2019 IN VITRO BIOLOGY MEETING ABSTRACT ISSUE

Plant Posters

P-2000

Effect of Light Quality and Intensity on Plant Growth and Essential Oil of *Lippia rotundifolia*. JOSÉ EDUARDO PINTO, Bety S. de Hsie, Ana Izabela S. Bueno, Alexandre A. de Carvalho, and Suzan Kelly V. Bertolucci. Departament of Agronomy, Medicinal Plants, Lavras University, C. P. 3037, Lavras, MG, CEP 37200-000, BRAZIL. Email: jeduardo@dag.ufla.br

Lippia rotundifolia Cham. (Verbenaceae) is an endemic plant of the Cerrado, aromatic, characterized by the presence of glandular trichomes in its leaves, rich in monoterpenes. In vitro propagation has been used in the multiplication of several species with medicinal properties with difficulty in the conventional propagation and to obtain a homogeneous material. Micropropagation is an alternative to production of medicinal plants; however some physics and chemistry factors can affect plantlet growth and its compounds. The light generally used for in vitro propagation is fluorescent lamps. However, these lamps contain very spread wavelengths that are of low quality for growth and development of plantlets. The influence of different light spectra and intensities were evaluated in an in vitro culture and essential oil compounds of L. rotundifolia. The treatments were: a) use of light emitting diode (LED) lamps in the white, red, blue, red/blue, 2red/1blue and 1red/2blue wavelengths, b) photosynthetic photon flux of 20, 54, 78, 88 and 110 μ mol m⁻²s⁻¹. The quality and intensity of light significantly influenced the in vitro growth and compounds of L. rotundifolia. The lowest light intensities (20 and 54 µmol m⁻²s⁻¹) and 2red/1blue presented better results in plant growth. Analysis by HS-GC-MS detected presence of myrcene, limonene and myrcenone in the plantlets developed in different intensity and quality of light. The production of volatiles constituents is highly influenced by the type of growing environment.

P-2001

Unraveling the Biosynthesis of Prenylated Stilbenoids in Peanut and Their Anti-inflammatory Activities In Vitro. ROKIB



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Stilbenoids are a non-flavonoid class of polyphenols that are important for their potential medicinal applications. Resveratrol is one of the most well-studied stilbenoid and several studies have described its anti-inflammatory, antioxidant and anticancer activities. Prenylated stilbenoids, which include arachidin-1 and arachidin-3, are produced to counteract biotic and abiotic stresses in peanut (Arachis hypogaea). Despite their importance to plant and human health, the biosynthesis of prenylated stilbenoids is still poorly understood. To address this issue, we are using the CRISPR/Cas9 gene editing technique to knockout two stilbenoid-specific prenyltransferase genes in peanut hairy roots. Wild type and knock-out hairy root lines of peanut cv. Tifrunner are being developed. HPLC analyses of the wildtype hairy roots treated with elicitors show the presence of prenylated stilbenoids such as arachidin-1, -2, -3, and -5 in the culture medium. Furthermore, the anti-inflammatory activity of the arachidins is being studied using mammalian cell culture. Our preliminary results suggest that the prenylated stilbenoids are not toxic to the cells. These studies will increase our overall understanding of the biosynthetic pathway of arachidins and carry important translational implications of the arachidins as anti-inflammatory compounds.

P-2002

Grafted *Aptenia cordifolia (L. f.)* Schwant Leaves as Perfusable Tissue Engineered Scaffolds. YUEQING WANG¹, Tanja Dominko², Glenn R. Gaudette², and Pamela J. Weathers¹. ¹Department of Biology and Biotechnology, Worcester Polytechnic Institute, MA and ²Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA. Email: ywang24@wpi.edu



that influence HDR efficiency are plant species, type of endonuclease, delivery method and length and type of repair template. In this chapter, the present knowledge of RNP-induced genome modifications in plants with an emphasis on homology directed repair will be summarized.

