However, EC increased by 52.8% in stressed plants over control. Total chlorophyll decreased by 81.28% while guaiacol peroxidase activity and MDA accumulation increased by 68.88% and 57.62 % respectively with 200 mM salt treatment in respect to control. The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and instability of the pigment protein complex. MDA and guaiacol peroxidase have been considered as the indicator of oxidative damage. Thus the toxic effect of NaCl owing to osmotic as well as ionic stress on growth and physiological parameters in *Camelina sativa* was evident from this study.



P8.10

Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency and antioxidant metabolism

M. Iqbal R. Khan and Nafees A. Khan
Department of Botany, Aligarh Muslim University, Aligarh-202 002. India
email: amu.iqbal@gmail.com

Influence of exogenously sourced ethylene (200 µL L^{-1} ethephon) in the protection of photosynthesis against 200 mg kg^{-1} soil each of nickel (Ni)-and zinc (Zn)-accrued stress in mustard (*Brassica juncea* L.) was investigated. Plants grown with Ni or Zn but without ethephon exhibited increased activity of 1-aminocyclopropane carboxylic acid synthase, and ethylene with increased oxidative stress measured as H_2O_2 content and lipid peroxidation compared with control plants. The oxidative stress in Ni-grown plants was higher than Zn-grown plants. Under metals stress, ethylene protected photosynthetic potential by efficient PS II activity and through increased activity of ribulose-1, 5-bisphosphate carboxylase and photosynthetic nitrogen use efficiency. Application of 200 µL L^{-1} ethephon to Ni- or Zn grown plants significantly alleviated toxicity and reduced the oxidative stress to a greater extent together with the improved net photosynthesis due to induced activity of ascorbate peroxidase and glutathione (GSH) reductase, resulting in increased production of reduced GSH. Ethylene formation resulting from ethephon application alleviated Ni and Zn stress by reducing oxidative stress caused by stress ethylene production and maintained increased GSH pool. The involvement of ethylene in reversal of photosynthetic inhibition by Ni and Zn stress was related to the changes in PS II activity, photosynthetic nitrogen use efficiency and antioxidant capacity was confirmed using ethylene action inhibitor, norbornadiene.



P8.11

Study reveals *Brassica juncea* Natural Resistance-Associated Macrophage Protein 4.1 (BjNRAMP4.1) as an interactor of G-protein coupled receptor like protein associated signalling

Ananya Marik, Gairik Mukherjee, Haraprasad Naiya, , Chinmay Saha & Anindita Seal
Dr. B. C. Guha Centre of Genetic Engineering & Department of Biotechnology, University of Calcutta, Kolkata, India.
email: aninditaseal@gmail.com

Brassica juncea (Indian mustard) is a broad spectrum heavy metal tolerant and accumulator plant. Its rapid rate of growth and easy maintenance makes it is an ideal model to study heavy metal homeostasis. Natural Resistance Associated Macrophage Protein family (NRAMPs) are evolutionarily conserved transporters regulating intracellular metal homeostasis (Fe, Mn etc). These proteins are reported to sense the metal ion levels, oxidative stress and regulate cellular metal intake and efflux. Several partial NRAMP genes were cloned and characterized from *Brassica juncea* .BjNRAMP 4.1 was selected as it showed elevated expressions under Fe and Mn deplete and excess conditions. To understand the signalling pathway and importance of BjNRAMP4.1, a Split Ubiquitin based Yeast Two hybrid screen was performed. 150 potential interactors were obtained and validated initially based on selective media and X-Gal assay. A lung seven transmembrane receptor-like protein showing similarities with G-protein coupled receptor was one of the strongest interactors of BjNRAMP4.1. The interaction has also been confirmed using Bimolecular Fluorescence Complementation which enabled us to visualize the interaction *in planta* and monitor its location.



P8.12

Identification of abiotic stress responsive With No Lysine kinase in rice

Manuka R. and Kumar K.
Department of Biological Sciences, Birla Institute of Technology & Science
K. K. Birla Goa Campus, Goa-403726, India
email: kundan@goa.bits-pilani.ac.in

With No Lysine (WNK) kinases are member of protein kinases, a subfamily of serine/theronine protein kinases related to the STE20/PAK-like family in which lysine residue in kinase subdomain II essential for the coordination of ATP in the active centre and conserved among all other kinases, is missing. The WNK serine-threonine kinases have been shown to be osmosensitive regulators and are critical for cell volume homeostasis in mammalian system. In plants the role and expression of WNK genes is largely unknown. However 10 WNK kinases has been identified in *Arabidopsis*, designated as AtWNK1-AtWNK10 and few of them has so far been characterized in regulating biological activity. Soybean, GmWNK1 has identified as a component of a regulatory pathway that is involved in ABA signalling and repression of lateral root formation by an ABA-dependent mechanism. Here, we identified nine WNK kinases in rice plant on the basis of missing lysine in the conserved kinase subdomain II and designated as OsWNK1-OsWNK9. The transcript analysis of *OsWNKs* under various abiotic stresses viz. salinity, drought, cold and heat revealed differential regulation pattern of *OsWNKs* genes at different time points, suggesting their probable role in various abiotic stresses. We further observed transcript level of *OSWNKs* genes in various rice organs, which showed different spatial expression patterns suggesting their role in various developmental processes in rice.



P8.13

Analysis of salt-stress induced modulation of ouabain-sensitive ATPase activity in sunflower seedling roots using a novel fluorescence imaging approach

Mukherjee S. and Bhatla S.C.
Laboratory of Plant Physiology and Biochemistry, Department of Botany, University of Delhi, Delhi 110 007, India
email: soumobios@gmail.com; bhatlasc@gmail.com

Exposure of sunflower seedlings to sodium ions in the growth medium leads to sodium accumulation in the cells of the roots employing a variety of mechanisms. Existence of Na^+/K^+ ATPase in plants has remained controversial until now. Present work demonstrates the activity of the said enzyme in the roots of sunflower seedlings subjected to 120 mM of NaCl. This has been achieved using 9-anthroylouabain which is fluorescent derivative of ouabain (a well known inhibitor of Na^+/K^+ ATPase). Spectrofluorometric analysis and confocal laser scanning microscopic imaging of 9-anthroylouabain binding with putative Na^+/K^+ATPase in the root tissue system confirms an upregulation of this enzyme in response to salt-stress, particularly in the meristematic region of the seedling roots. Inhibition of Na^+/K^+ ATPase activity by calcium ions and a parallel accumulation of sodium ions in the columella, epidermis and meristematic cells of the roots is also evident. Investigations on protoplasts isolated from roots further shows localization of ouabain-sensitive ATPase in the nuclear membrane, highlighting the possibility of sodium efflux from the nucleus. Thus it is evident that ouabain-sensitive ATPase activity is correlated with accumulation of sodium ions in various regions of the seedling roots and it is also modulated by calcium. The present findings have thus demonstrated modulation of Na^+/K^+ ATPase activity in salt-stressed seedling roots, using a novel approach.



P8.14

Physiological approaches for mitigating high temperature stress effects in rice

Chandrakala J. U., Ashish K. Chaturvedi and Madan Pal*
Division of Plant Physiology
Indian Agriculture Research Institute, New Delhi-110012, India
email: madanpal@yahoo.com

Rice is extremely sensitive to high temperature stress during the reproductive stage, which can decrease yield due to reduced pollen viability, and increased spikelet sterility. Potential of three signaling molecules *viz.* salicylic acid, calcium chloride and brassinosteroid explored through exogenous application at pre-anthesis stage in mitigating high temperature effects on pollen viability, spikelet fertility, grain yield and quality attributes in two contrasting rice genotypes (Pusa Sugandh-5 and Nerica L-44). Three different concentrations of salicylic acid (SA) (0.1, 0.25, 0.5 mM), calcium (Ca)(10, 50, 100 mM of $CaCl_2$) and brassinosteroid (BR) (0.5, 1, 1.5 ppm of 24-Epibrassinolide) were applied and plants was exposed to high temperature stress (36°C ±1.7°C) inside temperature tunnel. High temperature stress-induced physiological changes led to reduction in grain yield and quality in both the genotypes. Foliar application of signaling molecules showed variable mitigating reponse of high temperature stress on yield and quality of rice. Lower concentrations of SA and Ca and all three concentrations of BR were found effective to enhance pollen viability, spikelet fertility and grain quality in both the genotypes and decreased the concentration of peroxidation products, while higher concentrations of Ca and SA showed additive stress effects and caused more reduction than high temperature stress. The ameliorative effect of signaling molecule on heat stress was more pronounced in PS-5 compared to Nerica L-44. Amelioration effect of all three chemicals was attributed to induced activities of antioxidant enzymes viz. CAT, APX and SOD in PS-5 and GPX, APX and SOD in Nerica L-44. Higher expression of *HSP*101 in both the genotypes under high temperature and pre-treatment of signal molecules also suggests the role of these chemical in mitigation.However further studies are required to test the role of above signal molecules through pre-treatment of wide range of concentrations.



