In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-021-00567-5

Plant Posters

P-2000

Hash Rosin Yield of Distinct Batches of Micropropagated Cannabis Clones Flowered Outdoors Under Record Heat and High ETo Conditions. SAVANAH M. ST. CLAIR SENN, Norman Senn, Patrick Powell, and Giovanni Giannelli. CRTFD Plant Science, LLC. Los Angeles, CA. Email: savanah@crtfd.com

We report several Cannabis strains regenerated from meristem and harvested during a severe outdoor season in Southern California. Furthermore, we report the yields for the resulting cold water hash and rosin. Meristem-derived in vitro cloned plants of Sundae Driver, Garlic Cookies, Sunset Sherbet, Blue Apricot Sherbet, and Dante's Fire performed well during one of the hottest growing seasons on record in the San Fernando Valley (CNN). The most frequent ETo interval during the season was 0.234 to 0.273 (CIMIS). The maximum ETo value was 0.32 during the second week of June 2020. Plants were grown outdoors in garden beds and 5-gal containers of coco-perlite starting June and fed an organic nutrient regime with mycorrhizal infection. The season did not result in harvest for many growers due to fire and smoke damage. High temperatures ranged from −1 to 13 degrees above the average high during September (Accuweather). The hottest days in September were 113–115°F on September 5–6; overlapping with wildfires throughout California during August and September 2020 (CalFire). Plants were harvested week 7–8 ½ of the flower cycle. Three weeks prior to harvest bone meal was top-dressed. During the last two weeks of the growing season the plot was irrigated to field capacity. The hash yield for the tissue cultured batches was 3.8% on average; the maximum was 4.6% for Batch 2. Average rosin yield was 2.0% for tissue cultured batches and the Batch 2 mixture performed the best with a 2.6% rosin yield. Blue Sherbet was 25% damaged and some of the material had to be discarded. For Blue Sherbet, the hash yield was 1.7% and the rosin yield was 0.9%. The quality of the tissue culture-derived batches of hash was superior to the Blue Sherbet hash, which was not as resilient to the conditions. The resiliency of tissue cultured plants suggests an advantage for growers during extreme weather in the context of climate change. This may be related to adaptability of some cultivars to regeneration in vitro. These results highlight sustainability of fresh-frozen cold-water hash as a useful tool and contingency in a changing environment.

P-2001

An Improved Spectrophotometric Method to Detect Antimicrobial Efficacy of Plant Essential Oils In Vitro. SAVANAH ST. CLAIR SENN[1], Rosealie Vicuna[1], Daila Melendez[2], Karu Smith[1], Tracey Spann[3], Maryam Saraylou[4], and Jay Reichman[2]. [1]Los Angeles Pierce College Department of Agriculture Sciences; [2]Oregon State University College of Agriculture Sciences; [3]California State University, Northridge Department of Health Sciences; and [4]University of Khuzestan, Department of Agriculture and Natural Resources. Email: stclais@piercecollege.edu

Our previous work was concerned with testing essential oils for antimicrobial efficacy in petri plates. Some problems arise with the measurement and analysis of the area of inhibition. The measurement can be somewhat subjective, since the AOI is not always a perfect circle. A new method was employed here using spectrophotometry, employing optics to quantify the amount of bacterial growth via the level of turbidity of the sample. The clarity of the solution, which was a proxy for bacterial colonization levels, was measured as % transmission at 600 nm. Lemon balm (*M. officinalis*) and Tea tree (M. alternifolia) oil was tested against isolations from the Pierce College Farm. Plant material was harvested and air dried for 1 week. Soxhlet extraction was carried out for 3 cycles using reagent EtOH as the solvent. The extracts were subsequently dewaxed by running them through a Buchner funnel.

Bacterial colonies were selected from serial dilution plates from 4 different fields. Bacteria were cultured in 1.5 mL tubes of NB for 48 hours. Separate sterile glass tubes filled with 2 mL of nutrient broth were inoculated with 100uL of each NB culture. The glass tubes were treated with 20uL of plant oil. Transmittance at 600 nm

was measured after culturing at 20C overnight. The data was analyzed using R. The results of the ANOVA show that the type of bacteria is highly significant in the model in determining the transmittance at 600 nm ($F = 25.14$ on 78 d.f., $p < 10$^-5). Interaction between the essential oil treatment and type of bacteria was also significant at alpha<0.05. The results did not show that either treatment was more effective at controlling bacteria #1. For bacteria #2, both Lemon balm and Tea tree were effective. The Lemon balm treatment was significantly more effective versus the control treatment at significance alpha = 0.05. The Tea tree treatment showed a marginally higher transmittance level according to Tukey's HSD ($p = 0.06$). Finally, bacteria #4 showed a trend toward being more effectively controlled with Tea tree oil than the control; the result was marginally significant according to Tukey's HSD ($p < 0.1$).

P-2002

Detection of Azadirachtin, a Bioactive Triterpenoid from Tissue Culture Derived Neem Plants (*Azadirachta indica* A. Juss.) by Ultra-high-performance Liquid Chromatography. RAJENDRA ADAK and Rakhi Chaturvedi. Centre for Rural Technology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, INDIA. Email: rakhi_chaturvedi@iitg.ac.in

Azadirachta indica A. Juss. (family Meliaceae), commonly known as Neem, is an important medicinal plant, native to the Indian subcontinent, and Southeast Asia. It has been widely used in agriculture, environment protection and traditional medicine practices since ages. All parts of the neem tree, from leaves, stems, roots, flowers, fruits and seeds are the natural bio-factories of various biologically active natural compounds. In this study, a simple procedure has been described for screening and improved yield of a prominent triterpenoid, azadirachtin, from leaves of in vitro established plants of *Azadirachta indica.* In vitro lines of neem plant has been successfully developed from uninucleate microspores in anther cultures in the author's laboratory. Leaf samples were harvested from 2-year-old acclimatized plants and chemical analysis was done. Optimum separation of the triterpenoid was achieved by Ultra-High-Performance Liquid Chromatography (UHPLC) on a C_{18} column with methanol/water as mobile phase. With this route, 2.98 ± 0.05 mg/gm of azadirachtin was obtained from in vitro leaves. Compared to this, the leaves from parent plants contained 2.76 ± 0.00 mg/gm of azadirachtin. These elite lines could be used further for uniform production of targeted metabolites for industrial purpose.

P-2003

Development of an Isolation and Culture System for *Cannabis* Protoplasts. ADRIAN S. MONTHONY and A. Maxwell P. Jones. Department of Plant Agriculture, University of Guelph, 50 Stone Road East, N1G2W1, Guelph, Ontario, CANADA. Email: monthona@uoguelph.ca

Cannabis sativa L. tissue culture is a rapidly growing area of study and recently there has been increased interest in the application of plant biotechnologies to study secondary metabolite production, cellular and molecular biology, and to enhance breeding (Hesami *et al.* 2020). To date, few studies report the successful transformation of *Cannabis* and no regeneration of transformed *Cannabis* callus has been reported (Reviewed by Schachtsiek *et al.* 2018; Monthony *et al.* 2021). One such technique is the use of protoplasts for transient gene expression, transformation, and genome editing. In this process the cell walls are removed, which facilitates the introduction of foreign genetic material. The successful culture and induction of cell division in is critical for the development of protoplast transformation or genome editing systems, as has been demonstrated in other species (Davey *et al.* 2005). Unfortunately, the isolation and culture of *C. sativa* protoplasts has yet to receive much attention. While some studies have reported protoplast isolation, none have reported data on subsequent culture or regeneration (Morimoto *et al.* 2007; Lazič 2020). Here we present a method for the isolation, purification, and initial culture of *C. sativa* protoplasts. We compare protoplast isolation efficacy from various starting tissues, including seedling tissues and callus cultures. In addition, we explore the use of 2-aminoindan-2-phosphonic acid (AIP), a competitive inhibitor of the phenylpropanoid pathway, and its effect in reducing the accumulation of phenolic compounds in cell walls. The use of AIP in protoplast isolation systems has been well-documented in other recalcitrant species, such as *Ulmus americana,* where it facilitated efficient cell wall digestion, protoplast isolation and whole plant regeneration (Jones *et al.* 2012, 2015). Together our findings represent a modest starting point for the development of a protoplast-to-plant regeneration protocol, which can be used for the development of inter-specific hybridization or the regeneration of transgenic/edited *Cannabis.*

