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Plant Posters

P-2000

Silicon Supplementation and *Staphylococcus scuiri* SAT-17 Inoculation Improved the Morphogenesis and Physio-biochemical Patterns in *In Vitro* Grown Sugarcane. MUHAMMAD SOHAIL AKRAM, Raheel Parvez Abbasi, Naeem Iqbal, and Muhammad Azeem. Department of Botany Government College University, Faisalabad-38000, PAKISTAN. Email: sohailakram79@gmail.com

Sugarcane has great economic importance as the crop is source of sucrose, biofuel and industrially important products. Increased population demands a substantial increase in gross production of sugarcane. Silicon (Si) can stimulate natural defense mechanisms of plants through low molecular weight metabolites and assist in alleviating adverse effects of stresses. Its addition in culture medium proved beneficial for various crops (Sahebi et al. 2016). Plant growth promoting rhizobacteria (PGPRs) can enhance plant growth and PGPRs induced impacts on field grown plants have been well established; yet, their effects on *in vitro* plant growth are poorly known. Leaf roll explants of three sugarcane genotypes were used to induce callogenesis and regeneration using MS medium plus appropriate growth regulators for each growth phase (Akram 2012). Silicon (K_2SiO_3 or $CaSiO_3$) at a concentration of 0, 12.5, 25, 50 or 100 mg/L was added in the medium at all phases of culture. Inoculation of *Staphylococcus scuiri* SAT-17 (Akram et al. 2016) was done at regeneration or soil acclimatization step. The *in vitro* grown plants were raised in fields under natural conditions. Addition of K_2SiO_3 was more effective than CaSiO3. The explants cultured on 25 mg/L K_2SiO_3 amended medium showed 21% higher callogenesis frequency (CF) with 3.25% increase in weight in comparison to control. The least CF was recorded in the medium having 100 mg/L K_2SiO_3 which was 13 and 17% less than respective control (0 mg/L K_2SiO_3) and medium with 100 mg/L $CaSiO_3$, respectively. The calli initiated on 25 mg/L K_2SiO_3 or 12.5 mg/L CaSiO3 showed maximum regeneration when inoculated with SAT-17 at regeneration phase. Inoculation of SAT-17 at both regeneration and soil acclimatization stage, instead of any alone, induced maximum stability as revealed from plant vigor/growth and physio-biochemical (catalase-peroxidase activity, chlorophyll-phenolic content) attributes. The study results highlighted the significance of Si and PGPRs in *in vitro* growth of sugarcane and shall be beneficial for studies pertaining to sugarcane yield improvement.

P-2001

Direct Adventitious Shoot Bud Induction Integrated with Direct Somatic Embryogenesis: A New Method for Date Palm Micropropagation. C. SUDHERSAN, S. Jibi, L. Al-Sabah, and S. Al-Melhem. Biotechnology Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, KUWAIT. Email: schellan@kisr.edu.kw

Date palm (Phoenix dactylifera L.) is the major fruit crop for the Middle East and North African countries. It is highly adapted to the harsh climate and environmental conditions of the arid regions. It has thousands of cultivars developed through natural crossing and few selected ones are mass produced and cultivated. Traditional method of propagation is very slow and inefficient to meet the required number for establishing a commercial plantation. Therefore, an alternative method was developed via micropropagation using plant tissue culture technology during 1982. Several research laboratories worldwide established tissue culture laboratories and started date palm micropropagation research, Few laboratories succeeded and established commercial production centers. Kuwait Institute for Scientific Research (KISR) established a date palm tissue culture laboratory in the year 1995 and developed a protocol using somatic embryogenesis method for commercial-scale plant production. Half a million plantlets of 30 different date palm plantlets were produced and supplied to

the farmers from our laboratory. Clonal nature, early flowering and high yielding quality of the tissue culture derived date palms were confirmed through field evaluation. Long duration for the initial regeneration (6-12 m) and very low rate of somatic embryo to plantlet conversion (10%) are the major problems that controlled the cost of plant production. In order to solve these problems, we conducted a research study to develop a fast method of regeneration and 100% embryo conversion using selected date palm cultivars such as Barhi, Khlas, Khudhri, Madjhool, Suckari and Sigai. During our study, we used leaf primordia as initial tissue explants. Modified MS culture medium with auxin and cytokinins was used for the experiments. All the 6 date palm cultivars showed shoot but regeneration within 45 days. As a result of the study, we have developed a protocol for fast regeneration coupled with high rate of somatic embryo to plantlet conversion for the commercial-scale date palm plant production.

P-2002

Evaluation of Biochemical and Free Radical Scavengers of Landraces *Digitaria exilis* L. Under, Osmotic Stress.
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Digitaria exilis L. is an under-utilized crop with high nutritional and medicinal values. It is adapted to marginal land doing well in poor soil. The study was to investigate the mechanisms adopted by *D. exilis* to survive osmotic stress using biochemical markers. Landraces Dinat Iburua (DIN), Jakah Iburua (JI_1) and Jiw Iburua (JI_2) were collected from National Cereal Research Institute, Niger State. Two landraces NG/11/JD/061 and NG/11/JD/062 were collected from National Center for Genetic Resources and Biotechnology, Ibadan. Murashige and Skoog medium of about 1.2 litres was supplemented with poly-ethylene glycol 6000 to create osmotic conditions of -9.29 MPa, -13.93 MPa, -20.13 MPa -26.32 MPa, -32.51 MPa and 0 MPa. Sterilized seed were inoculated on the medium and placed in the growth room for four weeks. Proline accumulation was significantly high in all the osmotic stressed JI_1. Proline and APX activities were directly correlated thus, reinforced the survive ability of landrace JI_1 during the stress. Catalase (CAT) was also significantly induced in an osmotic stressed landrace JI_1 which synergistically improved the landrace. Proline was a source of reducing power hence; decreased lipid peroxidation was recorded during stress. As a result, above 50% of OH^-, H_2O_2 and NO radicals were scavenged. However, other landraces (DIN, NG061, NG062 and JI_2) showed variations in their responses to different levels of osmotic stress though not significant. Therefore, Landrace JI_1 possesses well-equipped free radicals quenching system that is protected by the accumulation of osmolyte proline thus, landrace JI_1 is an osmotolerant. Activities of CAT and SOD were stabilized against oxidative stress by proline.

P-2003

In Vitro Culture of *Thymus capitatus* L.: Development of New Shoots and Callus.
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In vitro multiplication of thyme species has been the subject of several studies in order to save endangered species or develops a reliable and reproducible multiplication technique with a fairly high multiplication rate. Aim of our work was to develop a protocol for multiplication of *Thymus capitatus*, in order to increase expansion of the culture, and induction of undifferentiated callus synthesis, useful as a starter for in vitro synthesis of secondary metabolites. For this purpose, nodal explants of *Thymus capitatus* were cultured on media containing different hormonal combinations. The medium which induced the most axillary budding during the initiation phase was MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/l BAP. The percentage of budding was however low, not exceeding 15%. Callogenesis was observed for all combinations and hormonal concentrations except for the hormone-free MS substrate. Rooting was obtained on MS medium containing 1 mg/l NAA; the percentage of rooting was 75%. Cotyledon and hypocotyl explants were isolated from seeds germinated on hormone-free MS substrate and were transplanted on MS medium, supplemented with different hormonal combinations. The highest percentage of callogenesis was obtained when the cytokinin / auxin ratio was lowest. Indeed, in media enriched with BAP 0.1 mg/l and NAA 3 mg/l, the percentage of callogenesis was around 100% for cotyledon explants and 100% for hypocotyl explants. The higest percentages of callogenesis was obtained with a level of NAA between 1 and 3 mg/l. Caulogenesis was induced when 1 mg/l BAP was added to the medium, regardless of the concentration of NAA. The emergence of adventitious buds was observed from the first week to the 8^{th} week. The hypocotylar explants seem more apt for caulogenesis. In addition, these buds have high rooting and survival rates which are high when transferred to the rooting and propagating medium. However, the percentages of caulogenesis remain low, not exceeding 25%.

P-2005

A Newly Designed Chamber for the Acclimatization of Coconut Plantlets Coming from *In Vitro*. ZHIHUA MU[1], Xuhong Guo[2], Julianne Biddle[1], Mike Foale[1], Zhiying Li[3], and Steve Adkins[1]. [1]School of Agriculture and Food Sciences, The University of Queensland, 8117A, Gatton Campus, Gatton, 4343 QLD, AUSTRALIA; [2]Lanzhou Highland Pumps Co., LTD, 41 Changxin RD, Lanzhou, Gansu, CHINA; and [3]Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, 496 Wenqing Ave, Wenchang, Hainan, CHINA. Email: uqzmu@uq.edu.au

Coconut (*Cocos nucifera* L.) is an important tropical palm worldwide, but decreasing fruit production cannot match the fast-growing demand the industry is facing. Production is declining because of aging palms, pests, pathogens and other factors and traditional breeding cannot produce sufficient numbers of high-quality plants. Hence, *in vitro* technologies, such as clonal propagation (somatic embryogenesis) and embryo culture are being studied to increase productivity and preserve unique coconut varieties. Although both techniques have been developed for coconut, improvements in the acclimatization process of the plantlets coming from *in vitro* have the potential to reduce the time required for production and to increase the number produced. To do this it is necessary to assist the development of the coconut plantlets during acclimatization with elevated CO_2 concentrations and suitable light intensities. A semi-automated acclimatization chamber was designed and tested on 5-6-month-old coconut plantlets *in vitro* with the aim of increasing their survival rate and wellbeing. In this semi-automated system, the CO_2 concentration, culture medium, light quality, and intensity and the relative humidity can be monitored and controlled. Preliminary experiments demonstrated that this acclimatization chamber can be applied successfully to the early growth of coconut seedlings produced from embryo culture. The results showed that the acclimatization chamber, provided with elevated CO_2 and enhanced light intensity, was of benefit to coconut plantlet growth and rapid developments. This acclimatization chamber was designed in Australia, built and tested in China. It was designed with the aim of being portable, cost-effective and with the potential to be introduced into the developing world where most of the coconut industry is located.

P-2006

Maintaining a Clonal Gene Bank of Cannabis Synthetic Seeds. ANGELO M. ALVAREZ and Savanah M. St. Clair. Los Angeles Pierce College, Test Tube Garden, CRTFD Plant Science, Northridge, CA 91324. Email: alvarezangelo96@gmail.com

Synthetic seed encapsulation combined with cold storage has shown to reduce the maintenance and space required to preserve clonal cannabis genetics (site lata and our old paper). Micro-cuttings (nodal sections or shoot tips) can be maintained in a stasis for up to 8 months without maintenance and regrown with a 15% regeneration rate. This study investigates a system for preserving and maintaining the synthetic seeds so the plant genetics can be stored longer than 8 months and recovered when needed. Maintenance of a synthetic seed library involves scheduled recovery of the plants in tissue culture. Recovered synthetic seeds are grown in vitro to produce fresh shoots which can be used to reintroduce the variety into synthetic seed storage. This recovery process is repeated every 6-8 months. The maintenance, space, and water usage is drastically reduced compared to maintaining genetics via potted mother stock plants.

P-2007

Propagation of Marijuana (*Cannabis sativa* L.) Plantlets from Meristems and Nodal Explants and Identification of Fungal Contaminants in Tissue Culture Using a PCR-based Assay. DANIELLE COLLYER[1,2], Samantha Lung[2], and Zamir K. Punja[2]. [1]Agrima Botanicals, Maple Ridge, British Columbia, CANADA and [2]Simon Fraser University, Department of Biological Sciences, Burnaby, British Columbia, CANADA. Email: dcollyer@sfu.ca

Plantlet production from meristems and nodal explants of seven strains of marijuana (Cannabis sativa L.) was investigated. Shoot development was assessed on Murashige and Skoog medium with Gamborg's B-5 vitamins, sucrose (2%), phytagel (3%), activated charcoal (1.5%), thidiazuron (TDZ) and naphthalene acetic acid (NAA) (MM medium) after incubation at 21-25 °C with an 18-hr photoperiod for six weeks. Shoots from meristem explants were transferred to fresh MM and height, number of axillary buds, and number of shoots were measured after four weeks. There were significant differences ($P < 0.01$) among strains, with greatest shoot growth observed in strains Moby Dick, CBD Therapy and Pennywise. Nodal explants, each containing an axillary bud, were incubated on MM for four weeks, after which growth was measured. Greatest growth was observed in strains Blue Deity and Sweet Durga; while contamination by fungi hindered explant survival for nodal explants, meristems were contaminant-free. A PCR-based method was developed to detect and identify the common fungal contaminants emerging from nodal explants. Up to 8 fungal genera were recovered, which include species of Penicillium, Fusarium, Trichoderma and Chaetomium. To

induce rooting of shoots, MM without TDZ or NAA supplemented with indole-3-butyric acid and silver nitrate or sodium metasilicate was used. Rooting was highest in strain Moby Dick (44%) with 6 mg/L of sodium metasilicate. Plantlet survival 2 weeks after transfer from tissue culture was 57% in hydroponic solution and 76% and 83% in peat or rockwool substrates, respectively. Plantlet production in marijuana is influenced by the strain and explant source, with meristems responding better when microbial contamination was present in the source plants.

P-2009

Basal Media Optimization for the Micropropagation and Callogenesis of Cannabis sativa L. S. R. G. PAGE, A. Monthony, and A. M. P. Jones. University of Guelph, Plant Agriculture, 50 Stone Rd. E, Guelph, ON N1G 2W1, CANADA. Email: spage01@uoguelph.ca

The propagation of Cannabis sativa L. using micropropagation is an emerging area in tissue culture for large scale production of clean planting material. However, existing protocols have been developed using a limited number of genotypes and are often not reproducible. To date, no universally applicable protocol for efficiently growing multiple cultivars in vitro exists. Previous studies reported MS + 0.5 μM TDZ as the optimal medium for cannabis micropropagation, however, our preliminary studies found that this resulted in excessive callus formation, hyperhydricity, low multiplication rates, and ultimately plant death in many cultivars. Following an initial screen of five different micropropagation basal salt mixtures, we determined that DKW produced the healthiest plants and that DKW also increased callus growth in auxin based (2,4-D) callus induction media. The current study was conducted to evaluate these observations across multiple genotypes by comparing multiplication rate and canopy area of explants grown on MS + 0.5 μM TDZ and DKW + 0.5 μM TDZ using five different cultivars. Callogenesis experiments using a range of 2,4-D concentrations (0-30 μM), also showed more vigorous callus growth on DKW than on MS. Explants cultured on DKW + 0.5 μM TDZ produced larger canopies with broader leaves and had an average multiplication rate that was 1.5x higher than explants grown on MS + 0.5 μM TDZ. While further improvements are likely possible through media optimization, this study represents an important step towards developing standardized tissue culture practices for the production of *in vitro* Cannabis in a nascent field of study.

