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Theme 1- Abiotic and Biotic Stresses

KEYNOTE

Beyond single genes: receptor networks underpin plant immunity

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A fundamental concept in plant pathology is that most plants are actively resistant to most pathogens and pests. Plants fend off their innumerable biotic foes primarily through immune receptors that detect the invading pathogens to trigger a robust immune response. The conceptual basis of such interactions was elegantly articulated by Harold H. Flor, who back in 1942 proposed the hypothesis that single genes in plants and pathogens define the outcome of their interactions. Flor's gene-for-gene model turned out to be hugely insightful and influential—it has, ever since the mid-twentieth century, helped to guide applied and basic research on disease resistance. However, recent findings are taking the field far beyond the simplified binary view of plant-pathogen interactions. Plants turned out to carry extremely diverse and plastic repertoires of immune receptors that are interconnected in complex ways. Conversely, plant pathogens secrete a diversity of virulence proteins and metabolites known as effectors, and pathogen genomics revealed hundreds of effector genes in many species. These effectors have evidently evolved to favor pathogen infection and spread, but a subset of them inadvertently activate plant immune receptors. The emerging paradigm is that dynamic webs of genetic and biochemical networks underpin the early stages of plant-pathogen interactions. In this talk, I will discuss our work on NLR networks and explore the implications of this systems view of plant-pathogen interactions. I postulate that Flor's intuitive gene-for-gene model is superseded by the systems view that plant immune receptors form networks with complex topology. These networks are defined by the uncoupling of plant pathogen perception from initiation of downstream signaling and immune response. Current work aims at decrypting the biochemical codes that define receptor network wiring. Ultimately, an improved knowledge of plant immune systems would enable optimal use and deployment of immune receptors in agriculture.

0–3

The alga that never read the literature-fastest growing photodamage tolerant alga isolated from desert crust

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The unparalleled performance of *Chlorella ohadii* clearly indicates that we lack essential information on its photosynthetic machinery and what sets its upper growth limits. When grown under optimal laboratory conditions or controlled outdoor conditions, this alga, recently isolated from one of the harshest environments (a biological desert sand crust), exhibits the fastest growth rates ever reported for an alga, division times shorter than 2 h were recorded. The cultures have very high photosynthetic rates and reach high cell densities ($1.3*10^9$ cells/mL). Unlike other photosynthetic organisms, *C. ohadii* productivity is unaffected by irradiances twice full sun light and the level of protein D1, encoded by a single gene, is hardly affected. Rather than succumbing to photodamage *C. ohadii* undergoes major structural and compositional changes (including development of pyrenoids, 2–3 fold increase of lipid and carbohydrate contents and a large rise in thylakoids abundance), emphasizing unique PSII functioning as well as highly efficient reductant utilization downstream of the photosynthetic reaction centers. *C. ohadii* may be used to clarify the processes that limit growth and productivity of photosynthetic organisms. Based on these remarkable capabilities we were able to explore several novel and uncharacterized aspects of algal growth under extremely high illumination, temperature, and desiccation, which are too damaging for current model organisms. Growth of batch cultures under continuous high light (3000 μmol photons m^{-2} s^{-1}) combined with metabolome analyses revealed a highly coordinated metabolic switch, supporting growth to higher densities than those achieved if abolished, and regulated by specific signaling molecules. RNA-Seq revealed regulation of genes networks under

changing light and trophic regimes, and provided novel insights on the mechanism underlying its exceptional photodamage resistance.

0–6

Antifungal plant defensins: mechanisms of action and engineering disease resistance

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Fungal pathogens impose major constraints on crop yields globally. Host defense peptides have evolved to protect plants from pathogen attack. Defensins are cysteine-rich antifungal peptides expressed in all plants. They exhibit potent antifungal activity and therefore have potential for use in transgenic crops for enhanced resistance to fungal pathogens. MtDef4 and MtDef5 are two sequence-divergent apoplast-localized defensins expressed in *Medicago truncatula*. MtDef4 is a monomeric defensin of 47 amino acids, whereas MtDef5 is a novel bi-domain defensin containing two monomeric domains linked by a 7-amino acid peptide. They differ from each other in sequence, net charge and hydrophobicity. MtDef4 inhibits the growth of several filamentous fungi including *Fusarium graminearum* at micromolar concentrations. In contrast, the bi-domain MtDef5 inhibits the growth of these fungi at submicromolar concentrations. Sequence motifs governing the antifungal activity of these defensins have been identified. MtDef4 and MtDef5 permeabilize the plasma membrane of fungal pathogens. They translocate into fungal cells, but use spatially distinct modes of entry and localize to different subcellullar compartments. MtDef4 and MtDef5 bind to different plasma membrane resident phospholipids in fungal cells and disrupt the plasma membrane. MtDef5, but not MtDef4, forms oligomers in presence of PIP, PI and PA. MtDef4 and MtDef5 exhibit different modes of antifungal action and show promise as novel antifungal agents in crops. Aflatoxins, secondary metabolites produced by *Aspergillus flavus,* are extremely toxic carcinogenic compounds. Aflatoxin contamination of peanuts poses a major threat to public health in sub-Saharan Africa and Asia. Transgenic peanut lines expressing MtDef4 have been generated. Peanut seeds expressing this defensin exhibit strong resistance to *A. flavus* and accumulate extremely low levels of aflatoxins. This is the first study to demonstrate highly effective biotechnological strategy for generating peanuts that are near-immune to aflatoxin contamination, offering a panacea for food safety for people in developing countries.

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Evaluation of Aluminum Toxicity using *Solanum macrocarpon* Seedlings

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Aluminum (Al) is the third most abundant element and most abundant metal in the earth's crust. It becomes biologically available in medium with pH ≤ 5.5. Al toxicity to plants is increasing as natural and anthropogenic factors like industrialization, urbanization, acid rain *etc.* are continually reducing soil pH in Sub Saharan Africa (SSA). In the delta oil region in Nigeria where gas flaring is a daily activity, acid rain is continually lowering the soil pH. *Solanum macrocarpon* plants where exposed to varying concentrations of $AlCl_3$ (control, 0.3, 0.6, 0.9, and 1.2) in mM/L. *S. macrocarpon* were germinated and grown in screen house conditions and samples were analyzed in the laboratory. Al treatments of 0.3, 0.6, 0.9, and 1.2 mM/L had significant ($p < 0.05$) effects on chlorophyll, peroxidase, fresh weight, dry weight, germination index, roots, shoots, leaves and height of the plants compared to the control. Toxicity increased with increased in $AlCl_3$ treatment concentration. Each treatment was replicated 28 times in perforated bags. The screen house experimental design was RCBD.

0–14

Application of biotechnological tools for crop protection of coffee varieties in Costa Rica

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Coffee (*Coffea arabica* L.) is one of the most important crops in the world and one of the main export products in several developing countries. This crop is susceptible to different diseases and pests. The coffee berry borer (CBB) (*Hypothenemus hampei* Ferrari) and leaf rust **(***Hemileia vastatrix***)** are two of the major threats for its production. Climatic conditions such

as soil salinity also limit coffee production sustainability and profitability. The control of CBB insect depends mostly on the application of synthetic insecticides, which are harmful to the environment. As a result, resistant varieties are one of the strategies to control this pest. However, genetic resistance to CBB is a feature that is not available in the coffee genetic pool of either *C. arabica* nor *C. canephora*. A suitable transformation vector with fruit tissue-specific promoter (CaEXP and CrLTP) for the expression of the entomopathogenic *cry10Aa* and *cy1Aa* genes from *Bacillus thuringiensis* was developed and genetic transformation of cell suspensions and leaves using *A. tumefaciens* with the genes *cry10Aa* and *cyt1Aa* was achieved. Moreover, crop improvement *via* mutagenesis represents a powerful alternative to increase genetic variability and accelerate breeding programs. In this sense, coffee embryogenic suspension cultures (ESC) and seeds were incubated with sodium azide (NaN_3) and ethyl methane sulfonate (EMS). Therefore, in the case of ESC the LD_{50} were determined for NaN_3 (5 mM for 15 min) and for EMS (185.2 mM for 120 min). Whereas, the LD_{50} values for the treatment of seeds with NaN_3 and EMS were between 50 and 75 mM and 2–3% *v*/v, respectively. The generation of coffee varieties resistant to CBB using transgenic technology and tolerance to leaf rust by chemical mutagenesis is very important and constitute strategic tools in order to offer coffee farmers alternatives to control these important pests.

Keywords: *Coffea arabica* L., Coffee berry borer (CBB), Leaf rust, Genetic transformation, Chemical mutagenesis

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Novel Insights into the Function of Dead Organs Enclosing Embryos of Angiosperms

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In angiosperms the embryo is dispersed from the mother plant surrounded by the remnants of the mother's reproductive organs such as indehiscent fruits and seed coats. For instance, in dicots, the integument layers will form the seed coats; in wild grasses the dispersal units consist of the caryopsis surrounded by the dead floral bracts. The maternally derived organs (MDO) of grasses are undesired in agriculture but their adaptive value has not been fully explored. We investigated the proposal that the MDO of seeds and dispersal units have been evolved to have a function other than physical embryo protection. We showed that, upon hydration, dead floral bracts of wild emmer wheat (*Triticum turgidum var dicoccoides*) store and release active hydrolases including nucleases and chitinases, which maintain activity years after the mother plant dies. Proteome and ICP analysis revealed multiple oxidative and pathogenesis stress related proteins and nutrients that are released upon hydration. Further analysis showed that although germination from the intact dispersal unit of wild emmer wheat was delayed, post germination growth was better than that of separated caryopses. In some of the studied dicot species, seed coats and pericarps exhibited microbial growth control activity of the seed coat extracts, even after years of storage in uncontrolled conditions. Thus, our study shows that the dead MDO enclosing the embryo stores active hydrolases and other substances that can engineer the micro environment of the germinating seed, support seed persistence in the soil, serve as a first line of defense during germination and increase seedling establishment.

P - 25

OsMADS57 coordinates transcription of its target OsWRKY94 and D14to switch organogenesis to cold defense

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Plants, unable to move, modify their development to adapt to their environment. In chilling stress a variety of signaling pathways are activated to protect plants from detrimental conditions. But little is known about how plants coordinate developmental patterns and stress responses at a molecular level. Our study revealed that, in rice, interacting transcription factors OsMADS57 and OsTB1 directly target the defense gene *OsWRKY94* and the organogenesis gene *D14* to trade off functions tolerance to cold. Overexpression of *OsMADS57* maintains rice tiller growth under chilling stress. OsMADS57 binds directly to the promoter of *OsWRKY94*, activating its transcription for the cold stress response, while suppressing its activity under normal temperatures. Moreover, *OsWRKY94* was directly targeted and suppressed by OsTB1 under both normal and chilling temperatures. *D14* transcription, however, was directly promoted by OsMADS57 for suppressing tillering under the chilling treatment, whereas *D14* was repressed for enhancing tillering under normal conditions. We demonstrated that OsMADS57 and OsTB1 conversely affect rice chilling tolerance *via* targeting *OsWRKY94*. Our findings highlight a mo-

lecular genetic mechanism coordinating organogenesis and chilling tolerance in rice, which supports and extends recent work suggesting that chilling stress environments influence organ differentiation.