P-2004

Zero-valent Iron Nanoparticle-induced Reactive Oxygen Species in *Fremyella diplosiphon*, a Biodiesel-Producing Cyanobacterium. SAMSON GICHUKI[1], Yavuz Yalcin[1] LaDonna Wyatt[1], Christian Jones[1], William Ghann[2], Jamal Uddin[2], Hyeonggon Kang[2], and Viji Sitther[1]. [1]Department of Biology, Morgan State University, 1700 East Cold Spring Lane, Baltimore and [2]Center for Nanotechnology, 2500 W

North Ave Coppin State University, Baltimore, MD. Email: sagic1@morgan.edu, viji.sitther@morgan.edu

Iron is important for various physiological processes in cyanobacteria; however, its bioavailability is a growth limiting factor. Nanofer 25s zerovalent iron nanoparticles (nZVIs) are proposed as "a new miracle" due to their bioavailability leading to enhanced growth, and unsaturated fatty acid methyl esters in *F. diplosiphon*, thus improving its biodiesel capability. In this study, reactive oxygen species (ROS) induced by nZVIs in *F. diplosiphon* strains, B481-WT (wild type) and B481-SD (engineered for enhanced lipids) was quantified using malondialdehyde (MDA) and 2′,7'-Dichlorodihydrofluorescein Diacetate (DCFH-DA) assays. We used Energy Dispersive X-ray Spectroscopy (EDS) combined with Transmission Electron Microscope (TEM) to determine the localization of nZVIs in *F. diplosiphon*. Cultures treated with 1.6 mg/L, 3.2 mg/L, 6.4 mg/L, 12.8 mg/L and 25.6 mg/L nZVIs were grown under continuous growth light for 15 days at 70 rpm. Culture not exposed to nZVIs served as the control. Three replicated treatments were maintained. On day 15, ROS was quantified using MDA and DCFH-DA assays, and data analyzed using one-way ANOVA and Tukey's honest significant difference test. Our results indicated that cultures treated with 3.2 mg/L, 12.8 mg/L and 51.2 mg/L nZVIs exhibited significantly higher MDA concentration compared to the untreated control and 1.6 mg/L nZVI-treated cultures. Results from DCFH-DA assay showed that ROS in all nZVI-treated cultures was significantly higher than the untreated control. TEM images demonstrated aggregation of variously sized nZVIs outside *F. diplosiphon* cells and EDS analysis indicated the presence of iron within cells. Future studies will be aimed to investigate the effect of nZVIs on gene expression via. Next generation sequencing. This study was supported by the National Science Foundation's Division of Engineering [CBET-1900966] and National Institute of General Medical Sciences [RL5GM118972] grants awarded to Morgan State University.

P-2005

Efficient Transformation and Gene Editing of a Public Maize Inbred Using Morphogenic Regulators. LENNERT IMPENS, Griet Coussens, Stijn Aesaert, Els Van Lerberge, Mansour Karimi, and Laurens Pauwels. Ghent University, Department of Plant Biotechnology and Bioinformatics, 9052 Ghent, BELGIUM and VIB Center for Plant Systems Biology, 9052 Ghent, BELGIUM. Email: lennert.impens@psb.vib-ugent.be

Plant transformation efficiency is a bottleneck for the application of gene editing in plants. In *Zea mays* (maize), a breakthrough technology uses co-transformation of morphogenic transcription factors BABY BOOM (BBM) and WUSCHEL (WUS) to induce direct somatic embryogenesis and increase transformation efficiency. However, ectopic expression of the morphogenic regulators results in developmental abnormalities in transgenic plants. Here, we reconstructed the BBM-WUS system using modular cloning and evaluated functionality in the public inbred line B104 that is widely used for maize transformation by the research community. We confirm that expression of the morphogenic genes under tissue- and stage-specific promoters leads to somatic embryo formation on the scutellum of zygotic embryos and translates to a marked increase in the number of transgenic lines, produced in a shortened time. However, most transgenics still suffered from the presence of the developmental regulators and were not fertile. The undesirable phenotype was positively correlated with T-DNA copy number. Use of constructs in which morphogenic genes are flanked by a developmentally controlled Cre/Lox recombination system led to reduced T-DNA copy number and fertile plants, while maintaining an increased transformation efficiency. Addition of CRISPR/Cas9 gene editing modules confirmed functionality for gene editing. Our modular vectors allow easy cloning for gene editing or transcriptional units for functional analysis. We anticipate that our vectors can be widely applied to increase the efficiency of maize transformation in an academic setting and will lead to the production of single copy high-quality events for downstream functional analysis.

P-2006

Scratching the Surface: The Critical Need for *In Vitro* Research on Exceptional Plants. VALERIE C. PENCE[1] and Emily Beckman[2]. [1]Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, 3400 Vine St., Cincinnati, OH 45220 and [2]The Morton Arboretum, 4100 Illinois Route 53, Lisle, IL 60532. Email: valerie.pence@cincinnatizoo.org

Exceptional plant species are those that, either through seed availability or seed physiology, are unable to be conserved in conventional seed banks and will require cryobiotechnologies for their *ex situ* conservation. A recent analysis of current data available on seed storage behavior has resulted in a Global Working List of Exceptional Plants, which illustrates the scope of the challenge of conserving these species. This list contains 780 species, 2% or less of the number of plants projected to be exceptional. Of these, 480 (62%) represent species without available seed or with recalcitrant (desiccation sensitive) seeds, species that are most likely to require *in vitro* protocols in order to implement cryo-storage procedures. A search of Web of Science of the most recent 500 articles on Micropropagation in the research areas of Plant Science, Agriculture, and Forestry was made to compare the families represented with those on the exceptional plant list. When the

top 10 families represented in the micropropagation search were compared with the 10 families having the most known exceptional species, there were only 3 in common: Arecaceae, Fabaceae, and Orchidaceae. Dipterocarpaceae, which had the most listed exceptional species, was absent from the search. While this search is not exhaustive and not all the species represented in these families correspond with the listed exceptional species, the comparison between these two lists at the level of family highlights the disparity between current research and the urgent need for *in vitro* studies focused on exceptional species. (Research supported in part by a grant from the Institute of Museum and Library Services.)

P-2007

Plant Preservative Mixture Increases the Percentage of Walnut Aseptic Shoots. SVETLANA KUSHNARENKO, Moldir Aralbayeva, Nazgul Rymkhanova, and Natalya Romadanova. Institute of Plant Biology and Biotechnology, Almaty, KAZAKHSTAN. Email: svetlana_bio@mail.ru

Walnut (*Juglans regia* L.) is one of the most economically important nut crops in the world. Initiation and maintaining contamination-free shoot cultures is difficult for many species especially for woody plants. Woody shoots used as explants for *in vitro* culture initiation are usually inhabited by numerous microorganisms not only on their surface, but also in the internal tissues. Cultivars of walnut from field collections have severe microbial and fungal contamination resulted in high loss of plant material during *in vitro* initiation. One-year shoots of walnut varieties Milotai 10 and Xin 2 were cut in the field at the end of January. Cuttings of 30–40 cm long were washed in soap water, treated with a dilute commercial bleach "Belizna", washed in tap water and were put into the vessel with ½ mineral components of Driver and Kuniyuki medium (DKW) for shoot growth stimulation. After two weeks, the swollen dormant buds were excised from the stem, were surface disinfected in 0.1% mercuric chloride for 10 min, washed with sterile water, and placed into the borosilicate glass tubes with DKW medium. Addition of Plant Preservative Mixture (PPM) (0.2% *v*/v) into the initial medium significantly reduce the number of contaminated explants and increased the percentage of green shoots. The indexing of *in vitro* shoots for endogenous bacterial infection using 523 detection medium resulted in 28.6% of Xin 2 variety' and 41.7% of Milotai 10 variety' aseptic shoots.