P8.15

Comparative Transcriptome Analysis of Signalling Regulators in Traditional and Modern Indica Rice Genotypes under Phosphorus Deficiency

Pandey B.K., Mehra P. and Giri J.
National Institute of Plant Genome Research,
Aruna Asaf Ali Marg, New Delhi-110067, India
email: bipinkbiotech@gmail.com, Jitender.giri@gmail.com

Phosphorus (P) is one of the most essential nutritional elements for the adequate growth and development of plants. Plants absorb P only in the form of orthophosphate $(PO_4)^{2-}$. But the availability of the soluble PO_4^{2-} in most of the soil world-wide is very poor for the optimum growth of plants. Therefore, plants have manoeuvred physiologically, biochemically and morphologically to alleviate the P deficiency through local and systemic signaling. Plants modify their Root System Architectures (RSA) through local signaling as well as remobilize and redistribute the internal Pcontent by systemic signaling during P deficiency. Phosphorusbeing the prime nutritional compound also behaves as a signal molecule. Various hormones like auxin, cytokinin, ethylene and sugars also participate in signaling during P deficiency so as to fine tune the gene regulatory system during Pstarvation. Through the advancement of technology and comprehensive knowledge of molecular players the genuine intricacy of P signaling is still to be explored. So, this present research work aims to reveal various components of local and systemic regulators in rice during P deficiency. To delineate these components for low P tolerance, two contrasting rice genotypes, Dular and PB1 have been studied for their behaviour under low P conditions. Our morphophysiological studies established Dular and PB1 as low P tolerant and sensitive genotypes, respectively. Comparative global transcriptome analysis of root and shoot tissues of both genotypes revealed the different adaptation mechanisms under P deficient conditions. Further, we found many novel genetic regulators involved in P deficiency. Noticeably, low P tolerant genotype, Dular showed higher induction of most of the signaling genes indicating its better P Starvation Response (PSR) as compared to low P sensitive modern genotype PB1.



P8.16

In silico analysis of riceascorbate peroxidase gene expression reveals its role in stress

Saurabh Pandey, Ganga Gupta and Tanushri Kaul
Plant Molecular Biology Group, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India.
email: saurabhpandey28@gmail.com

Diverse isoforms of plant antioxidant enzymes contribute to spatial and temporal fine tuning of cellular responses. In plants, ascorbate peroxidases (EC, 1.11.1.11) present a crucial line of defense against reactive oxygen species. Present study provides a comparative view of the functional attributes of major isoforms of ascorbate peroxidase expressed in rice. Different APX isoforms existed in discrete subcellular compartments in rice and their expressions were stress-regulated. Among the eight isoforms of APX described in rice; OsAPX1 and OsAPX2 were cytosolic, OsAPX3 and OsAPX4 were peroxisomal, whereas OsAPX5, OsAPX6, OsAPX7, and OsAPX8 were chloroplastic. Genome mapping analysis of promoter regions of different rice APX isoforms revealed the ten different cis-acting regulatory elements such as DRE/C- repeat, Myb binding, low temperature responsible (LTR), heat shock element (HSE), W1 box, wun motifs, ABRE, ARE, TCA and TC- rich in the region upstream of TATA box. The protein-protein interaction analysis revealed the association of cytosolic APX isoforms with Hsp 101, HSfA2, ZAT6, WRKY 33, WRKY40, WRKY25, BAP1, HSFB2A, HSFB1, DREB2, CBF2, STZ, MPK6, MPK4 and MSK1. Moreover, chloroplastic APX isoforms were associated with ZAT12, CM1, PRXID, PRXID, SAP12, SAP10, RBOHD, RD22, AB12 and AHA1. Comparative protein sequences of various isoforms of different plant species revealed two conserved motifs for haeme-ligand binding and peroxidase activity. Homology modeling of rice cytosolic APX revealed that these two signature motifs may play a crucial role during stress tolerance. Further gene expression data analysis revealed that salt stress led to an upregulation in the expression of cytosolic OsAPX1 in shoots, whereas expression of peroxisomal OsAPX4 and OsAPX6 and stromal OsAPX7 isoforms in roots. Results revealed functional diversity of APX genes/proteins in rice with highly coordinated expression of chloroplastic and cytosolic isoforms that formed the first line of defense via antioxidant defense pathway in response to abiotic stresses.



P8.17

Elucidating the Role of *Trichoderma harzianum* on Drought-Specific Expression of Genes in Different Genotypes of Rice (*Oryza sativa* L.)

Veena Pandey, Mohammad Wahid Ansari[1], Suresh Tula[1], Ranjan Kumar Sahu[1], Nandani Shukla[2], Gurdeep Bains, Subhash Chandra[4], Atul Kumar, Anil Kumar Gaur[3], Sudhir Kumar Guru, Deepti Shankhdhar, Shailesh Chandra Shankhdhar, Alok Shukla, Ramesh Chandra Pant[5], Narendra Tuteja[1] and Jitendra Kumar[2]
Department of Plant Physiology, [1]International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi – 110067, [2]Department of Plant Pathology, [3]Department of Molecular Biology and Biotechnology, [4]Department of Agronomy, G.B.Pant University of Agriculture & Technology, Pantnagar-263145

Rice is the most important staple food of the global population. It is susceptible to drought stress. Every year its production is affected drastically due to erratic monsoon and drought condition in India as well as in other parts of the world. In the present investigation, a biocontrol agent, *Trichoderma harzianum* (TH56) which has proved its potential in the biotic stress tolerance was taken for its effect on drought stress in rice genotypes namely Pusa Basmati 1, Pant Sugandh Dhan 17 and Kalanamak 3131. Experiment was performed in the pots with four seedlings per pot. Root-dip treatment of rice seedlings was done during transplanting i.e. 21 days after sowing. For root dip treatment of rice plants, *Trichoderma* isolate TH56 was grown in, *Trichoderma* Selective Media (TSM). Actively growing *Trichoderma* was added to autoclaved barnyard millet (*Echinocloa frumentacae*) grains (local name "Jhanghora"). Colonized Jhanghora grains were air dried and ground to fine powder. *Trichoderma* formulation was prepared by diluting spore powder with talcum powder. 1% carboxy methyl cellulose (CMC) was used as a sticking agent during treatment. Seedlings were planted in pots and their growth was observed daily. Drought stress was given at the time of maximum tillering and flowering with withdrawn of water for five days. Morphological and phenological behaviour of rice genotypes was compared with the control. During biochemical analysis, drought specific enzymes were evaluated. It was found that SOD was increased in *Trichoderma* treated rice genotypes as compared to control. MDA and proline levels were found to decrease in *Trichoderma* treated plants. These results confirm that *Trichoderma harzianum* might have acted in triggering signalling cascade in drought tolerance in the selected genotypes. Further, the expression analysis of drought specific genes i.e. aquaporins and dehydrins are underway to justify the present investigation.



P8.18

Variation in phytocystatin activity in mustard seeds is correlative with efficient antioxidant metabolism and protection of cadmium-inhibited photosynthesis

Tasir S. Per[1], Nafees A. Khan[1]*, Bilquees Bano[2] and Shumaila Khan[2]
[1]Plant Physiology and Biochemistry Division, Department of Botany, Aligarh Muslim University, Aligarh-202 002, India
[2]Department of Biochemistry, Aligarh Muslim University, Aligarh 202002, India
email:naf9@lycos.com

Phytocystatins (PhyCys) are ubiquitous inhibitors of cysteine proteases in plants. PhyCys activity as percent inhibition of papain activity was determined in three mustard cultivars (*Brassica juncea* L.), namely Roagro 5444, Roagro 4001 and Amruta. The activity of PhyCys was greatest in Roagro 5444 followed by Roagro 4001 and Amruta. These cultivars showed differential antioxidant metabolism and capacity to protect photosynthesis of cadmium (Cd)-treated (50 µM) plants. The oxidative stress (measured as content of H_2O_2 and lipid peroxidation) was maximum in Amruta and lowest in Roagro 5444. Activity of antioxidant enzymes, ascorbate peroxidase (APX) and glutathione reductase (GR) and glutathione (GSH) content were highest in Roagro 5444 and the lowest in Amruta. In contrast, activity of superoxide dismutase (SOD) was maximum in Amruta and minimum in Roagro 5444. Activity of ATP-sulfurylase and cysteine content were greatest in Roagro 5444 and lowest in Amruta, while reverse was observed for sulfur (S) content. These characteristics of Roagro 5444 helped in lesser reduction in photosynthesis and growth of Roagro 5444 than Amruta. The results are discussed in light of PhyCys activity in mustard and capacity of plants to protect photosynthesis under Cd stress. It may be said that, less reduction in photosynthetic attributes and growth in Roagro 5444 might be associated with their capacity to enhance antioxidant metabolism (GR activity and GSH content), ATP-S activity and activity of PhyCys; as PhyCys prevent protein breakdown from cysteine proteases.