0–28

Strategies for the elicitation of important anticancer secondary metabolites in cell cultures of Fagonia indica

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Fagonia indica is an important medicinal plant highly traded for its promising potential against breast cancer. The natural availability of this high-valued herb is not fulfilling the ever-increasing demand. Therefore, alternative production strategies should be developed. Our work is focused on developing elicitation strategies for enhanced production of many different anticancer polyphenolic compounds in *in vitro* cultures of *F. indica*. We have applied different abiotic stresses to cell cultures. In the first experiment, out of various plant growth regulators tested for elicitation, Thidiazuron induced maximum biomass, total phenolic content (202.8 μg gallic acid equivalent/mg) and total flavonoid content (191.03 μg quercetin/mg) in callus cultures of *F. indica*. In the second experiment, out of the different carbon sources (sucrose, glucose, fructose, and maltose) in various concentrations, sucrose induced the highest biomass and glucose at optimal levels induced the highest TPC in callus cultures followed by fructose after 42 days. Further, manipulation in the light regimens combined with the effects of PGRs and elicitors, significantly affected both primary and secondary metabolism. The highest TPC was observed in the Methyl Jasmonate (Me-J) treated dark-grown cell cultures. Similarly, the highest TFC was recorded in Me-J treated dark-grown samples from cell suspension cultures. The HPLC data showed an enhanced amount of important anti-cancerous secondary metabolites such as gallic acid, myricetin, caffeic acid, catechin, apigenin, plurglucinol, salicylic acid and ellagic acid in response to all these different elicitation strategies. Assessing the anticancer activities of extracts from these cultures on breast cancer cell lines such as MDA-MB-231 and MCF-7 showed promising results killing majority of the cells at a lower MIC value compared to control. Conclusively, Thidiazuron supplemented cell cultures treated with a disaccharide such as glucose and kept under dark condition in the presence of Me-J give the highest quantities of important anticancer secondary metabolites.

0–32

Characterization of sugarcane (Saccharum hybrids) mutants tolerant to imazapyr

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Imazapyr is effective against weeds such as *Cynodon* and *Rottboellia* species which are a major problem in sugarcane production. Imazapyr-tolerant sugarcane mutants of cultivar N12 were produced by chemical mutagenesis using ethyl methanesulfonate (EMS). The imazapyr concentrations that inhibited their acetolactate synthase (ALS) basal activity were 0.77–5.36 times greater than that of N12 control. Due to random mutations caused by EMS, this study aimed to compare the agronomic characteristics of three mutant genotypes (Mut 1, Mut 6, and Mut 7) with the non-mutated N12 control, and to sequence the *ALS* gene to identify the point mutations conferring imazapyr tolerance. The mutagenesis protocol had no effect on the number of stalks/plot, stalk height, fiber and sucrose content of the mutants. However, Mut 1 genotype was more susceptible to the lepidopteran stalk borer, *Eldana saccharina* than the non-mutated N12 (11.14 ± 1.37 and 3.89 ± 0.52% internodes bored, respectively), rendering Mut 1 less desirable for commercial cultivation. Various point mutations in the *ALS* gene were identified. A nucleotide change within the N12 *ALS* gene at position 1178 (C to T) resulted in a R287C mutation in Mut 1. In Mut 6, an A to G change at nucleotide position 1857 resulted in a N513D mutation, while a G to A change at nucleotide position 2184 imposed a S622 N mutation. The molecular dynamics simulations revealed that the S622 N mutation imposes an asparagine side chain clash with imazapyr, hence this mutation is likely effective in conferring imazapyr tolerance.

0–34

A fungal endophyte consortium counterbalances the negative effects of reduced nitrogen input on barley yield

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The use of chemicals to fertilize crops incurs economic and environmental costs and it is widely recognized that the current level of chemical fertilizer use is unsustainable in many intensive farming systems. Any methods that can reduce

fertilizer input and still maintain acceptable yields would be of great benefit to both the farmer and the environment. The use of beneficial endophytes as crop inoculants may go some way towards improving crop yields beyond that achievable using fertilizer increases alone. Field trials were conducted over two seasons on three contrasting field sites to test the effects of fungal endophytes from a wild barley relative on three barley cultivars (Mickle, Planet and Propino). Seeds were either untreated or dressed with a consortium of four endophyte strains, and three levels of nitrogen (N) were applied to both treatments: full N, 50% N and 0 N. On the field site with the lowest overall N input, the endophyte treatment with 50% N restored yield for 'Planet' to that associated with untreated plants receiving the full N input. On the same site and with the same cultivar, endophyte treatment increased yield by 15% under full N, and by a mean 12% for all three cultivars with 50% N input. Over both seasons and all three sites, the endophyte treatment increased yield for the cultivar Planet by a mean of 9%. A strong correlation was found between increased yield and each of low rainfall, greater evaporation, and greater number of degree days above the base. Furthermore, the efficacy of the endophytes was not removed by regular foliar fungicidal treatment. These results suggest that fungal endophytes can contribute to improving barley yield grown in low rainfall areas and under a range of fertilizer input regimes, provided that endophyte treatments are applied to compatible crop cultivars and sites.

P - 37

Agrobacterium-mediated transformation system for Chickpea (*Cicer arietinum* L. cv. PB-2008) using GUS gene expression.

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The purpose of this research study was to propose a set of conditions for a *Agrobacterium*-mediated transformation system for Chickpea (*Cicer arietinum* L. cv. PB-2008) which will ensure an optimal transient *gus* gene response. Three different types of explant (cotyledonary node, cotyledon and node) obtained from 7-day-old *in vitro* germinated seedling and one month-old calli derived from these explant types were treated for 20 or 30 min with bacterial inoculum (*A. tumefaciens* strain EHA101 in this case) with optical density (OD_{600}) of 0.8, 1.0 or 1.2. Potentially infected plant tissues were co-cultivated for 2, 3, 4 or 5 days. After an antibiotic treatment, plant tissues devoid of bacterial presence were subjected to *GUS* histochemical analysis for detection of transient expression of *gus* gene. Statistical analyzed data revealed that the combination of 1.0 OD_{600} with 5 days of co-cultivation and 20 min of infection caused cotyledonary node and cotyledon to respond optimally. For node explant, transient gene expression was optimized at 1.0 OD_{600} with 4 days of co-cultivation and 30 min of infection. Wounding of cotyledons was found to be effective in increasing the histo-chemically stained surface area. Optimization of transient gene expression by callus revealed that set of conditions required for optimal gene expression in calli didn't corresponded to those required for their respective explant type. Further optimization of this transient transformation by evaluating the effects of other factors on *GUS* response was recommended.

P - 41

Salicylic Acid Increases Freezing Tolerance of Spinach Leaves: mechanism explored through metabolite profiling and signaling

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To investigate the effect of salicylic acid (SA) on plant freezing tolerance (FT) 0.5 mM SA was applied through subfertigation to two-week old *Spinacia oleracea* L. 'Reflect' seedlings grown under ambient (non-acclimated) conditions (NASA). A week later NASA and fertigated controls (NA) plants were subjected to a controlled-temperature 'whole-plant freeze-thaw' cycle and injury was assessed visually and by ion-leakage. NASA leaves accumulated ~60% higher SA and were significantly more freeze-tolerant than NA. Comparative metabolomics revealed NASA leaves had higher trehalose, ascorbic acid, γ-tocopherol, proline, and leucine than NA leaves. However, lower mannose and aconitic acid contents were observed in NASA than NA leaves. To investigate effect of SA on FT and metabolism at warm (NASA) *vs.* cold conditions, plants were subjected to 9-d cold acclimation without or with SA-feeding (CA, CASA, respectively). CASA leaves were most freeze-tolerant followed, respectively, by CA, NASA, and NA treatments, and the improvement in FT by SA under cold treatment was twice that of warm treatments when compared at 'absolute' °C change in LT_{50}. CASA leaves accumulated higher concentrations of SA, compatible solutes and antioxidants than CA tissues. Pair-wise comparisons of NASA / NA, CA / NA, and CASA/NA indicate changes in trehalose, ascorbic acid, and aconitic acid were SA-specific and occurred to a greater extent in cold *vs.* warm conditions. Principle component analysis distinctly separated metabolic phenotypes for four conditions. These results indicate SA differentially affected FT and metabolism in warm *vs.* cold conditions. Additionally, 7 metabolites (5-oxoproline, fructose, glucose, maltose, proline, sucrose, and tartaric

acid) were quantitatively associated with the FT levels across four treatments. Experiments with excised leaves indicated that the beneficial effect of SA on FT was abolished when either H_2O_2- or NO-scavenger was added to SA as pretreatment, suggesting that SA-induced FT may be mediated by NO and H_2O_2 signaling.

0–51

Comprehensive functional characterization of CCCH tandem zinc finger protein genes in rice

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A number of zinc finger proteins are known to function in biotic and abiotic stress responses. In rice there are nine CCCH tandem zinc finger protein genes. The expression profile of tandem zinc proteins genes were analyzed. The expression of *OsC$_3$H$_{50}$* gene, a member of CCCH zinc finger proteins gene family, was induced by drought and NaCl. The expression of the other member genes were also regulated by different abiotic stresses. For sub-cellular localization, the coding regions were cloned in frame with Green Fluorescent Protein (GFP). OsC$_3$H$_{50}$-GFP protein localization was observed in the cytoplasm and nucleus. OsTZF1 was localized in cytoplasmic foci and at times in nucleus. The coding region of these genes were cloned adjacent to maize (*Zea mays*) ubiquitin promoter in pBI binary vector and transformed into *Agrobacterium tumefaciens* (EHA105). The regenerated putative transgenic plantlets were confirmed by Northern blot analysis. Transgenic rice plants overexpressing these genes driven by ubiquitin promoter displayed different phenotypes upon maturity. *Ubi*:*OsC$_3$H$_{50}$*-OX plants showed improved tolerance to drought stress and high-salt stress. Our comprehensive analysis of the CCCH tandem genes have shed light on their functions. The results revealed that these genes are involved in conferring abiotic stress tolerance in rice and they should be analyzed further for their specific functions in abiotic stress tolerance.

P - 60

Compact growth of ornamentals induced by ethanol treatment

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Three ornamental plant species *Euphorbia pulcherrima, Kalanchoë blossfeldiana* and *Tagetes erecta* were subjected to series of experiments aiming to manipulate their growth habit using ethanol treatment. The most common procedure to induce a compact growth in ornamental plants is the use of chemical growth retardants, which interfere with gibberellin biosynthesis. These chemicals are able to control stem length, but unfortunately they do not improve branching and in most cases delay flowering. Furthermore, due to the possible negative impact on the environment and human health the use of some chemical growth retardants has been restricted in several countries. The chosen model ornamental plants were watered with different ethanol concentrations ranging from 0 to 8% ethanol solution. In most cases ethanol concentrations of 1%–2% were most beneficial for the compact growth, short internodes, decreased fresh and dry weight. High concentrations of ethanol had a damaging effect, causing chlorotic and necrotic spots and even leading to plant death. Delay of flowering was not observed in *Kalanchoë blossfeldiana,* neither was delay of bract coloration in *Euphorbia pulcherrima* observed. In conclusion, ethanol treatment could be used as an alternative treatment for induction of compact growth. However, more investigations for particular plant species or cultivars are needed.