P-2008

In Planta Transformation in *Populus* and *Eucalyptus*. CATHLEEN MA, Greg S. Goralogia, and Steven H. Strauss. Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR. Email: mac@oregonstate.edu

In planta transformation seeks to transfer genes and regenerate transgenic plants under *in vivo* conditions. This method takes advantage of natural totipotentcy of specific plant tissues, and also seeks to manipulate totipotency by application of hormones and/or developmental regulator genes. By avoiding the need for customizing *in vitro* conditions during traditional plant transformation, *in planta* methods may also be less costly and more consistent among genotypes. In pilot studies, we tested a number of conditions to promote *in planta* regeneration or transformation with *Agrobacterium* using fluorescent reporter genes. Shoots, stems, and root crowns were wounded and treated with hormones and/or *Agrobacterium* containing transformation vectors, often including developmental regulator genes. Up to 20 wild genotypes of *Populus trichocarpa* and 30 genotypes from four eucalypt species and interspecies hybrids were tested in various experiments. We found that decapitated stems combined with cytokinin application promoted callus development in nearly all eucalypt genotypes tested and in 65% of poplar genotypes. No shoots were regenerated in the eucalypts, however, one-third of poplar genotypes regenerated shoots after 7 weeks. Development modifying genes that affect cytokinin, auxin, and ethylene signaling—as well as genes that promote shoot or root meristem development—were tested on decapitated stems using the dsRED reporter gene. After 11 weeks, about 40% of poplar and 20% of eucalypt genotypes produced transgenic callus when an ethylene inhibition gene was employed, and 45% of poplar and 35% of eucalypt plants were transformed when a construct with auxin/cytokinin related genes from *Agrobacterium* was employed. To date, transgenic shoots could not be induced. Results of ongoing experiments involving *in planta* transformation of these genes and several others will be reported. We thank industrial members of the GREAT Trees Research Cooperative at Oregon State University for financial support. Ma and Goralogia are co-senior authors.

P-2009

Genetic Transformation of Diverse Maize Inbred Lines via a BBM/WUS2 Morphogene-based "Quick Corn" Protocol. FRANK MCFARLAND, Lucas Gontijo Silva Maia, and Heidi Kaeppler. Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706 and Wisconsin Crop Innovation Center, University of Wisconsin, 8520 University Green, Middleton, WI 53562. Email: fmcfarland@wisc.edu

A major bottleneck in plant transformation has been genotype dependence of the most widely utilized systems to date. Recently, genotype-flexible transformation systems based on precise expression of specific morphogenic genes have been established and tested on several different plant species. In

maize, transformation of previously "non-transformable" genotypes was achieved via a system utilizing controlled expression of maize WUSHEL2 and BABYBOOM morphogenes. Early attempts by others to repeat those results in the same or different maize genotypes were often unsuccessful or resulted in significantly lower transformation frequencies than reported. Following protocol review and optimization, we tested the morphogene-based system for transformation of a diverse set of ten maize genotypes, including Ex-Plant Variety Protection Act inbred lines (ExPVPs) of historical and breeding importance, and sweet, flint and silage lines. At least six ears were evaluated for each genotype, with three embryo size ranges examined. PHP02, PHN46, PHK76, 3IIH6, Gaspé Flint, W606S, and P39 produced regenerable plantlets with the morphogenes at differing frequencies, while LH85 and LH182 did not. Transformation efficiency was genotype-dependent and embryo-size dependent, and ranged from 0.7% to 81%.

P-2010

Mining the Compact Bladderwort Genome for C*is*-regulatory Elements for Plant Transformation. JUBILEE PARK[1], Lynsey Kovar[2], Pete LaFayette[1], Wayne Parrott[1], and Jason Wallace[1]. [1]Institute of Plant Breeding, Genetics, and Genomics, University of Georgia, Athens, GA and [2]Inari Agriculture, West Lafayette, IN. Email: jubilee.park25@uga.edu

The advent of plant genetic engineering has brought about profound agricultural and scientific advances. Current technology can place many genes close together and insert them into a crop; however enhancer interference and read-through transcription often results in silenced or variable transgene expression. Enabling multigene transformations will be key for the future of trait stacking in commercial agriculture and for advancing plant synthetic biology. To do this, short, yet effective, cis-regulatory elements (CREs) such as promoters, terminators, transcriptional blockers, and insulators are needed to regulate transgene expression while keeping construct sizes compact. The aim of this research is to identify and test putative CREs from the bladderwort (Utricularia gibba) genome, one of the smallest known angiosperm genomes (82 Mb). Paired-end Illumina sequencing reads were used to create a de novo bladderwort genome, and 3' RNA sequencing data were used to find intergenic sequences flanked by genes with expression patterns consistent with CREs. Bioinformatic analysis yielded several hundred putative CREs, from which thirty-two were identified by filtering the list by length, fold change in expression of flanking genes, and consistency across datasets. These CREs were tested transiently in Nicotiana benthamiana using combinations of GFP, RFP, and RUBY to evaluate their effectiveness. The transient assays identified blocker/insulator sequences (average length 550 bp) that show the ability to reduce ectopic expression caused by the 35S promoter. These will be stably transformed into Arabidopsis thaliana and rice to confirm their effectiveness. If the results hold, it will demonstrate the possibility of systematically screening plant genomes for novel CREs to be used in plant biotechnology.

P-2011

Development of Next-generation Transformation Protocols for Switchgrass (*Panicum virgatum* L.). MONICA PRIAS[1], Pete LaFayette[1], Tim Chappell[1], and Wayne Parrott[1,2,3]. [1]Center for Applied Genetic Technologies, University of Georgia, Athens, GA; [2]Institute for Plant Breeding, Genetics and Genomics, University of Georgia, Athens, GA; and [3]Department of Crop and Soil Sciences, University of Georgia, Athens, GA. Email: mp41423@uga.edu

Genetic transformation of switchgrass, a bioenergy species, is important for functional genomics of yield and biomass quality. *Agrobacterium* overgrowth and the antibiotics used to control it can be detrimental to regeneration. Hence, we first focused on transformation using auxotrophic strains and evaluated the capacity of three auxotrophic strains to deliver the T-DNA from pCambia 1305.2, which harbors *GusPlus* and *hph*. Based on transient transformation of switchgrass and rice embryogenic calli, the auxotrophic strains kept their capacity to transfer T-DNA, and the EHA105-derived auxotroph for methionine was as efficient as its parental strain, but without overgrowing the tissues, so transgenic tissue is recovered more quickly when stable transformation is measured just 35–42 days post-inoculation, and 12% of the switchgrass explants already show hygromycin resistant calli. In contrast, transgenic callus does not appear with the prototrophic EHA105 until day 49. The same rapid rate is seen in rice, with only 8% of explants showing transgenic tissue 35 days after inoculation when the prototrophic strain is used, but 24% doing so when transformed with the auxotrophic versión. For regeneration, we overexpressed Baby Boom (*BBM*) and Wuschel2 (*WUS2*) to stimulate somatic embryogenesis in young leaves and stems from *in vitro*-grown switchgrass plants instead of from immature inflorescence explants from greenhouse-grown plants. While recovery of transgenic plants is being achieved, subsequent removal of BBM/Wus is still not optimized. With additional optimization, it should be possible to achieve transformation in a shortened time frame while using more readily available explants.

P-2012

Homeostasis Between L-Proline Biosynthesis and Degradation Is a Key Indicator for Salinity Tolerance in

Common Fig (*Ficus carica* L.). MONTHER T. SADDER, Ibrahim Alshomali, and Ahmad Ateyyeh. Department of Horticulture and Crop Science, Faculty of Agriculture, University of Jordan, Amman, 11942, JORDAN. Email: sadderm@ju.edu.jo

Salinity stress is still a major issue in current and future agriculture areas. Salinity stress is lifelong abiotic stress adversely affecting plant growth and development. In a previous study, we characterized common fig landraces contrasting in response to salinity tolerance. Our results align common fig to moderately tolerant threshold slop with a C_{50} range of 100 to 150 mM NaCl. In this study, we further investigated the accumulation levels of L-proline under salinity stress. Concurrently, degradation activity was determined by following expression levels of the common Fig. L-proline dehydrogenase (*FcPRODH*) gene via qRT-PCR. The data revealed a sharp decline in *FcPRODH* actively in the tolerant common fig genotype. This trend was coupled with elevated L-proline levels. The accumulation of L-proline was directly proportional to salinity stress levels (from 50 to 150 mM NaCl). On the contrary, the less tolerant genotype showed up-regulation of *FcPRODH* and lower L-proline levels. Interestingly, common fig mannitol dehydrogenase (*FcMTD*) showed parallel expression levels similar to *FcPRODH* levels under elevated stress for investigated genotypes. On the other hand, the leaf K/Na ratio showed a continuous decline along with elevated salinity stress levels with similar behavior for all genotypes. Therefore, homeostasis of osmoprotectants is an indispensable tool for the glycophyte common fig in salinity stress mitigation.

