Poster # 1 A FACILE SYNTHESIS OF [$^{15}N_4$]-SUBSTITUED PURINE DERIVATIVES FROM SIMPLE [^{15}N]-PRECURSORS (Chemical biology)

Jan Buček[1], Marek Zatloukal[1], Libor Havlíček[2], Lucie Plíhalová[1] and Karel Doležal[1]
[1]*Department of Chemical Biology and Genetics & Laboratory of Growth Regulators, Centre of the Region Haná or Biotechnological and Agricultural Research, Faculty of Science, Palacký University and Institute of Experimental Botany, Academy of Sciences of Czech Republic, Šlechtitelů 27, 78371 Olomouc-Holice, Czech Republic*
[2]*Isotope Laboratory, Institute of Experimental Botany ASCR, Vídeňská 1083,14220 Prague, Czech Republic*

Purine derivatives are widespread and highly biologically active compounds. Those natural occurring substances or its synthetic analogues can play diverse roles in many different biological processes, not only in plants (Davies P.J., 2010; Zatloukal M. *et al.*, 2008; Havlíček L. *et al.*, 1997; Szüčová *et al.*, 2009). To study these activities of cytokinin (CK) derivatives in detail, monitoring of their endogenous levels as well as metabolism is needed. For this purpose, substances substituted by stable nuclides together with robust and sensitive analytical methods are used (Novák O. *et al.*, 2008; Kakimoto T., 2001).

Here we report a fully functional synthesis leading from simple [^{15}N]-precursors to [$^{15}N_4$]-core substituted aromatic/isoprenoid CKs and/or N^6,C^2-[$^{15}N_4$]-core substituted purine derivatives respectively. In this work, general synthetic protocol has been optimized so 15 compounds have been consequently prepared. [$^{15}N_5$]-Adenine plays role as an important starting compound. Based on previously published methods (Ochai M. *et al.*, 1968; Horgan A., Scott I.A., 1987), its cyclization from [^{15}N]-formamide was significantly improved, so strongly increased yields and purity were achieved. A preparation process of compound located two steps further, [$^{15}N_4$]-6-chlorpurine, was subjected to the set of modification as well. In result, yields and purity were increased by 52 % and 14 %, respectively, in comparison with already published methods (Bendych A. *et al.*, 1954). By reaction of [$^{15}N_4$]- 6-chloropurine or [$^{15}N_4$]-2,6-dichloropurine with appropriate amines, six [$^{15}N_4$]-core substituted aromatic CKs, three [$^{15}N_4$]-core substituted isoprenoid CKs and one [$^{15}N_4$]-core substituted inhibitor of enzyme cytokinin oxidase/dehydrogenase (CKX) were prepared. All prepared compounds were subjected to HPLC-MS, HRMS and ^{1}H;^{13}C;^{15}N NMR analyses, so their identity and purity was proved sufficient for further applications. Moreover, presented approach can be applied for synthesis of any other desired isotopically substituted N^6-purine derivatives.

Except synthesis from [^{15}N]-formamide, [^{15}N]-ammonia was tested as starting compound due to its much lower price and better availability. According to preliminary tests this seems to be functional and ready to use.

Financial support was provided by the project GA16-04184S from the Czech Science Foundationas well as by the Ministry of Education, Youth and Sports, Czech Republic (Grant LO1204 from the National Program of Sustainability I.) and IGA_PrF_2016_011 from Palacký University of Olomouc, Czech Republic.

Poster # 2 A forward chemical genetic screen for small molecules that induce the promoter of UGT74E2, an Arabidopsis UDP-glucosyltransferase that affects auxin homeostasis and stress tolerance (Chemical biology)

Pavel Kerchev[1], Tom Van Der Meer[1] and Frank Van Breusegem[2]
[1]*VIB - Plant Systems Biology,* [2]*VIB-Department of Plant Systems Biology*

Arabidopsis URIDINE DIPHOSPHATE GLYCOSYLTRANSFERASE 74E2 (UGT74E2) glycosylates indole-3-butyric acid and modulates auxin homeostasis. *UGT74E2* transcripts are strongly induced by various stress conditions and its overexpression confers enhanced drought and salt stress tolerance. We speculated that the ability to chemically boost *UGT74E2* levels might be a promising way to conditionally control plant stress tolerance by mimicking the overexpressor phenotype. To look for small molecules inducing *UGT74E2*, we used a luciferase-based high-throughput screeining assay. The 1500-bp region upstream of the *UGT74E2* start codon was fused to a luciferase reporter and the resulting *pUGT74E2:LUC* plants treated with a chemical library of 10 000 compounds. To counterselect for chemicals that trigger a general stress response and thus nonspecifically induce *UGT74E2* expression, we used the chlorophyll fluorescence parameter photosystem II (PSII) maximum efficiency (Fv'/Fm') that is a stable and sensitive stress marker. Chemicals that negatively affected photosynthetic performance,

reflected by a Fv'/Fm' drop, were not retained as potential hits. Following a primary screen and two validation rounds, we isolated 17 hit compounds that induced the luciferase signal in *pUGT74E2:LUC* lines above the control threshold without impairing PSII maximum efficiency. The two most potent hit compounds shared a characteristic (2-furyl)acrylate moiety fused via an amide linkage to the rest of the molecule. The (2-furyl)acrylate core structure had been identified previously as an auxin transcriptional inhibitor and negatively affected mitochondrial function implying an interplay between mitochondrial function and auxin signalling. Two other structures with distinct chemistry conferred stress tolerance and are currently under investigation aimed at identifying their putative protein targets.

Poster # 3 A moss-specific diterpenoid hormone regulates protonemal growth and development (Hormone metabolism)

Hiroshi Kawaide[1], Sho Miyazaki[2] and Masatoshi Nakajima[2]
[1]*Tokyo University of Agriculture and Technology,* [2]*The University Tokyo*

Gibberellins (GAs) are a group of diterpenoid plant hormone and regulate growth of vascular plants. GAs are synthesized from geranylgeranyl diphosphate (GGDP) *via ent*-kaurene. In vascular plants, two monofunctional cyclases, *ent*-copalyl diphosphate (*ent*-CDP) synthase (*ent*-CPS) and *ent*-kaurene synthase (KS), are responsible for the ent-kaurene biosynthesis from GGDP. In contrast, bryophytes have bifunctional CPS/KS catalyzing two-step cyclization from GGDP to *ent*-kaurene *via ent*-CDP. Surprisingly, any GAs were not detectable in the model moss plant *Physcomitrella patens*, and no effect of exogenous application of GA on the growth of *P. patens* was observed. *ent*-Kaurene biosynthesis, however, is indispensable for regulation of protonemal growth in *P. patens*. Compared with wild type, *P. patens* mutants lacking *ent*-kaurene production showed abnormal responses under the blue-light. When protonemata of the wild type were incubated under blue-light, the chloronemal filaments grew in the opposite direction to the light source. In contrast, this avoidance was not observed in the *ent*-kaurene-deficient mutant. The chloronemal growth toward a blue-light source found in the mutant was suppressed by application of *ent*-kaurenoic acid, and the avoidance response was recovered.

Another moss plant, *Hypnum plumaeforme*, is known as a momilactone-producing moss plant. This moss has three bifunctional diterpene cyclases in which *CPS/KS* (*HpCPS/KS*) gene expression was observed during protonemal cell growth. Paclobutrazole (PBZ) treatment suppressed the differentiation of gametophytes from protonemal cells of *H. plumaeforme*, but exogenous application of *ent*-kaurenoic acid rescued the growth suppression by PBZ.

These results indicated that moss plants require *ent*-kaurene-derived and novel diterpenoid growth hormone for protonemal growth regulation and development, but the hormone is not GA but moss-specific compound like as a proto-type of GA.

Poster # 4 A novel root-knot nematode attractant is released from seeds through seed coat mucilage extrusion (Biotic interaction)

Allen Yi-Lun Tsai[1], Tetsuya Arita[1], Ryo Kurota[1] and Shinichiro Sawa[1]
[1]*Kumamoto University*

Root-knot nematodes (RKNs, *Meloidogyne incognita*) are ubiquitous plant pathogens that infects nearly all plant species and cause significant agricultural losses. RKNs are obligate parasites that infiltrate plant roots, feed on plant nutrients while inducing the eponymous root-knot galls at the feeding sites. Newly hatched RKN juvenile 2 (J2) larvae must migrate in the soil to locate and infect new hosts to continue growth and development. RKN J2s are thought to detect chemo- attractants in plant exudates to locate their hosts, however the identities and properties of these attractants have not been extensively characterized. Here we demonstrate that RKN J2s are attracted to Arabidopsis (*Arabidopsis thaliana*) seeds, suggesting seeds may secrete RKN attractants. Seeds from many angiosperms, including Arabidopsis, extrude a layer of gelatinous mucilage from the seed coat upon hydration. Mutant analyses suggest the extrusion of mucilage is required and sufficient for seeds to attract RKNs. However, extracted mucilage alone is unable to attract RKNs. Mutants with loosely attached

mucilage also does not show RKN attraction defects, suggesting the RKN attractant may be a distinct substance from mucilage. Interestingly, seeds from the glucomannan-deficient mutant *cellulose synthase-like A2* (*csla2*) and plant species with glucomannan-poor mucilage show reduced RKN attraction, implying glucomannan positively regulates RKN attraction. Lastly, wild-type seeds germinated in the presence of RKN J2s are indeed more prone to RKN infiltration than mucilage-deficient mutants. However this effect appears to be relevant only for a short period of time during seed germination before the root emerges. In summary, we propose an RKN attractant is extruded from seeds concurrently with the seed coat mucilage, which is perceived by RKN J2s to target their hosts. This study highlights a novel mechanism of RKN behaviour regulation, while suggesting the possibility for seed coat mucilage to be involved in pathogen interactions.

Poster # 5 A role for auxin during the later stages of legume seed development (Reproductive development)

John Ross[1], Laura Quittenden[1], Erin McAdam[1], Tobias Meitzel[2], Sam Cook[1], Marion Dalmais[3], Abdelhafid Bendahmane[3], Richard Thompson[4] and Jennifer Smith[1]

[1]University of Tasmania, [2]Leibniz Institute of Plant Genetics and Crop Plant Research, [3]Unité de Recherche en Génomique Végétale, [4]National Institute for Agronomic Research

We present evidence that auxin plays important roles in the later stages of seed development in the garden pea, *Pisum sativum*. The pea *tar2-1* mutant carries a loss-of-function mutation in the *PsTAR2* gene, which encodes a key auxin synthesis enzyme. Maturing *tar2-1* seeds are grossly deficient in auxin, strongly wrinkled when dry, and smaller than wild-type seeds. Since the seeds of the mutant are not auxin-deficient at the early stages of seed development, the *tar2-1* mutation offers a unique opportunity to specifically explore the role of auxin during the later stages. Introduction of *TAR2* as a transgene reversed the phenotypic effects of the *tar2-1* mutation, while a synthetic auxin partially rescued the mutant dry seed phenotype. Since the predominant auxin in maturing pea seeds is the chlorinated form of indole-3-acetic acid, we suggest that it may be 4-Cl-IAA that is the main auxin responsible for later seed development. Interestingly, *tar2-1* did not reduce the content of another auxin, phenylacetic acid (PAA) in maturing pea seeds, a result that does not support a recent theory that *TAR* genes are responsible for the formation of PAA. Our observations indicate that the normal development of maturing legume seeds depends on 4-Cl-IAA and/or IAA, potentially providing a new avenue for modifying seed development in grain legume crops.

Poster # 6 A Rotating View of Auxin Signaling (Hormone signaling)

Hongwei Jing[1†], Xiaolu Yang[1†], Jian Zhang[1], Xuehui Liu[2], Huakun Zheng[1], Guojun Dong[3], Jinqiang Nian[1], Feng Jian[1], Bin Xia[4], Qian Qian[3], Jiayang Li[1] & Jianru Zuo[1,5]

[1]State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101.[2]Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101. [3]State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006.[4]College of Life Sciences, Peking University, Beijing 100871.

[†]These authors contributed equally to this work.

Auxin is an essential phytohormone involved in various aspects of plant growth and development. Auxin signaling is mediated by a derepression mechanism through promoting the proteasomal degradation of the Auxin/INDOLE 3-ACETIC ACID (Aux/IAA) transcriptional repressors that directly inhibit the activity of AUXIN RESPONSE FACTORS. The degradation of Aux/IAAs is dependent on the auxin promoted interaction of Aux/IAAs with the auxin receptor TRANSPORT INHIBITOR RESPONSE1 (TIR1), an F-box E3 ubiquitin ligase, and the interaction is mediated by the highly conserved domain II of Aux/IAAs. Various gain-of-function mutations in domain II abolish the Aux/IAA-TIR1 interaction and thus cause the accumulation of excessive amount of Aux/IAAs, resulting in an auxin-resistant phenotype. Here, we show that the peptidyl-prolyl isomerization of rice OsIAA11 catalyzed by LATERAL ROOT-LESS2 (LRT2), a cyclophilin-type peptidyl-prolyl *cis-trans* isomerase highly conserved in the plant kingdom, directly regulates the stability of OsIAA11. NMR spectroscopy reveals that LRT2 efficiently catalyzes the *cis/trans* isomerization of OsIAA11. The *lrt2* mutation causes the reduced interaction of

OsIAA11 with OsTIR1 and consequently the accumulation of a higher level of OsIAA11 protein. Moreover, RNA interference-mediated knockdown of the *OsIAA11* expression partially rescues the *lrt2* mutant phenotype in lateral root development.
These results indicate that the cyclophilin-catalyzed peptidyl-prolyl isomerization promotes Aux/IAA degradation, thus illustrating a new regulatory mechanism of auxin signaling.

Poster # 7 A screen for AtD14 mutant proteins that can transmit the signal independently of strigolactone binding in Arabidopsis (Hormone perception)

Akane Sakurada[1], Mengmeng Cao[1], Weiqiang Li[2], Kiyoshi Mashiguchi[1], Yoshiya Seto[1], and Shinjiro Yamaguchi[1]
[1]Graduate School of Life Sciences, Tohoku University, [2]RIKEN Plant Science Center

Strigolactones (SLs) are a class of plant hormones that regulate shoot branching as well as being known as root-derived signals for both parasitic and symbiotic interactions. AtD14 is an α/β-hydrolase family protein, which is thought to be an SL receptor in Arabidopsis. It has been shown that AtD14 interacts with SMAX1-LIKE proteins (SMXL6, SMXL7), repressors of SL signaling, in an SL-dependent manner. However, it is still unclear what kind of change in the AtD14 protein is important for the interaction with SMXL proteins. To address this question, we tried to obtain AtD14 mutant proteins that can transmit the SL signal in the absence of SL. We overexpressed randomly mutagenized AtD14 cDNAs in the max4 atd14 double mutant plants that are defective in both SL biosynthesis and signaling. After screening 1200 transformants, we have identified an AtD14 mutant protein that is able to partially complement the max4 atd14 mutant phenotype without SL treatment. We are now testing the interaction of this AtD14 mutant protein with SMXLs using a yeast two-hybrid system to examine whether their interaction is SL-independent or not.

Poster # 8 A study on adaptive significance of gall formation for an aphid inducing galls on Japanese elm trees (Biotic interaction)

Mami Takei[1], Shinsaku Ito[2], Taichiro Ishige[3], Keisuke Tanaka[3], and Yoshihito Suzuki[4]
[1]United Graduate School of Agricultural Science, Tokyo University Agricultural Technology, [2]Deptartment Bioscience, Tokyo University of Agrculture and Technology, [3]NODAI Genome Research Center, Tokyo University of Agrgulture and Technology, [4]Department Bioresearch Science, Ibaraki University

Insect galls are abnormal plant tissues induced by insects. A number of hypotheses have previously been proposed for the adaptive significance of gall induction. They pur- port that galls provide gall-inducers with enhanced nutrition, or preferable residence space, protected from unfavorable biotic and abiotic stresses. In this study, we hypothe- sized that gall tissues have reduced defense responses as another trait of gall favorable for galling insects, based on the observation that galling insects are keeping healthy conditions in the galls. To test this hypothesis, galls induced by an aphid *Tetraneura nigriabdominalis* on the leaves of Japanese elm tree were used as experimental material. The production of volatile organic compounds (VOCs), which is a typical defense response to insect feeding, was compared between gall and leaf tissues. While hexenyl butanoate and perillene were identified as major VOCs from leaf tissue, and the amounts of the VOCs increased in response to jasmonic acid (JA) treatment, their amounts from gall tissue remained low regardless of JA treatment. Gall and leaf tissues showed com- parable biosynthetic ability of JA and JA-Ile in response to wounding. On the other hand, transcript levels of JA responsive genes, such as *LOX*, increased after JA treatment, whereas those in gall tissue stayed low. These results suggested that gall tissue is less responsive to jasmonates. To further clarify the difference of these tissues in terms of JA responsiveness, comprehensive gene expression analysis using RNA-Seq was performed. The result of the Gene Ontology analysis showed that the number of genes related to “response to stress” were fewer in gall tissue than in leaf tissue. All the results obtained in this study suggested that the gall tissue is less offensive than leaf tissue against the galling-aphids and offers favorable residential space to the residents.

Poster # 9 Abscinazole-E3M, a practical inhibitor of abscisic acid 8'-hydroxylase for use in drought tolerance improvement (Hormone metabolism)

Jun Takeuchi[1], Masanori Okamoto[2], Ryosuke Mega[2], and Yasushi Todoroki[1]
[1]Shizuoka University, [2]ALRC Tottori University

Catabolic inactivation of abscisic acid (ABA) is mainly controlled by CYP707A, which is the cytochrome P450 catalyzing the C8'-hydroxylation of ABA to 8'-hydorxy-ABA that is rapidly converted into more stable tautomer, phaseic acid. In a previous study, we developed a selective inhibitor of CYP707A, abscinazole-E2B (Abz-E2B), through structural modification of uniconazole that functions as an inhibitor of CYP707A in addition to gibberellin biosynthetic enzyme (CYP701A) (*Bioorg Med Chem* **2012**, 20, 3162). Treatment of Abz-E2B enhanced the drought tolerance of the plants through the temporary increase of endogenous ABA level and the induction of stomatal closure. Despite the potential value of Abz-E2B for improving the drought tolerance of plants, the synthesis yield 1% is too low to use as a practical application in agricultural field. Here, we designed four novel abscinazole compound series which has a simpler structure with a triple bond instead of a 1,2,3-triazole of Abz-E2B, and synthesized them as racemic mixture in 6 steps and 15–25% yields. After optically resolved with chiral HPLC, the activities of obtained optically active isomers were examined in enzyme inhibition assay and bioassay. All (−)-isomers showed stronger inhibitory activity against recombinant *Arabidopsis* CYP707A3 than (+)-isomers, and one of them exhibited a similar ligand profile to that of Abz-E2B, that is, the strong inhibitory activity to CYP707A3 and the weak inhibitory activity to rice CYP701A6. This compound, named abscinazole-E3M (Abz-E3M), enhanced the expression of ABA-responsive genes more strongly than Abz- E2B under the co-treatment with ABA. Moreover, treatment with Abz-E3M induced stomatal closure and improved drought tolerance in *Arabidopsis* and maize seedlings. These results suggested that Abz-E3M is more practical and effective inhibitor of ABA catabolic enzyme than Abz-E2B.

Poster # 10 Analysis of tissue-specific gene expression and hormone biosynthesis during tissue-reunion process in incised Arabidopsis flowering stem. (Abiotic stress)

Miyuki Nakanowatari[1], Kentaro Ogura[2], Maasa Banse[2], Keita Matsuoka[2], Emi Yumoto[2], Takao Yokota[2], Hisakazu Yamane[2], Shinobu Satoh[3], Masashi Asahina[1,2]
[1]Grad. Sch. Sci. & Eng., Teikyo Univ. 2: Dept. Biosci, Teikyo Univ. 3: Life & Environ Sci., Univ. Tsukuba.

We reported that the differential gene expression between incised and non- incised Arabidopsis flowering stems and their changes in expression during reunion process. Genes that were
up-regulated during the reunion process include those involved in cell division, cell wall modification, phytohormone-related gene and transcription factors (TFs). Among them, two plant-specific TF genes, ANAC071 and RAP2.6L, were abundantly expressed. ANAC071 was expressed at 1-3 days after incision exclusively in the upper region of the cut gap with concomitant accumulation of indole-3-acetic acid. In contrast, RAP2.6L was expressed at 1 day after incision exclusively in the lower region with concomitant deprivation of indole-3-acetic acid. The expression of ANAC071 and RAP2.6L were also promoted by ethylene and jasmonic acid, respectively. Our results suggested that plant-specific TFs differentially expressed around the cut position were essential for tissue reunion of Arabidopsis incised flowering stems, and were under opposite control by polar-transported auxin, with modification by the ethylene and jasmonic acid wound-inducible hormones. In this study, laser microdissection (LMD) was used to isolate epidermis/cortex, vascular, and pith cells from incised stem, and gene expression analyses were performed. QRT-PCR analyses showed that *IAA5,* auxin response protein, were abundantly expressed in the upper epidermis/cortex and vascular cells neighboring the wounded regions in 1 day after incision, similar to *ANAC071*. The *DAD1* and *LOX2*, involved in JA production, showed exclusive expression in the lower region in 1 day after incision. However, other JA biosynthesis/signaling genes and *ACS2*, ethylene biosynthesis genes, showed different temporal/spatial expression patterns. The endogenous hormone levels and expression of genes involved in cell division and cell wall modification during tissue-reunion process were also analyzed.

Poster # 12 Application of bioregulators of microbiological origin as new effective stimulants of *Triticum aestivum* L. regeneration *in vitro* Victoria Tsygankova, Elena Shysha, Anatoly Galkin, Alla Yemets, Yaroslav Blume, Galina Iutynska (Hormones & biotechnology)

Victoria Tsygankova[1], Elena Shysha[2], Anatoly Galkin[2], Alla Yemets[2], Galina Iutynska[3], and Yaroslav Blume[2]
[1]Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kiev, [2]Department of Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kiev, [3]Department of General and Soil Microbiology, Zabolotny Institute of Microbiology and Virology,National Academy of Sciences of Ukraine, Kiev

Impact of new bioregulators of microbiological origin: Avercom, Avercom nova-2, Violar and Phytovit, created on base of selected strains of soil streptomycetes at the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, on the initiation of callus formation and shoot organogenesis in the isolated tissue cultures in vitro of wheat (*Triticum aestivum* L.) of two varieties Yatran 60 and Zimoyarka was studied. The main components of these bioregulators are metabolism products of soil streptomycetes such as phytohormones, free fatty acids, amino acids, vitamins of the B group, and antiparasitic antibiotics. The isolated wheat tissues – segments of roots and hypocotyls were obtained from 5-day-old seedlings grown on the modified MS media supplemented with each bioregulator at the concentration of 10-50 µl/L. The initiation of callus formation was observed on the root and hypocotyl segments on the modified MS media supplemented with each bioregulator at the concentration of 20-30 µl/L in combination with 1-2 mg/L auxin 2,4-D. The shoot organogenesis was obtained from the callus cells formed on the hypocotyl segments on the modified MS media supplemented with each bioregulator at the concentration of 20-30 µl/L in combination with 1-2 mg/L auxin 2,4-D. The formation of shoots and leaf buds was observed on the callus cells obtained from the root segments on the modified MS media supplemented with each bioregulator at the concentration of 10-50 µl/L in combination with 1-2 mg/L cytokinin BAP. The direct shoot organogenesis was obtained on the root segments on the modified MS media supplemented with each bioregulator at the concentration 40 µl/L. The obtained shoots were rooted on the modified MS media supplemented with each bioregulator at the concentration of 10-50 µl/L in combination with 0,1-0,2 mg/L auxins IAA or NAA. Obtained results confirm the possibility of using of bioregulators of microbiological origin as new efficient stimulants for regeneration of *Triticum aestivum* L. *in vitro*.

Keywords: bioregulators of microbiological origin, *Triticum aestivum* L., shoot organogenesis *in vitro*

Poster # 13 Arabidopsis Cytokinin Response Factor 6 Represses Cytokinin Related Genes in Response to Oxidative Stress to Promote Tolerance (Abiotic stress)

Aaron M. Rashotte[1], Paul J. Zwack[1], Inge De Clercq[2,3,] Timothy C. Howton[4], H. Tucker Hallmark[1], Erika Keshishian[1], Andrej Hurny[5], Lenka Plačková[6], Ondřej Novák[6], Eva Benkova[5], M. Shahid Mukhtar[4], and Frank Van Breusegem[2,3]
[1]Department of Biological Sciences, Auburn University, [2]Department of Plant Systems Biology, VIB, [3]Department of Plant Biotechnology and Bioinformatics, Ghent University, [4]Department of Biology, University of Alabama at Birmingham, [5]Institute of Science and Technology Austria (IST Austria), [6]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science of Palacký University & Institute of Experimental Botany AS CR

Cytokinin is a phytohormone that is well known for its roles in numerous plant growth and developmental processes, yet it has also been linked to abiotic stress response in a less defined manner. *Arabidopsis thaliana* Cytokinin Response Factor 6 (CRF6) is a cytokinin-responsive AP2/ERF-family transcription factor that through the cytokinin signaling pathway plays a key role in the inhibition of dark-induced senescence. CRF6 expression is

also induced by oxidative stress and here we show a novel function for CRF6 in oxidative stress tolerance as well as identify downstream transcriptional targets of CRF6 that are repressed in response to oxidative stress. Analysis of transcriptomic changes in WT and *crf6* mutant plants treated with H_2O_2 identified CRF6-dependent differentially expressed transcripts, many of which were repressed, rather than induced. Moreover, many repressed genes also show decreased expression in 35S:CRF6 overexpressing plants. Together this suggests that CRF6 functions largely as a transcriptional repressor. Interestingly, among the H_2O_2 repressed – CRF6 dependent transcripts was a set of five genes associated with cytokinin processes: (signaling) ARR6, ARR9, ARR11, (biosynthesis) LOG7, and (transport) ABCG14. We have examined mutants of these cytokinin- associated target genes to reveal novel connections to oxidative stress tolerance across development. Additional examination of CRF6-DNA interactions indicates that CRF6 may regulate these targets both directly and indirectly. Furthermore, we will show changes in cytokinin levels after H_2O_2 treatment in WT, CRF6OE and mutant backgrounds in relation to stress processes. Together we believe that this shows that CRF6 functions during oxidative stress as a negative regulator to control this cytokinin-associated module of CRF6-dependent genes and establishes a novel connection between cytokinin and oxidative stress response.

Poster # 14 Arabidopsis gibberellin 2-oxidase7 catabolic properties revised (Hormone metabolism)

Maria João Pimenta Lange[1], Nils Kappe[1], and Theo Lange[1]

[1]*Braunschweig University of Technology, Institute of Plant Biology, Germany*

Gibberellin (GA) 2-oxidases are catabolic enzymes involved in the regulation of many GA regulated developmental processes in plants, including reactions to abiotic stresses. In plants 2-oxidases belong to the group of 2-oxoglutarate dependent dioxygenases that are encoded by a multigene families. Lee and Zeevaart (2005) proposed three structural classes of GA 2-oxidases: Class I enzymes are represented by AtGA2ox1, -2 and -3, and class II by AtGA2ox4 and -6. Both classes utilise C_{19}-GAs as substrates. The third, Class III, is represented by AtGA2ox7 and -8 and is active against C_{20}-GAs as substrates. However, exceptions to this classification were published recently for Class I GA 2-oxidases from cucumber and bread wheat that are also capable to oxidise C_{20}-GAs while bread wheat Class III GA 2-oxidases also converted C_{19}-GAs (Pimenta Lange et al. 2013, Pearce et at. 2015).

Here we re-characterize the class III AtGA2ox7 and show that it converts C_{20}-GAs GA_{12}, GA_{15}, and GA_{24} to GA_{110}, 2ß-hydroxyl GA_{15}, and 2ß-hydroxyl GA_{24}, respectively. Moreover, we show that this enzyme also converts C_{19}-GAs, as demonstrated by the oxidation of GA_9, at the 2α- position, to GA_{40}.

Lee and Zeevaart (2005) Plant Physiol. **138**, 243
Pimenta Lange et al. (2013) Phytochem. **90**, 62
Pearce et at. (2015) BMC Plant Biol. **15**, 130

Poster # 15 Are non-canonical strigolactones major players? (Hormone metabolism)

Koichi Yoneyama[1], Xiaonan Xie[1], Kaori Yoneyama[1], Takahito Nomura[1], Kohki Akiyama[2], and Christopher SP McErlean[1]

[1]*Utsunomiya University,* [2]*Osaka Prefecture University*

Natural strigolactones (SLs) are carotenoid-derived plant metabolites and can be classified into two groups based on their chemical structures. Strigol and related compounds that contain the ABC ring system connected to the butenolide D ring *via* an enol ether bridge are called canonical SLs. Since the discovery of carlactone (CL) as a biosynthetic precursor for SLs, germination stimulants structurally distinct from canonical SLs have been characterized. These non-canonical SLs include avenaol (black oat), heliolactone (sunflower), zealactone (maize), and methyl lotuslactonoate (*Lotus*) and are major SLs in some plant species like maize, black oat, and sunflower. In addition, CL and its oxidized metabolites such as carlactonoic acid (CLA) and methyl carlactononate (MeCLA) have been detected in tissues and root exudates from various plant species. These results suggest that non-canonical SLs may be major players as plant hormones and also as host recognition signals in the rhizosphere.

Poster # 16 Assessment of cytokinin based grain yield in wheat (*Triticum aestivum* L (Hormone genomics)

Laura E. Perry[1] and Neil Emery[1]
[1]*Trent University*

Bread wheat (*Triticum aestivum* L.) accounts for more than 20% of the current food supply and is one of the most important cereal crops produced today. Despite this, research on the parameters that have the potential to influence yield in wheat is lagging behind other important grain crops, which have seen increased yields in recent years. Thorough understanding of the regulatory function of plant growth hormones creates a chance to increase yield parameters in global cereal crops, including bread wheat. Cytokinins (CKs) are a group of hormones crucial for plant growth and development. They are of great importance in agriculture as they promote flowering and seed development, essential factors that impact yield. In this research, relative expression levels of 12 putative metabolic CK genes from multigene families (*Ta*IPT, *Ta*CKX, *Ta*Glu, and *Ta*ZOG) during stages of kernel development were analyzed in high- and low- yielding lines of spring wheat using qPCR. The same stages of seed maturation were analyzed through use of HPLC/MS-MS for the presence of 27 different CK metabolites. Several correlations were detected between the relative CK gene expression levels and quantified CK metabolite levels. Data on CK gene expression can help for better understanding of the distribution of CK active forms and their derivatives across the developmental stages of wheat kernels. The results of this project revealed that high-yielding cultivars of wheat differ significantly from the low-yielding lines in regards to their CK metabolite profiles and CK metabolic gene expression levels. Identification of these differences may lead to the development of a new yield marker and in consequence, more rapid implementation of high-yielding wheat cultivars in agriculture.

Poster # 17 Auxin receptors and seed development in pea (Hormone perception)

Ariane Gélinas-Marion[1] and John Ross[1]
[1]*University of Tasmania*

The Auxin F---Box receptors, TIR1 and the AFBs, have been defined predominantly in *Arabidopsis thaliana*. Broadening research to commercial crop species, such as the garden pea, *Pisum sativum,* can expand our knowledge of auxin function and its future agronomic potential in seed production. Five *AFB* genes have been isolated from pea: *PsAFB1A*, *PsAFB1B*, *PsAFB2*, *PsAFB5* and *PsAFB6*. The proteins encoded by the genes are highly conserved with little variation in the binding residues between the family members. Transcript quantification revealed that the five genes are expressed throughout the plant with varying level of expression depending on tissue type and developmental stage. One gene in particular, *PsAFB5*, is now thought to be the pea gene *RMS2* (Ligerot et al, 21st IPGSA meeting; Rameau et al, Auxin 2012), originally characterized for its role in shoot branching. Our data confirm that the *PsAFB5* gene has one mutation in each of the two available *rms2* mutants, resulting in amino acid substitutions in critical auxin binding sites of the protein. PsAFB5 shows a high degree of sequence similarity to the *Arabidopsis* proteins AtAFB4 and AtAFB5, shown to function as auxin receptors (Prigge et al 2016; G3 doi:10.1534/g3.115.025585). In *Arabidopsis*, null *afb4* alleles have no reported phenotypic abnormalities. Here, we show that the two *rms2* pea mutants exhibit altered reproductive phenotypes compared with their respective wild types, including reduced seed yield per pod, smaller seeds and dimpling of the cotyledons. We propose that our results demonstrate the effects of diminished auxin perception on pea seed development, and that the PsAFB5 receptor contributes to auxin response in the reproductive structures.

Poster # 18 Auxin regulation of ethylene biosynthesis in developing pea fruit (Hormone Interactions)

Charitha Jayasinghege[1], Jocelyn Ozga[1], Kosala Waduthanthri[1], and Dennis Reinecke[1]
[1]*Plant BioSystems Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta*

In pea, both auxins 4-chloroindole-3-acetic acid (4-Cl-IAA) and indole-3-acetic acid (IAA) occur naturally; however, only 4-Cl-IAA stimulates growth and GA biosynthesis, and inhibits ethylene response in deseeded

pericarps, mimicking in general the presence of seeds. To further understand the role of these two auxins in the differential regulation of ethylene biosynthesis
/response in the pericarp, 4-Cl-IAA and IAA effects on the ethylene biosynthesis gene expression, 1-aminocyclopropane-1-carboxylic acid (ACC) levels and ACC oxidase (ACO) enzyme activity in deseeded pea pericarps were studied. During ethylene biosynthesis, s- adenosylmethionine is converted to ACC by ACC synthase (ACS; *PsACS1*, *PsACS2*, *PsACS4* code for ACS). Ethylene is produced via the oxidation of ACC by ACC oxidase (ACO; *PsACO1*, *PsACO2* and *PsACO3* code for ACO). Although ethylene evolution was shown to be similar in 4-Cl-IAA- and IAA-treated deseeded pericarps, regulation of the transcript abundance profiles of *ACS* and *ACO* genes by 4-Cl-IAA was dramatically different than that of IAA in this tissue. 4- Cl-IAA increased deseeded pericarp *PsACS1* transcript abundance by 1800-fold (2 h after treatment), IAA induction was about 40-fold. Consistently, free ACC levels in 4-Cl-IAA-treated deseeded pericarp were more than 20-fold higher than IAA-treated fruits (12 h after hormone treatment). Both auxins increased pericarp *PsACO1* transcript abundance, but 4-Cl-IAA had a more pronounced effect (10-fold increase) than IAA (2-fold increase). 4-Cl-IAA decreased *PsACS4, PsACO2* and *PsACO3* transcript abundance to levels similar to that in pericarp with seeds, IAA did not. 4-Cl-IAA did not affect pericarp ACO enzyme activity; however, ACO enzyme activity was 1.5-fold higher in IAA-treated pericarps than non-treated pericarps. Based on these observations we propose that the differential effects of IAA and 4-Cl-IAA on ACC levels and ACO protein pool may play important roles in regulating ethylene action in developing pea fruit.

Poster # 19 AUXIN RESPONSE FACTOR ACTIVITY AS A GENETIC SWITCH TO CONTROL PLANT REGENERATION AND ORGANOGENESIS PROPERTIES (Hormones & biotechnology)

Wenzislava Ckurshumova[1], Naden Krogan[2], Adriana Caragea[1], Danielle Marcos[1], and Thomas Berleth[1]
[1]*University of Toronto,* [2]*American University,*

Combinatorial interactions of AUXIN RESPONSE FACTORs (ARFs) and Aux/IAA proteins relay auxin responses. Short-lived Aux/IAA proteins negatively regulate ARF activity, mediated by interaction domains III and IV. As a new tool to explore the systems properties of this regulatory network, we generated gain-of-function *ARF* genotypes by eliminating domains III and IV from the functionally well-characterized ARF MONOPTEROS (MP)/ARF5. This irrepressible *MP* version, MP*Δ*, accentuated auxin responses, altered organ patterning and dramatically stimulated shoot regeneration in Arabidopsis.
A glucocorticoid-inducible variant of *MPΔ* can be used as a powerful analytical and biotechnological tool to (a) identify downstream MP targets both molecularly and genetically, (b) deliberately manipulate leaf shape and vascular tissue patterns throughout the plant, (c) to influence ovule development and (d) to vastly increase de novo shoot formation from callus tissue. The fact that *MP*Δ activity triggers similar morphological changes and significantly enhanced shoot formation from calli in only moderately conserved angiosperms, indicates that this single gene activity is sufficient to alter important biotechnological traits in a vast diversity of angiosperms in ways not observed with any other transgene.
Target gene searches and genetic tests identify subclades of the *Aux/IAA* family as well as auxin transport facilitator genes as direct regulatory targets of MP. These regulatory links are critical constituents of interdependent feedback controls, whose mathematical descriptions enable computer simulations of *de novo* pattern generations during shoot and root regeneration as well as in the formation of individual organs.