P-2010

Passive CO_2 Fertilization and Humidity Regulation for Photoautotrophic Micropropagation of *Cannabis sativa* L. MARCO PEPE and A. Maxwell P. Jones. Department of Plant Agriculture, University of Guelph, Guelph ON, CANADA. Email: pepem@uoguelph.ca

Micropropagation shows high value to the emerging C*annabis* industry allowing large scale, aseptic maintenance and propagation of clonal lines. Current *in vitro* methods are characterized by low light intensity, high humidity, and limited CO_2 availability. These limitations can hinder emergence of the photosynthetic apparatus. Moreover, the presence of sucrose as a carbon source increases likelihood of microbial contamination and elicits mixotrophic plantlet responses, complicating acclimation upon *ex vitro* transfer. A passive photoautotrophic tissue culture system will reduce contamination, increase explant hardiness and lower the costs associated with active photoautotrophic culture systems. We propose a method integrating a Na_2CO_3/$NaHCO_3$ buffer for passive CO_2 release from within the culture vessel combined with a supersaturated salt solution to stabilize microclimate humidity. We hypothesize that passive CO_2 fertilization and humidity control in sucrose-free cultures will deter unfavorable culture induced phenotypes and promote the emergence of the photosynthetic apparatus. Preliminary data indicate that constrained photoautotrophic proficiency due to phenotypic responses can be overcome by manipulating abiotic conditions in cultures lacking hormones. Coupled to the cultures, CO_2 meters and relative humidity sensors test concentrations ranging from 600-2000 ppm, and 75-95 %, respectively, at increased irradiance levels to determine optimal synergy among photoautotrophic factors. Quantifiable responses include canopy surface area, root emergence, plantlet height, stomata conductance, chlorophyll content, and specimen weight. This passive photoautotrophic micropropagation method will ultimately allow *Cannabis* producers to maintain clonal populations for efficient transfer to production conditions.

P-2011

Development of HLV-free Lines from Founder Plants: A Path to Maintaining Long-term Cultures Remediated of HLV. C. LEAVITT, L. Rincon Mautner, E. Hsu, R. Saephan, and S. Healy. Node Labs Inc. Email: chris@nodelabsca.com

Producing disease-free stock is part of a comprehensive clean plant program, which includes removal of plant pathogenic microorganisms, virus and viroid particle elimination. Plant tissue culture micropropagation can effectively remove plant pathogens, but does not address virus and viroids, especially ones that are cryptic. Hop Latent Viroid (HLV) poses a difficult challenge to create virus-free lines because of its patchy distribution and difficulty of detection. Current techniques of low-resolution sampling of a larger specimen or sample set does not adequately demonstrate absence of the disease. We

have developed a novel tissue culture micropropagation strategy methodology that creates high-resolution testing of a 'founder plant', which is then used as a mother plant to produce generations of virus-free lines. We have implemented a clean plant program that incorporates this virus/viroid detection procedure and total eradication of pathogenic microorganisms, resulting in industry-leading plant quality that persists through mass propagation.

P-2012

NsD3, a Defensin from Nigella sativa, Confers High Resistance of Several Commercial Potato Varieties to Fungi and Bacteria. DENIS BELIAEV[1], Eugene A. Rogozhin[2], Alexei A. Meleshin[3], Dmitriy V. Tereshonok[1], Marina K. Derevyagina[3], Natalya O. Yuorieva[1], Ilina I. Tashlieva[1], Fevzi S. Djalilov[4], and Elena V. Voronkova[5]. [1]K. A. Timiryazev's Institute of Plant Physiology, Moscow, RUSSIA; [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, RUSSIA; [3]All-Russian Research Institute of Potato Farming by A. G. Lorh, Kraskovo, RUSSIA; [4]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy; and [5]Institute of Genetics and Cytology of the National Academy of Sciences of Belarus, Minsk, BELARUS. Email: bdv@ippras.ru

Defensins of black cumin N. sativa had been found to possess high and specific antimicrobial activities (Plant Physiology and Biochemistry (2011) 49:131-137) against fungi, Phytophthora infestans and bacteria. A published raw transcriptome of this species was assembled using Trinity software on Galaxy cluster courtesy of Indiana University (*Nucleic Acids Research*, Volume 46, Issue W1,2 July 2018, Pages W537–W544), and searched for sequences similar to the above defensins. The gene for peptide most similar to the above defensins was found in it by BLAST, cloned, named NsD3 and expressed in stably transformed potato plants of several Russian and Hungarian-bred potato varieties. The transgene expression in plants was driven by the CaMV 35S promoter and measured by qRT-PCR with Taqman probes for the transgene and cyclophillin as a reference potato gene. The greenhouse-grown transgenic plants were assayed for resistance to early blight by inoculation of detached leaves with suspension of Alternaria spp. (Agric. Biol. (1986) 21:85–88), and the times needed for appearance of disease symptoms (incubation period) and the lesion diameters were measured for juvenile, middle-aged and older plants. During their development, 18 out of 76 transgenic lines consistently had longer incubation periods and smaller lesions, and some lines did not demonstrate any symptoms at all. Tubers of the transgenic lines of the Skoroplodny variety were also tested for resistance to black leg Dickeya spp., since this variety is susceptible to this pathogen, and two lines resistant to early blight were also found to be resistant to black leg. The NsD3 peptide was detected in transgenic lines, and its gene was inherited in the next generation (T1). The progeny of crosses of the transgenic lines is being analyzed for the resistance to pathogens.

P-2014

Development of Indirect Shoot Organogenesis and *Agrobacterium*-mediated Transformation for *Antirrhinum majus.* ZHAOYUAN LIAN[1,2], Sandra Wilson[1,3], Jianjun Chen[1,2], Heqiang Huo[1,2], and Chi D. Nguyen[1,2]. Department of Environmental Horticulture, IFAS, University of Florida; [2]Mid-Florida Research & Education Center, Apopka, FL 32703; and [3]Department of Environmental Horticulture, PO Box 110675, Gainesville, FL 32611. Email: lianzhaoyuan@ufl.edu, hhuo@ufl.edu

Snapdragon (*Antirrhinum majus* L.) is a popular bedding and cut flower plant and has been widely used as a model in plant genetic studies due to its genetic diversity and well-established transposon mutagenesis system. The existing natural transposons in snapdragon genome has facilitated a fundamental understanding of plant development ever since, yet its glory as a model plant has been drastically faded with the advent of *Arabidopsis.* Nevertheless, snapdragon still remains a model for investigating floral organ identity and leaf and flower asymmetry. The whole genomic information of snapdragon is now available, but the lack of an efficient genetic transformation system becomes a limiting factor for its application in genetic and molecular research. In this study, we developed a simple method for regeneration of snapdragon and established an efficient system for genetic engineering this species through *Agrobacterium*-mediated transformation. Cotyledon and hypocotyl explants of two-week-old seedlings were cultured on MS medium supplemented with 2 mg/L ZT, 0.1 mg/L NAA, and 4 mg/L $AgNO_3$ (an ethylene inhibitor). Adventitious shoots were produced from 48% and 40% of explants, respectively. In the absence of $AgNO_3$, however, only 20% and 30% of explants produced the shoots. Interestingly, shoots induced from explants cultured on medium with $AgNO_3$ were derived from indirect shoot organogenesis, while, those induced without $AgNO_3$ were produced through direct organogenesis. Using the indirect shoot organogenesis, an *Agrobacterium*-mediated transformation system was

developed, which had the transformation frequency ranging from 1% to 7%. Resultant stable transgenic lines were confirmed by PCR. The developed regeneration and transformation system could have a significant impact on the investigation of floral asymmetry or scent compound biosynthesis and improve breeding efficiency through CRISPR/Cas9 genome editing.

P-2016

Isolation and Functional Characterization of Novel Citrus and Plum Fruit Promoters. J. G. THOMSON[1], K. Dasgupta[2], and R. Thilmony[1]. [1]Crop Improvement and Genetics, Western Regional Research Center, USDA-ARS, Albany, CA and [2]Citrus Research Board, Visalia, CA. Email: James.Thomson@ars.usda.gov

Promoters that confer expression in fruit tissues are important tools for genetic engineering of fruit quality traits, yet few fruit-specific promoters have been identified, particularly for citrus fruit development. In this study, we report five citrus fruit-specific/preferential promoters for genetic engineering. Additionally, we have characterized a novel fruit-preferential promoter from plum. Genes specifically expressed in fruit tissues were selected and their isolated promoter regions were fused with the *GUSPlus* reporter gene for evaluation in transgenic plants. Stable transformation in Micro-Tom tomato demonstrated that the candidate promoter regions exhibit differing levels of expression and with varying degrees of fruit specificity. Among the five candidate citrus promoters characterized in this study, the *CsSEP* promoter showed a fruit-specific expression pattern, while the *CsWAX* and *CsJuSac* promoters exhibited high fruit-preferential expression with strong activity in the fruit, weak activity in floral tissues and low or undetectable activity in other tissues. The *CsVO1, CsUNK* and *PamMybA* promoters, while exhibiting strong fruit-preferential expression, also showed consistent weak but detectable activity in leaves and other vegetative tissues.

P-2017

Can a Single Metabolic Gene Modulate a Rhizosphere Microbiome Through Exudation of Plant? EKATERINA N. BARANOVA[1], Alexander A. Gulevich[1], and Galina N. Raldugina[2]. [1]All-Russia Research Institute of Agricultural Biotechnology and [2]K. A. Timiryazev's Institute of Plant Physiology RAS, Moscow, RUSSIA. Email: greenpro2007@rambler.ru

Changing the expression of just one gene encoding a product, which copes with stress, often can indirectly alter the expression of other genes both related to other associated metabolic chains, and marginally linked. Thus, transgenic plants often have an altered phenotype and noticeable biomass changes. We found that the FeSOD and codA genes, expressed in transgenic tobacco plants, produce the changes in pigment composition, sugar transport, and some modifications of the root architecture. As we expected, these changes caused alterations in the cells of the root tissues including on the root surface (root cap, epidermal layer and the differentiation zone). Changes in the composition of root secretions modified the rhizosphere microflora. It was found in our study that as an intervention result of functioning of single gene to the metabolism of many plants under stress conditions or imitation of stress, the sensitive microbial community undergoes perceptible conversions. A significant change in the proportion of actinomycetes and non-spore-forming rod-shaped bacteria, micromycetes in the root zone of the soil of transgenic tomato and tobacco plants was revealed in comparison with control plants. Thus, genetically modified plants are able to influence the structure of the microbial community and the ratio between individual groups of prokaryotes. This work was supported by the Russian Foundation for Basic Research 19-016-00207.

P-2018

Development of an Efficient *Barley Stripe Mosaic* Virus-mediated CRISPR/Cas9 System for Gene Editing in Wheat. HUI CHEN[1], Bin Tian[2], Yan Liu[1], Harold N. Trick[2], and Guihua Bai[1,3]. [1]Department of Agronomy, Kansas State University, Manhattan, KS; [2]Department of Plant Pathology, Kansas State University, Manhattan, KS; and [3]Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS. Email: huichen98@ksu.edu, guihua.bai@usda.gov

Fusarium head blight (FHB) is a destructive wheat disease worldwide. Severe and frequent FHB epidemics that cause significant losses in grain yield and quality, which threaten world food security. Growing FHB resistant cultivars is an effective approach for FHB control. Disrupting susceptibility genes (S-genes) has proven to be a promising strategy to create new sources of resistance, whereas the newly developed CRISPR/Cas9 system can serve this purpose. However, because gene editing relies on genetic transformation and plant regeneration, and most recalcitrant wheat varieties have a low rate of callus induction and regeneration in tissue culture process, only a few wheat varieties can be used for conventional transformation. Therefore, a new efficient gene delivery method without transformation is urgently needed to deploy gene editing technology in routine wheat breeding and genetics research. We developed a novel *Barley stripe mosaic virus*(BSMV)-mediated CRISPR/Cas9 gene editing system in wheat that bypasses the transformation and tissue regeneration procedures. Using this new system, we successfully knocked out a

FHB susceptible allele of *TaHRC_S*(reticulum histidine-rich calcium-binding protein), a causal gene for FHB resistance QTL *Fhb1*, in both spring wheat variety 'Bobwhite' and hard winter wheat variety 'Everest'. The edited mutants had significantly lower FHB severity than non-edited controls, confirming that loss-of-function mutation in *TaHRC_S*increases FHB resistance in different backgrounds. The new system provides an efficient tool for targeted gene editing and will facilitate application of the CRISPR/Cas9 technology in molecular breeding and validation of gene functions in wheat.

P-2021

Development of SPACE Tomatoes: Small Plants for Agriculture in Confined Environments. MARCUS HARLAND-DUNAWAY[1], Andres Narvaez[2], Martha Orozco-Cardenas[2], and Robert Jinkerson[1,3]. [1]Department of Botany and Plant Science, University of California Riverside, Riverside, CA; [2]Plant Transformation Research Center, University of California Riverside, Riverside, CA; and [3]Department of Chemical and Environmental Engineering, University of California Riverside, Riverside, CA. Email: mharl003@ucr.edu

Agriculture has been expanding into built environments like vertical urban farms and will be needed for any long-term colonization of space. In both urban and space settings, the cultivation of plants for food can have many beneficial impacts such as providing a year-round, local source of fresh fruit and vegetables, increasing food quality, and food security. However, both of these environments have limited space for the cultivation of crop plants. This has often limited plants grown in these systems to leafy green vegetables such as lettuce. Increasing the diversity of plants suitable for built confined environments would increase nutritional opportunities, and the diversity of fresh food options. In this study, using CRISPR-Cas9 genome editing, we modified a gene in tomato that has altered its development to be more suitable for built environments. These tomato plants, called Small Plants for Agriculture in Confined Environments (SPACE), have several traits that make them ideal for cultivation in confined environments: (1) small size, (2) a greater ratio of food to non-edible biomass(leaves), and (3) the ability to produce fruit faster. These traits facilitate the growth of SPACE tomatoes via indoor farming systems or onboard the International Space Station in NASA's Vegetable Production System (Veggie). The SPACE tomatoes' harvest index can be up to 70% in the homozygous plants. We have ongoing efforts to engineer the SPACE tomatoes to increase carbon uptake and nutrition.

