In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-020-00453-6



# Plant Contributed Papers

**P-1001**

Localized Regulation of Hybrid *SN19* and GUS Genes Expression Driven by Pathogen Related Promoters in Potato. HUSSEIN ABDULLAH AHMED AHMED[1], Serkan Uranbey[1], Guray Akdogan[1], Sancar Fatih Ozcan[2], Cengiz Sancak[1], and Sebahattin Ozcan[1]. [1]Department of Field Crops, Faculty of Agriculture, Ankara University, 06100 Ankara, TURKEY and [2]Central Research Institute for Field Crops, Ministry of Agriculture and Forestry, Ankara, TURKEY. Email: hahmet@agri.ankara.edu.tr

The present study was focused on localized regulation of hybrid *SN19* (*cry1*Ba-domain I–III and *cry1*Ia-domain II) and GUS reporter genes driven by pathogen-related AoPR1 promoter isolated from wounded *Asparagus officinalis* cells, PR1a (pathogenesis-related protein 1a gene promoter) isolated from tobacco and the constitutive CaMV 35S promoter (widely used to express recombinant proteins in plants) on different organs of transgenic potato plants. Potato leaf disc explants and *Agrobacterium tumefaciens* GV2260 or EHA 105 strains were used in transformation experiments. GUS reporter gene expression driven by AoPR1 and PR1a promoters was specifically localized and increased on wounded sites of leaves, stems and flowers in transgenic tobacco, and leaves, stems, stolons, and micro tubers of transgenic potato plants in both salicylic acid, and wounding treatments. However, GUS gene expression by AoPR1 was undetectable or present in extremely low amounts in non-wounded tissues, in comparison with the levels found in CaMV 35S plants. Moreover, transgenic potato plants carrying a hybrid *SN19* gene regulated by AoPR1 promoter showed higher levels of *cry* gene expression at wounded tissues than CaMV 35S controlled *SN19* and exhibited 100% mortality for *Leptinotarsa decemlineata* and *Tuta absoluta* Meyrick insects.

**P-1002**

Accelerating Optimization of Genome Editing in Sugarcane. AYMAN EID[1,2], Chakravarthi Mohan[2], Sara Sanchez[1,2], Duoduo Wang[1,2], and Fredy Altpeter[1,2]. [1]DOE Center for Advanced Bioenergy and Bioproducts Innovation, Urbana, IL and [2]University of Florida, Gainesville, FL. Email: amohamedeid@ufl.edu, altpeter@ufl.edu

Sugarcane (*Saccharum* spp. hybrid) provides 80% of the world's sugar and 26% of its bioethanol and is one of the most productive crops due to its superior light conversion, water and nitrogen use efficiencies. Adding additional value streams will be facilitated by metabolic engineering and genome editing of sugarcane. Therefore, sugarcane is a prime candidate feedstock to fuel the emerging bioeconomy. Genome editing by sequence specific nucleases (SSN) is revolutionizing crop improvement and is a very promising technology for vegetative propagated polyploid crops of complex genome like sugarcane. Among SSNs, CRISPR\Cas9 and other RNA guided nucleases have been repurposed for genome engineering applications. Despite the flexibility and efficiency of the RNA guided nucleases, several technological challenges remain to achieve specific and efficient multiallelic editing of the highly polyploid sugarcane genome. Therefore, our objectives are to develop a rapid and simple approach to detect CRISPR/Cas9-based alterations in the sugarcane genome and adapt alternative CRISPR/nucleases to sugarcane editing to overcome PAM site limitations. Recombinant DNA vectors were constructed using traditional restriction enzyme cloning and Golden Gate assembly. Single guide RNA's were confirmed by in vitro cleavage assays prior to biolistic co-transfer with Cas9 nuclease and selectable *npt*II expression cassettes. Regenerated plants were analyzed phenotypically. Target amplicons were analyzed by restriction enzyme digestion and Sanger sequencing. Targeted mutagenesis of genes involved in chlorophyll biosynthesis resulted in an easily distinguishable phenotype. We will present data describing drop-out frequency caused by co-delivery of two sgRNA's as well as validation of multi-allelic targeted mutations by Sanger sequencing. Our data confirm a superior approach that efficiently generated distinct phenotypes due to reduced

chlorophyll content. This will facilitate the assessment of alternative delivery strategies and RNA guided nucleases to overcome PAM site limitations.