Poster # 20 BES1 down-regulates Abscisic Acid 2 to increase seed size in *Arabidopsis thaliana* (Hormone Interactions)

Seung-Hyun Son[1], Ji Hyun Youn[1], Ji Eun Lee[1], Jeehee Roh[1], Hyeon Soong Yeom[1], Hwanhee Jang[1], Sunyoung Kim[1], and Seong-Ki Kim[1]
[1]*Department of Life Science, Chung-Ang University*

Abscisic acid 2 (ABA2) catalyzes the conversion of xanthoxin to ABA aldehyde in ABA biosynthesis in Arabidopsis. Expression of *ABA2* was down-regulated by brassinosteroids (BRs), and up-regulated by a BR biosynthetic inhibitor. *ABA2* expression was altered in BR-related mutants, especially reduced in *bes1-D* and increased *in bes1*, suggesting that *ABA2* is down-regulated by BRs *via* BES1 transcription factor in BR signaling. EMSA and ChIP revealed that BES1 directly binds to E- box sequences in the promoter region of *ABA2*. GC-MS and ELISA analysis showed that endogenous level of ABA is reduced in *bes1-D* seeds, while increased in *bes1* seeds compared to that in wild type seeds, indicating that BES1-induced down-regulation of *ABA2* plays a role to maintain endogenous level of ABA in Arabidopsis seeds. Embryo development of *bes1-D* was delayed than that of wild type. At late stage of embryogenesis, expression of regulatory genes to determine seeds size such as *SHB1, IKU1/2, MINI3, AGL62, KLU* and *ARF2* was enhanced in *bes1-D*.The patterns for embryogenesis and expression of the regulatory genes is quite similar to those in *aba2* to determine size of seeds. Compared to wild type, seed size of *aba2* and *bes1* was increased and decreased, respectively. *bes1xaba2* showed increased seed size which was shown in *aba2*, determining that BES1-mediated BR signaling is an up- stream process for ABA signaling in determination of seed size. Taken together, BR signaling *via* BES1 down-regulates *ABA2* expression to reduce ABA contents, which leads to increase size of seeds in Arabidopsis.

Poster # 22 Biochemical characterization of LATERAL BRANCHING OXIDOREDUCTASE involving strigolactone biosynthesis in Arabidopsis (Hormone metabolism)

Kaori Yoneyama[1] , Phillip Brewer[2], Kohki Akiyama[3], Xionan Xie[1], Yoshiya Seto[4], Shinjiro Yamaguchi[4], Koichi Yoneyama[4], Christine Beveridge[2], and Takahito Nomura[1]
[1]Utsunomiya University, [2]The University of Queensland, [3]Osaka Prefecture University, [4]Tohoku University

Strigolactones (SLs) previously identified as host recognition signals for both root parasitic plants and symbiotic arbuscular mycorrhizal fungi, and have been classified into a new group of plant hormones optimizing plant growth and development. Natural SLs are carotenoid-derived compounds consisting of butenolide rinf (Dring) linked by an enol ether bridge to a less conserved moiety. D ring is essential for all activities. Carlactone (CL) is the first endogenous precursor for SLs carrying the D ring. In Arabidopsis, MORE AXILLARY GROWTH (MAX1), a cytochrome P450 monooxygenase, converts CL to carlactonoic acid (CLA). Herein, we report that LATERAL BRANCHING OXIDOREDUCTASE (LBO), a 2-oxoglutarate and Fe (Ⅱ)-dependent dioxygenase, converts methyl carlactonoate (MeCLA) to a novel oxidized compound.

Poster # 23 Biocommunication between flowering plants and insects through pollen fluorescence (Biotic interaction)

Shinnosuke Mori[1], Mari Kawakami[1], Masanori Oishi[1], Hiroshi Fukui[2], Katsumi Goto[3], Junko Tsukioka[3], Masahiro Osakabe[1], Nariaki Sugioka[1], Masayuki Sakuma[1], and Nobuhiro Hirai[1]
[1]Kyoto University, [2]Kagawa University, [3]Kyoto Pharmaceutical University

Pollen often shows blue fluorescence under UV light. Anthers usually extend beyond the corolla, and genes in pollen are exposed to the risk of damage from the solar UV light. Fluorescent compounds may protect genes from UV energy through emission of heat and fluorescence. Another function may be attracting pollinating insects. Although biocommunication between plants and insects through flower fluorescence has been discussed for decades, fluorescent compounds in pollen have not been identified, and the use of fluorescence as an informative cue has remained unclear. The objective of this study was identification of the fluorescent compounds, and examination of their ecological function by behavioral experiments employing honeybees.
Plants with pollen showing strong blue fluorescence were screened. Fluorescent compounds from pollen of *Plantago lanceolata*, *Dipsacus fullonum*, *Scabiosa atropurpurea*, *Fatsia japonica, Prunus persica*, and *Allium fistulosum* were isolated, and identified as acteoside and its *cis*-isomer, 3,5-dicaffeoylquinic acid, chlorogenic acid, chlorogenic acid and 3,5-dicaffeoylquinic acid, 1-*O*-feruloyl-βD-glucose and its *cis*- isomer, and L-tryptophan, respectively. The major fluorophores of these compounds are caffeoyl and feruloyl groups with

fluorescence emission maxima at 458 nm and 419 nm. These wavelengths correspond to the blue color, suggesting that these compounds are responsible for pollen fluorescence. These compounds are known as antioxidants. Fluorescent compounds would protect pollen genes not only by absorbing UV-harmful energy, but also scavenging UV-induced reactive oxygen species.
We examined whether honeybees are attracted by chlorogenic acid adsorbed on filter paper under sunlight. The results showed that honeybees can perceive the fluorescence and be significantly attracted by it. The fluorescent compounds in pollen might originally generate to protect genes from UV light in the initial evolution process, and later insects might use them as a guide to pollen as food. Pollen fluorescence may have contributed to the co-evolution between plants and insects.

Poster # 24 BSS1 as a negative regulator for brassinosteroid signaling that was identified by chemical biology using Brz. (Hormone signaling)

Takeshi Nakano[1,7], Setsuko Shimada[1,2], Tomoyuki Komatsu[1,3], Ayumi Yamagami[1], Miki Nakazawa[4], Minami Matsui[2], Hiroshi Kawaide[3], Masahiro Natsume[3], Hiroyuki Osada[5], and Tadao Asami[6,7]
[1]Gene Discovery Research Group, RIKEN Center for Sustainable Resource Science, [2]Synthetic Genomics Research Team, Biomass Engineering Program Cooperation Division, RIKEN Center for Sustainable Resource Science, [3]United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology (TUAT), Saiwai-Cho, Fuchu, [4]RIKEN Genome Science Center, Tsurumi, Yokohama, [5]Chemical Biology Group, RIKEN Center for Sustainable Resource Science, Wako, [6]Department of Applied Biological Chemistry, The University of Tokyo, Yayoi, Bunkyo-ku, [7]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST)

Brassinosteroids (BRs) play important roles in plant development and response to environmental cues. BIL1/BZR1 is a master transcription factor in BR signaling, but the mechanisms that lead to the finely tuned targeting of BIL1/BZR1 by BRs are unknown. Here, we identified BSS1 (BRZ-SENSITIVE-SHORT HYPOCOTYL1) as a negative regulator of BR signaling by chemical biology analysis using Brz, a specific BR biosynthesis inhibitor. The *bss1-1D* mutant, which overexpresses *BSS1*, presented a Brz-hypersensitive phenotype in hypocotyl elongation. *BSS1* encodes a BTB–POZ domain protein with ankyrin repeats, known as BLADE ON PETIOLE 1 (BOP1), which plays an important role in regulating leaf morphogenesis. The *bss1-1D* mutant exhibited an increased accumulation of phosphorylated BIL1/BZR1 and a negative regulation of BR-responsive genes. Punctate BSS1/BOP1-GFP was increased by Brz, and the formed puncta were diffused by BR treatment in the root and hypocotyl. The direct interactions between BSS1/BOP1 and BIL1/BZR1 or BES1 were identified. The large protein complex formed between BSS1/BOP1 and BIL1/BZR1 could only be detected in the cytosol. The nuclear BIL1/BZR1 increased in *BSS1/BOP1*-deficient background and decreased in *BSS1/BOP1*-overexpressed background. Our study suggests that the BSS1/BOP1 protein complex inhibits transport of BIL1/BZR1 to the nucleus from the cytosol and negatively regulates BR signaling. (1)

(1) Shimada et al., Plant Cell (2015) 27: 375-390

Poster # 25 C2-substituted aromatic cytokinin sugar conjugates delay the onset of senescence by maintaining the activity of the photosynthetic apparatus (Chemical biology)

Ondrej Plihal[1], Hana Vylicilova[1], Lucie Plihalova[1], Alexandra Husickova[1], Lukas Spichal[1], and Karel Dolezal[1]
[1]CRH, Palacky University

Cytokinins are plant hormones with biological functions ranging from coordination of plant growth and development to the regulation of senescence. A series of 2-chloro-N^6- (halogenobenzylamino)purine ribosides was prepared and tested for cytokinin activity in detached wheat leaf senescence, tobacco callus and *Amaranthus* bioassays. The new compounds showed significant activity, especially in delaying senescence in detached wheat leaves. The newly prepared derivatives were also tested in bacterial receptor bioassays using both monocot and

dicot members of the cytokinin receptor family. Most of the derivatives did not trigger cytokinin signaling *via* the AHK3 and AHK4 receptors from *Arabidopsis thaliana* in the bacterial assay, but some of them specifically activated the ZmHK1 receptor from *Zea mays* and were also more active than the aromatic cytokinin BAP in an *ARR5::GUS* cytokinin bioassay using transgenic *Arabidopsis* plants. Whole transcript expression analysis was performed using an *Arabidopsis* model to gather information about the reprogramming of gene transcription when senescent leaves were treated with selected C2-substituted aromatic cytokinin ribosides. Genome-wide expression profiling revealed that the new halogenated derivatives induced the expression of genes related to cytokinin signaling and metabolism. They also prompted both up- and down-regulation of a unique combination of genes coding for components of the photosystem II (PSII) reaction center, light-harvesting complex II (LHCII), and the oxygen-evolving complex, as well as several stress factors responsible for regulating photosynthesis and chlorophyll degradation. Chlorophyll content and fluorescence analyses demonstrated that treatment with the new derivatives increased the efficiency of PSII photochemistry and the abundance of LHCII relative to DMSO- and BAP-treated controls. These findings demonstrate that it is possible to manipulate and fine-tune leaf longevity using synthetic aromatic cytokinin analogs.

Poster # 26 Characterization and gene functional study of a novel albino mutant in *Arabidopsis** (Hormone signaling)

Na Li[1] and Junxian He[1]
[1]*School of Life Sciences, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, New Territories*

Chloroplasts are one type of most important organelles in plant cells. The major role of chloroplasts is to conduct photosynthesis but they are also responsible for the synthesis of starch, amino acids, lipids and pigments. Therefore, chloroplasts are crucial for plant's normal life. However, how chloroplast is developed and how its function is regulated currently remain largely unknown.

Brassinosteroids (BRs) are a group of steroid plant hormones that regulate many aspects of developmental and physiological processes in plants, including cell elongation, cell proliferation, photosynthesis, and responses to stress. Early studies on BR mutants suggest that BRs may be involved in chloroplast development. However, how these hormones regulate chloroplast development is poorly understood.

From a recent genetic screen for new BR signaling mutants in *Arabidopsis using* T-DNA activation tagging, we identified a seedling lethal and albino mutant *bzs20*, which has severe defects in chloroplast development and accumulating chlorophylls. Gene cloning results indicated that the *BZS20* gene encodes a chloroplast-localized protein that has not been characterized before. Mutant complementation using the cloned *BZS*20 gene completely rescued the *bzs20* albino phenotype, suggesting that the cloned *BZS20* is the gene responsible for the mutant phenotype. Collectively, our preliminary results suggest that BZS20 may define a novel regulator of chloroplast development in *Arabidopsis*. In the future we will perform further detailed phenotypic characterization to this mutant and study the *BZS20* gene function in chloroplast development. Since the mutant was identified from a BR-related mutant screen, we will also explore whether the *BZS20* gene plays a role in BR-regulated chloroplast development.

*This work was supported by the General Research Fund (CUHK Codes 464412 and 14121915) and an AoE grant (AoE/M-05/12) from the Research Grants Council (RGC) of Hong Kong, and the Direct Grants from the Chinese University of Hong Kong [toJ.H.].

Poster # 27 Chemical regulation of photorespiratory hydrogen peroxide-induced cell death. (Chemical biology)

Tom van der Meer[1,2], Pavel Kerchev[1,2] and Frank Van Breusegem[1,2]
[1]*Department of Plant Systems Biology, VIB, Ghent University,* [2]*Department of Plant Biotechnology and Bioinformatics, Ghent University*

Protection of crops against adverse climate conditions, such as drought or heat, is essential to ensure a sustainable year to year yield. Characteristic of abiotic stress responses is the increased metabolic flux through the photorespiratory pathway which in turn leads to an accumulation of ROS. Although the major enzymatic steps of the photorespiratory pathway are well described, little information is available on the functional significance of photorespiration beyond carbon recycling and the importance of ROS as a signaling molecule in stress responses. A key component in the pathway is the peroxisomal catalase activity, which removes H_2O_2 produced as a byproduct of the oxidation of glycolate to glyoxylate. In this way the plant maintains the cellular redox homeostasis which in turn governs the perception and execution of stress responses. A high-throughput chemical screen using a catalase2 (cat2) deficient *Arabidopsis thaliana* mutant was used to identify 34 small molecules, from an extensive chemical library, able to alleviate the negative effects of photorespiration-induced oxidative stress. One of the hit compounds was characterized as an inhibitor of sirtuin activity. Sirtuins are NAD+-dependent protein deacetylases and are emerging as important regulators of stress responses. Using this novel chemical sirtuin inhibitor and a combination of metabolomics, transcriptomics and in vitro enzymatic assays we studied the role of plant sirtuins in adverse environmental conditions.

Poster # 28 Chemical screening of novel strigolactone agonists that specifically interact with D14 proteins.
(Hormone perception)

Rei Yasui[1], Yoshiya Seto[1], Hiroyuki Kasahara[2], and Shinjiro Yamaguchi[1]
[1]Graduate School of Life Sciences, Tohoku University, [2]The Global Innovation Research Organization, Tokyo University of Agriculture and Technology

Strigolactones (SLs) are a class of plant hormones that regulate shoot branching as well as known as root-derived signals for parasitic and symbiotic interactions. We have examined the interaction between SL and its possible receptor of rice, DWARF14 (D14), by the differential scanning fluorimetry (DSF) analysis. DSF allows us to evaluate the protein-ligand interactions by measuring the shift of the protein melting temperature. Previously, it was reported that the melting point of a petunia D14 orthologue, DAD2, shifts to lower temperature in the presence of SL in the DSF analysis. Therefore, we assessed the melting point of D14 and its ortholog in *Arabidopsis*, AtD14, upon SL addition by the same method, resulting in a decrease in their melting points. In this study, we carried out a chemical screening from a library consisting of 10,000 compounds using the DSF method, and found that 23 compounds can induce the melting temperature shift of both D14 and AtD14. Because these compounds are considered as strong candidates for novel SL agonists, we next checked their biological activities for parasitic plant seeds and *Arabidopsis*. As a result, six compounds only stimulated the germination of parasitic plant, *Striga hermonthica*, in a dose-dependent manner, although their activity was much weaker than that of the SL analog GR24. In contrast, one compound inhibited both hypocotyl elongation and shoot branching in an AtD14-dependent manner in *Arabidopsis* and could not stimulate the germination of *Striga hermonthica*. Our data suggested that this chemical is a novel SL agonist that targets D14 proteins.

Poster # 29 Comparison of cytokinin metabolism kinetics of two distinct Arabidopsis ecotypes through experimental and computational techniques (Novel methods and technology for hormone research)

Hosek P.[1], Hoyerova K.[1], Dobrev P. I.[1], Kiran N. S.[2], Zizkova E.[1], Motyka V.[1]
[1]Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, [2]Department of Molecular Biology and Radiobiology, Faculty of Agronomy, Mendel University in Brno

Until now, almost 70 cytokinin derivatives have been described in higher plants, suggesting how complex the network of interconversions determining the concentration of particular cytokinin forms have to be. Because of such complexity we have previously used mathematical methods for interactive visualisation and analysis of experimental data obtained after exogenous application of four major cytokinins: trans-zeatin, cis-zeatin, dihydrozeatin, and isopentenyladenin in two-hour time span on *Arabidopsis thaliana* Col-0. The metabolic system was divided into four subsystems according to particular incubations and independent

multicompartment mathematical models of these subsystems were then constructed. Multiple Monte Carlo optimization of the models was carried out providing estimates of kinetic parameters of major reactions. Subsequent sensitivity analysis and statistical analysis provided further insight into parameter importance and reliability of the estimates. To reveal whether the metabolic machinery determining the concentration of particular cytokinin forms in A.t.
Col-0 is universal for the whole specie or whether the network of reactions is rather specific and might be a subject to adaptational changes, we have analysed distinct *Arabidopsis thaliana* ecotype Van-0 in order to obtain a comparison of kinetic parameter estimates of the major reactions of cytokinin metabolism between Col-0 and Van-0.

Supported by the Czech Science Foundation (16-14649S and 16-19557S).

Poster # 30 Conformational changes of DAD2 are an important mechanism in the signal reception of strigolactone Lee (Hormone perception)

Hui Wen Lee[1,2], Richard D Newcomb[1,2], Kimberley C Snowden[2]
[1]School of Biological Sciences, University of Auckland, [2]Plant and Food Research

The ability of plants to regulate development and architecture requires the coordination of various endogenous growth regulators (plant hormones) with responses to environmental stimuli. Strigolactones (SLs) are a class of plant hormones that regulate developmental growth through the modulation of shoot branching, root development, stem secondary growth, leaf senescence and flower development. DAD2, the petunia receptor for SLs, is an α/β-hydrolase fold protein with a canonical catalytic triad in the cavity active site. DAD2 hydrolyses SLs, undergoes a conformational change and interacts with its signal transduction partner PhMAX2A, a central component of SL signalling to inhibit branching in plants. There is little understanding on the exact role of DAD2 in this mechanism and how this mechanism leads to changes in axillary branching. Therefore, a mutagenesis approach was used to uncouple the hydrolytic activity of DAD2 from its ability to interact with PhMAX2A and to determine the role of DAD2 conformational change and enzyme activity in SL signal transduction. Random mutagenesis was used to identify DAD2 mutants that are able to interact with PhMAX2A in the absence of SL. Site-specific mutations were generated for various conserved amino acid residues predicted to be important for conformational shifts and/or interaction with PhMAX2A, and/or catalysis. Data will be presented from yeast two-hybrid assays to investigate the interaction between the DAD2 mutants and PhMAX2A. The DAD2 mutants were expressed, purified and their biological function tested *in vitro*. Subsequent experiments are in progress to elucidate the biological activity of these DAD2 mutants in the inhibition of axillary branching in plants.

Poster # 31 Control of extra- and intracellular cytokinin levels in cyanobacteria and algae (Hormone metabolism)

Eva Žižková[1], Martin Kubeš[1,2], Pavel Přibyl[3], Jan Šimura[2], Klára Hoyerová[1], Lenka Záveská Drábková[3], Ondřej Novák[2,1], Petre Dobre[1], Václav Motyka[1]
[1]Institute of Experimental Botany CAS, [2]Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, [3]Institute of Botany CAS

Cytokinins (CKs) are plant hormones essential during plant lifespan, e.g. in processes of cell division, embryogenesis, *de novo* organogenesis and senescence. In higher plants, bioactive CK pool is controlled through the metabolic pathways including biosynthesis, translocation, metabolic interconversions, inactivation and degradation. For non-vascular plants, however, metabolic data for CKs are rather scarce.
A comprehensive screen of endogenous CK metabolites in cyanobacteria and algae revealed substantially different metabolic profiles in comparison to higher plants. The prevalence of bioactive and phosphate CK forms together with only inconsiderable or even missing storage and inactive CKs, *O*- and *N*-glucosides, in selected cyanobacteria and algae strains arose the question of regulation of CK homeostasis in lower plants. Additionally,

detection of CK 2-methylthioderivatives at substantial concentrations suported the idea of their involvement in CK homeostasis control in non-vascular plants as well (Žižková *et al*., submitted).
Futher, we followed metabolization of unlabelled and radiolabelled CK *trans*-zeatin (*trans*Z) in cell suspensions of four cyanobacteria and algae species, *Chroococus minutus*, *Chlorococcum elbense*, *Klebsormidium flaccidum* and *Scenedesmus obliquus*. Generally, the treatments with both unlabelled and radiolabelled *trans*Z revealed its relatively intense metabolization with increased formation of several well-known as well as some unknown CK forms. Interestingly, no accrue of CK *O*- and *N*-glucosides and no activity of CK oxidase/dehydrogenase, the enzyme catalyzing CK degradation in higher plants, were found. Our data indicate a different strategy for CK homeostasis control between lower and higher plants. Possibilities of CK pool regulation in evolutionary older organisms will be discussed.

Supported by the Czech Science Foundation (16-14649S and 15-22322S), the Postdoctoral Fellowship from the Czech Academy of Sciences (EZ, MK), the Ministry of Education, Youth and Sports of the Czech Republic (LO1204, LD15137) and the long-term research development project (RVO 67985939).

Poster # 32 Cross-talk between brassinosteroid and ethylene: regulation of sugarcane ACC synthase in response to brassinosteroid Kieber (Hormone Interactions)

Jose Sergio Soares[1], Eduardo Purgatto[2], Joseph Kieber[3], and Marcelo Menossi[1]
[1]Departamento de Genética, Evolução e Bioagentes, Universidade Estadual de Campinas, [2]Departamento de Alimentos e Nutrição Experimental, Universidade de Sao Paulo, [3]Department of Biology, University of North Carolina at Chapel Hill

Sugarcane (*Saccharum hybrids*) is a very efficient biomass producer and has the capacity to accumulate high concentrations of sucrose in the stem. Ethylene is a phytohormone that plays an important role in the regulation of growth and sucrose accumulation in sugarcane. The rate limiting step of ethylene biosynthesis is mediated by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACS). In several hormone biosynthesis and signaling pathways, protein turnover has emerged as a common regulatory element. These proteins may function as cross-talk points, interconnecting hormone signaling pathway with molecular mechanisms regulating various aspects of plant physiology. Brassinosteroids (BR) are a class of steroid hormone that function as a master regulators of plant growth and belong to a group of factors influencing ethylene biosynthesis through the regulation of ACS protein stability. In this research project we will investigate the effects of brassinosteroid in ethylene biosynthesis via sugarcane ACS regulation. In our first results, etiolated rice wild-type seedlings and *in vitro* wild-type sugarcane plants showed an increase in ethylene production in the presence of brassinolide (BL). And to determine if ethylene production by BR is the result of an increase in ACSs stability we have been using rice protoplasts to measure the stability of HA-tagged ACS under the control of the ubiquitin promoter in the presence of BL. The elucidation of ACS regulation mechanism via brassinosteroid signaling will provide significant knowledge not only in sugarcane growth and ripening, but also for plant in general.

Keywords: Protein turnover, sugarcane, ethylene, brassinosteroid, ACC synthase, plant growth, ripening.

Poster # 33 Cytokinin hypersignaling reprograms maize leaf patterning (Vegetative development)

Michael Muszynski[1], Sivanandan Chudalayandi[2], James Cahill[1], Anna Rogers[2], and Michael Scanlon[3]
[1]University of Hawaii at Manoa, [2]Iowa State University, [3]Cornell University

Organized, patterned growth is essential for normal multicellular development. We are using the maize leaf as a model to understand the molecular signals contributing to pattern formation. Our study of the semi-dominant, leaf patterning maize mutant *Hairy Sheath Frayed1* (*Hsf1*) indicates cytokinin (CK) is one of those signals. In *Hsf1* leaves, the normal positioning of a proximal sheath separated from a distal blade by the auricle and ligule, is

altered, such that proximal tissues (sheath, auricle and ligule) also develop in the distal blade.
Analysis of *Hsf1* indicates specific missense mutations in the cytokinin receptor gene, *Zea mays Histidine Kinase1 (ZmHK1*), underlie this phenotype and result in CK hypersignaling in developing leaf primordia giving rise to these outgrowths with proximal identity we call prongs. To gain insight into how CK hypersignaling reprograms leaf patterning, we used laser microdissection RNA-Seq analysis on developing prongs. Transcriptomic analysis revealed about 900 differentially expressed (DE) genes in *Hsf1* prong margins compared with wild type margins. Among the DE genes, we find an over-representation of specific transcription factors and hormone pathway genes. This set of DE genes is remarkably similar to the suite of genes involved in organ formation. To test the idea that organogenesis genes contribute to prong formation, we have combined *Hsf1* with mutations in some of the DE genes and analyzed their epistatic interactions.
Double mutant plants showed enhancement of the *Hsf1* phenotype, confirming the predicted role the DE genes play in prong formation. These results suggest that CK hypersignaling in the distal blade triggers the misexpression of downstream pathway genes which specifies a new type of organ with proximal identity – the prong.

Poster # 34 Cytokinin production by *Methylobacterium* organophillum and their promoting effect on growth and physiology of soybean (*Glycine max*) (Biotic interaction)
Anna Kisiala[1] and Neil Emery[1]
[1]*Trent University*
Plant-symbiotic *Methylobacteria* possess the unusual ability to utilize one carbon metabolites (i.e methanol) as their sole energy source. To stimulate methanol production in the host plant, *Methylobacteria* secrete the phytohormones cytokinins (CKs). An extensive collection of *Methylobacterium* isolates from publically available depositories were screened using HPLC- MS/MS for their ability to produce CKs under *in vitro* growth conditions. Interestingly, analysis revealed significant differences between the *Methylobacterium* strains with regards to CK levels and types. Comparison of the CK hormone profiles from the different bacteria isolates suggested that a strain of *Methylobacterium organophillum* was the most likely to have a growth promoting effect on plants, and therefore, it was chosen to investigate its effect on soybean seedling in a greenhouse experiment.
Methylobacterium organophillum was introduced to soybeans using two methods of the inoculant delivery, of which the soil treatment was the more effective compared to seed imbibition prior to planting. To evaluate the growth promoting effect of *Methylobacterium*, 3-week-old seedlings of three tofu soybean lines were screened for important biometric and physiological parameters. Plants inoculated with *Methylobacterium* showed considerably higher shoot and root length, increased biomass production, larger leaf size, higher chlorophyll and carotenoids content, and enhanced photosynthesis and transpiration rate compared to non-treated controls. The enhanced growth seen in *Methylobacterium* treated plants is often attributed to increased CK levels in plant tissues. The results of our HPLC-MS/MS analysis support this proposal based on an elevated CK level detected in the leaves of bacteria inoculated soybean seedlings compared to control plants.

Poster # 35 Cytokinin Response Factors 1 & 2 Response to Abiotic Stress During Germination (Abiotic stress)
Erika Keshishian[1], Paul Cobine[1], Ondřej Novák[2], Lenka Plačková[2], and Aaron Rashotte[1]
[1]*Auburn University,* [2]*Palacký University & Institute of Experimental Botany*
Cytokinin is a plant hormone known to be involved in growth and development, regulating such processes as response to light, maintenance of apical dominance, and onset of senescence. A sub-family of the AP2/ERF transcription factors called Cytokinin Response Factors (CRFs) has been shown to be a side branch of the cytokinin signaling pathway and is conserved across all land plants. In addition to playing a role in the canonical cytokinin processes, CRFs seem to be linked to several different abiotic stress responses. In Arabidopsis, CRFs can be divided into four distinct phylogenetic clades that appear to have differing functional roles in cytokinin signaling and stress responses, including Clade I CRFs: CRF1 and CRF2. CRF1 and CRF2 are transcriptionally cytokinin-inducible genes that we have also shown are regulated in the presence of high salt and osmotic stress

conditions. Although many cytokinin signaling pathway genes including the CRFs are functionally redundant, we find differences in stress responsiveness between CRF1 and CRF2. Detailed examination of seedling growth and developmental parameters, as well as germination findings under different salt, osmotic, and cytokinin treatments will be presented. In addition, qPCR and promoter::GUS expression of both CRF1 and CRF2 in tissues will be shown in response to cytokinin and abiotic stress treatments, indicating unique profiles and potential different functional roles of these genes.

Poster # 36 Cytokinin–ethylene interaction in wood formation (Hormones & biotechnology)

Juha Immanen[1], Jorma Vahala[1], Airi Lamminmäki[1], Kaisa Nieminen[1], Ykä Helariutta[1], and Jaakko Kangasjärvi[1]
[1]*Department of Biosciences, University of Helsinki, Finland*

Plant hormones cytokinins and ethylene are important regulators of wood formation. We have shown that overexpression of cytokinin biosynthetic enzyme (Arabidopsis ISOPENTENYLTRANSFERASE 7, AtIPT7) under a wood-directed promoter in hybrid aspen (*Populus tremula* × *tremuloides*) resulted in increased stem growth rate under greenhouse conditions. We have also shown that exogenously applied ethylene (by closed flow-through cuvette system for two weeks) on an internode of wild type hybrid aspen clone stimulated stem diameter growth two-fold compared to control. In line with this, overexpression of specific *Populus* Ethylene Response Factors (ERFs) enhanced wood formation. Intriguingly, application of exogenous ethylene on the stem of AtIPT7 overexpressor with enhanced cytokinin biosynthesis stimulated stem diameter growth four-fold, which was substantially more than in ethylene-treated wild type. This indicates that ethylene and cytokinins can have a synergistic effect to stimulate wood formation, and that transgene technology may have potential to increase wood biomass production even more significantly by simultaneous overexpression of cytokinins and specific ERFs under wood-directed promoter. We have generated both single and double-transgenic hybrid aspen lines in which cytokinin biosynthesis and ethylene signaling are stimulated separately or together. The single transgenics are in a field experiment and the double transgenic lines are being tested under greenhouse conditions. *In vitro* observations suggest that root formation of the double-lines may be affected. This suggests that simultaneous enhancement of cytokinins and ethylene pathways affect the whole plant growth in more complex manner compared to the experiment where ethylene is exogenously applied merely on a stem of hybrid aspen. Additionally, modifications in cytokinins and ethylene pathways most likely have effects on balances of other hormones such as abscisic acid and auxin.

Poster # 37 Cytokinins in poplar (Hormone metabolism)

David Kopečný[1], Pavel Jaworek[2], David Zalabák[3], Ondrej Plíhal[3], and Petr Tarkowski[2,4]
[1]*Department of Protein Biochemistry and Proteomics, Palacký University Olomouc, Faculty of Science, Centre of the Region Hana for Biotechnological and Agricultural Research,* [2]*Central laboratories and Research Support, Palacký University Olomouc, Faculty of Science, Centre of the Region Hana for Biotechnological and Agricultural Research,* [3]*Department of Molecular Biology, Palacký University Olomouc, Faculty of Science, Centre of the Region Hana for Biotechnological and Agricultural Research,* [4]*Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Centre of the Region Haná for Biotechnological and Agricultural Research*

Cytokinins (CK) are phytohormones derived from adenine with fundamental role in the regulation of plant growth and development. They occur naturally either with isoprenoid or aromatic side chain, however the second ones are quite rare and less studied. We performed screening for aromatic CKs in 13 *populus* species by UHPLC-MS/MS. One of the few plants confirmed to contain aromatic CK is *Populus* x *canadensis* (cv. *Robusta*), the organism used as a model in presented work. Mass spectrometric data show that cell production of phenolics and aromatic cytokinins drops by 60% in 14 months of cultivation (t- zeatin/NAA). While the endogenous levels of other ARCKs show transient increase after daybreak, the levels of tRNA derived *o*-topolin remain unchanged. With the intention to explore possible link between biosynthesis of both CK subgroups, we cloned and sequenced all nine isopentenyl transferase genes (*IPTs*) found in the poplar genome and analyzed their expression by qPCR. For example,

transcripts of *IPT3*, *IPT5a* and *IPT5b* were more abundant in leaves than those of *IPT6a* and *IPT6b*. These genes code for the key enzymes of isoprenoid CK biosynthesis. To explore the poplar *IPTs* further, they were further subcloned into *Escherichia coli* expression vectors to obtain pure proteins for the kinetic measurements. All the genes were also subcloned into pMDC7 vector under estradiol inducible promoter for transformation of *A. thaliana*. The difference in *Arabidopsis* CK levels before and after induction should indicate whether the corresponding proteins are functional *in vivo*.

This work was supported by grant 15-16888S from the Czech Science Foundation.

Poster # 38 D6 PROTEIN KINASE is required at the plasma membrane to mediate PIN-dependent auxin transport (Hormone transport)

Inês C. R. Barbosa[1], Melina Zourelidou[1], Benjamin Weller[1], Ingo Heilmann[2] and Claus Schwechheimer[1]
[1]Technical University of Munich, [2]University Halle-Wittenberg,

Polar auxin transport through PIN (PIN-FORMED) auxin efflux carriers is essential for the spatio-temporal control of plant development. We have previously shown that auxin transport in *Arabidopsis thaliana* is dependent on the serine/threonine kinases of the D6PK (D6 PROTEIN KINASE) family. D6PKs are polarly localized at the plasma membrane of many cells where they co-localize with PINs. D6PKs activate PIN-mediated auxin efflux through phosphorylation at conserved serine residues in the PIN proteins. We have previously shown that D6PK is transported independently from PINs to the plasma membrane and that PIN phosphorylation at the plasma membrane is essential for PIN activity.

The mechanisms controlling D6PK association with the plasma membrane remained to be identified. Here, we show that D6PK binds polyacidic phospholipids *in vitro* and associates with the basal plasma membrane but also with root hair initiation sites in a PIP5K-dependent manner *in vivo*. We identify a motif in D6PK required for phospholipid binding, proper PIN3 phosphorylation and for auxin transport-dependent tropic growth. Thus, the phospholipid-dependent plasma membrane recruitment emerges as a mechanism for the control of D6PK and polar auxin transport in plants.

Using PIN1 phosphosite-specific antibodies, we examined the distribution of PIN1 phosphorylation at the plasma membrane and during trafficking. We can show that PIN1 phosphorylation and hence PIN1 activity is dependent on the presence of BFA- sensitive protein kinases at the plasma membrane. As opposed to established models, we do not find PIN1 phosphorylation to be a reliable indicator for PIN1 polar distribution. Based on our findings, we argue that the established models for PIN1 polarity control are insufficient.

Stanislas T, Hüser A, Barbosa ICR, Kiefer CS, Brackmann K, Pietra S, Gustavsson A, Zourelidou M, Schwechheimer C, Grebe M **Nature Plants** 2015
Barbosa IC, Schwechheimer C. **Curr Opin Plant Biol** 2014
Zourelidou M, Weller B, Barbosa IC, Willige BC, Fastner A, Streit V, Port SA, Colcombet J, de la Fuente van Bentem S, Hirt H, Kuster B, Schulze WX, Hammes UZ, Schwechheimer C. **Elife** 2014
Barbosa IC, Zourelidou M, Willige BC, Weller B, Schwechheimer C. **Dev Cell** 2014
Willige BC, Ahlers S, Zourelidou M, Barbosa IC, Demarsy E, Trevisan M, Davis PA, Roelfsema MR, Hangarter R, Fankhauser C, Schwechheimer C. **Plant Cell** 2013
Zourelidou M, Müller I, Willige BC, Nill C, Jikumaru Y, Li H, Schwechheimer C. **Development** 2009

Poster # 39 Deciphering the role of DELLA in sugarcane: physical interactions with PIF and EIN3/EIL1 proteins (Hormone signaling)

Rafael Tavares[1], Edgar Peiter[2], Prakash Lakshmanan[3], and Marcelo Menossi[1]
[1]*Functional Genome Laboratory, Department of Genetics, Evolution and Bioagents, Institute of Biology, State University of Campinas – UNICAMP,* [2]*Plant Nutrition Laboratory, Institute of Agricultural and Nutritional Sciences, Martin Luther University of Halle-Wittenberg,* [3]*Sugar Research Australia (SRA), Indooroopilly*

The worldwide growing demand for sustainable energy production has become one of the major challenges for agriculture in this century. Sugarcane has a high efficiency in fixing CO_2 into carbohydrates and is a promising source of biofuels. Despite the simple model of carbon partitioning in sugarcane, there is a lack in the understanding the molecular mechanisms of the source-sink regulation. Conversely, the use of plant growth regulators, such as gibberellin (GA) biosynthesis inhibitors and ethylene precursors (Ethrel) has been a common practice in sugarcane fields to promote growth arrest, sucrose accumulation, earlier maturation, and to delay flowering. Despite the opposing roles of ethylene and gibberellin in plant growth - ethylene inhibiting and gibberellin stimulating growth – there is one protein, DELLA, which may be a regulation hub between these two hormones. Furthermore, DELLA is also known to interact with PIF proteins, which mediate the growth dynamics in the diurnal light-dark cycle and are essential regulators for high temperature and sucrose-induced stem elongation in plants. In the present work we have cloned the sugarcane genes encoding DELLA, PIFs and EIN3/EIL1 proteins. Multiple sequence alignment allowed us to identify all conserved domains in the sugarcane sequences. By using a yeast two hybrid assay, we have identified interactions of ScRGA with ScPIF3/ScPIF4 and ScEIN3/ScEIL1. Interestingly, the DELLA domain from ScRGA also showed transactivation activity in this GAL4-based assay, as previously described for RGA and GAI from Arabidopsis. Using a bimolecular fluorescence complementation (BIFC) assay, these protein-protein interactions were confirmed *in planta* and localized in the cell nucleus. These results provide the first insights into the involvement of DELLA in the coordination of sugarcane growth.