P-2013

Doubled Haploid Production in Melon by Isolated Microspore Culture. JOHN CHEN, Elise Vanek, and Mark Pieper. HM. Clause, 9241 Mace Blvd, Davis, CA 95618. Email: John.chen@hmclause.com

A first reporting of melon (*Cucumis Melo L*) doubled haploid (DH) derived via isolated microspore culture (IMC) is elucidated. As a target to develop a highly efficient system for producing DH of Melon by IMC, this work builds on a published Patent No. 20180213736 (Chen *et al.* 2016). Practical application of an IMC process requires a high throughput approach that would have utility in combining DH with molecular and genomic selection. Key parameters from donor plant growth, microspore staging, and culture media have been found paramount to a successful proof of concept. A mix of 1n, 2n and polyploid plantlets are produced without incorporation of anti-mitotic doubling chemicals. Confirmation of DH fertility and microspore origin are reported.

P-2014

Leaf Culture and Regeneration in Different Sweetpotato (*Ipomoea batatas* L.) Genotypes. KEDONG DA[1,2], Felicia Shepard[2], Christie Almeyda[3], Kenneth Pecota[2], Wusheng Liu[2], and Craig G. Yencho[2]. [1]Plant Transformation Lab, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27607; [2]Department of Horticultural Science, North Carolina State University, Raleigh, NC; and [3]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC. Email: kda@ncsu.edu

Sweetpotato (*Ipomoea batatas* L.) is one of the world's most important food crops. Great achievements have been made on yield and starch content breeding in sweetpotato by using conventional breeding methods. However, due to its highly heterogeneous, polyploid genetic background, it is difficult to continuously improve the desirable characteristics in a genotype using conventional breeding alone. Emerging gene editing technologies are much needed to enable further trait improvement for enhanced stress tolerance and disease resistance in sweetpotato. High efficiency plant in vitro regeneration system is a prerequisite for gene editing in sweetpotato. Here, we report two plant regeneration methods for efficient induction of leaf regeneration by using the first three open leaves harvested from the 15-day-old in vitro cultures on modified MS medium [MS inorganic salts (Murashige and Skoog 1962) containing 0.6 mM myo-inositol, 5.0 μM thiamine-HCl, 10.0 μM nicotinic acid, and 5.0 μM pyridoxine-HCl]. A one-step method was used to test the effects of various concentrations of the auxins 1-Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA), and 2,4-Dichlorophenoxyacetic acid (2,4-D) individually, while a two-step method was used to test the effects of 2,4-D followed by regeneration on zeatin riboside medium. We found that shoot induction was influenced by the type and concentration of auxins. NAA was the best for adventitious shoot induction in the one-step method. Plant regeneration can also be induced from the cultured leaves by the two-step method. Regeneration efficiencies, defined as the number of shoot-developing explants out of the total number of explants tested, reached 100% for the best genotypes. Significant genotype-dependent responses were observed in all the growth regulators evaluated. Shoot regeneration was achieved in all tested genotypes within two months. Our results led to the establishment of optimized in vitro regeneration protocols for the economically important sweetpotato genotypes Beauregard94–14, NC04–531, NCP13–0030, and Jewel.

P-2015

Transcript Profiling Reveals the Role of Phytosulfokine Kinase Receptor Gene Family Members in Development and Stress Response in *Oryza sativa*. PREETI NAGAR[1],

Ashish Kumar[1], Muskan Jain[1], Sumita Kumari[2], and Ananda Mustafiz[1].[1]Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi, INDIA and [2]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, INDIA. Email: preetinagar87@gmail.com

Phytosulfokine Receptor (PSKR) is a transmembrane LRR-RLK family protein with a binding site for small signaling peptide, phytosulfokine (PSK). PSK-PSKR mediated signaling helps to relay the information responsible for coordinating normal growth and development and also plays a role in defense responses. But the role of PSKR in various abiotic stresses are yet to be explored. In this study, it was shown that PSKRs might have a role in signal transduction pathways related to abiotic stress responses. Genome-wide analysis of phytosulfokine receptor gene family has led to the identification of fifteen putative members in the *Oryza sativa* genome. RNA-seq data analysis of *OsPSKR* genes showed that these genes were differentially expressed in different tissues and responded specifically to heat, salt, drought and cold stress. Furthermore, the real-time quantitative PCR for fifteen *OsPSKR* genes revealed temporally and spatially regulated gene expression corresponding to salinity and drought stress. Our results provide useful information for a better understanding of *OsPSKR* genes and provide the foundation for additional functional exploration of the rice *PSKR* gene family in development and stress response.

P-2016

Metabolic Engineering of Energycane for Hyperaccumulation of Lipids in Vegetative Biomass. VIET DANG CAO[1], Guangbin Luo[1], Baskaran Kannan[1], Hui Liu[2], John Shanklin[2], Stephen P. Long[3], and Fredy Altpeter[1]. [1]University of Florida, Gainesville, FL; [2]Brookhaven National Laboratory, Upton, NY; and [3]University of Illinois at Urbana-Champaign, Urbana, IL. Email: Presenting author caodangviet@ufl.edu, Corresponding author altpeter@ufl.edu

Metabolic engineering to achieve hyper-accumulation of lipids [e.g. triacylglycerol (TAG)] in the vegetative tissues of high biomass crops is a promising strategy to improve lipid yields for biofuel production. Energycane is an ideal feedstock for this approach due to its superior biomass production and persistence. In this study, a multigene expression construct was used to elevate the production of free fatty acids, catalyze their conversion into TAG and prevent TAG hydrolysis. This construct was transferred into energycane callus, using the biolistic particle delivery system. Presence of transgenes in the regenerated plants were confirmed by PCR. A combination of TLC and GC-MS analysis revealed that the TAG contents in transgenic leaf tissue was elevated more than 18-fold compared to wild-type energycane. Currently, we are propagating the highest TAG producing energycane plants for field trials which are scheduled to begin under USDA-APHIS permit in April 2021 and will allow analysis of lipid and biomass production.

P-2017

Enhanced Salt Tolerance in *CsSOS2* Overexpressing Transgenic Carrizo Citrus Rootstocks. LAMIAA M. MAHMOUD[1,2], Jude W. Grosser[1], and Manjul Dutt[1]. [1]Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850 and [2]Pomology Department, Faculty of Agriculture, Mansoura University, 35516 Mansoura, EGYPT. Email: lamiaa.mahmoud@ufl.edu

The commonly used citrus rootstocks are sensitive to salt and cannot be utilized to grow citrus crops in saline soils. Carrizo citrange, a popular rootstock widely adapted in Florida is highly salt sensitive. To improve salinity tolerance in Carrizo, several transgenic lines overexpressing the *Citrus sinensis* salt overly sensitive 2 gene (*CsSOS2*) under the control of the constitutive d35 promoter were produced. Gene expression analysis identified four transgenic lines with enhanced *CsSOS2* expression in the leaves and roots. All lines where phenotypically similar to wild type control plants. There were significant differences between the transgenic lines and wild type at 100 mM NaCl salt stress. Three lines were observed to be highly tolerant to 100 mM NaCl salt stress based on several evaluation criteria. The tolerant lines had increased chlorophyll *a*, *b* and total chlorophyll compared to wild type. Furthermore, we observed an increase in the relative water content (RWC), proline content and the activity of antioxidant enzymes (*CsPOD1*, *CsPOD2*, *CsAPX2*), and decrease in the electrolyte leakage (EL) percentage and malondialdehyde (MDA) concentrations in these transgenic lines compared to the wild type control. These results suggest that the *CsSOS2* gene plays a vital role in modulating salt tolerance in citrus. Overexpression of this gene can enhance salinity tolerance in Carrizo citrange rootstock and could be a key biotechnological tool to improve the salinity stress tolerance in citrus rootstocks.

P-2018

Intra-genomic Gene Targeting in Maize Using Inducible CRISPR-Cas9. PIERLUIGI BARONE, E. Wu, B. Lenderts, A. Anand, W. Gordon-Kamm, S. Svitashev, and S. Kumar. Corteva Agriscience, Johnston, IA 50131. Email: piero.barone@corteva.com

The use of CRISPR-Cas9 as a tool for targeted mutagenesis and genomic knockouts is now common practice for numerous crops. However, its efficiency for gene targeting (GT) via

homologous recombination (HR) remains still low. HR-mediated GT comprises the generation of DNA double-strand break (DSB) in the genomic target site synchronized with the supply of a donor DNA repair template containing flanking regions of homology. Here, we report an intra-genomic approach in maize using a heat-shock inducible Cas9 expression that results in high-frequency, selectable marker-free GT. Our T-DNA vector contained a single guide RNA, an inducible Cas9 expression cassette, a donor DNA repair template flanked by Cas9 cut sites matching the genomic target site, and morphogenic genes (*Bbm* and *Wus*). In addition, the donor DNA repair template was placed between a promoter and a selectable marker (SM) coding sequence, rendering the SM non-functional. The first step of the process is the generation of the donor locus by random integration of the T-DNA in the genome. Heat-shock induction of Cas9 expression during early stages of maize transformation results in 1) DSB at the genomic target site, 2) the mobilization of the repair template intra-genomically, and 3) activation of the SM upon donor template excision within the donor locus. The expression of the selectable marker gene provides a preferential selection for plant regeneration from cells with released donor template and potential GT. This method generated up to 4.7% targeted insertion of the donor sequence into the target locus in T0 plants. Analysis of T1 progeny confirmed Mendelian inheritance and segregation of the GT-modified target locus.