P8.19

"Lipidomics"- an approach to assess the plasticity of the membrane for ecological adaptation in Eastern Himalayan mosses

Roy Chowdhuri S.and Poddar-Sarkar M.
Chemical Signal & Lipidomics Lab, Department of Botany (Centre for Advanced Study), University of Calcutta, Kolkata-700019, India.
email: sumedha.roy.c@gmail.com

Mosses having poikilohydric adaptive nature, have been chosen for its simplicity in tissue differentiation pattern, efficient osmoregulatory mechanism, highly developed chemo-signalling pathway and for production of numerous secondary metabolites to develop defence mechanism. Plasma membrane is the immediate cellular component for sensing climatic stress to respond frequently or infrequently to changing environment. Membrane phospholipids, an integral part of the plasma membrane, play a major role in stress signalling and eco-adaptation. Considering these aspects, the physiology and biochemistry of the membrane phospholipid of Eastern Himalayan (EM) mosses collected from different altitude have been studied. In order to study-whether altitudinal differences and temperature variation cause any kinds of qualitative and quantitative changes in their membrane fatty acid composition for combating the climatic stress, we have undertaken lipidomics approach to ascertain the plasticity of membrane fatty acid (FA) composition by analysing 24 fatty acids with the help of Gas Chromatography and Mass spectrometry (GCMS). We hypothesised that under climatic condition of EH no direct correlation between degree of unsaturation of membrane FA and increasing altitude and with decreasing temperature can be drawn. The fluidity and plasticity of the membrane for combating cold stress and for adaptation is dependent on physiological status of the plant itself which is guided by its many synergistically-gene-regulated sequential events like activation of calmodulin-kinase-dependent signalling pathway and for enzymatic activity for production of desaturase classes of enzymes. Thus, cyclic alteration of saturated & unsaturated fatty acid by transient opening and closing of specific Ca^{++} channel and by up regulation and down regulation of the desaturase families may be reflected through ecologically adapted species diversity. Our findings on moss families, namely, Dicranales, Pottiales, Bryales and Hypnales of EH, collected from altitude 4700ft – 11800 ft with a temperature variation from ± 15◦C to <0◦C strengthen this view for plant's inherent thermobalance capacity.



P8.20

Proteome and S-nitrosoproteome analysis suggest a cross talk between nitric oxide and cold stress signalling

Ankita Sehrawat, Ravi Gupta, Bhavana Sharma, Renu Deswal
Department of Botany, University of Delhi, Delhi-110007, India
email: rdeswal@botany.du.ac.in

Plants protect themselves from cold stress by cold acclimation which involves changes in transcriptome, proteome and metabolome. Effect of cold stress was analysed in the secretome of *Hippophae rhamnoides* (Seabuckthorn) using a 2-D-MS approach. Identification of cold stress induced targets by n-LC-MS/MS showed that 47% proteins were involved in signalling, redox and defense related functions. Besides, antifreeze activity analysis showed formation of hexagonal ice crystals and inhibition of ice recrystallization, showing the presence of antifreeze proteins (AFPs). Antifreeze activity was also detected in field grown Seabuckthorn leaf and berry. In addition to the altering the proteome, cold stress also increased nitric oxide production (NO, 2 fold) and thiol pool (1.27 fold) in *Brassica juncea* secretome. S-nitrosylation (a NO based post-translational modification) was analyzed in cold treated *B. juncea* secretome by biotin switch assay, neutravidin-agarose affinity chromatography and MALDI-ToF-ToF. About 45% of the targets showing increased S-nitrosylation were involved in stress/redox/signaling related functions. Interestingly, identification of targets of "ascorbate-gluthathione cycle", "mustard oil bomb" and proteolysis, suggest the role of S-nitrosylation in regulating redox and defense responses. To sum, present study provides novel evidence of NO mediated cold stress signaling. Additionally, proteome analysis further expanded horizons of this novel signaling mechanism.



P8.21

Synthesis of phytochelatins and antioxidants in response to lead accumulation in Indian mustard (*Brassica juncea* L.)

Chandra Shekhar Seth
Department of Botany, University of Delhi, Delhi-110007, India
email: seth_bhu@yahoo.co.in

The phytotoxic effects of lead (Pb) and its detoxifying responses in Indian mustard was investigated by considering effect on biomass, photosynthetic pigments and protein level as gross effect along with the lipid peroxidation for oxidative stress. Synthesis of phytochelatins and antioxidants (enzymatic and non-enzymatic) were particularly measured to find out the detoxification strategy in plants during accumulation. Plants were exposed to Pb concentration (0, 15, 30, 60, 120 µM) for 28 and 56 days. Plants accumulated substantial amount of Pb in different plant parts (root and shoot), the maximum being in roots (2040 µg g^{-1} dw after 56 d at 120 µM). Lead induced oxidative stress, which was indicated by increase in MDA content in relation to metal concentration and exposure periods. The key antioxidative enzymes *viz.*, superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (GPX, EC 1.11.1.7) ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) showed stimulation except catalase (CAT, EC 1.11.1.6) which showed declining trend. Initially, an enhanced level of cysteine, glutathione and non-protein thiols was observed, which depleted with increase in exposure concentration and duration. The metal binding peptides i.e. phytochelatins induced significantly at 15 µM Pb in roots and at 30 µM Pb in leaves however it is decreased in roots at 60 µM Pb, which may be correlated with reduced level of GSH, probably due to reduced GR activity, which exerted increased oxidative stress as also evident by the phenotypic changes like browning of roots and slight yellowing of leaves. Thus, besides synthesis of phytochelatins, availability of GSH and concerted activity of GR seem to play a central role for Indian mustard to combat oxidative stress caused by metal and to detoxify it. Plants ability to accumulate and tolerate high amount of Pb through enhanced level of PCs and various antioxidants suggest it to be a suitable candidate for phytoremediation.



P8.22

Comparative analysis of drought stress responsive genes in contrasting rice cultivars

Pratikshya Borah, Eshan Sharma, Amarjot Kaur, Sanjay Kapoor and Jitendra P. Khurana
Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology,
University of Delhi South Campus, New Delhi, India
email: eshansharma15@gmail.com

Drought is a major environmental stress that adversely affects rice production due to its high water requirements. However, genetic variations available within traditional accessions of rice and its wild relatives offer a wide range of alleles for drought tolerance. Here we present physiological and genome wide expression analysis for one-week-old seedlings of contrasting rice cultivars, drought tolerant Dhagaddeshi and sensitive IR20. We have identified 2,963 and 3,115 genes uniquely up and down-regulated in Dhagaddeshi as compared to 474 and 558 genes in IR20, respectively, after 3 hours of water deficit stress. However, the number of genes that express differentially in both these cultivars is fairly comparable after 6 h of drought stress. At physiological level, Dhagaddeshi seedlings showed higher cell membrane stability and differential ABA accumulation pattern under water deficit stress. In an attempt to mine stress responsive genes underlying quantitative trait loci (QTLs), the microarray expression data was overlaid with known QTLs from publicly available databases and published literature pertaining to observed physiological traits of the contrasting cultivars in this study. By localization of differentially expressed gene loci (DEG) to their known positions on physical genome of rice, we could identify 11 genomic blocks with significant differential gene expression and five of these regions enriched in DEGs co-localized with QTLs being analyzed. Further, integration of the microarray expression data with metabolic pathway data available at Gramene database highlighted number of pathways involved in phytohormones, secondary metabolites, osmolytes, amines and amino acid biosynthesis in response to stress. Few stress responsive genes underlying QTLs, genomic blocks and metabolic pathways were validated by qPCR and may serve as candidate genes for exploring their role in drought response in rice.