Poster # 40 DELLA, IDD and SCL3 cooperate in the gibberellin feedback system (Hormone signaling)

Hideki Yoshida[1], Ko Hirano[1], Makoto Matsuoka[1], and Miyako Ueguchi-Tanaka[1]
[1]*Nagoya University*

DELLA protein is a key negative regulator of gibberellin (GA) signaling. Although possessing strong transactivation activity, DELLA lacks a DNA-binding domain. Therefore, a model has been proposed in which DELLA acts as a transcriptional coactivator with another transcription factor or factors containing a DNA-binding domain. Here, using yeast hybrid screenings, we identified five members of INDETERMINATE DOMAIN (IDD) protein family which bind physically to both DELLA and the promoter sequence of the GA-positive regulator *SCARECROW-LIKE 3* (*SCL3*), which previously was characterized as a DELLA direct target gene. Transient assays using Arabidopsis protoplasts demonstrated that a luciferase reporter controlled by the *SCL3* promoter was additively transactivated by REPRESSOR of ga1-3 (RGA) and IDDs. Phenotypic analysis of transgenic plants expressing AtIDD3 (one of the 16 IDDs in the *Arabidopsis* genome) fused with the plant-specific repression domain (SRDX) supported the possibility that AtIDD3 is positively involved in GA signaling. In addition, we found that SCL3 protein also interacts with IDDs, resulting in the suppression of its target gene expression. In this context, DELLA and SCL3 interact competitively with IDD proteins to regulate downstream gene expression. From these results, we propose that a coregulator exchange system between DELLA (as coactivator) and SCL3 (as corepressor) regulates the expression of their downstream targets to control the GA signaling pathway.

Poster # 41 DELLA-GAF1/IDD2 COMPLEX REGULATES GIBBERELLIN HOMEOSTASIS AND SIGNALING (Hormone signaling)

Jutarou Fukazawa[1], Takeshi Ito[1], and Yohsuke Takahashi[1]
[1]*Hiroshima University, Higashi-Hiroshima*

Gibberellins (GAs) are important phytohormone for plant growth and development. DELLA proteins act as

repressors of the GA signaling pathway. DELLAs are rapidly degraded in the presence of GA. Recently it have been reported that DELLAs inhibit transcription factors in several signaling through protein-protein interaction. Mechanism of GA-dependent transcription has been explained by the titration of transcriptional activators with DELLA and their release through degradation of DELLA in response to GA. However, the effect of GA on genome-wide expression is predominantly repression, suggesting the existence of unknown mechanisms. Recently we identified a DELLAs binding transcription factor, designated GAI-ASSOCIATED FACTOR1 (GAF1) and revealed a new role of DELLAs as transcriptional coactivator. GAF1/IDD2 also interacts with corepressor TOPLESS RELATED (TPR). DELLAs and TPR act as coactivator and corepressor of GAF1, respectively. To investigate the biological role of DELLAs binding to GAF1, we generated several transgenic plants. The overexpression of a mutant version of GAF1 that cannot bind DELLA in *ga1-3* partially rescue dwarf and low-germination phenotypes without affecting DELLA levels. The Arabidopsis plants that overexpressed GAF1 and mutant exhibited GA related phenotypes. These data indicates that target genes of GAF1 involved in plant growth and development. We have been searching for target genes of DELL-GAF1 complex.

Poster # 42 Design, synthesis and biological activity of new brassinosteroid analogues with phenyl group in the side chain (Hormone Interactions)

Miroslav Kvasnica[1], Jana Oklestkova[1], Vaclav Bazgier[1], Petra Korinkova[1], Jaromir Mikulik[1], and Jenny Russinova[2]
[1]Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR, [2]Department of Plant Systems Biology, VIB

Brassinosteroids (BRs) are polyhydroxylated steroidal plant hormones that play pivotal role in the regulation of various plant growth and development processes including cellular expansion and proliferation, vascular differentiation, fertility and senescence[1]. In addition to this, BRs have the ability to increase resistance to plants against various biotic and abiotic stresses[2]. These attributes make them good candidates for application in agriculture to improve growth and yield, but the major constraints to use BRs at large scale in the fields is their high cost. However, recent progress in the chemical synthesis of new biologically active brassinosteroids analogs leads to overcome this economically restriction.

We prepared a series of new brassinosteroid derivatives with *p*-substituted phenyl group in the side chain (Fig.1). Phenyl group was chosen based on successful molecular docking of designed structures into the active site of BRI1 using AutoDock Vina. Some compounds showed promisingly very strong interaction with BRI1 receptor. Plant biological activity was establish using the pea inhibition biotest and Arabidopsis root sensitivity assay and then was compared with natural brassinosteroids. Differences in the production of plant hormone ethylene were also observed in etiolated pea seedlings after treatment of brassinosteroid analogues.

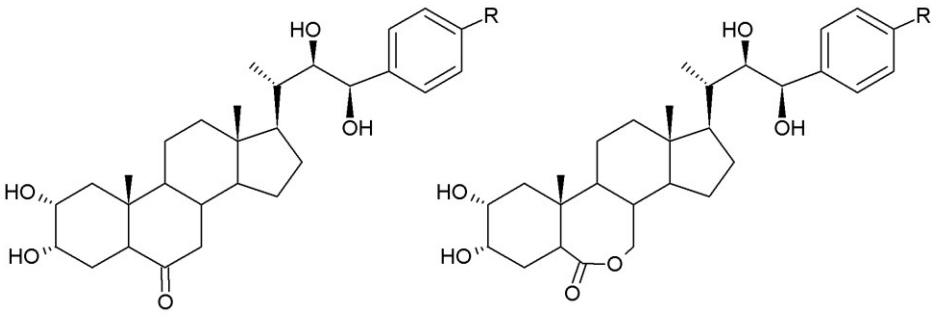

Fig.1.: General structures of new brassinosteroids analogues.

REFERENCES

(1) Oklestkova, J.; Rárová,L.; Kvasnica, M.; Strnad, M. *Phytochemistry Reviews.* **2015**, *14*, 1053-1072.
(2) Fariduddin, Q.; Yusuf, M.; Ahmad, I.; Ahmad, A. *Biologia Plantarum.* **2014**, *58*, 9-17.

This work was supported by projects of the Ministry of Education, Youth and Sports CR NPUI LO1204 and by grant GJ15-08202Y of the Grant Agency of the Czech Republic.

Poster # 43 Determining the mechanism of the type-A ARRs in the cytokinin signaling pathway in Arabidopsis thaliana (Hormone signaling)

Carly Shanks[1], Chia-Yi Cheng[2], and Joseph Kieber[1]
[1]University of North Carolina Chapel Hill, [2]J. Craig Venter Institute
Cytokinin is a plant hormone that regulates many aspects of growth and development, including meristem maintenance, senescence, and vascular development. Cytokinin is perceived by a receptor histidine kinase, which activates a multi-step phosphor-relay cascade. The type-A Arabidopsis response regulators (ARRs) are primary cytokinin response genes that are phosphorylated by the Arabidopsis histidine phosphotransfer (AHP) proteins and negatively regulate cytokinin signaling. The mechanism underlying the negative regulation of cytokinin signaling by the type-A ARRs is not clear, but likely involves interaction with target proteins, possibly in a phospho-dependent manner. A yeast two-hybrid screen using phosphomimic type-A ARRs as bait, we identified candidate type-A ARR interacting proteins. The goal of this study is to characterize the interaction of the type-A ARRs with these targets proteins. We used co- immunoprecipitation assays to determine the specificity of the interaction and the role of type-A ARR phosphorylation. To determine the function of the target proteins in cytokinin signaling, we examined mutants for the interacting proteins. We also explored the biological significance of the interaction by examining the type-A ARR effect on target protein stability, localization and activity.

Poster # 44 Development of compounds showing ethylene like activity (Chemical biology)

Masashi Suzuki[1], Yuko Kengoyama[1], Tsubasa Mizuno[1], Yusaku Imamura[1], Nobutaka Kitahata[1,2], Keiko Abe[1], Shinji Okada[1], and Tadao Asami[1]
[1]The University of Tokyo, [2]Tokyo University of Science
Ethylene is a phytohormone that promotes fruit maturation and leaf senescence, influences seedling morphogenesis, and induces germination of *Striga*, a root parasitic weed, but ethylene is not optimized for the use in agriculture because regulation of its concentration in the field is difficultdue to its diffusibility. Ethephon and ACC are used as ethylene generating agent and ethylene precursor. Since both of these compounds finally generate ethylene, these are not able to solve the problem. And these compounds are expensive. Thus, novel ethylene regulators are required to solve these problems. We have identified HJ2 showing ethylene like activity. Dark-grown Arabidopsis seedlings under HJ2 treatment indicated the triple response, a typical ethylene overproduced phenotype, whereas ethylene like activity of HJ2 is much less than that of ACC. We report here the activity improvement of HJ2 as a lead compound.
Arabidopsis was germinated in dark including structural derivatives of HJ2. In this bioassay, we found that KUT15 had more than four times higher ethylene like activity than HJ2. KUT15 induced the expression of ethylene responsive genes. Dark-grown wild type co-applied with KUT15 and an ethylene biosynthesis inhibitor showed triple response, suggesting that KUT15 was not activator of ethylene biosynthesis. On the contrary, dark-grown *ein2*, an ethylene insensitive mutant, applied only with KUT15 did not show triple response. From these, we assume that KUT15 is a positive regulator of an ethylene signal. KUT15 has a higher activity for inducing germination of *Striga* than ACC, suggesting that KUT15 may be used as a germination stimulant of *Striga*.

Poster # 45 Developmental changes in vegetative and reproductive growth in sunflower (*Helianthus annuus* L.) as influenced by exogenous application of plant growth hormones (Reproductive development)

NAGARATHNA, T.K[1], GAYITHRI, M[1], SHADAKSHARI, YG[1], MURALI, K[1] and PUNITH, H.V.[1]
[1]University of Agricultural Sciences, GKVK campus

Influence of manipulating vegetative growth on partitioning of photosynthates to seeds was studied in sunflower by manipulating hormones by exogenous application of plant growth regulators. The major production constraints in sunflower are poor seed filling and seed set, lack of uniformity in open pollinated varieties leading to production instability, excessive vegetative growth and lack of photosynthetic activity at the time of seed filling and improper translocation of photosynthates. To improve the productivity, translocation of photosynthates from vegetative organs to seed is very important. In our study, source size and sink capacity were manipulated by applying 240ppm Tri Iodo Benzoic Acid (TIBA) in combination with 120ppm of naphthalene acetic acid (NAA), 150ppm benzyle adenine (BA) and 3000ppm cycocel at start bud stage. In addition to this, higher dose of nitrogen, humic acid and micronutrient mixtures (0.3% zinc + 0.2% manganese + 0.5% boron + 0.5% iron) were also tried. Plants treated with cycocel, TIBA and NAA are anti gibberellin agents leads to deficiency of gibberellins in the plant and reduce the plant height by blocking the conversion of geranyl pyrophosphate to copalyl pyrophosphate in the gibberellins synthesis. However, they increased the rate of seed filling which in turn increased the productivity by 33 to 36 percent. The increased percent of filled seeds may be attributed to corresponding increase in translocation of photosynthates from source to sink during seed filling period. Application of cycocel significantly increased the seed yield by 40%. There was a significant reduction in plant height, which minimized the distance between the source and sink hence the rate of translocation was enhanced between source leaves and the sink. It also showed higher total dry matter (TDM) accumulation since it is found to increase RUBP carboxylase activity which in turn increases the rate of photosynthesis and dry matter partitioning. Overall result from this set of treatments indicated, that there is a possibility of increasing yield up to 40 per cent either by increasing source size or by manipulating sink capacity thereby improving translocation. When the source size was reduced by removing a few basal and middle leaves, at a critical stage of the crop (ray floret stage), yield was drastically affected due to reduction in the available functional leaf thus the photosynthetic rate. These results indicate a major role played by green leaves and their function in increasing productivity. Overall results from our study indicates that there is a possibility of increasing seed yield up to 40% either by increasing source size or by manipulating sink capacity thereby improving translocation by exogenously applying plant growth hormones.

Poster # 46 Diterpenoid Momilactones Exhibit Broad Range of Growth-Inhibitory Action upon Various Organisms (Biotic interaction)

Keisuke Tomita[1], Yasuhiro Matsuo[2], Makoto Kawamukai[2], Ken-ichiro Hayashi[3],
Hideaki Nojiri[1], and Kazunori Okada[1]
[1]Biotechnology Research Center, The University of Tokyo, [2]Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, [3]Department of Biochemistry, Okayama University of Science

Momilactones are allelopathic substances produced by some species of *Oryza* genus and the moss *Hypnum plumaeforme*. There has been a report that momilactone inhibits growth of some plants and mosses as an allelopathic substance under experimental condition, and that a rice knockout mutant defective in momilactone production causes decreased allelopathic activity to lowland weeds grown in rice paddies in natural environment. In addition, cytotoxic and antitumor activities of momilactone B against human cells have also been reported with induction of apoptosis and G1 arrest in the cell cycle. However, mechanisms how momilactones inhibit the growth of other plants or microorganisms are largely unknown.

In order to elucidate the mode of action of momilactones, we first tested sensitivity to momilactone B on some plant species and yeasts (fission yeast and budding yeast). The results showed that micromolar to submicromolar of momilactone B (0.5 ~ 2 μM) inhibited the growth of all organisms tested except for some *Poaceae* plants (rice and wheat) and budding yeast, suggesting that the action of momilactones has a broad species range, but there might be a resistance system to momilactones in the distinct organisms such as rice, wheat and budding yeast. As momilactone B arrests the cell cycle and induces apoptosis in mammalian cells, we speculate that a disorder of cell cycle, possibly due to G1 arrest, may also have taken place in such momilactone-sensitive organisms. To gain

insight into the resistance mechanism of momilactone B to these organisms, screenings of genetic factors that confer resistance to momilactones are being performed using rice FOX *Arabidopsis* lines, which overexpress about 4000 rice full-length cDNAs in *Arabidopsis*, and an *Schizosaccharomyces pombe* mutant library in which 3,400 loci are individually replaced with the G418 resistant marker.

Poster # 47 Effect of colonization with a bacterial endophyte on strigolactone signaling in rice (Biotic interaction)

Hideo Nakashita[1], Miyuki Kusajima[1], Takamasa Mori[1], Tadao Asami[2], and Hiromoto Yamakawa[3]
[1]*Fukui Prefectural University,* [2]*The University of Tokyo,* [3]*National Agriculture and Food Research Organization*

Plants are known to interact with various kinds of fungal and bacterial endophytes. Some endophytes have given the useful functions, such as growth promotion, disease resistance, and drought tolerance, to the host plant. *Azospirillum* sp., isolated from rice in Japan, can induce disease resistance in rice plants and promote plant growth.

Endophytic colonization of rice by *Azospirillum* sp. increased tiller number and yield. To elucidate the mechanism of plant growth promotion by *Azospirillum* sp., acceleration of tillering was characterized. The plastochron of the host plant was not influenced by colonization with *Azospirillum* sp., suggesting that the increase in tiller number is due to the tiller bud outgrowth that is inhibited by strigolactone (SL). The involvement of SL in the plant growth promoting effect of *Azospirillum*-colonization was examined by using synthetic SL biological analog 4-Br debranone (4BD).

One-weak-old rice seedlings (*Oryza sativa* cv. Nipponbare) were pretreated with *Azospirillum* (2x10^7 cfu/ml) 3 d before a soil drenching with 4BD (30 µM), and then tiller number, leaf number and height were measured. Treatment with 4BD suppressed the tillering in control plants, whereas it did not have any influence on the tillering in *Azospirillum-colonized* plants. These results suggested that signal perception of SL was influenced by the colonization with an endophytic bacterium *Azospirillum* sp. Further investigation for the molecular mechanisms is in progress.

Poster # 48 Effect of Compost and Hormones (Auxin and Gibberellin) on the Chlorophyll and phytochemical content of Jatropha (Hormones & biotechnology)

Idowu Odeleye[1]
[1]*University of Ibadan*

Due to effect of climate change on agricultural production and human health, alternative source of energy is now being sought globally. *Jatropha curcas* is one of the crops that are being promoted for production of biodiesel. The growth and yield of *Jatropha curcas* therefore needs to be enhanced. Effect of hormones and compost was evaluated on the growth and yield of *Jatropha curcas*. This experiment was carried out at the Department of Crop Protection and Environmental Biology, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria. The treatments were 0, 100 ppm, 150 ppm, 200ppm, of auxin and gibbrellin respectively with 10 t/ha, 20 t/ha and 40 t/ha of compost made from *Tithonia diversifolia* and poultry manure in ratio 3:1. The pots were laid out in a completely randomized design and replicated four times. Plants were sampled and assessed for growth and yield parameters. Statistical analysis of the results obtained was done using analysis of variance (ANOVA) and means separated using Student-Newman-Keuls (SNK) at $P<0.05$. The result of the experiment shows that gibberellins 150 ppm significantly increased the stem height, leaf area and other yield attributes of Jatropha. Likewise, auxin 150 ppm significantly increases the number of leaves, chlorophyll content of leaves and the root fresh and dry weight. Growth and yield of *Jatropha curcas* was improved by application of compost (20t/ha) though were not significantly different from control ($P<0.05$). Application of compost 20 t/ha combined with 150 ppm of either auxin or gibberellin can be recommended as adequate for optimum performance of *Jatroha curcas.*

Keywords: Auxin, Gibberellin, Jatropha, Compost, Yield

Poster # 49 Effect of transcription factor *OsHRT1* on the regulation of seed dormancy in rice (Reproductive development)

Heng Xu[1] and Ying Zhu[1]
[1]*Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences*

Pre-harvest sprouting (PHS) is an important agricultural trait in crop that leads to serious loss of yield and quality. Via RNA-seq we identified several endosperm-specific transcription factors responsive to high temperature during the grain-filling stage. Mutation in one of them, *oshrt*1 (high temperature responsive transcription factor 1) mutant, showed PHS under high humidity conditions before harvest. Compared with wild type, *α-amylose* genes, such as *Amy1A*, *Amy3C*, *Amy3D* were greatly upregulated in immature *oshrt1* seeds. *OsHRT1* gene expression was greatly induce by ABA treatment but was only slightly affected by GA treatment. *oshrt1* mutant seems more sensitive to ABA compared with wild type, and the ABA responsive genes, such as *LEA3*, *RAB21*, were reduced in *oshrt*1 mutant compared with the wild type under ABA treatment. Further data showed that the level of ABAin *oshrt1* mutant was higher than that in the wild type, which is most likely due to the feedback of inhibition of ABA signaling in *oshrt1*. Therefore, *oshrt*1 mutant significantly promoted pre-harvest sprouting by impairing abscisic acid (ABA) signal pathway and decreasing amylase genes expression. All aboved results suggested that *OsHRT1* may be involved in the regulation of seed dormancy through ABA signal pathway. Further study on *OsHRT1* and other key components in this process will help us to elucidate the molecular mechanism of seed dormancy and control the preharvest sprouting in rice.

Keywords: rice, seed dormancy, pre-harvest sprouting

Poster # 50 Effects of auxin, cytokinin and paclobutrazol on vegetative growth of *Solanum tuberosum* L. ' Tedjo MZ' cutting Kumala Dewi, Ummi Darrist (Vegetative development)

Kumala Dewi[1] and Ummi Mahmudah Darist[2]
[1]*Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan,* [2]*Alumni of Faculty of Biology, Universitas Gadjah Mada, Jl.Teknika Selatan*

The demand for good quality of seed potato (*Solanum tuberosum* L.) in Indonesia increases every year, however only several big companies and few farmers can produce it. This research was aimed to evaluate the vegetative growth of potato spout cutting treated with auxin, cytokinin and paclobutrazol. Potato tubers of variety 'Tedjo MZ' that have sprouting were selected and each sprout was cut aseptically and then soaked in auxin of 100 ppm for 15 minutes. Sprout cuttings were then planted in a plastic pot containing a mix of soil and compost (3: 1= v/v) and placed under a screen house. Cytokinin of 0 ppm (control), 25 ppm, 50 ppm or 100 ppm was applied at week 3 and 4 after sprouts cutting were planted. Paclobutrazol of 0 ppm (control) or 12.5 ppm was applied at week 6. Growth parameters observed were plant height, leaf number and leaf area, chlorophyll content, root number and root length, index stomata, activity of nitrate reductase and weight of new tubers. The results showed that auxin and cytokinin application of 25 ppom or 50 ppm increased plant height, leaf number leaf area and weight of new tuber. Additional of paclobutrazol reduced index stomata but it did not alter the weight of new tubers.

Keywords: Solanum tuberosum L. 'Tedjo MZ', auxin, cytokinin, paclobutrazol, sprout cutting, vegetative growth.

Poster # 51 Effects of Cytokinin on Adventitious Root Formation in *Arabidopsis thaliana* (Vegetative development)

Dennis Mathews[1] and Kaylyn Bergquist[1]
[1]*University of New Hampshire*

The formation of adventitious roots is part of the normal developmental process for some plants while for others it is only initiated in response to stress. The ability to generate rooted plants from stem or leaf cuttings is a valuable technique utilized in agriculture and horticulture. Application of exogenous cytokinin has been shown to inhibit primary root elongation as well as the formation of lateral and adventitious roots. This interaction has been studied extensively in primary and lateral roots but much less in adventitious roots.
We have examined the effect of cytokinin on formation of adventitious roots from the hypocotyl of root---excised Arabidopsis seedlings. Exogenous cytokinin application as well as plants containing mutations that alter cytokinin signaling were utilized to study the effects of this hormone on the developmental timing, initiation frequency and elongation rate of adventitious roots. Since it has been shown that auxin signaling and auxin transport contribute to adventitious root formation, we have also attempted to examine the effects that cytokinin may have on auxin signaling and/or transport during adventitious root formation.
Enhanced cytokinin signaling had the greatest impact on the number of adventitious roots initiated, which was reduced. Mutants with reduced cytokinin signaling were less sensitive to exogenous cytokinin. However, mutants severely deficient in cytokinin signaling were significantly delayed in adventitious root formation and exhibited altered locations of adventitious root emergence. These mutants also appeared to have reduced auxin signaling as evidenced by auxin reporter activity.

Poster # 52 EFFECTS OF INDOLE-3-ACETIC ACID ON GROWTH PARAMETERS OF CITRULLUS LANATUS (THUNBERG) MATSUM. AND NAKAI Moses Osawaru, (Vegetative development)

Moses Osawaru[1] and Matthew Ogwu[1,2]
[1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, [2]Keimyung University, Center for International Education and Development

Citrullus lanatus (Thunberg) Matsum. and Nakai of the family Cucurbitaceae, commonly referred to as watermelon is one of the most economically useful exotic fruit vegetables. It has numerous potentials in food security and poverty alleviation. Effects of Indole-3-Acid (IAA) on the plant height, leaf, branching and flower production were studied in order to evaluate the optimum concentrations for growth and development. The study was conducted at the Experimental Field of University of Benin, Nigeria using sun dried seeds from four varieties of the plant labelled A, B, C and D, collected from National Horticultural Research Institute, Nigeria based on morphological characteristics. The synthetic IAA was obtained from Nigerian Institute for Oil Palm Research and through serial dilution, five different concentrations were obtained; 1, 5, 10, 15 and 0 ppm IAA. Foliar application was done twice at two weeks interval from when plants were 2 WAP. Results for plant height showed that as IAA concentration increases plant height decreases. The optimum concentration suitable for plant height from the study was 5 ppm IAA for varieties B, C and D and 1 ppm IAA for variety A. Significant mean plant height were recorded 6 WAP (varieties B, C and D), 8 WAP (variety B) and 10 WAP (variety A). More so, 1 ppm IAA was observed as the optimum concentration for leaf production in variety A, 5 ppm IAA for variety C and 10 ppm IAA for varieties B and D respectively. Significant mean number of leaves were only obtained from variety A (4 and 12 WAP). Results obtained for number of branches suggest that 1 ppm IAA is optimum for variety A and 10 ppm is optimum for varieties B, C and D. Although flower production was poor during the study, high flower production were recorded for plants in 15 ppm IAA at 6 WAP for all the varieties. The mean values of number of branches and flowers were not significant for all the varieties. This work establishes the inducing effects of different IAA treatments thus the commercial production of this plant may be enhanced with IAA.

Keywords: Watermelon (*Citrullus lanatus*), Indole-3-Acetic acid, Plant growth and Development, Plant Physiology

Poster # 53 Effects of phosphate and other plant hormones on strigolactone production (Hormone Interactions)

Kaori Yoneyama[1], Koichi Yoneyama[1], and Takahito Nomura
[1]*Utsunomiya University*
Strigolactones (SLs) released from plant roots induce germination of root parasitic plants and hyphal branching of symbiotic arbuscular mycorrhizal fungi. In plants, SLs or their metabolites act as plant hormones regulating shoot and root architecture. Other functions of SLs as plant hormones have recently been reported. It is widely accepted that SL production is regulated by mineral nutrients especially phosphate (Pi). Our previous studies demonstrated that exogenously applied IAA to the shoot apices increased 5-deoxystrigol levels in sorghum root tissues under Pi deficiency but not under Pi fertilized. By contrast, exogenously applied cytokinin to the roots suppressed SL levels in rice root tissues. Effects of phosphate and other plant hormones on strigolactone production will be discussed in details in relation to endogenous plant hormone levels and expression levels of related genes.

Poster # 54 ELUCIDATING THE ROLE OF ETHYLENE IN MANGO MALFORMATION (Hormone Interactions)
Archana Singh[1], Vinai Kumar[1], Alok Shukla[1], Mohd.Wahid Ansari[2], Ramesh Pant[1] and Gurdeep Bains[1]
[1]*G.B.Pant University, Pantnagar,* [2]*Zakir Hussain Delhi College, University of Delhi*
Mango malformation a major disorder of mango causes heavy economic losses. Several claims have been put forth for the cause of malformation but the exact cause and control of the disorder remains unclear. The probable causes of malformation include both biotic and abiotic stresses. The tendency of biotic and abiotic stress to promote the production of ethylene by plant tissues has proven to be so universal that the term “stress ethylene” has been coined. Some of the symptoms of malformation such as leaf epinasty, suppression of apical dominance, disturbance in natural orientation of shoots and panicles, increased gummosis resemble the effects caused by increased ethylene. Therefore, stress induced ethylene may be the major etiologic agent causing malformation. The present investigation was carried out to elucidate the effect of ethrel (ethylene releasing compound) on morphological, physiological and biochemical aspects of mango malformation. Different concentrations of ethrel i.e. 100 ppm and 200 ppm were applied to different mango cultivars namely Amrapali, Dashehari, Mallika, Langra and Bombay Green. It was found that ethrel treatment decreased the number of hermaphrodite flowers, fruits, panicle length, yield, antioxidant activity and RWC but increased percent malformation,MDA, total phenols, proline, chlorophyll and carotenoid content as compared to control. In scanning electron micrographs it was observed that ethrel (200 ppm) inhibited opening of healthy buds and the healthy buds were less differentiated with fused sepals and petals. The healthy flowers treated with 200ppm ethrel showed poorly developed reproductive organs.

Keywords: Mango malformation, ethrel, ethylene.

Poster # 55 Endogenous auxin regulates region of the adventitious shoot formation on internodal segments in ipecac (Vegetative development)
Imari Koike[1], Koichiro Shimomura[1], Hiroyuki Kasahara[2], Ken-ichiro Hayashi3, and Mikihisa Umehara[1]
[1]*Graduate School of Life Sciences, Toyo University,* [2]*RIKEN CSRS,* [3]*Department of Biochemistry, Okayama University of Science*
A plant cell can divide, differentiate and regenerate to a whole plant even after their prior differentiation to specific cell type in a mature plant. Exogenously applied phytohormone stimulates adventitious shoot formation in most plant species. In ipecac (*Cephaelis ipecacuanha* A. Richard), however, adventitious shoots are formed on apical region of internodal segments without phytohormone treatment. In this work, we observed that the shoots were derived from epidermis cells of internodal segments without callusing and can regenerate complete plants. A vascular bundle of an adventitious shoot connected to that of an internodal segment, the shoot grew up vigorously. When the biggest shoot was removed, other shoots started to grow up as break the apical dominance. Thus, we analyzed indole-3-acetic acid (IAA) levels in internodal segments using LC/MS-MS to evaluate endogenous phytohormone dynamics during adventitious shoot formation. IAA was highly accumulated in basal

region of internodal segments. To investigate effects of auxin polar transport on adventitious shoot formation in ipecac, we applied auxin transport inhibitors, 2,3,5-triiodobenzoic acid (TIBA) and N-(1 naphthyl) phthalamic acid (NPA). IAA levels decreased in basal region of internodal segments, and adventitious shoots were observed on whole segments by TIBA treatment. Vascular bundles were not observed in the adventitious shoots on internodal segments treated with TIBA. Adventitious shoots were formed, but the results were confirmed by NPA treatment. Auxinole (α-[2,4-dimethylphenylethyl-2-oxo]-IAA), TIR1 auxin receptor antagonist, also induced adventitious shoot formation on wider range of the segments. These results suggest that auxin regulates region of the adventitious shoot formation on internodal segments and stimulate connection of a vascular bundle from an adventitious shoot to that of an internodal segment.

Poster # 56 Endogenous ent-kaurenoic acid-metabolite regulates the differentiation of *Physcomitrella patens* (Hormone metabolism)

Sho Miyazaki[1], Mariho Hara[1], Hiroshi Kawaide[2], Ken-ichiro Hayashi[3], Tadao Asami[1] and Masatoshi Nakajima[1]

[1]Department of Applied Biological Chemistry, The University of Tokyo, [2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology, [3]Department of Biochemistry, Okayama University of Science

Gibberellins (GAs) are a group of diterpene-type phytohormones biosynthesized from geranylgeranyl diphospate (GGDP) *via ent*-kaurenoic acid (KA). While a moss (*Physcomitrella patens*) has the partial biosynthetic pathway toward GA; only from GGDP to KA, any GA has not found in the moss. After our characterization of the related substances, it was elucidated that in the moss, an *ent*-kaurene synthase (PpCPS/KS) catalyzes the tandem metabolisms from GGDP to *ent*-kaurene *via ent*-CDP, and that an *ent*-kaurene oxidase catalyzes a metabolism from *ent*-kaurene to KA. In our previous studies with mutants, we showed that the loss of the PpCPS/KS function leads to the suppression of protonemal differentiation under the continuous red-light, and the phenotype was fully complemented by the application of either *ent*-kaurene or KA, but not by that of GA4. By using a bioassay system with the *Ppcps/ks* mutants, we identified the biologically-active metabolite(s) derived from KA. This suggests that the substance(s) should regulate the normal development of the moss. Its structural identification is now proceeding.

Poster # 57 Endogenous Phytohormone Accumulation in the Cecidogenic Insects of *Solidago Altissima* (Biotic interaction)

Peter Andreas[1] and Neil Emery[1]

[1]Trent University

Tall Goldenrod *(Solidago altissima)* is the host plant to several cecidogenetic insects that induce the development of plant galls. In order to understand the involvement of phytohormone metabolism in cecidogenetic behavior, several galling insects were evaluated in this work, namely: *Eurosta solidaginis, Gnorimoschema gallaesolidaginis, Rhopalomyia solidaginis* and *Epiblema scudderiana*. Levels of endogenous cytokinins (CKs) and abscisic acid (ABA) were measured in the host galls and in control plant tissues, larval insects, and mature adults using the technique of liquid-chromatography-electrospray ionization-tandem-mass-spectrometry (HPLC-(- ESI)MS/MS). Analysis of broad spectrum of CK forms revealed highly elevated levels of these phytohormones in larval tissue compared to other tissue types examined. The larval CK profiles obtained for *E. solidaginis* and *R. solidaginis* suggest dominance of de novo CK biosynthesis pathway (methylerythritol phosphate pathway) in these two species. The prevailing isoprenoid CK types found in larvae included both precursors and active forms such as t*rans*-Zeatin riboside-5'- monophosphate, trans-Zeatin riboside, *trans*-Zeatin, and storage forms such as t*rans*-Zeatin riboside-O-glucoside.

Poster # 58 Environmental regulation of the strigolactone pathway in petunia (Vegetative development)

Bart Janssen[1], Revel Drummond[1], Luke Luo[1], Susan Ledger[2], Carla Oplaat[3], and Kimberley Snowden[1]
[1]Plant & Food Research Institute of New Zealand, [2]University of Sydney, [3]ClearDetections NL

In response to changes in the environment plants are able to alter their development, such responsiveness has proven to be a successful evolutionary trait. We manipulated both light quality (the red: far red ratio) and phosphate availability in petunia such that the axillary bud at node seven varied from a deeply dormant state to a rapidly growing one. We measured phenotypic responses and quantified SL-related gene transcripts. Our results suggest that TCP type transcription factors play roles in the SL signaling pathway both before and after the reception of the SL signal at the bud. We also show that the abundance of the receptor transcript is regulated by the light quality such that axillary buds growing in added red light have greatly reduced receptor transcript abundance over those growing in far-red light. This suggests a mechanism whereby the impact of any SL signal reaching axillary bud seven is modulated by the responsiveness of these cells to the signal.

Poster # 59 Ethephon *versus* AVG response on sugarcane ripening: sucrose yield, growth, and hormonal status (Chemical biology)

Camila P. Cunha[1], Claudiana M. Santos[2], Marcelo A. Silva[3], and Marcelo Menossi[1]
[1]Universidade Estadual de Campinas (UNICAMP), [2]Universidade Federal de Alagoas (UFAL), [3]Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP)

The regulation of sucrose accumulation at higher rates in sugarcane culms has not been unraveled. However, several hormone-based growth regulators (ripeners) are used to manipulate sucrose yield and productivity; hence, representing potential tools to further our understanding of this complex process. Among those chemicals, ethylene is successfully applied to sugarcane fields, anticipating ripening and/or inhibiting flowering. In order to characterize the ethylene response on sucrose yield, growth, and hormonal status, we evaluated two contrasting sugarcane cultivars (early and mid-late maturing) subjected to ethephon (ethylene-releasing compound) and AVG (ethylene biosynthesis inhibitor) before the onset of ripening. The experiment was a randomized complete block design with six replicates, in which traits were evaluated in a time-series. The patterns of shoot growth and sugar accumulation were significantly altered upon ethephon treatment, probably contributing to high productivity at harvest (total recoverable sugar, up to 6% increase depending on the cultivar). On contrary, morphological changes were less pronounced in AVG-treated plants, although yield traits tended to be lower as the stationary phase of sucrose accumulation curve was reached faster. The early-maturing cultivar was prone to flowering due to the favorable climatic conditions: ethephon slightly repressed, while AVG significantly induced the emergence of inflorescences. Additionally, senescence-related hormones, such as cytokinin and abscisic acid (ABA), were induced in at least one time-point in leaves; whereas, auxin (IAA) was repressed by ethylene in both leaf and culm tissues. For those hormones, the late-maturing cultivar showed an opposite pattern when exposed to AVG. Besides, ethephon-induced changes in salicylic acid (SA, leaf) and ABA (internode) showed a distinct pattern between cultivars. Our results demonstrate that AVG antagonizes the typical ethephon response on sucrose yield and flowering, including changes in the hormonal status probably interfering with growth and regulation of sugarcane ripening.

Poster # 60 Evaluating the target selectivity of two new auxin herbicides (Hormone perception)

Justyna Prusinska[1], Mussa Quareshy[1], Veselina Uzunova[1], Jared Bell[2], Paul Schmitzer[2], and Richard Napier[1]
[1]University of Warwick, [2]Dow AgroSciences LLC

Auxins are the basis of one of the most successful classes of herbicide with a commercial history dating back 50 years. The family of auxinic herbicides is broadly---based, is variously selective, has a number of chemical scaffolds and incidences of tolerance have not been widespread or pervasive. Hence there is an interest in novel, commercially relevant actives. The target site for auxins, the auxin receptor known as TIR1, was identified in 2005 and its crystal structure was solved in 2007. Following these discoveries, we are expressing and purifying

AtTIR1 and other members of the TIR1 family as the basis of compound activity screens, especially AtAFB5. We are also using structure---led strategies to describe in detail the binding sites and the mechanism of selectivity for auxins. Details of our assays will be presented along with a mode---of---action analysis of new arylpicolinates from Dow AgroSciences. The results will demonstrate our progress towards novel chemistries and the structural basis of selectivity.