P-2022

CRISPR/Cas9 Under Inducible Expression Systems. ZAHRA ALIZADA[1,2], Bhuvan Pathak[2], Soumen Nandy[2], Shan Zhao[2], and Vibha Srivastava[1,2]. [1]Cell and Molecular Biology Program and [2]Crop, Soil and Environmental Sciences Department, University of Arkansas, Fayetteville, AR. Email: Zalizada@uark.edu

Constitutive expression of Cas9 leads to a higher editing efficiency; however, it also increases the chances of off-target mutations. Thus, transient expression of Cas9 is a desirable approach to achieve higher targeting efficiency, and to curb the off-target effects. We have previously shown that heat inducible expression of Cas9 had an editing efficiency of 45% upon heat treatment, and the heat-shock induced mutations were inherited by the next generation. In this study, we used cold-inducible promoter (rd29a) for driving Cas9 expression, and tested the editing efficiency of the cold-inducible Cas9 on the GUS transgene loci. The expression analysis of Cas9 before and after cold-shock suggested that it has ~ 2.5x expression compared to room temperature and ~1000x lower as that of constitutive expression of Cas9. The study of the cold-shock induced mutations by Cas9 is currently being studied. The results of the ongoing project will be presented at the meeting.

P-2023

Plant Transformation and Genome Editing Systems for Hexaploid Wheat. BIN TIAN[1], Hui Chen[2], Guihua Bai[2,3], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan KS; [2]Department of Agronomy, Kansas State University, Manhattan KS; and [3]Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS. Email: btian@ksu.edu, hnt@ksu.edu

Bread wheat (*Triticum aestivum* L.) is one of the most widely cultivated crops in the world. The agronomic traits of wheat cultivars need continuously to be improved to meet the demand for the ever-increasing human population. CRISPR-based gene editing technology has vividly increased its application in many organisms including crop plants, providing a powerful tool for genetic improvement of crop traits of interest through breeding. However, the application of this technology in wheat is largely lagged behind other crops. The hexaploid genetic structure, large genome size, and the recalcitrant tissue culture of most wheat cultivars are major bottlenecks for the utilization of this CRISPR-based gene editing in wheat. Athough gene editing efficiency in polyploid wheat using conventional transformation method is relatively low compared to other crop plants, such as rice, maize, and soybean,

we have obtained multiple mutant lines with the targeted gene disrupted via biolistic transformation of the spring wheat cultivar "Bobwhite". The mutant lines showed improved resistance to Fusarium head blight (FHB), which confirmed the wild-type *TaHRC* as the susceptible allele for FHB in the *Fhb1* QTL, a locus that confers the resistance to FHB. To improve the transformation efficiency and expand editable of recalcitrant wheat cultivars, we conducted transient and/or stable co-expression of morphogenic genes such as *Baby Boom* (*Bbm*) and *Wuschel2* (*Wus2*) with target genes in a hard winter cultivar, "Jagger" that was difficult to be transformed using the conventional method. The regeneration efficiency was significantly increased when *Bbm* and/or *Wus2* genes were co-expressed with the target transgene compared to controls with the target transgene alone, demonstrating that the use of morphogenic genes will facilitate plant genome editing in recalcitrant wheat cultivars.

P-2024

Establishment of an Efficient Indirect Somatic Embryogenesis System for Sweet Sorghum Cultivars Using Immature Inflorescence. LOGAYN T. ABUSHAL[1,2], Shireen K. Assem[3], and Walid M. Fouad[1,2]. [1]Graduate Program of Biotechnology and [2]Department of Biology, School of Science and Engineering, American University in Cairo, New Cairo, Cairo 11835, EGYPT and [3]Agricultural Genetic Engineering Research Institute, Agricultural Research Centre, Giza, EGYPT. Email: logayn@aucegypt.edu

Sweet sorghum (high-sugar varieties in *Sorghum bicolor* L. Moench) is a substantial agronomic crop because of its multi-product usage as food, fiber, feed, sugar, and a potential biofuel. In Africa, sweet sorghum has the advantage to supersede sugarcane and maize because of its low water requirement and high tolerance to both biotic and abiotic stresses. However, the productivity of sweet sorghum cannot keep pace with the increasing food, feed, and energy demands mainly due to the delay in crop improvement. Therefore, the main objective of this research is to establish an efficient *in-vitro* regeneration system for sweet sorghum as the first step toward its molecular improvements. Immature inflorescences explants form three cultivars, Rex, Sugardrip, and Ramada were collected from field growing plants, surface sterilized, and then cultured on five different callus induction media. Five different types of callus were generated within few weeks; only the embryonic callus was selected to be transformed into four different shoot-regeneration media. While Sugardrip showed the highest embryonic callus induction frequency of 94% followed by Ramada with 76% frequency on MS medium supplemented with 6 mg/L 2,4-D + 0.2mg/L kinetin, Rex showed the highest embryonic callus induction frequency of 77% on MS medium supplemented with 4 mg/L 2,4-D + 0.2mg/L kinetin. The best shoot induction frequency (38-51%) was observed over Rex and Sugardrip when the embryonic callus was cultured on MS medium supplemented with 1mg/L TDZ. The average number of regenerated shoots per callus regenerated on TDZ-supplemented media was between 1-5 shoots. However, Ramada had its best regeneration frequency (93%) on MS medium supplemented with 0.5 mg/L NAA + 1 mg/L BAP. The regenerated shoots were grown on a half-strength MS medium for rooting, where 100% of the regenerated explants developed roots within 3-4 weeks. Finally, regenerated plants were transferred to the greenhouse for further evaluation. The successfully developed sweet sorghum regeneration system will be used to establish gene-transfer protocol.

P-2025

Developing Healthier Oils and Other Food Ingredients Through Genome Editing. A. DA SILVA CONCEICAO. Calyxt, Inc., 2800 Mount Ridge Road, Roseville, MN, 55113. Email: alexandre.dasilvaconceicao@calyxt.com

Soybean oil has historically been partially hydrogenated to enhance its oxidative stability in order to increase shelf life and improve frying characteristics. This process, however, creates trans-unsaturated fatty acids, or trans fats. The discovery that dietary trans fats increase the risk of several health issues led the FDA to ban the use of partially hydrogenated oils, in processed foods, by all food manufacturers from June 18, 2018. Using its cutting-edge plant breeding techniques, Calyxt developed a soybean trait that has produced oil with a fatty acid profile that contains approximately 80% oleic acid, 20% less saturated fatty acids compared to commodity soybean oil, and zero trans fats per serving. TALEN® technology is a precise genome editing tool that creates targeted cleavage of specific chromosomal sequences leading to knockout mutations at specific loci. To date, other crop species, such as wheat, potato and canola, have been edited by Calyxt to produce healthier foods and food ingredients.

P-2026

Integration of Field Assessment and In Vitro Biology Techniques for Improvement of Forage Quality in Sweet Sorghum. WALID M. FOUAD[1,2], Muziri Mugwanya[2], Logayn T. Abushal[2], and Shireen Assem[3]. [1]Biology Department and [2]Biotechnology Graduate Program, The American University in Cairo, Cairo, EGYPT and [3]Agricultural Genetic Engineering Research Institute, Agricultural Research Centre, Giza, EGYPT. Email: wfouad@aucegypt.edu

Sorghum is among the top five cereal crops grown in the world, and it is among the most efficient plants in the conversion of solar energy with high water-use efficiency.

Therefore, widely found in the drier areas of Africa, Asia, the Americas, and Australia. Several types of sorghum offer great versatility in use, including grain sorghums (for unleavened or gluten-free bread, porridge, or alcohol), forage sorghums (for pasture, hay, and silage), sweet sorghums (for syrups and biofuel). There has been an increasing interest in sweet sorghum as forage and biofuel feedstock since the juices extracted from stalks are high in sugars that are efficiently digested by animals and fermentable for biofuel. We have evaluated the forage quality of the Sudan grasses and sweet sorghum cultivars at different planting and harvesting time points for their suitability for animal consumption. Results of fiber fraction and nutrient composition analysis for plants harvested at 75 and 90 days after sowing (DAS) indicated that Sugar Drip (SD) had the lowest NDF and ADF and lignin compared to other cultivars ($P < 0.05$). Results of in vitro digestibility at 90 DAS indicated that SD had the highest forage quality compared to other varieties. Following the field evaluation, three sweet sorghum varieties were selected, including SD to establish a somatic regeneration system as the first step toward the establishment of a genetic transformation system, and hence, the opportunity for its molecular improvement. Different explants were collected from field-grown plants and cultured on MS medium supplemented with different plant growth regulators (PGR) for callus induction. The three varieties have generated embryogenic callus at various rates depending on the concentration of the PGR. The embryogenic calli were then transferred on shoot-regeneration medium supplemented with four different combinations of PGR. Detailed results on field evaluation and the impact of PGR on regeneration efficiency will be presented.

P-2027

Transformation Improvement in Wild Black Cottonwood *(Populus trichocarpa)*: Effects of Developmental Regulators, Surfactants, and Other Factors. Michael Nagle, CATHLEEN MA, Ekaterina Peremyslova, and Steve Strauss. Department of Forest Ecosystems and Society, Oregon State University, Corvallis OR. Email: mac@oregonstate.edu

As part of a GWAS (genome-wide association study) to discover the genes that control plant regeneration and transformation in *Populus trichocarpa*, we investigated numerous factors that influence transformation rate, and thus could provide the best conditions for GWAS of transformation rate in over 1,000 genotypes. These factors included the *Agrobacterium* inducer acetosyringone (AS), high auxin preconditioning, *Agrobacterium* concentrations, and selected surfactants and developmental regulators such as *WUSCHEL* genes. Through studies of a sample of twenty wild genotypes, we found that most of the factors studied had appreciable effects on recovery of GFP-expressing callus or shoots. For example, 100 μM AS in the co-cultivation medium improved recovery of transgenic shoots up to 22%, with the impacts varying widely among genotypes. Pre-cultured stem explants were bigger and also showed less necrosis than did explants without pre-culture, thus enhanced transgenic callus and shoot production by an average of 15 % and 2.5 %, respectively. The use of the surfactant "Break-Through" at a rate of 0.02-0.03% during cocultivation improved transformation frequencies up to 3% in three-quarters of the tested genotypes. The antioxidant lipoic acid at 5-10 μM during both callus and shoot regeneration improved shoot regeneration up to 24% and transformation up to 9%, but only was helpful for half of the tested genotypes. High concentrations of 2, 4-D combined with 2iP improved transformation efficiencies over other auxin:cytokinin combinations tested in two-thirds of the genotypes, improving transformation up to 27%. Several different constitutively expressed *WUSCHEL* genes that were tested inhibited shoot regeneration, though two poplar HOMEOBOX genes tended to improve regeneration of transgenic callus or shoots. These and additional results will be presented, and their importance for plant transformation methods discussed.

P-2028

Physiological and Molecular Responses for Long Term Salinity Stress in Common Fig (*Ficus carica* L.). MONTHER T. SADDER[1], Ibrahim Alshomali[1], Ahmad Ateyyeh[1], and Anas Musallam[2]. [1]Department of Horticulture and Crop Science, Faculty of Agriculture, University of Jordan, Amman, 11942, JORDAN and [2]National Agricultural Research Center, PO Box: 639, Baq'a 19381, JORDAN. Email: sadderm@ju.edu.jo

Salinity stress is increasingly becoming a major challenge for current and expanding agriculture areas. Unlike temporal abiotic stresses, plants are usually exposed to salinity stress for an entire lifespan. Therefore, a long term study (10 weeks) of continuous salinity exposure was investigated for three common fig landraces (Zraki, Mwazi, and Khdari). Both relative water content and chlorophyll content decreased with elevated salinity stress, while stem length barely changed. The most prominent decline was observed in root biomass. The data would align common fig to moderately tolerant threshold slop with a C_{50} range of 100 to 150 mM NaCl. A high and significant correlation was evident between root biomass and chlorophyll content (85%). Concurrently, differential expression of putative salinity responsive genes in

common fig were determined; signal peptide peptidase-like 2B (*FcSPPL2B*), dehydration responsive element binding protein (*FcDREB*), calcineurin B-like protein (CBL)-CBL-interacting serine/threonine-protein kinase 11 (*FcCIPK11*), sorbitol dehydrogenase (*FcSORD*) and dehydrin (*FcDHN*). The data will be presented for each gene in respect of its potential role in salinity stress mitigation. The combined physiological and molecular data would conclude Zraki as the most salinity tolerant genotype. The major implication of the data emphasizes the tremendous genotype by environment (salinity stress) interaction in common fig.

P-2029

Identification and Functional Characterization of Soybean Proteinase Inhibitor Genes in Defense Against Herbivores by Overexpression in Soybean and Arabidopsis. MST SHAMIRA SULTANA[1], Mitra Mazarei[1], Reginald J. Millwood[1], and C. Neal Stewart Jr.[1,2]. [1]Department of Plant Sciences, University of Tennessee, Knoxville, TN and [2]Center for Agricultural Synthetic Biology, University of Tennessee Institute of Agriculture, Knoxville, TN. Email: msultana@vols.utk.edu, nealstewart@utk.edu *(corresponding author)*

Proteinase inhibitors are widely distributed in plants and are known to play a protective role against pests. Soybean contains two major proteinase inhibitor classes known as Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI). These proteinase inhibitors inactivate trypsin enzyme, which is the main digestive enzyme in pests. The utilization of soybean proteinase inhibitors in different crops has been shown the level of resistance against insects. Despite the progress in the identification of soybean proteinase inhibitors, the functional analysis involved in soybean defense is still unknown. We aimed to investigate the role of soybean proteinase inhibitors in plant defense against insects and nematode. Based on sequence similarity, in addition to the three known proteinase inhibitors *KTI1*, *KTI2* and *KTI3*, we further identified and characterized the novel *KTI4*, *KTI5*, *KTI7*, and *BBI5* genes in soybean. The endogenous expression patterns of these genes varied in different plant tissues (leaf, stem and seed) as determined by qRT-PCR. In order to examine the functional role of these genes in plant defense, a total of seven individual genes were overexpressed in soybean and Arabidopsis. Stable transgenic plants were developed via *Agrobacterium*-mediated transformation method. Homozygous transgenic lines will be analyzed for level of gene expression, enzymatic activity, insect feeding and nematode infection assay. Our study will provide further insights into the potential involvement of proteinase inhibitors in plant defense. Understanding the enzymes involved in plant defense response would lead to devise some practical solutions for enhancing crop resistance to biotic stress.