**P-1003**

Towards Effective Biolistics-mediated Transformation in Hornwort (*Anthoceros agrestis*) to Unlock Genes Involved in Plant-cyanobacteria Symbiosis. A. GUNADI[1], X. Xu[1], F. W. Li[1], and J. Van Eck[1,2]. [1]Boyce Thompson Institute, Ithaca, NY and [2]Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY. Email: ag895@cornell.edu

Hornworts (Anthocerophyta) are one of only a few land plants to have evolved the ability for mutualistic symbiosis with cyanobacteria. Unlike other nitrogen-fixing symbionts, cyanobacteria can independently perform photosynthesis, requiring less input from the plant host. Unlocking the genes involved in hornwort-cyanobacteria symbiosis, therefore, holds great potential to improve the productivity of agricultural crops. *In vitro* methods for the culture of the haploid gametophyte of *Anthoceros agrestis* with and without cyanobacteria symbionts have been previously developed. Our recent transcriptome analysis of *A. agrestis* have identified 7 potential genes involved in the symbiotic relationship. However, functional characterization of genes and pathways involved in hornwort-cyanobacteria symbiosis are limited by the absence of reliable transformation and genome editing methodologies. To bridge this gap, we have recently identified critical parameters for boosting the growth of *A. agrestis* and for effective particle bombardment-mediated transformation. Two weeks after culture, addition of 2% sucrose into the culture medium improved tissue growth by more than two-fold compared to growth on medium without sucrose. Additional parameters, such as green fluorescent protein (GFP) driven by the native hornwort elongation factor 1-alpha (EF1a) promoter, optimized target distance, and tissue culture conditions resulted in greater than 470 GFP-expressing cells at 120 h post bombardment. Our efforts to further improve transformation and genome editing tools for *A. agrestis* will pave the way towards unlocking the secrets of plant-cyanobacteria symbiosis.

**P-1004**

Cytokinins Improve Shoot Regeneration Efficiency in Two Indian Cotton (*Gossypium hirsutum* cv. Narashima and *G. arboreum* cv. AKA-7) Cultivars. SAMEENA E. TANWIR[1], Mohd. Akmal[2], Neera B. Sarin[3], and Jawaid A. Khan[2]. [1]Citrus Research and Education Center, University of Florida, Sanford, FL; [2]Plant Virus Laboratory, Department of Biosciences, Jamia Millia Islamia, New Delhi-110025, INDIA; and [3]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA. Email: simitanwir@gmail.com

In the present investigation, regeneration of the two elite Indian cotton cultivars *Gossypium hirsutum* L. cv. Narashima and *Gossypium arboreum* L. cv. AKA-7 is reported using hypocotyl, cotyledon and shoot tip explants. The explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with twelve different combinations and concentrations of 6-benzyl amino purine (BAP) and kinetin (KIN), and the shoot regeneration was investigated. High shoot regeneration (86 and 90%) as well as shooting efficiency (83.8 and 85%) was obtained with shoot tip explant on medium containing BAP (1 mg/l) and KIN (1 mg/l) in cv. AKA-7 and Narashima, respectively. Generally, almost in all treatments where BAP and KIN were applied alone or in combinations, shoot tip explants gave higher shoot regeneration efficiency percentage than hypocotyl and cotyledon explants. Elongation of multiple shoots was achieved on MS medium supplemented with GA3 (0.1 mg/l). Shoots successfully rooted on MS hormone free medium followed by hardening and acclimatization of regenerated plantlets. Fully developed cotton plants were obtained only after eight weeks of *in vitro* culture. The described protocol is highly efficient, rapid and reproducible and could be used for genetic transformation of cotton plants. Critical factors affecting regeneration such as different explants and growth regulators were studied in order to optimize the micropropagation protocol providing future application in genetic transformation. The regeneration system was optimized from shoot-bud induction to rooting of shootlets, and their hardening and establishment in the nursery. These systems could be used for clonal propagation and transformation of desirable genes for generating cotton transgenic plants having high agronomic traits.

**P-1005**

Reprogramming of Stem Cell Activity to Convert Thorns Into Branches in *Citrus*. FEI ZHANG[1], Pascale Rossignol[1], Tengbo Huang[1], Yewei Wang[1], Alan May[2], Christopher L. Dupont[3], Vladimir Orbović[4], and Vivian Irish[1,2]. [1]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520; [2]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520; [3]J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037; and [4]Citrus Research and Education Center, University of Florida/IFAS, 700 Experiment Station Road, Room 103,