P8.23

Transcriptional expression analysis of potato tuberization in response to heat stress

Anupama Singh, Sundaresha, S., Krishan G. Thakur., Brajesh Singh and Vinay Bhardwaj
Central Potato Research Institute, Shimla - 171001, Himachal Pradesh, India
email: anupama_58@yahoo.co.in

Potato (*Solanum tuberosum* L.) is the third largest global food crop after wheat and rice, with the advantage that its tubers have a yield per cultivated area superior than the cereal grains. Tuberization occurs in response to certain climate conditions. Short days and cool night temperatures promote tuber formation. This strong dependence of environmental conditions has made necessary the selection of potato varieties to be able to adapt to diverse climate conditions and temperatures of the different production areas. In the present investigation we focused on the analysis of gene expression in two potato cultivars, Kufri Surya and Kufri Chandramukhi that present contrasting tuberization behavior at elevated night temperatures. Plants were grown under non inducing conditions for 30 days and were then subjected to temperature treatments (24^0C Day/ 20^0C Night) and (24^0C Day/ 20^0C Night). Using whole genome expression array for potato with probes targeting 39,031 protein coding genes, 1,153 genes were significantly upregulated with more than two fold change in tolerant cultivar K. Surya on 21st day of stress treatment whereas 3,012 genes were significantly upregulated with more than two fold change on 14th day of stress. Highly expressed genes included genes involved in signal transduction pathways, lipid signaling and protein homeostasis like Zn finger protein, proline rich protein , histones, DNA binding protein calmodulin myb and myc transcription factors. Apart from these genes, several genes involved in flowering and tuberization like sugar transporter potato constans, multicystatin, GA regulated protein, flowering promoting factor also exhibited a high fold change . In addition, several genes unique to heat stress library with unknown function, were identified, providing new candidate genes for investigation of heat stress responses in potato.



P8.1

Protective role of exogenously applied proline on photosynthetic inhibition by salt stress through changes in proline and antioxidant in mungbean cultivars

Syeed S. and Khan N. A.
Plant Physiology and Biochemistry Division,
Department of Botany, Aligarh Muslim University, Aligarh – 202002, India
email: shabinasyeed@gmail.com

Salinity is one of the major environmental factors limiting crop productivity. We investigated the influence of exogenous Proline (Pro) in the protection of photosynthesis against 100mM NaCl stress in Punt mung (salt-tolerant) and T44 (salt-sensitive) cultivars of mungbean (*Vigna radiata* L.) at 30 days after sowing (DAS). The cultivar T44 grown with NaCl but without Pro exhibited lesser Pro metabolism, GSH/GSSG, water relations, PS II activity, photosynthesis and growth with increased accumulation of Na^+ and Cl^- and oxidative stress than Punt mung. Exogenous application of Pro to plants without salt increased GSH/GSSG, water relations, PS II activity, photosynthesis and growth and decreased ion content and oxidative stress, but to a greater extent in Punt mung than T44. Application of 20mM Pro protected photosynthetic potential by efficient PS II activity and alleviated salt stress by increasing Pro production through the increase in γ-glutamyl kinase (GK) and decrease in proline oxidase (PROX) activity, resulting in promotion of water relations and maintained increased photosynthesis more prominently in Punt mung. The results suggest that Pro interacts with PS II activity and antioxidant metabolism to alleviate the adverse effects of salt stress on photosynthesis in mungbean.



P9.1

Functional complementation of rice blast resistance gene Pi54 using RNA interference approach

Arora Kirti[1], Rai AK[1], Narula A[2], Sharma TR[1*]
[1] National Research Centre on Plant Biotechnology, IARI, Pusa Campus, New Delhi, 110012,
[2] Department of Biotechnology, Jamia Hamdard, Hamdard Nagar, New Delhi, 110062
email: chugh.kirti29@gmail.com; trsharma@nrcpb.org

Pathogenic microbes secrete effector proteins into host tissue to cause infection and disease. Rice blast is a devastating disease caused by Magnaporthe oryzae which is an intracellular, hemibiotrophic fungal pathogen. Pi54 gene confers resistance to rice blast disease and is effective against different Magnaporthe oryzae isolates. Post transcriptional gene silencing (PTGS) by RNA interference (RNAi) has commonly been used for functional analysis of novel genes through transient and stable suppression of gene function in plants. In order to functionally validate the Pi54 gene, we performed RNAi, a reverse genetics approach for the knockdown of Pi54 gene. In order to to develop RNAi construct, PCR amplified gene product was cloned into Gateway pENTR/D-TOPO cloning vector and LR clonase reaction was performed to recombine entry clone with destination vector.pANDA-□. pANDA-□ vector carried kanamycin and hygromycin resistance markers for plant transformation. Agrobacterium mediated transformation was performed to transform resistant transgenic japonica rice line TP-Pi54-2. Putative transformants were confirmed by PCR amplification with GUS linker, Pi54 gene and ubiquitin promoter specific primers. Significant reduction in Pi54 transcript levels in PCR positive RNAi plants were observed by quantitative real time PCR. TP-Pi54-2-RNAi lines will be subjected to screening for resistance against virulent strains of M. oryzae.



P9.2

Serine rich peptide domain regulates the activity of AtMTM1 towards ATX1

Akanksha Nagpal[1], Frantisek Baluska[1]& Zoya Avramova[2]
[1]IZMB, Institute of Cellular and Molecular Botany, University of Bonn, Germany
[2]University of Nebraska School of Biological Sciences, Nebraska, USA
email: akankshakhatri1@gmail.com

AtMTM1 is a member of the myotubularin family which belongs to a unique sub-group of large family of dual-specificity serine–threonine phosphatase (DSP) that is highly conserved among eukaryotes. AtMTM1 cleaves the D3 phosphate from PtdIns3P and PtdIns(3,5)P2, forming PtdIns5P and PtdIns respectively. AtMTM1 contains a conserved PH-GRAM domain (Pleckstrin- Homology, Glucosyltransferases, Rab-like GTPase Activators and Myotubularins), a RID, CC domain as well as the catalytic domain along with SID. It has been reported that the loss of AtMTM1 is associated with an altered tolerance to dehydration stress. Furthermore, over-expression of AtMTM1 is known to sequestrate nuclear ATX1 (The plant trithorax factor) in the cytoplasm via the PHD domain of ATX1. But, little is known about the molecular mechanisms regulating AtMTM1. In this work, isoforms of AtMTM1 with and without serine rich peptide domain located beside SID peptide (AtAAF) were fused to fluroscent marker GFP under the control of a 35S promoter, and used to transiently transform cells of *Nicotiana benthamiana* leaves by infiltration with *Agrobacterium tumefaciens*. Moreover, subcellular localization of both AtMTM1 and AtAAF was also analyzed. The effect of ABA has been checked on the subcellular localization of AtMTM1 and AtAAF. Co-expression studies of AtAAF with ATX1 or PHD of ATX1 has been studied as diminution of ATX1 activity affects a variety of cellular signals which in turn affects plant growth.



P9.2

Molecular Characterization and expression analysis of a wound-responsive promoter cloned from *Arabidopsis thaliana*

Gupta N. C., Jain P. K., Bhat S. R. and Srinivasan R.
National Research Centre on Plant Biotechnology, New Delhi-110012. India
email: guptanc@nrcpb.org

An Arabidopsis mutant line T90, exhibiting a stem-specific and wound-responsive *GUS* expression was identified from a population of *Arabidopsis thaliana* (L.) tagged with a promoterless *β-glucuronidase* (*GUS*) in the T-DNA. Southern analysis confirmed the presence of a single copy of T-DNA in this line. Histochemical and fluorometric GUS assay revealed that the promoter of this gene is induced upon wounding specifically in stem tissues whereas non-inducible expression was present in pollen grains. No expression was detected in the leaves and root tissues. The insertion flanking sequence from right border of T-DNA was cloned by TAIL-PCR and the insertion was found in the third chromosome in 5′ UTR region of fatty acyl-CoA reductase 6 (*FAR6*)gene. RT-PCR analysis revealed that the gene is expressed predominantly in stem tissue, with very low expression in floral organs. Semi-qRT-PCR confirmed that the expression in stem tissues is induced by wounding. The 5′ promoter deletion analysis in transgenic Arabidopsis revealed that the 304 bp upstream sequences were ineffectual to drive *GUS* expression in wounded stem tissues. The addition of further upstream sequences (510, 958 and1400 bp) resulted in enhanced and extended expression of the *GUS* throughout the mature stem tissues after wounding.The observations suggest that the *FAR6 gene* may be involved in the defense response of wounding or pathogens that damage the plant mainly through the stem. In all the deletion lines of Arabidopsis non-inducible expression of the GUS in pollen grains remains unaltered which indicates the presence of two different modules in FAR6 promoter controlling inducible and non-inducible GUS gene expression. This work provides a promoter element that could be used to engineer the insect-resistant transgenic plants.