P-2030

Developing an Expression Vector for Easy Establishment of Stable Plant Cell Lines to Produce Recombinant Proteins. JIANFENG XU[1] and Uddhab Karki[2]. [1]Arkansas Biosciences Institute and [2]Molecuar Biosciences Program, Arkansas State University, Jonesboro, AR 72401. Email: jxu@astate.edu

"Molecular farming" in plant cells has emerged as a promising and feasible production platform for therapeutic proteins. Tobacco Bright Yellow 2 (BY-2) cell is especially favored for the development of large-scale processes because of its rapid growth, easy transformation, and simple and inexpensive nutritional requirements. However, it is difficulty to establish and maintain a stable cell culture with consistent expression of target proteins in high yields, which is reflected by the observation that selected BY-2 cell lines from initial transformation frequently lost target protein expression after subcultures. This is due to the heterogeneity of primary regenerated calli and subsequent genetic and epigenetic changes, such as transgene silencing and recombination events which diminish or even abolish protein production. To overcome this problem, we developed a new binary expression vector, termed pGFP+ that allows co-expression of proteins of interest with a reporter protein–green fluorescence protein (GFP). While the proteins of interest are targeted for extracellular secretion, the reporter protein is retained within cytoplasm. This way, the GFP green fluorescence could be used as a visual marker to monitor the protein expression during subculture of the calli by a handheld NIGHTSEA BlueStar flashlight with filter glasses. It was found that there was a high heterogeneity of fluorescence intensity in the cultured calli and the expression of proteins of interest positively correlated with the intensity of green fluorescence. With the selection of the brightest fluorescent sector of the calli for each subculture, stable and homogeneous cell lines exhibiting consistent protein expression could be finally generated. This method has been used to screen and establish sable BY-2 cell lines producing different therapeutic proteins, including human stem cell factor, erythropoietin, interleukin-3 and fibroblast growth factor-2. It provides a simple and reproducible approach to establish highly productive plant cell lines used in research and commercial production.

P-2031

Bioengineering of Herbal (*Piper longum* L.) Leaf Extract Mediated Silver Nanoparticles and Their Biomedical Applications. RENUKA YADAV and Veena Agrawal.

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Nanobiotechnology has opened up new vista in biomedical research due to their small size and targeted delivery. Present investigation highlights the green synthesis of silver-nanoparticles (AgNPs) using aqueous leaf extract of *Piper longum* to explore its reducing properties. Optimum conditions for the synthesis of AgNPs were 1 mM $AgNO_3$, 60 ± 2 °C for 120 min at pH 6. UV-Vis spectrum of biosynthesized AgNPs showed a maximum absorption peak at 420 nm. FE-SEM and HR-TEM micrographs showed the spherical shape AgNPs with mean diameter size of 28.8 nm. Crystalline nature of AgNPs was confirmed by XRD pattern. Their zeta potential was −24.5 mV, confirming high stability. Total yield of AgNPs was 41.92 ± 0.09 ppm using AAS. Aforesaid AgNPs and leaf extract prepared in different solvents such as hexane, ethyl acetate, methanol, chloroform and aqueous were assessed for their bio-efficacies. The AgNPs revealed the strong and dose-dependent cytotoxicity against Human cervical cancer cell line (HeLa) showing IC_{50} value being 5.27 μg/mL after 24h. The AgNPs were also able to induce DNA fragmentation in the cells, indicating apoptosis. AgNPs showed enhanced antioxidant (IC_{50}-67.56μg) and radical-scavenging (IC_{50}-198.8μg) activities as compared to crude extracts. Besides, an efficient larval mortality was also recorded with AgNPs against 3rd instar larvae of *Anopheles stephensi* having LC_{50} and LC_{90} value of 8.969 and 16.102 ppm, respectively followed by *Aedes aegypti* (LC_{50}-14.791 and LC_{90}-28.526 ppm) and *Culex quinquefasciatus* (LC_{50}-18.662 and LC_{90}-40.903 ppm) after 72h of exposure. This is the first report showing strong anti-tumorous and larvicidal activity of *P. longum* leaf extract mediated AgNPs against HeLa cells and mosquito vectors causing dengue, malaria and filariasis. Thus, we suggest that AgNPs derived using *P. longum* leaf extract can be bioprospected further for the management of these hazardous health diseases.

P-2034

Encapsulation of Individual Plant Protoplasts in Mechanically Tunable Hydrogel Microspheres. MATTHEW GRASSO and Philip Lintilhac. Department of Plant Biology, University of Vermont, Burlington, VT. Email: msgrasso@uvm.edu

Microfluidic platforms are emerging as powerful and unique tools in plant research. The major advantage of these devices is their ability to precisely control the chemical microenvironment of single specimens while seamlessly integrating with high resolution imaging systems. This allows researchers to track biological responses to changes in microenvironment with high temporal resolution. The miniaturized and controllable nature of these devices has also led to their application in studies on plant cell biomechanics as they can be used to mechanically stimulate cells with much greater sensitivity than conventional techniques. Due to the current lack of tools suitable for manipulating the stress-mechanical environment of individual plant cells, further development of microfluidic platforms for this application will benefit the field. In this work a multi-phase (oil/water), droplet-based microfluidic device was used to capture individual *Nicotiana tabacum* c.v. BY-2 protoplasts in hydrogel microspheres. By manipulating the properties of the hydrogel matrix, the chemical and mechanical environment of encapsulated cells can be modified. Hydrogel encapsulated protoplasts can be cultured for several days during which time they go through the stages of regeneration, elongation, and division. They can also be easily prepared for high resolution live-cell imaging, and hydrogel microspheres are permeable to fluorescent stains and dyes. This method contributes to a growing body of droplet-based microfluidic plant studies as well as microfluidic studies addressing problems of cellular biomechanics. Further development of this technology can lead to novel studies in plant cell biology including areas of biomechanics, protoplast technology, and single-cell resolution studies.

P-2035

Comprehensive Analysis and Regulation of Starch Biosynthesis and Degradation Genes in Rice (*Oryza sativa Japonica*). PETER JAMES ICALIA and Vibha Srivastava. Cell and Molecular Biology Program and Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR. Email: pjicalia@uark.edu

Starch biosynthesis and degradation in plants is very complex and highly regulated. The distribution of the starch biosynthetic processes at the different time of the day due to the light and dark phases of photosynthesis and interplay of genes is not fully understood. Our research aims to understand these processes to produce rice with consistent grain yield and quality across environments. We evaluated genes that could be responsible for starch biosynthesis and degradation by analyzing patterns of gene expression at the mRNA level, enzyme and metabolic activities as influenced by environmental temperature, genetic variation, stages of development, time of the day and point of reproductive development, where plants are subject of stressful conditions. Preliminary results showed that there is downregulation of *AGPL4* (Glucose-1-Phosphatre Adenylyl Transferase Large Subunit), *GBSSII* (Granule Bound Starch Synthase) and *SSIIa* (Starch Synthase IIa) for the rice lines known to have lower grain yield and quality. Genes screened for starch degradation were *RGG2*

(Guanine Nucleotide-Binding Protein Subunit Gamma 2), *GF14F* (14-3-3 Like Protein), *AMY1a* (Amylase 1A), *AMY3a* (Amylase 3a) and *AMY3b* (Amylase 3b), which all showed an increased pattern of expression for lines known to generate poor grain yield in stressful conditions. Initial data also suggest that expression patterns of these genes are affected by the increased temperature at night, which generally spiked-up expression of starch degradation genes, and lowered the expression starch biosynthesis genes. These expression patterns will be correlated with photosynthetic and enzyme activities to fully explain their mechanisms. Constitutive promoters and transcriptional enhancers could be used to upregulate genes positively associated with starch biosynthesis while genes that promotes degradation of starch could be knocked-out using the CRISPR/Cas9 system.

P-2036

Characterization of Douglas-fir *LEAFY COTYLEDON1* (*PmLEC1*) Gene Expression During Zygotic and Somatic Embryogenesis. M. VETRICI[1], D. P. Yevtushenko[1], and S. Misra[2]. [1]University of Lethbridge, Department of Biological Sciences, Lethbridge, Alberta, CANADA and [2]University of Victoria, Centre for Forest Biology, Victoria, British Columbia, CANADA. Email: mariana.vetrici@uleth.ca, dmytro.yevtushenko@uleth.ca

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is an economically valuable conifer with a superior growth rate and desirable wood qualities. The ability of Douglas-fir to adapt to climate change and mitigate its impact on the environment has greatly increased its commercial use globally. Rapid multiplication of Douglas-fir superior genotypes can be achieved through somatic embryogenesis (SE) of desired clones *in vitro*, and is essential in all tree improvement programs. However, the molecular mechanism of embryogenesis in conifers is poorly understood and remains an obstacle to the improvement of current SE protocols. In angiosperms, the *LEAFY COTYLEDON1* (*LEC1*) gene is critical to both zygotic and somatic embryo formation. In the present study, we isolated the *LEC1* homologue from Douglas-fir, designated as *PmLEC1*. Phylogenetic analysis revealed that the PmLEC1 protein is more closely related to AtLEC1 rather than to AtL1L from Arabidopsis. Sequence analysis of the *PmLEC1* gene revealed a 5′ UTR intron containing several binding sites for transcription factors, such as ABI3, VP1 and AGL15, that are associated with somatic embryogenesis in angiosperms. Quantitative RT-PCR analysis of *PmLEC1* expression in zygotic embryos demonstrated a unique, alternating pattern of expression with the highest levels during early stages of embryogenesis. This was similar to the expression pattern of this gene during SE. Western blot analysis revealed that the temporal pattern of PmLEC1 protein accumulation during seed development correlated with its transcriptional levels up to the seedling stage. In addition, *PmLEC1* expression was up-regulated in mature, stratified seeds treated with 2,4-epibrassinolide, sorbitol, mannitol, or NaCl, which may serve as a strategy for the induction of SE from mature tissues. These findings will contribute to our understanding of embryogenesis in conifers and may lead to advances in the development of novel protocols for SE in Douglas-fir and, possibly, other plant species.

P-2037

Flow Cytometry Application for Monitoring the Fate of Crop Plant Meristem Post Ploidy Status Change. HUIXIA WU and Dan Stessman. Corteva Agriscience™, 8305 NW 62nd Ave. Johnston, IA 50131. Email: huixia.wu@corteva.com

Measuring ploidy level is important for doubled haploid (DH) breeding programs in different crop species. The direct method of counting chromosome numbers using root tips is laborious as only limited numbers of cells are analyzed and root apices do not necessarily reflect the ploidy in shoot meristems. Indirect methods such as flow cytometry, stomata length, guard cell chloroplast number, and phenotypic observation have been used for evaluating the ploidy of regenerants via androgenesis. These methods have also been used to assess ploidy status of material derived from the maternal in vivo haploid induction system. During the maternal DH process, the desired ploidy change from haploid to diploid in the shoot meristem tissue is of critical importance to ensure successful development of a reproductive system. According to a recent article (Molenaar et al. 2019), there is a low correlation between ploidy status by indirect measure at an early growth stage and the success of DH in maize. Understanding the possible fate of the haploid cells after they have been subjected to ploidy change would shed light on improving the DH process. Among indirect methods, flow cytometry allows us to obtain detailed information about the existence and proportion of cells with different ploidies when cells undergo change. Here we provide an example on how mixoploid interpretations can be drawn using flow cytometry.

P-2039

Agrobacterium-mediated Transformation of *Brassica napus* Internodal Segments. UYEN CAO CHU[1], Kari Johnson[1], Yinghong Li[1], Sandeep Kumar[1], Leila Lachkar[2], and Todd J. Jones[1]. [1]Corteva Agriscience™, 8305 NW 62nd Ave, Johnston, IA 50131 and [2]University of Burgundy, FRANCE. Email: uyen.chu@corteva.com

Agrobacterium-mediated transformation of canola (B. napus) via hypocotyl segments has been a commonly used

method for the past 30 years. While the hypocotyl-based method is well established, it is a prolonged procedure and not ideal for a production transformation setting. We have developed an *Agrobacterium*-mediated transformation method based on epicotyl and stem segments (internodal) that is efficient, rapid and amenable for high-throughput transformation and genome editing. The method has been successfully implemented in multiple canola lines and appears to be genotype independent. Internodal segment transformation has been used to generate transgenic events, CRISPR/Cas9-mediated frameshift gene knockouts, gene dropouts, as well as homology-directed gene insertions.

P-2041

Expression of the Poplar *FER-LIKE Iron Deficiency-induced Transcription Factor 1* gene (*PtFIT*) in Raspberry Does Not Consistently Respond to Iron Deficiency. WENHAO DAI and Changhyeon Kim. Department of Plant Sciences, North Dakota State University, Fargo, ND 58108. Email: wenhao.dai@ndsu.edu

Iron deficiency chlorosis (IDC), mainly caused by insufficient iron uptake and transport, limits plant growth and development, causing millions of dollars loss yearly. Genes in the ferric reductase oxidase (FRO) and iron regulated transporter (IRT) families play important roles in iron uptake and transport in plants. Expression of these genes is knowingly regulated by a group of Fe-deficiency inducible transcription factors including the *FER-like iron deficiency-induced transcription factor* (*FIT*). We cloned a *FIT* gene from an iron deficiency resistant tree of the European aspen (*Populus tremula*) (*PtFIT*). Expression of the *PtFIT* gene increased iron uptake in transgenic lines of another *Populus* species (*P. canescens* × *P. grandidentata* 'C16'). In this study, the *PtFIT* gene was transferred into a purple raspberry (*R. occidentalis* × *R. idaeus*) cultivar 'Amethyst' using *Agrobacterium*-mediated transformation method. The gene transformation was confirmed in *12 transgenic lines using polymerase chain reaction (PCR). Expression of the PtFIT gene in transgenic raspberry lines was evaluated under the iron deficiency or sufficiency condition using real-time quantitative PCR method.* Results indicated that the *PtFIT* gene expressed in transgenic raspberry plants; however, the level of expression was different among transgenic lines. Moreover, the *PtFIT* gene showed an inconsistent response to iron deficiency in which the expression of *PtFIT* was either up-regulated or down-regulated by iron deficiency or its expression was not affected by the iron status in the plant. This research will help understand the role of the *FIT* genes in iron metabolisms in plants, which may lead to mitigation of iron deficiency chlorosis and enhancement of iron accumulation in plants.