P-2019

Neither Nutrient Nor Carbohydrate Enrichment of a Liquid Overlay Improved In Vitro Growth or Ex Vitro Survival of Hybrid Almond (*Prunus*) and Pistachio (*Pistacia*) Rootstocks. MIRIAM ESPINOZA and Micah E. Stevens. Sierra Gold Nurseries, Yuba City, CA. Email: miriam@sgtrees.com, micah@sgtrees.com

Significant work has been done to breed the *Prunus* rootstock SG1™ and *Pistacia* rootstock UCB-1 so that they possess superior agronomic traits compared to traditional seedling rootstock, which has led to the demand for hundreds of thousands of each selection every year. Although clonal propagation via rooting hardwood cuttings is possible for SG1™ this option is not available for UCB-1, and so tissue culture provides a powerful tool to supplement the production of these rootstocks. Unfortunately, SG1™ and UCB-1 suffer extensive losses ex vitro during acclimatization. Therefore, we sought to elucidate the role a liquid overlay, a common technique to increase growth, in the vitro propagation of these rootstocks. We tested three carbohydrates at three concentrations with or without nutrients and found the addition of nutrients to during the elongation phase as a liquid overlay did not significantly influence in vitro growth or acclimatization success of either SG1™ or UCB-1. However, carbohydrate type provided in the overlay was shown to have affected ex vitro survival in the absence of supplemental nutrients. UCB-1 had a survival rate of 54% when 22 g/l of sucrose and no nutrients were in the overlay compared to when nutrients were added at this sucrose concentration, 44%. SG1™ when supplemented with sucrose had the highest mean survival, 24.9%, compared to glucose or maltose, 15.5% and 17.6% respectively, but not significantly different than an overlay lacking nutrients and carbohydrates. We also performed a nutrient analysis of tissue two weeks after overlay and there was only a limited effect of carbohydrate type and nutrient presence on nutrient accumulation in SG1™ tissue. Nutrient status of the microshoots was not indicative of ex vitro survival. Our results indicated additional nutrients were seemingly superfluous and provided no in vitro or ex vitro benefit, suggesting the growth response seen as a result of the use of a liquid overlay is from the addition of more carbohydrates.

P-2020

In Vitro Regeneration Systems of Three Elite *Rosa hybrida* Cultivars. DAVIS HARMON[1], Darren Touchell[2], Kedong Da[1], Wusheng Liu[1], and Tom Ranney[2]. [1]Department of Horticultural Science, North Carolina State University, Raleigh, NC and [2]Mountain Crop Improvement Lab, North Carolina State University, Mills River, NC. Email: ddharmo2@ncsu.edu

Rose (*Rosa hybrida* L.) is one of the most economically important horticultural crops. Thousands of cultivars have been developed through conventional breeding for improved disease resistance, unique flowering traits, and diverse commercial applications. Transgenics and precision breeding provide augmented approaches to introduce novel traits for rose improvement. The recovery of modified plants typically depends upon the development of efficient regeneration protocols either through embryogenesis or organogenesis. Due to high heterozygosity, regeneration in rose is highly genotype dependent (Burrell et al., 2006). The goal of this study was to develop regeneration systems for three cultivars, Oso Easy Italian Ice® 'CHEWNICEBELL', Carefree Beauty™ 'Bucbi', and Ringo All Star® 'ChewEyesUp', to provide a platform for future genetic improvement. Regenerative callus induction was explored by culturing leaf explants on MS media supplemented with 2,4-D or 2,4,5-T. For 'Bucbi' and 'CHEWNICEBELL', 2,4-D at 10 μM produced the greatest amount of regenerative calli, while 2,4-D and 2,4,5-T at 5 μM were superior for 'ChewEyesUp'. In an additional study, the effect of carbohydrate source on regenerative callus formation was investigated. Production of regenerative callus for 'CHEWNICEBELL' and 'ChewEyesUp' was greater on sucrose, while glucose was superior for 'Bucbi'. Additional studies are exploring the

effects of cytokinins on the recovery of somatic embryos and shoots from regenerative callus. Initial results suggest a genotype-dependent response influenced by both concentration and source of plant growth regulators (PGR) and carbohydrates. These protocols will provide a platform to expanded opportunities for the genetic enhancement of selected modern roses cultivars.

P-2021

Determination of Total Terpenoid Content in In Vitro Developed Haploid Neem Plants (*Azadirachta indica* A.Juss.). DHILIPHAN MADHAV M C and Rakhi Chaturvedi. Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, INDIA. Email: rakhi_chaturvedi@iitg.ac.in

Neem (*Azadirachta indica* A.Juss.), an evergreen medicinal tree is native to the Indian subcontinent and possess excellent pharmacological and insect repellent properties mainly due to the presence of terpenoids containing complex 5C isoprene as building block. Their bioactivities range from insect antifeedant, antifungal, antibacterial to antipyretic, anti-inflammatory and anticancer activities. In this study, the total terpenoid content was determined from leaves of field acclimatized 5-year-old mature haploid neem plant developed under in vitro conditions from microspores (young pollen grains) in anther cultures in the authors' laboratory. Total terpenoid content was estimated using linalool as standard reagent. In 200 µl methanol extract of leaves, 1.5 ml of chloroform was added. After vortexing the mixture, 100 µl of sulfuric acid was added. The reddish brown precipitate, obtained after incubation of reaction mixture, was utilized after redissolving its small amount in 1.5 mL of methanol (*v*/v) to read the absorbance at 538 nm using spectrophotometer. Total terpenoid content was calculated as linalool equivalents (mg/g) using the regression equation of the Linalool standard curve ($y = 0.004x - 0.0613$, $r^2 = 0.99$). Spectrophotometric determination of total terpenoid content is an screening process, compared to other analytical techniques. The elite haploid plant accummulated the highest amount of terpenoids $275.7 \pm 1.$ (mg leq/g dry weight of leaves)as compared to the parental line (control) that contained 217.9 ± 0.75(mg leq/g) of terpenoids. Hence, it is valued to develop elite neem plants that could be utilized for higher terpenoid production to meet the commercial value.

P-2022

Influence of Bacterial Strains on the Peroxidase Activity of *Sorghum bicolor* (L.) Moench Under Conditions of Moisture Deficit. E. ABDURASHYTOVA, T. Melnichuk, S. Abdurashytov, and A. Egovtseva. FSBSI "Research Institute of Agriculture of Crimea" Simferopol, REPUBLIC OF CRIMEA. Email: elvi-jadore@mail.ru

It is known that an increase in peroxidase activity is an indicator of plant resistance to adverse factors. The response of plants to inoculation with selected microorganisms increases the endurance of *Sorghum bicolor* in conditions of moisture deficit. The study was carried out on the influence of introduced bacterial strains on the adaptation indices of *S. bicolor* plants in the active phase of development - the ear-raising stage. Plant seeds were treated with a bacterial suspension by three strains. There are *Paenibacillus polymyxa* P (plants protection from phytopathogens), *Lelliottia nimipressuralis* 32–3 (growth stimulation and phosphate mobilization), *Agrobacterium tumefaciens* 204 (participates in nitrogen nutrition). Inoculation was carried out with a bacterial suspension (1:100) at the rate of 2% by weight of seeds. As a result of the experiment the indicator of sorghum water deficit was established at high level (21.8–28.9%) in the conditions of 2018 and 2019 and the medium (16.6–17.4%) - in 2020. The activity of peroxidases was determined in leaves by the photocolorimetry method. Two-factor analysis of variance ($p \leq 0.05$) confirmed the effect on the peroxidase activity: strains - 20.4%, year conditions - 60.2%, mutual influence of the year and strains - 13.9%. According to experimental data, the use of bacteria introduced into the plant's rhizosphere contributed to an increase the enzyme activity on 3.9% in 2018, 47.6% in 2019 and 23.2% in 2020. The activity in variant without treatment was 7.7; 8.2; 5.6 µmol oxidized guaiacol / g raw plant tissue per minute in 2018, 2019 and 2020 respectively. The obtained research results confirm the influence of the introduced microorganisms on the seed on the adaptability of *S. bicolor* to the conditions of stress caused by arid conditions.