P-2027

In Vitro Micropropagation and Conservation of a Desert Medicinal Plant, *Lycium shawii* Roem. & Schult. TALAAT AHMED and Walid Kriaa. Environmental Science Center, Qatar University, Doha 2713, QATAR. Email: t.alfattah@qu.edu.qa

Lycium shawii Roem. & Schult.is a desert medicinal plant that is also known as Awsaj or Desert Thorn. It belongs to the family Solanaceae and can be found in the Arabian peninsula as well as some areas in Africa. It is an erect, spreading, intricately branched, very spiny shrub with about 3-meter height. Local people use it as food and medicine. Current study describes a micropropagation protocol of Lycium shawii through tissue culture techniques. Fresh leaves were collected and rinsed using 20% sodium hypochlorite for 15 minutes followed by rinsing in distilled water for three times. Leaf segments were cultured on Murashige and Skoog (MS) medium supplemented with 2,4dichlorophenoxy acetic acid (2,4-D), indole 3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthoxyacetic acid (NOA) with four concentrations (0.5, 1.0, 1.5 and 2.0 mg/ 1) for callus induction . Both 2,4, D and NOA formed the highest callus production where 95.6 and 86.7 % of cultured leaf discs produced viable callus, respectively. The lowest callus induction rates were obtained by IAA and IBA where 79.4 and 68.5 % of cultured leaf segments produced callus, respectively. Calli were transferred to free hormone 0.5 X MS medium for somatic embryogenesis. After three weeks, somatic embryos were obtained and sub-cultured at halfstrength MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP) for shoot regeneration. 0.5 X MS media supplemented with 1 mg/l BAP was the best treatment for multiple shoot induction and produced 12.4 ± 0.15 shoots. For rooting of the obtained shoots, 0.5 X MS medium supplemented with different concentrations of α -naphthalene acetic acid (NAA) was used. The best result was obtained using 0.5X MS medium with 2.0 mg/l NAA, where 90% of the regenerated shoots developed roots with an average of 5.2 roots per shoot within five weeks. The plantlets with healthy root systems were gradually acclimatized in greenhouse using cocopeat. The current established micro-propagation protocol should be useful for conservation as well as mass propagation of this important medicinal plant.



P-2028

Biotization of *In Vitro* Plants with Endophytic Beneficial Bacterium (PGP_invit) to Understand Plant-bacteria Interaction. Nil Türkölmez¹, Merve Albayrak¹, Maria Batool¹, Doğa Selin Kayıhan², Hamit Ekinci¹, Özlem Akkaya¹, Mine Gül Şeker¹, Ceyhun Kayıhan³, Fatma Aydınoğlu¹, and YELDA ÖZDEN ÇIFTÇI¹. ¹Gebze Technical University, Department of Molecular Biology, 41400, Kocaeli, TURKEY; ²Nanobiz Technology Inc, METU, Technopolis, Gallium Block, 06800, Ankara, TURKEY; and ³Başkent University, Department of Molecular Biology and Genetics, Ankara, TURKEY. Email: yelda75@yahoo.com

With the recent advances in DNA sequencing and metagenomic analysis, the importance of microbiome to many aspects of human health together with plant development is highlighted and just starting to be appreciated. Moreover, the advent of these new molecular technologies allows the identification of uncultured endophytic microbes that may have beneficial influence to plant growth. Recently a putatively endophytic beneficial bacterium (PGP invit) was isolated and characterized in the long-term in vitro cultured microshoots of fraser photonia (Photinia × fraseri Dress) (Gül Seker et al., 2017). The aim of this study is not only to confirm the positive influence of PGP invit on in vitro plant development, but also to understand plant-bacteria interaction by using metagenomic and transcriptomics approaches. Our results show that co-culture of this bacterium with shoot tips, leaves, seeds and seedlings of different plant species provided improvement of plant regeneration and development in plant tissue culture conditions. Moreover, as this positive influence of bacterium was detected in both tobacco and fraser photinia, it is not host-dependent. In addition, it produces plant growth regulators (auxin and gibberelic acid) and fixes nitrogen. Hence, these results will provide a deeper understanding of endophytic beneficial bacteria and their contribution to plantbacteria interaction.

P-2029

CRISPR-Cpf1 Editing and Hairy Root Evaluation of Gene Targets to Obtain Low Phytate Soybean. JESSICA CARRIJO¹, Peter LaFayette², Giovanni R. Vianna³, Francisco Aragão^{1,3}, and Wayne Parrott². ¹Department of Molecular Biology, Brasilia, University Brasilia, BRAZIL; ²University of Georgia, Athens, GA; and ³Embrapa, Brasilia, BRAZIL. Email: jessicarrijo@gmail.com