P-2042

In Vitro Regeneration for Genetic Transformation of Avocado and Lettuce. SERENE KOUDSI[1], Patricia Manosalva[2], Robert Jinkerson[3], and Martha L. Orozco-Cárdenas[1]. [1]Plant Transformation Research Center, University of California, Riverside, CA; [2]Plant Pathology and Microbiology Department, University of California, Riverside, CA; and [3]Department of Chemical and Environmental Engineering, University of California, Riverside, CA. Email: serene.koudsi@yahoo.com

Genetic engineering and gene editing are high-value technologies that can be used to improve productivity, quality, and resistance to biotic and abiotic stresses of avocado (*Persea americana*) and lettuce (*Lactuca sativa*). Therefore, these technologies can have a direct positive impact on the growers, consumers, and the environment. For instance, by reducing the use of pesticides and/or increasing the water efficiency of crops. Efficient *in vitro* regeneration systems for avocado and lettuce have been established as the first critical step for the application of genetic engineering or gene editing. For avocado, immature embryos were isolated from fruits about 1.5–2.0 cm in length. Isolated immature embryos were cultured on Murashige and Skoog (MS) medium supplemented with sucrose, myo-inositol, thiamine-HCl, agar, and picloram. After four months, regenerated globular embryos were transferred to a hormone-free MS medium with vitamins, ascorbic acid, and citric acid. For lettuce, an efficient *Agrobacterium-mediated* transformation system has been developed. Cotyledons were cut from seedlings germinated after seven days, inoculated with the *Agrobacterium* strain GV3101 transformed with the pBI121 binary vector containing the β-glucuronidase (GUS) reporter gene. The cotyledons were inoculated with a concentration of 10^9 *Agrobacterium* cells/ml for a period of 30 minutes without wounding. For lettuce, the established methodology resulted in an efficiency of transformation of 86%. An efficient and reliable *in vitro* regeneration system for both avocado and lettuce, and transformation for the latter, has therefore been established.

P-2043

Improvement of *Agrobacterium*-mediated Transformation in Rice. MICHELLE TJAHJADI and Myeong-Je Cho. Innovative Genomics Institute, University of California, Berkeley, CA. Email: michelle.tjahjadi@berkeley.edu, mjcho1223@berkeley.edu (*corresponding author)*

Plant transformation is an under-appreciated but important field of study, required to support crop science research to feed an ever-increasing world population in an ever-changing climate. Great strides have been made in the last 30 years to advance plant tissue culture and transformation knowledge,

however, challenges still abound. In this study, we investigated three methods of improving Kitaake transformation: 1) *Agrobacterium* strain comparison, 2) prevention of *Agrobacterium* overgrowth, and 3) modification of regeneration medium. We compared transformation efficiency of three *Agrobacterium* strains and methods to prevent their overgrowth in culture. Ten weeks after transformation with pAct:DsRed-pAct:Hyg, there is no significant difference in the number of calli expressing DsRed after infection with EHA105, AGL1, or LBA4404. For *Agrobacterium* inhibition, we find that a combination of 250 mg/ml cefotaxime, 250 mg/ml timentin, and 1% plant preservative mixture (PPM) is able to prevent *Agrobacterium* overgrowth with no adverse effects to callus proliferation. This combination is preferable to cefotaxime alone in preventing *Agrobacterium* overgrowth in tissue culture. For regeneration of transgenic plants, we show that an MS-based medium with increased sucrose, and the addition of BAP and NAA is able to regenerate more plants at least two weeks faster than an N6-based medium. Together, results from our study significantly improve transformation frequency and will help advance science research in rice.

P-2044

Optimizing a High-throughput Gene Editing Pipeline at the Texas A&M Crop Genome Editing Lab. NIKOLAOS TSAKIRPALOGLOU, Nancy Wahl, Oneida Ibarra, Endang Septiningsih, and Michael J. Thomson. Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843. Email: ntsakirp@tamu.edu

Gene editing using CRISPR/Cas technology promises to greatly accelerate crop improvement. However, several bottlenecks need to be addressed to realize the full potential of this approach. Texas A&M AgriLife Research recently set up a new facility, called the Crop Genome Editing Lab (CGEL), to address these challenges and provide a gene editing service for crop improvement. Initial studies have focused on using rice as a model crop to optimize gene editing strategies. The CGEL facility is working closely with plant breeders and other core facilities on campus to optimize a high-throughput crop gene editing pipeline. Initial research activities in the lab have been funded internally by the Texas A&M Presidential X-Grant Initiative (a 2-year $500K project to optimize a high-throughput gene editing pipeline using rice as a model system), and initial service activities were funded through a Texas A&M AgriLife Research Gene Editing Seed Grant program for gene editing across major crops, including wheat, sorghum, and cotton. Currently, projects across multiple crops are in progress to provide breeding and research groups with a rapid gene editing pipeline to test candidate genes in their programs, with the ultimate goal of developing nutritious, high yielding, stress-tolerant crops for the future.

P-2045

An Effective Surface Sterilization Protocol to Eradicate Adult Hemp Russet Mites from Cannabis sativa. ANGELO ALVAREZ[1], Savanah St. Clair[1], and Norman Senn[2]. [1]Los Angeles Pierce College, Test Tube Garden, CRTFD Plant Science, Northridge, CA 91324 and [2]CRTFD Plant Science, LLC. Los Angeles, CA. Email: alvarezangelo96@gmail.com

An effective method to eradicate the russet mite pest for cannabis tissue culture is investigated in this research. The disinfectant agents: bleach, alcohol, and hydrogen peroxide were diluted at different rates and tested for surface sterilizing cannabis plant tissues. Optimal disinfectant concentrations and exposure times for effective eradication was observed. Ineffective treatments were also observed. The russet mite pest or its eggs showed to survive some disinfectant treatments and could live inside tissue culture without obvious detection. Russet mite pest populations were monitored under magnification (40-80x) while exposed to diluted bleach (10%, 20% v/v), hydrogen peroxide (1.5%, 3% v/v), and isopropyl alcohol (50%, 70% v/v). The time required to eradicate the russet mite was recorded for each treatment. Plant tissue health was also monitored and taken into consideration for each treatment. It was observed that hydrogen peroxide and alcohol were most effective. Bleach treatment on its own did not eradicate the russet pests under the duration of it becoming phytotoxic.

P-2046

In Vitro Growth and Accumulation of Antioxidant Compounds in *Urtica dioica* L. Plantlets Under Different Natural Ventilation Systems. SUZAN KELLY V. BERTOLUCCI, Adriane Duarte Coelho, Diene Xavier Araújo, Camila Knopp de Souza, Alexandre Alves de Carvalho, Erica Alves Marques, and José Eduardo B. P. Pinto, Department of Agronomy, Medicinal Plants, Lavras University, C. P. 3037, Lavras, MG, CEP 37200-000, BRAZİL. Email: suzan@ufla.br

Urtica dioica L. (Urticaceae) is a species rich in vitamins and minerals, known in folk medicine as being effective in treating the symptoms of arthritis, diabetes, rheumatism, and benign prostatic hyperplasia. This study aimed to evaluate the use of natural ventilation system (NVS) in the growth and accumulation of secondary compounds in *U. dioica* plantlets. Two types of explants (nodal and apical segments) with one pair of leaves were cultured in the absence and presence of sucrose (30 g L^{-1}) in four different culture systems: conventional culture system (without membranes) with one membrane (NVS1), two (NVS2) and four porous membranes (NVS4). After 40 days, growth, leaf area, dry matter, photosynthetic pigments, total phenolic compounds, total flavonoids and

antioxidant activity were evaluated. The natural ventilation system was superior to the conventional one regarding the parameters of growth and dry matter production. The best results of micropropagation were observed with the use of apical segments in culture media supplemented with 30 g L^{-1} of sucrose and culture in NVS1 or NVS2. Apical segments in NVS1 and NVS2, without the addition of sucrose, favored leaf dry weight production. The addition of sucrose in the culture medium increased the accumulation of flavonoids by 3.1 times. NVS2 and NVS4 favored the accumulation of photosynthetic pigments. Sucrose in the culture medium promotes rooting, accumulation of flavonoids and enhances the antioxidant activity. The use of natural ventilation system favors *U. dioica* growth and micropropagation.

P-2047

Comparing Liquid and Semi-solid Culture Systems for the Micropropagation of *Cannabis sativa*. REBECCA BRADLEY and Andrew Maxwell Phineas Jones. University of Guelph, Department of Plant Agriculture, 50 Stone Road East, Guelph, ON, N1G 2W1, CANADA. Email: rbradl04@uoguelph.ca

Micropropagation of *Cannabis sativa* provides the cannabis industry with a means for large scale propagation of disease/pest free planting material and a safe approach for genetic storage with minimal space requirements. However, the cost of tissue culture makes it difficult to adopt into industry without a fully optimized system. Existing methods published for *C. sativa* utilize a semi-solid media for micropropagation, but in preliminary experiments plant growth and multiplication rates were low and nutrient deficiencies were observed. Many plant species benefit from liquid culture, in part because it provides a more uniform nutrient supply that is not limited by the diffusion rate through the gel. We hypothesized that *C. sativa* would grow quicker and display fewer deficiency symptoms in a liquid culture system. To test this, a semi-solid culture system was compared to various liquid systems to determine their impact on plant growth and multiplication. The liquid systems included a rocker based temporary immersion system as well as a self replenishing static thin layer culture system. While liquid culture resulted in increased plant growth, the tissues were extremely hyperhydric and subsequent growth was impaired. In an attempt to reduce hyperhydricity while benefiting from the increased growth from liquid, various support systems to prevent leaves from being in direct contact with the liquid were tested. This resulted in relatively rapid plant growth with fewer signs of hyperhydricity. Overall, this research demonstrates that liquid culture with the use of a support system has potential to improve micropropagation of *C. sativa*.

P-2048

Physical Factors Increased Quantity and Quality of Micropropagated Apical Shoots of *Cannabis sativa L.* in a Repeated Harvest System. RYAN MURPHY and Jeffrey Adelberg. Clemson University, Department of Plant and Environmental Sciences, E143 Poole Agricultural Center, Clemson, SC 29634. Email: rsmurph@clemson.edu

Sub-culture in micropropagation is the most labor intensive and costly process for shoot tip culture. Shoot tip culture is a preferred method to propagate clean, vegetative stock plants of *Cannabis sativa* L. *In-vitro* clones of *C. sativa* 'US Nursery Cherry 1,' were micropropagated at four different light intensities (25 – 200 μmol m^{-2} s^{-1} PPFD provided by 2 red:1ble LED light) in vented or non-vented vessels in factorial arrangement, to observe shoot tips harvested without sub-culturing for four repeated 2-week cutting cycles. The quality of the micro-cuttings was determined by rooting for 2-weeks in an Oasis® phenolic foam saturated with ½ strength Hoagland's medium under 200 μmol m^{-2} s^{-1} PPFD. The number of cuttings increased over the four cycles, with the most cuttings produced in ventilated boxes at a moderately high (appx 100 μmol m^{-2} s^{-1} PPFD) light intensity, and all of the micro-cuttings from optimal light and ventilation rooted *ex vitro*. Cuttings became smaller (by mass) in the second cutting cycle although, mass could be restored with proper light. Light intensity (appx. 100 μmol m^{-2} s^{-1} PPFD) and ventilation increased the quantity of micropropagated shoots over four cycles of repeated harvest and all of the shoots were of good quality to be rooted *ex vitro*. The labor efficiency of micropropagation by shoot tip culture could be improved by using a multi-cycle cutting process which would allow the same material to be repeatedly cut instead of sub-cultured. Shoot tip production from successive cycles was variable, but improved over successive cycles with the appropriate physical factors, when compared to the standard, once-over system in common use.

P-2049

In Vitro Co-culture System for *Prunus spp.* and *Armillaria mellea* in Oasis® IVE Phenolic Foam. JACQUELINE NAYLOR-ADELBERG, Jeffrey Adelberg, Sarah Miller, Ksenija Gasic, Guido Schnabel, Patricia Bryson, Christopher Saski, and Gregory Reighard. Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634. Email: jnaylor@clemson.edu

Armillaria and *Desarmillaria* spp. are causal agents of a devastating root-borne disease of peach. Breeding resistant rootstock requires a reliable screening tool. An in vitro co-

culture screen designed for almond was modified by replacing agar-gelled medium with a phenolic foam, rooting plants in a larger vessel, and combining resistant and susceptible varieties in the same vessel to adjust for non-uniform inoculum load. Eight of the ten *Prunus* rootstocks tested (peach, plum, peach × plum, and choke cherry) rooted at rates that would allow effective use of this assay. Roots grown in an aerated condition in the foam had better differentiation than roots grown in anoxic conditions in agar. An Armillaria root rot susceptible peach rootstock, 'GF 305', was cultured for 15 weeks in phenolic foam in the same vessel with a resistant peach plum hybrid, 'MP 29', without a decline in plant health. When the same pair of genotypes was subjected to *Armillaria mellea* at week 5, declining plant quality and mortality were much more pronounced on the peach, than the peach-plum hybrid. Eight weeks of fungal co-culture with the *Prunus* plantlets produced the clearest distinctions between susceptible and resistant genotypes. This method allows peach genotypes that are difficult to micropropagate, to be screened alongside other *Prunus* genotypes. The difference during a uniform challenge with the *A. mellea* fungus recapitulates resistant/susceptible reactions. The phenolic foam-based co-culture method will work on many *Prunus spp*. of potential use in rootstock breeding.