Poster # 61 Evolution of biosynthetic gene clusters of diterpene phytoalexins in rice (Biotic interaction)

Tomonobu Toyomasu[1], Koji Miyamoto[2], Matthew R. Shenton[3], Morifumi Hasegawa[4], Hiroshi Kawaide[5], Wataru Mitsuhashi[1], Hisakazu Yamane[2], Nori Kurata[3] and Kazunori Okada[6]

[1]Faculty of Agriculture, Yamagata University, [2]Department of Bioscience, Teikyo University, [3]Plant Genetics Laboratory, National Institute of Genetics, [4]College of Agriculture, Ibaraki University, [5]Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, [6]Biotechnology Research Center, The University of Tokyo

Plants frequently possess operon-like gene clusters for specialized metabolism. Cultivated rice *Oryza sativa* produces antimicrobial diterpene phytoalexins represented by phytocassanes and momilactones, and the majority of their biosynthetic genes are clustered on chromosomes 2 and 4, respectively. These labdane-related diterpene phytoalexins are biosynthesized from geranylgeranyl diphosphate via *ent*-copalyl diphosphate or *syn*-copalyl diphosphate. The two gene clusters consist of genes encoding diterpene synthases (CPS and KSL) and chemical-modification enzymes including P450s (CYP76M, CYP71Z, CYP99A, MAS). In contrast, biosynthetic genes of gibberellins, which are labdane-related phytohormones, are scattered throughout the rice genome similar to other plant genomes. The mechanism of operon-like gene cluster formation remains undefined despite previous studies in other plant species. Here we show evolutionary insight into the rice two gene clusters by comparison with wild *Oryza* species. Comparative genomics and biochemical studies using AA genome lineage wild rice species, including *O. barthii*, *O. glumaepatula*, *O. meridionalis* and *O. rufipogon* (the progenitor of Asian cultivated rice) indicate that gene clustering for biosynthesis of momilactones and phytocassanes had already been accomplished before rice domestication. The same strategy using the BB genome lineage species *O. punctata*, the distant FF genome lineage *O. brachyantha* and an outgroup species *Leersia perrieri* suggest that prototype of the phytocassane biosynthetic gene cluster on chromosomes 2 was already present in the common ancestor of the *Oryza* species. On the other hand, the momilactone biosynthetic gene cluster on chromosomes 4 evolved within *Oryza* before the divergence of the BB genome via assembly of ancestral genes of the cluster member genes.

Poster # 62 Exogenous nitric oxide mitigates paraquat toxicity in Brassica *napus* L. seedlings through the modulation of antioxidant defense and glyoxalase systems (Abiotic stress)

Mirza Hasanuzzaman[1,2] , Masayuki Fujita3 and Hirosuke Oku[1]

[1]Molecular Biotechnology Group, Center of Molecular Biosciences (COMB), Tropical Biosphere Research Center, University of the Ryukyus, [2]Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, [3]Laboratory of Plant Stress Responses, Department of Applied Biological Science, Faculty of Agriculture,Kagawa University

Nitric oxide (NO) is an important signaling molecule with diverse physiological and biochemical functions including protection against abiotic stresses in plants. To investigate the possible regulatory role of NO in relation to the changes in ascorbate (AsA), glutathione (GSH) levels as well as the activities of antioxidant and glyoxalase pathway enzymes, the rapeseed (*Brassica napus*) seedlings were grown in semi-hydroponic medium. A set of 10- days-old seedlings was pretreated with 500 µM sodium nitroprusside (SNP – a NO donor) for 24 h. Three doses of paraquat (62.5, 125 and 250 µM) were imposed separately as well as in SNP-pretreated seedlings and grown further for 48 h. Paraquat resulted in oxidative stress as evidenced by increased lipid peroxidation (MDA, malondialdehyde), H_2O_2 content $^{\bullet-}$ and O_2 generation rate, higher lipoxygenase (LOX) activity and MG

level. Paraquat toxicity decreased plant biomass, leaf chlorophyll (chl) and relative water content (RWC); increased proline (Pro); decreased ascorbate (AsA); increased endogenous GSH and glutathione disulfide (GSSG) content; increased the GSH/GSSG ratio; increased ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), superoxide dismutase (SOD) and glutathione *S*-transferase (GST) activities; decreased activities of dehydroascorbate reductase (DHAR) and catalase (CAT). Activities of glyoxalase I (Gly I) was not changed but glyoxalase II (Gly II) increased due to paraquat. Compared to paraquat alone, exogenous SNP supplementation with paraquat reduced LOX activity, improved chl, endogenous GSH, GSH/GSSG ratio and activities of SOD, APX, MDHAR, DHAR, GR, GPX, Gly I and Gly II which helped to reduce oxidative stress and MG toxicity. Exogenous SNP had little effect in combination with higher dose of paraquat. These results indicate that the exogenous application of NO donor increased the tolerance of the rapeseed plants to paraquat-induced oxidative damages by enhancing their antioxidant defense and glyoxalase systems.

Poster # 63 Exploring regulatory network of rice tiller angle through dynamic transcriptome analysis of shoot gravitropism (Hormone genomics)

Ning Zhang[1]
[1]*Chinese Academy of Sciences*

Poster # 64 Exposure brassinosteroid and brassinosteroid mimics continually improve photosynthesis in rice subjected to heat stress (Abiotic stress)

Weerasin Sonjaroon[1], Jutiporn Thussagunpanit[1], Kanapol Jutamanee[1], Lily Kaveeta[1], Ornusa Khamsuk[1] and Apichart Suksamrarn[2]
[1]*Department of Botany, Faculty of Science, Kasetsart University,* [2]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University*

Until now, the role of brassinosteroids have studied on physiological processes, especially effect to photosynthesis in plants, also could alleviate effect of abiotic and biotic stress. Nevertheless, the extraction of natural BRs from explants mostly produces low quantities, difficult to isolate and too expensive for large-scale applications. To solve this problem, our group looked for the new chemical substances which were structurally and functionally similar to brassinosteroids. The brassinosteroid mimics are 7,8-dihydro-8α-20- hydroxyecdysone (DHECD) and 7,8-dihydro-5α,8α-20-hydroxyecdysone (αDHECD). To investigate application of brassinosteroids and brassinosteroid mimics on photosynthesis in rice subjected to heat stress. In this experiment, we continually applied water, EBR, DHECD and αDHECD on rice leaves at the reproductive growth stage every other day for 4 times.
Then, rice was exposed to two different temperatures, which were 30/26˚C day/night as normal temperature and 40/30 ˚C day/night as high temperature, in a greenhouse for 7 days. EBR, DHECD and αDHECD were steadily effective in increasing the net photosynthetic rate, stomatal conductance, Intercellular CO_2 concentration and transpiration rate at normal temperature from 0 day to 7days of experiment. Under heat stress, EBR and DHECD steadily enhanced the net photosynthetic rate, stomatal conductance, Intercellular CO_2 concentration and transpiration rate. While, αDHECD was the best in supplement the net photosynthetic rate in rice at heat stress. The results indicated that the foliar application of EBR, DHECD and αDHECD during reproductive growth could enhance photosynthetic performance under high temperature stress. Furthermore, DHECD and αDHECD, a BR mimic were a good candidate for improving photosynthesis on rice under heat stress similar as EBR, a synthetic BR.

Keywords: epibrassinolide, 7,8-dihydro-8α-20-hydroxyecdysone, 7,8-dihydro-5α,8α-20- hydroxyecdysone, stomatal factor, high temperature

Poster # 65 Expression patterns of gibberellin and jasmonate signaling genes during wheat seed germination (Hormone signaling)

Nguyen Nguyen-Tran[1] and Belay Ayele[1]
[1]*University of Manitoba*

Seed dormancy and germination are regulated by several plant hormones including abscisic acid (ABA), gibberellins (GA), ethylene and jasmonates and their interaction as well. To elucidate the role of GA and jasmonate signaling in the dormancy and germination of wheat seeds, the transcript levels of genes encoding GA and jasmonate (JA) signaling was measured in dormant and non-dormant wheat seeds before and after imbibition. The GA signaling genes including *reduced height* (*RHT*), *spindly* (*SPY*) and *GA-regulated MYB transcription factor* (*GAMYB*) exhibited no differential expression between dormant and non-dormant seeds in both dry and imbibed states, implying their regulation at post-transcriptional level. In contrast, the expression of *GID* tended to be lower in non-dormant seeds than dormant seeds during imbibition, suggesting the independence of GA signaling on GID level. The expressions of JA signaling genes including *CORONTAINE INSENSITIVE* (*COI*), *MITOGEN ACTIVTED PORTEIN KINASE6* (*MPK*) and *MYC TRANSCRIPTION FACTOR* (*MYC*) were compared between dormant and non-dormant seeds; similar expression pattern was observed for *COI* and *MYC* genes between the dormant and non-dormant dry and imbibed seeds. A specific gene encoding MPK, which acts as a negative regulator in JA signaling, exhibited downregulation in imbibed non-dormant seeds, suggesting its role in seed dormancy regulation.

Poster # 66 F-Box Protein as Signal Transducer of Plant Growth Regulators - A Review (Hormone signaling)

Nairanjana Bera[1], Sukumar Sarkar[2] and Ananya Bagchi[3]
[1]*Food Corporation of India,* [2]*Hooghly Mohsin College,* [3]*Agricultural and Food Engineering Department*

F-box proteins (FBPs), the largest class of ubiquitin ligases, represent one of the fastest evolving gene/protein families in the plant kingdom as intracellular signal transducer by recruiting themselves in crucial corners during development of plant life and their interaction with surrounding ecosystems. The FBP superfamily could be separated into several subfamilies characterized by different C-terminal protein-protein interaction domains originated by a major wave of expansion and thereby selection in nature. Currently, many F-box proteins have been identified in plants which are controlling the crucial processes in different stages of plant life cycle starting from embryo development throughout seedling development, root formation, floral organogenesis and finally during senescence. In addition, FBPs play crucial role in expression of pathogenicity of fungal pathogen, as well as in signal transduction during functioning of plant growth regulators. In this regard, attempt of a thorough scrutiny reveals the interaction of FBPs on plant-environment interaction along with their specific activities for example TIR1 for Auxin, SLEEPY1 and SNEEZY in Gibberellin signaling, EBF1 and EBF2 for ethylene, COII for jasmonates, DOR for abscisic acid and MAX2 for Strigolactone and Karrikin signaling.

Keywords: F-box proteins, signal transducer, TIR1, SLEEPY1, SNEEZY, EBF1, EBF2, COII, DOR, MAX2

Poster # 67 Feedback regulation of YUCCA gene expression in Auxin biosynthesis through SCF$^{TIR1/AFB}$ complex (Hormone signaling)

Shin Takato[1], Akiko Sato[1], Masashi Suzuki[1], Yusuke Kakei[1], Ken-ichiro Hayashi[2], Ayako Nakamura[1], Kazuo Soeno[3], and Yukihisa Shimada[1]
[1]*Kihara Institute of Biology, Yokohama City University,* [2]*Department of Biochemistry, Okayama University of Science,* [3]*NARO/WARC*

Indole-3-acetic acid (IAA) is the most abundant auxin, which is biosynthesized from Tryptophan (Trp). Endogenous level of IAA must be precisely regulated to maintainplantgrowthanddevelopment.In*Arabidopsis thaliana,*Indole-3-pyruvic acid (IPyA) pathway was proposed as a major auxin biosynthesis pathway. In this pathway, Trp aminotransferase *(TAA1/TARs)* converts Trp to IPyA, and *YUCCA (YUC)* catalyzes the conversion

from IPyA to IAA. We have previously demonstrated that exogenous application of auxin to *Arabidopsis* seedlings resulted indown-regulation of expression of auxin biosynthesis genes, in a feedback manner. In this study, we investigated signal transduction pathway to regulate *YUC* gene family expression in response to auxin and the signal transduction inhibitor. Exogenous application of α-(phenylethyl-2-one)-indole-3-acetic acid (PEO-IAA), an inhibitor of *Transport Inhibitor Response 1 (TIR1)* to *Arabidopsis* seedlings resulted in up-regulation of *YUC* gene expression. We also investigated endogenous IAA levels of auxin-signal transduction mutants and PEO-IAA-treated wild-type seedlings. Increased levels of endogenous IAA as compared to the wild type were observed in both experiments. These results suggest that the local homeostasis of endogenous IAA level is regulated in a feedback regulatory manner, and Aux/IAA and $SCF^{TIR1/AFB}$ complex mediate this regulation.

Poster # 68 Functional analysis of CLE16 and CLE17 in Arabidopsis using the CRISPR/Cas9 system (Hormone signaling)

Chie Shimaoka[1], Yasuka Yamaguchi[1], Shinichiro Sawa[1], and Takashi Ishida[1]
[1]*Graduate School of Science and Technology, Kumamoto University*

Intercellular signaling contributes to the various aspects of plant development. But the details of these biological relevance don't be clear. To reveal them, we focus on CLE collected as a homolog of CLV3. Arabidopsis has 32 kinds of them. They have highly conserved CLE domain near the C-terminus, and encodes peptides that produce a final ligand consisted of 12 amino acids, followed by the initiation of signaling cascades.
In this study, we try to generate mutants of CLE which expresses at the root tips, especially CLE16 and CLE17. Now, there is no data reported to success generating the mutant of Columbia. For a method, we use CRISPR/Cas9 system. As the results, we could success to induce error and got mutant inserted a base pair by transformed using induced-vector. Moreover, we got double homozygous mutants. Also we are going to study how affect synthetic peptide to a living plants.

Poster # 69 Functional Analysis of Cytokinin-Dependent Targets of Cytokinin Response Factor 6 (Hormone signaling)

Ariel M. Hughes[1], Alyssa M. Parish[1], Paul J. Zwack[2], Paul A. Cobine[1], and Aaron M. Rashotte[1]
[1]Auburn University, [2]Duke University

Cytokinin is a plant hormone essential to plant growth and response. Cytokinin Response Factor 6 (CRF6) is a cytokinin responsive AP2/ERF transcription factor known to be involved in regulation of several cytokinin controlled processes such as senescence with additional links to abiotic stress. Recent work studying CRF6 function has been focused on its regulation by oxidative stress pathways, yet less is known about its functions in a cytokinin related context.
Here we provide the first identification of downstream targets genes of CRF6. To do this we performed transcriptome analyses (using Affymetrix ST1.0 Arabidopsis microarrays) in the presence and absence of cytokinin on WT and a *crf6* mutant, as well as a CRF6OE (overexpression line). Genes lacking differential expression (DE) in the *crf6* background in the presence of cytokinin treatment were identified as potential CRF6-dependent cytokinin regulated targets. The majority of CRF6-dependent genes are repression by cytokinin, suggesting that CRF6 functions as a negative regulator of cytokinin response as previously predicted. A subset of these targets was selected for further phenotype analysis and verification by qPCR. While CRF6-dependent target gene function appears somewhat varied, several members show an interconnected relationship to potassium (K^+). Potassium transport/movement has been linked to plant response to ROS damage from abiotic stresses and CRF6 has similar links. We further investigated this connection between cytokinin regulation and *in planta* ionomic levels by using CRF6-target mutants (with reduced transcript levels, similar to CRF6OE). Inductively-Coupled Plasma: Optical Emission Spectrophotometry (ICP-OES) analysis will be paired with examination of cytokinin phenotypes and transcripts in relation to CRF6 backgrounds to further show functional analysis of CRF6. This may suggest a mechanistic cytokinin action in response to abiotic stress, rather than a

feedback loop simply altering cytokinin levels or sensitivity that has previously been explored.

Poster # 70 Functional analysis of hormone transporting NRT1/PTR family (NPF) proteins (Hormone transport)

Takafumi Shimizu[1], Hidenori Matsui[1,2], Gang-Su Hyon[1], Hirofumi Nakagami[1], Mitsunori Seo[1]
[1]RIKEN Center for Sustainable Resource Science, [2]Graduate School of Environmental and Life Science, Okayama University

Elucidation of localization and transport of plant hormones is indispensable for better understanding of their physiological roles. For example, jasmonoyl-isoleucine (JA-Ile) that plays key roles in plant growth regulation and immunity is transiently accumulated at local stressed tissue, and then transported long-distance in plant. One possible strategy to reveal the mechanisms that regulate such plant hormone dynamics is identification and characterization of plant hormone transporters. We have established modified yeast two-hybrid systems with the JA-Ile receptor COI1 and JAZ, the abscisic acid (ABA) receptor PYR/PYL/RCAR and PP2C, and the gibberellin (GA) receptor GID1 and DELLA, to detect JA-Ile, ABA, and GA transport activities of proteins expressed in the yeast cells. By using this method, we found that relatively large numbers of Arabidopsis NRT1/PTR FAMILRY (NPF) proteins could transport JA-Ile, ABA, and/or GA. Currently, we are analyzing physiological functions of these plant hormone transporting NPFs. We found that mutants defective in one of the NPFs with JA-Ile transport activity were more susceptible to the plant pathogen *Hyaloperonospora arabidopsis* (*Hpa*) than the wild type. This suggests that the NPF might regulate disease resistance against *Hpa* through the transport of JA-Ile. On the other hand, mutants defective in one of the ABA- transporting NPFs exhibited higher leaf surface temperature compared to the wild type, suggesting that ABA transport by the NPF might be required for appropriate stomatal functions. We are now conducting more detailed analysis of the in vivo functions of the NPFs.

Poster # 71 Functional studies of brassinosteroid-induced anthocyanin accumulation in plant responses to low N stress (Abiotic stress)

Jian Liang[1] and Junxian He[1]
[1]School of Life Sciences, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong,

Nitrogen (N) is an important nutrient element of plants. Lacking N will affect plantgrowth even survival. To adapt to low N, plants have evolved a set of adaptive responses, such as deduction of growth, reduction of photosynthesis, and accumulation of anthocyanins.

Anthocyanins are a branch of flavonoids with protective effects to plants under certainoxidative stress conditions. For example, anthocyanins have been reported to scavenge ROS in plants grown under high light condition. Under nutrient stress conditions such as N & P depletion, plants also accumulate large amount of anthocyanins, however, the functions of the increased anthocyanin accumulation remain obscure.

Brassinosteroids (BRs) are steroidal phytohormones that play important roles in plant responses to different stresses. Recently, we found that BR could increase plant tolerance to low N stress and enhance low N-induced anthocyanin accumulation. These results suggest that BR may increase plant tolerance to low N partially through the enhanced anthocyaninaccumulation.

To understand whether and how anthocyanins increase plant tolerance to low N, we are investigating the effects of low N- and BR-induced anthocyanin accumulation on ROS production. First, we quantified the ROS production as well as enzymatic and non-enzymatic antioxidants in different BR and anthocyanin mutants and the preliminary results indicated that anthocyanins and BR treatment could compromise ROS production under low N condition. Next we will perform more molecular and biochemical studies to see whether BR and anthocyanins affect gene expression and signaling pathways related to low N stress.

This work was supported by the General Research Fund (CUHK Codes 464412 and 14121915) and an AoE grant (AoE/M-05/12) from the Research Grants Council (RGC) of Hong Kong, and the Direct Grants from the Chinese University of Hong Kong.

Poster # 72 Functions of carotenoid cleavage dioxygenase 8 in Micro-Tom (Hormone Interactions)

Shoko Hasegawa[1], Yoshihiro Okabe[2], Tohru Ariizumi[2], Hiroshi Ezura[2], and Mikihisa Umehara[3]
[1]Graduate School of Life Sciences, Toyo University, [2]Fac. Life Envir. Sci., University of Tsukuba, [3]Graduate School of Life Sciences, Toyo University, Dept. Appl. Biosci., Toyo University

Strigolactones (SL) not only induce germination of root parasitic plants but also inhibit shoot branching. Shoot branching is an important trait that affects number and quality of fruits. Infection of root parasitic plants (*Orobanche aegyptiaca*) causes agricultural damage in tomato (*Solanum lycoperiscum* L.) cultivation in Europe. Thus, tomato cultivars are expected to be resistant to root parasitic plants. If fruits of SL defective mutants show quantity and quality like that of wild type (WT), it is useful for breeding of resistant plants to *Orobanche aegyptiaca*, and contributes to solve agricultural damage. In the past study, carotenoid cleavage dioxygenase 8 (*Slccd8*) defective mutants were found in Micro-Tom by targeting induced local lesions in genomes (TILLING) method. *Slccd8*-defective mutants showed excess branching phenotype and exogenously applied SL suppressed the excessive shoot branching. Next, to examine whether the branching was inhibited by endogenous SLs, we performed grafting experiments between a WT and a *Slccd8* mutant. Shoot scions of *Slccd8* mutants grafted onto WT rootstocks restored number of branching in shoot scions of *Slccd8* mutants. We quantified orobanchol levels in root exudates and roots using LC-MS/MS. In addition, we performed germination assay using a root parasitic plant, *Orobanche minor*. Orobanchol was not detected in *Slccd8* mutants and *Slccd8* mutant root exudates did not induced the germination, indicating that SL levels were very low in *Slccd8* mutants. Furthermore, we investigated the number of fruits, fruit weight, fruit diameter, number of seeds, flowering time and Brix/acid ratio. There was no significant difference in the evaluated fruits traits between WT and *Slccd8* mutants, but flowering time of *Slccd8* mutants was slower than that of WT. *Slccd8* mutants might be useful as a new tomato line resistant to the parasitic plants.

Poster # 73 Fungal derived CKs and their role in the Ustilago *maydis*- *Zea mays* pathosystem. (Biotic interaction)

Erin Morrison[1], RJ Neil Emery[1], and Barry Saville[1]
[1]Trent University

Fungal derived phytohormones play a key role in regulating plant-pathogen interactions, however deciphering the separate contribution of the pathogen from the plant during infection has been difficult. Recent studies have examined the importance of key fungal CK genes and their role in certain plant-pathogen interactions. Here we examine the control and role of fungal CKs during the *Ustilago maydis-Zea mays* pathosystem. *U. maydis*, the causative agent of corn smut, is capable of producing CKs. During disease development dramatic tumours are formed on aerial portions of the corn plant composed of both fungal and plant tissue. The sole isopentenyltransferase (IPT) gene was identified in *U. maydis* and when deleted resulted in strains no longer capable of producing CKs. These deletion strains elicited fewer and smaller tumours than wild type infections. High performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI MS/MS) was used to detect and quantify phytohormone levels in infected tissue. This revealed that key hormone changes previously identified in wild type infection were not present in deletion strains; suggesting that CK production by *U. maydis* is required for the altered phytohormone profile in infected tissue relative to uninfected tissue. Mining the *U. maydis* genome also identified genes coding putative CK signaling and biosynthesis proteins. A model is presented illustrating how *U. maydis* CK production may impact tumour development.

Poster # 74 GA-3 signaling: ubiquitin-proteasome system allows plant growth through breakdown of DELLA proteins (Hormone signaling)

Je-an Cedric M. Cruz[1]
[1]*Department of Biology, Faculty of Science, York University*

The purpose of the study is to show the effectiveness of gibberrellic acid (GA-3) as a plant hormone. The main focus would be on the underlying biological or cellular mechanisms as to why it helps contribute to growth in plants. The study was done through soaking the *Phaseolus vulgaris* seeds in water for more than half a day. These seeds were planted in a mixture of soil and sand with water. The pots used were placed inside a plastic bag and stored in a warm dark place for four days. When the seeds began to grow, the plastic was removed and the pots were placed again in a warm sunny place. After another four days, the seeds grew taller. The five healthiest plants were selected for further observation using the plant growth hormone. These plants were labeled numerically from one to five and their initial stem measurements were written down. GA-3 was applied using a cotton swab to the stems and leaves of plants being placed in one pot while the other one remained treated with water. These pots were observed for another week and measurement of each was also done from first day to eighth day.

Results showed that that the plants were mostly influenced by GA-3 hormone. Evidently, stem elongation existed. Once this happens through GA-3 application, the plants would develop longer internodes than of the controls. DELLA proteins are components of GA signaling. These proteins are referred to as repressor of plant growth. The presence of GA makes the proteins to be degraded in order to allow growth in plants through 26S proteasome pathway and E3 ubiquitin ligase or collectively known as ubiquitin-proteasome system. More specifically, GA-GID1-DELLA complex forms in the existence of GA to GID binding. Thus, it represses the function of DELLAs.

Poster # 75 Genetic approaches to studying IPyA-independent routes of auxin biosynthesis. (Hormone metabolism)

Anna Stepanova[1], Javier Brumos[1], Jeonga Yun[1], David Bullock[1], and Jose Alonso[1]
[1]*Department of Plant and Microbial Biology, North Carolina State University*

Plant hormone auxin is a key growth regulator that controls numerous developmental processes throughout plant's life. While most of the bioactive auxin, indole-3-acetic acid (IAA), is synthesized from tryptophan (Trp) via indole-3- pyruvic acid (IPyA), other parallel IPyA-independent branches of auxin biosynthesis have been proposed to also contribute to the IAA pools. To elucidate the role of IPyA-independent routes of auxin production, we are taking advantage of an auxin- overproducing mutant *sur2* in which the flow of metabolites via IPyA-independent branches of the auxin pathway is dramatically enhanced. Using a combination of forward, reverse and chemical genetic approaches, we screened for phenotypic suppression of the high-auxin phenotypes of *sur2.* While reverse genetics failed to implicate any of the putative auxin biosynthesis genes in *sur2*-triggered IPyA- independent auxin production, screening of a chemical library led to the identification of over 30 compounds that reverse the high-auxin defects of *sur2*.

Forward mutant screens yielded several genetic suppressors, including four alleles of *rus1,* a mutant with a defect in a novel plastid envelope-localized protein. *rus1* mutant accumulates less Trp than wild-type plants and many of the *rus1* phenotypes are reversed upon tryptophan supplementation. Remarkably, the Trp biosynthetic machinery in this mutant is fully functional, but Trp biosynthesis is nevertheless dampened due to feedback inhibition of the rate-limiting step of the pathway by the end-product, Trp. We hypothesize that *rus1* is defective in the export of Trp from plastids, where Trp is produced, to the cytoplasm and the accumulating Trp feedback-inhibits further synthesis of this auxin precursor in the plastids, leading to the observed *rus1* Trp deficiency. To test this hypothesis, we are employing a FRET Trp sensor to evaluate Trp levels and distribution in *rus1*. Our studies are shedding fresh light on the roles of Trp and IPyA-independent auxin biosynthesis in generating adequate pools of IAA.

Poster # 76 Gibberellin 3-oxidase 1 is essential for pollen maturation in Rice (Hormone metabolism)

Kyosuke Kawai[1], Sayaka Takehara[1], Makoto Matsuoka[1], and Miyako Ueguchi (Tanaka)[1]
[1]*Nagoya University*

Gibberellins (GAs) are essential for regulation many developmental processes in plants including seed germination, stem elongation, pollen maturation and the induction of flowering. GA3-oxidase (GA3ox) is a key enzyme in GA biosynthesis and catalyzes the conversion of GA9 to bioactive GA4. Two GA3oxs were isolated from rice, named OsGA3ox1 and OsGA3ox2 to date. OsGAox2 is expressed in various organs in rice, for example leaves, root and so on. We investigated their enzyme reactions, and found that OsGA3ox1 largely converted GA9 into GA7 other than the typical conversion of GA9 into GA4, while OsGA3ox2 mainly converted GA9 to GA4, and the conversion of GA9 into GA7 was much less occurred. GA7 is considered to be a more effective bioactive GA compared with GA4, because of its structural properties resistant to inactivation by GA2oxidase.-GA7 has double band between carbon atoms 1 and 2, thus GA7 can not be metabolized by OsGA2oxidase.
Then, we performed promoter GUS analysis to investigate in which organs OsGA3ox1 is expressed in rice, and we revealed that the gene expression of OsGA3ox1 was specifically localized in early-maturated rice pollens, in which the accumulation of starch is started. Furthermore, we generated the knockout mutant of OsGA3ox1, to clarify the role of OsGA3ox1 in planta using CRISPER/Cas9 system. The mutant pollens were not stained with iodine starch reaction, while those from wild-type rice plant were stained. These observations suggest that OsGA3ox1 is involved in the accumulation of starch and sterile in early maturated stage of pollen.

Poster # 77 Gibberellins increase seed sink strength in *Pisum sativum* L. (Reproductive development)

Kosala Waduthanthri[1], Jocelyn Ozga[1], Harleen Kaur[1], and Dennis Reinecke[1]
[1]*Plant BioSystems Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta*

Gibberellins (GAs) control many aspects of plant development including seed growth and development. The involvement of GAs during pea (*Pisum sativum*) seed development was studied by comparing a GA deficient mutant *lh*-2 (mutation affecting ent-kaurene oxidase) and a GA-overexpressor line (TG1) with their respective isogenic controls. The *lh*-2 mutation largely affects pea seed growth and development and results in seed abortion (Swain et al, 1997). The GA-overexpressor line constitutively expresses *PsGA3ox1* (*LE*; a fully functional wild-type GA 3β-hydroxylase gene that encodes GA 3β-hydroxylase that converts GA20 to GA1) in a semi- dwarf *lele* pea line (*le-1*; single base-pair mutation in *PsGA3ox1*) (Reinecke et al., 2013, Plant Physiol 163:929). We observed delayed differentiation and reduced cell expansion in the seed coat layers and cotyledonary storage parenchyma cells of *lh-2* compared to the LH line. With respect to photoassimilate partitioning, starch accumulation (8-12 days after anthesis; DAA) in the seed coat and mobilization of seed coat starch to the embryo (16-20 DAA), as well as starch accumulation in the cotyledons, were dramatically reduced of the *lh-2* mutant compared to LH. In the GA-overexpressor line TG1, early cell differentiation and enhanced cell expansion were observed in the seed coat compared to the transgenic control. Starch accumulation in the seed coat was greater at 10 DAA and mobilization of seed coat starch to the embryo was enhanced from 16 to 20 DAA in TG1 compared to the transgenic control. Greater cell expansion and starch accumulation in the cotyledonary storage parenchyma cells was correlated with an 11 to 34% larger seed size at maturity in TG1 when compared to the transgenic control. These data support that GAs regulate specific aspects of seed coat and embryo development, and as a result, can modify photoassimilate partitioning into the developing embryo.

Poster # 78 High throughput chemical screening using an Arabidopsis ShHTL7 system to identify strigolactone signaling agonists (Chemical biology)

Asrinus Subha[1], Shigeo Toh[2], Duncan Holbrook-Smith[1], Peter McCourt[1]
[1]University of Toronto, [2]Nagoya University

Strigolactones (SLs) are a newly discovered collection of terpenoid-derived small molecule hormones that control germination and shoot architecture in Arabidopsis. Unfortunately, SLs have been co-opted as a germination cue enabling parasitic plants of the genus *Striga* to coordinate their life cycles with their host plants. For example, *Striga hermonthica* has evolved strong seed dormancy, which is broken only when they sense SLs extruded from host roots. Upon germination, *Striga* attaches to the host root and depletes it of nutrients. In sub-Saharan Africa alone, *Striga* has infested up to two-thirds of the arable land affecting over 100 million people in 25 countries. Chemical control has mostly followed the idea of making SL mimics that germinate *Striga* in the absence of a host resulting in the death of these obligate parasites. High throughput chemical screening (HTS) could identify potent and inexpensive SL analogs that germinate *Striga* seed. However, screening directly for compounds that germinate *Striga* seeds is problematic because *Striga* seeds vary greatly in their SL responsiveness, and therefore are not amenable to consistent high throughput screening methodologies. The identification of *ShHTL7* as a picomolar sensitive SL receptor in Arabidopsis allows the design of such a system. I have developed a germination assay using Arabidopsis *ShHTL7* seeds in a 96 well format for HTS of SL agonists and have screened approximately 4000 compounds and identified 5 leads that germinate this line. More importantly, of the 5 leads found from HTS, one compound was found to initiate germination of the obligate parasitic plant *Striga hermonthica.*

Poster # 79 Identification of a sorgomol synthase, that converts 5-deoxystrigol to sorgomol in sorghum (Hormone metabolism)

Masaharu Mizutani[1], Shunsuke Ishiwa[1], Hideyuki Suzuki[2], Hirosato Takikawa[1], and Yukihiro Sugimoto[1]
[1]Kobe University, [2]Kazusa DNA Research Institute

Strigolactones (SLs) are carotenoid-derived plant metabolites that function as signaling molecules, both endogenously as phytohormones and exogenously as rhizospheric infochemicals. All naturally occurring SLs share a core structure comprising an ABC-ring system linked to a 2'*R*-configuration butenolide D-ring. Since the identification of the first SL, strigol as a germination stimulant for seeds of the root parasitic weed *Striga lutea*, over 15 structural variants were isolated. It is likely that the substituents giving rise to the diversity of SLs are introduced in the already-formed parent SLs, 4-deoxyorobanchol (4-DO) and its stereoisomer 5-deoxystrigol (5-DS). The conversion of □-carotene to orobanchol via carlactone and 4-DO has been established in rice. However, the conversion of carlactone to 5-DS and subsequent oxidized metabolites, such as strigol and sorgomol, remains to be elucidated. We previously demonstrated that the high sorgomol producing sorghum (cv. Sudax) converted exogenously applied 5-DS to sorgomol by introducing a hydroxyl group at C-9. The conversion was achieved by microsomal preparations obtained from sorghum roots and inhibited by uniconazole-P, a cytochrome P450 inhibitor. In this study, we conducted comparative transcriptome analysis using roots of the sorghum cultivar grown in phosphorous rich and deficient media, where sorgomol production is down- and up-regulated, respectively. Candidate P450 genes were selected based on similarity of their expression profile to genes homologous to the SL biosynthetic genes *D27*, *CCD7*, *CCD8* and *MAX1*. Functional analyses of the candidate genes led to molecular identification of SbCYP-SM1 as a sorgomol synthase that catalyzes conversion of 5-DS to sorgomol.

Poster # 80 Identification of carlactonoic acid methyltransferase in *Arabidopsis* (Hormone metabolism)

Yuta Onozuka[1], Naoki Kitaoka[1], Takaya Kisugi[1], Kohki Akiyama[2], Kiyoshi Mashiguchi[1], Yoshiya Seto[1], and Shinjiro Yamaguchi[1]
[1]Graduate School of Life Sciences, Tohoku University, [2]Graduate School of Life and Environmental Sciences, Osaka Prefecture University

Strigolactone (SL) functions not only as an allelochemical for symbiosis and parasitism in the rhizosphere, but also as a phytohormone to inhibit shoot branching. SL is a group of terpenoid lactones, which are biosynthesized from carotenoids. Recently, carlactone (CL) and carlactonoic acid (CLA) were identified as biosynthetic precursors for SLs. In addition, a methyl ester derivative of CLA, methyl carlactonoate (MeCLA), was identified as an endogenous SL-like compound in *Arabidopsis*. Moreover, a possible SL receptor, AtD14, was proved to interact with MeCLA, but not with CL and CLA *in vitro*. These results suggest that MeCLA acts as an active hormone that inhibits shoot branching in *Arabidopsis*. Although *Arabidopsis* P450, MAX1, was revealed to catalyze the conversion of CL to CLA, the enzyme converting CLA to MeCLA has yet to be identified. In this study, we aimed to identify CLA methyltransferase and searched for proteins having CLA methylation activity from methylansferase families of *Arabidopsis*. As a result, we successfully found a promising candidate that can efficiently catalyze CLA methylation. The *Arabidopsis* knockout mutants of this candidate gene showed an increased shoot branching phenotype like SL biosynthetic mutants. Moreover, analysis of endogenous CLA and MeCLA levels in those knockout mutants showed that the CLA content increased, while the MeCLA content decreased, compared with those of wild type. These results indicate that the candidate is the main enzyme catalyzing CLA methylation in *Arabidopsis*. Now we are analyzing transport of CLA-related compounds from roots to shoots in detail by grafting experiments.

Poster # 81 Identification of essential amino acid residues in KAI2/HTL in Arabidopsis (Hormone perception)

Tomoki Akatsu[1], Yu Morimoto[1], Yoshiya Seto[1], Kiyoshi Mashiguchi[1], and Shinjiro Yamaguchi[1]
[1]Tohoku University

Karrikins are smoke-derived compounds that promote seed germination. It has been suggested that karrikins are perceived by the KARRIKIN INSENSITIVE 2/HYPOSENSITIVE TO LIGHT (KAI2/HTL) family of α/β-fold hydrolases, which is paralogous to the strigolactone (SL) receptor DWARF14 (D14) family. Recent studies have shown that *kai2* displays various phenotypes such as delay of seed germination and an elongated hypocotyl in Arabidopsis, while *atd14* does not. Moreover, *KAI2* promoter-driven *AtD14* expression is unable to complement the *kai2* phenotypes. These observations indicate that KAI2 perceives an endogenous hormone-like molecule that is distinct from SL in Arabidopsis. In this study, we performed a genetic screen for *kai2*-like mutants with elongated hypocotyls to obtain novel components in the KAI2 pathway. We found that all *kai2*-like mutants identified from EMS-mutagenized M2 seedlings were allelic to *kai2* and *kai1*/*max2*, which are defective in KAI2 and an F-box protein, MAX2, respectively. Seven amino acid substitutions were identified from six new *kai2* alleles. Because these amino acid residues are highly conserved not only in KAI2 proteins but also in D14 proteins, it is likely that they play crucial roles in signal perception or transduction of an endogenous karrikin-like compound. In this presentation, we will show hydrolase activities and physical interactions with downstream components of mutated KAI2 proteins identified by the genetic screen.

Poster # 82 Identification of novel regulators of the auxin biosynthesis during embryo development in Arabidopsis (Reproductive development)

Souad Mroue[1] and Hélène Robert Boisivon[1]
[1]*CEITEC – Central European Institute of Technology*

Morphogenesis in the early *Arabidopsis* embryo depends on auxin biosynthesis, transport, and responses (Moller and Weijers, 2009). A recent report by Robert *et al.,* 2013 showed that a tight spatio-temporal regulation of IPyA-dependent auxin biosynthesis is crucial for the establishment of the embryonic apical-basal axis. During early embryogenesis, a basal auxin production in the suspensor is required for apical embryo patterning. Later, from globular stage on, auxin is produced in the most apical cells of the proembryo, triggering basal polarization of PIN1 and specification of the basal end of the embryo axis as future root.