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*Citrus* is one of the most popular fruit crops, with great economic and health value. However, *Citrus* breeding through traditional approaches can be difficult and time consuming. Here we present a functional genetics approach with the aim of *Citrus* tree improvement. Most *Citrus* species produce thorns that affect harvesting efficiency and the thornless trait is a breeding objective. We have characterized thorn development, and have shown that *Citrus* thorns differentiate in a tip-to-base fashion based on both analysis of cell cycle markers and lignification. In addition, we demonstrated that *Citrus* thorn primordia express the stem cell markers *STM* and *WUS* in a dynamic fashion. Through a comparative genomics approach, we identified genes that were differentially expressed in limes with or without thorns. Among them, *THORN IDENTITY 1 (TI1)* and *THORN IDENTITY 2 (TI2),* were highly expressed in developing thorns. Disruption of *TI1* and *TI2* function by multiplex genome editing using a highly efficient CRISPR/Cas9 system fully converted thorns into branches. We further demonstrated that TI1 can directly bind to the *WUS* promoter and repress its activity in thorn development. Shifts in the timing and function of components of this gene network can account for the evolution of *Citrus* thorn identity. Modulating this pathway can alter plant architecture and could be used to improve crop yields. Our functional genetics approach provides a powerful resource that can be broadly applied for many aspects of *Citrus* biology, including developing solutions for challenges affecting the *Citrus* industry, such as combatting Huanglongbing (HLB) disease.

**P-1006**

A Comparative Analysis on the Crop Performance in *Cannabis sativa* Between Tissue Cultured Propagules Versus Propagules from Tissue Cultured Stock. NORMAN SENN, Savanah St. Clair, Angelo Alvarez, and Maryam Saraylou. CRTFD Plant Science, LLC. Los Angeles, CA. Email: Normansenn80@gmail.com

Cannabis sativa cultivars vary in resistance to vitrification. This has led to challenges in particular for production and preservation of clone-only varieties with therapeutic value. Cannabis hybridizes readily and new varieties are constantly in demand. Clones can be produced rapidly by traditional means. In order to cut costs and shorten the timeline, producers have taken on strategies such as providing traditional clones of tissue cultured mother plants. There have been few studies comparing the output from clonal individuals initiated in vitro versus the output of ex vitro clones arising from tissue cultured stock plants to validate this approach. This study makes a comparison on the crop performance utilizing clones directly from tissue culture, and cuttings from a tissue cultured mother plant. In this study we will analyze the differences in yield, required veg. Time, flower time, pathogen resistance and trichome and terpene output in two cannabis varieties.

**P-1007**

Flower Power: A Rapid *In Vitro* Regeneration Protocol from *In Vitro Cannabis sativa* Inflorescences. ADRIAN S. MONTHONY and A. Maxwell P. Jones. Department of Plant Agriculture, University of Guelph, 50 Stone Road East, N1G 2 W1, Guelph, Ontario, CANADA. Email: monthona@uoguelph.ca

The legalization of recreational cannabis (*Cannabis sativa L.*) in North America has driven the need for large-scale propagation of disease free, chemically defined clones. Currently, cannabis is propagated using stem cuttings from mother plants, which can occupy up to 15% of commercial production space and are susceptible to pests and diseases. *In vitro* growth of cannabis allows for rapid clonal propagation of axenic plants for research and germplasm storage, but published methods are few and multiplication rates can be low. A recent study from our lab explored the regenerative potential of cannabis inflorescences, using greenhouse grown flowers. Subsequent studies have shown that morphologically normal plants can regenerate from *in vitro* florets in the absence of plant growth regulators (PGRs). Since cannabis inflorescences are comprised of compact internodes, they represent a meristem-dense region with a high multiplication potential. We hypothesized that micropropagation using inflorescences can enhance multiplication rates in *C. sativa* over existing vegetative methods while providing an alternative approach to culturing day neutral (autoflowering) plants and some photoperiod sensitive genotypes. We explored the use of PGRs (BAP and TDZ) to enhance the multiplication rate of explants regenerated from individual or groups of florets and assessed the incorporation of inflorescences into alginate-based synthetic seeds. This study provides a framework for the rapid multiplication of clonal explants using *C. sativa* inflorescences. Our findings have the potential to facilitate the automation of cannabis micropropagation while providing a roadmap to overcoming the challenges associated with tissue culture of day-neutral cannabis cultivars.