Phytate (InsP6) is the main form of phosphorus in soybean seeds, accounting for more than 85% of the total phosphorus. However, phytate is considered to be an antinutritional factor

because it limits the availability of some essential nutrients. such as iron, zinc, magnesium, and calcium. In addition, monogastric animals, including humans, lack phytases in their digestive tract, so they cannot digest the InsP6 present in seeds. Thus InsP6 decreases the nutritional value of the seeds. Furthermore, as InsP6 is not digestible by monogastrics, it is largely excreted rather than absorbed, leading to phosphorus accumulation in soils and the consequent risk of phosphorus water pollution. Therefore, the generation of low phytic acid (lpa) plants can overcome these limitations. The metabolic pathway responsible for phytate production is the inositol phosphate pathway, which is involved in different regulatory processes during plant development. Therefore the choice of the targets to knock requires caution, as knockouts in some part of the pathway may influence other processes. Candidate genes are being evaluated using a two-step process. First, CRISPR-Cpf1 is being used to knock out inositol phosphate pathway genes in hairy roots and validate the effectiveness of the target sequences being used. Next, stably edited soybean plants will be obtained, and evaluated based on the level of phytate. Two candidate genes were chosen, and targets identified based on their target/off-target scores > 0.6. Vectors were constructed in two different configurations to compare the efficiency of a conventional system, with separate promoters for each component, vs a simplified system with a single promoter for all components. The cassettes are under the control of a Gmubi3 promoter and contain GFP to aid in the detection of the transformed roots. The editing efficiency of the target genes is being evaluated by sequencing.

P-2030

Characterization of Transgenic Tobacco Expressing Archaeal Thioredoxin Reductase B. QUASHAWN CHADWICK, Christopher Cotter, and Ayalew Osena. Department of Biology, University of North Carolina at Greensboro, Greensboro, NC. Email: chadwickquashawn@gmail.com, alosena@uncg.edu

Changes in global climates in the last century have put unprecedented stress on the growth and development of agricultural products. Higher temperatures and longer, more frequent heat waves push agricultural goods, such as corn (*Zea mays*), cotton (*Gossyptium hirstum*), and soybeans (*Glycine max*) beyond their evolutionarily adapted limits. Abiotic stresses such as heat induce oxidative stress in plants. The thioredoxin system is ubiquitous among organisms as a defense mechanism against oxidative stress. In extremophiles, the thioredoxin system has not been well characterized, and their heterologous expression *in planta* has not been attempted, however, some reports suggest that and these enzymes play an important role in extromophile survival. Our research team is currently focusing on improving plant resilience to heat stress by expressing archaeal thioredoxin reductases *in planta*. The overall goal of this project was to improve crop resilience to environmental stressors such as heat. *Pyrococcus furiosus* Trx B was codon optimized and expressed in transgenic tobacco (*Nicotiana benthamiana*) under the control of heat-inducible barley *HSP17* gene promoter using *Agrobacterium*-mediated transformation. We have obtained six transgenic lines which are being evaluated using molecular and biochemical studies. The transgenic lines are expected to be more resilient to higher temperatures and other abiotic stresses. Novel traits demonstrated in model tobacco will be transferred to other economically important crops.

P-2031

Enabling Genome Editing Through the Delivery of Editing Reagents into Soybean Meristem Explants. YURONG CHEN, Dafu Wang, Xudong Ye, and Annie Saltarikos. Bayer Crop Science, Plant Biotechnology, 700 Chesterfield Parkway West, Chesterfield, MO 63017. Email: yurong.chen@bayer.com

Genome editing technologies based on zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALLENs) and clustered regularly interspersed short palindromic repeats (CRISPR-Cas9 or Cpf1) systems have promised to be very powerful tools for gene function analysis and crop improvement. These genome-editing reagents can be delivered to plant cells in the forms of DNAs or ribonucleoproteins (RNPs). One of the critical factors to enable the successful genome editing is the development of efficient and genotype independent delivery systems for these genome-editing reagents. Current status and future opportunities in developing efficient genome editing delivery system in soybean meristems will be presented and discussed.

P-2032

Investigating the Effects of Peroxiredoxin from *Pyrococcus* furiosus on Heat Stress Tolerance in Model Tobacco. CHRISTOPHER COTTER and Ayalew Ligaba-Osena. Department of Biology, University of North Carolina at Greensboro, Greensboro, NC. Email: cjcotter@uncg.edu, alosena@uncg.edu

During times of abiotic stress such as increased heat, light, salinity, or drought, plant cells generate increased concentrations of reactive oxygen species (ROS) which are toxic to the cells. Plants produce antioxidant enzymes, such as peroxiredoxins (Prx). The Prxs may also be implicated in modulating redox signaling during development and adaptation to decrease ROS concentrations, but the capacity of the plant mesophilic Prx may be limited. This research aims to