P9.3

Glyphosate and sulphonyl urea tolerant "Swarna" rice for sustainable weed management, high water-use efficiency and enhanced yield

Dhiren Fartyal, Aakrati Agarwal, M. K. Reddy and Tanushri Kaul
Plant Molecular Biology Lab, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India.
email: tanushri@icgeb.res.in

Rice is cultivated in waterlogged fields primarily to suppress weeds in lowland cultivation. Similarly, in upland rice cultivation, weeding in early stages of crop growth is imperative for acquisition of yield-potential. Efficacy of this method is contingent to prompt availability of water at imperative stages of rice crop in the growing season. Burgeoning populations in developing economies of Asia lead to reduced accessibility of water for rice production. Simultaneously, yield losses caused by weeds worldwide are reported at an average of 10-20 %. Manual weeding over large cultivated areas is cost-, time- and labor-intensive. Moreover, in early stages weeds are indistinguishable from crop plants posing a challenge for manual weeding. Introduction of chemical weed control for realizing superior and sustainable productivity is relevant. Annually, 10,000 tons of toxic herbicides are consumed by conventional cropping in Indian agriculture. Amongst them non-selective herbicides are mainly administered prior to sowing and/or pre-emergence of crops. Restricted pliancy in application-schedule and their residual-effect on the subsequent crop generations limits herbicide-based weed management. We have generated double herbicide tolerant "Swarna" rice lines with binary mode of action via site-directed mutagenesis of rice genes encoding for EPSPS-synthase (EPSPS) and acetolactate synthase (ALS) to confer herbicide tolerance in rice. Pyramiding of EPSPS and ALS genes using site-specific homologous recombination obviates segregation of EPSPS and ALS in subsequent generations. Transgenic lines were confirmed by PCR and genome walking analyses. Two transgenic lines exhibited high dose-tolerance for specific herbicides (2-8 mg/L) in covered fields. This approach shall empower farmers to restrain numerous weed species having strikingly variable life cycles and survival mechanisms via systematized rotation of two broad-spectrum non-selective systemic herbicides: glyphosate (for spraying) and sulphonyl urea (for broadcasting) with remarkably distinct mode of action. This strategy would forestall development of herbicide resistant weeds and insure high water-use efficiency, thereby boosting rice productivity.



P9.5

A GH3 gene, *SlGH3.2*, is required in the regulation of various auxin-mediated responses during tomato (*Solanum lycopersicum*) development

Thula Sravankumar[1], Ashima Khurana[2], Arun K. Sharma[3] and Rahul Kumar[1*]
[1]Repository of Tomato Genomics Resources, Department of Plant Sciences, University of Hyderabad, Hyderabad, 500046, India
[2]Zakir Husain Delhi College, Botany Department, University of Delhi, New Delhi, 110002, India [3]Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, 110021, India
email:rksl@uohyd.ernet.in; rahulpmb@gmail.com

The growth hormone auxin (indole-3-acetic acid, IAA) has profound effects on plant growth and development from embryogenesis to senescence. Several classes of auxin-related genes such as ARFs, Aux/IAAs and SAUR have been implicated in auxin-controlled diverse responses; however, precise functions of many of these genes and underlying mechanisms remain unknown. In this study, we have investigated role of Gretchen-Hagen3 (GH3) family gene, *SlGH3.2*, in tomato. This class of proteins is involved in the maintenance of auxin homeostasis by converting excessive free auxins to their conjugated forms. *SlGH3.2* genomic sequence comprised of three exons and two introns and also encoded a 598 aa long ~68 kDa protein. A total of 669 structural variants were identified for this gene in 11 tomato wild relatives, with highest variants observed in *S. huaylense*, however only one variant each was observed in two inbred lines, including Ailsa Craig and Moneymaker. Transcript profiling of *SlGH3.2* showed that its transcripts accumulate at high levels in roots, buds, flowers and red-ripe fruits. Ripening-associated induction in its expression was found to be lost in chronologically similar-staged *ripening-inhibitor* (*rin*) fruits. Further, its transcription was found to be under the control by many phytohormones, including auxin, ethylene, gibberellins and salicylic acid and abiotic stresses such as cold and salt. Analysis of its promoter architecture revealed presence of most of these hormone- and stress-associated *cis*-regulatory elements. Additionally, many light signaling *cis*-elements were also present in its promoter. We generated *35S::GH3.2* RNAi lines and found varied levels of its inhibited transcripts in different transgenic tomato plants. Light grown transgenic tomato seedlings exhibited reduced number of lateral root formation and shorter hypocotyls whereas roots of these plants were found to be longer than that of wilds type plants. Additionally, enhanced accumulation of anthocyanin in the roots of transgenic plants was observed. Mature transgenic plants developed more side shoots, however internode length of the main shoot was found to be reduced in these plants. Thus, preliminary results clearly indicate that main shoot was found to be reduced in these plants. Thus, preliminary results clearly indicate that *SlGH3.2* gene regulates plant architecture *via* maintaining the auxin homeostasis in different tissues in tomato.



P9.6

Localization of hydrogen peroxide in glandular trichomes and its effect on artemisinin production in *Artemisia annua* colonized by Arbuscular mycorrhizal fungi *Glomus intraradces*

Mandal S., Singh V. P., and Kapoor R.
Department of Botany, University of Delhi, Delhi - 110 007. India
email: kapoor_rupam@yahoo.com

Arbuscular mycorrhizal symbiosis is the most prevailing type of beneficial bio-trophic intracellular association between plant roots and soil borne fungi. This association provides the host plants with a horde of beneficial effects such as nutrient absorption, protection against water, salt and heavy metal stress. It also improves plant's immune system by activation of various defense responses such as induction of secondary metabolite that can protect the plant from pest and pathogen. *Artemisia annua* plants also produce one such secondary metabolite (artemisinin) that provides protection against herbivory and pathogen attack. It was reported that root colonization by AM fungi favoured production of artemisinin, in the above ground parts of the host plant. However, very little is known about the mechanism that the symbiotic partner employs to manifests such effects. Artemisinin is biosynthesized through terpenoid pathway. The last step of artemisinin biosynthesis i.e. the conversion of dihydroartemisinic acid to artemisinin is non-enzymatic (as a photo-oxidative reaction) and uses H_2O_2 molecule to get converted into a stable compound artemisinin. Mycorrhizal fungi also produces H_2O_2 molecule during the development of AM-symbiosis in the roots of host plant. Till now, no study has been carried out to correlate the increased concentration of H_2O_2 molecule with that of increased production of artemisinin in mycorrhizal plants. In the present study a fluorescent dye named 2,'7'-dichlorodihydrofluoroscein diacetate (DCFH2-DA) was used to monitor the concentration of H_2O_2 molecules in glandular trichome of *A. annua* colonized by AMF. Confocal laser scanning microscopy (CLSM) revealed that mycorrhizal plants exhibited significantly higher concentration of H_2O_2 molecules than that of control plants. This increase in H_2O_2 concentration in mycorrhizal plants was also supported by increased concentration of dihydroartemisinic acid and the final product artemisinin. Mycorrhizal inoculation also augmented the transcript profiles of artemisinin biosynthesis genes that are required for artemisinin biosynthesis.



P9.7

Pyramiding genes of "ascorbate-glutathione pathway" for conferring multiple abiotic stress tolerance in rice

Subramanyam Reddy, Maheedhar Reddy, M. K. Reddy and Tanushri Kaul
Plant Molecular Biology Laboratory, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India
email:csreddy@icgeb.res.in

Multiple abiotic stresses lead to generation of oxidative stress or reactive oxygen species (ROS) in cells propounding as a major limiting factor in plant productivity. ROS is ubiquitously produced in cells at low levels during normal growth conditions. However, in stress metabolic perturbations are concomitant with high ROS production and the innate anti-oxidative machinery is incapable to scavenge the excess ROS. Excessive ROS causes peroxidation of membrane lipids, membrane disintegration and eventually cell death. In plants, crucial ROS-scavenging enzymes of ascorbate-glutathione pathway are instrumental in combating oxidative stress by deactivation of ROS released during multiple redox reactions. In order to eliminate or alleviate excess ROS production during adverse stress conditions, we propose to overexpress the crucial enzymes of "ascorbate-glutathione pathway" that include superoxide dismutase (SOD) ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) controlled by different stress-inducible rice promoters in rice plants. These promoters are utilized for their high expression levels of all transgenes in rice. All the gene cassettes have been pyramided in a single binary vector by site-specific homologous recombination. Marker free strategy (mutated rice acetolactate synthase) was employed for agro-mediated transformation of this multi-gene construct in to rice crop. Putative T_0 transgenic rice lines were analyzed for transgene integration by genome walking. Expression of transgenes in these transgenic lines was confirmed by RT-PCR. Leaf disc assay with methyl viologen (100 µM) revealed that these transgenic lines were tolerant to oxidative stress. Moreover, no morphological anomaly was observed in the T_0 transgenic lines.