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Physiological and Biochemical Characteristics of Some Clematis Cultivars Cultured *Ex Situ* and *In Vitro*

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To assess the possibility of adaptation and drought resistance development in some *Clematis* L. cultivars, under the growth conditions of southern Russia, structural and metabolic features were analyzed under the pressure of hydrothermal stress. At the same time, some aspects of morphology, anatomy and physiology in *Clematis* regenerants were studied under *in vitro* culture. *Ex situ*, water deficit ranging from 10.4 to 18.2% developed at total water content of 68.2 to 75.3% (coefficient of variation – 2.1-8.2%). After 24 h exposure, the cultivar 'Nikitskiy Rozoviy' did not reach the sublethal water deficit but water loss was 29.6% (compared to 33.6–45.9% in the other cultivars). Leaves were found to be xeromorphic, possessing a thickened cell wall in the epidermal cells, in addition to the presence of a cuticular layer, trichomes, multiple stomatal apparatus (anomocyte type), and dense palisade tissue (palisade index 0.42–0.54). The fluorescence induction parameters indicated the normal state of the assimilating tissues ($(F_m-F_{st})/F_m = 0.54–0.68$ a.u.). Protective compound content was high: 23.16–52.20 μg/g proline, 26.53–36.04 mg/100 g ascorbic acid, 1989–2176 mg/100 g phenolic compounds, 445–767 mg/100 g flavonols. Enzyme activity was low: catalase 0.43–9.35 $gO_2/g \cdot min$, superoxide dismutase 14.12–18.17 units/g,

polyphenol oxidase – 0.033-0.075 units/g. Microshoots, obtained *in vitro,* had bifacial, hypostomatic leaf blades with 3–4 rows of differentiated mesophyll (palisade index 0.29–0.37), with a single-layer epidermis and 1–2-celled trichomes. Number of stomatal apparatus was 58–136/mm^2 (maximum – in the cultivar 'Madame Julia Correvon'). The total water content was 82.5–89.1%. The partial photoinhibition – $(F_m\text{-}F_{st})/F_m = 0.48\text{–}0.58$ a.u. was determined, especially in the cultivar 'Alpinist'. The content of phenolic substances, flavonols and proline in plants under *in vitro* conditions was lower than in intact plants. Enzymes activity and ascorbic acid concentration, with the exception of the cultivar 'Alpinist', were higher. This study was funded by the research grant N 14–50-00079 of the Russian Science Foundation.

0–79

Methyl jasmonate/salicylic acid enhanced flavonoid production and change metabolites profile in *Thevetia peruviana* cell culture

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Thevetia peruviana (Apocynaceae) is an ornamental shrub that grows in America, where is known for its cardiac, anti-microbial, and anti-oxidant properties. Cell suspension cultures of *T. peruviana* were established to promote the production of phytochemicals with potential pharmaceutical value. Cells were treated with the elicitors methyl jasmonate (MeJA) and salicylic acid (SA) to stimulate flavonoids production. Elicitors were added to cultures during the exponential growth phase, at optimized concentrations (3 μM MeJA, 300 μM SA and 3 μM-MeJA/300 μM-AS). Cells were harvested 24 h post-elicitation, over six days. Biomass from the different treatments and control (culture without elicitor), were analyzed to determine the total flavonoid content (TFC) using the spectrophotometric method based on $AlCl_3$ complexation, near infrared spectroscopy (NIR) and multivariate statistical analysis. Flavonoid profiles were determined by HPLC. Highest TFC were achieved with MeJA and MeJA/SA at 72 h post-elicitation (7.22 ± 1.17 and 5.70 ± 0.48 mg quercetin equivalent (QE)/Dry Weight (DW)) and with SA at 48 h post-elicitation (4.84 ± 0.27 mg QE/DW). Principal component analysis (PCA) separated the samples into four groups referring to each different treatment. NIR spectral data were pre-processed using Standard Normal Variate transformation (SNV), and Multiplicative Scattering Correction (MSC) and then used to generate a partial least squares (PLS) model to determine the TFC, obtaining a correlation coefficient of 0.79 and a prediction error of 14%. PCA with HPLC data from a single wavelength (280 nm) showed that MeJA and SA generated differences in the metabolite profile of cell cultures. Treatment with MeJA/SA increased the production of dehydroquercetin at 72 h (MeJA: 1.27 ± 0.12 mg/DW; control: 0.37 ± 0.1 mg/DW). This is an antioxidant flavonoid with potential chemopreventive action. This is the first report of dehydroquercetin in *T. peruviana*, demonstrating its potential as a source of metabolites with pharmaceutical value.

0–83

Local adaptation in the extremophile *Eutrema salsugineum*: Exploring the role of putative lncRNAs

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Eutrema salsugineum is a halophyte that is tolerant to abiotic stresses such as nutritional deficiencies, cold temperatures, and drought, and has been used as a model plant for stress tolerance research. Most studies have focused on the Shandong ecotype, a natural accession of *E. salsugineum* from the temperate region of Shandong, China. However, an *E. salsugineum* population from Yukon, Canada, has recently emerged as a novel source for insights on the stress response of this species. The Yukon ecotype is naturally found in a subarctic, semiarid environment, and displays different molecular and phenotypic changes when exposed to stressors compared to the Shandong ecotype. The variation in stress response may indicate local environmental adaptations in each *E. salsugineum* ecotype. RNASeq libraries of both Shandong and Yukon cabinet grown plants exposed to two sequential drought conditions were obtained, along with RNASeq data of naturally found Yukon plants sampled during a year of drought. Using transcriptome information obtained by RNASeq, assembled transcripts were input into a custom ensemble machine learning algorithm, CREMA, to identify putative long non-coding RNAs (lncRNAs). Using this data, we predict that both Shandong and Yukon ecotypes will express unique lncRNAs that regulate genes required for tolerating local environmental stress. We also predict that Yukon plants found in their natural environment will have elevated

expression of these lncRNAs and may express unique "field" Yukon lncRNAs due to constant challenges by multiple stressors. As lncRNAs have been previously associated to abiotic stress in plants, their rapid evolution and gene regulatory functions may also play important roles in how two ecotypes of the same species have potentially adapted to their own natural environments.

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Response of Wheat Genotypes to Excess Boron Estimated by *In vitro* Culture

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Boron (B) is a metalloid that is essential for different plant functions. When present in suboptimal amounts it causes nutritional deficiency, while in excess it may become toxic. Boron toxicity is an environmental problem in areas throughout the world causing significant decrease in crop yield. The objective of this study was to evaluate boron tolerance of wheat genotypes using mature embryo culture. The analysis involved 79 recombinant inbred lines of the International Triticeae Mapping Initiative (ITMI) population and three Serbian varieties with known boron tolerance (Pobeda - sensitive (S), Balerina - medium tolerant (MT), and Nevesinjka - tolerant (T)). The evaluation was performed on a modified MS medium to which 15 mM of boric acid was added. The control medium contained no excess B. Fresh callus weight (FCW) was measured after one month of cultivation. Also, reductions of fresh callus weight (RFCW) at boron concentration of 15 mM, in relation to the control, were calculated. Fifteen genotypes expressed low callusing ability at the control medium (FCW ≤ 10 g) and they were excluded from further analyses. The rest of the genotypes were classified into three groups with different levels of boron tolerance. Genotypes with RFCW below 50.0% were considered as tolerant, from 50.1 to 70.0% as medium tolerant and above the 70.1% as sensitive. The most of the genotypes had MT (24) or S (38) reactions to excess boron, which was in agreement with reactions of the parent genotypes of the ITMI population, Synthetic (S) and Opatha (MT). Only four ITMI lines exhibited tolerant reactions to excess B, probably due to mutations or somaclonal variations, which occurred during the cultivation. The level of tolerance of Serbian cultivars was confirmed in this study. The results have shown that boron tolerance of wheat genotypes is detectable at the cellular level in the *in vitro* culture.

P - 91

Evaluation of F2:3 rice population for tolerance to seedling stage salt stress

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This study investigated $F_{2:3}$ rice population of BG90–2 and Jarmissa for tolerance to salt stress as seedlings. 242 progenies, 2 parents and 2 checks were grown under two salt stress regimes: (0.15 dSm^{-1} (non-stress) and 12 dSm^{-1}(stress), scored on a scale 1–9 (IRRI, 1996) and evaluated by six parameters: leaf and root development, height of plant, root length, shoot dry weight and shoot dry weight after 21 days. Progenies were grouped into highly tolerant (0), tolerant (1), moderately tolerant (8), susceptible (192) and highly susceptible (45) based on SES scores. Ordinal regression analysis showed 123 and 127 progenies out of the 246 entries showed more tolerance to salt compared to FL478 (tolerant check) and Jarmissa (tolerant parent). Genotype by environment interactions were highly significant ($p \leq 0.01$) for shoot height and root number and significant ($p \leq 0.05$) for other traits. Heritability ranged from 26 to 46% and 62.65 to 83.14% under stress and non-stress conditions. Positive correlations were observed between shoot dry weight and root length (R^2 = 0.551), root length and root number ($R^2 = 0.633$) root number and shoot height ($R^2 = 0.709$) under salt stress. Principal components (PC 1 and PC 2) together explained 99.60 and 99.43% of the variations under stress and non-stress and showed high association with shoot and root dry weights. Progenies BGJ 4, BGJ 30 and BGJ 25 combined good shoot and root dry weight traits have been selected as parental lines.

P - 95

Overexpression of PagSAP5 improves drought stress tolerance in transgenic poplars (*Populus alba* × *P. glandulosa*)

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Members of the stress-associated protein (SAP) gene family are considered important in trees because they induce abiotic stress tolerance. However, the specific functions of these genes have not been clearly elucidated. Here, we provide molecular and physiological characterizations of *PagSAP5*, a novel member of the SAP gene family, in the hybrid poplar (*Populus alba* × *P. glandulosa*). The PagSAP5 protein contains an A20 and an AN1 zinc-finger domain at the N- and C-

terminal, respectively. *PagSAP5* showed differential regulation in response to various abiotic stresses such as drought, salt, cold, and abscisic acid (ABA) treatments. Compared to wild type, *PagSAP5* overexpression lines showed increased tolerance to drought stress, while RNAi lines in which *PagSAP5* expression had been knocked down showed increased drought sensitivity. *PagSAP5* overexpression lines undergoing drought stress also showed increased stomatal closure and increased expression of ABA biosynthesis-related genes, such as 9-cis-epoxycarotenoid dioxygenase and abscisic acid-deficient 3, compared to wild type. This suggests that overexpression of *PagSAP5* in the transgenic poplar confers drought tolerance *via* rapid, ABA-mediated stomatal closure. These results indicate that *PagSAP5* is a good candidate for engineering drought stress tolerance in woody plants.

0–103

Bracing for Impact: Engineering aerial roots to withstand climate change

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Environmental fluctuations, *e.g.* storms, can impart intense forces on objects in their path. Indeed, plants are faced with the mechanical challenge of staying upright, while withstanding these dynamic external forces. In agriculture, the failure of plants to stay upright is called lodging. Depending on the crop and growth environment, lodging can account for between 5 and 66% of yield losses worldwide, and lodging is likely to increase with increased frequency of extreme weather events. When faced with the challenge of feeding a growing world population, mitigating crop losses due to lodging is one area in which rapid production gains can be made. Lodging can be classified into stalk lodging and root lodging depending on the point of mechanical failure, with root lodging suggested to be more prevalent worldwide. In some crops, specialized aerial roots called brace roots are hypothesized to play a key role in anchorage and the prevention of root lodging. We are taking a structural engineering approach to define the contribution of brace roots to plant stability. From structural engineering, we know that there are two key features to building stable structures: the arrangement of the building materials and the mechanical properties of the building materials. To extrapolate these features into plants, we have obtained field-based above- and below-ground root phenotyping data from a diverse germplasm to define the arrangement of building materials. In addition, we are subjecting brace roots to bending tests to define the mechanical properties of the building materials. This information is being integrated into structural engineering models to determine the contribution of brace roots to plant stability. These experiments are among the first to define the diversity of brace root architecture and mechanical properties in maize, which is critical to understanding the significance of these specialized roots in plant stability under fluctuating environments.

