ABSTRACT VOLUME

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LECTURES

L13.1

A model system for multiplex carrot genome editing with constitutively expressing Cas9 and Cas12a proteins

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Technologies based on clustered-regularly interspaced short palindromic repeats (CRISPR) and associated proteins enable precise gene editing at desired location in genome. They rely on the introduction of a short RNA and the gene coding for a Cas9 or Cas12a protein of nucleolytic activity into host cell. These guided by RNA proteins target DNA at site homologous to RNA sequence and generate DNA double-strand breaks, which are then repaired by the host mechanism leading to DNA sequence alterations. Unlike other methods, the CRISPR technology is relatively effective, fast and easy for application. This tool can be used in order to improve plants tolerance to unfavorable environmental conditions and in consequence influence on crop yield. However to achieve high performance of genome editing it is crucial to effectively deliver nucleolytic proteins or their genes and guide RNA (gRNA) molecules as well as to design correct gRNAs to prevent off-target modification. Thus, the goal of this work was to develop a model system suitable for comparison of different gRNA in cells where Cas9 or Cas12a are constitutively expressed.
For this purpose, we conducted Agrobacterium-mediated transformation of carrot callus tissues and introduced genes coding for Cas9 and Cas12a variants but not gRNAs. After few months of culture on a selection medium, only well growing callus lines were chosen for further analyses. Transgenic events were confirmed by PCR with pairs of primers specific for hygromycin resistance gene and for regions of each Cas9 or Cas12a gene. Positively verified callus lines were subjected to RT-qPCR to estimate Cas9 or Cas12a expression level.
In consequence, we created a unique set of callus lines with stable expression of Cas9 or Cas12a protein. These lines may serve as a model system for genome editing after delivery of only gRNAs with sequences designed to target desired DNA sites.
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L13.2

Rumex tianschanicus x Rumex patientia an energy plant well adapted to moderate climate conditions

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Nowadays, biofuels are desirable energy sources in the vision of depleting non-renewable energy sources. Rumex tianschanicus x Rumex patientia is a hybrid of the English spinach (R. patientia L.) – maternal plant and sorrel Tien Shan (R. tianschanicus A. Los.) – paternal plant. This plant is characterized by rapid biomass growth and because of that it is used as biofuel (pellets, briquettes), and also has many other applications due to the nutrients and vitamins content. Complete protocol of in vitro micropropagation of hybrid sorrel has been obtained and used during the study. Higher biomass growth in the hybrid is observed than in the parent line. To determine the reason, the physiological studies of the photosynthetic apparatus of parent and hybrid plant lines and the hybrid obtained from in vitro culture were performed during the first year of cultivation in field conditions. The parameters of gas exchange and the degree of hydration were analyzed. The results showed statistically significant differences between the tested plant lines. In the case of the hybrid regenerated in vitro, the lowest gs and E, and the highest Pn and WUE were observed, what is characteristic for plants with high biomass growth. The analyses concerning the water content and photosynthetic pigments are in progress.

L13.3

Allelopathic effects of industrial hemp (Cannabis sativa L.) on antioxidant enzymes activity of soybean seedlings

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Fibre or industrial hemp (Cannabis sativa L.) have been cultivated for thousands of years as a fibre, seed or dual-propose crop. Industrial hemp is currently witnessing a revival as a rich source of secondary metabolites. Several studies showed that hemp could be used in pest control. It has been documented that hemp could suppress weeds, harmful nematodes and soil pathogens and also could have allelopathic effects on some field crops (inhibitory or stimulatory). The aim of this study was to examine the effects of ethanol extracts of industrial hemp on activity of antioxidative enzymes, activity of enzymes of polyphenolic metabolism and intensity of lipid peroxidation in seedlings of soybean (Glycine max (L.) Merr.). This survey was also conducted in order to evaluate the allelopathic activity of hemp extract on content of phenolic compounds and antioxidant capacity of soybean seedlings. In the experiments the 70% ethanol extracts of dried flowering buds of hemp (cv. Helena) was applied in 0.5, 1.0 and 2.0% concentration on seeds of soybean. Ethanol solution (70%) was used as a control. Experiment was performed according to ISTA recommendations (2017). After eight days plants were harvested and activity of antioxidant enzymes (catalase, superoxide dismutase and peroxidase), enzymes of polyphenolic metabolism (phenylalanine

ammonia lyase and polyphenol oxidase) and intensity of lipid peroxidation was measured in phosphate buffer extracts of whole seedlings. Content of total phenolics and tannins and antioxidant capacity was measured in extracts of soybean seedlings with 70% methanol. Extract of hemp decreased activity of phenylalanine ammonia lyasein soybean seedlings and also decreased content of total phenolic and antioxidant capacity. Treatments with ethanol extract of firbe hemp did not affect activities of antioxidative enzymes and did not induce lipid peroxidation in soybean seedlings tissues.
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L13.4

Polish isolate of baculovirus LdMNPV-BNP activate cover infection increasing speed of kill of susceptible forest pest.