I am using a reverse genetics approach to investigate the upstream transcriptional regulators of the embryonic auxin production and identify the TFs involved in the control of auxin biosynthetic gene expression patterns. In this context, I am characterizing the function of L-AFL transcription factors (LEC1, LEC2, L1L, FUS3, ABI3 and ABI4) during early embryogenesis.The relationships between the TFs and the auxin biosynthetic genes are being investigated using genetic interaction studies, such as studying the expression pattern of the auxin homeostasis and distribution in the *tf* loss and gain of function backgrounds and performing multiple mutant combination analysis. In addition, analysis of embryo phenotypes of *tf* loss and gain of function mutants is in progress. In order to study the expression patterns of these TFs during embryogenesis, transcriptional and translational fusions are beinggenerated.

Poster # 83 Identification of strigolactone-response genes and their interactions with cytokinin and decapitation in the regulation of shoot branching (Hormone signaling)

Stephanie Kerr[1], Milos Tanurdzic[1], and Christine Beveridge[1]
[1]*University of Queensland*

Strigolactone (SL) is a key inhibitory hormone of shoot branching in plants. DWARF53 (D53) in rice and members of the SUPPRESSOR of MAX2---LIKE (SMXL) protein family in Arabidopsis have been identified as targets of the SL receptor complex. These targets are proposed to suppress SL---response genes in the absence of SL. In order to identify transcriptional targets of the SL signaling complex for the regulation of shoot branching in pea, we used RNA---Seq techniques to identify genes that are transcriptionally regulated by the synthetic SL, GR24, in *Pisum sativum* buds over a six hour time---frame. More than 20 genes were identified as SL---responsive and were validated using qPCR. These genes included previously identified SL---responsive genes, such as *BRANCHED1 (BRC1)* and members of the *SMXL* gene family, as well as genes not previously implicated in SL signaling.

The plant hormone cytokinin (CK) acts antagonistically to SL to promote shoot branching. The transcription factor *BRC1*, a SL---responsive gene involved in the regulation of shoot branching, also responds to SL and CK in an antagonistic manner. We tested response of the SL---responsive genes we identified to CK and found that at least seven genes were also antagonistically regulated by CK, further supporting a shared signaling pathway between the two hormones in the regulation of shoot branching. Similar to CK, decapitation of the growing shoot tip also leads to increased shoot branching and decreased expression of *BRC1*. Further examination of our SL---responsive genes found that at least five genes are also regulated by decapitation, implying that decapitation may also signal, at least partially, through a common pathway to both SL and CK.

Poster # 84 Identifying phytohormones involved in metal-specific root architecture responses (Abiotic stress)

Stefanie De Smet[1], Tony Remans[1], Jaco Vangronsveld[1], and Ann Cuypers[1]
[1]*Hasselt University*

To survive in a changing environment, plants possess a high level of plasticity, especially at the root system. Since plant roots are the first contact point with contaminants, their positioning is the first factor determining metal uptake and subsequent plant growth.

We found that roots of *Arabidopsis thaliana* plants exposed to cadmium (Cd), copper (Cu) or zinc (Zn) showed different morphological responses. Homogeneous exposure to Cd or Cu stimulated lateral root density, while

excess Zn inhibited lateral root development and elongation. Using a split-root system we studied the ability of the root system to colonise control medium when part of the root system was exposed to metal stress. This heterogeneous exposure to excess Cd and Cu diminished root growth in metal-containing zones and stimulated growth in control zone, indicating a redistribution response. Exposure to excess Zn, however, caused a systemic inhibition of both lateral and primary root growth also in the control zone.
Since root development is strongly connected with the action of phytohormones, and several reports indicate altered phytohormone concentrations or gradients during metal stress, we postulate that phytohormones are also involved in the metal-specific root growth responses. We analysed root responses to Cd, Cu or Zn of several phytohormone signalling mutants, including the abscisic acid insensitive *abi4-1,* ethylene insensitive *ein2-1* and strigolactone signalling mutants *d14-1* and *max2-1*. The Cu-induced increase of lateral density was absent in *abi4-1.* ABI4 and EIN2 were found to be involved in the inhibition of lateral emergence and elongation respectively after homogeneous Zn exposure. The redistribution response to Cd was less apparent in *ein2-1.* D14 may be involved the systemic inhibition induced by Zn and in the effects of Cd stress on lateral elongation. *Max2-1* showed higher systemic stimulation of lateral length by Cd, but was less prone to the local inhibition by Zn.

Poster # 85 Impact of hormones on cell death induced by cellulose biosynthesis inhibitors (Hormone signaling)

Fatima Awwad[1] and Nathalie Beaudoin[1]
[1]*Centre Sève, Biology Department, University of Sherbrooke*

Thaxtomin A is a phytotoxin produced by *Streptomyces scabiei*, the causative agent of common scab disease of potato. This toxin has been shown to inhibit cellulose synthesis. Treatments of *Arabidopsis* cell suspensions with thaxtomin A and other cellulose biosynthesis inhibitors (CBI) such as isoxaben perturb the cell wall and activate a specific form of programmed cell death (PCD) that can be distinguished from the hypersensitive cell death. We have found that CBI-induced cell death could be altered by dose-specific hormone treatments and that calcium efflux is essential for this kind of response. More specifically, CBI- induced cell death could be prevented by auxin and by some auxin transport inhibitors like NPA and TIBA. Interestingly, adding salicylic acid, jasmonic acid or abscisic acid to CBI-treated cells also altered the mortality rate in *Arabidopsis* suspension cells. This study provides a deeper comprehension of hormonal importance in response to CBI and may give us some hints on how to improve plant protection against common scab.

Poster # 86 In vivo physiological responses in one-year-old Merwilla plumbea originally treated with different cytokinins (Hormones & biotechnology)

Mack Moyo[1], Adeyemi Aremu[1], Karel Dolezal[2], and Stephen Amoo3
[1]*University of KwaZulu-Natal,* [2]*Palacký University & Institute of Experimental Botany AS CR,* [3]*Agricultural Research Council*

Since their discovery in the 1950s, cytokinins (CKs) have become integral, almost indispensable, components of plant growth media in micropropagation. However, despite the extensive use of CKs in plant tissue culture, there is a paucity of knowledge on the longevity of their physiological effect post *in vitro* growth phases. The aim of this study was to evaluate the long- term physiological effect of *in vitro*-applied CKs (including a novel *meta*-topolin derivative) after one year of *ex vitro* growth in *Merwilla plumbea*. Quantification of endogenous auxins and phenolic compounds in one-year-old *M*. *plumbea* plants was done using UHPLC-MS/MS. Exogenous CKs had a significant carry-over effect on *M*. *plumbea* after one year of *ex vitro* growth. *M*. *plumbea* plants treated with *meta*-topolin tetrahydropyran-2-yl (*m*TTHP, 1.0 µM) had significantly more leaves/plant, higher leaf area/plant, leaf fresh weight and bulb diameter. Varying concentrations of free indole-3-acetic acid (IAA), oxindole-3-acetic acid (OxIAA) and indole-3-acetyl-L-aspartic acid (IAA-Asp) were detected in leaves of plants originally treated with CKs whereas these compounds were not detected in control plants. Both IAA conjugates are critical for maintenance of IAA homeostasis in plant cells. Likewise, a CK type and dose- dependent response was observed on levels of various phenolic acids and flavonoids. Overall, the results of the current study demonstrated the in

planta longevity of CKs applied during the *in vitro* growth phase of *M*. *plumbea*, and their long-term residual influence on regulating auxin biosynthesis and homeostasis, phenolic metabolism as well as various growth parameters. Our study provides profound insights into the post-*in vitro* residual effects of exogenous CKs, most notably *m*TTHP, on *M*. *plumbea* physiology after one year of *ex vitro* growth. Further research should determine whether or not these effects are generic using combinations of physiological, biochemical and molecular approaches in universal model plants.

Poster # 87 Inducing effect of low molecular weight nitrogen-containing heterocyclic compounds on direct shoot organogenesis of *Linum usitatissimum* L. i*nvitro* (Hormones & biotechnology)

Victoria Tsygankova[1], Oleg Bayer[2], Yaroslav Andrusevich[1], Vladimir Sergeevich Brovarets[1], Alla Yemets[2], and Yaroslav Blume[2]

[1]Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, Kiev, [2]Department of Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Kiev

Linum usitatissimum L. (flax) is one of the oldest economically important crops used in biotechnology as a source for bioactive compounds. Flax fiber and seed have industrial significance as a source of cellulosic fiber for textile and paper industry, and seed oil for pharmaceutical, cosmetic and food industry. The elaboration of new effective techniques of *in vitro* regeneration of flax cultivar (*Linum usitatissimum* L.) is very actual problem today. Impact of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds synthesized in the Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine on *in vitro* shoot organogenesis of *Linum usitatissimum* L. was studied. It was shown that nitrogen-containing heterocyclic compounds derivatives of pyridine, pyrimidine, pyrazolotriazinone and isoflavones at the concentration 10^{-8} M/l of the MS medium revealed high stimulating activity on direct shoot organogenesis from hypocotyl explants of *Linum usitatissimum* L. that exceeds almost one and a half - twice the activity of 0.05 mg/l 1-Naphthaleneacetic acid (NAA) and 1 mg/l 6- benzylaminopurine (BAP) supplemented in the control MS medium. Obtained results confirm perspective of using of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds as new effective substitutes of traditional growth regulators for regeneration of *Linum usitatissimum* L. *in vitro*.

Keywords: screening of new plant growth regulators, nitrogen-containing heterocyclic compounds, pyridine, pyrimidine, pyrazolotriazinone and isoflavones, *Linum usitatissimum L*., direct shoot organogenesis *in vitro*.

Poster # 88 Insect-producing auxin involved in insect gall induction (Hormone metabolism)

Yoshihito Suzuki[1], Hiroyoshi Suzuki[1], Chiaki Yokohama[1], Shinji Nagata[2], and Tadao Asami[2]

[1]Ibaraki University, [2]The University of Tokyo

A variety of insect species induce galls on host plants. Insect galls are abnormal plant tissues, which are used for food and habitation by galling insects. Several studies have implicated phytohormones in insect-induced gall formation. However, it has not been determined whether insects can synthesize phytohormones. LC-MS/MS analyses indicated that extremely high concentrations of cytokinins and IAA are contained in the gall-inducing sawfly (Pontania sp.). Feeding experiments demonstrated the ability of sawfly larvae to synthesize IAA from tryptophan (Trp). The abnormally high concentration of *t*-zeatin riboside in the gland fluid strongly suggested cytokinin biosynthesis in sawfly, too. Some auxin and cytokinin-inducible genes are expressed significantly higher in gall tissue than in leaves, suggesting the action of insect-derived phytohormones.
The other galling insect such as a gall midge (*Rhopalomyia yomogicola*) an aphid (*Tetraneura nigriabdominalis*) also showed higher concentrations of cytokinins and IAA than their host plants, suggesting that insect-producing IAA and cytokinins are generally involved in gallinduction. Furthermore, we found that the silkworm, a non-

galling insect, also contains a small amount of IAA and produces IAA *de novo* from Trp. The detailed metabolic analysis using IAA-biosynthetic intermediate candidates knownin plants and microbes revealed that indole-3-acetaldoxime (IAOx) is the major intermediate. The pathway Trp -> IAOx -> IAA was shown to also operate in galling sawfly. Screening of a chemical library identified a inhibitor for the latter step commonly active to sawfly and silkworm enzymes, and another inhibitor which was much effective on sawfly than silkworm. We also obtained preliminary result showing that the step Trp -> IAOx is limiting IAA production in silkworm, and sawfly has a more efficient conversion system for the step. Galling insects might have evolved pre-existing IAA producing system in insects to be more efficient one.

Poster # 89 Interactions between ethylene, gibberellins, and brassinosteroids in the development of rhizobial and mycorrhizal symbioses of pea (Biotic interaction)

James B. Reid[1], Eloise Foo[1], Erin L. McAdam[1] and James L. Weller[1]
[1]*School of Biological Sciences, University of Tasmania*

The regulation of arbuscular mycorrhizal development and nodulation involves complex interactions between the plant and its microbial symbionts. We used the recently identified ethylene-insensitive *ein2* mutant in pea to explore the role of ethylene in the development of these symbioses. We showed that ethylene acts as a strong negative regulator of nodulation, confirming reports in other legumes. Double mutants produced by crosses between *ein2* and the severely gibberellin-deficient *na* and brassinosteroid-deficient *lk* mutants showed increased nodule numbers and reduced nodule spacing compared with the *na* and *lk* single mutants, but nodule numbers and spacing were typical of *ein2* plants, suggesting that the reduced number of nodules in *na* and *lk* plants is largely due to the elevated ethylene levels previously reported in these mutants. We showed that ethylene can also negatively regulate mycorrhizae development when ethylene levels are elevated above basal levels, consistent with a role for ethylene in reducing symbiotic development under stressful conditions. In contrast to the hormone interactions in nodulation, *ein2* does not override the effect of *lk* or *na* on the development of arbuscular mycorrhizae, suggesting that brassinosteroids and gibberellins influence this process largely independently of ethylene.

Poster # 90 Investigating SMAX1 Degradation in Response to Karrikins and Strigolactones (Hormone signaling)

Nicholas Morffy[1] and David Nelson[1]
[1]*University of Georgia*

Karrikins (KAR) and strigolactones (SL) are two recently identified classes of plant signaling molecules. SLs are endogenous plant hormones that regulate a number of growth processes including shoot branching. KARs are found in smoke generated by burning plant material and impact germination and seedling responses to light. While much is known about the mechanisms of SL signaling, very little is known about the mechanisms of KAR signaling.

Genetic and biochemical evidence suggest that the SL receptor, D14, binds to SMXL6 or SMX7 and allows it be marked for degradation by the F-box protein MAX2. MAX2 and D14 act as positive regulators of SL signaling, while SMXL6, SMXL7, and SMXL 8 are negative regulators.

Interestingly, KAR signaling also requires MAX2, as well as a paralog of D14 called KAI2. Genetic evidence places SMAX1, a paralog of SMXL6 and SMXL7, downstream of MAX2, and SMAX1 negatively regulates KAR signaling phenotypes. It is unknown if SMAX1 is degraded in the presence of KAR, of it interacts with KAI2 in a KAR dependent manner.

Here we show that transiently expressed SMAX1, but not SMXL7, is degraded in response to KAR in tobacco leaves. However, both SMAX1 and SMXL7 are degraded by the synthetic SL 5- deoxystrigol (5DS), but to different degrees.

In addition, we have undertaken bi-molecular fluorescent complementation (BiFC) analysis to determine if SMAX1 interacts with KAI2. We show that SMAX1 interacts with both KAI2 and D14, and that the catalytically

inactive forms of both receptors are not necessary for this interaction. SMXL7 also interacts with both KAI2 and D14 in tobacco leaves. Interestingly, leaves expressing SMXL7 and KAI2 show stabilized signal in the presence of 5DS, while the leaves expressing SMXL7 and D14 do not. KAR treatment also fails to destabilize the SMXL7 and KAI2 interaction in tobacco leaves.

Poster # 91 Investigating the role of XERICO in GA-ABA crosstalk during germination and stress response (Abiotic stress)

Eliana Vonapartis[1], Carina Carianopol[1] and Sonia Gazzarrini[1]
[1]*Department of Cell and Systems Biology, University of Toronto*

Plant hormones are small molecules whose signaling pathways interact, together forming a complex crosstalk network that regulates the plant's response to developmental signals and environmental stressors. Although hormone crosstalk is central to various aspects of plant biology, it is a phenomenon that is poorly understood despite much recent progress. XERICO (XER) is a DELLA-induced putative RING E3 ubiquitin ligase that has been found to play a role in increasing intracellular abscisic acid (ABA) levels, thereby enhancing abiotic stress tolerance. Since its precise mode of action remains unexplored to date, we aim to understand the role of XER in GA-ABA crosstalk during stress response and germination, a developmental phase transition that is tightly regulated to ensure seedling survival. As a first step in characterizing XER function, transient expression assays of its RING-mutated form in *Nicotiana benthamiana* show that it localizes to the chloroplast envelope and cell periphery. In contrast, the wild-type XER protein shows very low expression in transient assays, suggesting that it may be subjected to fast turnover. Subcellular localization of XER *in vivo* is currently being investigated. To shed light on XER involvement in hormone signaling at the molecular level, several potential *XER* upstream regulators as well as downstream targets have been isolated using high-throughput yeast one-hybrid (Y1H) and two-hybrid (Y2H) approaches, respectively. Lastly, overexpression lines have been generated to study its *in planta* function and to confirm the Y1H and Y2H results. This study will provide important insight as to how an E3 ligase integrates the GA and ABA signaling pathways, ultimately affecting plant response to environmental stress.

Poster # 92 Jasmonates biosynthesis in Arabidopsis seeds is distinct from its wound-induced pathway in leaves (Hormone metabolism)

Ehsan Khodapanahi[1] and Eiji Nambara[1]
[1]*University of Toronto*

Phytohormone jasmonates (JAs) regulate seed dormancy and germination in Arabidopsis. Our current knowledge on JAs biosynthesis pathway is owned to the research which is primarily focused on leaves and wounding tissues. However, how JAs are synthesized in seeds is yet elusive. This research reports a variation in Arabidopsis ecotypes in the JA-Ile level, the major bioactive form of JAs in plants. An instance of such variation is found in the Col ecotype, where there is an accumulation of JA-Ile in dry seeds. In comparison, the Cvi ecotype has very low or no deposit of the compound in (dry) seeds. The Mendelian segregation of this metabolic phenotype in Col x Cvi F2 indicates that the lack of JA-Ile in Cvi seeds is due to a defective single locus. Currently, I am mapping a candidate locus to identify the gene responsible for the JA-Ile deposit variation between the Col and Cvi ecotypes.

Poster # 93 Jasmonoyl-L-isoleucine is required for the production of a flavonoid phytoalexin but not diterpenoid phytoalexins in ultraviolet-irradiated rice leaves (Hormone signaling)

Koji Miyamoto[1], Isami Enda[1], Toshiki Okada[1], Yumiko Sato[1], Kohei Watanabe[1], Tomoko Sakazawa[1], Emi Yumoto[1], Kyomi Shibata[1], Masashi Asahina[1], Moritoshi Iino[2], Takao Yokota[1], Kazunori Okada[3], Hisakazu Yamane[1]

[1]*Department of Biosciences, Teikyo University,* [2]*Botanical Gardens, Osaka City University,* [3]*Biotechnology Research Center, The University of Tokyo*

Rice produces low-molecular-weight antimicrobial compounds called phytoalexins in response to not only pathogen attack but also abiotic stresses. Rice phytoalexins are composed of diterpenids and a flavonoid. Recent research indicated that endogenous jasmonyl-L-isoleucine (JA-Ile) is not necessarily required for the production of diterpenoid phytoalexins in blast-infected or CuCl2-treated rice leaves. However, JA-Ile is required for the accumulation of the flavonoid phytoalexin, sakuranetin, Ultraviolet (UV) irradiation also induces the phytoalexin accumulation in rice leaves. However, the roles of JA-Ile in UV-induced phytoalexin production in rice remain unknown. To investigate such roles, we first measure the levels of JA-Ile, and its precursor jasmonic acid (JA), upon UV-irradiation. As a result, the levels of JA and JA-Ile peaked between 1 and 2 h after UV-irradiation in rice leaves. We also found that the mRNA levels of jasmonate biosynthetic genes, such as *OsAOS1*, *OsAOS2*, *OsAOC* and *OsOPR7*, were increased 1 h after UV-irradiation. Furthermore, we found that jasmonate biosynthesis mutants of rice, such as *cpm2*/*osaoc* and *osjar1*, produced diterpenoid phytoalexins but not sakuranetin in response to UV, indicating that JA-Ile is required for the production of sakuranetin rather than diterpenoid phytoalexins in UV-irradiated rice leaves.

Poster # 94 Karrikin singling in arbuscular mycorrhiza development (Biotic interaction)

Samy Carbonnel[1], Markus Kolodziej[1], Veronica Basso[1], Maximilian Griesmann[1], Trevor Wang[2], and CarolineGutjahr[1]

[1]*LMU Munich, Faculty of Biology, Genetics,* [2]*John Innes Center, Metabolic Biology, Norwich Research Park*

Arbuscular mycorrhiza (AM) is a symbiotic association between most land plants and glomeromycotan fungi that is based on the mutual exchange of nutrients between the two partners. AM colonization is initiated by a molecular dialog involving plant strigolactones and fungal signals called Myc factors. After a successful recognition, AM fungi form a hyphopodium at the root surface and penetrate into the root. Then they develop intercellular hyphae along the root axis and form branched arbuscules inside cortex cells. Increasing evidence shows the fundamental importance of phytohormones in fine-tuning the colonization process. Recently, the rice alpha-beta hydrolase D14L has been shown to be required for early recognition of AM fungi (Gutjahr et al. 2015, Science). D14L in association with the F-box protein MAX2, is involved in the perception of karrikin, a butenolide compound found in smoke. Additionally, to the role in AM symbiosis, this complex regulates seedling morphogenesis in Arabidopsis. We study the role of karrikin signaling in AM symbiosis in the model legume *Lotus japonicus.* Phenotypic analysis of *Lotus japonicus d14l* and *max2* mutants revealed a reduction of AM colonization, indicating conservation of karrikin receptor complex function with respect to AM symbiosis in dicotyledons. Progress in understanding the role of the *Lotus japonicus* karrikin receptor complex in AM symbiosis will be presented.

Gutjahr C., Gobbato E., Choi J., Riemann M., Johnston M.G., Summers W., Carbonnel S., Mansfield C., Yang S.Y., Nadal M., Acosta I., Takano M., Jiao W.B., Schneeberger K., Kelly K.A and Paskowski U. (2015). Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. *Science* **350**, 1521-1524.

Poster # 95 Karrikin-based degradation system as a tool to identify KL (Hormone signaling)

Aashima Khosla[1] and David Nelson[1]
[1]*University of Georgia*

Karrikins (KARs) are smoke derived compounds that are recognized by the receptor KAI2. There is no evidence to date that karrikins are present in plants. Therefore, the endogenous ligand of KAI2 (KL) is unknown. Identification of low abundance KL will require sensitive and highly specific assays. Preliminary studies from our lab show that in tobacco SUPPRESSOR of MAX2 1 (SMAX1) protein is rapidly degraded following KAR treatment. Here we describe the design of quantitative *in vivo* biosensors for KAR/KL that are based upon ligand- triggered proteolysis of SMAX1. Alongside, we are interested in identifying a "degron" domain(s) that is necessary and sufficient for KAR-induced degradation of SMAX1. For this purpose, various *SMAX1* deletion variants were made and tested for KAR induced degradation in tobacco. We will present our preliminary results suggesting that the C-terminal region is sufficient for ligand- induced degradation. Future work involves reducing the putative degron domain to a minimal size by further deletions and tests for stability. Identification of degron will undoubtedly aid in designing best sensors that are useful for high throughput screening of compound library to detect KL and to study KL distribution and dynamics in plants. Furthermore, the identification of KL would open new research prospects for the plant hormone field and agriculture alike, as this may represent a new plant hormone.

Poster # 96 Karrikins delay soybean seed germination by mediating abscisic acid and gibberellin biogenesis under shaded conditions (Hormone metabolism)

Yongjie Meng[1] and Kai Shu[1]
[1]*Key Laboratory of Crop Ecophysiology and Farming System in Southwest China (Ministry of Agriculture), Sichuan Engineering Research Center for Crop Strip Intercropping System, Institute of Ecological Agriculture, Sichuan Agricultural University*

Karrikins (KAR) are a class of signal compounds, discovered in wildfire smoke, which affect seed germination. Currently, numerous studies have focused on the model plant Arabidopsis in the KAR research field, rather than on crops. Thus the regulatory mechanisms underlying KAR regulation of crop seed germination are largely unknown. Here, we report that KAR delayed soybean seed germination through enhancing abscisic acid (ABA) biosynthesis, while impairing gibberellin (GA) biogenesis. Interestingly, KAR only retarded soybean seed germination under shaded conditions, rather than under dark and white light conditions, which differs from in Arabidopsis. Phytohormone quantification showed that KAR enhanced ABA biogenesis while impairing GA biosynthesis during the seed imbibition process, and subsequently, the ratio of active GA4 to ABA was significantly reduced. Further qRT-PCR analysis showed that the transcription pattern of genes involved in ABA and GA metabolic pathways are consistent with the hormonal measurements. Finally, fluridone, an ABA biogenesis inhibitor, remarkably rescued the delayed-germination phenotype of KAR-treatment; and paclobutrazol, a GA biosynthesis inhibitor, inhibited soybean seed germination. Taken together, these evidences suggest that KAR inhibit soybean seed germination by mediating the ratio between GA and ABA biogenesis.

Keywords: Karrikin, shade, seed germination, ABA, GA, soybean.

Poster # 97 MAKR2 sets up the pace of the root gravity response by controlling PIN2 trafficking (Hormone signaling)

Maria del Mar Marquès-Bueno[1], Laia Armengot[1], Thomas Stanislas[1], Matthieu Plattre[1], Vincent Bayle[1], and Yvon Jaillais[1]

[1]*Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon*

Gravitropism is the growth of plant according to the gravity vector, a process that is coordinated by differential auxin accumulation on either side of the bending organ. During the last decade, visualization of auxin accumulation and/or activity by synthetic reporters such as *DR5* and *DII* revealed the dynamics of differential auxin accumulation during the gravitropic response, notably in the root. In addition, both genetic and cell biological observations showed that auxin influx (AUX1) and efflux (PIN2, PIN3, PIN7) carriers are involved in this process. We recently identified MAKR2 (MEMBRANE ASSOCIATED KINASE REGULATOR 2) as a pivotal regulator of the timing of the response to gravity, MAKR2 activity acting like a rheostat that controls the speed at which the root will bend. We notably found that MAKR2 overexpression slows down the gravitropic response in a dose dependent manner, while *makr2* loss-of-function mutants responds faster. MAKR2 is an intrinsically disordered protein that is targeted to the plasma membrane by a membrane hook region that binds to phosphatidylinositol-4-phosphate. In addition, MAKR2 is expressed in both epidermis and cortex cells, where it colocalizes with PIN2. Importantly, both *makr2* mutants and overexpressing lines have altered auxin asymmetry and PIN2 trafficking during the gravitropic response. We will discuss a model that integrates membrane kinase signaling and polar auxin transport in setting up the pace of the gravity response.

Poster # 98 Mechanism of polyamine-induced aluminum toxicity tolerance in mung bean plants: A study on antioxidant defense and methylglyoxal detoxification systems (Abiotic stress)

Kamrun Nahar[1,2], Mirza Hasanuzzaman[3], and Masayuki Fujita[1]

[1]*Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University,* [2]*Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University,* [3]*Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University*

Being a potent toxic compound aluminium (Al) induces inhibition of cell elongation and cell division in root tip, imposes injurious effects on cell wall, plasma membrane, cytoskeleton and nucleus. Aluminium signaling is also responsible for the mitochondrial pathway of cell death. Although polyamines (PAs: putrescine, Put; spermidine, Spd; and spermine, Spm) have been identified as important signaling molecules very few studies focused on roles of PAs in Al toxicity tolerance. Present study investigates the roles of exogenously applied Spd (0.3 mM) in alleviating Al ($AlCl_3$, 0.5 mM, 48 h and 72 h) induced injury in mung bean seedlings (*Vigna radiata* L. cv. BARI Mung-2). Aluminium toxicity induced oxidative damage overproducing reactive oxygen species (ROS; H_2O_2 and $O_2^{\bullet-}$), increasing lipoxygenase activity and membrane lipid peroxidation. The toxic compound methylglyoxal (MG) also overproduced under Al stress. In order to circumvent the Al-induced oxidative stress, enzymatic and non-enzymatic antioxidant pathways were activated by exogenous Spd application. Exogenous Spd increased ascorbate (AsA) and glutathione (GSH) content, AsA/DHA (ratio of ascorbate and dehydroascorbate), GSH/GSSG (ratio of glutathione and glutathione dissulfide), activity of APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), GR (glutathione reductase) and CAT (catalase) in antioxidant system pathway which reduced ROS production and oxidative stress under Al stress. Spd-induced improvement of GSH pool and Gly II activity alleviated injurious effects of MG. Exogenous Spd positively modulated the endogenous PAs level. Regulating the osmoprotectant molecule proline, Spd improved plant water status under Al stress. Exogenous Spd has been proved to be potent to prevent the breakdown of Al-induced photosynthetic pigment and to improve growth performances of mung bean seedlings.

Poster # 99 Mode of action of a gibberellin mimic, AC94377, in *Arabidopsis* (Chemical biology)

Kai Jiang[1,3], Masato Otani[1,3], Hiroaki Shimotakahara[1], Jun-min Yoon[1], Seung-Hyun Park[1], Tsuyoshi Ohta[1], Hidemitsu Nakamura[1], Masatoshi Nakajima[1], Tadao Asami[1,2]

[1]Graduate School of Agricultural and Life Sciences, The University of Tokyo, [2]Department of Biochemistry, King Abdulaziz University, Jeddah, Saudi Arabia, [3]These authors contributed equally to this work

Gibberellin (GA) is a major plant hormone that plays many roles in the regulation of plant growth and development, and is widely used as a plant growth regulator in agricultural production. However, many factors limit the agronomic application of GA to crops. For instance, GA causes some unfavorable changes in plant growth, resulting in taller plants that are prone to lodging. Furthermore, although the cost of producing GA_3 is lower than the cost of producing GA_4 (albeit higher than the production cost of general synthetic plant growth regulators), until now, GA_3 could only be produced by fungi. The demand for function-limited GA mimics that are easily synthesized at low cost is steadily increasing, and such chemicals will expand the practical applications of GA signaling to agricultural purposes.

AC94377 has been reported to display GA-like activity in various weeds and crops. However, the mode of action of its GA-like activity at the molecular level is still unknown. Our report in this conference demonstrates for the first time that AC94377 is a GID1 agonist with selectivity for a specific subtype of GID1 *in vivo* and *in vitro*. Further studies of AC94377 are expected to extend our understanding of GA signaling when it is mediated by different subtypes of the GID1 receptor. As a bona fide agonist of GID1, AC94377 could also be used to develop even more-effective agonists or antagonists with selectivity for specific GID1 in structure–activity relationship studies, using an assay system similar to that used in our study.

Poster # 100 Molecular basis for ENOYL-COA HYDRATASE2 roles in IBA-to-IAA Conversion (Hormone metabolism)

Samantha Powers[1], David A. Korasick[2], Joseph M. Jez[1], and Lucia C. Strader[1]

[1]Washington University in St. Louis, [2]University of Missouri

In *Arabidopsis thaliana*, conversion of the auxin precursor indole-3-butyric acid (IBA) to the active auxin indole-3-acetic acid (IAA) is necessary for proper growth and development. IBA-to- IAA conversion occurs by peroxisomal β-oxidation. ENOYL-COA HYDRATASE2 (ECH2) is a protein likely involved in the IBA-to-IAA conversion pathway and *ech2* mutants are resistant to the effects of IBA, likely because of this conversion defect. Despite the importance of ECH2, the exact reaction in the multistep IBA-to-IAA pathway catalyzed by ECH2 remains unknown. We have determined the ECH2 crystal structure and found that it folds into a canonical hot dog domain. Consistent with the proposed function of ECH2 in peroxisomal β-oxidation of IBA-to-IAA, proteins containing a hot dog domain typically have either thioesterase or enoyl-coA hydratase activity. Using the ECH2 crystal structure, we modeled potential substrates in the ECH2 binding pocket to determine possible ECH2 roles in the IBA-to-IAA conversion pathway. By uncovering the ECH2 structure, we hope to better understand the molecular mechanism of ECH2 and the role it plays in IBA-to-IAA conversion.

Poster # 101 Nanoparticle effect on phytohormone dynamics and transcriptome of Arabidopsis plants (Abiotic stress)

Radomira Vankova[1], Premysl Landa[1], Petre Dobrev[1], Sylva Prerostova[1], Vojtech Knirsch[1], Alena Gaudinova[1], and Tomas Vanek[1]

[1]Institute of Experimental Botany AS CR

Nanoparticles represent an intensively developing area, with versatile use, e.g., in cosmetics or dyes. Thus, their release into the environment seems to be inevitable. Until now, the attention has been focused predominantly on their impact on humans, mammals or fish. However, after spreading into environment, nanoparticles may significantly affect plants, and being up-taken, they can accumulate in the food chain. In order to provide a

complex analysis of the impact of nanoparticles on plants, the effect of nZnO on the growth, hormonal pools and gene expression was followed in *Arabidopsis thaliana*. The increasing concentration of nZnO gradually suppressed plant growth as well as biosynthesis of the growth promoting hormones cytokinins and auxins in apices. In contrast, *cis*-zeatin and its riboside were elevated by higher nanoparticle concentrations in roots. Higher nZnO concentrations resulted in up-regulation of the stress hormone abscisic acid in apices and leaves. Production of the stress hormone salicylic acid was stimulated in leaves and roots. In contrast, the other stress hormone jasmonic acid (as well as its active metabolite jasmonate isoleucine) was suppressed in the presence of nanoparticles. Transcriptome analysis showed up-regulation of the expression of genes involved in stress response (e.g., to salt, osmotic stress or water deprivation), while genes involved in cell organization and biogenesis (e.g., tubulins, arabinogalactan proteins) and DNA or RNA metabolism (e.g., histones) prevailed in the down-regulated transcripts. Thus, stimulation of the antioxidant system as well as of general stress responses and down-regulation of genes related to cell division were found. The achieved results indicate that different nanoparticle concentrations specifically affect plant hormone pools as well as transcriptome and subsequently the physiological state of plants.

Acknowledgement. The work was supported by MEYS CR, project nos. LD14120 and LD15093.

Poster # 102 New lead compounds for regulating ethylene signaling (Chemical biology)

Nobutaka Kitahata[1], Hiroki Hayase[2], Hiroko Shimizu-Yumoto[3], Masashi Suzuki[2], Masayoshi Nakayama[3], Kazuyuki Kuchitsu[1], and Tadao Asami[2]
[1]Tokyo University of Science, [2]The Tokyo University, [3]National Agriculture and Food Research Organization

The gaseous hormone ethylene regulates many physiological and developmental processes in plants. To identify chemicals with ethylene-like activity, we performed a phenotype-based screen of commercially available chemical libraries. From a total of 2,000 compounds screened, we found one compound that induced the triple response phenotype, named HJ2. We designed and synthesized 27 derivatives of HJ2 and found a chemical with a more potent activity, named C17. C17 showed activity in the presence of ethylene biosynthesis inhibitors and had no effect on ethylene content. On the other hand, C17 did not induce the triple response phenotype in ethylene insensitive mutants *etr1-1* and *ein2*. C17 activity was inhibited by ethylene perception inhibitor STS. Moreover, C17-treatment gradually induced the transcription of ethylene-responsive genes, although on a different time course from 1-aminocyclopropane-1-carboxylic acid. These results suggest that C17 activates ethylene signaling. Furthermore, we screened for inhibitors of the ethylene response and found a compound that suppressed the short-root phenotype of ethylene overproducer mutant *eto1*, named C18. C18 had no effect on ethylene content and did not exhibit the activity in constitutive ethylene response mutant *ctr1*. These results suggest that C18 inhibits ethylene signaling. These chemicals would be useful tools for agriculture and chemical genetics.

Poster # 103 NIN-LIKE PROTEIN 8 regulates ABA catabolism during nitrate- promoted seed germination in *Arabidopsis* (Hormone metabolism)

Dawei Yan[1], Vanathy Easwaran[1], Vivian Chau[1], Masanori Okamoto[2], and Eiji Nambara[1]
[1]University of Toronto, [2]Totter University; Japan Science and Technology Agency

Seeds respond to multiple different environmental stimuli and these environmental factors in turn regulate seed germination. Nitrate is a germination stimulator for many plant species. Despite its importance, it remains unclear as to how seed responds to nitrate in the control of seed germination. Here, we report that the *Arabidopsis* NIN-LIKE PROTEIN 8 (NLP8) is essential for nitrate-promoted seed germination. Loss-of-function mutants of *NLP8* lacked the ability for nitrate-promoted seed germination. *NLP8* functions even in thenitrate reductase-deficient mutant background, and this mechanism is conserved amongst *Arabidopsis* accessions. NLP8 reduced abscisic acid (ABA) levels in a nitrate-dependent manner through directly binding to the promoter of

CYP707A2 encoding an ABA catabolic enzyme. Overexpression of *CYP707A2* in *nlp8* mutant background led to nitrate-independent seed germination. This indicates that the NLP8-mediated induction of *CYP707A2* is essential for the nitrate- promoted seed germination.