P9.8

Understanding the role of a putative protein kinase from *Arabidopsis* in plant defense response

Sardar A. and Chattopadhyay D.
National Institute of Plant Genome Research, New Delhi- 110067. India
Email: atish_sardar@yahoo.co.in

Protein Kinases are key regulators of cell function that constitute one of the largest and most functionally diverse gene families. By adding phosphate groups to substrate proteins, they direct the activity, localization and overall function of many proteins, and serve to orchestrate the activity of almost all cellular processes. Kinases are particularly prominent in signal transduction and co-ordination of complex functions. The diversity of essential functions mediated by kinases is shown by the conservation of some 50 distinct kinase families between yeast, plant and animal kinomes. In plants, members of several major protein kinase families, including the SNF1-related protein kinase-2 and -3 (SnRK2 and SnRK-3) subfamilies, the calcium-dependent protein kinase (CDPK) family, the mitogen activated protein (MAP) kinase family, the glycogen synthase kinase (GSK)- 3/shaggy family and the receptor-like protein kinase (RPK) family are involved in vast areas of hormone-mediated signaling covered include ABA-mediated responses to osmotic stress, wounding and pathogen attack, as well as ethylene and cytokinin signaling pathways. Till date only two proteins kinases have been reported to be associated with plant defense response, that is, calcium-dependent protein kinases (CDPKs) and mitogen activated protein kinases (MAPKs). In a microarray experiment with *Arabidopsis* seedlings treated with flg-22, a bacterial flagellin-derived 22-amino acid peptide, a putative protein kinase was identified downregulated. Our primary investigation showed that *Arabidopsis* protein kinase *knock-out* (*Atpk-/-*) plants were less susceptible in response to biotrophic pathogen *Pseudomonas syringae* pv. tomato DC 3000 (*Pst*DC3000) in comparison to the wild-type plants. The expression of several genes involved in the salicylic acid-mediated defense response pathway also justified and established this observation. We aim to elucidate the role of *AtPK* in plant defense response.



P9.9

Synergistic effect of potato class I chitinase and mung bean defensin gene on defense signaling against blister blight in transgenic tea

SinghH. R.[1], Deka M.[2] and Das S.[3]
1. Department of Biotechnology, Tea Research Association, Tocklai, Jorhat-785008, Assam, India
2. Department of Biological Sciences, Gauhati University, Assam, India
3. Institute of Bioresources and Sustainable Development, Takyelpat, Manipur, India
email: jit_eini@yahoo.co.in

For enhancing and broadening plant's natural defense mechanism against blister blight disease *Agrobacterium*-mediated co-transformation of tea, *Camellia sinensis* [L.] O. Kuntze, was developed. The objective was set to control the growth of blister blightpathogen, *Exobasidium vexans* by simultaneous expression of *Solanum tuberosum* class I chitinase and *Vigna radiata* defensin genes. The genes of interest were cloned individually into pCAMBIA 1301 vector under the control of the constitutive 35S-promoter from cauliflower mosaic virus and co-introduced into *A. tumefasciens* (LBA4404). By antibiotic selection 3.41% of the transformants were selected and twelve plantlets were confirmed by PCR about transgene incorporation. In four plantlets transcription level of transgenes was confirmed by real time-PCR. Performance test of resistance against *E. vexans* with selected T0 generation transgenic tea by antifungal bioassay of detached leaves showed that the transgenic plantlets exhibited higher degree of resistance to the pathogen infected than that of the non-transgenic plantlet. The present results indicate that the generation of transgenic tea is possible using co-transformation system.



P9.10

P-protein forisome from *Pisum sativum* functions in sealing of wounds and confers mode of recovery from biotic stress

Srivastava V. K., Raikwar S, Patti K. M., Tula S., Tuteja R., and Tuteja N.
Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India
email: vineet@icgeb.res.in

Forisomes are ATP-independent mechanically active crystalloid phloem proteins encoded by *SEO* (sieve element occlusion) gene family unique to Fabaceae. To protect against loss of photo-assimilate-rich phloem sap, plants have evolved several mechanisms to plug phloem sieve tubes in response to damage. Forisomes are localized in sieve tubes of phloem tissue and proposed to plug sieve plate after wounding and help in preventing loss of nutrient rich photoassimilate. Aphids (*Myzus persicae*) infest many plants and cause damage by depriving them of nutrients and by transmitting many viral diseases. Furthermore the productivity is limited by the removal of sap and alterations in source–sink patterns. In this study we have cloned and characterized forisome from pea (*Pisum sativum*) and overexpressed forisome intobacco. To demonstrate the role of forisome in wound sealing mechanism, the exudate rate analysis was conducted, which shows reduced leakage of photoassimilates in overexpression lines as compared to wild type plants. The role of forisomes in recovery of plant by sealing of wounds from biotic stress caused by aphids (*M. persicae*)) was established by bioassay experiments, which demonstrates improvement in physiological and gas exchange parameters in transgenic line as compared to WT. Our results suggest that forisome from *P. sativum* has a role in wound sealing by preventing loss of nutrient rich photoassimilate and provides recovery from biotic stress. The present study provides the first direct evidence of the function of P-protein forisome from *P. sativum* in wound sealing and in providing recovery after aphid infestation in transgenic tobacco.



P10.1

Effect of salicylic acid and its esters on stomatal closure and increase in reactive oxygen speices, nitric oxide in *Pisum sativum* guard cells

Srinivas Agurla, Gunja Gayatri, Mallikarjuna Rao Puli and Agepati S. Raghavendra
Department of Plant Sciences, School of Life SciencesUniversity of Hyderabad, Hyderabad 500046, India
email: agurlasrinivas02@gmail.com

The stomata play a major role in controlling gas exchange between plant and the surrounding atmosphere and loss of H_2O via transpiration into the atmosphere. Besides this, stomata can defend plants from pathogen attack, by preventing its entry into the plant. The opening and closing of stomatal pores are brought about by changes in turgidity of surrounding guard cells, stomata being open when guard cells are turgid and closed when guard cells are flaccid. Several compounds induce stomatal closure, such as plant hormones (abscisic acid, ABA; methyl jasmonate, MJ), ethylene (ET), and elicitors (Flg22, harpin and chitosan), and polyamines (putrescine, spermine and spermidine. Among these, ABA is well studied phytohormone inducing stomatal closure and modulating several signalling components. It has been suggested that many signalling components like reactive oxygen species (ROS), reactive nitrogen species (NO), Ca^{2+}, cytosolic pH, sphingolipids, phospholipids and MAP kinases are involved in ABA induced stomatal closure.We studied the effect of three phenolic compounds on stomatal closure in *Pisum sativum*: salicylic acid (SA), and its esters such as acetylsalicylic acid (acetyl-SA) and methyl salicylate (meSA). In comparison with ABA, meSA was more effective in promoting stomatal closure than SA. In contrast, acetyl-SA could only cause partial stomatal closure. We have further monitored the levels of two signaling components like ROS and NO in guard cells, in response to SA, acetyl-SA and meSA by using fluorescent probes like H_2-DCFDA and DAF-2DA. The levels of ROS and NO in guard cells were increased significantly in response to SA, acetyl-SA and meSA. Our results suggest that ROS and NO function as signaling components during stomatal closure in *Pisum sativum* induced by salicylic acid and its two esters, acetyl-SA and meSA.