P-2023

Complementation of Long-Distance Movement with Escherichia Phage MS2 Coat Protein in Plants. T. V. GASANOVA and E. V. Skurat. Lomonosow Moscow State University, Faculty of Biology, Department of Virology, 119234, Moscow, RUSSIA. Email: tv.gasanova@gmail.com

Tobacco mosaic virus (TMV) is the most studied plant virus with single-stranded positive RNA (6395 nt), encoding four proteins, assembled with coat protein molecules (CP) (17,5 kDa). TMV is the appropriate object for creating TMV-based vectors including those intended for the chimeric particles assembly. We replaced the coat protein from prokaryotic virus to the rod-shaped plant virus. In our laboratory, the *Escherichia* phage MS2 coat protein CP MS2 (14 kDa) was cloned into pCambia TMV-based vector with replacement of its own CP and the insertion of CP MS2 gene was checked by sequencing. Then *Agrobacterium tumefaciens* cells strain GV3101 were transformed with the obtained binary plasmid containing viral vector TMVCPMS2 genome

under control of plant transcription promoter for subsequent agroinfiltration of *Nicotiana benthamiana* leaves. On the 10th day post inoculation (d.p.i.) we observed systemic infection, necrotization of petioles of infiltrated and nearby leaves. After 20 days stems of all plants were necrotized. Non-encapsidated viral RNA is not capable to spread through the plant vascular system. In our experiments we observed the complementation of the plant CP TMV function that allow to pack TMV RNA to icosahedral viral particles by CP MS2 even without hairpin operator signal for the assembly of *Escherichia* phage MS2 *in vivo* and evade sieve elements of *N. bethamiana* plants. Previously, we demonstrated that long distance movement occurred only when TMV genome was packed in capsid. These capsids can be rod-shaped, formed with CPs of Barley Stripe Mosaic Virus and Poa Semilatent Virus or icosahedral, formed with CPs (P3) of Potato Leafroll Virus (Pechnikova *et al.*, 2015; Skurat *et al.,* 2017). Coat proteins of other plant viruses such as Tobacco Rattle Virus, Potato Virus A, Potato Virus X, Potato Virus Y, Beet Yellows Virus were unable to pack TMV genome and no long distance movement was observed. Thus for the first time the cross-kingdom complementation between plant virus and bacteriophage coat protein was demonstrated.

P-2024

Cell Selection to Increase Deicing Reagents Resistance. EVGENY ALEKSANDROVICH GLADKOV [1] and Olga Victorovna Gladkova[2]. [1]K. A. Timiryazev Institute of Plant Physiology RAS, IPP RAS and [2]Moscow State University of Environmental Engineering (Moscow State University of Mechanical Engineering), Moscow, RUSSIA. Email: gladkovu@mail.ru

Soil salinization is an important ecological problem for urban ecosystems and agroecosystems. Deicing reagents are the priority pollutants of the city of Moscow. Sodium chloride is one of the main deicing reagents. NaCl is limiting the spread of lawn grass. Therefore it is important to obtain plants resistant to NaCl 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 to develop a cell selection technology for obtaining resistant *Agrostis stolonifera* plants to NaCl. The addition of 1% NaCl caused a 2-fold decrease in callus growth. The survival rate of calli was 68% at 1% NaCl, 56% at 2% NaCl after the first passage. A significant decrease in the regenerative capacity of calli was observed at 2% NaCl. Resistant cells were selected after 1–4 subcultivation of callus on selective medium contained 1% NaCl. 30% of calli survived after two passages on a medium with 1% NaCl, after three passages - 21%, after 4 passages - 16% of calli. Cultivation of calli for 3 and 4 passages leads to a decrease in the regenerative capacity. It is preferable to use 2 passages. Regeneration and root formation were performed on Murashige and Skoog medium containing 1% NaCl too in order to increase the probability of producing of resistant plants. The most of the tested plants produced from NaCl-resistant cells were more tolerant to 1% NaCl than original plants. Most of the studied regenerants retained their decorative qualities at 1% NaCl. The tolerance is remained in next generation. NaCl-tolerant plants showed more resistance to other deicing reagents ($MgCl_2$ and $CaCl_2$). Thus, we have developed a technology for obtaining plants resistant to sodium, magnesium and calcium chlorides.

P-2025

Cell Selection to Increase Cadmium and Copper Resistance. OLGA VICTOROVNA GLADKOVA[1], EVGENY ALEKSANDROVICH GLADKOV[2,3], and Olga Nikolaevna Gladkova[1]. [1]Moscow State University of Environmental Engineering (Moscow State University of Mechanical Engineering), RUSSIA; [2]K. A. Timiryazev Institute of Plant Physiology RAS, RUSSIA; and [3]Moscow Institute of Physics and Technology, Moscow, RUSSIA. Email: olgav.gladkova@mail.ru, gladkovu@mail.ru

Heavy metals are one of the priority pollutants of anthropogenic ecosystems. Copper and cadmium are some of the most toxic metals for urban plants. The object of our study was a lawn grass *Agrostis stolonifera* L. Cadmium and copper are limiting the spread of *Agrostis stolonifera*. The aim of the work was to develop a cell selection technology for obtaining resistant plants of *Agrostis stolonifera* to cadmium. Plants resistant to one environmental factor may be cross-resistant to another. Also the aim of this work was to assess the resistance to copper of the next generation descendants of cadmium - resistance regenerant of *Agrostis stolonifera*. Callus of *Agrostis stolonifera* was obtained from germinated seeds on Murashige-Skoog (MS) medium with 3 mg/l 2,4-dichlorophenoxyacetic acid. To select tolerant clones, *Agrostis stolonifera* calli were cultivated on MS modified medium with 2,4-dichlorophenoxyacetic acid and $CdCl_2$. Cadmium has shown high toxicity *in vitro* to *Agrostis stolonifera*. Most calli died at 30 mg/l cadmium. A concentration of 10 mg/l Cd was selected as selective one for calli. We used a stepwise cell selection scheme. After cultivation for one month, calli were transplanted for further cultivation in MS medium with cadmium. The concentration of cadmium increased at the stage of regeneration to 20 mg/l and rooting to 30 mg/l. We have obtained plants that are resistant to cadmium. Cadmium-tolerant plants showed more resistance to copper compared to the original plants at all the tested concentrations (50–100 mg/l). Copper resistance may be related to possible synthesis of metallothioneins. However, the increase in plant resistance to copper was not very significant. The

tolerance to cadmium is remained in next generation. The descendants of the regenerants showed resistance to 30 mg/l of cadmium.

P-2026

TMV-based Virus Vector Carrying Cucumovirus Coat Protein Gene Forms Stable Infectious Icosahedral Particles in Systemic Leaves of *Nicotiana benthamiana*. T. V. GASANOVA, E. V. Skurat, and P. A. Ivanov. Lomonosov Moscow State University, Faculty of Biology, Department of Virology, Leninskie Gori 1–12, 119234, Moscow, RUSSIA. Email: tv.gasanova@gmail.com

Previously, a new Moscow isolate of the plant cucumovirus Gayfeather mild mottle virus (GfMMV-Mo), belonging to the family *Bromoviridae*, was isolated and characterized in our laboratory. To test the possibility of using GfMMV-Mo in some "green" biotechnology applications, we cloned the coat protein (CP) gene from GfMMV-Mo into a viral vector based on the cDNA copy of Tobacco mosaic virus (TMV), replacing its own CP with the desired one. Then *Agrobacterium tumefaciens* cells were transformed with the obtained binary plasmid containing viral vector $TMVCP_{GfMMV}$ genome under control of plant transcription promoter for subsequent agroinfiltration of *Nicotiana benthamiana* leaves. On the 10th day post inoculation (d.p.i.), symptoms similar to those of GfMMV-Mo were developed: deformation and twisting at the edges of the leaf blade, yellow chlorosis and light clearing of the veins, but $TMVCP_{GfMMV}$ vector did not cause plant growth retardation. Viral particles were isolated from systemic leaves and analyzed in SDS-PAGE. The presence of the coat protein (24 kDa) was confirmed by the Western blotting with antibodies to both GfMMV-Mo and related cucumovirus Tomato aspermy virus (TAV). Electron microscopy revealed icosahedral particles of about 28 nm in size, comparable in diameter to GfMMV-Mo particles, without obvious signs of virion instability and RNA release from the capsid. Reinoculation with either purified particles of the recombinant virus or a plant extract from infected systemic leaves of *N. benthamiana* made it possible to see the development of a viral infection and symptoms similar to those of wild-type infection. Asessment of the assembly of particles from the recombinant CP GfMMV-Mo obtained earlier in *E. coli* (Gasanova *et al.,* 2020) and $TMVCP_{GfMMV}$ showed significant advantages in the efficiency of assembly, the number of virions, and the ease of purification in favor of plant-based preparation. Thus, vector $TMVCP_{GfMMV}$ can be the basis for the creation of genetically and/or chemically modified icosahedral particles or nanocontainers with new technological properties.