Poster # 104 Novel roles of Arabidopsis molybdenum cofactor sulfurase ABA3 in environmental stress responses (Abiotic stress)

Shunsuke Watanabe[1], Yuji Sawada[1], Masami Yokota Hirai[1] and Mitsunori Seo[1]
[1]*RIKEN Center for Sustainable Resource Science*

It is well known that Arabidopsis molybdenum cofactor sulfurase ABA3 has a pivotal role in adaptation to environmental changes since sulfuration of Mo cofactor is required for the activities of several enzymes involved in phytohormone homeostasis. For example, the Arabidopsis aldehyde oxidase AAO3 catalyzes the oxidation abscisic aldehyde to produce abscisic acid (ABA), which is an essential biomolecule for plant responses to environmental stimulus. In addition, xanthine dehydrogenase (XDH), which is the first committed and rate-limiting enzyme of purine degradation pathway, also plays an important role in the regulation of ABA homeostasis; a major purine metabolite allantoin promotes the regeneration of ABA from ABA-glucose conjugates. These suggest that ABA3 coordinately controls multiple metabolism pathways contributing to stress responses. Intriguingly, it has been reported that ABA3 mutants exhibited pleiotropic phenotypes that are not observed in other typical mutants defective in ABA and purine metabolisms, suggesting that not only ABA and allantoin but also other metabolites synthesized by Mo enzymes have some biological activities. To identify metabolic pathways regulated by ABA3, we investigated the impact of ABA3 disruption on transcriptome and metabolome of Arabidopsis. Genome-wide gene expression analysis showed that the expression of 268 genes was changed more than 2-fold in *aba3-1* compared with both wild type and the typical ABA deficient mutant *aba2-2* when subjected to PEG-induced osmotic stress. Widely targeted metabolome analysis detected 29 compounds whose endogenous levels were higher or lower in *aba3-1* than wild type and *aba2-2*. We will discuss hidden roles of ABA3 in stress responses based on our findings.

Poster # 105 Nucleoside N-ribohydrolases and adenosine kinases in maize *(Zea mays)* (Hormone metabolism)

David Kopečný[1], Radka Končitíková[1], Eva Hájková[1], Armelle Vigouroux[2], Martina Kopečná[1], David Zalabák[3], Ondřej Plíhal[3], and Solange Moréra[2]
[1]*Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University,* [2]*Institute for Integrative Biology of the Cell, CNRS-CEA-Univ. Paris-Sud, Université Paris-Saclay,* [3]*Department of Molecular Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University,*

Nucleosides and nucleobases can be recycled to nucleoside monophosphates, which is also known as salvage pathway preserving an energy, which would otherwise be needed for *de novo* synthesis. In plants, both uridine kinases and uracil phosphoribosyl transferases act on the pyrimidine salvage pathway. In purine salvage, an important role has been shown for adenosine kinases (AK, E.C. 2.7.1.20) and adenine phosphoribosyltransferases. Purine and pyrimidine nucleosides are hydrolyzed by nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-) to corresponding nitrogenous bases and ribose. There are two NRH subclasses in the plant kingdom; one preferentially targets the purine ribosides while the other is more active towards uridine and xanthosine. We focused on purine metabolism and interconversion of cytokinins, which are N^6-substituted adenine/adenosine derivatives. We analyzed spatial and temporal expression of all five *NRH* and three *ADK* genes present in maize (*Zea mays*). Transcripts of *ADK2* were the most abundant of all three ADKs in all organs and developmental stages. Transcripts of *NRH1a*, *NRH2a* and *NRH3* genes are the most abundant in leaves. ADKs are monomeric enzymes while NRHs are dimeric. NRHs impose a strict specificity for the ribose moiety while residues interacting with the nucleobase highly vary. We combined a site-directed mutagenesis approach with kinetic and structural analyses

to study nucleoside binding sites in two NRHs, namely NRH2b and NRH3. The crystal structures of both NRHs were solved at 1.75 and 2.51 Å resolution, respectively. Five NRH2b variants and seven NRH3 variants were studied in detail. Replacement of the active-site tryptophan and lysine residues reduced the specific activity but increased hydrolysis of cytokinin ribosides isopentenyl adenosine and *trans*-zeatin riboside.

This work was supported by grant 15-22322S from the Czech Science Foundation and grant IGA_PrF_2016_024 from Palacký University.

Poster # 106 Nutrient acquisition strategies are allosterically regulated by a conserved quorum-sensing molecule to prime and modulate xylem pericycle pole cells for lateral root development (Vegetative development)

Margaretha J. van der Merwe[1], David Secco[1], Jens Kossmann[2] and James Lloyd[2]
[1]*Australian Research Council Centre of Excellence in Plant Energy Biology, University of Western Australia,*
[2] *Institute of Plant Biotechnology, Department of Genetics, Merriman Avenue, Stellenbosch University*

The autotrophic and sessile nature of plants means that they need to respond to abiotic stresses in a finely tuned manner to grow and survive. Metabolites play an important role during these adaptations, either as direct modulators, or as biochemical indicators of the pathways activated. Plants have evolved from relatively simple unicellular organisms, which have a remarkable adaptability to respond to their environment through metabolite-modulated quorum-sensing mechanisms. Evidence presented here suggest that at least one of these ancient bacterial sensing molecules have been retained in higher plants, and forged new metabolic networks and connectivity to enable root-dependent nutrient uptake strategies. Elucidation of the molecular mechanism demonstrated that the sensing and signaling pathway primes root founder cells in order to influence the local cellular adaptations for lateral root development. This contributes to the impressive array of phenotypic plasticity in root development upon nutrient-limited growth conditions, and is currently being explored to understand the plant's ability to sense and respond to its changing environment.

Poster # 108 Omic approaches to shed light on apple fruit development and ripening (Reproductive development)

Francesca Populin[1], Giulia Eccher[1], Mirko Moser[2], Elisa Asquini[2], Javier Miret[3], Mickaël Maucourt[4], Agnes Gacel[4], Duyen Prodhomme[5], Sylvain Guyot[4], Annick Moing[5], Sergi Munne-Bosch[3], Azeddine Si-Ammour[2], and Alessandro Botton[1]
[1]*University of Padova - Dept. Agronomy Food Natural resources Animals and Environment,* [2]*Edmund Mach Foundation,* [3]*Universitat de Barcelona - Department of Plant Biology,* [4]*INRA, MetaboHUB, IBVM,* [4]*Chargé de Recherches, Equipe PRP,* [5]*INRA, UMR1332 Biologie du Fruit et Pathologie*

Omic approaches to shed light on apple fruit development and ripening Apple (*Malus domestica* L. Borkh) is one of the most economically relevant fruit crops in the world, and Italy is among the most important apple producers. Nowadays the scientific interest on this species is growing exponentially, from the point of view of both basic and applied research. Apple is being increasingly considered as a model species for studying fruit development and many other related process, such as the ripening syndrome. Despite the availability of large collections of biological data, which are frequently focused either on early development or on the ripening phase, only a few information are available for what concerns the intermediate stages (cell expansion and maturation) and the transition between different phases of fruit development. Furthermore, this information was collected in different years and from diverse genotypes, and sometimes with contradictory results. For these reasons, "omic" approaches have been adopted to shed light on the regulatory network of apple fruit development (cv. Golden delicious) along the whole fruit developmental cycle, including both the main phases (cell division, cell expansion, maturation and ripening) and the transitions. Sample collection started from fruit set (immediately after pollination) up to the ripening *in planta*. Transcriptional, metabolomic and hormonal profiling analyses

were performed and results integrated through a dedicated bioinformatic pipeline. Correlative networks were built as a starting point to understand the molecular and metabolic events governing the apple fruit development. Moreover, a new sampling protocol was set up to shed light on the progression of the ripening syndrome through the fruit. Unexpected internal gradients in terms of transcripts, metabolites, and hormones were observed, suggesting that the maturation-ripening syndrome proceeds according to an internal gradients following a "starter signal" that is next to be identified in experiments that are currently being carried out.

Poster # 109 On the presence of *nos*F gene in the four aquatic and terrestrial *Nostoc* strains revealing antifungal and trypsin inhibitor activity Ramezan-Ali Khavari-Nejad, Taher Nejadsattari (Chemical biology)

Bahareh Nowruzi [1], Ramezan-Ali Khavari-Nejad[1, 2], and Taher Nejadsattari[1]
[1]*Department of Biology, Science and Research Branch, Islamic Azad University,* [2]*Faculty of Biological Sciences, Kharazmi University*

Cyanobacteria are one of the most widespread microorganisms on earth, and they are found in almost all ecosystems, from fresh and marine water to terrestrial environments. *Nostoc* strains received a great deal of attention as prolific producers of bioactive secondary metabolites which are highly toxic to humans and other animals. Discovery of several dead dogs, mice, ducks, and fish around paddy fields, prompted us to study the toxic compounds and characterize novel bioactive natural products from four *Nostoc* strains isolated from aquatic and terrestrial paddy fields. Results of molecular analysis demonstrated that the only two aquatic strains contain the *nosF* gene and a remarkable antifungal activity was identified in the methanolic extracts. While trypsin inhibitor was detected from two terrestrial strains. Based on the results, this study suggests that methylproline-containing compounds in two aquatic strains might account for the death of these animals. This case is the first documented incident of toxicity from aquatic cyanobacteria related intoxication in dogs, mice, and aquatic organisms in Iran.

Keywords**:** Natural bioactive compound; *Nostoc*; Toxicity; Antifungal; *nos*F gene

Poster # 110 OsHRT3, a high temperature responsive transcription factor, is involved in pre-harvest sprouting in rice (Reproductive development)

Hua Zhang[1], Min Yu[1], and Ying Zhu[1]
[1]Institute of Virologyand Biotechnology, Zhejiang Academy of Agricultural Sciences

Seed dormancy is an important agricultural trait closely related with yield and grain quality in crop. However, the mechanisms regulating dormancy remain unclear. Here, we identified a transcript factor *OsHRT3* involved in the regulating pre-harvest sprouting in rice. Expression analysis results showed that *OsHRT3* was specifically expressed in rice endosperm and highly induced by high temperature. Transgenic rice plants with RNA interference (RNAi) of *OsHRT3* exhibited pre-harvest sprouting phenotype and the expression of *α-amylase* genes were significantly up-regulated in immature RNAi seeds. Over-expression (OE) of *OsHRT3* displayed a GA deficient phenotype, such as dwarf, late-flowering, low fertility, and germination rate of OE seeds was much lower than that of the wild type (WT). The level of bioactive gibberellic acid (GA) precursors of OE plants was detected lower than that of the WT, and exogenous GA could partially rescue the GA deficient phenotype. Expression of GA-20-oxidase (*GA20ox*), a key gene for GA biosynthesis, was found down-regulated in OE plants, but up-regulated in RNAi plants. Subcellular localization assay showed that OsHRT3 protein was mainly localized in nucleus. However, no trans-activation activity of OsHRT3 can be detected by using yeast one hybrid system**.** Thus, *OsHRT3* should be an important transcript factor involved in the regulation of seed dormancy, and it might act as a transcription repressor of *GA20ox* in developing rice seeds. Further study on this protein and other key components in this process will help us to elucidate the molecular mechanism of seed dormancy and control the pre-harvest sprouting in crop.

Keywords: rice, pre-harvest sprouting, endogenous hormone, regulation mechanism

Poster # 111 Overexpression of bacterial halogenases in Arabidopsis thaliana to generate new chlorindol-3-acetic acids (Hormone Interactions)

Antje Walter[1], Jutta Ludwig-Müller[1], Lorenzo Caputi[2], and Sarah E. O'Connor[2]
[1]TU Dresden, [2]John Innes Centre

Over the last few decades nat ural products became more import ant for many areas of medicine, pharmacology and agriculture. Many important antibiotics and anticancer agents are based on natural products. The introduction of a halogen (halogenation) into natural products can improve the bioactivity and bioavailability. It is already know n that the halogenated plant growth hormone 4-chlorindole-3-acetic acid (4-Cl-IAA) show s a higher activity than indole-3-acetic acid. The introduction of a halogen in the metabolism of medically important plants could lead to a variety of novel plant metabolic products w ith improved properties. A regioselective incorporation of a halogen atom can be achieved by using bacterial flavin-dependet halogenat es. We use the model plant *Arabidopsis thaliana t o* introduce halogen atoms at various positions to generate halogenated indole acetic acids and related compounds.

Poster # 112 Phosphorylation of FUSCA3 by SnRK1 is required for reproductive development and seed vigor at high temperatures in Arabidopsis (Abiotic stress)

Aaron Chan1,2, Carina Carianopol[1,2], Allen Tsai[1,2], Kresanth Varathanajah[1] and Sonia Gazzarrini[1,2]
[1] Department of Biological Sciences, University of Toronto, [2] Department of Cell and Systems Biology, University of Toronto

Seed maturation is orchestrated by a network of transcription factors, which directly regulate hormone biosynthesis or signaling and are themselves regulated by hormones. For example, the B3 domain FUSCA3 (FUS3) promotes ABA biosynthesis, and ABA in turn positively regulates FUS3 by an unknown mechanism (1). Recently, the catalytic subunit of the SnRK1 kinase complex, AKIN10, was shown to phosphorylate FUS3 (2). SnRK1/AMPK/Snf1 kinases are energy sensors conserved among eukaryotes and are activated during stress to inhibit growth and conserve energy. Interestingly, AKIN10 is dephosphorylated and inactivated by PP2C phosphatases, which are negative regulators of ABA signaling (3). To understand the role of AKIN10-mediated FUS3 phosphorylation we first analyzed AKIN10 expression pattern. AKIN10-GFP is expressed throughout embryogenesis and during germination, and its localization pattern overlaps with that of FUS3. In agreement with this, we show that FUS3 is phosphorylated by AKIN10 during both embryogenesis and germination, suggesting their interaction is biologically relevant. We generated transgenic plants that express FUS3 phosphonull (S>A) and phosphomimic (S>D) constructs. Both constructs can rescue the *fus3-3* mutant under normal growth conditions; however, the FUS3 phosphonull construct is unable to rescue *fus3-3* at high temperatures. Both embryonic and vegetative growth of FUS3 phosphonull plants are affected at high temperature, resulting in reduced seed yield, desiccation intolerance, lower seed vigor and seedling survival. This data indicates FUS3 phosphorylation by AKIN10 plays an essential role in seed development and seed vigor at high temperature.

1. ***Gazzarrini et al.*** (2004) The transcription factor FUSCA3 controls developmental timing in Arabidopsis through the hormones gibberellin and abscisic acid. *Dev. Cell*, **7**, 373–385.
2. ***Tsai and Gazzarrini*** (2012) AKIN10 and FUSCA3 interact to control developmental phase transitions and lateral organ development in Arabidopsis. *Plant J.* **69**, 809–821.
3. ***Rodrigues et al.*** (2013). ABI1 and PP2CA phosphatases are negative regulators of Snf1- related protein kinase1 signaling in Arabidopsis. *Plant Cell*. **25:** 3871-3884.

Poster # 113 Physiological role of strigolactones in rice leaves (Hormone Interactions)

Yusuke Yamada[1], Akira Oikawa[2], Kazuki Saito[3], Koichiro Shimomura[1], Shinjiro Yamaguchi[1], and Mikihisa Umehara[1]
[1]*Graduate School of Life Sciences, Toyo University,* [2]*Fac. Agric., Yamagata Univ., RIKEN CSRS,* [3]*RIKEN CSRS*

Strigolactones (SLs) act as plant hormones that regulate leaf senescence as well as shape plant architecture. Previously, we found that SLs accelerate leaf senescence in response to phosphate (Pi) deficiency. Leaf senescence is a critical event for nutrient translocation from old leaves to young tissues. Regulation of leaf senescence by SLs contributes to nutrient translocation in rice. In this work, to investigate when SLs are produced during leaf senescence under Pi deficient and sufficient conditions, we analyzed expression levels of SL-related genes in third leaves by qRT-PCR. In addition, we also performed comprehensive analysis of primary metabolites using CE-MS in wild type (WT) and *dwarf* (*d*) mutants to evaluate whether SLs affect regulation of primary metabolism. Expression levels of SL biosynthetic genes (*D10, D17, D27* and *OsMAX1* homologues such as *Os900, Os1400* and *Os1500*) were elevated in response to progression of leaf senescence under Pi deficient condition. In SL signaling genes, *D3* mRNA levels decreased under Pi sufficient condition, whereas *D14* mRNA levels did not change between Pi deficient and sufficient conditions. In addition, CE-MS analysis demonstrated that amino acid, especially Gln and Asn levels decreased in *d* mutants compared with WT under both Pi conditions. We analyzed expression patterns of Gln synthetase (GS)/Glu synthase (GOGAT) cycle and Asn metabolism-related genes in rice leaves by qRT-PCR. Among genes we checked, *GS1;1* levels were significantly reduced in all *d* mutants regardless of Pi conditions, resulting in reducing Gln and Asn levels in *d* mutants. GS1;1 is a key enzyme of Gln synthesis in senescent leaves. We suggest that SL signaling is required for leaf senescence regulation and Gln and Asn synthesis in rice leaves.

Poster # 114 PIN-mediated auxin efflux- its regulation and biochemical properties (Hormone transport)

Ulrich Hammes[1], Astrid Fastner[1], Martina Kolb[1], Melina Zourelidou[2], and Claus Schwechheimer[2]
[1]*University of Regensburg,* [2]*TU Munich*

The development and morphology of vascular plants is critically determined by synthesis and proper distribution of the phytohormone auxin. The directed cell-to-cell distribution of auxin is achieved through a system of auxin influx and efflux transporters. PIN-FORMED (PIN) proteins are proposed auxin efflux transporters, and auxin fluxes can seemingly be predicted based on the - in many cells - asymmetric plasma membrane distribution of PINs. We used a heterologous Xenopus oocyte system to show that PIN-mediated auxin transport is directly certain kinases of the Arabidopsis AGCVIII kinase family. We demonstrated that D6PK and PINOID, two AGCVIII kinases, display a differential phosphosite preference. We further determined the substrate specificity and kinetics of PINs in the heterologous system. Our data suggest that the properties of different PINs differ significantly and models of auxin transport are mor complex than previously thought.

Poster # 115 PLANT MEDIATED OXIDATION OF GR24 (Hormone metabolism)

Jakub Koristka[1], Rostislav Halouzka[1], Sanja Cavar Zeljkovic[1], and Petr Tarkowski[2]
[1]*Palacky University,* [2]*Crop Research Institute*

Strigolactones (SLs), as the newest class of phytohormones, they play a key role in the regulation of plant architecture, play a part in the rhizosphere as host detection signals for root parasitic plants (genus *Striga* and *Orobanchaceae*, as well as a branching factor for arbuscular mycorrhizal fungi.

All naturally occurring SLs share a common structure composed of a tricyclic lactone (the ABC part) connected via an enol ether bridge to a methylfuranone group (the D-ring). Their biosynthetic pathway is very complex and involves many enzymes in process of bioconversion to final signalling products which are exuded to rhizosphere in extremely low quantities. Since it is difficult to obtain reasonable amount of natural occurring SLs for their studies, they were replaced by synthetic analogues with a simpler structure but similar biological activity.

The study is focused on oxidation of GR24 by different plants species, such as pea, maize, tomato, etc. For this purpose, we developed a simple and efficient method for the isolation and purification of GR24 and its oxidation

products. Final analysis was carried out on UHPLC-MS/MS, and further supported by IR and Raman spectroscopy. We detected oxidised form of parent compound GR24, i.e. 4- hydroxy-GR24.

Poster # 116 Pleiotropic effects of spermidine on plant development due to altered expression of polyamine transporters (Hormone transport)

Sheaza Ahmed[1], Jigar Patel[1], Menaka Ariyaratne[1], and Paul Morris[1]
[1]*Bowling Green State University*

Spermidine is an essential plant metabolite, but apart from its role as a substrate needed for the hypusination of the translation elongation factor eIF-5A, little is known about how changes in the levels of spermidine mediate changes in gene expression. To evaluate the role of the spermidine-preferential transporter, *AtPUT5* (polyamine uptake transporter) on polyamine homeostasis, we created transgenic lines overexpressing a homologous gene from rice (*OsPUT1*) using both the CaMv and the PUT5 promoter. Under long days, wild type plants flowered at about 23 days while flowering was delayed by more than 2 weeks in the two over-expressing lines. These plants also had larger leaves, thicker stems and produced more flowers and seeds than wild type plants. In contrast, *put5* knockouts flowered 3-4 days earlier than wild type plants, produced fewer flowers, and had wispier stems that tended to droop. HPLC analysis of polyamine levels in the rosette leaves showed the delay of flowering was correlated with elevated spermidine and spermidine conjugate levels at two weeks. *CaMV::OsPUT1* but not *PUT5::OsPUT1* plants were also significantly delayed in senescence. Constitutive expression of the heterologous gene was correlated with sustained high levels of spermidine and spermidine conjugates in the rosette leaves at four and six weeks after planting. Although overexpression of PUTs results in the hyperaccumulation of spermidine and spermidine conjugates in the leaves, transient expression of GFP- tagged *AtPUT5* and *OsPUT1* in *N. benthamiana* shows that these transporters are localized to the ER. Thus it is possible that cytosolic levels may actually be lower. Earlier research in another lab has shown has shown that decreased expression of deoxyhypusine synthase, and thus lower levels of active eIF-5A, produces plants with similar phenotypes to those having *CaMv::OsPUT1* constructs. Experiments now in progress, will assess whether PUT transporters are functional, and are capable of sequestering spermidine in the ER.

Poster # 117 Potential for using a rhizospheric bacterium to control bacterial canker of tomato (*Solanum lycopersicum L.*) (Biotic interaction)

Yoko Takishita[1], Jean-Benoit Charron[1], and Donald Smith[1]
[1]*McGill University*

Bacterial canker disease, caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is one of the most destructive diseases of tomato. The disease can cause substantial crop losses and has become a serious concern for both field and greenhouse tomato production. Existing strategies, such as chemical treatments, have shown only limited efficacy in controlling this disease. Biological control of *Cmm* is a viable alternative to chemical controls, especially in an organic system. Our laboratory isolated several rhizospheric bacteria and found a strain that showed antagonistic activity against *Cmm in vitro*. The objectives of this research are to examine the degree of disease control exhibited by the biocontrol strain, and to study the activities of defense-related enzymes and the gene expression of defense-related metabolites in the tomato plants treated by the biocontrol strain. We conducted growth chamber experiments where two-week-old tomato seedlings were treated with our biocontrol strain by soil drench, followed by *Cmm* inoculation by needle-injection at the stem. We monitored the disease progress for three weeks, and sampled stems to estimate the *Cmm* population inside the tissue. We found that treatment with our biocontrol strain delayed the disease progress of bacterial canker as compared to the control treatment. Further experiments will be conducted to study the expression of genes related to production of plant hormones, such as salicylic acids and jasmonic acids, and the activities of defense-related enzymes, such as phenylalanine ammonia lyase, to explore the control mechanisms exerted by our biocontrol strain.

Poster # 118 PREPARATION AND BIOLOGICAL ACTIVITY OF KINETIN LIKE AROMATIC CYTOKININS Martin Hönig (Hormones & biotechnology)

Martin Hönig[1], Lucie Plíhalová[1], Lukáš Spíchal[1], Jiří Voller[1], Vladimír Kryštof[1] and Karel Doležal[1]
[1]*Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University*

Kinetin, a compound from aromatic cytokinin family, exhibits various anti-oxidative properties (Olsen, 1990). Kinetin delays leaf senescence in plants, protects DNA against oxidative damage and inhibits oxidative and glycoxidative protein damage (Verbeke, 2000). Together with its ability to delay age-related characteristics in human fibroblast cells and induce keratinocytes differentiation, kinetin has been utilized in anti-ageing skin care (McCullough, 2002). Since 1955 some kinetin derivatives have been prepared to enhance its biological activity. In 2008, 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine (Pyratine) was reported as more active in anti-senescence bioassay. In human clinical studies Pyratine also reduces signs of photodamaged skin, such as fine wrinkles, roughness and mottled hyperpigmentation significantly more efficiently than kinetin.

New pyratine-like derivatives were prepared as potential substances for cosmetics. Standard routes of preparation *via* nucleophilic substitution of 6-chlorpurine by appropriate amines were modified to obtain compounds in high purity and decent yields. Prepared derivatives were screened in variety of bioassays in plants, such as *Amaranthus caudatus* betacyanin test, *Tobacco* callus biotest and detached *wheat* leaf senescence (chlorophyll retention) biotest. These above mentioned bioassays revealed cytokinin activity of prepared derivatives. Potential anticancer activity and cytotoxity on animal cells were studied on tumor cells lines K-562 and MCF7. Toxicity of the compounds was also tested on BJ human fibroblast cells. Biological activity of these derivatives was put into relation with their structure.

Supported by the Ministry of Education Youth and Sports, Czech Republic (grant ED3.1.00/14.0302 PRE-SEED New technologies in chemistry and biology at UP and project Effective Transfer of Palacký University Olomouc Results into Praxis.

Poster # 119 Purification and characterization of (3*Z*):(2*E*)-hexenal isomerase and its application to enhance the production of (2*E*)-hexenal (Abiotic stress)

Mikiko Kunishima[1], Yasuo Yamauchi[1], Masaharu Mizutani[1], Masaki Kuse[1], Hirosato Takikawa, and Yukihiro Sugimoto[1]
[1]*Kobe University*

Green leaf volatiles (GLVs) are semiochemicals that modulate plant-insect and plant- plant interactions. Emission of GLVs, including (2*E*)- and (3*Z*)-hexenal, is enhanced by wounding in plants. Recently (2*E*)-hexenal was reported to induce expression of transcription factors *viz HSF*s, *DREB2A* and *ZAT*s. Furthermore, *Arabidopsis thaliana* treated with (2*E*)-hexenal exhibited enhanced abiotic stress tolerance. Conversion of (3*Z*)-hexenal to (2*E*)-hexenal had been proposed although the enzyme catalyzing the conversion remained unidentified. In this study (3*Z*):(2*E*)-hexenal isomerase (HI) was purified and characterized. Preliminary screening of crude enzyme preparationsobtained from fruits and leaves of several Solanaceous plants, including paprika (*Capsicum annum*), indicated conversion of (3*Z*)-hexenal to (2*E*)-hexenal. HI in paprika fruits (CaHI) was purified to homogeneity. SDS and Blue-Native PAGE indicated that CaHI is a homotrimeric protein of 110 kDa composed of 35 kDa subunits. The enzyme exhibited high activity at acidic and neutral pH, whereas the activity was negligible at pH9 or above. Based on internal amino acid sequence, determined by Edman analysis, the full length cDNA was obtained. Phylogenetic analysis showed that CaHI belongs to the cupin superfamily, homologous HIs are ubiquitous in planta and that three catalytic amino acid residues (His, Lys, Tyr) are conserved among the HIs. Enzymatic isomerization of (3*Z*)- hexenal conducted in deuterium oxide and subsequent analysis of the product by ^{1}H-NMR showed incorporation of a deuterium atom at the C4 position of (2*E*)-hexenal. Results of further experiments showed inhibition of enzymatic conversion of (3*Z*)-hexenal to (2*E*)- hexenal by 3-hexyn-1-al and a significantly

higher accumulation of (2*E*)-hexenal in transgenic tomato plants, expressing CaHI, than in the wild type. The results propose a pivotal role for HI in (2*E*)-hexenal production in planta and suggest that transgenic lines and inhibitors are potent tools to elucidate the role of (2*E*)-hexenal in abiotic stress.

Poster # 120 Quantitative proteomic analysis of auxin signaling during seedling development (Hormone genomics)

Dior Kelley[1], Zhouxin Shen[2], Steven Briggs[2], and Mark Estelle[2]
[1]Iowa State University, [2]University of California, San Diego

Auxin induces rapid gene expression changes throughout plant development. How these transcriptional responses translate to changes in protein abundance is not well characterized. Using an iTRAQ (isobaric tags for relative and absolute quantification) based quantitative proteomics approach we identified auxin regulated proteins in seedlings, roots and hypocotyls and at three different time points (30 min, 120 min and 3 hours). Proteins were identified and quantified as a single step using tandem mass spectrometry (MS/MS). These profiling experiments detected 4,701 proteins from seedling tissue, 6,740 proteins from root tissue and 4,145 proteins from hypocotyl tissue. Within each of these data sets 988 (seedlings), 198 (roots) and 744 (hypocotyl) proteins showed significant differential abundance between auxin treatment and controls. Comparisons between these datasets showed little overlap, suggesting that auxin proteomes exhibit both temporal and spatial specificity. In order to test this hypothesis of tissue specificity directly we selected approximately two dozen proteins that changed significantly in abundance upon auxin treatment for functional characterization. For these studies we focused on proteins that had not previously been linked to auxin pathways. Currently, insertion mutants in the corresponding genes are being evaluated for various auxin related phenotypes. These data provide the most extensive description of auxin-regulated proteomes to date and is an excellent resource for identifying novel downstream signaling components related to auxin-mediated plant growth and development.

Poster # 121 Reduced MTA Metabolism Affects Thermospermine Regulation of Auxin and Lipid Pathways (Vegetative development)

Benjamin Tremblay[1], Maye Saechao[1], Ishari Waduwara-Jayabahu[1], Hitoshi Sakakibara[2], and Barbara Moffatt[1]
[1]University of Waterloo, [2]RIKEN Yokohama Institute

5'-Methylthioadenosine nucleosidase (MTN) catalyzes the recycling of 5'- methylthioadenosine (MTA), a by-product of multiple biosynthetic pathways including those producing polyamines. In *Arabidopsis thaliana* MTN is encoded by two genes: *MTN1* (At4G38800) and *MTN2* (At4G34840). Double mutants that fail to adequately metabolize MTA experience feedback inhibition of MTA-producing enzyme activities, including polyamine synthases. This leads to a complex phenotype in a severe MTN mutant including disturbed vasculature as well as infertility. We used microarray analysis to determine the impact of MTN deficiency in this line. Here we report on our functional testing of the transcriptional changes identified in lipid and hormone metabolism related genes and their potential association to the polyamine content in this and a more moderate MTN mutant. Thermospermine was of particular interest because it has been shown to negatively regulate auxin signalling and affect vascular development. We determined the impact of MTN-deficiency on thermospermine regulation using the auxin sensitive reporter *DR5rev:GFP* and the SCF(TIR/AFB) inhibitor PEO-IAA. The impact of thermospermine mis-regulation on lipid metabolism was explored using the lipid stain Nile red and the lipid inhibitor fumonisin B1. Current work is aimed at determining the impact of MTN-deficiency on auxin and brassinosteroid homeostasis. We are also evaluating an RNAseq analysis of these two mutants to identify the transcriptional changes that are common to both.

Poster # 122 Regulation of diterpenoid phytoalexin biosynthesis mediated by the stress-inducible transcription factor DPF in rice (Hormone signaling)

Yumu Tabuchi[1], Ryo Tsutsumi[1], Keiichiro Nemoto[2], Tatsuya Sawasaki[2], Masaki Mori[3], Koji Miyamoto[4], Hisakazu Yamane[4], Nobutaka Mitsuda[5], Hideaki Nojiri[1], and Kazunori Okada[1]

[1]Biotechnology Research Center, The University of Tokyo, [2]Proteo-Science Center, Ehime University, [3]Disease Resistant Crops Research Unit, National Institute of Agrobiological Sciences, [4]Department of Biosciences, Teikyo University, [5]Research Institute of Genome-Based Biofactory, National Institute of Advanced Industrial Science and Technology

Phytoalexins production is one of the various defense responses in plants. DPF, a blast-induced bHLH-type transactivator, is responsible for the inductive production of diterpenoid phytoalexins (DPs). Previous study has shown that DPF transcriptionally activates expression of all of the DP biosynthetic genes and the promoter of the DP biosynthetic gene *OsCPS2* with dependence on N-box (CACGAG) in the promoter region. The expression of *DPF* in rice was shown to be induced after variety of stresses, including blast fungus infection, heavy metal stress, and jasmonic acid (JA) treatment, which is known to affect phytoalexin productions in rice. In this study, we focus on the mechanism of transcriptional regulation of DPF and downstream target genes for phytoalexins in JA signaling.

Promoter analysis of *DPF* revealed that 0.40 kbp to 0.35 kbp upstream region from the translational start site harboring G-box (CACGTG), a binding site of bHLH transcription factor, was most likely responsible for the JA inductive expression of *DPF*. Hence, transcription factors that bind to the G-box were screened by yeast one hybrid system. We found that OsMYC2 known as a central regulator in JA signaling binds to this region, suggesting that JA-inductive expression of *DPF* is likely to be directly regulated by OsMYC2 in JA signaling. We also investigated the regulation of downstream phytoalexin gene *OsCPS2* by DPF. Possible direct binding of DPF to the N-box in the *OsCPS2* promoter was experimentally supported by *in vitro* interaction using wheat germ cell-free protein in AlphaScreen system. Interestingly, however, western blot analysis exhibited predominant localization of DPF in cytoplasm but not in nucleus. These results imply that DPF might be translocated into nucleus under specific conditions, subsequently leading to up-regulation of *OsCPS2* expression. We are currently investigating whether DPF is translocated into nucleus in response to JA.

Poster # 123 Regulation of hormones, lipids and proteins in soybean V3 stage trifoliates by bacterial compounds Lipo-chitooligosaccharide and Thuricin 17 (Vegetative development)

Sowmyalakshmi Subramanian[1] and Donald Smith[1]

[1]Department of Plant Sciences, MacDonald campus, McGill University

Lipo-chitooligosaccharide (LCO) and Thuricin 17 (Th17) have been shown to stimulate plant growth in soybean under optimal conditions, with V3 stage the most responsive stage visually and quantitatively. This study explores the effects of LCO and Th17 on the regulation of phytohormones, lipids and proteins in soybean trifoliates at the V3 stage. Soybean plants at V3 stage were treated (root drench) with 10^{-6} M LCO and 10^{-9} M Th17 and the trifoliates collected at 24 h and 36 h post treatment. Lyophilized samples were quantified for phytohormones viz. IAA, cytokinins, gibberellins, abscisic acid; salicylic acid and jasmonic acid and conjugates using UPLC-ESI/MS-MS and lipids using GC. Total cytokinins showed no change between treatments or time points and total ABA was lower in both the treatments as compared to control at both 24 and 36 h. Total IAA with IIA-Asp and IAA-Glu conjugates were 15.21% higher in LCO and 31.11% higher in Th17 treatments than 36 h control plants. Th17 treatment increased total free SA by 65.89% at 24 h and total free JA by 74.85 % at 36 h. Amongst lipids studied, stearic acid levels increased significantly in LCO treated samples while homo γ-linolenic acid levels decreased significantly in Th17 treated samples at 36 h. Using LTQ-Orbitrap Velos, 1880 proteins were identified in the samples. Some of the key upregulated proteins in Th17 treated samples in comparison with control and LCO treatments were ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, ribulose-1,5-bisphosphate carboxylase small subunit, ATP synthase β-subunit and chlorophyll a/b binding protein type II

at both 24 and 36 h while rubisco activase was up-regulated at 36 h. This finding in part suggests that LCO and Th17 trigger different responses in soybean at 24 and 36 h post treatment and that Th17 appears to invoke stronger responses, probably promoting better plant growth.

Poster # 124 RNA-Seq analysis of radish (*Raphanus sativus* L.) agrobacterial tumours (Biotic interaction)

Alexander Tkachenko[1], Alexander Predeus[2], Irina Dodueva[1], and Lyudmila Lutova[1]
[1]*Laboratory of Plant Genetic and Cell Engineering, Saint-Petersburg State University,* [2]*Bioinformatics Institute*

Radish (*Raphanus sativus* L.) is a vegetable crop of great economic importance that is grown worldwide. Being a close relative to the model flowering plant, *Arabidopsis thaliana*, radish provides an opportunity to be used as a model for studying developmental processes in plants, such as meristem formation. Our studies were aimed to elucidate the early events of agrobacterial tumor formation with particular focus on signaling events that may be linked to the emergence of a new meristem.
As a source of plant material we used genetic collection of radish inbred lines of Saint Petersburg State University's department of Genetics and Biotechnology. In order to study processes in emergent irregular meristem in plants inoculated by *Agrobacterium tumefaciens* C58, we used RNA sequencing technique to compare inoculated and mock-treated plant tissues of two radish inbred lines differing from each other by the trait of spontaneous formation of tumor on the taproot. We obtained 146.3 million 2x100bp reads and utilized this data to *de novo* assemble transcriptome of radish with different transcriptome assemblers (Trinity, SOAPdenovo-Trans, Trans-AbySS). We then performed analysis of genetic variants distinct between the two radish lineages, as well as analysis of differential expression (using DEseq2 package) between inoculated and non-inoculated plant tissues harvested five days after inoculation. We annotated sets of differentially expressed genes (DEG) using GO terms and gene set enrichment analysis. Among genes upregulated in inoculated plants we found significant enrichment in GO terms related to stress response and cell wall formation (GO:0009628, GO:0042546, GO:0010410, GO:0010411). Downregulated genes were enriched in carboxylic acid metabolic process term (GO:0019752). A subset of DEGs was verified by qPCR analysis of additional biological replicates.

The project is supported by RSF grant № 16-16-100 11.

Poster # 125 Role of *Oryza sativa* pseudo-histidine phosphotransfer proteins in responses to abiotic stress
(Abiotic stress)

Yu-Chang Tsai[1] and Chia-Yun Lee[1]
[1]*Department of Agronomy, National Taiwan University*

Cytokinin two-component signaling involves in several important plant development and stress responses. Several studies have illustrated histidine phosphotransfer proteins (AHPs) mediate the stress tolerance in *Arabidopsis* and rice. AHP6, an inhibitory pseudohistidine phosphotransfer protein (PHP) in *Arabidopsis*, interacts with auxin signal and regulates vascular development. Here, we analyzed the role of rice OsPHPs in response to different abiotic stress. Three OsPHPs were identified in rice and highly expressed in seedling and booting stages. In response to the cytokinin treatment, the transcription of *OsPHP2* and *OsPHP3* were down-regulated in rice seedlings. *OsPHP3* expression was found to be induced significant by drought and salt stress, while expression of *OsPHP1* was repressed. *Osphp3*-insertion mutants were isolated and hypersensitive to the salt stress. Loss of OsPHP3 function is also impaired in the heat tolerance. Additional role of OsPHPs in response to the environmental stimuli will be presented.