**P-1008**

Tomato (*Solanum lycopersicum* L.) Class II Glutaredoxin Mutants Generated Via CRISPR/Cas9 System Are Susceptible to Multiple Abiotic Stresses. T. KAKESHPOUR[1], T. Tamang[1], Q. Wu[2], J. Park[1], and S. Park[1]. [1]Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS and [2]Institute of

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Global environmental change and rapid population growth make transgenic technologies necessary for crop improvement. CRISPR/Cas9 system can be precisely designed to generate multiplex genome editing, providing a powerful tool for studying functions of gene families in plants. Glutaredoxins (GRXs) are low molecular weight oxidoreductases that are involved in oxidative stress responses; however, despite their importance, their function in plants has not been well understood. In this study, we successfully designed and applied pYLCRISPR/Cas9 multiplex vector system to edit *Solanum lycopersicum* class II glutaredoxins (*SlGRXS14, S15, S16, and S17*) and used mutant plants to study physiological functions of knock-out genes. Our genotyping data showed highly efficient gene editing in T0 plants that were genetically inherited to T1 and T2 generations. Transgene-free single and multiple null mutants in T3 were evaluated under control, heat, drought, chilling, cadmium and short photoperiod stress conditions. In standard conditions, single and multiple mutants did not show any phenotypic differences compared to wild-type plants. Unsuccessful attempts to find any single, double, triple or quadruple mutant lines containing *S15* null mutants suggests that complete loss of function of *S15* is embryonic lethal for tomato. However, upon exposure to different abiotic stresses, wild-type and mutant plants showed significant phenotypic differences that were easily distinguishable. Phenotyping data suggested that function of GRX gene family is critical for plant's survival under abiotic stress conditions, making them attractive targets for crop improvement.

**P-1009**

Evaluation of miRNA Mediated Networking and Feedback Against Drought, Heat and Combined Stress Tolerance in Potato (*Solanum tuberosum* L.). ARSLAN ASIM, Ufuk Demirel, Allah Bakhsh, and Zahide Neslihan Ozturk Gökçe. Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, TURKEY. Email: arslanasim92@gmail.com

miRNA's have their own significance to play against abiotic stress. The main aim of this study is to find out miRNA's and their target genes related to response and/or tolerance to drought, heat and drought + heat stresses through comparison of sensitive and tolerant potato cultivars and to investigate their roles with transgenic approach. Drought tolerant Unica and sensitive Russet Burbank cultivars were used for comparison of miRNA transcriptomes obtained with new generation sequencing. Aiming this goal, both potato cultivars were treated with drought, heat and heat + drought stresses and total 314 (104 novel and 210 known) miRNAs were identified. Target genes of all miRNAs were defined with bioinformatic analyses and 24 potential miRNAs specific to potato variety and/or abiotic stress treatment were selected where stress dependent changes in gene expression levels of 14 miRNAs and their targets were proven with qRT-PCR. Depending on the qRT-PCR results and roles of their target genes, novel_miR8 (target; mitochondrial transcription termination factor family protein), novel_miR105 (target; zinc finger family protein), stu-miR156d-3p(target; phospholipid-transporting ATPase), stu-miR160a-5p (target;auxin response factor ARF16),stu-miR162a-3p(target; endoribonuclease Dicer homolog1),stu-miR172b-3p(target;AP2 transcription factor SIAP2d) and stu-miR398a-5p (target; photosystemII core complex proteins psbY, chloroplast) were selected for transgenic studies. Although transgenic plants were obtained for five miRNAs, transgenic plant lines and their mini tubers were obtained for both potato cultivars. Transgenic plants having over-expression of miRna,s grown from tubers were treated with drought, heat and heat + drought stresses to investigate the effects of miRNA transformation in abiotic stress tolerance level. At the end, promising transgenic potato lines with diverse behavior against stresses were achieved. These transgenic lines are believed to have an impact on sustainability of potato cultivation in changing climate.

**P-1010**

High Yields Secretion of Human Erythropoietin from Tobacco Cell for Ex Vivo Production of Red Blood Cells. UDDHAB KARKI[1,2] and Jianfeng Xu[1]. [1]Arkansas Biosciences Institute and [2]Molecular Biosciences Program, Arkansas State University, Jonesboro, AR 72401. Email: Uddhab.karki@smail.astate.edu

Human blood transfusion is crucial in healthcare, which entirely depends on the human donors. Ex vivo generation of clinically available red blood cells (RBCs) from hematopoietic stem cells (HSCs) offers a promising solution to overcome the challenges associated with current use of donor blood, including shortage of blood supply and risk of transfusion-transmitted infections. However, ex vivo expansion and differentiation of HSCs into RBCs requires large quantities of hematopoietic growth factors/cytokines, particularly erythropoietin (EPO) that is a key cytokine responsible for effective erythropoiesis. High-quality functional EPO is increasingly demanded for fundamental research and clinical applications. Plant cell culture is an emerging alternative bioproduction platform for therapeutic proteins, as it offers advantages in safety, scalability and cost over other eukaryotic and prokaryotic systems. However, low protein productivity and secretion is a common bottleneck preventing the commercialization of