P-2051

In Vitro Growth and Anatomy of *Lippia dulcis* Cultivated Under Natural Ventilation System. JOSÉ EDUARDO PINTO, Taina Teixeira Rocha, Diene Xavier Araújo, Alexandre Alves de Carvalho, Suzan Kelly V. Bertolucci, Fernanda Naiara S. Ribeiro, Maria de Fátima Santos, and Manuel Losada Gavilanes. Department of Agronomy, Medicinal Plants, Lavras University, C. P. 3037, Lavras, MG, CEP 37200-000, BRAZİL. Email: jeduardo@ufla.br

Micropropagation is a technique of plant tissue culture widely used in the production of medicinal plants. *Lippia dulcis* is an aromatic species that has a potential therapeutic use. The objective of this study was to evaluate the effects of the natural ventilation system (NVS) on vegetative growth, anatomical characteristics and photosynthetic pigment production of *L. dulcis* plantlets grown *in vitro*. Nodal segments were inoculated into flasks with 50 mL of MS culture medium with conventional culture system (without membranes) with one membrane (NVS1), two (NVS2) and four porous membranes (NVS4). After 45 days, parameters of vegetative growth, anatomical characteristics and photosynthetic pigments were evaluated. The NVS4 treatment provided plantlets with higher growth, greater accumulation of photosynthetic pigments and anatomically more organized tissues. Only the accumulation of phenolic compounds was more abundant in the plantlets cultured in NVS1. Therefore, the natural ventilation system with four membranes offered the best conditions for the growth and development of *L. dulcis* plantlets.

P-2053

Effects of Co-applying Nitrogen Fertilizers and *Streptomyces* on Chlorophyll Content in Spinach Leaf. MARYAM SARAYLOU[1], S. St. Clair[2], H. A. Nadian[1], and N. Enayat Zamir[3]. [1]Ramin University of Agricultural Sciences and Natural Resources, IRAN; [2]Pierce College, CA; and [3]Chamran University, IRAN. Email Maryam.sarayloo64@yahoo.com

Chlorophyll is vital for photosynthesis as it helps to channel the energy of sunlight into chemical energy. With photosynthesis, chlorophyll absorbs energy and then transforms water and carbon dioxide into oxygen and carbohydrates. N is the most important elements in chloroplast pigments. The level and kind of fertilizer play an important role in yield and quality of plants. We aimed to find a correlation between two kinds of nitrogen fertilizers (urea and potassium nitrate) with chlorophyll content in *Spinach* as a highly consumed vegetable. *Spinach* plants were grown in plastic pots filled with 3 kg of soil per pot with N fertilizers. Also, we combined this experiment with co-applying *Streptomyces* as a plant growth promoting rhizobacteria. About 0.5 g of fresh leaves were prepared and 80% acetone was used as extraction solvent. Analytical determination of total chlorophyll (a+b) was performed with spectrophotometer at 663nm and 645nm, respectively. The results showed that the content of chlorophyll in the leaf of *Spinach* seedling treated by levels of 100 and 200 ppm of KNO3 had no significant difference, but when the nitrogen fertilizer level 200 ppm combined with *Streptomyces* inoculation, chlorophyll content improved significantly. There was a significant correlation in chlorophyll content between *spinach* treated by Urea and KNO_3. We came to this conclusion that spinach treated by N-NH4 had better yield due to chlorophyll content as there is a positive correlation between chlorophyll content and yield.

P-2054

Water Deficit Increases Recombinant Protein Production and Hydroxyproline-o-glycosylation in Tobacco Transient Expression. CRISTOFER CALVO-SANCHEZ[1,3], Argelia Lorence[1,4], Jianfeng Xu[1,2], and Maureen C. Dolan[1,3]. [1]Arkansas State Biosciences Institute, [2]College of Agriculture, Engineering & Technology, [3]Department of Biological Sciences, and [4]Department of Chemistry and

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Plant-based recombinant protein production is emerging as a promising approach with significant advantages in cost and safety over other expression systems. One of the leading plant-based platforms for recombinant protein production is a transient *Agrobacteria*-mediated expression system in *Nicotiana benthamiana*. Despite the advantages of plant recombinant proteins, the most important bottleneck that limits the commercialization is the low protein yields. Plants have a unique type of O-glycosylation that has potential to enhance the stability and solubility of recombinant proteins expressed using plant platforms. Specifically, target gene sequences are fused with a sequence to code for hydroxyproline-O-glycosylated peptide (HypGP) tags. To understand the underlying mechanism of HypGP modification process of recombinant expressed proteins, we explored the impact of water deficit on *N. benthamiana* for increasing expression and recovery of the HypGP-tagged EGFP. We used a non-destructive high throughput plant phenotyping system (HTPP) to assess plant fitness for recombinant protein production under water deficit and normal conditions. We found water deficit for two weeks decreases biomass and leaf water content, but it does not impaired photosynthetic efficiency of *N. benthamiana* plants. In addition, water deficit increases the *in planta* accumulation of the two HypGP-tagged EGFP glycoforms in the transient expression system. The HTPP system may provide a valuable tool in monitoring water deficit status in the plants to ensure increased recombinant protein production and enhanced Hyp-O-glycosylation of HypGP-tagged recombinant proteins.

P-2055

Plant Tissue Culture as a Continuous Source of Bioactive Compounds from Rare Medicinal Plants in Qatar. TALAAT AHMED[1], Mohammed Alsafran[2], and Amjad Shraim[3]. [1]Environmental Science Center, Qatar University, Doha 2713, QATAR; [2]Central Laboratories Unit (QUCLU), Qatar University, Doha 2713, QATAR; and [3]Chemistry and Earth Sciences Department, Qatar University, Doha 2713, QATAR. Email: t.alfattah@qu.edu.qa

Plant tissue culture technique can be used as an alternative source to whole plant cultivation to produce valuable natural bio-products. An efficient in-vitro culture system of three desert medicinal plant species (*Convolvulus pilosellifolius Desr, Prosopis cineraria and Glossonema varians*) at different stages of differentiation (callus and organized shoot cultures) was established to test their abilities to accumulate bioactive molecules compared with intact plant growing in nature. Results indicated that in *Glossonema varians* the MS media supplemented with 0.5 mg/L, 1.0 mg/L and 2.0 mg/L of 2,4-D with NAA combination were the best medium for callus induction from cotyledons and root tissues. Furthermore, callus produced in medium supplanted with IBA enhanced production of the regenerated plantlets. In addition, the study suggested that supplemented MS media with combination of hormones of auxin and cytokines for callus initiation produced high quantity and quality callus. Qualitative analysis by HPLC method showed a significant difference between callus and leaves extracts. It appeared from the number of peaks that determine the number of compounds in each mixture, which concluded that callus extract has 28 peaks; also, leaves extract HPLC has 28 peaks. Each one differs in the concertation of the common compound. Regarding, *Convolvulus pilosellifolius Desr* the highest callus induction (100%) was obtained from leaves on MS media with 0.5 mg/L (2, 4-D + BAP) combination. HPLC results showed that callus and leaves extracts gave different extraction yield and differences in the intensity of the resulted peaks. In case of *Prosopis cineraria*, results showed that the maximum callus induction was under 1 mg/L of 2, 4-D. In addition, there were significant differences in peaks intensities of both callus and leaves, which propose the variation in the amount of metabolites for each sample. To avoid the need of relying on wild plants, plant tissue culture is an excellent alternative for production of the bioactive compounds, as it does not depend on the geographical environments.

P-2057

Production of Diosgenin, An Important Bioactive of Constituent of *Costus Pictus* Through Hairy Root Cultures. AMMANA S. RANI. Dept. of Botany, Osmania University, Hyderabad-500007, INDIA. Email: sabitaammana@yahoo.com

Recently, there is a growing demand for plant based drugs, causing a heavy pressure on existing resources resulting in depletion and extinction of many plants from their natural habitat. Plant cell and tissue culture has emerged as a powerful tool for conservation, large-scale multiplication and *in vitro* production of wide range of secondary metabolites. *Costus pictus D.Don* (Family: *Costaceae*), commonly known as insulin plant, is one of the important medicinal plants of India. *Costus* species possess many medicinal properties and used in treatment of diabetes, jaundice, asthma, blood pressure, skin care etc. Rhizome is the major source of diosgenin, which acts as anti-diabetic. Hairy roots, induced by genetic transformation with *Agrobacterium rhizogenes* are gaining importance for *in vitro* production of plant bioactive metabolites. When bacterium infects, the T-DNA is transferred and integrated into host plant, resulting in hairy root production. Hairy roots are negatively geotropic, branched, grow rapidly on phytohormone free medium and accumulate lot of secondary

metabolites. In the present study, hairy roots were induced from different explants of *C.pictus* by infecting with *A. rhizogenes*. Different time intervals of co-cultivation (24, 48, 72 hrs.) and concentrations of bacterium (0.2-1.0 Optical Density) was standardized. PCR analysis of hairy roots was done to confirm genetic transformation of *A.rhizogenes.* Hairy roots were multiplied in large number by sub-culturing onto hormone free media. These were harvested, dried and extracted with the solvents. The diosgenin content in hairy roots was quantified by HPLC analysis. This study is useful in production of Diosgenin from *C.pictus,* an important product of industrial and pharmaceutical applications.

P-2058

Somatic Embryogenesis Regulators in *Medicago truncatula*. ELINA POTSENKOVSKAIA, Varvara Tvorogova, Maria Lebedeva, and Ludmila Lutova. Saint Petersburg State University, Department of Genetics and Biotechnology, 7/9 Universitetskaya emb, 199034, Saint Petersburg, RUSSIAN FEDERATION. Email: epots556@gmail.com

Somatic embryogenesis (SE) is widely used in biotechnology as a regeneration process conditioning many plant transformation methods, artificial seeds obtaining and zygotic embryogenesis studying. The main objective of this research is the search of new SE regulators among genes belonging to *WOX* and *NF-Y* family and analysis of action mechanisms of chosen genes in *Medicago truncatula,* the legume model object. In the previous study, we have demonstrated that SE can be stimulated with MtWOX9-1 protein from WOX family. *MtWOX9-1* overexpressing calli transcriptome analysis revealed gene groups, which can be stimulated by MtWOX9-1. These groups include some genes from *NF-Y* family, among which *MtNF-YB10* gene is presented. This gene is closely related to *Arabidopsis thaliana LEC1*, which has a lot of different functions, related to embryogenesis. MtNF-YB10 can be a part of heterotrimeric transcription factor NF-Y, which includes NF-YA, B and C subunits and binds to the CCAAT boxes in promoters. The interactions of MtNF-YB10 with other NF-Y subunits, demonstrating high expression levels during SE, were analyzed by yeast two-hybrid system. Our next step will be to confirm the revealed interactions in planta. Currently, we also obtain plants with loss of function of the studied genes using CRISPR technology to assess their role in SE. This work was supported by Russian Science Foundation project no. 16-16-10011 and the grant from Russian Foundation for Basic Research no. 20-016-00124.

P-2059

Whole Genome Sequencing of *Bacillus* sp. B5 - Plant Growth Promoting Rhizobacterium. S. ABDURASHYTOV[1], T. Melnichuk[1], E. Andronov[2], E. Abdurashytova[1], A. Egovtseva[1], and E. Chirak[2]. [1]FSBSI "Research Institute of Agriculture of Crimea" Simferopol, REPUBLIC OF CRIMEA and [2]FSBSI "All-Russian Research Institute of Agricultural Microbiology" Saint-Petersburg, RUSSIA. Email: asuleyman83@rambler.ru

Many microorganisms belonging to the genus *Bacillus* have a positive effect on various plants. The Crimean collection of microorganisms of the Research Institute of Agriculture of Crimea contains a number of strains of this genus with growth-promoting activity. Therefore, the aim of our work was to evaluate the effectiveness of the strain of *Bacillus* sp. B5 and to sequence its genome to identify genes that stimulate plant growth. Strain *Bacillus* sp. B5 was isolated in 2018 from the rhizosphere of *Triticum aestivum* L., variety Ermak (southern chernozem, 45°31'37" N 34°09'41" E). The strain was isolated from the apical part of the root of wheat, free from soil, grown in a vessel of Leonard plants. Strain *Bacillus* sp. B5 increases the mass of roots and shoots from 31.5% to 62.5%, yield productivity – to 12.8% by interacting with various varieties of wheat. A total of 1,997,668 reads were received. They were processed in the CLC Genomic Workbench. The genome of *Bacillus* sp. B5 was assembled in 66 contigs with the N_{50} 161041 bp. The range of G+C content (34.9%) and the total length of the obtained contigs (5808458 bp) are comparable with other strains of the same genus from the GenBank NCBI. The genome is annotated in RAST (Rapid Annotation Subsystem Technology) under the number *Bacillus* sp. B5: 1428.1083. The proportion of identified subsystems was 42%. 5916 coding sequences combined into 482 subsystems were identified. *Bacillus* sp. has 110 genes responsible for RNA synthesis, 112 protections against stress groups of genes for, 4 groups of genes responsible for the biosynthesis of plant hormones (auxins) according to the available recognized subsystems. Currently, the most of the genes of the studied microorganism have to be recognized that can reveal its new features, including the interaction with the partner wheat plants.