0–105

Engineering of Jasmonate Biosynthesis Pathway in Wheat

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Jasmonates are plant hormones, which regulate plant adaptation processes in constantly changing environments, protect plants against insect herbivores and phytopathogen infection. Modulation of jasmonate-related signaling system activity with the aim of improvement of plant tolerance to unfavorable environment is one of the promising approaches in modern biology and agriculture. Here we report on the development of transgenic bread and emmer wheat events, expressing genes coding for two key enzymes of jasmonate biosynthesis pathway. The sequences of allene oxide synthase (AtAOS) and oxophytodienoate reductase (AtOPR3) from *Arabidopsis* were placed under control of constitutive Ubi promoter/intron and expressed in wheat plants. More than 80 transgenic wheat plants were generated through micro projectile bombardment, including the events transformed with the both heterologous sequences. The expression of transferred genes was observed in 59 primary transgenic wheat plants, the analysis of transgene inheritance was carried out, and homozygous T1-T2 transgenic progenies were obtained. In spite of difficulties caused by low transformation efficiency during transfer of AOS gene and possible gene silencing in subsequent generations, homozygous plants expressing AtAOS and AtOPR3 genes have been found and now are being already tested for the tolerance to environmental stresses. In addition, an RNAi construct was designed to suppress (knock down) allene oxide synthase (AOS) genes in wheat, and introduced using a biolistic bombardment protocol into genome of hexaploid and tetraploid wheats. Physiological tests performed on homozygous transgenic plants overexpressing AtAOS gene and wild type plants treated with methyl jasmonates demonstrated the protective role of this hormone under low temperature stress conditions. Obtained results inspire hope for the possible improvement of wheat cold and freeze tolerance by means of alteration of jasmonate biosynthesis pathway activity. The work was supported by the Russian Science Foundation, grant No 16–14-10,155.

P - 112

A PyMPV17, Pyropia (Rhodophyte) homolog of the human MPV17 enhances abiotic stress tolerance in Chlamydomonas

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Pyropia yezoensis is the most cultured marine red algae (Rhodophytes) in Asia and is extremely desiccation tolerant. *PyMPV17* is a desiccation stress response gene isolated from *P. yezoensis*. The *PyMPV17* shares an amino acid sequence homology with human *MPV17*, which is the inner mitochondrial membrane and associated with mitochondrial DNA depletion syndromes. *MPV17* homologs are found in all eukaryotes including yeast and zebra fish, and mutants in the gene cause different phenotypes. The molecular function of the MPV17 protein has remained unclear. The yeast ortholog of *MPV17*, the *SYM1* mutant, cannot grow on an ethanol-containing medium at 37 °C. Fluorescence of the PyMPV17-GFP fusion proteins such as MPV17 and SYM1 were detected in the mitochondria. The expression of *PyMPV17* in *sym1* knock-out yeast cells complements the ethanol growth defect at 37 °C, suggesting that the *PyMPV17* is a functional ortholog of *SYM1*. The *PyMPV17* shows an up-regulation in transcription under desiccation in gametophytes of *P. yezoensis*. Transcription of the *PyMPV17* gene is also increased by H_2O_2 and ABA treatments. Transformed *Chlamydomonas* sp. overexpressing the *PyMPV17* gene grow better than control cells with an empty vector on agar plates containing mannitol. These results suggest that the *PyMPV17* contributes to the tolerance mechanism for osmotic stress in *Pyropia*. This is the first study on the physiological function of a *MPV17* homolog in plants and contributes to understanding the functions of the *MPV17* gene better.

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Effects of fullerenol on antioxidant enzyme activity and gene expression in sugar beet under drought

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In the wake of changes in global climate, drought stress has become the main cause of a significant crop yield reduction. At the same time, irrigation is not applied in many areas due to increases in production costs. Therefore, new approaches based on promoting plants' adaptation to drought may be more viable solution. There are reasons to believe that fullerenol ability to form hydrogen bonds with water molecules makes this nanoparticle a potential intracellular water depot, which can be used if osmotic stress occurs. As a part of an extensive study of fullerenol influence on reaction of sugar beet plants exposed to water deficit, this research was conducted with the aim of detecting changes in the activity of antioxidant enzymes and transcriptional profiles of the genes coding for those enzymes. Foliar application of 0.01 (F1 treatment) and 0.001 (F2 treatment) nmol fullerenol solution was performed after four months of sugar beet plant growth in greenhouse. Moderate (20–30%) and severe (10–20%) water deficit regimens were used with a control of 60–70% soil water capacity. Moderate drought effected catalase activity in the F0 treatment, resulting in increased enzyme activity in comparison to F1 and F2 treatment. In contrast, severe water deficit negatively influenced catalase activity in F0 and F2 plants when compared with F1. Ascorbate peroxidase activity increased under water stress in F1 treatment. The enzyme activity was not affected in F0 and F2 treatments under both water deficit regimens in comparison to the control. Only severe drought increased the activity of glutathione peroxidase in plants treated with fullerenol. The selected candidate genes differed in relative gene expression among applied fullerenol treatments and water regimens. Although further studies are necessary to elucidate the effect of fullerenol on sugar beet plants, the present results indicate its usefulness in improving plant adaptation to drought.

0–135

Secondary metabolite scopoletin controls crop disease

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Scopoletin has multiple beneficial properties that support its use in agriculture or as a nutraceutical. In plants, scopoletin contributes to the response to biotic and abiotic stress. However, successful application of coumarin in plant protection has not been demonstrated so far. We show that scopoletin accumulates in *Arabidopsis thaliana* leaves during postinvasion defense to the Asian soybean rust (SBR) fungus *P. pachyrhizi*. However, scopoletin is absent from leaves of the soybean host even upon SBR infection or after exposure to abiotic stress. Spray application of scopoletin to soybean leaves provided SBR protection by interference with the

formation of *P. pachyrhizi* preinfection structures rather than by activation of endogenous plant defense pathways. Consistent with its pivotal function in scopoletin biosynthesis, constitutive expression of Arabidopsis *Feruloyl-CoA 6'-hydroxylase 1* (*AtF6'H1*) enabled efficient production of scopoletin in transgenic plant cell cultures. Moreover, high levels of scopoletin and its glucoside scopolin in *AtF6'H1*-overexpressing soybean plants reduced their susceptibility to different fungal pathogens. Our results show that spray-application and genetically engineered accumulation of scopoletin are two promising strategies for plant disease control.

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Potential role of plant DSS1(V) gene in defense against oxidative stress

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DSS1 protein has a crucial role in persistency of genome integrity, regarding its involvement in BRCA2 mediated repair through DNA recombination. Also, DSS1 takes part in protein homeostasis, participating in the 26S proteasome biogenesis. Two genes encoding DSS1 protein have been revealed in the *Arabidopsis thaliana* genome: *DSS1(I)* and *DSS1(V)*, indicating its diverse biological functions. Novel role of DSS1 protein has been proposed recently. Apparently, DSS1 is able to bind oxidized proteins and therefore mark them for degradation through ubiquitin-proteasome systems. In order to elucidate plant DSS1 function, mature *Arabidopsis* plants grown hydroponically were submitted to oxidative stress induced by hydrogen peroxide or methyl viologen. The level of lipid peroxidation was measured as an indicator of oxidative stress. Expression profiles of the *DSS1(V)* gene and protein were analyzed using real time PCR and Western blot. An increasing trend of lipid peroxidation was detected in plants exposed to stated stress factors. The tested concentrations of methyl viologen or hydrogen peroxide did not cause significant changes in the level of *DSS1(V)* expression in the leaves. Particularly dramatic increase of *DSS1(V)* gene and protein expression was detected in the roots treated with 10 mM hydrogen peroxide. With an aim to further clarify functions of plant *DSS1(V)* gene we have selected an *Arabidopsis* homozygous line with T-DNA insertion in *DSS1(V)* gene and further characterized mutant plants. Gene expression analysis revealed that mutant plants show 75% lower levels of *dss1(V)* mRNA than the wild type. Also, *dss1(V)* mutants were slightly more sensitive to the stress and grow more slowly compared to wild type *Arabidopsis*.

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Responses of six aspen genotypes to water deficit stress during shoot culture *in vitro*

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Genetic variation in growth response to water deficit stress (WDS) is a basis for evaluation of draught-tolerant genotypes. In this study, explants (10–12 mm apical shoot segments) of six aspen genotypes, representing *Populus tremula* and hybrids between this species and *P. tremuloides*, were tested in Parafilm-sealed glass tubes (150 × 20 mm) for growth responses under WDS conditions. To induce WDS, polyethylene glycol (PEG-6000) was added to a hormone-free nutrient medium at the concentrations of 50, 100, and 150 g/l. After six weeks of culture, the *in vitro*-grown plants were sampled for the analyses of fresh and dry mass, chlorophyll content, and leaf stomata number. Other measurements taken for the study included plant height, leaf area, shoot and root number per plant, and root length. The results revealed a significant interaction between plant genotype and PEG-6000 concentration in the medium in respect of shoot and root growth and morphology, of the total shoot biomass, and of the total amount of pigments. Two of the six tested genotypes were distinguished by their ability to adapt to WDS *in vitro*. It should be noted that the shoots of these two genotypes, grown under *in vitro*-induced WDS, also showed increased recovery after their transfer onto a PEG-free medium.

Keywords: aspen, hybrid, *in vitro,* nutrient medium, polyethylene glycol, water deficit stress

0–143

From microspore to embryo: how to get stressed and survive to tell

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Microspores can be induced to change their developmental pathway and form haploid embryos *in vitro*. This process, known as microspore embryogenesis (ME), is generally induced by one or more stress treatments. ME

can be easily induced in some species, *e.g. Brassica napus*, while other species are recalcitrant, including the model species Arabidopsis and members of the Solanaceae. In *B. napus,* this change of developmental pathway is commonly induced by a heat stress. After the stress treatment, some microspores start to divide, other adopt a pollen-like development or arrest. Induced cells can either continue to form haploid embryos or arrest and die. We have observed that the fate of these dividing microspores is related to their stress response. Our data indicate that the induced microspores that do not follow further embryo development show endoplasmic reticulum (ER) accumulation (related to ER stress), high lytic activity, accumulation of starch and lipids and loss of cell adhesion. They become callus-like structures, and eventually undergo premature cell death. In embryo-like structures, all these processes are also present but at lower levels, which contributes to survival and conversion into differentiated embryos. Using cellular and molecular approaches we are studying the effect of several stress-related compounds in embryo yield and their role in the occurrence of the above mentioned features in different genotypes with different ME responses. Our aim is to link these cellular processes with stress response and cell fate in different genotypes, in an attempt to understand the underlying mechanisms, design ways to modulate these processes and eventually increase the number of cells committed to embryogenesis.

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Improving Germination and Seedling Flooding Tolerance for Direct Seeding in Rice

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To cope with global climate change that threatens food security, and to meet the world's high demand on food supply in the next few decades, there is an urgent need to integrate advanced biotechnologies into conventional breeding programs in order to accelerate the breeding process. Direct seeding, by which seeds germinate and seedlings grow under water, has increasingly become a popular cultivation method in many rice growing areas, especially in Asian countries, due to the advantages of reduction in labor, production costs and water use, and the ease of large-scale mechanization. However, most rice varieties are extremely sensitive to anaerobic conditions during germination and early seedling growth stages. This has been the major obstacle to promote direct rice seeding widely. To improve anaerobic germination and seedling growth of elite rice cultivars for use in direct seeding cultivation, we have taken several multidisciplinary approaches that include: (1) Development of a gene in the O_2 deficiency regulatory pathway as a functional marker for marker-assisted selection (MAS) breeding. (2) Identification and investigation of genes selected from QTL and transcriptomic analyses that may control seedling flooding tolerance. (3) Characterization of T-DNA activation/knockout-tagged rice mutants for functional validation of genes conferring flooding tolerance. Several O_2 deficiency-induced genes have been identified, and rice mutants corresponding to these candidate genes are in the process of phenotyping. Differential expression of some of these genes correlates with the degree of flooding tolerance in various rice varieties, and these genes will be evaluated as markers for the MAS breeding. Progress on these studies will be presented in the conference.