this platform. To overcome these bottlenecks, we expressed EPO with a designer peptide tag, termed (SP)20 consisting of 20 tandem repeats of a "Ser-Pro" motif. This de novo designed tag is expected to direct extensive Oglycosylation on each Pro residue in plant cells and function as a molecular carrier in boosting extracellular secretion and stability of EPO. Tobacco codon-optimized EPO gene with the (SP)20 tag attached at either N-terminal or C-terminal region was stably expressed in tobacco BY-2 cells. BY-2 cell secreted EPO products were purified with hydrophobic interaction chromatography and Ni + affinity chromatography for their function assay. The yield of (SP)20-tagged EPO was significantly higher than the EPO without the tag. The in vitro expansion and differentiation of hematopoietic stem cell (CD34+ cells) was established to test the plant cell-produced EPO products. This research develops a new plant cell-based platform for high yield production of EPO, facilitating manufacturing of HSCs-derived RBCs at large scale for clinical applications.

**P-1011**

*In Vitro* Plant Metabolism of Plant Protection Products. LEONIE HILLEBRANDS[1], Marc Lamshoeft[1], Andreas Lagojda[1], Andreas Stork[1], and Oliver Kayser[2]. [1] Bayer AG Division CropScience, Monheim am Rhein, GERMANY and [2]Technical University Dortmund, Dortmund, GERMANY. Email: leonie.hillebrands1@bayer.com

Development and registration of the most promising and safest plant protection product (PPP) needs the determination of its metabolic behavior in plants to elucidate their nature of residues by using $^{14}$C-labeled compounds. This data is even in early research and development phase very helpful and an *in vitro* screening test would be an option. Based on these results a first risk assessment for human and environment can be implemented as soon as possible. Therefore, it was checked if a short-term *in vitro* tool with plant callus cultures and usage of non-radioactive labeled PPP's can be utilized instead of a long-term study with entire *in planta* systems. It is known that callus cultures can be used for different biotechnological approaches. One is, to apply PPP's to predict their metabolic behavior in e.g. wheat, soybean, oilseed rape, mays and *Cannabis sativa* L. (Mumma RO, Hamilton RH. J. Toxicol. Clin. Toxicol. 1982;19(6–7):535–55). Analysis of the plant extract takes place by means of high-resolution mass spectrometry. Experiments with four well-known and non-labeled PPP's showed, two weeks after the application of 10 μM, almost all well-known metabolites. Tebuconazole, Fenhexamid, Flurtamone and Metalaxyl-M exhibits qualitative similar metabolic degradation products in all *in vitro* test system as observed in the intact *in planta* system. Subsequent to the initial oxidation step, conjugations with sugars as well as non-enzymatic degradation was observed. The results confirm, that this assay is capable to conduct a metabolic profile and a preliminary pathway in callus cultures after two weeks with small quantities of the applied PPP's. It can support the rapid identification of the major metabolites of PPP's. Moreover, the assay can be used to identify the metabolic degradation of PPP's in non-target plant species (e.g. *Cannabis sativa* L.). Finally, preliminary risk assessments of metabolites are possible in non-crop plant species without sophisticated whole *in planta* experiments. We recommend this *in vitro* assay as a screening tool to characterize the metabolism of PPP's in plants.

**P-1012**

RNA-Seq Analysis of Fruit Retention-regulated Gene Expression and Its Variation by Hexanal in 'Honeycrisp' (*Malus domestica Borkh.*). KARTHIKA SRISKANTHARAJAH[1], Erika DeBrouwer[1], Alan Sullivan[1], Gopinadhan Paliyath[1], and Jayasankar Subramanian[2]. [1]Department of Plant Agriculture, University of Guelph, Guelph, ON N1G2W1, CANADA and [2]Department of Plant Agriculture, University of Guelph-Vineland Station, 4890 Victoria Ave N, Vineland, ON L0R2E0, CANADA. Email: sriskank@uoguelph.ca

'Honeycrisp' is a premium apple fruit variety with high market potential due to its crunchy texture and unique flavor. Despite these favorable organoleptic qualities and consumer demand, Honeycrisp possesses numerous challenges such as preharvest fruit drop (PFD), quality decline during storage and bitter pit. Preharvest fruit drop is typical at the beginning of the harvest and increases with the extended harvesting period, resulting in up to 50% loss of matured fruits. Ethylene from the ripening fruit stimulates the formation of the abscission layer in the stem resulting in abscission. Hexanal, an inhibitor of the phospholipase-D enzyme (PLD), has shown promising results in controlling fruit drop and thus extend the shelf life of many fruits. However, underlying molecular mechanisms of controlling fruit drop by hexanal is not fully understood. Here we studied the molecular changes that occur in the fruit stalk, specifically at the abscission zone, as a result of hexanal application using a transcriptomic approach in Honeycrisp. To do this Honeycrisp trees were treated with hexanal formulation 30 and 15 days before the commercial harvest. Abscission zone (AZ) samples were collected from treated and non-treated fruits grown in two commercial orchards in the Niagara region of Ontario, Canada. RNA was isolated from AZ and were sequenced using Illumina Next-Seq 500. Apple genome project (GDDDH13) version 1.1 used as the reference genome. Approximately 35,000 genes were detected in each sample, with the average read per sample of 28,500,000. Currently, we are analyzing the differentially expressed genes (DEGs) due to hexanal spray. In addition to transcript