P-2060

Assessment of Rhodanese in Ripe & Unripe Pawpaw (*Carica papaya* L) Seeds and Mesocarp. A. A. AJIBOYE[1] and M. D. Ajiboye[2]. [1]Department of Plant Science and Biotechnology, Federal University Oye Ekiti, Ekiti State, NIGERIA and [2]Department of Plant Biology, Osun State University, Osogbo, Osun State, NIGERIA. Email: abiodun.ajiboye@fuoye.edu.ng

Rhodanese was extracted from the mesocarp and seed of ripe and unripe pawpaw *Carica papaya*. Rhodanese is an enzyme which primary function is the protection of the electron

transport system from the deleterious effect of cyanide by catalyzing the conversion of the cyanide to thiocyanate, a less toxic compound. This research work was targeted at assessing the activity of rhodanese in ripe and unripe paw-paw seeds and mesocarp. The pawpaw was peeled, the mesocarp and seed were homogenized and then sieved. The presence of the enzyme was tested by putting 0.1ml of the crude sample and 0.5ml of Bradford reagents in a test tube and the readings were taken with the spectrophotometer. The presence of rhodanese was confirmed and then its activities were tested for under different pH, temperature and different sulphur donating compounds like, Mercaptoethanol, $Na_2S_2O_3$, Sodium metabisulphite, Ammonium sulphate, $(NH_4)_2SO_4$. At temp 70°C the ripe paw-paw mesocarp had its optimum activity and had its lowest activity at 100°C while the unripe pawpaw mesocarp had its optimum activity at temp 50°C and its lowest activity at 100°C. The ripe paw-paw seed had its optimum activity at 40°C and lowest at 90°C and 100°C while the seed had its optimum activity at temp 60°C and lowest at100°C. Under different pH the optimum activity for the ripe paw-paw mesocarp was at pH6 and no activity at pH9 and pH10 and the unripe pawpaw mesocarp had its own optimum activity at pH6 and showed no activity at pH 8, pH 9 and pH 10. The ripe pawpaw seed it had its optimum activity at pH7as it lowest was at pH 4 while the unripe paw-paw seed had its optimum activity at pH6 and the lowest was at pH4. This study confirmed the presence of rhodanese in the seed and mesocarp of ripe and unripe pawpaw, therefore the consumption of pawpaw can help to reduce the toxicity of cyanide to a less toxic thiocyanate. Paw-paw was confirmed to be a good source of rhodanese and its consumption can be advised and as it has been known to be a source of rhodanese, it can serve as a supply of rhodanese to the body.

P-2061

Cell Selection to Increase Zinc Resistance. EVGENY ALEKSANDROVICH GLADKOV and Olga Victorovna Gladkova*. K. A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, RUSSIA. Email: gladkovu@mail.ru

Lawn grasses – a basis of a grassy cover of cities. Zinc is a priority soil pollutant in major cities of Russia. Zinc is limiting the spread of lawn grass. Therefore it is important to obtain plants resistant to zinc especially in urban conditions. The object of our study was a lawn grass – *Agrostis stolonifera* L. *Agrostis stolonifera* forms the highest quality lawn. The aim of the work was the development of technology for obtaining resistant plants of *Agrostis stolonifera* to zinc. Callus of *Agrostis stolonifera* was obtained from germinated seeds on Murashige-Skoog medium with 3 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), the sucrose content was 30 g/l, casein hydrolyzate – 500 mg/l, agar-agar – 7 g/l. To select tolerant clones, *Agrostis stolonifera* calli were cultivated on MS modified medium with 2,4-D and ZnSO4 * 7H2O. In all experiments, data are calculated in terms of Zn content. A concentration of 300 mg/l Zn was selected as selective. Resistant cells were selected after 1-3 subcultivation of callus on selective medium contained 300 mg/l zinc. It is preferable to use 2 subcultivation. Regeneration and root formation were performed on Murashige and Skoog medium containing 300 mg/l zinc too in order to increase the probability of producing of resistant plants. The most of the tested plants produced from zinc -resistant cells were more tolerant to zinc than original plants. The test plants fully retain high decorative quality when planted in soil contained 450 mg/kg zinc. The tolerance is remained in next generation. *Comment. Part of the work was done at Moscow State University of Mechanical Engineering (ealier – Moscow State University of Environmental Engineering), which is currently reorganized.

P-2062

Antibacterial Activity of Sea Colewort Suspension Cultures Against Entomopathogenic Bacteria. ANASTASIIA KRYZHKO[1], Igor Bugara[2], Aleksandr Omelchenko[2], and Viktoria Gorelova[1]. [1]Research Institute of Agriculture of Crimea, Simferopol; RUSSIA and [2]Taurida Academy (structural subdivision) of V. I. Vernadsky Crimean Federal University, Simferopol, RUSSIA. Email: solanum@ukr.net

Nowadays, the development of plant protection methods based on using the bioagents with complex high insecticidal and antifungal activity is an urgent problem. That's why the study of interactions between the suspension culture of sea colewort (*Crambe maritima L.)* as an antagonist of phytopathogens and *B. thuringiensis* as an entomopathogen, is of interest as a complex method of plant protection. It is shown that the intensity of influence of antimicrobial substances of *C. maritima* suspension cultures on *B. thuriengiensis* entomopathogenic strains depends both on the composition of the nutrient media and bacterial strain properties and resistance. *C. maritima* suspension cultures were obtained on Murashige and Skoog mediums without $CaCl_2$, which was differ in the content of auxin and cytokinin. It was found that the strains of *B. thuriengiensis* 792, 888, 0578 and 0428 are the most resistant to antimicrobial substances of the *C. maritima* suspension culture. Bacterial cultures of strains 0162, 0279 and 994 are exposed to the action of antimicrobial substances of suspension culture. The influence of *C. maritima* suspension on these strains in comparison with the corresponding control variants is shown in change of dynamics of passing by *B. thuriengiensis* the phases of physiological development.

Acceleration of development dynamics was a characteristic for the strains 0162 and 0279, and slowdown of development - for the strain 994. Thus, the research has allowed to identify the strains of *B. thuriengiensis*, which is promising for use against leaf-eating pests in agrocenoses of the popular crop *C. maritima*, which has a high antibacterial activity. The obtained results open up the prospects for the development of a new generation of biological crop protection preparations based on the complex using of *C. maritima* suspension culture as an antagonist of phytopathogens and entomopathogenic bacteria *B. thuringiensis*.

P-2063

Reproduction of the Endemic Rare Species *Crataegus pojarkovae* Kossych with a Culture of Isolated Embryos. ANASTASIIA KRYZHKO. Research Institute of Agriculture of Crimea, Simferopol, RUSSIA. Email: solanum@ukr.net

Crataegus pojarkovae Kossych is a rare endemic species listed in the Red book of Crimean Republic since 2015. This species of hawthorn is decorative and can be used in urban gardening. Its fruits (up to 2,5-3 cm in diameter) have valuable nutritional and medicinal properties. However, since the natural renewal of the *C. pojarkovae* population is low due to irregular fruiting, low seed germination and a long period of their germination (280-400 days), it is advisable to develop methods for its *in vitro* reproduction. Seeds with embryos were used as explants. Using the sterilization with 0,8 % $AgNO_3$, 1,5 min., 70 % ethanol, 30 sec. allowed to get up to 70-85% of aseptic explants. Seeds with embryos were exposed to cold stratification at +4 °C for 3 days and then placed on hormone-free nutrient Smirnov agar. The control explants were immediately placed on the nutrient medium. In an *in vitro* culture of isolated embryos on a Smirnov nutrient medium with cold stratification the greenening of cotyledons and the beginning of morphogenesis were observed after 4-5 days. First pair of true leaves 2,1 mm in length appeared after 11-12 days. In the control, the greenening of cotyledons was noted after 5-6 days. Then the development of explants in control was stopped. Obtained seedlings were transplanted to Smirnov nutrient medium supplemented with 1 mg/l indolylacetic acid for root formation induction. *In vitro* seedlings development continued in 43-48 days. Compared with the natural development of hawthorn Poyarkova seedlings, *in vitro* development accelerated in 7-8 times. Thus, a method of the endemic rare species *C. pojarkovae* reproduction based on the culture of isolated embryos from mature seeds has been developed.

P-2064

Studying of *Origanum Vulgare L. In Vivo* and *In Vitro* Collection. ELENA MYAGKIH and Olga Yakimova. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. Email: origanum.science@mail.ru

Origanum vulgare L. is a valuable medicinal and aromatic plant widely used in medicine, cosmetics, and food industry. There are 48 cultivars and varieties of *O. vulgare* in the collection of FSBSI "Research Institute of Agriculture of Crimea". They vary considerably in such morphological and biological parameters as plant height, flower colour, inflorescence shape, dates of the phenological phase beginning, as well as in economically valuable traits, i.e. the yield of green mass (1.05-4.67 t/ha), mass fraction of the essential oil (0.05-1.55 % of the raw mass), amount of essential oil (0.33-316.3 kg/ha), and its component composition (phenolic, without phenolic, terpineol, and other chemotypes). This collection is of considerable interest for breeding work as a donor of various genetic traits. Providing the maintenance of the collection is a time-consuming process that depends on many factors. The creation of an *in vitro* deposited collection is a promising method for source material storage. This method has several advantages compared to traditionally grown *in vivo* gene pool collection. The development of clonal micropropagation technique is the basis for the creation of an *in vitro* collection. Previously, the main stages of *in vitro* micropropagation that provide a high multiplication index of up to 1:95 per subcultivation were optimized, as well as the adaptation to *in vivo* growth conditions for a number of *O. vulgare* samples. To create an *in vitro* collection, we studied the effect of the cold storage duration on the three genotypes of *O. vulgare* (g26, 78, and cultivar Slavnitsa). We planted microcuttings in MS medium supplemented with 0.5 mg/l BAP, which is optimal for the second stage of clonal micropropagation, then placed them (microcuttings) in conditions of low positive temperatures (+ 4-6 °C) and illumination of no more than 1,000 lx. Studies have shown that the frequency of explants development was 79-100% after 6 months of deposition (depending on the sample). The maximum adaptation to cold showed sample g26.

P-2065

Successful Management of Secondary Metabolite Biosynthesis of Essential Oil Plants Using Unmodified Antisense Oligonucleotides in a *Lavandula angustifolia* Mill. Model. V. V. OBEREMOK[1,2], K. V. Laikova[1,3], R. Z. Useinov[1], N. V. Gal'chinsky[1], K. A. Yurchenko[1,2], Yu. S. Khokhlov [2], I. A. Novikov[1,3]. [1]V. I. Vernadsky Crimean Federal University, Simferopol, CRIMEA; [2]Nikita Botanical Gardens – National Scientific Centre Russian Academy of Sciences, Yalta, CRIMEA; and [3]Research Institute of Agriculture of Crimea, Simferopol, CRIMEA. Email: genepcr@mail.ru

Lavender essential oil is used in a variety of human activities. The quality and yield of lavender oil depends on both the origin of the raw materials and the processing technology. We have developed a unique technology that allows targeted manipulation of the expression of genes of essential oil crops, thereby controlling the biosynthesis of secondary plant metabolites, including those of lavender. One preparation, LAVAN-11 (the 5'-CCATCTCGAAC-3' fragment of the antisense strand of the linalool synthase gene of *Lavandula angustifolia*), was tested on *L. angustifolia* during its flowering period in late June and early July 2019 in Crimea. The preparation (concentration: 200 mg/L) was sprayed onto flowering plants at a concentration of 50 pmol/cm^2; 4 days after treatment, samples were taken to obtain the essential oil, which was then analyzed by gas chromatography. The experiment was performed in triplicate. The analysis showed a targeted change in the chromatographic profile of ether lavender; significant changes were observed exclusively for linalool and linalyl acetate. After treatment with LAVAN-11, linalool decreased on average 6.14±0.85% (43.52±0.51 vs. 37.39±0.99, respectively) and linalyl acetate increased 8.64±2.02% (21.91±0.73% vs. 30.55±3.18%, respectively) compared with the control. The yield of essential oil after treatment with our preparations increased by 0.225±0.096% (1.7±0.29% vs. 1.925 ±0.095%, respectively) on average for LAVAN-11 compared with the control. Thus, treatment with the preparation not only improved the quality of the essential oil, but also increased its yield.

P-2066

The Influence of Growth Characteristics of Agrobacterium (*Agrobacterium Tumefaciens)* on the Effectiveness of Agrobacterium-mediated *Thalictrum Minus* L. Cell Culture Transformation. ELENA ALEKSANDROVNA OSIPOVA. K. A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, RUSSIA. Email: eleang@mail.ru

The cell culture of the medicinal plant *Thalictrum minus* L. is capable of synthesizing berberine. Berberine exhibits antimicrobial, choleretic, antitumor activity, lowers blood cholesterol promising for neurodegenerative diseases. An agrobacteria-mediated transformation method *Agrobacterium tumefaciens* containing cointegrative vector pGV 3850 was used. The plazmida is characterized by the presence of target gene *ipt* encoding key enzyme of cytokinins biosynthesis, isopentenyl transferase, under the control of promoter 35S of cauliflower mosaic virus and selective gene *nptII* encoding enzyme neomycin phosphotransferase conferring tolerance to kanamycin under the control of NOS promoter. Evaluated the influence of growth characteristics of agrobacteria on the effectiveness of the method 1) in a liquid medium LB and modified MS with stirring 100 rpm for three days; 2) on the same media with the addition of agar stationary for 7 days. In the liquid medium during the first day, an exponential growth of agrobacteria was observed. The cell density increased 10 times. On an agarized medium, the increase in cell mass was also during the first day, by 1.6 – 2.4 times. During the transformation, an agrobacterium was used every three days of cultivation in a liquid medium and were cocultivated with callus for one two three days in a liquid medium. Next, sown on agar medium. Elimination with cefotaxime 500 mg/l; callus selection on kanamycin 90 mg/l.The highest transformation efficiency was with agrobacterium, in the exponential growth phase, in a liquid medium, 10% for kanamycin, 90% for PCR. When coculturing only on an agar medium, the efficiency is 1% for kanamycin, 10% for PCR. Six PCR-stable cell lines were evaluated. Compared to the initial callus, the content of alkaloids was 54% higher in only one cell line. Growth, by contrast, was reduced by 31%. In two lines, the content of alkaloids was 44–46% lower; the growth corresponded to the initial callus. Alkaloids and growth in the other three cell lines did not differ.