P10.2

Enzymatic scavenging of reactive oxygen species accompanying sodium chloride-induced stress in sunflower seedlings

Jain P., Neha, Kaur H., Arora D. and Bhatla S.C.
Laboratory of Plant Physiology and Biochemistry, Department of Botany, University of Delhi, Delhi- 110 007, India
email:; bhatlasc@gmail.com

Enhanced production of hydrogen peroxide (H_2O_2) in seedling roots and cotyledons in response to salt stress is scavenged through temporal, qualitative and quantitative modulation of the activities of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR) and heme oxygenase (HO-1). Roots possess more total SOD activity as compared to cotyledons. Cotyledons and roots possess two and three SOD isoforms, respectively. SOD activity remains unaltered by NaCl (120 mM) stress. Peroxidase (POD) activity increases in roots in response to NaCl (120 mM) stress, with maximum activity in 4-day old seedlings. It shows a reverse trend in cotyledons. Two major and some minor POD isoforms are evident in root tissue while only one isoform is observed in cotyledons. Western blot analysis, however, shows more POD isoforms at different developmental stages of sunflower seedlings. Their expression is not significantly modulated by salt stress. A significantly reduced titre of glutathione content and glutathione reductase (GR) activity is observed in roots, which remains unaffected by NaCl stress. Zymographic analysis shows gradual increase in GR activity in cotyledons and its reduction in roots with advancing age of seedlings. Biliverdin, a product of HO-1 action on heme, is believed to play a crucial role in controlling H_2O_2 levels. Heme oxygenase (HO-1) activity is initially more in 2 d old seedling cotyledons. Salt-stress does not bring about any significant change in HO-1 activity in light-grown seedling cotyledons. Enzyme activity is, however, more in salt-stressed seedling cotyledons at later stages of growth (4 and 6 d) in dark-grown seedlings. Western blot analysis reveals two isoforms of HO-1 at the three stages of seedling growth, irrespective of NaCl stress. Another HO-1 isoform is, however, induced in 6 d old seedling cotyledons in presence of NaCl. Induction of lateral roots is observed in control and in seedlings grown in presence of zinc protoporphyrin (ZnPPIX; an inhibitor of HO-1 activity) in the absence of NaCl in the growth medium. The present findings thus highlight significant variations in the activity of the ROS scavenging enzymes in sunflower seedlings.



P10.3

Understanding of calcium ion regulation and ROS-interacting enzymes in *Anabaena* sp. PCC 7120 under varied calcium chloride levels

Savita Singh and A. K. Mishra
Laboratory of Microbial Genetics,Department of Botany,Banaras Hindu University,Varanasi-221005, India

Calcium is a well known second messenger both in eukaryotes as well as prokaryotes owing to its favourable chemical nature such as binding strength, ionization potential and kinetic parameters. New roles played by calcium in biological systems are constantly been identified. Although a voluminous literature is available pertaining to role of calcium in eukaryotes however, knowledge about prokaryotes particularly the cyanobacteria in this regard is scanty. The present study focuses on the involvement of calcium ion in the regulation of cellular processes in terms of influx and efflux of calcium ion from the cytosol along with changes in intracellular levels of Na^+ and K^+ during exogenous supply of calcium salt. An assessment of ROS-interacting enzymes such as SOD, catalase and peroxide along with changes in protein expression by means of SDS-PAGE has been undertaken. A dual nature of calcium ion was evident. Calcium chloride concentration of 10 mM was found to be inhibitory while 100 mM proved lethal for growth. SOD, catalase and peroxidase activity was found to be maximum for 100 mM and minimum for 1mM of exogenously supplied calcium salt. SDS-PAGE analysis revealed a difference in protein banding pattern with changes in calcium chloride regime. Thus, a correlation among various diverse factors like calcium ion concentration, ROS-interacting enzymes and protein expression has been observed which suggests an indirect evidence of calcium ion participation in regulating basic metabolism of *Anabaena* sp. PCC 7120 cells.



P10.4

Correlation between accumulation of reactive oxygen species, their enzymatic scavenging mechanisms, Ca^{+2} and modulation of protein kinase C activity during seed development in sunflower

Thakur A. and Bhatla S. C.
Laboratory of Plant Physiology and Biochemistry, Department of Botany, University of Delhi, Delhi 110007, India
email: anita19accenture@gmail.com; bhatlasc@gmail.com

A gradual dehydration of developing cotyledons and accumulation of oil bodies in the cells is evident during seed development in sunflower. The content of reactive oxygen species decreases as the seeds mature. Nitric oxide content, however, does not change appreciably. Thirty days after anthesis, superoxide dismutase activity is maximal, thereby highlighting its scavenging role at the mid stage of seed development. Elevation of glutathione reductase activity is evident subsequently. Glutathione reductase and peroxidase activity exhibit noteworthy temporal differences during seed development. Analysis of protein kinase C (PKC) activity, undertaken by the use of specific fluorescent probe (Fim-1) and its inhibitors (staurosporine and bisindolymaleamide) reveals an increase in PKC activity with seed maturation and its calcium modulation. Use of calcium chelating agents (BAPTA) and subsequent lowering of protein kinase C activity shows the positive modulatory role of calcium on PKC. Immunolocalization analysis by Western blot technique shows two bands representing PKC which are upregulated both with seed maturation and calcium. Present investigations provide new information on a possible correlation between reactive oxygen species, calcium ions, enzymatic scavenging of reactive oxygen species and 'conventional' PKC activity.



P11.1

Isolation and Functional Characterization of Heterotrimeric G-Proteins from *Brassica rapa*

Gulab Chand Arya and Naveen C. Bisht
National Institute of Plant Genome ResearchAruna Asaf Ali Marg, New Delhi-110067, India
email:garya56@gmail.com

Heterotrimeric G-proteins are guanine nucleotide (GTP and GDP) binding proteins that regulate many aspects of physiological development and cell signaling in animals as well in plants. In plants, heterotrimeric G-proteins are widely studied in model plants Arabidopsis and rice. Various loss/gain of functional studies revealed that G-proteins are involved in many biological processes like hormone signaling, stomatal opening and closing, resistance against necrotrophic fungi, controlling shape and size of leaf and seeds and root development. However, our understanding about important roles played by G-proteins in regulating fundamental growth and developmental processes from any polyploid crop is limited. The current study was initiated to investigate the role of heterotrimeric G-proteins in controlling various agronomic traits of *B. rapa*, an economically important crop of *Brassica* genus. In initial part of this study, we performed the isolation of yet complete inventory of heterotrimeric G-protein complex from *B. rapa* (AA genome), which is one of the progenitor genome of two major *Brassica* oilseed crops, *B. juncea* (AABB genome) and *B. napus* (AACC genome). A total of nine genes encoding one Gα, three Gβs and five Gγ subunits were isolated from *B. rapa*. We further performed analysis on their expression pattern across different tissue types representing various developmental stages and protein interaction specificities between different G-protein subunits using yeast based assays. A series of over-expression and knock-down constructs has been recently developed for Gα, Gβ and Gγ subunit gene using Agrobacterium mediated genetic transformation. The gain/loss of function of these G-protein subunit genes will be subsequently analyzed to study the role of G-protein genes in controlling various agronomical important traits in *Brassica* species.



P11.2

Glucosinolates in plant fitness

Augustine, R. and Bisht, N. C.
National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110067, India.
email: rehna.nipgr@gmail.com

Glucosinolates (GSL) or mustard oil glucosides are nitrogen and sulphur containing plant secondary metabolites specific to Brassicaceae family. Upon tissue disruption, these compounds come in contact with myrosinases (β-thioglucoside glucohydrolase), and are hydrolysed to products which are biologically active and impart various biological functions. Since GSL metabolism has evolved as a result of plant–environment interactions, impact of various environmental stimuli including herbivore attack, wounding and hormone treatments on content and profile of GSL were studied. Glucose and wounding significantly elevated GSL accumulation whereas negative correlation exists between SA concentration and glucosinolate accumulation. Different components of GSL behaved differently under various stress conditions. We observed that there was a drastic induction of 4-ME, an indolic GSL in wounded plants. We also dissected the molecular basis of these findings by studying the expression pattern of one of the key regulators of GSL biosynthesis pathway namely *BjMYB28* under various stimuli and hormone treated conditions using qRT-PCR and Promoter:*uidA* fusion lines. Real-time expression analysis in *B. juncea* showed that the expression of *BjMYB28* paralogs was significantly induced by wounding and glucose treatment, and down regulated in presence of elicitors such as SA and MeJA which are known to be involved in biotic stress response. The observations were further confirmed in Pro$_{BjMYB28}$:*uidA* lines. Our study also shows that GSL profile within leaves seems to be highly partitioned and under the coordinated control of four *BjMYB28* paralogs. The GSL accumulation in mid-vein of leaf is largely controlled by *BjMYB28-4*, whereas GSL profile of the outer lamina of the leaf is under the coordinated control of all four *BjMYB28* paralogs. Our data clearly reflect a high degree of transcriptional regulatory complexity of *BjMYB28* paralogs across plant development and in response to various environmental stimuli.