profiling, hexanal treated fruits showed lower ethylene production, reduced PLD activity and an increased total soluble solid throughout the storage. Timely application of hexanal will help to extend the fruit retention and improve postharvest storage of Honeycrisp.

**P-1013**

Marker Assisted Selection of Elite Genotypes in Some Medicinal Plants, Their Micropropagation, Isolation and *In Vitro* Elicitation of Anticancerous Compounds. VEENA AGRAWAL. Medicinal Plant Biotechnology & Applied Research Lab., Department of Botany, University of Delhi, Delhi, INDIA. Email: drveena_du@yahoo.co.in, dragrawaldu@gmail.com

Biochemical and molecular markers employing HPLC and DNA fingerprinting have been proved beneficial in the selection of elite germplasm accumulating high content of bioactive compounds in some medicinal plants e.g., *Cullen corylifolium*, *Plumbago zeylanica* and *Holarrhena antidysentrica*. *In vitro* regeneration and genetic fidelity have been achieved in such plants using the selected explants for their large scale plantation and commercial application. For enhancement of the bioactive compounds, the calluses were raised using selected explants on $MS/B_5$ medium containing various plant growth regulators e.g., BA, IBA and TDZ in different concentrations (0.1,1,5 &10 μM) either individually or in combination. *In vitro* enhancement of psoralen, daidzein & genistein, biomolecules was achieved through cotyledon-callus of *C. corylifolium* augmented with chitosan or salicylic acid. Maximum psoralen content (14.8–fold) was achieved with 5 μM salicylic acid over control. Daidzein and genistein enhanced up to 11.2 and 6.21–fold with 50 and 100 mg/L chitosan, respectively. An enormous increment of 12.08–fold plumbagin content occurred when a combination of 50 mg/L chitosan +100 mg/L yeast extract was used in the root derived callus of *P. zeylanica* . In case of conessine, phenylalanine proved best for its elicitation in *H. antydysentrica* green bark derived callus. An increment up to 6.67–fold conessine occurred with 200 mg/L phenylalanine. The biomolecules have been isolated and characterized using NMR, HR-MS and FT-IR techniques. Green synthesis of nanoparticles was done using aqueous extract of elite plant part. Bioefficacy study was done against different cancer cell lines and mosquito vectors causing malaria, filarial and dengue.

**P-1014**

Effect of BAP and TDZ on Direct Shoot Organogenesis in Coconut (*Cocos nucifera* L.). EVELINE YEE YAN KONG[1], Julianne Biddle[1], Sisunandar Sudarma[2], Bart Panis[3,4], and Steve W. Adkins[1]. [1]School of Agriculture and Food Sciences, The University of Queensland, AUSTRALIA; [2]Universitas Muhammadiyah Purwokerto, INDONESIA; [3]Bioversity International, BELGIUM; and [4]Department of Biosystems, KU Leuven, BELGIUM. Email: eveline.kong@uqconnect.edu.au

Coconut is an important tropical crop grown by more than 11 million subsistence farmers in more than 90 coconut producing countries. However, due to its aging populations, biotic and abiotic threats, the industry needs a massive 3 billion palm replanting program in the next 5 years to meet the growing demand for coconut products. This activity will be difficult due to the current shortage of planting materials, especially elite cultivars, so a rapid clonal propagation method is urgently needed. Over the past 60 years, clonal propagation in coconut has only been achievable through somatic embryogenesis because coconut shoot tips do not have an apparent capacity to produce vegetative lateral bud outgrowths. However, direct organogenesis, a less focused on plant regeneration pathway for coconut, could be another clonal propagation system for coconut. Direct organogenesis, which eliminates a callus phase, lowers the possibility of somaclonal variation and therefore, offers a more secure plantlet regeneration pathway. In addition, it is more time efficient as compared to somatic embryogenesis. This paper provides insights into the potential use of direct organogenesis as a clonal propagation method for coconut. This is further supported by the documented success of direct shoot regeneration of oil palm, which like coconut, does not propagate vegetatively to produce offshoots and can only have a single shoot per palm (non-branching palm) under natural environmental conditions. In general, plant growth regulators (PGRs) have a significant impact on the rate of direct shoot regeneration depending on the type and concentration of PGRs used. As cytokinins have been shown to have significant effects on promoting the growth and proliferation of axillary or adventitious shoots, their effects on direct shoot organogenesis of coconut is outlined in this paper. The influence of various concentrations of 6-benzylaminopurine (BAP) and thidiazuron (TDZ) on the growth of both coconut shoot tips and young inflorescence tissues is reported.