P-2067

Comparison of Growth, Anthocyanin Content and Antioxidant Enzyme Activities of Black Carrot Hairy Root Cultures Grown in Different Aeration Systems. Gregorio Barba, Carlos Martínez-Vasallo, José Ramón Acosta, José Antonio Hernández, and ABEL PIQUERAS. Department of Plant Breeding. CEBAS(CSIC). 30100 Murcia, SPAIN. Email: piqueras@cebas.csic.es

Transformation with the naturally occurring soil borne *Rhizobium rhizogenes* is a biotechnological breeding method independent of recominant DNA techniques. which allows the development of characteristic "hairy roots" (HRs) at the infection site upon the transfer, integration and expression of bacterial transfer DNA (T-DNA) into the plant. Axenic culture of HRs offers several advantages: raipid and high-density growth in hormone-free medium, genotipic stablity of the derived HRs lines and production of specialized metabolites comparable to those of the wild type plants. In this study, an anthocyanin-accumulaing HR line from R. rhizogenes-mediated transformation of black carrot (*Daucus carota ssp. sativus var. atrorubens*) was grown in 500 mL flasks containing 200 ml 1/2 MS for 4 weeks at 25°C in the presence of

light. Aireation was provided by agitation on orbital shaker (100 rpm) (system A) or by an air bubbling system (system B). The growth and key physiological parametrs were compared for both aireation systems. At the enf of the growing period, fresh weight (fw) was similar in both systems, reaching 32,5±8,7 and 34.3±1,2 g for systems A and B respectivelly. However, the total anthocyanin content was substantially increased in system A (62,3±3,8 μg g^{-1} fw) than in B (6.3±3,8 μg g^{-1}fw). Likewise the activities of peroxidase, superoxide dismutase and ascorbate peroxidase were higher in system A. This results could be attributed to a moderate oxidative stress caused by mechanical damage in the oscillatory shaker, which in system A would índuce the accumulation of anthocyanin as well as the increased activity of antoxidant enzymes.

P-2068

Development of Tissue Culture Methods for *Brachycome Iberidifolia.* ILINA IGOREVNA TASHLIEVA and Evgeny Aleksandrovich Gladkov. K. A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, RUSSIA. Email: ii_tash@mail.ru

Brachycome iberidifolia Benth. is a annual plant in the family Asteraceae. It grows 25 to 40 cm tall, with branched stems and highly-divided leaves and daisy flowers. It is a popular and easily-grown garden plant. The aim of this study was to establish a novel *B. iberidifolia* cell culture that could be utilized for various research and biotechnological applications. Seed were cultured on Murashige and Skoog (MS) medium and Gambourg (B5) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin for callus induction. Studies have shown that the frequency of callus induction increased with an increase in the concentration of 2,4-D from 1 to 6 mg/l on B5 media, at these concentrations B5 was more effective than MS. At a concentration of 8-10 mg/l 2,4-D, the frequency of induction on medium B5 were decreased, while on MS it increased. The addition of kinetin to media with 2,4-D 2 ml/l resulted in an increase in the frequency of callus formation. The greatest callus formation of *B. iberidifolia* was on media: B5 with 6 mg/l 2,4-D and 2 mg/l kinetin (56.1%) and MS with 8 mg/l 2,4-D and 2 mg/l kinetin (52.3%). Callus was light yellow or light green in color, medium density. Period of subcultivation was 21-26 days. With an increase in cultivation time, the callus became dark and was observed tissue necrosis. MS basal medium supplemented with 15 mg/l sucrose was optimized as shoot induction medium. As growth regulators, we considered a combination of 6-benzylaminopurine (BAP) and α-naphthalene acetic (NAA). The highest frequency of shoot formation was observed on the nutrient medium with a combination of 2 mg/l BAP and 0.1 mg/l NAA acid (62.3%). The formation of shoots was after 2-3 passages. For root formation plants were transferred to a fresh solution 1/2MS with 0.1 mg/l NAA and secured to filter paper. After roots were established in vitro, plants were transferred directly to soil.

P-2069

Peculiarities of *Canna* × *Hybrida Hort.* ex Backer Micropropagation *In Vitro*. ARZY TEVFIK. Research Institute of Agriculture of Crimea, Simferopol, Kievskaya str., 150, RUSSIA. Email: tevfik.arzy@yandex.ru

Canna garden (*Canna* × *hybrida hort*. ex Backer) is a perennial herb often used in the design of gardens and parks. This crop has large leaves and a long flowering period. The aim of the work was to reveal the ways of morphogenesis during cultivation of different explants *in vitro* and to develop biotechnological methods of micropropagation for promising canna cultivars. Experiments were carried out in the Nikita Botanical Gardens. The implementation of the vegetative buds morphogenetic potential is mainly carried out through direct organogenesis by the adventive shoots formation. After the mass formation of meristemoid clusters, their separation and plant regeneration were revealed. Histological studies confirmed the meristemoids formation during the cultivation *in vitro* of canna explants. The optimal concentration of TDZ at the micropropagation stage of the canna cultivars 'Dar Vostoka' and 'Suevia' was 1.27 mg/l, of the 'Livadia' – 1.91 mg/l. To activate the regeneration processes, the meristemoids were placed in MS medium with 1.5 mg/l BAP and 1.5 mg/l IAA. After stratification (5 ± 1 °C, without light for 60 days) of isolated 'Dar Vostoka' embryos and cultivation them under standard *in vitro* conditions (35 days), seedlings were formed. The ability of the somatic embryos formation in the tissues of canna cv. 'Dar Vostoka' zygotic embryo cotyledon was shown. The use of a mixture of perlite and soil as a substrate makes it possible to obtain 75% of plants *in vivo*.

P-2070

Clonal Micropropagation of *Thymus Vulgaris* L. *In Vitro*. ARZY TEVFIK and Natalia Yegorova. Research Institute of Agriculture of Crimea, Simferopol, Kievskaya str., 150, RUSSIA. Email: tevfik.arzy@yandex.ru

Thymus vulgaris L. is a valuable aromatic and medicinal plant. Thyme essential oil and raw materials contain biologically active substances with antiseptic, antioxidant, expectorant, analgesic and immunomodulatory effects. The aim of the work was to study the peculiarities of explant

morphogenesis *in vitro* culture and to develop *T. vulgaris* clonal micropropagation method. Shoot tips and stem segments with a node (8.0-10.0 mm) isolated from two-year plants (breeding sample No. 20841) were introduced in aseptic culture. It was established that the addition of cytokinin (BAP, TDZ, kinetin) to the MS culture medium caused different explant morphogenetic reactions. BAP and TDZ stimulated formation of the large number small (2.0–4.0 mm), vitrified microshoots (4.0–26.5 pcs./explant), but kinetin – normal shoots with a 17.0–20.0 mm length. The culture medium for the explant introduction was optimized – MS with 1.0 mg/l kinetin and 1.0 mg/l GA_3. The effectiveness of 2 propagation methods application – the multiple shoot formation and microcutting induction was revealed. The maximum multiplication index (12.8) at the second stage of micropropagation was obtained on MS culture medium with 1.0 mg/l kinetin. The effect of culture vessel type, the growing cycle duration and the subcultures number on the multiplication index was shown. The maximum frequency of rooted microshoots (98.3%) and the number of the roots (7.7 pcs./explant) were noted on MS culture medium with 1.0 mg/l IBA. The conditions of *in vivo* adaptation and the substrate (peat and perlite 1:1), which ensures the survival 89.5% of *T. vulgaris* microplants, were selected.

P-2071

Investigation of *Melissa Officinalis* L. Callusogenesis During Long-term Cultivation *In Vitro*. OLGA YAKIMOVA and Natalia Yegorova. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. Email: olyyakimova@yandex.ru

Melissa officinalis L. is a medicinal and essential oil plant widely used in medicine. To increase the breeding work efficiency on the creation of high-oil cultivars it is advisable to use cell engineering methods. The development of many biotechnologies is based on the study of the callus induction features and optimization of its long-term subcultivation method. The aim of the study was to investigate the effect of various factors on the callusogenesis of *M. officinalis* and to analyze the cytophysiological parameters of the somatic cell population in the growing cycle. To obtain callus, explants of the leaf, stem, petiole, hypocotyls and cotyledons of *M. officinalis* cultivars Citronella, Sobornaya and Krymchanka were used. The efficiency of callusogenesis depended on the hormonal composition of the culture medium, explant type, origin of the donor plant and cultivation duration. The maximum induction frequency (59.5-92.9%) and growth of callus were on MS medium supplemented with 1.0 mg L^{-1} 2.4-D and 0.5 mg L^{-1} BAP. For long-term callus cultivation, it is necessary to use the nutrient medium of the same composition. When cultivating callus during 19 passages, the growth index varied from 3.7 to 13.7, depending on the cultivar and passage. Analysis of dynamics of some callus cytophysiological parameters (callus mass and density, viability and the ratio of different cell types) made it possible to establish the characteristics of cell population changes in the growing cycle. The duration of the main phases of the growth cycle has been determined. The lag phase lasted from 1^{st} to 10^{th} day; exponential - from 10^{th} to 20^{th} day; linear - from 20^{th} to 35^{th} day. The stationary phase began after 35 days of cultivation. The conducted studies will improve the method of callus tissue cultivation of *M. officinalis* cultivars.

P-2072

The Effect of Long-term Subcultivation on Rooting In Vitro of Some Lamiaceae Species, OLGA YAKIMOVA, Margarita Zagorskaya, and Natalia Yegorova. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. Email: olyyakimova@yandex.ru

Representatives of the Lamiaceae family (*Origanum vulgare* L. and *Mentha* spp.) are widely used in medicine, aromatherapy, perfumes, cosmetics and food industries. To increase the efficiency of breeding in order to create highly productive varieties of the studied plant species, it is advisable to use biotechnological techniques, including the method of clonal micropropagation, which allows to quickly propagate new genotypes with economically valuable traits. The plant explants used *Origanum* (sample g31) and *Mentha* spp. (sample 2.8.14 - line S1 of the collection form *M. spicata*, is used in interspecific crosses as a donor of high oil content. Previously, we developed the main stages of *in vitro* micropropagation of varieties and breeding samples of these plants, providing a high reproduction rate (in Oregano up to 1:94,5; in Mentha up to 1:24,7 per subculture). An important step in micropropagation is rooting *in vitro*. The maximum frequency of rooting of Oregano microplants was noted on MS medium supplemented with 1.0 mg/L IBA, and Mentha on MS medium without the addition of growth regulators. During studying the process of morphogenesis of long-cultivated microplants (9 subcultures), a decrease in the frequency of root formation in the studied genotypes was noted. It was shown that, in 5 subcultures of *O. vulgare* microplants, the rooting frequency varied from 80,0% to 85,9%, and by the 9th this indicator decreased to 55,1%. At the same time, an increase in the length of the roots by 1,5 times and a decrease in their number by 1.9 times were noted. While in sample 2.8.14, during 5 passages, the rooting frequency was 91,3 - 95,2%, and by the 9th subculture, this parameter

tended to decrease (to 85,0%). With an increase in the number of subcultures, the number and length of roots decreased by 1,2 and 1,8 times, respectively.

P-2073

Obtaining Essential Oil Rose Hybrids Using Biotechnological Methods. NATALIA YEGOROVA, Irina Stavtzeva, and Victor Zolotilov. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. Email: yegorova.na@mail.ru

The main task of essential oil roses breeding is to obtain hybrids combining high winter hardiness, yield and quality of essential oil. However, it is difficult to obtain hybrid plants because of postgamous non-compatibility. The aim of the study was to develop methods for creating essential oil rose hybrids using embryo culture and clonal micropropagation *in vitro*. In crosses were used cultivars and breeding samples obtained with the participation of *Rosa gallica* L., *R. damascena* Mill., *R. alba* L. The frequency of hybrid seedling formation in the embryo culture was maximum when crossing cultivars 'Lada' and 'Kazanlykskaya' (71.4%) and minimum – when crossing 'Aura' and 'Raduga' (13.7%). For accelerated propagation of hybrids, we used clonal micropropagation. The seedlings obtained *in vitro* were divided into stem segments with a node and cultivated on MS medium with 0.5 mg L^{-1} BAP, 0.1 mg L^{-1} GA_3 and 2.0% glucose. During micropropagation, the induction of adventitious shoots and their microcutting were used. The multiplication index depended on the hybrid and the number of subcultures. An analysis of hybrids (*R. alba* × *R. damascena* cv. 'Kazanlykskaya') micropropagation during 6 subcultures showed that the maximum multiplication index was in 3-4 subcultures. The best ability to propagation *in vitro* showed hybrid No. 37-14 (multiplication index up to 18.5), and the minimum – No. 37-19 (multiplication index up to 9.5). Microshoots were rooted *in vitro* on ½ MS culture medium containing 0.5 mg L^{-1} NAA with a frequency of 8.3 to 100.0% depending on the hybrid. After *ex vitro* adaptation, hybrid plants were transferred to the field for further research.

P-2074

Long-term Clonal Micropropagation *In Vitro* of Peppermint. MARGARITA ZAGORSKAYA and Natalia Yegorova. Research Institute of Agriculture of Crimea, Simferopol, Kievskaya str., 150, RUSSIA. Email: zagorskayamargo@gmail.com

The aim of the work was to study the effect of three-year clonal micropropagation *in vitro* on the morphometric parameters of peppermint explants. The axillary buds of the Ukrainskaya Perechnaya cultivar (*Mentha piperita* L. × *Mentha spicata* L.) were introduced into *in vitro* culture. Explants were cultured on an optimized Murashige and Skoog nutrient medium supplemented with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} IAA in a climatic chamber. The obtained microshoots were cut every 40 – 50 days. During 20 subcultures (passages), morphometric parameters as the number and length of shoots, the number of nodes, the number and length of roots etc. were analyzed. The number of shoots was multiplied by the number of nodes on the shoot to determine the multiplication index. It was shown that microcutting of axillary and adventitious shoots developing from explants should be used for *in vitro* propagation of peppermint. In the first subculture, the number of shoots was 2.3 pieces/explant with a length 36.8 mm. The shoot length reached its maximum (56.0 mm) in the fourth subculture. However, by the 20th passage, this parameter reduced a half (27.5 mm). The maximum number of shoots was noted in the 8th and 9th passages (3.8 and 4.0, respectively). The multiplication index during micropropagation over three years was the highest in the ninth subculture (12.8). During micropropagation shoots formed roots, starting from passage 1 was noted. In the first passage, the root formation frequency was 72.2%; by passage 5 it decreased to 21.0%; by 20th — it increased to 70.0%. Thus, it was concluded that peppermint is capable of long-term propagation *in vitro*. The multiplication index in most passages reached 6.9 – 8.7. After 2-3 years of subculturing, growth inhibition or shoot development abnormalities were not noted. During *ex vitro* adaptation,the frequency of microshoots survival was 94.3 – 97.2%.