0–148

Improvement of plant stress tolerance through modulation of the oxylipin pathway in Arabidopsis and wheat

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Lipid-derived signaling molecules oxylipins are known regulators of plant responses to biotic and abiotic stresses. Apparently, the oxylipin pathway emerged in the process of evolution as an instrument for removing oxygenated fatty acids, the formation of which in plant cells is accelerated under stress conditions. Later this system evolved into a multi-branched pathway where diverse metabolites with different biological functions are produced. Allene oxide synthase (AOS) and 13-hydroperoxide lyase (13-HPL) are enzymes of two dominant branches of the oxylipin pathway, competing for 13-hidroperoxide of linolenic acid formed in chloroplasts. Jasmonates, cyclopentenone octadecanoids produced in the AOS branch (12-oxophytodienoic acid, jasmonic acid and its derivatives), share structural similarity with animal eicosanoids, regulate plant growth and development, flower formation, gene expression, fertility, photosynthesis and stress responses. HPL catalyzes formation of 6-carbon metabolites and 12-carbon oxo-acids from 13-hydroperoxides of linolenic acids. HPL branch produced volatile metabolites, major components of green leaves aroma, were shown to be involved in plant protection against insects and pathogens. Our long-standing research demonstrates that the genetic manipulations

resulting in alteration of oxylipin pathway activity is a powerful tool for modulation of plant tolerance to variety of stresses, biotic and abiotic. As exemplified by transgenic *Arabidopsis* and wheat plants with altered oxylipin profile, modulation of AOS and HPL branches activity affects plant responses to biotic challenges, such as fungal pathogens and insects, and abiotic stresses, such as drought, waterlogging, excessive light and suboptimal temperatures. A set of evidences, showing that genetic manipulation of the oxylipin pathway is a useful instrument for generation of plants with desired tolerance to unfavorable environmental conditions, will be presented.

0–158

Ilex paraguariensis Reconfigure the Root Transcriptome and Metabolome in Response to Drought

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Ilex paraguariensis is a subtropical tree cultivated in south of Brazil, northeast of Argentina, and east of Paraguay. The occurrence of drought episodes during the active phase of the annual growth cycle leads to leaf abscission and yield reduction. RNA-seq, gas chromatography-mass spectrometry, and spectrometry assays were used to elucidate a global understanding of how roots respond to dehydration. A total of 190,474 unigenes and 265,309 transcripts were obtained from 74,911,111 pair-ends reads (2x100bp) from 6 libraries. Among them, 30.6% were associated with a Gene Ontology (GO) term. The comparative analysis of transcriptomes between the control and each stressed sample revealed that 8046 transcripts were differentially expressed [DETs, FDR <0.001; $\log_2$(Fold Change) ≥ |1|]; among them, 3020 were up-regulated and 5026 repressed by drought. GO functional enrichment revealed over-represented hormone signaling pathway, trans-acting elements families, cellular amino acids metabolism and development of root anatomical components involved in response to drought. Along with, 27 metabolites including amino acids, carbohydrates, and organic acids varied their concentration in response to stress. These results provide a comprehensive overview of how *I. paraguariensis* responds to dehydration at transcriptome and metabolome levels which may help to understand molecular mechanisms associated with drought response.

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PtDRG1, desiccation response gene from Pyropia tenera, exhibits chaperone function and enhances abiotic stress tolerance

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Pyropia spp. are commercially valuable marine red algae that grow in the intertidal zone. They are extremely tolerant to desiccation stress. We have previously identified and reported desiccation response genes (*DRGs*) based on transcriptome analysis of *P. tenera.* Among them, *PtDRG1* encodes a polypeptide of 22.6 kDa that is located in the chloroplast. PtDRG1 does not share sequence homology with any known genes deposited in public database except some genes of several red algae species. Transcription of *PtDRG1* gene was upregulated by osmotic stress induced by mannitol or H_2O_2 as well as desiccation stress, but not by heat. When *PtDRG1* was over-expressed in *Escherichia coli* or *Chlamydomonas* sp., transformed cells grew much better than control cells under high temperature as well as osmotic stress induced by mannitol and NaCl. In addition, PtDRG1 significantly reduced thermal aggregation of substrate protein under heat stress condition. These results demonstrate that PtDRG1 has a chaperone function and plays a role in tolerance mechanism for abiotic stress. This study shows that red algae have unknown stress proteins such as PtDRG1. They play a role in stress tolerance of red algae as stress proteins with chaperon function such as dehydrin in green plants.

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Co-integrating Two Insect Resistant Genes in the transgenic Sugarcane Exploiting AMT

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Sugarcane (*Saccharum sp*. hybrids) is a highly productive C4 grass used as the main source of sugar and more recently to produce biofuel. Transgenic sugarcane with improved agronomic and value added traits has been reported mostly *via* biolistic gene transfer. We routinely transform established callus cultures from sugarcane with *Agrobacterium*-mediated transformation (AMT) by using immature leaf trans-sections or trans-sections with callus initiation as target in order to

reduce the time in tissue culture. This will accelerate the production of transgenic sugarcane and likely enhance the performance of the transgenic plants. With the optimized AMT, marker gene hpt or nptII driven by 35S, and genes of interest (GOI) *sck* and *cryIAc* driven by promoters ubi1 (or actin) which enhancing sugarcane insect resistance, were integrated into two T-DNA independently facilitating knock out of the selection marker gene *via* self-seed setting as the T-DNA separation in progenies for the biosafety. The GOIs and selection maker gene hpt or nptII integrated randomly into sugarcane genome owing to the two separate T-DNAs. The GOIs expressions in the transgenic plants were confirmed by either molecular analysis or bioassay.

0–171

Unlocking how an extraordinary plant pigment combats salt stress

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Salinity tolerance is very important to food production and environmental sustainability given that arable land area affected by high salinity has increased due to climate change, irrigation practices, desertification, flooding, and other causes. As a result it is useful to identify different mechanisms responsible for salt tolerance in plants. Our previous studies showed that foliar betacyanin pigments are associated with salinity tolerance in *Disphyma australe*, a halophyte capable of growing under high salt conditions. However the molecular mechanism responsible for this process has not been explored. To address this we have conducted large scale RNAseq analysis to help develop a comprehensive understanding of salt tolerance in *D. australe*. The results obtained from this study should generate new insights on alternative salinity tolerance mechanisms in halophytes, and could inform the development of novel biotechnological approaches to improving agricultural productivity in salinity affected areas.

0–173

Expression pattern analysis of ACC synthase and EREBP genes in response to drought stress

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Chickpea (*Cicer arietinum* L.) one of the most important grain-legume crops, is grown in more than 45 countries, mostly in arid and semiarid zones. Plants respond and adapt to water deficit at both the cellular and molecular levels. A large number of genes has been described that respond to drought at the transcriptional level and the mechanisms of the molecular response to water stress in higher plants has been analyzed by studying the expression of genes responding to drought and other abiotic stresses. The expression pattern of *ACC* synthase and *EREBP* genes in two chickpea genotypes MCC 283 and MCC80 in the different growth stages under drought stress were investigated. For drought treatment, soil-grown 30 day-old (vegetative growth stage), 60 day-old (early pod visible) plants were subjected to progressive drought by withholding water for 2, 4, and 6 days and untreated plants were used as control. RNA was extracted from leaf and then cDNA was synthesized. RT-qPCR analysis of *ACC* synthase and *EREBP* expression using specific primers showed different expression patterns in different stages of both chickpea genotypes. Differential expression of *ACC* was observed in both genotypes in various phonological stages and its timing, duration and intensity of drought treatments. The expression levels of *EREBP* in both genotypes were increased significantly from 2 to 6 days of water deficit in vegetative and early pod visible stages. The increase in *ACC* synthase and *EREBP* expression in the drought treatment for both genotypes in the vegetative growth stage and early pod visible might be an adaptation to overcome the stress condition, supplying energy for growth and survival, thus helping the plant to survive.

Keywords: Chickpea, Drought stress, RT- PCR, Gene expression

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Characterization of small heat shock protein (PtsHSP) in marine red algae, Pyropia tenera (Bangiales, Rhodophyta)

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Small heat shock proteins (sHSPs) are a family of heat stress proteins identified in all organisms. It is suggested that they evolved very early in life history. The sHSPs are very diverse in higher land plants. Some seed plants have more than 30 individual sHSPs that consist of distinct subfamilies. Green algae have only a subfamily. However little is known about sHSP in red algae. We analyzed transcriptome and draft genome sequence of the *P. tenera,* and identified a total of 8 small heat shock protein genes (PtsHSPs), which are containing an α-crystallin domain. Amino acid sequence and expression pattern of the PtsHSPs were analyzed. The PtsHSP19.6 respond to mainly heat stress, but did not show significant sequence

homology with any known sHSP except the α-crystallin domain. When *PtsHSP19.6-GFP* construct was introduced into protoplast of *Nicotiana benthamiana*, GFP signals were detected at several small granules. Gel filtration and native gel electrophoresis show that PtsHSP19.6 forms a polymer of large molecular weight. When the *PtsHSP19.6* was overexpressed in *E. coli,* the transformed bacteria cells exhibited much higher growth than those of the wild type under high temperature. These results indicate that *PtsHSP19.6* can confer heat tolerance ability. These data will provide information on sHSPs in red algae, and elucidate the differences in structure and function between and within the diverse sHSPs.

0–179

Nuclear migration is a morphological marker for acquisition of salt stress resistance *in vitro*

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In vitro selection for salt resistance has focused on cellular and genetic mechanisms using selected NaCl-tolerant plant cell cultures. The biology and genetic diversity of early plantlet growth as well as the effects of salinity stress on plant morphology have been extensively studied. To date, only the effects of salinity on cell size have been examined while those on cell morphometry have not. We produced calli of *Medicago truncatula* Gaertn. R108 capable of resisting up to 350 mM NaCl following the application of a step-up recurrent selection strategy. Additional assessments of cell suspensions derived from such calli revealed that the surface area of the resistant cells and of their nuclei were reduced compared to non-selected tissues when NaCl concentrations in the medium increased. We also subjected cell suspensions of tobacco (*Nicotiana tabacum* L. BY2) to the same increasing NaCl concentrations for 8 days. Cell and nuclear size also decreased, consistent with signs of plasmolysis, and cells elongated as NaCl concentration increased, but none of these parameters were reliable in explaining cell survival and growth at high NaCl concentrations. However, for both species, nuclei of resistant cells migrated from the centre of the cytoplasm to a peripheral position close to the cell walls and could thus become a novel and reliable morphological marker of acquisition of salinity tolerance *in vitro*.