Łukasz Rąbalski

A new potential species of baculovirus (LdMNPV-BNP) isolated from a dead gypsy moth caterpillar was found in the Biebrzanski National Park in Poland. Here, we examined its biological activity, structure, genetic content and phylogeny. The ability of this species to kill pest larvae in a relatively short time (LT50 for 2nd instar caterpillars is approximately 9 days for a dose of 2x107 OBs/ml) highlights the possibility for its use as a biopesticide. Next-generation sequencing of LdMNPV-BNP revealed gene content (e.g., photolyase) that is not present in any LdMNPV isolate sequenced to date. We found that when larvae from USA were infected we were not able to detect LdMNPV-BNP in its body. Polish isolate only activate cover virus multiplication which in the end cause better usefulness as biopesticide

POSTERS

P13.1

Biological treatment of toxic waste effluent generated upon hydrogen fermentation of sugar beetroot pulp with the yeast Yarrowia lipolytica.

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Hydrogen is regarded as a pro-ecological fuel and environment-friendly energy carrier which generates much less pollution than the other sources of energy. Due to its high heating value (120 kJ/g) and low density it is highly competitive compared to solar, wind or geothermal energies and even conventional fuels (coal, gasoline). There are several methods of hydrogen production for industrial-scale implementation, namely petrochemical cracking, water electrolysis and splitting, or coal-based processes. These methods, however, often require additional energy input from fossil fuels and have many technological limitations.
Hydrogen can also be generated upon biomass degradation, carried out with thermo-chemical and biological processes. Microbiological bioprocess known as dark fermentation is one of the most efficient and economically viable way of biohydrogen production. Its greatest advantage is the possibility of using organic wastes as substrates. Nevertheless, this method itself generates highly contaminated (mostly by phosphorus and organic matter) post-fermentation liquid, which is environmentally hazardous when discharged without proper treatment involving detoxification, purification, and elimination of eutrophic elements – biogens.
The aim of the study was to characterize the effluent generated upon mesophilic fermentation of sugar beetroot pulp and to evaluate its selected parameters: content of biogens, organic matter and microbiological toxicity. The work then focused on elaboration of effective tools for optimized microbial treatment of the digester supernatant.
The experiments showed that post-fermentation liquid exhibited very high levels of COD (chemical oxygen demand) of 41760 mgO2/L, as well as elevated concentration of (mainly inorganic) phosphorus (1152 mg/L); the latter being 200-times higher than Polish legal norm (5 mg/L). Toxicity tests involving different species of yeasts: Trichosporon sp., Saccharomyces cerevisae, Yarrowia lipolytica, Hansenula polymorpha, and Candida sp. proved strongly toxic properties of the effluent. The only yeast able to survive and proliferate (population density increased 10-times within 7 days of incubation) was Y. lipolytica. Supernatant treatment with this yeast enabled to reduce 73% of COD (to 11 600 mg/L) and 47% of phosphorus content (to 739.26 mg/L). We propose that among the factors responsible for high effluent toxicity are fatty acids or their derivatives that remain in digesters after biohydrogen production. We also conclude that Yarrowia lipolytica, an oleaginous yeast capable of performing unique lipid and fat metabolism, is a suitable candidate with application potential towards biological treatment of recalcitrant wastewaters, especially those generated at conditions of mesophilic dark fermentation.

P13.2

Application of natural plant extracts in reproduction and cryopreservation of bleeding heart

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Lamprocapnos spectabilis (bleeding heart) is valued both on the horticultural and pharmaceutical markets. Despite its great popularity, information on the in vitro tissue culture technology in this species is limited. There is also little knowledge on the application of natural plant extracts in tissue culture of ornamental plants. The aim of this study was to evaluate the utility of plant extracts; i.e. obtained from the coconut pulp, as well as oat, rice and sesame seeds; in micropropagation and cryopreservation of Lamprocapnos spectabilis 'Gold Heart' and 'White Gold'. Modified Murashige and Skoog medium (1962) was fortified with 10% (v/v) plant extracts for a 10-week-long micropropagation cycle via single-node method and during a 7-day preculture in the encapsulation-vitrification cryopreservation protocol. It was found that the addition of plant extracts did not increase the propagation ratio, although rice extract stimulated a more abundant formation of callus in 'White Gold'. Sesame extract, on the other hand, suppressed the development of the explants of both cultivars analysed. The addition of plant extracts into the preculture medium also did not increase the survival rate of the cryopreserved explants (sesame and oat extracts even decreased this parameter), although coconut extract stimulated more intensive proliferation of shoots after rewarming of samples.