Poster # 126 Role of plant hormones in flower and fruit development in tomato (Reproductive development)

Irina Panizel[1], Sayantan Panda[1], Asaph Aharoni[1], and Hagai Yasuor[2]
[1]Department of Plant Sciences, The Weizmann Institute of Science, [2]Department of Vegetable and Field Crops, Gilat Research Center, Agricultural Research Organization

Hormones play a pivotal role in most physiological processes in plants. The aim of this research was to elucidate the role of plant hormone from different chemical families during flower and fruit development. In order to elucidate which components participate in the hormone homeostasis and possible interaction of the different phytohormones we used transcriptome analysis to identify hormone-related genes using Illumina digital gene expression technology and by comparing the hormone metabolome using analytical hormone profiling method, in which the analytes are concentrated from plant extracts and separated by chemical properties using consecutive SPE followed by analysis on UPLC-ESI-MS/MS. The gene expression and metabolome were determined in different flower organs (sepals, petals, stamen, pollen, stigma and ovary) and fruit (embryo, seeds, placenta, jelly, pericarp+peel) during different developmental stages under control conditions. The hormonal metabolome data shows differential metabolome expression between flower organ, fruit organs and between tomato genotypes. The hormone content analysis shows a complicated spatial and temporal expression pattern of the hormonal metabolome during flower organ and fruit development. Such dynamic expression patterns might point on the important role of hormone homeostasis and interaction during developmental events. We are in the process of identifying transcripts for the different hormones biosynthesis, metabolism, transport, responsive genes, and response factors. More research is needed in order to clarify the hormone interactions during these developmental events.

Poster # 127 Root-derived active cytokinins regulate shoot growth in Arabidopsis (Hormone transport)

Asami Osugi[1], Mikiko Kojima[2], Yumiko Takebayashi[2], Takatoshi Kiba[2], and Hitoshi Sakakibara[3]
[1]NAGOYA University, [2]RIKEN, [3]RIKEN, Nagoya University

In order to optimize plant growth, organ-to-organ communication is necessary. Various signaling molecules such as RNAs, proteins and plant hormones are transported between distant organs as communication signals. Vasculature plays a central role in shoot-root translocation of the signals, including plant hormone cytokinins (CKs). Since major forms of CK in vascular sap are riboside-type precursors (CK-ribosides), it has been believed that plants use CK-ribosides as communication forms. However, vascular sap also contains a certain amount of active CK, whose role in the communication has not been investigated. Our recent study shows that root-derived active CKs play a significant role in some aspects of shoot growth regulation. Feeding experiment using labeled active CK (tZ) confirmed that active CK could be translocated as is from root to shoot. Grafting between wild-type (WT) root and *log* septuple mutant (*logS*) shoot, whose ability to generate active CKs is severely impaired, showed that some (but not all) phenotypes were recovered in *logS* shoot grafted on to WT root. These results suggest that root-derived active CKs regulate shoot growth independently of LOG function. We will discuss the functional difference between active CKs and CK-ribosides based on additional grafting experiments using CK transport and metabolism mutants.

Poster # 128 Screening of chemicals that affect protonemal differentiation of a moss *Physcomitrella patens* (Hormone perception)

Masatoshi Nakajima[1], Che-Dong Yang[1], Sho Miyazaki[1], Seung-Hyun Park[1], Masato Otani[1], Hiroshi Kawaide[2], Ken-ichiro Hayashi[3], Tadao Asami[1]
[1]University of Tokyo, [2]Tokyo University of Agriculture and Technology, [3]Kayama University of Science

In a moss *Physcomitrella patens*, any gibberellin has not been detected, whereas the early steps for its biosynthesis are physiologically required in it. Namely, we previously reported that the mutant lines for its *ent*-copalyl diphosphate synthase/*ent*-kaurene synthase (*Ppcps/ks*) which starved for *ent*-kaurene, showed a significant inhibition of their protonemal differentiation. And the inhibition was fully recovered by the

application of either *ent*-kaurene or *ent*-kaurenoic acid, but not by that of gibberellins. So it strongly suggests that any *ent*-kaurenoic acid metabolite different from gibberellins should regulate the process of the differentiation. We are very interested in the regulation of its signal perception and transduction, and the identification of the unknown metabolites from *ent*-kaurenoic acid is now proceeding. To support this approach, we performed the screening of the chemicals that promoted the differentiation of the mutants from the commercially-available chemical library, those of which are expected to share the signal transduction via the perception of the physiologically-active *ent*-kaurenoic acid metabolite.

Poster # 129 Seed and auxin regulation of TIR1 auxin co-receptors in pea fruit (Reproductive development)

Charitha Jayasinghege[1], Jocelyn Ozga[1], and Dennis Reinecke[1]
[1]*Plant BioSystems Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta*

Pea fruit development requires the presence of seeds. Removal of seeds from young pea fruit leads to fruit senescence and abscission. Treatment of deseeded pericarps with naturally occurring pea auxin 4-chloro-indole-3-acetic acid (4-Cl-IAA) mimics the role of seeds and rescues pea pericarp growth, and the other naturally occurring auxin indole-3-acetic acid (IAA) does not. To examine if the differential effect of these auxins on pea pericarp growth might be mediated in part through TIR1/AFB auxin co-receptors, we cloned the pea homologs of the TIR1 genes, functionally characterized these gene in Arabidopsis, and determined the effects of each auxin on *PsTIR1a* and *PsTIR1b* transcript abundance in pea pericarp assays. To determine whether the protein products of the putative pea auxin co-receptor genes *PsTIR1a* and *PsTIR1b* were functional, we introduced these *PsTIR1* genes driven by the *AtTIR1* promoter into *Attir1-10* and *Attir1-10afb2-3* plants and showed that the expression of each gene restores the normal auxin response to mutant seedling root growth. In pea, seed removal elevated pericarp *PsTIR1b* transcript abundance. Application of 4-Cl-IAA to deseeded pea pericarps reduced the transcript abundance of *PsTIR1b*, but IAA application did not. Deseeding and auxin application minimally affected pericarp *PsTIR1a* transcript abundance. The auxin signaling pathway was functional in deseeded pericarps treated with either 4-Cl-IAA or IAA, as DR5-GUS gene expression was detected in deseeded pericarps (from DR5-GUS expressing pea plants) treated with either auxin. Auxin application also appeared to minimally affect *PsTIR1a* or *PsTIR1b* transcript abundance in 4 days after imbibition pea root tips, epicotyls and plumules. These data suggest that pericarp *PsTIR1b* gene expression is modulated by seeds and 4-Cl-IAA, but not IAA. It is possible that seed and 4-Cl-IAA modulation of the auxin co-receptor pool (PsTIR1b in this case) is part of the mechanism for regulation of auxin response in pericarp tissue.

Poster # 130 Selection and characterization of chemicals that modulate the effect of high temperature on abscisic acid and gibberellin action in Arabidopsis seeds (Hormone Interactions)

Naoto Kawakami[1], Shota Higashi[1], Tomoki Kishigawa[1], Shizuka Kojo[1], Seiya Chikamatsu[1], Motoki Yamaguchi[1], and Otowa Kameoka[1]
[1]*Department of Life Sciences, Meiji University*

Suppression of seed germination by ambient high temperature during summer is essential for winter annual plants to germinate and establish seedling growth in autumn. High temperature enhances abscisic acid (ABA) biosynthesis, and ABA suppresses expression of gibberellin (GA) biosynthesis genes in Arabidopsis seeds. In this study, we took chemical genetic approach to find the factors which are involved in the hormone regulation at high temperatures. We screened chemical libraries containing 28,679 compounds, and selected 27 chemicals which inhibit germination at permissive warm temperatures (ca. 30°C) but do not inhibit germination at 22°C. Most of the selected chemicals inhibit germination of ABA deficient mutant seeds, but seven out of the 27 chemicals showed little effect on the mutant seeds. In addition, five out of the seven chemicals enhanced expression of ABA biosynthesis genes, *ZEP* and *NCED9* at 30°C but not at 22°C in the imbibed seeds. Three out of the five chemicals suppressed expression of GA biosynthesis gene, *GA3ox2* at 30°C but not at 22°C. We also selected eight chemicals which induce germination at non-permissive high temperatures (ca. 34°C). One of the eight chemicals induced germination of the seeds in the presence of GA biosynthesis inhibitor, paclobutrazol

or prohexadione at 22°. Other two chemicals showed only a little effect on germination of karrikin insensitive mutant seeds (*max2* and *kai2/htl*) imbibed at non-permissive high temperatures. These chemicals may be valuable to identify novel factors that are involved in the regulation of seed germination and hormone action in response to high temperature.

Poster # 131 Selection of Arabidopsis germination mutants that have defect in abscisic acid and gibberellin regulation at high temperature (Hormone Interactions)

Masanori Kaji[1, 2], Shione Kawano[1], and Naoto Kawakami[1]

[1]Department of Life Sciences, Meiji University, [2]Technology Development Center, Sumika Agrotech Co., Ltd.

Germination of winter annual seeds is inhibited by ambient high temperature during summer, and this enables the seeds to germinate in autumn. High temperature suppresses germination of Arabidopsis seeds by up-regulating abscisic acid (ABA) biosynthesis genes and down-regulating ABA catabolism and gibberellin (GA) biosynthesis genes. We have found that *PIL5* and *SOM* are involved in the hormone gene regulation at high temperature in a specific light condition. To further identify genes that are responsible for the regulation of ABA and GA levels in response to the super-optimal temperature, we first selected 31 lines of high temperature resistant germination mutants from more than 32,000 lines of ethyl methanesulfonate mutagenized populations of Arabidopsis. The seeds of nine out of the 31 lines showed almost normal ABA sensitivity and GA requirement for germination. The seeds of the three lines (TRL14-3, TRL19-2, LE23-4) out of the nine did not increase the transcript level of an ABA inducible gene, *EM6* in response to high temperature. The seeds of the three lines greatly reduced the ability of regulating not all but some of the ABA metabolism and GA biosynthesis genes in response to high temperature. These results suggest that the three lines have defect in the regulation process of ABA and GA levels in response to high temperature in Arabidopsis seeds. The mutation of TRL19-2 was mapped on the bottom arm of chromosome 2, and we are now in the course of fine mapping.

Poster # 132 Single amino acid substitutions modify the composition and activity of jasmonate-related transcription factor complexes (Hormone signaling)

Jonas Goossens[1], Debora Gasperini[2], Gwen Swinnen[1], Robin Vanden Bossche[1], Edward E. Farmer[2], Laurens Pauwels[1] and Alain Goossens[1]

[1]Department of Plant Systems Biology, VIB, Department of Plant Biotechnology and Bioinformatics, Ghent University, [2]Department of Plant Molecular Biology, University of Lausanne

Jasmonate (JA) is an important phytohormone involved in several developmental processes and in plant defense, activating the production of specialized metabolites. Central in the primary signaling of the JA pathway is the key transcription factor (TF) MYC2 which is repressed by a family of 13 JAZ proteins in the absence of JA. Here we successfully generated two superactive constructs of Arabidopsis MYC2 by substituting amino acids in its sequence to modulate its regulation. Both variants of MYC2 were able to escape from interaction with nearly all JAZ proteins, leading to increased transactivation activity. Overexpression of these constructs in Arabidopsis resulted in an activation of the JA and abscisic acid response. The same strategy is now implemented to further characterize PEAPOD2 (PPD2), a protein that is closely related to the JAZ, and a negative regulator of meristimoid division. Single changes in the amino acid sequence of PPD2 induce interaction with JA- related bHLH TFs. Furthermore, we have molecular evidence that PPD2 confers repressing activity to one of these bHLH-type candidates. PPD2 was generally assumed to be a DNA-binding protein, however, our findings suggest a new model in which PPD2 mediates transcriptional repression in an indirect manner via interaction with a JA-related TF. Currently, this model is being further validated *in planta*. In summary, our results underscore the possibility of modulating the activity of TF complexes by changing the interaction strength of the proteins involved. This strategy can contribute to current knowledge about TF complexes and lead to the creation of superactive TFs, which can ultimately be used for metabolic engineering of medicinal plants or crops.

Poster # 133 SMALL ACIDIC PROTEIN 1 (SMAP1) and SCF^{TIR1} ubiquitin proteasome pathway act in concert to regulate 2,4-dichlorophenoxyacetic acid-mediated alteration of actin in Arabidopsis root (Hormone signaling)

Maho Takahashi[1], Kana Umetsu[1], Takumi Higaki[2], Yutaka Oono[3], Elison B. Blancaflor[4], and Abidur Rahman[1]
[1]*Iwate University,* [2]*The University of Tokyo,* [3]*Japan Atomic Energy Research Institute,* [4]*The Noble Foundation*

2,4-dichlorophanoxyacetic acid (2,4-D), a functional analogue of auxin, is used as an exogenous source of auxin as it evokes physiological responses like the endogenous auxin, Indole-3-acetic acid (IAA). Previous molecular analyses of the auxin response pathway revealed that IAA and 2,4-D share common signaling pathways to elicit downstream physiological responses. However, recent findings with 2,4-D specific mutants suggested that 2,4-D and IAA might also use distinct pathways to regulate Arabidopsis root growth. Using genetic and cellular approaches, here we demonstrate that the distinct effects of 2,4-D and IAA on actin filament organization partly dictate the differential responses of roots to these two auxin analogues. 2,4-D but not IAA altered the actin structure in long term and short term assays. Analysis of the 2,4-D specific mutant *aar1-1* revealed that Small Acidic Protein 1 (SMAP1) acts as a positive regulator for the 2,4-D-induced alterations of actin. The ubiquitin proteasome mutants, *tir1-1* and *axr1-12*, which show enhanced resistance to 2,4-D compared with IAA for root growth inhibition, were also found to have less disrupted actin filament networks after 2,4-D exposure. Consistently, chemical inhibitor of the ubiquitin proteasome pathway mitigated the disrupting effects of 2,4-D on the organization of actin filaments. Roots of the double mutant *aar1-1 tir1-1* also showed enhanced resistance to 2,4-D-induced root growth inhibition and actin degradation compared with their respective parental lines. Collectively, these results suggest that the effects of 2,4-D on actin filament organization and root growth is mediated through synergistic interactions between SMAP1 and SCF^{TIR1} ubiquitin proteasome components.

Poster # 134 Stress induced formation of axillary buds in sunflower (*Helianthus annuus* L.) due to interactive effect of auxin to cytokinin ratio (Hormone Interactions)

Nagarathna, T.K[1] and Shadakshari, Y.G.[1]
[1]*University of Agricultural Sciences, GKVK campus*

An experiment was conducted to study the effect of stress on axillary bud initiation and its growth in sunflower. Nine different treatments including nutrient stress, moisture stress and their combinations were imposed. During the experimental period, plants were examined for the presence or absence of buds. At the end of the experiment, the number and percentage of plants exhibiting axillary buds and average number of buds in different treatments were computed. During vegetative growth of plants, observations were recorded on number of leaves per plant, plant height, SPAD chlorophyll meter readings and leaf area were measured. Five plants each with and without axillary buds were selected to estimate plant growth hormones *viz.,* auxin, cytokinin and reducing sugar (glucose). These plants were cut at each node at leaf axil and kept in a beaker separately containing methanol for growth hormones and ethanol for reducing sugar. Nodes were allowed to diffuse overnight. This diffusate was collected to quantify growth hormones by cucumber root inhibition bioassay using 2, 4-dichloro phenoxy acetic acid for auxins and cucumber cotyledon expansion bioassay using benzyle adenine for cytokinins. Dintro salicylic acid method was followed for reducing sugar estimation. The experimental results indicated that there was a significant difference in axillary bud production. Per cent axillary buds were more in plants with nutrient stress (53.27%) followed by early moisture stress (48.93%) and with their combination of treatment (38.03%). Late moisture stress did not have any influence on bud growth. At harvest, leaf, stem and flower heads were separated and their dry weight was taken to get total dry matter (TDM). Mean leaf and dry weight was more in plants with axillary buds. Hence the TDM was more in those plants (106.34g/pl), whereas not much difference was observed in plants without axillary buds (39.73g/pl) when compared to plants with axillary buds (35.43g/pl). Data on growth hormones indicated that, auxin concentration was high in first node and it was reduced towards the base of the plant in plants with axillary buds. It was vice versa with cytokinin content. Cytokinin concentration was more near the base and less towards the top of the plant where auxin content was high. The growth hormone concentration was not altered in plants without axillary buds

wherein, auxin content was maintained through out the plant system due to its basipetal movement which inhibited bud out growth. These results suggest that alteration in the ratio of auxin and cytokinin may induce axillary bud growth under stress conditions. Reducing sugar (glucose) content was shown to be high in plants with axillary buds (140.34ug/bud) and it was less in plants without axillary buds (65.1134ug/bud). Especially top most nodes showed more glucose content. This result indicates that plants with axillary buds may be producing more photosynthates, hence the increase in TDM. The three years data on growth hormones suggests that, auxin to cytokinin ratio may get altered during stress. When auxin content reduces, cytokinin content gets activated, leading to the growth of axillary buds due to more availability of photosynthates, which accounts for high TDM. The results of these experiments also indicate that, the plants with axillary buds may not affect the grain yield as the required photosynthates have already been translocated to the main sink (flower head).

Poster # 135 Stress responses of the biosynthetic genes for momilactones in chemical defence in the moss *Hypnum plumaeforme* (Biotic interaction)

Kazunori Okada[1], Hiroshi Kawaide[2], Koji Miyamoto[3], Sho Miyazaki[4], Masahiro Natsume[2], Hideaki Nojiri[1], Masatoshi Nakajima[4], Hisakazu Yamane[3], Hiroshi Nozaki[5], and Ken-ichiro Hayashi[5]

[1] Biotechnology Research Center, The University of Tokyo, [2] Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3 Department of Biosciences, Teikyo University, 4 Department of Applied Biological Chemistry, The University of Tokyo, 5 Department of Biochemistry, Okayama University of Science,

Momilactones, which are diterpenoid phytoalexins with antimicrobial and allelopathic functions, have been found only in rice and the moss *Hypnum plumaeforme*. Although these two evolutionarily distinct plant species are thought to produce momilactones as a chemical defence, the momilactone biosynthetic pathway in *H. plumaeforme* has been unclear. Here, we identified genes encoding *syn*-pimara-7,15-diene synthase (HpDTC1) responsible for the first step of momilactone biosynthesis and momilactone A synthase (HpMAS) involving the last step of the pathway in the moss. HpDTC1 is a bifunctional diterpene cyclase that catalyses a two-step cyclization reaction of geranylgeranyl diphosphate to syn-pimara-7,15-diene. HpMAS shares 53 % amino acid identity with rice orthologue OsMAS and shows dehydrogenase activity to catalyze conversion from *syn*-pimara-7,15-diene-olide to momilactone A. Both *HpDTC1* and *HpMAS* transcriptions were up-regulated in response to abiotic and biotic stress treatments. In this study, we further focus on the regulation of *HpDTC1* under various stress conditions including treatment by $CuCl_2$, chitosan, jasmonates, and infection of a pathogen *Botrytis cinerea*. *HpDTC1* promoter-GUS analysis in transgenic *Physcomitrella patens* showed similar transcriptional responses as *H. plumaeforme* to these stresses, suggesting that a common response system to stress exists in mosses. Regarding response to jasmonates, jasmonic acid (JA), a potent signalling molecule for inducing plant defences, could not activate *HpDTC1* expression. In contrast, 12-oxo-phytodienoic acid, an oxylipin precursor of JA in vascular plants, enhanced *HpDTC1* expression and momilactone accumulation, implying that as-yet-unknown oxylipins could regulate momilactone biosynthesis in *H. plumaeforme*. These results demonstrate the existence of an evolutionarily conserved chemical defence system utilizing momilactones and suggest the molecular basis of the regulation for inductive production of momilactones in *H. plumaeforme*.

Poster # 136 Strigolactone Promotes Light-adapted Development in Arabidopsis thaliana Depending on STH7 Function (Hormone signaling)

Jutiporn Thussagunpanit[1], Yuko Nagai[1], Takeshi Nakano[2], Hidemitsu Nakamura[1], and Tadao Asami[1]

[1] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, [2] RIKEN Advanced Science Institute

Light-adapted development in plant is controlled by light signals and multiple phytohormones. Strigolactones (SLs) have been reported to regulate photomorphogenesis. Moreover, previous report showed that SLs could up-regulate *STH7*, encoding a transcription factor belonging to the double B-box zinc finger subfamily. In this study, we used wild type, 3 lines of *STH7*- overexpressing (*STH7ox-2, ox-3* and *ox-5*) and 3 lines of functionally defective *STH7* (*STH7- SRDX; STH7-SRDX-2, SRDX-4* and *SRDX-5*) mutants to investigate a role of SL on light-adapted growth of *Arabidopsis thaliana.* Firstly, the addition of GR24, a synthetic SL, reduced hypocotyl elongation of wild type under weak light condition. Moreover, all of *STH7ox* mutants showed the decrease in hypocotyl length by GR24 treatment under weak light, but all of *STH7- SRDX* mutants did not. Next, *Arabidopsis* were grown under darkness for a week and transferred to light for de-etiolation and grown for 12 hr. After that cotyledons were cut for chlorophyll and anthocyanin extraction. The results showed that *STH7ox-3* and *ox-5* significantly increased the total chlorophyll and anthocyanin contents when de-etiolated with light. Lastly, *Arabidopsis* wild type, *STH7ox-3* and *STH7-SRDX-5* were selected to examine the expression level of photosynthesis related genes. *Arabidopsis* were grown under darkness for 4 days and then incubated with 1/2MS media with or without GR24 for 12h or 24h, respectively. GR24 treatment significantly increased *LHCB1*, encoding the light-harvesting chlorophyll a/b binding protein, and *rbcS*, encoding the small subunit of rubisCO, especially in *STH7ox-3* and wild type at 24h- incubated with GR24. Furthermore, *STH7-SRDX-5* showed the lowest *rbcS* transcription level at 12h-incubated with GR24 implying that *STH7* might be important for photosynthesis related genes expression. Based on the above results, we conclude that SL induces light-adapted development of *Arabidopsis* depending on STH7 manner.

Poster # 137 Structural and functional analysis of gibberellin 2-oxidase in rice (Hormone metabolism)

Sayaka Takehara[1], Kyosuke Kawai[1], Bunzo Mikami[1], Makoto Matsuoka[1], and Miyako Ueguchi (Tanaka)[1]

[1]Nagoya University

Gibberellins (GAs) are plant hormones that play a key role in plant growth and development, including seed germination, stem and root growth, flower induction and fruit set. A major catabolic pathway for GAs is initiated by a 2β-hydroxylation reaction catalyzed by gibberellin 2-oxidases (GA2oxs). This enzymes belong to the 2-oxoglutarate (2OG)-Fe oxygenase superfamily. We show here that a crystal structure of OsGA2ox3 complexed with 2OG and GA_4 substrate. In overall structure, disulfide-linked dimer have taken tetramer structure of two side-by-side dimer. GA_4 was present at each of the four active site of OsGA2ox3. Surprisingly, GA_4 was also observed between the dimer and the dimer. The present study aimed to clarify the relationship between the formation and the enzyme activity of tetramer structure.

Gel filtration and BiFC analysis detected tetramer form. These findings suggest that it has taken a maltimer structure even in *planta* as well as in *vitro.* In addition, a tetramer and monomer structure were isolated using gel filtration and investigated their enzyme activity. These results provide an idea that increasing GA4 concentration is able to form tetramer and increases reaction rate, such as to promote the GA metabolism. Moreover, we generated the knockout mutant of *OsGA2ox3*,to clarify the role of OsGA3ox1 *in planta* using CRISPER/Cas9 system. The mutant stems had more extension compared with wild-type rice plant.

Poster # 138 Structure-based development of novel potent antagonists of soluble ABA receptors (Chemical biology)

Naoki Mimura[1], Jun Takeuchi[2], Naoki Asahina[2], Noriaki Kawashima[2], Masanori Okamoto[3], Toshiyuki Ohnishi[1,2,4], and Yasushi Todoroki[1,2,4]

[1]Graduate School of Science and Technology, Shizuoka University, [2]Department of Agriculture, Shizuoka University, [3]ALRC, Tottori University, [4]RIGST, Shizuoka University

Abscisic acid (ABA) signals are believed to be transduced through the soluble ABA receptors PYR/PYL/RCARs (PYLs). PYLs change their conformation depending on ABA binding to inhibit the clade A protein phosphatases 2Cs (PP2Cs), which are negative regulators of ABA signaling. On the basis of the crystal structures of PYL-ABA-PP2C complex, Takeuchi *et al.* developed a PYLs antagonist, 3'-hexylsulfanyl-ABA (AS6), which binds PYLs with an affinity comparable to that of ABA, inhibits ABA-induced PYL-PP2C interactions through direct steric hindrance by its *S*-hexyl chain, and consequently although not completely blocks plant ABA responses (*Nat Chem Biol* **2014**, *10*, 477-482). In this study, we developed more potent PYLs antagonists than AS6 in focusing on the 4'-position of ABA that is involved in key interactions between PYL-ABA and PP2C.

We designed and synthesized novel ABA analogs (named as PANs) with an aromatic ring linked by the *O*-propynyl chain at the 4'-position of ABA. The aromatic ring was introduced to not only inhibit PYL-PP2C interactions but also enhance binding affinity to PYLs by making the □-□ interaction with the phenyl ring of PYLs-Phe that interacts with the indole ring of PP2Cs-Trp, which forms a water-mediated hydrogen bond with the 4'-carbonyl oxygen of ABA, in PYL-ABA-PP2C complexes. We tested phenyl, methylphenyl, methoxyphenyl, acetylphenyl and trifluoromethylphenyl rings as the aromatic ring. As expected, all the PANs bound almost 10-fold more tightly to PYL5 and suppressed the ABA-induced PYL-PP2C interactions and the ABA-induced inhibition of Arabidopsis seed germination and seedling growth more greatly than AS6 did. Moreover, PAN with a methylphenyl ring, PANMe, strongly alleviated also the thermoinhibition of Arabidopsis seed germination.

Poster # 139 Studies on the effects of a pesticide isoprothiolane on root growth (Hormone Interactions)

Miyuki Kusajima[1], Mai Inoue[1], and Hideo Nakashita

[1]Fukui Prefectural University

Isoprothiolane (diisopropyl-1, 3-dithiolan-2-ylidenemalonate) is a pesticide effective against rice blast disease and have been used practically in the fields. In addition, isoprothiolane was reported to regulate the growth of rice root. The micromolar levels of isoprothiolane promoted the growth of seminal root, whereas the higher concentrations prevent the growth. The previous report indicated the involvement of ethylene and auxin, however, was lacking for evidences at the molecular level. Then we analyzed the effect of isoprothiolane on the root growth in Arabidopsis, indicating that the elongation of primary root was enhanced at 12.5 µg/ml but was inhibited at the concentration higher than 37.5 µg/ml. Analyses using phytohormone-related mutants in combination with some phytohormone biosynthesis inhibitors indicated that jasmonate biosynthesis is also important for the root growth promotion by isoprothiolane. In addition to the primary root, the influence of this chemical on root hair was analyzed, indicating that isoprothiolane inhibited the elongation of root hair in a dose dependent manner. Analyses using phytohormone-related mutants indicated that the effect on root hair is independent of jasmonate, ethylene and auxin. The detailed mechanisms of the effects of isoprothiolane on root growth were under investigation.

Poster # 140 Study the function of EIN2 in the nucleus in ethylene response (Hormone signaling)

Fan Zhang[1], Likai Zhang[1] and siddharth rode Hong Qiao[1]
[1]*University of Texas at Austin*

My research is focus on how plant hormone affect plant growth and plant abiotic stress response. Ethylene is one of the most important plant hormones. It plays pleotropic roles in many aspects of plant life such as seeds germination, hypocotyl elongation, cell expansion, stem cell maintenance and adaption to stress conditions. Molecular genetic dissection has revealed that ethylene signaling pathway is function in a liner manner from the perception by the receptors on the ER membrane to EIN3 dependent transcriptional regulation in the nucleus. A model for hormone signaling has emerged in which the perception of ethylene by the receptors alters the activity of CTR1, which in turn, by an unknown mechanism, functions to relieve repression of EIN2, resulting in activation of EIN3/EIL1-dependent transcription and the activation of an ethylene response. EIN2, an ER membrane resident protein, is the key regulation of ethylene signaling, lose of its function cause completely ethylene insensitive phenotype. A few years ago, the study demonstrated that the F-box proteins ETP1 and ETP2 via 26S proteasome negatively regulate the levels of EIN2. Recently study showed that EIN2 triggers its endoplasmic reticulum (ER)– to–nucleus translocation. ER-tethered EIN2 shows CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) kinase–dependent phosphorylation. Ethylene triggers dephosphorylation at several sites and proteolytic cleavage at one of these sites, resulting in nuclear translocation of a carboxyl-terminal EIN2 fragment. These findings uncover a mechanism of subcellular communication whereby ethylene stimulates phosphorylation-dependent cleavage and nuclear movement of the EIN2-C' peptide, linking hormone perception and signaling components in the ER with nuclear-localized transcriptional regulators. The discovery of the functional mechanism of EIN2 opens a new avenue for fully understanding ethylene regulations. In this project, we focus on the function of EIN2 in the nucleus and its regulation in ethylene response.

Poster # 141 Sulfate deficiency stimulates strigolactone production in rice (Vegetative development)

Masato Shindo[1], Hanako Nakamura[2], Yusuke Yamada[1], Koichiro Shimomura[1], and Mikihisa Umehara[3]
[1]*Graduate School of Life Sciences, Toyo University,* [2]*Department of Applied Biosciences, Toyo University,*
[3]*Graduate School of Life Sciences, Toyo University, Department of Applied Biosciences, Toyo University*

Strigolactones (SLs) are a class of plant hormones that shape shoot branching as well as communication signals for parasitism and symbiosis in the rhizosphere. It has been reported that all-*trans*-β-carotene is converted into a precursor of SLs, carlactone by sequential reactions of DWARF27 (D27), D17, and D10. *OsMAX1* encodes cytochrome P450 that converts from carlactone to SLs. SLs bind to the receptor, D14 and form a complex with D3 for SL signaling. It has been known that plants produce SLs in roots in response to nitrogen and/or phosphate deficiencies. To evaluate SL levels in other mineral deficiency in rice, we cultivated rice seedlings in hydroponic culture media without nitrogen, phosphate, potassium, sulfate, calcium, magnesium or iron. SL levels increased under sulfate deficiency in addition to phosphate and nitrogen deficiencies. In combination of nitrogen, phosphate and sulfate deficiencies, SL levels were slightly elevated under both phosphate and sulfate deficiencies compared with phosphate deficiency alone. We investigated the relationship among leaf senescence, shoot branching and SL production under sulfate deficiency. Chlorophyll contents and number of outgrowing tiller buds decreased in both wild-type and SL mutants under sulfate deficiency, indicating that SL did not directly regulate leaf senescence and shoot branching. To explore how to produce SLs under sulfate deficiency, we performed expression analysis of SL-related genes in sulfate sufficient and deficient conditions. *D27* strongly expressed under sulfate deficiency, reduced on sulfate supply. Other SL biosynthetic genes, *D10*, *D17* and *OsMAX1* levels did not change between sulfate deficient and sufficient conditions. In contrast, *D14* and *D3* levels increased by sulfate supply. Our results revealed that increased SL level under sulfate deficiency is due to marked expression of *D27*.

Poster # 142 Tea major polyphenolic (−)-epigallocatechin-3- gallate and its preventive pathways to inhibit neurodegenerative disease (Chemical biology)

Ananya Bagchi [1], Dillip Kumar Swain[2], and Analava Mitra[3]

[1]Senior research scholar, Agricultural and Food Engineering department IIT Kharagpur, [2]Associate Professor, Agricultural and Food Engineering department IIT Kharagpur, [3]Associate Professor, School of Medical Science and technology, IIT Kharagpur

In terms of central nervous system function phenolic compounds react with neurotransmitter systems in human brain. It is found that in animal models, a wide range individual and combined flavonoids that generate in medicinal plant extracts have tranquillizing and anti- anxiolytic effects via direct interacting to GABAA receptors (Ren et al., 2010), cognitive improvement via antagonistic GABAA receptor binding cholinergic receptor up regulatory effect (Kim et al., 2007), and antidepressant property via monoamine oxidase inhibition . In tea (*camellia sinensis*) the major polyphenolic found is Epigallocatechin-3-gallate can inhibit acetylcholinesterase enzyme and can reduce the free radicals generated in Alzheimer's and many other neurodegenerative disorders.(−)-Epigallocatechin-3- gallate (EGCG) can decrease β-amyloid (Aβ) levels and plaques via increase of the non-amyloidogenic α- secretase proteolytic pathway in amyloid precursor protein overexpressing mice model (Rezai-Zadeh et al., 2008). (Amo et al., 2012) proved that EGCG interferes with the aromatic hydrophobic core of Aβ oligomer and inhibits its neurotoxicity in Alzheimer's disease. So it can be said that small molecule EGCG induced structural change of the β-amyloid (Aβ) can be used as new perspectives for neurodegerative diseases especially in Alzheimer's disease drug development.

Keywords: (−)-epigallocatechin-3-gallate, neurodegerative, acetylcholinesterase enzyme, Alzheimer's disease, cholinergic receptor

Poster # 143 Temperature-dependent control of flowering by the gibberellin pathway (Vegetative development)

Thomas Regnault[1], René Richter[2], Carina Behringer[1], Quirin Ranftl[1], Ulrich Lutz[1], Julia Diener[1], Markus Schmid[3], and Claus Schwechheimer[1]

[1]TU Munich, [2]MPI Cologne, [3]UPSC

The switch from vegetative development to flowering is a major transition in the life cycle of plants and changes in flowering time are strongly influenced by environmental factors. In Arabidopsis, the experience of cold temperatures delays growth and flowering. At the molecular level, this growth restriction results in gibberellin (GA) catabolism that leads to the accumulation of DELLA proteins, major plant growth and flowering time repressors of the GA signalling pathway. DELLAs inhibit the activity of a range of transcription factors by sequestration, hence forming a complex unable to bind on the promoter of target genes. We have discovered that DELLA proteins repress the MADS-box transcription factor AP1 (APETALA1) by direct physical interactions and that this repression can be relieved by GA treatment. In Arabidopsis, *AP1* expression confers floral meristem identity to the shoot apex and its overexpression leads to dramatic early flowering, suggesting a role as integrator of flowering inductive stimuli. We further identified two Arabidopsis accessions with elevated *AP1* expression already during the early stages of development and that show physiological behaviour supporting the notion that AP1 may promote early flowering in these plants in cold temperature. Based on these data, we propose that the repression of AP1 by DELLA proteins in the cold is relieved by GA when plants grow in warmer ambient temperatures thereby constituting a novel regulatory mechanism for the control of flowering time.

Keywords: Arabidopsis, DELLA, APETALA1, flowering, gibberellin

Poster # 144 The (oxalato)aluminate complex as an antimicrobial substance protecting the "shiro" of Tricholoma matsutake from soil microorganisms (Biotic interaction)

Katsutoshi Nishino[1], Misao Shiro[1], Kazuya Oizumi[1], Ryuki Okura[1], Toru Fujita[2], Akiyoshi Yamada[3], Chihiro Tanaka[1], Takahiro Sasamori[4], Norihiro Tokitoh[4], and Nobuhiro Hirai[1]

[1]Graduate School of Agriculture, Kyoto University, [2]Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center, [3]Shinshu University, [4]Institute for Chemical Research, Kyoto University

Mycorrhiza is a symbiotic organ consisting of plant roots and fungi. The plant is supplied with nitrogen and mineral nutrients such as phosphorus, potassium, and water from fungi, and the fungi receive photosynthetic sugars such as glucose and fructose from the plant through mycorrhiza. *Tricholoma matsutake*, a basidiomycete, forms ectomycorrhizas with *Pinus densiflora* as the host tree. Its fruiting body, "matsutake" in Japanese, is an edible and highly prized mushroom, and it grows in a circle called a fairy ring. Beneath the fairy ring of *T. matsutake*, a whitish mycelium-soil aggregated zone, called "shiro" in Japanese, develops. The front of the shiro, an active mycorrhizal zone, functions to gather nutrients from the soil and roots to nourish the fairy ring.

Bacteria and sporulating fungi decrease from the shiro front, whereas they increase inside and outside the shiro front. In 1966, Ohara demonstrated that the shiro front exhibited antimicrobial activity, showing that the shiro front produces an antimicrobial substance. However, the antimicrobial substance has remained unidentified for 50 years.

We have purified the antimicrobial substance from the shiro front, and identified it by an X-ray analysis as the (oxalato) aluminate complex which is known as a reaction product of oxalic acid and aluminum phosphate to release soluble phosphorus. We examined the distribution of mycelia of *T. matsutake*, the (oxalato)aluminate complex, antimicrobial activity, microorganisms, and soluble phosphate in the shiro area. The results clearly showed a good correlation between the distribution of the (oxalato)aluminate complex and that of the other items. The (oxalato)aluminate complex may protect the shiro from microorganisms, and contributes to its development.