0–181

Role of a Stress-induced Intrinsically Disordered Protein on the Establishment of Drought Tolerance in Rice

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Plant roots are the first organ to perceive water-deficit stress, but how the root system adapts to the unfavorable environment is still unclear. In rice, an intrinsically disordered protein with nearly 40% proline, REPETITIVE PROLINE-RICH PROTEIN (RePRP), is induced by water-deficit stress and abscisic acid (ABA) preferentially in the root elongation zone. Ectopic expression of RePRP confers a 'short but heavy' root phenotype, resembling the effect of water-deficit stress or ABA treatment; this phenotype is reduced in RePRP RNA-interference knockdown transgenic rice, which suggests that RePRP is sufficient and necessary for water-deficit stress or ABA-repressed root development. RePRP interacts with the highly ordered cytoskeleton components, actin and tubulin, both *in vivo* and *in vitro*. The binding of RePRP reduces the abundance of actin filaments and impairs non-cellulosic polysaccharide transport to the cell wall. RePRP also reorients the microtubule network, which leads to disordered cellulose microfibril organization in the cell wall. The cell wall modification inhibits the elongation of root cells and promotes biomass accumulation in the 'heavy' root, which facilitates plant survival under adverse conditions. We demonstrate a novel role of intrinsically disordered proteins controlling cell expansion *via* an 'order-by-disorder" mechanism for development of 'short-but-heavy' roots as an adaptive response to water deficit in rice.

0–183

Cytomics and morphometry of tobacco cells expressing the C-terminal domain of the clathrin heavy chain

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Tobacco plants and cells constitutively expressing the C-terminal domain of clathrin heavy chain, called the Hub domain, have been obtained. Clathrin is the major protein forming a lattice-like coat surrounding endocytic vesicles.

Thus, the Hub domain is a negative dominant for the onset of endocytosis but may also modify other cellular traits such as wall synthesis and ploidy level. Here we aimed at characterizing leaves of *Nicotiana tabacum* (cv. Xanthi) but also *N. tabacum* cell suspensions and callus tissues (cv. Xanthi and cv. BY-2) that express the Hub domain. Flow cytometry has been used for characterization of cells and tissues in terms of ploidy level, genome size and AT-GC ratio. For that purpose, tissues and cells were stained with the A-T specific dye 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) to assess their ploidy level, with propidium iodide (PI) to assess their genome size, and with chromomycine A3 to assess their G-C content. In addition, we intended to examine the genetic transformation effects on cell morphometry by analyzing length, width, surface area and shape coefficient of cells from callus and suspensions. Hub expression resulted in a higher genome size of Xanthi plants and of callus of Xanthi and BY-2, but its effects were variable for cell suspensions; the A-T level increased in callus but decreased in plants and was variable in cell suspensions. In terms of morphometry, Hub expressing cells tended to be more elongated. Implications of these findings for stress resistance and endocytosis are discussed.

0–191

Trace Elements and Antioxidant Analysis of Lemongrass Growing in Different Concentrations of Tannery Sludge

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Lemongrass (*Cymbopogon flexuosus* Stapf.) is an aromatic plant, which is a member of family Poaceae. It is a multi-harvest crop which can be helpful in phytoextraction. Due to its ability to uptake metal, it is also known as hyper-accumulator. Through several harvests soil contaminants can be extracted effectively in order to improve soil quality. This research was carried out to assess phytoremediation potential of *Cymbopogon flexuosus*. Lemongrass was grown in different amendments of tannery sludge in soil (*i.e.* 0, 5, 10 and 15%). Plants were harvested twice, after 30 and 60 days of the experiment. It was noticed that metal uptake was high in roots when compared to shoots *i.e.*, in case of control (0%) of tannery sludge in soil, uptake of metals like Cr, Cd, Cu, was observed as 293, 278, 54 mg kg^{-1} respectively in root and 141, 127 and 38 mg kg^{-1} respectively was estimated in shoot. Uptake of these heavy metals induces morphological changes in plants *i.e.*, reduction in number of leaves, roots seedling length and other parameters were takes place. Along with these morphological changes due to heavy metal uptake, amount of free radicals in the plants increases with increasing concentrations of tannery sludge in the soil which induces antioxidants to eliminate the free radical produced under metal stress. Thus, antioxidant activity enhances with increase in free radicals due to heavy metal uptake might be a reason to increase the growth of plants under stress.

0–196

Multistep interplay of glutathione with salicylic acid and ethylene to combat inevitable environmental stress

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Glutathione (GSH) is a nearly ubiquitous, abundant non-protein tripeptide thiol compound found in both prokaryotes and eukaryotes. GSH is know to play a role in plant defense, in addition to its substantial role in stress tolerance and antioxidant signaling. However, how GSH interacts with other established signaling molecules in a rather complicated defense signaling network is unknown. To this end we tried to untangle the interaction between GSH, salicylic acid (SA) and ethylene (ET) to combat environmental stress. For that a transgenic *Arabidopsis thaliana* line *viz. AtEcs*, exhibiting enhanced GSH content as confirmed by HPLC analysis, has been developed. Transgenesis was confirmed by Southern Blot and Western blot analysis of *AtEcs* lines. Transcriptomic and proteomic profiling of *AtEcs* lines identified the genes and proteins altered at enhanced GSH condition and probably regulated by GSH as well. Several SA related genes *viz. PR*1*, GLS, MAPKK, NPR*1, *etc.* and proteins *viz.* HSP 70, ADC, NBS-LRR, CA, PR10 *etc.* were identified. QRT-PCR analysis further confirmed the expression level of SA-related genes in *AtEcs* line. Interestingly, in addition to SA-related transcripts, 1-*aminocyclopropane-1-carboxylate oxidase* (*ACC oxidase*), a key enzyme of ET biosynthesis, was identified as well. Fascinatingly, induction of 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) was also noted in the proteomic profiling of *AtEcs* line, corroborating with the transcriptomic profiling. Together, our data revealed that GSH is involved in multiple steps crosstalk through ET and SA to combat environmental stress *in planta.*

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Interactive Effect of pH and Temperature on Germination of Two Indian Wheat Varieties

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Wheat production is adversely affected by extreme temperature and pH. These abiotic stresses individually or in

combination result in low leaf photosynthetic rate, increased embryo abortion and lower grain number; hence lower grain yield of wheat. Acidic soil enhances mobility of heavy metals where by reducing the availability of essential metal ion content which are required for the proper growth and development of the plants. Two, seven day old wheat genotypes (*T. astevium*; HI 1544 and *T. durum;* HD 8737) were evaluated for nine different combinations comprised of three different pH (4.0, 5.0, 6.0) and temperature (10 °C, 20 °C, 30 °C) conditions, supplemented with quarter strength Hoagland solution in a static hydroponic system. *T. durum* had high protein and low proline content compared to *T. astevium* suggesting the good adaptability of *T. durum* under varying pH and temperature conditions. Based on the statistical analysis for various parameters such as chlorophyll content and nitrate reductase (NR), pH 6 at 20 °C proved to be optimal condition for the growth of *T. durum*. But, in HD 8737 relatively low proline and high protein content were observed under the pH 5 at 20 °C combination. Thus, it has been suggested that HD 8737 can be robust when cultivated under acidic conditions.

Keywords: *Triticum aestivum. Triticum durum, pH, Temperature*

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Genome-wide expression profiling of unique glyoxalase III genes in soybean reveal the differential transcriptional regulation

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Reactive carbonyl species, such as methylglyoxal (MG) and glyoxal are very toxic in nature and can inactivate various cellular macromolecules such as DNA, RNA, and protein by forming advanced glycation end products. The conventional glyoxalase pathway with two enzymes, glyoxalase I (GLY I) and glyoxalase II (GLY II), detoxify MG into D-lactate with the help of reduced glutathione (GSH). However, DJ-1/PfpI domain(s) containing DJ-1/Hsp31 proteins do the same in a single step, and is thus termed as "glyoxalase III (GLY III)". A comprehensive genome-wide analysis of soybean identified eleven putative GLYIII proteins with DJ-1/PfpI domain encoded by seven genes. Most of these proteins are found to be localized in mitochondria and chloroplasts. In spite of the same function, differential evolution pattern was observed in the case of Hsp31 and DJ-1 proteins. Expression of GmDJ-1B, GmDJ-1A, and GmDJ-1D2 transcripts were found to be constitutive in all tissues and developmental stages. However, the developmentally less expressive members GmDJ-1C1 and GmDJ-1C2 transcripts were upregulated in response to ozone, heat, and drought stresses. Transcript profiling with qRT-PCR revealed the strong substrate specific upregulation of all GmDJ-1 in response to exogenous MG exposure. A member of the GmDJ-1 family, GmDJ-1D1, maintained upregulation in response to all studied stresses, including salinity, dehydration, oxidative and exogenous ABA. This study identifies some novel tissue-specific and abiotic stress-responsive GmDJ-1 genes that could be investigated further.

0–230

Pollen analysis made easy

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Success of doubled haploid production, interspecific crosses, breeding strategies, and seed production depends highly on pollen quality which is affected by genetic and environmental factors. The recently developed microchip-based impedance flow cytometry (IFC) detects changes in the electrical properties of cells and enables a reliable, non-destructive analysis of pollen. Within a single measurement it is now possible to discriminate between dead, viable, and pollen with germination capacity. This technique allows us to identify plants with distorted pollen development, to screen for abiotic stress tolerance, to improve and develop pollen storage protocols, and to optimize F1 hybrid seed production. These and other applications will be presented.

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Screening of IFVCNS Rapeseed Varieties for Lead and Cadmium Tolerance *In Vitro*

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Remediation methods allow the removal of metals from contaminated soil, and phytoremediation, a technology for cleaning contaminated soil and waste material by plants, is becoming increasingly used. *Brassica napus* L., as one of the main oilcrops and high-biomass producing species, is becoming more and more interesting for the use in phytoextraction as it is proved to be tolerant to higher concentrations of heavy metals. Rapeseed breeding program at Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS), comprises creation spring and winter varieties, as well as hybrids. Commercial spring and winter varieties, that already possess good potential for biomass production,

were screened for their tolerance to lead (Pb) and cadmium (Cd) *in vitro*. Screening was done on MS medium supplemented with different concentrations of Pb and Cd. The effect of heavy metals on tested genotypes was determined by measurement of fresh and dry weight of root and aboveground part after 14 days of culture. Variety Banaćanka was found to be the most tolerant to the applied concentrations of Pb since there were no significant changes in the growth and biomass accumulation in all treatments except one. The similar results were obtained with Cd, where Banaćanka and Slavica showed good tolerance to all applied Cd concentrations. As Banaćanka has already been found to be resistant to the increased concentrations of nickel, it could be recommended for further use in phytoremediation studies.

0–260

A tripartite feedback of WHIRLY1, SA and H2O2 affects leaf senescence, cell death in Arabidopsis

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Dually located proteins have been suggested to hold a novel role resembling that of reactive oxygen species (ROS) and metabolites in integrating the metabolic, hormonal, and environmental signals of retrograde signaling. However, the underlying detail mechanisms of these signal integrations remain to be demonstrated. Here we found that overexpressing *pWHY1* and *nWHY1* altered the gene expression profiles of enzymes related to hydrogen peroxide (H_2O_2) and salicylic acid (SA) syntheses and produced different phenotypes. A knockout mutation in *WHY1* brought forward the first H_2O_2 peak and SA peak by one week during plant development due to alteration of the expression of *PAL* and *PRX33* by nWHY1. On the contrary, H_2O_2 treatment increased the accumulation of WHY1 in plastids and decreased in the nucleus, while methyl salicylate (MeSA) treatment increased WHY1 in the nucleus and decreased in plastids. H_2O_2 treatment increased the expression of *WRKY53* and *PAL*, producing a senescent phenotype. MeSA treatment increased the expression of *WRKY53*, *WRKY33*, and *peroxidase,* producing a cell death phenotype at late stages. Both effects were dependent on WHY1. Furthermore, CHIP-PCR data showed that H_2O_2 treatment enhances H3K9ac enrichment and RNAPII recruitment at the *WRKY53* promoter, resulting in promote *WRKY53* expression owing to decrease of nWHY1. Therefore, our results suggest that a tripartite feedback loops including dual located WHY1, SA and H_2O_2 as integrated retrograde signals promote WRKY53 mediated leaf senescence and WRKY33 mediated cell death in *Arabidopsis*.