P-2027

The Influence of *Bacillus thuringiensis* 0271 and 0371 Strains Cry-proteins on the Biofilm Formation of Bacterial Tomato Cancer Causative Agent. ANASTASIIA KRYZHKO. Research Institute of Agriculture of Crimea, Simferopol, RUSSIA. Email: solanum@ukr.net

B. thuringiensis Δ-endotoxins exhibit antibiotic activity against of some aerobic microorganisms. The simultaneous manifestation of insecticidal and antagonistic *B. thuringiensis* properties significantly enhances the regulatory role of this bacterium in natural ecosystems, as well as increases the bioinsecticides effectiveness. The bacterium *Clavibacter michiganensis* subsp. *michiganensis* is the causative agent of bacterial tomato cancer, a quarantine plant disease in many countries all over the world. Thus, the study of the *B. thuringiensis* var. darmstaiensis 0271 and *B. thuringiensis* var. *thuringiensis* 0371 Δ-endotoxins (cry-proteins) effect on biofilm formation of phytopathogenic bacteria *C. michiganensis* subsp. *michiganensis* 0239 and 0240 strains seems relevant. The analysis of cry proteins effect on the plankton cells and biofilms formation was carried out according to the method of O'Toole G. A. (1998). 200 μl of bacterial culture with $OD_{600} = 0.01–0.02$ density was introduced into the culture plate wells in LB medium containing cry-proteins at 100, 50, 25, 10 and 5 μg / ml concentrations and grown for 48 hours. *B. thuringiensis* 0271 and 0371 contain cry1A proteins. Cry-proteins of the strain 0271 contribute to the suppression of *C. michiganensis* 0240 biofilm growth by an average of 80%, and strain 0371 by 41%, regardless of the tested concentration. Both of *B. thuringiensis* strains did not significantly affect the growth of the biofilm of *C. michiganensis* strain 0239. *B. thuringiensis* 0371 Cry-proteins actively inhibit the growth of plankton culture of *C. michiganensis* 0239 and 0240 strains at a concentration of 10–100 mcg / ml (up to 68–70%). Cry-proteins of the strain *B. thuringiensis* 0271 did not have an impact on the development of *C. michiganensis* plankton culture. Thus, cry1A proteins are able to inhibit the growth of biofilms and plankton culture of *Clavibacter michiganensis* subsp. *michiganensis*.

P-2028

Antibacterial Activity of *Crambe maritima* L. Callus Cultures Against Phytopathogenic Bacteria. IGOR BUGARA[1], Aleksandr Omelchenko[1], Lolita Shvedova[1], Svetlana Zhaldak[1], and Anastasiia Kryzhko[2]. [1]Taurida Academy (structural subdivision) of V. I. Vernadsky Crimean Federal University, Simferopol, RUSSIA and [2]Research Institute of Agriculture of Crimea, Simferopol, RUSSIA. Email: bia.05@mail.ru

Crambe maritima L. is a protected species of Brassicaceae, which grows in the littoral zone. It's leaves and seeds contain potentially antimicrobial glucosinolates and flavonoid glycosides. It is worth studying the antimicrobial activity of plant extracts and callus cultures against phytopathogenic bacteria for the development of biological plant protection products. In our work, we have shown an investigation the *C. maritima* aqueous extracts of leaves and callus cultures effect on the phytopathogenic bacteria *Xanthomonas campestris* growth dynamics during cultivation in 96-well plates. For preparing the aqueous extracts, dried plant material was mixed with distilled water at a ratio of 1:20, boiled for 15 minutes, centrifuged, filtered through a 0.2 μm sterile filter, transferred into sterile tubes and stored in a refrigerator at 4°C. The bacterial culture after 48 hours of cultivation in a liquid nutrient medium, was added into the wells of the culture plate in an amount of 200 μl, mixing with 10 μl, 20 μl, 30 μl and 40 μl of leaf extract or callus culture extract. Callus culture extract was obtained from leaf explants on MS nutrient medium containing 2 mg/L 2,4-D, 0.5 mg/L 6-BAP and 0.5 mg/L kinetin. The growth dynamics of *Xanthomonas campestris* was determined by cultivating the plate for 60 hours at 28°C and measuring the optical density at 620 nm every 25 minutes. As a result, the *Xanthomonas campestris* optical density was 1.00 ± 0.02. In the variants of experiment with the leaf extract concentrations 10 μL, 20 μL, 30 μL, and 40 μL, the optical density values were (1.47 ± 0.02, 1.17 ± 0.01, 1.14 ± 0.005, 0.99 ± 0.004) vs. (0.92 ± 0.008, 0.87 ± 0.04, 0.81 ± 0.002, 0.75 ± 0.002) for callus culture. The leaf extract not only showed no antibacterial activity, but also stimulated the growth of a bacterial culture at concentrations of 10 μl, 20 μl, 30 μl. The callus culture of *C. maritima* showed a significant antibacterial effect at all tested concentrations.

P-2029

Complete Genome Sequence of the *Agrobacterium radiobacter* 204, Obtained Using Oxford Nanopore Technologies Sequencing. YELIZAVETA PUZANOVA[1], Suleiman Abdurashytov[1], Tatiana Aksenova[2], Tatiana Melnichuk[1], Kirill Gritsevich[1], and Aleksandr Pinaev[2]. [1]Laboratory of Molecular Genetics, Proteomics and Bioinformatics in Agriculture FSBSI "Research Institute of Agriculture of Crimea" Simferopol, Republic of Crimea, UKRAINE and [2]FSBSI "All-Russian Research Institute of Agricultural Microbiology" Saint-Petersburg, RUSSIA. Email: liza.puzanova1996@gmail.com

The strain *Agrobacterium radiobacter* 204 is a bioagent of a microbial preparation. It has a stimulating effect on the germination of seeds of various agricultural crops and increases their yield. The purpose of this study was to carry out its whole genome sequencing and study the genetic characteristics of the *A. radiobacter* 204. The strain is deposited in the All-Russian collection of non-pathogenic microorganisms for agricultural purposes ARRIAM and the Crimean collection of microorganisms (USU No. 507484). We used an overnight liquid culture of the microorganism grown at 27 ° C to isolate DNA. DNA was isolated using the GeneGet Genomic DNA kit (ThermoFisher, USA). The gene library of purified DNA was prepared according to the 1D native barcoding genomic DNA protocol recommended by the manufacturer (Oxford Nanopore, GB). Libraries were sequenced on a MinION device according to the manufacturer's instructions on flowcell R9.4. The research was performed using equipment of the Core Centrum 'Genomic Technologies, Proteomics and Cell Biology' in ARRIAM. Sequencing of library yielded a total of Reads N50 / N90: 5238/1216. Reads were assembled in Flye v. 2.6. The *A. radiobacter* 204 genome was assembled into 4 contigs: 3 circular and 1 linear, respectively 3,042,390 bp, 47,124 bp, 11,826 bp and 1,925,541 bp. The G + C range of composition (59.5%) and the total length of the obtained contigs (5,027,044 bp) are comparable to other strains of the same species from the NCBI GenBank. The genome is annotated in RAST (Rapid Annotation Subsystem Technology) under the number: 358.312. The share of identified subsystems was 27%. Revealed 6012 coding sequences, which were combined into 339 subsystems. According to the available subsystems, *A. radiobacter* 204 has 112 groups of genes for protection against stress, 31 groups for resistance to certain heavy metals, and 4 groups of genes responsible for the biosynthesis of plant hormones (auxins). At the moment, it is necessary to recognize most of the genes of the studied strain of the microorganism, which may reveal new features, including interaction with plants.