Poster # 145 The Arabidopsis DAG1 transcription factor controls the dormancy/germination developmental switch acting on the balance of ABA and GA (Hormone metabolism)

Riccardo Lorrai[1], Alessandra Boccaccini[1], Veronica Ruta[1], Annie Marion-Poll[2], Danuše Tarkowská[3], Miroslav Strnad[3], Paolo Costantino[1] and Paola Vittorioso[1]

[1]Sapienza Università di Roma, [2]INRA, [3]Palacký University

The phytohormones abscisic acid (ABA) and gibberellins (GAs) play opposite roles in controlling the plant developmental switch between seed dormancy and germination: ABA establishes dormancy during late stages of seed maturation, while GAs release dormancy and promotes germination, thus counteracting the effects of ABA.

We had shown that the transcription factor Dof AFFECTING GERMINATION 1 (DAG1) negatively regulates seed germination, and it represses the GA biosynthetic gene *GA3ox1* during germination by directly binding to its promoter. We also observed an increase of the ABA catabolic gene *CYP707A2* in *dag1* seeds, suggesting that DAG1 may regulate this gene.

In order to dissect the molecular mechanisms underlying maturation, dormancy and germination of seeds, our research focused on the study of the role of DAG1 in controlling the balance between GA and ABA during these developmental processes. In addition, this research aimed to identify new targets of DAG1, and to elucidate the functional and molecular relationship between DAG1 and other factors involved in these processes.

Our results point to DAG1 as a key player in the control of the developmental switch between seed dormancy and germination, acting on the balance between ABA and GAs.

Poster # 146 The bZIP transcription factor VIP1 regulates ABA degradation in Arabidopsis seed germination (Hormone metabolism)

Lisza Duermeyer[1] and Eiji Nambara[1]
[1]*University of Toronto*

Water is an essential environmental factor that is required for the successful initiation and completion of seed germination. Germination is a process that occurs in the imbibed and non-dormant seed, by which the radicle breaks through the seed-covering layers to grow into a seedling. To activate this process, the seed must 'sense' multiple environmental cues such as water, and respond by activating large transcriptional and hormonal changes. One phytohormone - abscisic acid (ABA) – must be degraded, as it is a negative regulator of germination. *CYP707A2*, a member of the cytochrome P450 *CYP707A* family of ABA 8'-hydryoxylases, is rapidly upregulated in response to water, acting as the primary ABA degradation enzyme during seed imbibition. Here we show evidence that the bZIP transcription factor virulence E2 interacting protein 1 (VIP1) is a regulator of seed imbibition, and ABA catabolism in the Arabidopsis seed. *vip1* mutants show significantly delayed seed imbibition, while overexpression of VIP1 has an opposite effect on seed imbibition in the wild-type. In addition *vip1* mutants show an abnormal seed coat, and high ABA content and low *CYP707A2* expression at 6 hr post-imbibition. *vip1* mutants also show delayed seed germination, and increased sensitivity to mannitol and PEG treatment. These results indicate that VIP1 regulates seed imbibition processes, which influence germination potential.

Poster # 148 The Cyanobacterium Synechocystis Contains a Functional Ethylene Receptor (Hormone signaling)

Randy Lacey[1] and Brad Binder
[1]*University of Tennessee*

Ethylene is a plant hormone that plays a crucial role in the growth and development of plants. It is generally assumed that ethylene receptors are only found in plants. In a search of sequenced genomes, we found that many bacterial species contain putative ethylene receptors. We provide data that *Synechocystis* sp. PCC 6803, has a functional receptor for ethylene, Ethylene response1 (SynEtr1). We first show that SynEtr1 directly binds ethylene. Second, we demonstrate that application of ethylene to Synechocystis cells or disruption of the *SynEtr1* gene affects several processes including phototaxis, type IV pilus biosynthesis, PSII levels, biofilm formation, and spontaneous cell sedimentation. These data lead to a model where SynEtr1 is inhibiting down-stream signaling and ethylene inhibits SynEtr1 which is similar to the inverse-agonist model of ethylene receptor signaling proposed for plants. Thus, ethylene receptors likely originated over 1 billion years ago. Previous research showed that SynEtr1 contains a light responsive phytochrome-like domain. Therefore, SynEtr1 is a bifunctional receptor that mediates responses to both light and ethylene. These results show that the perception of ethylene is more wide- spread than previously thought.

Poster # 149 The effect of strigolactones on the rhizome formation and the ability of strigolactone biosynthesis in *Oryza longistaminata* (Vegetative development)

Naoki Kitaoka[1], Takashi Kumaki[1], Mikihisa Umehara[1], Tomoya Furuta[2], Stefan Reuscher[2], Kiyoshi Mashiguchi[1], Yoshiya Seto[1], Motoyuki Ashikari[2], and Shinjiro Yamaguchi[1]
[1]*Graduate School of Life Sciences, Tohoku University,* [2]*Bioscience and Biotechnology Center, Nagoya University*

Strigolactones (SLs) are a class of plant hormones that regulate many physiological events in plants. SLs inhibit shoot branching by inhibiting the outgrowth of axillary buds. Considering that rhizomes also grow out from axillary buds, SLs may have a negative effect on rhizome formation. To examine the effects of SLs, we have been using *Oryza longistaminata* (OL), a wild---rice species that propagates by rhizomes. Firstly, the effect of exogenous SLs was evaluated by observing rhizomes after treatment with GR5, a synthetic SL analog. Plants treated with GR5 had shorter rhizomes compared with mock---treated plants. Next, the ability of SL biosynthesis in OL was examined by analyzing the contents of 4---deoxyorobanchol (4DO) and orobanchol in root extracts and root exudates. The root exudates were also tested for the germination---inducing activity on the seeds of root parasitic

weeds. The results from these experiments suggested that OL was unable to produce SLs or only produced trace levels of SLs. The endogenous contents of SL biosynthetic intermediates in OL were analyzed. The contents of carlactone (CL) in OL shoots were almost the same as those in *Oryza sativa* L. cv. Nipponbare (OS), and the contents of CL in OL roots were about half of those in OS. The contents of carlactonoic acid (CLA) in OL were much lower than that in OS. CL is converted to 4DO and CLA by cytochrome P450s belonging to the CYP711A subfamily. It was suggested that those cytochrome P450(s) primarily contributed to the low abundance of SLs in OL. This hypothesis was supported by quantitative analysis of 4DO and CL in chromosome segment substitution lines (CSSLs) carrying chromosome segments derived from OL in the genetic background of OS.

Poster # 150 The gene expression pattern during the critical point of the callus formation in *Arabidopsis thaliana* (Hormone genomics)

Zhenhua Feng[1] and Jian Zhu[1]
[1]*Department of Molecular and Cell Biology, School of Life Science and Technology, Tongji University*

More and more researches show that almost all plants have the callus induction potential, the plant cells could keep the potency of reverting back to a meristem state, and it means that they have the ability of dedifferentiation and obtaining the new cell fate.
Herein, *Arabidopsis* seeds were treated with artificial auxin for several days and then transplanted in normal culture. The continuous hormone stimulation for 7 days is enough for induction the callus. Beyond 7 days, the seedlings would generate the callus, even the normal culture would not relieve symptom. If the lack of 7 days, the seedlings can restore to normal differentiation (to form root, stem and leaf) while the hormone removes. Overall, many transcription factors (TFs) that related to stem cell or meristematic tissue enriched in our screened data. Some genes in the “meristem initiation” and “stem cell maintenance” and “meristem development” clusters were up-regulated after the hormone stimulation for 7 days. Besides, the development-related TFs are also enriched in the callus formation process. Also a large number of genes associated with the response to salt stress, water stress, and chitin stress. It suggests that there is a certain correlation between response to stress and callus induction (cell dedifferentiation). Exactly, the genes that induced by auxin in the seedlings are enriched in the response to stimulation, stem cell related and certain development. Intriguing, many genes of stress-response were up-regulated before 5 days when hormone was present. However, the high expression patterns did not enrich before 7 days when the seedlings were in the hormone-free medium. Therefore, it suggests that stress-response genes have been involved in the early proceeding of callus formation. The stress-response genes may inhibit the differentiation and assist cell re-enter the static state, making cells easier to dedifferentiate or split.

Poster # 151 THE INVOLVEMENT OF ENDOGENOUS PLANT HORMONES IN THE REGULATORY NETWORK OF FATTY ACID BIOSYNTHESIS IN SOYBEAN SEED (Reproductive development)

Quoc-Thien Nguyen[1], R.J. Neil Emery[1] and Suresh Narine
[1]*Environmental & Life Sciences Graduate Program, Trent University,*

The activities of phytohormones during the reproductive phase have been partially clarified in seed physiology while the biological role of plant hormones in oil accumulation during seed development has been investigated in part only. In this research, fatty acid (FA) contents and hormone profiles, including abscisic acid (ABA) and cytokinins (CKs) of seed samples in four different stages and comparing six soybean varieties have been investigated in order to examine the hypothesis that the endogenous plant hormones play important roles in FA production in soybean seeds. The FA contents increased significantly during this period while the hormone concentrations gradually declined towards the seed physical maturation. However, the interactions between FA contents and hormone profiles were complex and went beyond linear correlations. Hormone metabolism in the earlier stages of seed maturation period demonstrated numerous robust relationships with FA accumulations, as derived from several simple and multiple regression models in the determination of different FA contents. Evaluation of the effects of exogenous ABA and *trans*-Zeatin (*t*Z) on FA biosynthesis has revealed that ABA

appears to be involved in the accumulations of unsaturated FAs while *t*Z participated in the synthesis of saturated and unsaturated FAs. Notably, the alterations of FA synthesis differ according to what exogenous hormone concentrations could be used.

Keywords: Soybean, Seed Development, Cytokinin, Abscisic Acid, Fatty Acid.

Poster # 152 The O-GlcNAc transferase, SECRET AGENT, positively regulates gibberellin signaling in tomato (Hormone signaling)

Vai Lor[1] and Neil Olszewski[1]
[1]*University of Minnesota*

O-linked N-acetylglucosamine (O-GlcNAc) transferases (OGTs) affect protein activity by transferring GlcNAc onto Serine and Threonine residues of proteins. *Arabidopsis thaliana* OGT SECRET AGENT (SEC) O-GlcNAcylates DELLA proteins and is predicted to positively regulate gibberellin (GA) signaling by negatively regulating DELLA activity. Here we report the results from studies investigating the function of SEC in tomato. Tomato has two SEC genes and expression of both genes were suppressed using the microRNA-induced gene silencing (MIGS) system. The majority of the primary transgenic lines carrying the MIGS construct were dwarves. Lines exhibiting the strongest dwarfism were infertile but it was possible to obtain seeds from less severely dwarfed lines. The dwarf phenotype, reduced SlSEC1 and SlSEC2 RNA abundance and the MIGS construct co-segregated in the progeny of the fertile lines. The dwarfism of the fertile lines was completely rescued by application of GA3 indicating that SlSEC affects GA signaling. Since the MIGS construct partially reduce SEC RNA levels, we have created a CRISPR/Cas9 induced SlSEC1 loss of function allele and are attempting to generate SlSEC2 loss of function alleles. Homozygous *slsec1* plants are indistinguishable from wild type suggesting that SlSEC1 and SlSEC2 function redundantly or that only SlSEC2 has a role in GA signaling.

Poster # 153 The Regulation of GAs Biosynthesis During the Photoperiodic Response in Aspen (Vegetative development)

José Alfredo Zambrano[1], Shashank Sane[1] and Ove Nilsson[1]
[1]*Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences*

Gibberellins (GAs) are one of the major hormones regulating plant growth, such as stem elongation, germination, dormancy, flowering and fruit development. In trees, the GAs are key regulators of shoot growth and have been suggested as important controllers of short day (SD) induced growth cessation. It has been shown that Gibberellin 20-oxidase (GA20ox) overexpressing hybrid aspens (*Populus tremula x tremuloides)* did not form buds in SD. By contrast, GA20ox antisense transgenic plants exhibited earlier bud set compared to wild type plants.

The aim of our work is to investigate how GA biosynthesis is affected by the photoperiodic signaling pathway in the regulation of growth cessation, bud set and bud break. We analyzed the expression levels of the GA-metabolism genes (*GA2ox, GA3ox* and *GA20ox*) in *Populus FLOWERING LOCUS T1 (PtFT1)*, *PtFT2* and *PtGIGANTEA (PtGI)* RNAi transgenic plants in Long day (LD) and SD, respectively.

We observed downregulation of *GA20ox* and *GA3ox* in the LD-grown *PtFT* and *PtGI* RNAi transgenic plants. However, upregulation of *GA20ox* and *GA3ox* was observed in SD-grown plants, where both of the RNAi lines showed earlier bud set compared to the wild type. Expression of *GA2ox*, encoding a bioactive GA deactivating enzyme was downregulated in both LD and SD.

The second part of the project will be the absolute quantification of the different GAs metabolites in *FT* and *GI* RNAi lines and the results will be discussed.

Poster # 154 The role of cytokinins during de *novo* organogenesis in *Arabidopsis thaliana* (Hormone Interactions)

Markéta Pernisová[1], Martina Matonohová[1] and Jan Hejátko[1]
[1]*CEITEC - Central European Institute of Technology and Laboratory of Functional Genomics and Proteomics, National Centre for Biomolecular Research, Faculty of Science, Masaryk University*

Plant cells are able to undergo a postembryonic *de novo* organogenesis, thus effectively regenerate plant organs. The long known phenomenon of *in vitro* plant regeneration suggests the principal role of two phytohormones, cytokinin and auxin. The root or shoot identity of newly formed organs was shown to be dependent on concentration ratios of these two phytohormones.

To study the role of cytokinins in *de novo* organogenesis, we used hypocotyl explants system. We performed deep morphological analysis of primordia structure in WT, transgenic lines with depleted endogenous cytokinins (*Pro35S:AtCKX2* and *Pro35S:AtCKX3*) and in cytokinin receptor mutants (*ahk)* during the first five days of cultivation. In general, the highest number of root primordia was observed on medium with auxin only, while increasing cytokinin concentration led to decreasing number of primordia and disorganization of their structure. The resistance to the increasing concentration of exogenous cytokinin was detected in *Pro35S:AtCKX2* and *Pro35S:AtCKX3* lines followed by *ahk* mutant lines. Measurement of cytokinin content reflected changes in endogenous cytokinin levels during *de novo* organogenesis. Moreover, our results suggest the role of cytokinins in controlling primordia identity.

Supported by CEITEC 2020 (LQ1601) and GP14-30004P.

Poster # 155 The role of gibberellin in plant response to drought (Hormone Interactions)

Ido Nir[1], Hagai Shohat[1], and David Weiss[1]
[1]*Institute of Plant Sciences and Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*

Previous studies have shown that reduced gibberellin (GA) level or signal promote plant tolerance to environmental stresses, including drought, but the underlying mechanism is not yet clear. Here we studied the effects of reduced and enhanced GA activity on tomato plant tolerance to drought, as well as the mechanism responsible for these effects. To reduce the levels of active GAs, we generated transgenic tomato plants overexpressing the *Arabidopsis GA methyl transferase 1* (*AtGAMT1*) gene. For inhibition of GA signaling we generated transgenic tomato plants overexpressing the gain-of-function DELLA mutant genes *Δ-rga* from Arabidopsis and its tomato homolog *Δ-procera* (*Δ-pro*). Tomato plants overexpressing *AtGAMT1*, *Δ-rga* or *Δ-pro* exhibited typical GA-deficiency/signal-inhibition phenotypes, including dwarfism, dark green leaves and increased *GA20oxidase* expression. The transgenic plants also exhibited increased tolerance to drought stress: they were able to maintain high leaf water status for a longer time under transient drought conditions, due to reduced whole- plant transpiration. The reduced transpiration can be attributed to reduced stomatal conductance. To understand the mechanism by which GA affects stomatal conductance, we studied the response of the DELLA loss-of-function mutant *pro* to drought. *pro* plants exhibited a wilty phenotype i.e., they were more sensitive to drought and lost water more rapidly than control plants. Microscopic examination of *pro* stomata revealed much larger pores than control stomata and slower stomatal closure during the day. Additionally, *pro* stomata exhibited reduced sensitivity to ABA, whereas *Δ-pro* stomata exhibited increased sensitivity to ABA. Taken together, the results suggest that DELLA activity promotes ABA activity and therefore stomatal closure and whole-plant transpiration.

Poster # 156 The Serine Threonine protein kinase Long Primary Root (LPR) mediates ethylene regulation of plant root growth in *Arabidopsis* (Hormone signaling)

Wei Yang[1] and Junxian He[1]
[1]*School of Life Sciences and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong*

Roots are important organs for plants to absorb water and nutrients from soil. Growth and development of plant roots are modulated by environment factors and internal hormone cues. However, how these factors coordinate to regulate plant root growth and development remains largely unknown. From a recent phosphoproteomic study, we identified a previously uncharacterized protein kinase, which we named Long Primary Root (LPR), with significant phosphorylation state changes under nitrogen starvation and recovery conditions. LPR contains a eukaryotic Serine/Threonine kinase domain at its N-terminus. Loss of *LPR* function by T-DNA insertion resulted in increased primary root growth whereas over-expression of *LPR* led to reduced root growth compared to the wild type. Furthermore, the *lpr* mutant had lowered sensitivity to ACC, a direct precursor of the plant hormone ethylene, reduced root hair formation, but expanded elongation zone of roots. These results suggest that LPR may mediate ethylene-regulated plant root growth under nitrogen-limiting conditions. From a LPR-based yeast two-hybrid screen, several LPR-interacting proteins were identified, which include the ethylene receptor ETR1 and Constitutive Triple Response 1 (CTR1), further supporting LPR's role in ethylene-regulated plant root growth in response to nitrogen. In the future, we will study how LPR interacts with these proteins to mediate nitrogen- and ethylene-regulated plant root growth.

This work was supported by the GRF grants (464412 and 14121915) and an AoE grant (AoE/M- 05/12) from the Research Grant Council of Hong Kong, the Shenzhen Science & Technology Research & Development Funding - Peacock Scheme, and the Direct Grants from The Chinese University of Hong Kong.

Poster # 158 Time course transcriptomics reveal overlaps in auxin and ethylene transcriptional networks in Arabidopsis roots (Hormone Interactions)

Gloria Muday[1], Amy Olex[2], Alexandria Jones[1], Sheena Gayomba[1], and Joelle Vidi[1]
[1]*Wake Forest University,* [2]*Virginia Commonwealth University*

Auxin and ethylene synergistically inhibit root elongation while ethylene antagonizes the positive effect of auxin on Arabidopsis lateral root formation. We have utilized genetic and molecular approaches to identify mechanisms by which lateral root formation is reduced by ethylene and enhanced by auxin. We performed genome wide transcriptional profiling in roots of light grown seedlings treated with auxin (IAA) or an ethylene precursor (ACC) over an extended time course (from 0.5 to 24 hrs) to better understand the molecular mechanism for crosstalk between these two hormones. Analysis of transcript accumulation profiles identified primary response genes, including transcription factors (TFs) and signaling proteins, and the more slowly accumulated transcripts encoding proteins that control growth and development. We identified 1,246 and 449 genes that changed consistently in response to IAA or ACC, respectively, with an overlap of 139 genes. This analysis provided insight into the time course of gene expression changes for genes previously identified as primary auxin- or ethylene-responsive genes, and identified novel genes with informative patterns of induction or repression. We are further defining the ethylene signaling networks that control root development, examining root phenotypes in ethylene receptor and TF mutants. To further define the auxin transcriptional networks, we are focusing on the transcriptional networks controlled by two Auxin Response Factors, ARF7 and ARF19. To dissect the transcriptional network in ARF19, which is the most rapidly induced TF after IAA treatment, we have completed an RNA-Seq analysis of the *arf19* mutant. The identified ARF19-dependent transcripts include TFs and cell wall remodeling proteins, whose role in lateral root development have been tested by mutant analysis. (Supported by NSF IOS 0820717 and USDA NIFA 2009-65116-20436).

Poster # 159 To branch or not to branch – Is Tre6P the answer to the question? (Vegetative development)

Franziska Fichtner[1], François F Barbier[2], Regina Feil[1], Mark Stitt[1], Christine A Beveridge[2] and John E Lunn[1]
[1] Max Planck Institute of Molecular Plant Physiology, [2] School of Biological Sciences, and The Queensland Alliance for Agriculture and Food Innovation, The University of Queensland

The metabolite trehalose 6-phosphate (Tre6P) is a crucial phosphorylated intermediate in the synthesis of trehalose, used by all plants as a specific signal of sucrose availability. As such, Tre6P acts as a negative feedback regulator of sucrose keeping its concentration close to the cellular optimum. Thus, Tre6P has a large influence on plant metabolism, growth and development. Little is known, however, about specific effects of Tre6P in different cell and tissue types, especially in relation to key processes such as shoot branching.

Earlier studies have shown that sucrose plays a crucial role in triggering bud outgrowth of decapitated pea plants. We measured metabolites in axillary buds of decapitated pea (*Pisum sativum* cv. Torsdag) plants, and observed that Tre6P starts to rise within hours after decapitation in the second axillary bud, indicating a potential role for Tre6P in the sucrose driven initiation of bud outgrowth. Following this lead, we studied Arabidopsis mutants with constitutive and tissue-specific changes in Tre6P content, finding plants with high Tre6P, from over-expression of Tre6P synthases, have increased shoot branching, whereas lowering Tre6P by over-expression of Tre6P phosphatases has the opposite effect.

We propose a role for Tre6P as an important factor in the control of shoot branching, acting as a signal for the sucrose status and thus energy availability of the plant.

Poster # 160 Transcriptional changes by treatment of auxin inhibitors (Chemical biology)

Yusuke Kakei[1], Yosuke Ishida[1], and Yukihisa Shimada[1]
[1]Yokohama City University Kihara Institute for Biological Research

The most abundant naturally occurring auxin is indole-3-acetic acid (IAA). Its biosynthesis, catabolism, polar transport and signaling have been extensively studied. Chemical genetics using micromolecules that are similar to auxins has been widely applied to analyze the function of auxin. Auxinole, PEO-IAA and PCIB are inhibitors for auxin signaling. NPA and TIBA have been used as inhibitors of auxin transport. Mechanisms included in the gravitropism, light tropism and the function of auxin transport in these tropisms have been analyzed using NPA. Many inhibitors for auxin biosynthesis have been developed recently. The first auxin-biosynthesis inhibitors, aminoethoxyvinylglycine (AVG) and L-aminooxyphenylpropionic acid (L-AOPP) were reported as inhibitors for Arabidopsis TAA1/TARs, which synthesize IPyA from tryptophan. L-Kynurenine and Pyruvamine (PVM) are more efficient inhibitors for TAA1/TARs. YUCASIN, BBo and PPBo inhibit YUCCAs. YUCCAs convert the IPyA to IAA. In this study, the transcriptional changes caused by inhibitors PEO-IAA, NPA, PCIB, AVG, L-AOPP, L-Kynurenine, PVM1169 and PPBo and that caused by IAA were compared. The treatment of PEO-IAA, L-Kynurenine, PPBo showed similar transcriptional change and the changes were highly correlated to each other. These changes were also negatively correlated with the transcriptional changes in response to IAA treatment. On the other hand, gene expressional changes caused by the other inhibitors were less or not correlated with that of IAA. These results show that PEO-IAA, L-Kyurenine and PPBo affected similarly to the function of auxin. We will also present our new transcriptome database, Arabidopsis thaliana transcriptome correlation analysis tool (AtCAST3: http://*atpbsmd.yokohama-cu.ac.jp/cgi/atcast/home.cgi*), which is developed to compare transcriptome of your interest to the other public transcriptome data, such as hormone inducible transcriptional changes.

Poster # 161 Transcriptional Networks Modulating Root Developmental Responses to Ethylene in Arabidopsis (Hormone signaling)

Alexandria Harkey[1], Justin Watkins[1], Daniel Lewis[1], David John[1], Jim Norris[1], Amy Olex[2], Jacquelyn Fetrow[3], and Gloria Muday[1]
[1]Wake Forest University, [2]Virginia Commonwealth University, [3]University of Richmond

The gaseous hormone ethylene reduces primary root elongation and the initiation of lateral roots, while increasing formation of root hairs. To identify transcriptional controls of these effects, we performed a 24-hour time-course microarray. A robust statistical analysis identified 449 Arabidopsis root transcripts that responded profoundly and consistently to treatment with the ethylene precursor 1- aminocyclopropane-1-carboxylic acid (ACC). Many of these transcripts encode transcription factors, which show a variety of expression patterns, including activation or repression at early (0.5-2 hrs), middle (4-8 hrs), and late (12-24 hrs) time points. This suggests a network in which earlier TFs regulate the expression of later TFs, and thereby control temporal patterns of gene expression. We have generated Bayesian next-state models of TF networks which predict hierarchical relationships wherein one TF controls synthesis of other downstream TFs. We have T-DNA insertion mutants for 22 of 26 TF genes, and are using qRT-PCR to test these networks by demonstration that mutations in early TFs alter synthesis of transcripts encoding downstream TFs and asking whether these TF mutants have altered root development. One interesting TF which we have identified is LATERAL ROOT PRIMORDIUM1, which is rapidly induced in our microarray dataset and is expressed in lateral root primordia. Two *lrp1* T-DNA insertion mutants show increased lateral root numbers and have altered levels of other downstream TFs, suggesting that LRP1 is a primary TF that participates in the ethylene inhibition of lateral root development. Additionally, we are testing the hypothesis that transcripts with similar patterns are regulated by common TFs by searching for conserved motifs within clusters of co-regulated transcripts using computational tools such as MEME and the JASPAR database. By combining computational approaches with experimental validation, we hope to make the connection between transcriptional events and ethylene induced changes in root development. (Supported by NSF IOS 0820717)

Poster # 162 Transcriptome changes associated with seed dormancy and dormancy loss in GA-insensitive *sly1-2* mutant of *Arabidposis thaliana.* (Hormone signaling)

Camille M. Steber[1, 2] and Sven K. Nelson[2, 3]
[1]USDA-ARS, Wheat Genetics, Health and Quality Unit, [2]Washington State University, Molecular Plant Sciences Program and the Dept of Crop and Soil Science, [3]USDA-ARS, Plant Genetics Research Unit, University of Missouri

Transcript profiling was used to characterize gene expression changes associated with the seed dormancy and its loss in the highly dormant GA-insensitive *sleepy1-2* (*sly1-2*) mutant. Because many genetic mechanisms contribute to seed dormancy, it is difficult to precisely define specific pathways contributing to dormancy control. *SLY1* encodes an F-box protein required for GA-triggered proteolysis of DELLA repressors of seed germination. Thus, the *sly1-2* mutant can be used to specifically look at transcriptome differences associated with dormancy as a consequence of failure to destroy DELLA proteins. GA signaling appears to play a major role in controlling protein translation associated gene expression in seeds (8-25% of differentially regulated mRNAs). The *SLY1* gene was required both for the decrease in translation-associated transcripts during seed development, and for the increase in translation-associated transcripts with dormancy loss through after-ripening.

The *sly1-2* seed dormancy phenotype is partially relieved either by the natural dormancy-loss through dry after-ripening (51% germination) or by overexpression of the GA receptor *GA-INSENSITIVE DWARF1b* (*GID1b*) (74% germination). Neither of these dormancy-breaking processes causes DELLA protein proteolysis. Only 23 genes showed changes in accumulation with *GID1b-OE*, whereas 4594 genes showed altered expression with *sly1-2* after-ripening over two imbibition timepoints. Thus, a strong change in germination potential can result from a small change in gene transcription.

Poster # 163 tRNA isopentenyltransferases - humble biosyntetic enzymes for cytokinins or robust global regulators? (Vegetative development)

Silvia Gajdosova[1], Marketa Pernisova[2], Milada Ciamporova[3], Jan Hejatko[2], and Vaclav Motyka[4]
[1]Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, [2]Functional Genomics and Proteomics of Plants, CEITEC - Central European Institute of Technology and National Centre for Biomolecular Research, Masaryk University, [3]Department of Plant Physiology, Institute of Botany, Slovak Academy of Sciences, [4]Institute of Experimental Botany, The Czech Academy of Sciences, v. v. i.

Cytokinins (CKs), structural isopentenyladenines, are important class of phytohormones involved in plant growth and development. Arabidopsis thaliana contains two classes of isoprenoid CK biosynthetic enzymes, ATP/ADP and tRNA isopentenyltransferases (IPTs) according to nature of substrate containing adenine which is isopentenylated. Two known tRNA IPTs, AtIPT2 and AtIPT9, were identified in Arabidopsis.
The two mutants were isolated and described to some extent previously. a*tipt9* as well as *atipt2 9* were prone to chlorosis, *atipt2* phenotypically resembled wild type. All tRNA IPT mutants contained reduced isoprenoid CK levels. It is known, that exogenous cytokinins induce chloroplast development and supply of exogenous CK usually rescues CK deficient. In this study, mutant hypocotyl explants subjected to organogenesis induction by supplemented exogenous CK and auxin unveiled interesting outcomes. Tissue from seedlings containing mutation in *AtIPT9* gene was neither able to restore its alterations in organogenesis nor chlorosis. Also other phenotypes were incongruent with common symptoms of cytokinin deficiency. Malfunctioning human and bacterial tRNA IPTs cause many adverse defects. Human disrupted homologue was confirmed to result in generalised disorder of mitochondrial protein synthesis and bacterial research also indicated tRNA IPT role in respirational processes. Sequence of AtIPT9 contains mitochondrial targeting motif too and observed pleiotropic alterations might be attributed to negatively-affected tRNA-related processes.
In light of these information it can be assumed that plant tRNA IPT, AtIPT9, may perform more profound role in tRNA related processes in mitochondria rather than in production of free tRNA-CKs and hormonal homeostasis.
Project was supported by the Pavol Jozef Šafárik University VVGS-2013-116 116 (SG), VEGA1/0046/14 (SG), the Grant Agency of the Czech Republic project GP14-30004P (VM, MP), 16- 14649S (VM) and by Ministry of Education, Youth and Sports Czech Republic, project CEITEC 2020 (LQ1601) (MP, JH).

Poster # 164 Tryptophan-independent auxin biosynthesis contributes to early embryogenesis in *Arabidopsis* (Hormone metabolism)

Bing Wang[1], Jinfang Chu[1], Tianying Yu[1], Qian Xu[1], Xiaohong Sun[1], Jia Yuan[1], Guosheng Xiong[1], Guodong Wang[1], Yonghong Wang[1] and Jiayang Li[1]
[1]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

Auxin plays essential roles in plant growth and development including embryogenesis, architecture formation, cell polarity and tropic growth. IAA can be synthesized through the precursor tryptophan (Trp) known as the Trp-dependent IAA biosynthetic pathway. However, IAA may also be synthesized through a proposed Trp-independent IAA biosynthetic pathway. Our recent studies on indole synthase (INS) presented the first genetic and biochemical evidence for the long-speculated Trp-independent pathway. The *Arabidopsis ins* mutant is defective in Trp-independent auxin biosynthetic pathway and that auxin synthesized through this spatially- and temporally-regulated pathway contributes significantly to the establishment of the apical-basal axis, which profoundly affects the early embryogenesis in *Arabidopsis*. To further identify critical genes in the Trp-independent IAA biosynthetic pathway and/or early embryogenesis, we carried out a genetic screen for embryo lethal mutants in the *trp2-301* background, which is a knock-down mutant of *Trp synthase* β1 (*TSB1*). More than 300 plants with small and pale ovules were considered as candidate mutants. Further studies through whole genome re-sequencing, primary mapping, complementation test, genetic analysis, and metabolism quantification will facilitate the characterization of molecular mechanisms underlying Arabidopsis embryogenesis and the Trp-independent IAA biosynthetic pathway.

Poster # 165 UNDERSTANDING THE ROLE OF GIBBERELLIN SIGNALLING IN WHEAT ANTHER DEVELOPMENT DURING HEAT STRESS (Reproductive development)

Matt Audley[1], Steve Thomas[1], Andy Phillips[1], Peter Hedden[1] and Zoe Wilson[2]
[1]*Rothamsted Research,* [2]*University of Nottingham*

High temperature stress during male reproductive development in wheat reduces fertility and leads to loss of yield. With the frequency of unseasonal, extreme weather events coinciding with floral development predicted to increase across major wheat producing regions, this investigation of hormonal control of anther development targets candidate genes for improved stress tolerance.

Wheat anther development is vulnerable to high temperature stress due to the sensitivity of the tapetum cell layer. Gibberellin biosynthesis and signalling is required for functional anther development in model species, particularly initiation of programmed cell death in the tapetum. Wheat orthologs of two rice transcription factors implicated in tapetum cell death, GAMYB and bHLH141, have been identified. Reverse-genetics approaches to characterising their functions in anther development have produced *Tabhlh141* and *Tagamyb* lines by RNAi silencing and Targeted Induced Local Legions In Genomes (TILLInG). Investigation of mutant phenotypes is ongoing. Yeast 2-hybrid assays support an interaction between TabHLH141 and the GRAS regulatory domain of the wheat DELLA RHT-D1A. Further assays attempting to establish if TabHLH141 and TaGAMYB are part of the GA-signalling network in anthers downstream of RHT-1 are ongoing.

Abiotic stress modulates multiple, overlapping hormone signalling pathways. Therefore, analysis of RNAseq transcriptome and global hormone profiling of anthers during high temperature stress was also carried out. Using differential gene expression analysis with ontology enrichment and total hormone concentrations, further candidate genes for investigation of hormone signalling responses to high temperature stress during anther development will be identified.

Poster # 166 Wheat ABA biosynthetic and catabolic genes: insights into their role in seed dormancy (Hormone metabolism)

SeungHyun Son[1], Vijaya Chitnis[1], Tran-Nguyen Nguyen[1], and Belay Ayele[1]
[1]*University of Manitoba*

Abscisic acid (ABA) is among the plant hormones implicated in the control of seed dormancy. The 9-*cis*-epoxycarotenoid dioxygenases (NCEDs) and ABA 8'-hydroxylase (ABA8'OH) enzymes catalyse the biosynthesis and catabolism of ABA, respectively, and are known to play important roles in regulating seed ABA level. This study identified three copies of *NCED2* in hexaploid wheat (*TaNCED2*; *TaNCED2A*, *TaNCED2B and TaNCED2D*) and characterized the role of *TaNCED2* in seed dormancy by expressing the A genome copy in Arabidopsis. The expression of *TaNCED2A* in wild type Arabidopsis using 35S promoter led to the presence of higher seed ABA level in imbibed seeds and a delay in germination relative to that observed in the wild type seeds, although the ABA level declined markedly during imbibition in both wild- type and *TaNCED2A* expressing seeds. The study also examined the role of the B genome copy of *cytochrome P450 monooxygenase 707A1* (*TaCYP707A1B*), which encodes ABA8'OH, in regulating seed ABA level and dormancy through complementation of the *cyp707a1 cyp707a2* double mutant and overexpression in wild type Arabidopsis. The *cyp707a1 cyp707a2* seeds complemented with *TaCYP707A1B* exhibited a substantial decrease in ABA level and recovery in germination. Similarly, the *TaCYP707A1B* expressing wild type seeds exhibited a decrease in ABA level and earlier germination phenotype. Examining the spatiotemporal expression patterns of *TaNCED2* and *TaCYP707A1* in wheat indicated that these genes are mainly expressed in seeds, and the difference observed in their expression pattern between dormant and non-dormant wheat seeds implies the physiological roles of the two genes in the regulation of seed dormancy in wheat.

Poster # 167 Interaction between SnRK1 and ABA signalling pathways in *Arabidopsis thaliana* (Abiotic stress)

Carina Carianopol[1], Shelley Lumba[1], Peter McCourt[1], and Sonia Gazzarrini[1]
[1]*University of Toronto*

Plants have developed intricate mechanisms to enable survival and propagation under non-optimal conditions. Regardless of the type of stress, energy deprivation occurs through decreased photosynthesis and respiration rates. In plants, the SnRK1 (Sucrose non-fermenting Related Kinase 1) members are the orthologs of the yeast Snf1 and mammalian AMPK energy sensors, and are activated under metabolic stress to achieve energy homeostasis. The catalytic subunit of the SnRK1 complex, AKIN10, has been proposed to link stress, sugar and developmental signals to regulate plant metabolism, energy balance, growth and therefore survival of the plant under stress. Despite the importance of SnRK1 activation during metabolic stress, the role of SnRK1 in regulating energy homeostasis and plant development is not yet fully understood. Recent work indicates that the SnRK1 and ABA signaling pathways share common transcriptional targets during stress response, although the mechanism of this crosstalk is unclear. Thus, the aim of this project is to investigate how AKIN10 and ABA interact to regulate growth, development and stress response in Arabidopsis thaliana. A high-throughput yeast two-hybrid (Y2H) screen using AKIN10 against 258 ABA-regulated genes was performed, resulting in 39 AKIN10 interactors. Potential AKIN10 targets were reconfirmed by one-on-one Y2H and include 5 previously characterized AKIN10 targets, metabolic and regulatory proteins (kinases, phosphatases, transcription factors etc.) and stress-related proteins. A subset of 14 interactors consisting of genes that are all up- regulated in response to ABA, cold stress, salt stress, osmotic stress and drought stress was chosen for further study. Since these genes cluster together in their expression in response to different stresses, they may form a signaling network and act together with SnRK1 to ameliorate the effect of stress on plant growth.