**P-1015**

Temperature Effects on Recovery and Growth of Shoot Tips of *Quercus virginiana* After Liquid Nitrogen Exposure. V. C. PENCE. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org.

Of the 91 species of oaks (*Quercus* spp.) in the U.S., 30% are of conservation concern, and at least 20% of the 500 species globally are thought to be at some risk. *Ex situ*

conservation of oaks is complicated by the fact that oak seeds (acorns) are desiccation sensitive and cannot survive the conditions of drying required for conventional seed banking. Thus, embryo and tissue cryopreservation are thought to be the most effective alternatives for long-term banking of oak germplasm. Shoot cuttings of the common *Quercus virginiana* (Southern live oak), *Q. suber* (cork oak), *Q. gambelii* (Gambel's oak) and the critically endangered *Q. hinckleyi* (Hinckley's oak) were used to initiate cultures from seedlings germinated in the greenhouse and shoot propagating cultures were established and maintained on Woody Plant medium with 0.2 mg/L BAP, 3% sucrose, and 0.25% Gelzan. Shoot tips of all 4 species were cryopreserved using the droplet vitrification method. A low level of survival was observed through LN exposure in all except *Q. gambelii. Q. virginiana* was then used to examine the effects of growth and recovery on the same medium under three temperature conditions: 26°C, 21°C, and an alternating 26°C/15°C 16:8 hr cycle (AT). Survival of shoot tips through cryopreservation was similar for 26°C and AT (41%, 55%), while there was a significant decrease in recovery at 21°C (21%). However, outgrowth of the surviving shoot tips was significantly improved by recovery at AT, compared with 26°C. This suggests that a cooler night temperature can contribute to improving the recovery and growth of oak shoot tips after LN exposure.

**P-1016**

Multivariate Adaptive Regression Splines: A Potential Method for Tissue Culture Data Analysis. MELEKSEN AKIN[1], Sadiye Peral Eyduran[1], Sezai Ercişli[2], and Barbara M. Reed[3]. [1]Igdir University, Agricultural Faculty, Department of Horticulture, Igdir, TURKEY; [2]Atatürk University, Agricultural Faculty, Department of Horticulture, Erzurum, TURKEY; and [3]USDA-ARS-Retired, National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333. Email: akinmeleksen@gmail.com

Multivariate Adaptive Regression Splines (MARS) is a non-parametric regression algorithm that can capture complex linear and non-linear relationships between explanatory and response variables by a sequence of spline functions without requiring the restrictive distributional assumptions of the general linear models. The MARS approach is appropriate to analyze both quantitative and qualitative multi-dimensional data sets.The MARS algorithm shows the contribution of each input on the outcome in a transparent mathematical equation, therefore produces relatively easy to interpret results compared to the other commonly used machine learning techniques. The relative importance of each explanatory variable on the response is expressed by a series of piecewise linear splines. The endpoints of the splines are termed knots. Each knot defines the end of one data section and the beginning of another, thus detecting the regions of relationship change between the input and output variables. The resulting piecewise splines provide great flexibility to the generated MARS model through bends and thresholds, consequently allowing departures from linearity. This study aims to provide a gentle introduction to the MARS algorithm through the "earth" package in R software and to inspire the future application of this promising approach on highly dimensional *in vitro* data.

**P-1017**

Improvement of Protoplast Proliferation for Efficient Genotype-independent Gene Editing. R. WELSCH[1], S. Pandey[1], A. Moradi[1], J. Dawson[2], R. Uhl[3], and K. Palme[1,4]. [1]Institute of Biology II, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Sonnenstrasse 5, 79104 Freiburg, GERMANY; [2]Department of Computer Science and Electrical Engineering, University of Rostock, Ulmenstrasse 69, 18057 Rostock, GERMANY; [3]TILL ID GmbH, Am Klopferspitz 19a, 82152 Planegg/Martinsried, GERMANY; and [4]BIOSS Center for Biological Signaling Studies, Albert-Ludwigs-University of Freiburg, Schaenzlestrasse 18, 79104 Freiburg, GERMANY. Email: ralf.welsch@biologie.uni-freiburg.de