P-2030

Influence of Genotype on the Micropropagation of Thymus Species *In Vitro*. ARZY TEVFIK, Natalia Yegorova, and Maria Kovalenko. Research Institute of Agriculture of Crimea, Simferopol, Kievskaya str., 150, RUSSIA. Email: tevfik.arzy@yandex.ru

Most species of the genus *Thymus* L. have a wide range of uses due to the high content of biologically active substances. Biotechnological methods of micropropagation make it possible to obtain a healthy plant material, preserve biodiversity by creating genebank *in vitro* and increase the efficiency of breeding process through the accelerated propagation of valuable samples. The aim of the work was to study the influence of the genotype on the explant morphogenesis during clonal micropropagation of different thyme species. When introduced *in vitro* culture the stem segments with a node of *Thymus vulgaris* L. (samples No. 20841 and No. 00003), *T. serpullum* L., *T. tauricus* Klokov et Des.-Shost, *T.* ×

citriodorus (Pers.) Schreb. and *T. caucasica* Willd. were used. It was established that the greatest morphogenetic potential was possessed by *T. tauricus* explants, in which the number of shoots was 5.4–11.7 pcs. Per explant (depending on the culture medium composition). The minimum number of shoots (1.2–2.0 pcs. per explant) was observed in *T. vulgaris* and *T.* × *citriodorus*. The optimal culture medium for introduction *in vitro* of *T. serpullum* was MS with 1 mg/l BAP, for other species – MS with 1 mg/l kinetin and 1 mg/l GA_3. At the 2nd stage of micropropagation adventive shoot formation and microcutting of shoots were used. The culture media for this stage were determined: hormone-free MS medium (*T.* × *citriodorus*), MS with 1 mg/l kinetin (*T. vulgaris*, *T. caucasica* and *T. tauricus*), MS with 1 mg/l kinetin or BAP (*T. serpyllum*). The maximum multiplication index was in *T. tauricus* (16.8) and the lowest in *T.* × *citriodorus* (2.0). Culture media for *in vitro* rooting, which ensured the rooting rate from 84.6% (*T. serpyllum*) up to 100% (*T. vulgaris* and *T. tauricus*) were identified. The conditions for *ex vitro* adaptation allowing to obtain 77.2–89.5% of viable microplants were revealed. The obtained results formed the basis for the development of micropropagation technique for different species of the genus Thymus.

P-2031

Optimization of Conditions for Obtaining of *Origanum vulgare* L. Plant-Regenerants *In Vitro*. OLGA YAKIMOVA, Natalia Yegorova, and Elena Myagkih. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. 295453. Email: oly.yakimova@yandex.ru

Origanum vulgare L. is a valuable essential oil plant widely used in medicine, perfumery, cosmetic and food industries. Therefore, breeding work is being actively carried out to create high-oil and high-yielding varieties of *O. vulgare*. To increase the efficiency of this process, it is advisable to use cell technologies for creating new genotypes with economically valuable traits. Such biotechnological techniques are based on the regeneration of plants in tissue and organ culture. The aim of this work was to study the features of the morphogenesis induction from oregano callus cultures *in vitro*. The influence of the culture medium composition (12 modifications of MS medium), genotype (variety Slavnitsa, hybrid samples h26 and h31) on the calluso- and morphogenesis from oregano bud explants was studied. On the tested modifications of MS medium, the formation of morphogenic and non-morphogenic calli was noted. In this case, the development of buds and shoots occurred from morphogenic primary callus. The maximum frequency of morphogenic primary callus induction in sample h31 (25,0%) was noted on the MS culture medium supplemented with 1.0 mg/l NAA and 0,5 mg/l kinetin, and in the variety Slavnitsa (20,0%) and sample h26 (38,9%) - on MS medium with 1,0 mg/l NAA and 2,0 mg/l TDZ. For callus subculturing, MS medium with 1,0 mg/l NAA and 2,0 mg/l TDZ was used. When non-morphogenic callus was cultivated on this medium, no buds regeneration was observed. Shoot regeneration was noted only from calli with morphogenic structures in subsequent subcultures. The conditions for micropropagation and rooting of shoots *in vitro* (with a frequency of 63,2–100%, depending on the genotype) and *ex vitro* adaptation (with a frequency of 40,0–76.5%) were developed. The obtained regenerants were planted in the nursery of the initial material for further analysis by the complex of economically valuable traits. The conducted research is the basis for the development of biotechnology methods for *in vitro* production of oregano breeding material.

P-2032

Somaclonal Variability of Regenerants from Callus Culture of *Salvia sclarea* L. NATALIA YEGOROVA and Irina Stavtzeva. Research Institute of Agriculture of Crimea, Kievskaya str., 150, Simferopol, RUSSIA. Email: yegorova.na@mail.ru

Clary sage (*Salvia sclarea* L.) is one of the most valuable essential oil plants grown in Russia, France, Tunisia, the USA, etc. Essential oil, sclareol, fatty oil and other valuable products used in medicine, perfumery and food industry are obtained from its plant raw materials. To increase the efficiency of breeding, it is promising to use cell engineering methods, in particular, the induction of somaclonal variability in callus cultures. The aim of this work was to develop a method for obtaining plant-regenerants from calli of *S. sclarea* and their analysis. In studies, we used clary sage cultivars – C-785, Taigan, Ai-Todor, C-1122 and obtained *in vitro* plants. As a result of the research, the peculiarities of the influence of the genotype, culture medium composition, explant type, and passage on the induction of morphogenesis in the callus were established. The culture medium for induction of morphogenesis in sage callus (MS with 1.0 mg/L NAA and 0.5 mg/L BAP) was optimized. The possibility of plant regeneration (with a frequency of up to 32–98%) during 6–10 passages in callus cultures obtained from buds and stem segments was shown. When growing regenerants in the field, we revealed that up to 12.5% of samples compared to the initial cultivar were with deviations in morphology (changes in the shape or color of leaves and inflorescences, and others). For all the quantitative traits studied, the range of variability in plant-regenerants significantly exceeded the variability within a cultivar. The analysis of economically valuable traits of seed progeny derived from calli regenerants (R_1 – R_3) was carried out at the main stages of the breeding process. The promising samples that exceeded the initial cultivars in yield, essential oil

content and drought resistance were identified. Based on a long-term study of regenerants, a new clary sage cultivar Selinzh was created. It exceeded the standard (cultivar Taigan) in inflorescences yield and essential oil harvest by 1.2 and 1.4 times, respectively.

P-2033

Effect of Culture Conditions on Mint Conservation *In Vitro*. MARGARITA ZAGORSKAYA and Natalia Yegorova. Research Institute of Agriculture of Crimea, Simferopol, Kievskaya str., 150, RUSSIA. Email: zagorskayamargo@gmail.com

The availability of a wide range of initial material is an important condition for successful plant breeding. The Research Institute of Agriculture of Crimea has been creating essential oil cultivars of mint for many years, and for this purpose, a field collection of cultivars, interspecific hybrids, polyploids, and breeding samples is maintained. In this regard, it is important to develop an alternative biotechnological method for conservation the genotypes of this valuable essential oil plant. The aim of this work is to study the illumination effect on the development of explants during the mint deposition *in vitro.* The research was based on the Diana cultivar (*Mentha canadensis* L. interline hybrid). Explants (stem segments with one node) were cultivated on MS culture medium with 1.0 mg/L BAP and 0.5 mg/L IAA. The conservation was carried out at 4–6°C without or with the illumination of 0.6 klx. It was found that after a year of deposition, there was the same number of viable explants under both illumination modes (73.3–76.6%). The formation of additional shoots (1.1–1.6 pcs/explant) along with the root formation (78.2–94.4%) was observed. The number of shoots and their length were larger under illumination. However, the frequency of rhizogenesis and the root length were greater when cultivated without illumination. To analyze the regrowth after a year of cold storage, the shoots were cut and the microcuttings were cultivated at 26°C, with the illumination of 2–3 klx with a 16-hour photoperiod. After deposition, the number of developing explants reached 90%, and the number of shoots was 5.1 pcs/explant. Morphometric parameters did not differ significantly or were higher in explants after storage in the dark. The deposition under illumination resulted in up to 56.6% of hyperhydrated shoots. The multiplication index after storage at 0.6 klx was 8.9, and after deposition in the dark - 16.9. The data obtained indicate the mint collection deposition expediency at 4–6°C without illumination. Using the developed method, 30 mint cultivars and samples were placed for long-term storage *in vitro*.