P11.3

Potential Involvements of Arabidopsis Glutamate-Receptor-Like Receptors in Endocytosis

Weiland M., Mancuso S. and Baluska F.
International Laboratory of Plant Neurobiology, University of Florence, Florence – 50121. Italy
Institute for Cellular and Molecular Biology, University of Bonn, Bonn – 53115. Germany
email: baluska@uni-bonn.de

Glutamate receptors emerge to be an essential element for almost every living organism since they were found not only in animals but also in bacteria and plants. The twenty glutamate receptor-like receptors of *Arabidopsis thaliana* (AtGLRs) are involved in processes ranging from light perception, via root growth control, up to the regulation of stomata. Recently, GLRs have been demonstrated to play roles in plant defense reactions. These involvements are most likely achieved by the productionof calcium signatures due to the conductance capacities of the AtGLRs. Like their animal counterparts, AtGLRs seems to be gated by several amino acids, among them glutamate and glycine. Since brain glutamate receptors of animals are best known for their participation in neurotransmission, we had a closer look on possible effects of low concentrations (about 100 □M) of L-glutamate and glycine on endocytosis/vesicle trafficking in Arabidopsis roots. By the treatments with brefeldin A (BFA) and endocytic tracer FM 4-64, it is possible to observe the formation of BFA compartments and to deduce, by the size of these compartments, on rates of endocytosis and endocytic vesicle recycling. Our data show that applications of small amounts of glutamate and glycine lead to increased endocytotic events. Also some AtGLR knockout lines exhibit a reduced activity in term of endocytosis and endocytic vesicle recycling. These results show potential involvement of AtGLRs in endocytosis within the transition zone of Arabidopsis roots.



P11.4

Pisum sativum G-protein beta subunit interacts with small pathogenesis-related cysteine rich protein to regulate stomatal functions

Bhardwaj D[1,3]., Lakhanpaul S[1]., Sharma S[2]., Narayanan L.[2] and Tuteja N.[3*]

[1]University of Delhi, Delhi, India

[2] Bioinformatics infrastructure facility, Sri Venkateswara College, Benito Juarez Road, New Delhi, India

[3]International Centre for Genetic Engineering and Biotechnology, New Delhi, India

email: narendra@icgeb.res.in

G-protein beta subunit has been shown to play a role in coping with heat stress and fungal resistance in the plants. In order to dissect the pathway involved in providing heat and fungal resistance, interaction studies of *Pisum sativum* G-protein beta subunit (PsGβ) were performed using yeast-two-hybrid assay. Seven proteins that reoccurred in the repeated experiments were identified and found to be strong interactors on the basis of β-galacosidase and ONPG assay. Out of seven, *Pisum sativum* defensins gene *PsDef*, a cysteine- rich small pathogenesis-related protein, was randomly selected for further confirmation through one to one gene interaction using Y2H, *in vitro* co-immunoprecipitation and bioinformatic studies. Interestingly, both PsGβ and PsDef showed co-expression after the treatment of heat and MeJA. They also got co-localised on the guard cell membrane when transiently expressed in the Tobacco leaves after co-infiltration and MeJA treatment. We propose a model in which Methyl jasmonate induced interaction of PsGβγ and PsDef controls the functioning of stomata with the involvement of guard cell channel proteins.



P11.5

Elucidation of *N*-acylethanolamine pathway and its physiological role in *Physcomitrella patens*

Kilaru A[1]., Sante R[1]., Swati S[1]., Kinser B[1]., Miller C[1]., Shiva S[2]., and Welti R[2].

[1]Department of Biological Sciences, East Tennessee State University, Johnson City, TN, USA; [2]Division of Biology, Kansas State University, Manhattan, KS, USA

email: kilaru@etsu.edu

N-acylethanolamines (NAE) are fatty acid ethanolamides that are ubiquitous among eukaryotes and activate a network of signaling pathways. Specifically, in mammals, *N*-arachidonylethanolamide (NAE 20:4) or anandamide is a ligand for cannabinoid receptor and acts as neuromodulator for a variety of physiological processes, including appetite, mood and memory, pain sensation, and protection against stroke. Interestingly, while only C12-C18 NAEs occur in seed plants, NAE 20:4 and its metabolites were identified in a nonseed early land plant, *Physcomitrella patens*. To this extent, putative genes, which likely encode for the enzymes that participate in NAE metabolism, were also identified using *in silico* analysis and are being cloned and characterized. Furthermore, selective lipid profiling revealed that NAEs and their precursors *N*-acylphosphatidylethanolamines were abundant in the late gametophyte stage of moss. The existence of NAEs in a bryophyte, along with anandamide, which was not previously observed in seed plants, and its metabolic machinery, suggests the possibility that NAEs in moss may play a role that is beyond what is understood in seed plants. In *Arabidopsis*, NAEs participate in abscisic acid (ABA) dependent or independent seed germination and seedling development and stress responses. In moss, NAE and ABA also negatively affect the growth of the gametophyte; the effect was, however, more severe with anandamide than with NAE 12:0. Here, a comparative study of the role of NAEs in seed and seedless species is discussed. The highly conserved nature of NAE metabolism and its function as modulator to growth and multiple stress signals implicates an evolutionary significance to NAE pathway in plants.



P11.6

Molecular interaction studies of *Brassica* annexin with methyl jasmonate

Vartika Mathur[1] and MNV Prasad Gajula[2]
[1]Department of Zoology, Sri Venkateswara College, Benito Juarez Marg, New Delhi-110021
[2]Centre for Agricultural Bioinformatics, IASRI, Pusa, Library Avenue, New Delhi-110012
email: vartika_m@yahoo.com

Brassica juncea (Family: Brassicaceae) is known to alter/produce various structures and compounds when confronted with unfavourable environment. At molecular level, a series of events takes place simultaneously in plants to combat stress, as well as for damage repair. A recent study showed the induction of the gene expression of *AnnBj4*, an annexin in *B juncea*, due to an increase in the level of cytosolic calcium (Ca^{2+}) following application of methyl jasmonate (Jami et al., 2010). However the molecular mechanism implicated in the behaviour of plant signalling and defined role of methyl jasmonate in triggering gene expression still remains unclear. In the present study, we applied molecular modelling, docking, and molecular dynamics simulations to probe the interaction of methyl jasmonate with annexins. Discovery studio was used for docking, and MD simulations were carried out for 30ns on docked protein by using Gromacs simulations suite. Our preliminary results showed that the methyl jasmonate forms bonds with key active site residues of the protein and stabilizes the complex. Additionally, hydrogen bond mapping reveals the course and intensity of molecular interactions. Our results support the fact that annexins are involved in a wide range of intra- and intermolecular cellular processes, most of which are directly triggered with the conserved ability to bind to small metabolites such as methyl jasmonate.



P11.7

Fatty acid derived oxylipin signalling molecules in some Eastern Himalayan mosses analysed by headspace sample enrichment probe-gas chromatography mass spectrometry (HS-SEP-GC-MS)

Mitra S.* and Poddar-Sarkar M.
Department of Botany (Centre for Advanced Study), University of Calcutta,
35, Ballygunge Circular Road, Kolkata-700019, India
email: souvikmitra1687@yahoo.com

The present study is based on extraction and characterization of oxylipin signalling molecules of mosses using headspace volatile (HSV) analysis technique. Oxylipin comprises diverse volatile bioactive compounds generated by oxygenation of polyunsaturated fatty acids (PUFAs) and subsequently modified by downstream enzyme-mediated metabolic pathway. Studies on characterization of these metabolites have recently been emphasized due to their significant roles as chemical signals involved in stress response and innate immunity. In contrast to higher plants and algae, the studies on phytooxylipins in mosses are still in infancy although mosses are evolutionary very significant for its early terrestrial emergence. In addition, their unique fatty acid composition and their ability to resist themselves against pathogens indicate the importance of oxylipins in this plant group. Three mosses- *Brachymenium ochianum* Ganguloe, *Bryhegeonium consanguineum*(Thwaites & Mitt.) Wilp. and *Barbula hassua* Mitt. collected from different altitude of Darjeeling hill range of Eastern Himalayas were studied for the first time for oxylipin generation. The HSVs were extracted by a high capacity sample enrichment probe (SEP) followed by characterization using GC-MS. No HSV was identified from intact plantlets whereas the mechanically damaged plantlets released a diverse array of volatiles. Thirty-one HSVs were identified which includes alkanes, alkenes, saturated and unsaturated aldehydes, alcohols with carbon chain length from C6 to C18. In all three mosses, hexanol and 7-octen-4-ol were observed as major alcohols along with moderate content of other volatiles such as hexanal, 2-octen-1-ol, nonanal, nonanol. Besides, some acyclonic oxylipins such as 2-decyn-1-ol, 1-dodecyne which are not very common in higher plants were also detected. Analysis of fatty acid content revealed □-linolenic acid and linoleic acid as highly abundant PUFAs along with appreciable amount of arachidonic acid and eicosapentenoic acid in all cases. Therefore, unlike higher plants, eicosapolyenoic acids (C20 PUFAs), along with octadecapolyenoic acids (C18PUFAs) might be the potent precursors of oxylipin in these representative genera.



plant
Signaling & Behavior