Public concerns against genetically modified plants (GMO) might be cleared by modern gene editing (GE) technologies expected to be considered as non-GMO in many countries. However, applicability of GE relies heavily on efficient proliferation of gene-edited protoplasts which is also desirable to achieve for *in vitro* propagation techniques. The factors affecting protoplast proliferation are numerous and protoplasts from a large number of species are recalcitrant and thus fail to enter cell division upon isolation. Using high-throughput automated microscopy coupled with image analysis, we have developed a systematic approach which allows optimizing protoplast proliferation efficiency through various approaches. Proliferation events in immobilized protoplasts are identified by the development of proliferation-selective dyes, exploitation of fluorescent marker proteins as well as the identification of cellular properties specifying proliferating cells. The methods developed to quantify protoplast proliferation allow targeted improvements of the proliferation rates by adjusting media components and hormone levels, applying epigenetic reprogrammers and using transient expression of key genes. Results obtained from Arabidopsis as a model and from sugar beet and poplar

as exemplary taxa with high agronomical values are promising regarding achieving a genotype-independent proliferation system applicable to a large variety of other taxa.

**P-1018**

CRISPR-Cas12b Genome Engineering Systems in Plants. Meiling Ming[1], Qiurong Ren[2], Changtian Pan[1] , Yao He[2], Yingxiao Zhang[1], Shishi Liu[2], Zhaohui Zhong[2], Jiaheng Wang[2], Aimee A. Malzahn[1], Jun Wu[3], Xuelian Zheng[2], Yong Zhang[2], and YIPING QI[1,4]. [1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742; [2]Department of Biotechnology, School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu 610054, CHINA; [3]Centre of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, CHINA; and [4]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850. Email: yiping@umd.edu

Cas12b is a type V-B CRISPR effector RNA-guided DNA endonuclease. Like Cas12a, Cas12b recognizes T-rich PAMs and generates staggered DNA double strand breaks. While Cas12a only requires crRNA to function, Cas12b requires both crRNA and tracrRNA or their engineered fusion known as single guide RNA (sgRNA). This feature resembles the CRISPR-Cas9 system and enables guide RNA engineering, making Cas12b advantageous over Cas12a in certain genome engineering applications. Here, we describe our recent efforts on developing a new plant genome engineering platform based on Cas12b. We first compared multiple Cas12b orthologs of different bacterial origins for genome editing in rice, an important crop. Among them, we identified a potent ortholog for targeted mutagenesis, which was further demonstrated in multiplexed genome editing in stable transgenic lines. Next, we engineered three Cas12b based repressors and showed that they could mediate targeted transcriptional repression at different levels. Finally, we compared over a dozen transcription activation systems based on Cas12b in plants. We found the most potent transcription activation system relies on both Cas12b protein and engineered sgRNAs for the recruitment of different transactional activators. With the demonstration of Cas12b for genome editing, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), our work comprehensively establishes Cas12b as the third promising CRISPR system, after Cas9 and Cas12a, for plant genome engineering.

**P-1019**

Improvement of Gene Delivery and Mutation Frequencies in the CRISPR-Cas9 Wheat Genomics System. MYEONG-JE CHO[1], Jaclyn Tanaka[1], Snigdha Poddar[1,2], Bastian Minkenberg[1], and Brian Staskawicz[1,3]. [1]Innovative Genomics Institute, [2]Department of Molecular and Cell Biology, and [3]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94704. Email: mjcho1223@berkeley.edu

The discovery of the CRISPR-Cas9 gene editing system has revolutionized the field of plant genomics. Compared to previous technologies, CRISPR-Cas9 allows for site-specific gene edits for extensive improvements in crops like wheat (*Triticum aestivum* L.). Wheat is a moderately recalcitrant species for tissue culture and genetic transformation. It is utilized as a protein and carbohydrate source more than any other crop in developing countries. As global wheat consumption increases, it is important to focus efforts on wheat advancement through genome editing. Despite the advantages of ease in designing gRNA and the relative low cost of the CRISPR-Cas9 system, there are still hurdles to overcome in low mutation frequencies, specifically in hexaploid bread wheat. Currently, wheat genomics projects require a high output of $T_0$ transformants in order to generate the desired mutations. In conjunction with gene delivery and transformation efficiency, the mutation rate bottleneck has the potential to slow down advancements in genome editing of wheat. In this study, we report our findings of efforts to increase gene mutation rates in the wheat cultivar Fielder in order to make CRISPR-Cas9 a more direct and stable gene editing system via biolistics.