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A novel OsDHN-FKBP complex trigger ABA mediated pathway to impart drought tolerance in rice

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Rice is an agronomically important crop globally, but abiotic stress, particularly drought, reduces its yield drastically. To meet the demand of increasing world population within a changing environment we need to develop strategies which would help in developing rice varieties that can grow in semi-arid and arid conditions. Therefore, we screened the putatively drought-resistant gene *OsDHN-RAB16D*. Constitutive overexpression of nuclear as well as cytoplasmic localized SK2 type *OsDHN-RAB16D* imparts drought resistance in the homologous system. We hypothesized that, compared to the wild type, overexpressing lines are perform better under water deficit. We observed that lines overexpressing the *OsDHN-RAB16D* gene have improved tolerance against drought, osmotic stress, exogenous ABA and ABA+PEG treatment. On exposure to water stress, overexpressing lines maintain membrane integrity and lower electrolyte leakage. Overexpressing lines also accumulate less reactive oxygen species (ROS) due to enhance CAT-B activity and increases the lignification of the primary cell wall of the cortical region, sclerenchyma layer and stele region of adventitious rice roots. Further *OsFKBP* and *OsDHN-RAB16D* protein-protein interaction suggest that possible signaling pathways activate the downstream ABA-responsive stress-related gene to impart drought tolerance to overexpressing lines. Thus, overexpressing lines harboring *OsDHN RAB-16D* type dehydrin provide drought and osmotic stress tolerance by activating a number of defense responses and in turn get activated when exposed to a stress condition.

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Molecular taxonomy: a modern approach to plant Identification, segregation and programming for conservation

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Sundarbans is the largest mangrove forest in the world and has the highest species richness. Unfortunately very meager work has been executed in terms of establishing the molecular identities and phylogenetic relationships among the true mangroves of Sundarbans. Manual and environmental adversities have proven disastrous for some economically important plant

species including *Heritiera fomes, Nypa fruticans, Xylocarpus mekongensis* and *X. granatum* in the Indian part of the Sundarbans. Unlike morphological or phenotypic markers, molecular markers are not prone to environmental influences and thus provide vital information towards the prioritization of areas for conservation. DNA Barcoding is the art of using a short DNA sequence for species identification. Mitochondrial cytochrome oxidase I (COI) gene has been universally accepted as the DNA Barcode for most of the animals, but the much slower mutation rate in plant mitochondrial genome impedes it's use as a universal plant barcode. The Consortium for the Barcode of Life - Plant Working Group recommended a core-barcode consisting of portions of two plastid regions, rbcL+ matK, to be supplemented with additional markers if required. In this current work, all 22 available true mangrove taxa were collected from different sites of the Sundarbans forest and the candidature of rbcL, matK, ITS, ycf1, trnH-psbA, atpF-atpH and psbK-psbI as the potential DNA barcode were tested. The study showed that none of the primers used could alone distinguish all the samples to species level, rather, maximum resolution obtained was to family level. The multilocus approach yielded best result for rbcL+matK+ITS, where complete segregation of the samples could be achieved to species level. The interspecific and intraspecific genetic diversity distribution is non-overlapping and it created a Barcoding Gap. With the immense credibility of the DNA Barcoding in the field of ecological forensics, the outcome of this work can be helpful for the natural resource administrators and regulators to monitor the illegal trade.

0–278

Pyramiding of transgenic Pm3 alleles in wheat results in improved powdery mildew field resistance

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Allelic *Pm3* resistance genes of wheat confer race-specific resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*, *Bgt*) and encode nucleotide-binding domain, leucine-rich repeat (NLR) receptors. Transgenic wheat lines overexpressing alleles *Pm3a, b, c, d, f,* and *g* have previously been generated by transformation of spring wheat cultivar Bobwhite and tested in field trials, revealing varying degrees of powdery mildew resistance conferred by the transgenes. Here, we tested four transgenic lines each carrying two pyramided *Pm3* alleles, which were generated by crossbreeding of lines transformed with single *Pm3* alleles. All four allele-pyramided lines showed strongly improved powdery mildew resistance in the field compared to their parental lines. The improved resistance results from the two effects of enhanced total transgene expression levels and allele-specificity combinations. In contrast to leaf segment tests on greenhouse-grown seedlings, no allelic suppression was observed in the field. Plant development and yield scores of the pyramided lines were similar to the mean scores of the corresponding parental lines, and thus, the allele pyramiding did not cause any negative effects. On the contrary, in pyramided line, Pm3b × Pm3f normal plant development was restored compared to the delayed development and reduced seed set of parental line Pm3f. Allele-specific RT qPCR revealed additive transgene expression levels of the two *Pm3* alleles in the pyramided lines. A positive correlation between total transgene expression level and powdery mildew field resistance was observed. In summary, allele pyramiding of *Pm3* transgenes proved to be successful in enhancing powdery mildew field resistance.

0–284

Progress and Challenges for deploying RNAi Resistance to Cassava brown streak disease

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Cassava brown streak disease (CBSD) is endemic to East and Central Africa and threatens cassava production and food security for millions of farmers in West Africa. Robust resistance to CBSD is not available to African farmers at this time. Collaborative research with the National Agricultural Research systems in Uganda and Kenya has developed RNAi technology to combat CBSD by expressing siRNAs from an inverted repeat construct consisting of fused coat protein (CP) sequences from the causal pathogens Cassava brown streak virus and Uganda cassava brown streak virus. Confined field trials in Uganda and Kenya have confirmed very high levels of resistance to CBSD in transgenic cassava plant lines expressing high levels of CP-derived siRNAs. Best performing lines presented approximately 2% of storage roots showing damage from CBSD, compared to greater than 95% loss of storage roots to the disease in non-transgenic plants of the same cultivar. Product development and deployment of GM crops remains challenging for the public sector. Before

farmers can benefit from RNAi-derived resistance to CBSD, regulatory dossiers must be prepared, submitted and reviewed by regulatory authorities. Field trials designed to collect regulatory safety performance data from lead RNAi cassava plant lines are ongoing in Uganda and Kenya. Progress towards deploying cassava with RNAi resistance to CBSD will be presented, together with discussion of remaining technical, regulatory and product acceptance challenges.

0–295

Use of EMS mutagenesis for development of forage brassicas with Tolerance to Sulfonylurea Herbicides

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Brassica oleracea (forage kale) lines with increased tolerance to the sulfonylurea herbicide chlorsulfuron were developed through EMS mutagenesis of seeds. Field analysis of advanced breeding lines developed from the original mutants confirmed tolerance to the sulfonylurea herbicide Telar®. The sulfonylureas are a class of herbicide used extensively for selective weed control in arable crops. The mode of action of sulfonylureas is to inhibit the chloroplast-localized enzyme AHAS (AcetoHydroxyAcid Synthase), a key enzyme in the formation of branched chain amino acids. This leads to a deficiency in these amino acids and the accumulation of the AHAS substrate, 2-ketobutyrate, which is toxic. Point mutations in the AHAS gene can result in herbicide-tolerant phenotypes by altering the herbicide binding site while retaining the catalytic activity of the enzyme. Five conserved domains are implicated in resistance to AHAS inhibitors across a range of naturally occurring and artificially generated resistant genotypes. Molecular analyses of the conserved domains of the AHAS gene indicate a proline to leucine substitution in Domain A is responsible for the resistance.

0–296

Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4

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Banana (*Musa* spp.) is a staple food source for more than 400 million people worldwide. More than 40% of world production, and virtually all the export trade, is based on the triploid AAA genome cultivar Cavendish. Cavendish banana is currently under threat from a highly virulent soil-borne fungus, *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4), the causal agent of Fusarium wilt TR4. This lethal disease has devastated Cavendish plantations in South East Asia, Australia and Mozambique and continues to move globally. The disease poses a major threat to commercial banana production worldwide. There is no effective chemical control for TR4 and no commercially acceptable TR4 resistant banana cultivars have been identified. Here we report the identification of a resistance gene (*RGA2*) from a wild TR4-resistant diploid banana and the production of transgenic Cavendish with resistance to TR4. In a 3-year field trial in northern Australia, three lines of transgenic Cavendish transformed with *RGA2* had infection rates of less than 20% with one line remaining completely disease free. *RGA2* levels strongly correlated with TR4 resistance with highest *RGA2* expression observed in the immune line. Interestingly, *RGA2* homologs were also identified in wildtype Cavendish but their expression was found to be significantly lower (tenfold) than in the immune transgenic line. The presence of such gene homologs and the recent development of an efficient CRISPR/Cas9 gene editing platform for Cavendish banana is an attractive avenue to pursue for the future development of non-GM Cavendish resistant to TR4.

0–330

Up-regulation of Helicase enhances abiotic stress tolerance in canola (*Brassica napus*)

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Canola is the second largest oilseed crop worldwide used for human and animal feed. Canola cultivation has grown rapidly over the past five years owing to a high demand for canola oil and meal and is now Australia's third largest broad-acre crop. Australia is the world's second largest exporter of canola seed. With rising global demand for canola for food and non-food applications, its production is expected to increase by 40% by the year 2025. However, impending global climatic changes are predicted to hamper crop productivity. Salinity, drought and high temperatures are major environmental factors that limit agricultural yields. In this context, genetic improvement of crops for abiotic stress tolerance is vital to maintaining our food supply. Helicases, an important class of DEAD-box protein family are primarily known to unwind duplex nucleic acids to perform many housekeeping activities. These highly conserved enzymes play an essential role in several cellular processes including RNA metabolism and regulation of gene expressions. Here we report the development of abiotic stress tolerant canola lines by heterologous overexpression of a DNA/RNA helicase gene.

0–346

In vitro ecotoxicity assessment of leachates from agricultural biodegradable plastic and paper mulches on plants

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The use of biodegradable plastic and paper films for agricultural mulching (BDMs) has arisen as an eco-friendly alternative in response to the increasing concerns for the unresolved disposal of non-biodegradable polyethylene plastic mulches. BDMs can be biodegraded directly in the soil, avoiding plastic waste accumulation. However, during the process until total biodegradation in the humid soil, they may release a variety of chemicals, organic compounds or heavy metals, which could interact and modify the soil ecosystem. The environmental safety of the compounds released needs to be addressed to prevent potential undesired effects in the future, especially on cultivated plants. Official guidelines for ecotoxicity assessment of chemicals on plants are mostly based on Petri dish or soil-pot assays, which provide reliable but limited information. In contrast, *in vitro* culture enables, among others, (i) accurate control of external factors, (ii) visualization of root morphology, (iii) testing a high number of individuals. In previous studies, *in vitro* tests were sensitive to reveal effects on plant development of some chemicals present in biodegradable mulches. In the present work, we study the effects of leachates from eight BDMs in the *in vitro* development of lettuce and radish plantlets. The leachates were incorporated to MS culture media and seeds from lettuce and radish were seeded. Plantlet development was monitored and four weeks after germination, biomass and stress markers (proline, catalase and peroxidase) were determined. In lettuce, one of the BDMs leachates reduced plant biomass and highly increased proline, and most BDMs increased catalase and/or peroxidase activity. Furthermore, two leachates induced dramatic changes in root morphology. In radish, biomass and stress markers were also altered by some of the BDMs leachates. Overall, the *in vitro* culture test has shown to be a suitable tool to detect potential effects of chemicals released from BDMs on plant development.

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The OsPP2Ac-2 as a Negative Regulator on Stresses Tolerance and Plant Development in Rice

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Both forward genetics and reverse genetics approaches are used in this study to clarify the possible physiological function of protein phosphatase 2A catalytic subunit isoform 2 (OsPP2Ac-2) in rice. A previously selected T-DNA insertional mutant line has been analyzed to contain multiple T-DNA insertions in which one of the insertions was tagged at the OsPP2Ac-2 gene. To isolate a single insertional line, the mutant line was backcrossed with wild type (Tainung 67; TNG67) and their progenies were further analyzed. The KoPP2Ac-2 homologous line was identified by genotyping analysis, GUS staining and Southern blot analysis. The expression of the OsPP2Ac-2 gene was down-regulated under stress treatment at seedling stage. The plasmid overexpressing OsPP2Ac-2 genes driven by the ubiquitin promoter were constructed and a total of 14 T_0 transformants (OxPP2Ac-2) were further identified. OxPP2Ac-2 lines were more sensitive but KoPP2Ac-2 knockdown line was more tolerant to osmotic stress compared with TNG67. The plant morphological traits at different growth stages were further observed in wild type and Ko- or Ox- lines. We concluded that OsPP2Ac-2 genes may act as a negative regulator in rice by affecting downstream cell growth genes and stress responsive genes.

0–354

Potato virus Y was detected outside the cell death zone in the hypersensitive response-conferred resistance

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One of the main types of resistance to potato virus Y (PVY) is hypersensitive response (HR)-conferred resistance, which restricts virus spreading and includes induction of cell death, manifested as necrotic lesions. While it is known that salicylic acid is the key component in the orchestration of the events restricting viral spread, the exact function of cell death in resistance is still unknown. Our results show that PVY can be detected outside the cell death zone in *Ny-1*-mediated HR in potato, observed as individual infected cells or small clusters of infected cells outside the cell death zone. We confirmed that the cells at the border of the cell death zone harbor viable PVY that is able to reinitiate infection by exploiting the features of temperature dependent *Ny-1*-mediated resistance. We studied the dynamics of both cell death zone expansion and occurrence of viral infected cell islands outside it. We compared the response of Rywal plants to their transgenic counterparts, impaired in SA accumulation, where the lesions occur but the spread of the virus is not restricted. We showed that PVY can be present outside the cell death zone in all developmental stages of lesions. Additionally, we measured the dynamics of lesions expansion in both genotypes. We showed that while rapid lesion expansion is observed in SA-depleted

plants, virus spread is even faster. On the other hand the majority of lesions slowly expand also in HR-conferred resistance opening the possibility that the infected cells are eventually engulfed by cell death zone. We suggest that the HR cell death is separated from the mechanisms which lead to PVY restriction in *Ny-1* genetic background. We suggest that HR should be regarded as a process where the dynamics of events is crucial for effectiveness of viral arrest albeit the exact mechanism conferring the resistance remains unknown.

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OsMADS57, a Modulator between Organogenesis and Cold Defense in Rice

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Plants modify their development for environment adaptation. Up to now, little was known about how plants coordinate development and stress responses at a molecular level. We demonstrate that interacting transcription factors OsMADS57 and OsTB1 directly target the defense gene *OsWRKY94* and the organogenesis gene *D14* to trade off the functions controlling/moderating rice tolerance to cold. Overexpression of *OsMADS57* maintains rice tiller growth under chilling stress. OsMADS57 binds directly to the promoter of *OsWRKY94*, activating its transcription for the cold stress response, while suppressing its activity under normal temperatures. In addition, *OsWRKY94* was directly targeted and suppressed by OsTB1 under both normal and chilling temperatures. *D14* transcription was directly promoted by OsMADS57 for suppressing tillering under the chilling treatment, whereas *D14* was repressed for enhancing tillering under normal condition. We demonstrated that OsMADS57 and OsTB1 conversely affect rice chilling tolerance *via* targeting *OsWRKY94*. Our findings highlight a molecular genetic mechanism coordinating organogenesis and chilling tolerance, in accordance with recent work suggesting that cold environment has effect on organ differentiation.

0–363

An Investigation of Rhizobacterial Volatile Organic Compounds to Promote Growth and Genetic Transformation in Potato

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The use of volatile organic compounds (VOC's) to promote plant growth has become a very active area in the field of plant-microbe relations in recent years. The vast array of VOC's identified from rhizobacterial isolates have shown great potential as an alternative to current plant protection products, many of which are under ever-increasing restrictions on use, especially within the European Union. An isolation campaign of soil rhizobacteria with plant growth-promoting (PGP) activities from across the south coast of Ireland yielded 120 bacterial isolates. Of these, 50 were selected for biochemical analysis to determine their respective PGP properties. Commonly reported PGP activities such as production of hydrogen cyanide, ammonia and siderophores, phosphate solubilization and VOC production were observed. To determine VOC contribution to plant growth, six isolates were selected for further analysis. Solid phase micro-extraction GC/MS analysis revealed a number of VOC's associated with PGP and growth-inhibition of fungal phytopathogens such as 2, 3-butanediol, 2, 5-dimethylpyrazine and 2-nonanone. These isolates which hail from genera such as *Bacillus*, *Serratia* and *Pseudomonas* were shown to promote growth of *Solanum tuberosum* cv. 'Golden Wonder'. A Preliminary analysis also reported that VOC's may increase the efficiency of transient genetic transformation *via Agrobacterium tumefaciens*. Whole-transcriptome analysis will be employed to identify genes and their associated regulation which may be involved in the modulation of plant growth and transient genetic transformation.

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Interaction of the pathogen

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'*Candidatus* Phytoplasma mali' ('*Ca. P. mali*') is an intracellular plant pathogenic bacterium, that resides in the phloem of its plant host and is the causative agent of apple proliferation (AP). AP results in a variety of symptoms such as witches'-broom formation, reduced vigor and it affects the size and quality of the crop leading to high economical losses in many European countries. Since phytoplasmas do not possess a cell-wall, their membrane proteins are in direct contact with the host cell. This makes membrane proteins a valuable target for phytoplasma research. In our study three membrane proteins of '*Ca. P. mali*' were analyzed in regard to protein-protein interaction and induction of symptoms in the plant host by yeast-two-hybrid screens and *Arabidopsis thaliana* plants recombinantly expressing these phytoplasma membrane proteins. We found that the hemolysin like-protein interacted with atToc33, a GTPase located on the outer envelope of *A. thaliana* chloroplasts and involved in the protein import. The Sap11-like protein of '*Ca. P. mali*' was shown to induce crinkled leaves and siliques as well as a more bushy appearance and smaller rosettes in *A. thaliana*, similar to Sap11 of '*Ca. P. asteris*' strain AY-WB. Furthermore, Sap11 was shown to be transported into the nucleus *via* passive diffusion. The

results from these studies help to understand how the pathogen '*Ca. P. mali*' interacts with the host plant and thus induces symptoms during infection. In a further step disruption of these interactions might be a helpful approach to prevent infection or at least reduce symptoms.

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Effects of precipitation amount on ecosystem carbon exchanges in Inner Mongolia grassland ecosystem

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The grassland ecosystems of Inner Mongolia are located in arid and semi-arid areas, which are sensitive to precipitation change. General circulation models (GCM) predict that precipitation will increase in this region in the future. The amount of precipitation change may have an impact on the ecosystem's carbon exchange. However, the response of ecosystem carbon exchange to precipitation increase, especially the threshold and inflection, remain poorly understood. A 2-year experiment was conducted to detect the effect of precipitation amount on ecosystem carbon exchange, using fully-controlled rainout shelters in an Inner Mongolia grassland ecosystem. The experiment had 9 precipitation treatments, which were 100 mm, 150 mm, 200 mm, 275 mm (historical long-term average), 300 mm, 350 mm, 400 mm, 450 mm and 500 mm. With the increase in precipitation, net ecosystem exchange (NEE), ecosystem respiration (ER) and gross ecosystem productivity (GEP) all increased significantly, but followed a nonlinear trajectory. Precipitation reduction had stronger impacts on three parameters relative to precipitation increase. Community biomass production was the primary factor in influencing ecosystem carbon exchanges.

0–378

Effect of oyster's shells soil amendment on *Theobroma cacao* seedling growth and resistance against Phytophthora megakarya (causal agent of black pod disease) in nurseries

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Application of organic amendments such as chitinous and calcium sources have been proposed as a strategy for the management of diseases caused by soil borne pathogens. The aim of this study was to evaluate the ability of heat-treated and non-treated oyster shell powder amendment to enhance cocoa seedling growth and induce resistance against *Phytophthora megakarya* under nursery conditions. After twelve weeks of growth, heat-treated oyster shell powder soil amendment at 1% *w*/w significantly increased plant height, leaf number, leaf area, dry shoot and root weight when compared to non-treated oyster shell powder at 5% w/w and chemical fungicide treatment. Similarly, chlorophyll rate and stomatal resistance were also increased. The results showed that oyster shell powder raised soil pH significantly and decreased the *Phytophthora megakarya* load of the soil suspension by 82%. Leaf inoculation showed the weakest disease severity index (highest level of resistance) recorded in plants treated either with heat-treated or non-treated oyster shell powder. Therefore, total phenolic compounds and total native proteins contents were greater in either healthy or infected leaves of cacao plants treated with heat-treated oyster shell powder compared to those treated with non-treated oyster shell powder. This suggests that these compounds are involved in disease resistance. In fact, the higher induction levels of polyphenoloxidase, chitinase, peroxidase and β-1,3-glucanases activities recorded in leaves from plants treated with heat-treated oyster shell powder play an important role in the adaptation of cacao plant to *P. megakarya* infection. These findings demonstrated that heat-treated oyster shell powder could be used as biofertilizer and biofungicide to improve the quality of cocoa seedling production and protect the plant against *Phytophthora megakarya.*

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Interaction of the pathogen 'Candidatus Phytoplasma mali' with the plant host

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'Candidatus Phytoplasma mali' ('*Ca. P. mali*') is an intracellular plant pathogenic bacterium, that resides in the phloem of its plant host and is the causative agent of apple proliferation (AP). AP results in a variety of symptoms such as witches'-broom formation, reduced vigor and it affects the size and quality of the crop leading to high economical losses in many European countries. Since phytoplasmas do not possess a cell-wall, their membrane proteins are in direct contact with the host cell. This makes membrane proteins a valuable target for phytoplasma research. In our study three membrane proteins of '*Ca. P. mali*' were analyzed in regard to protein-protein interaction and induction of symptoms in the plant host by yeast-two-hybrid screens and *Arabidopsis thaliana* plants

recombinantly expressing these phytoplasma membrane proteins. We found that the hemolysin like-protein interacted with atToc33, a GTPase located on the outer envelope of *A. thaliana* chloroplasts and involved in the protein import. The Sap11-like protein of '*Ca. P. mali*' was shown to induce crinkled leaves and siliques as well as a more bushy appearance and smaller rosettes in *A. thaliana*, similar to Sap11 of '*Ca. P. asteris*' strain AY-WB. Furthermore, Sap11 was shown to be transported into the nucleus *via* passive diffusion. The results from these studies help to understand how the pathogen '*Ca. P. mali*' interacts with the host plant and thus induces symptoms during infection. In a further step disruption of these interactions might be a helpful approach to prevent infection or at least reduce symptoms.