ACTA BIOLOGICA CRACOVIENSIA

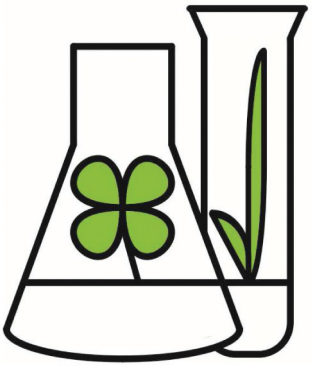
SERIES BOTANICA

Vol. 61 suppl. 1 2019

ABSTRACTS

XI Conference
"*In Vitro* Cultures In Biotechnology and Plant Physiology"

December 4–6, 2019
Kraków, Poland



Polish Academy of Sciences – Krakow Branch
Polish Academy of Arts and Sciences

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

The Official Publication
of the Biological Commission of the Polish Academy of Sciences – Cracow Branch
and the Jagiellonian University, Cracow

DEVOTED TO PLANT ANATOMY, MORPHOLOGY, CYTOLOGY, GENETICS, KARYOLOGY, EMBRYOLOGY, TISSUE CULTURE, PHYSIOLOGY, BIOCHEMISTRY, BIOSYSTEMATICS, MOLECULAR PHYLOGENETICS AND PHYLOGEOGRAPHY, PHYTOCHEMISTRY

ESTABLISHED 1958

The edition of this supplement is financed by the Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences

EDITORIAL NOTE

The abstract texts have been printed as received, and no proofreading or corrections have been made. Thus, the contents of the abstracts are entirely the responsibility of the contributors. In the Index, the names of authors (in alphabetical order) are accompanied by the respective page numbers.

Prepress: Dom Wydawniczy ELIPSA, ul. Inflancka 15/198, 00-189 Warszawa,
tel. 22 635 03 01, e-mail: elipsa@elipsa.pl, www.elipsa.pl

Managing editor: Monika Tuleja
Copy editor: Maria Czereyska-Kościelniak

ACTA BIOLOGICA CRACOVIENSIA Series Botanica on the Internet
The home page of *Acta Biologica Cracoviensia Series Botanica* can be found at
https://abcbot.pl
Webmaster: Piotr Osyczka

The Acta Biologica Cracoviensia Series Botanica is participating in CrossCheck System's users in order to ensure that the content published is original and trustworthy.

Primary version of the journal is printed version

Indexed in *AGRICOLA (National Agricultural Library) AGRIS, AGRO, CABI databases (Agroforestry Abstracts, Botanical Pesticides, CAB Abstracts, Crop Physiology Abstracts, Crop Science Database, Dairy Science Abstracts, Field Crop Abstracts, Forest Products Abstracts, Forestry Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Maize Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Growth Regulator Abstracts, Potato Abstracts, Rice Abstracts, Seed Abstracts, Soils and Fertilizers Abstracts, Soybean Abstracts, Sugar Industry Abstracts, Wheat, Barley and Triticale Abstracts), CNPIEC, EBSCO Discovery Service, Elsevier – SCOPUS, Foodline Science, FSTA – Food Science & Technology Abstracts, Google Scholar, Index Copernicus, J-Gate, Microsoft Academic Search, Naviga (Softweco), Polish Scientific Journals Contents, Primo Central (ExLibris), Summon (Serials Solutions/ProQuest), TDOne (TDNet), Thomson Reuters – Biological Abstracts, Thomson Reuters – BIOSIS Previews, Thomson Reuters – Current Contents/Agriculture, Biology, and Environmental Sciences, Thomson Reuters – Journal Citation Reports/Science Edition, Thomson Reuters – Science Citation Index Expanded, WorldCat (OCLC)*

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

Section Editors

XI Conference
"*In Vitro* Cultures In Biotechnology and Plant Physiology"

December 4-6, 2019
Kraków, Poland

Conference organized by:

Polish Academy of Sciences
The Franciszek Górski Institute of Plant Physiology
Department of Biotechnology

In co-operation with

Pedagogical University in Krakow
Institute of Biology

Conference sponsored by:

Polish Academy of Sciences
The Franciszek Górski Institute of Plant Physiology
www.ifr-pan.edu.pl

Pedagogical University in Krakow
www.up.krakow.pl

BioLike

www.biolike.com.pl

A&A BIOTECHNOLOGY
innovating life science

A&A Biotechnology

Scientific and Organizing Committee

Polish Academy of Sciences
The Franciszek Górski Institute of Plant Physiology
Department of Biotechnology

Zbigniew Miszalski, Director of the Institute
Franciszek Janowiak, Deputy Director for Scientific Research
Izabela Marcińska
Franciszek Dubert
Edyta Skrzypek
Ilona Czyczyło-Mysza
Marzena Warchoł
Kinga Dziurka
Katarzyna Juzoń

Pedagogical University in Krakow
Institute of Biology

Kazimierz Karolczak, Rector of Pedagogical University
Maria Filek, Head of Department of Biochemistry, Biophysics and Biotechnology

Technical assistance team

Polish Academy of Sciences
The Franciszek Górski Institute of Plant Physiology

Jakub Adamski
Piotr Calik
Anna Kramarz
Agnieszka Jarocka-Wadowska
Marta Pilipowicz
Kamila Kapłoniak
Anna Maksymowicz
Agnieszka Janas

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

CONTENTS

Volume 61 suppl. 1, 2019

Oral presentations

Posters

ORAL PRESENTATIONS

Epigenetics adaptations in response to the environmental stresses in plants

Elżbieta Bednarska-Kozakiewicz, Katarzyna Niedojadło

Faculty of Biological and Veterinary Sciences, Department of Cellular and Molecular Biology, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland
e-mail: ebedn@umk.pl

Plants growing in the changing and very heterogenous environments are exposed to many biotic and abiotic stresses. To deal with adverse conditions, plants have envolved epigenetic mechanisms to respond to the environmental factors. DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs are included in the gene regulation and adaptation of plants to the environmental stress. Recent studies indicate that environment-related covalent modifications of DNA and histones can be passed only during mitosis or also during mitosis and meiosis. In the first case epigenetic changes induced by environmental factors are reset in the each generation. This phenomenon is called developmental epigenetic. The example of the developmental epigenetic is vernalization, the process which mediates in the adaptation of plants to the temperature. It is known that the vernalization markers are reset during sexual reproduction, so flowering is dependent on the exposure to cold in each generation. Vernalization involves the epigenetic silencing of floral repressor gene (*FLC)*. In regulation of *FLC* expression are involved especially FRIGIDA protein and histone methylation enzymes (methyltransferases and demethylases). During vernalization in the locus *FLC* a changes in the pattern of histone markers are observed. The cold exposure leads to progressive reduction of H3K4 and H3K36 methylation associated with active chromatin and increase of H3K27 methylation associated with silenced chromatin. It is interesting that DNA in the locus *FLC* is poorly methylated and it does not change during cold period. The vernalization memory persists through *in vitro* culture. Therefore the cells from vernalizing plants can be regenerated into plants that flower without cold period.

Transgenerational inheritance of the epigenetics modifications it is transgenerational epigenetics. There are two factors that allow the plants to have greater potential for heritable epigenetic regulation versus animals. First, the plant germline cells are differentiating from somatic cells and they carry epigenetic markers of sporphyte and second – there is no complete demethylation of DNA during early embryogenesis. The example of environmentally induces transgenerational inheritance in plants is the appearance of new phenotypes (resistant) in the response to external stress such as pathogenesis, salinity, drought. The heritability of epigenetic modyfications that regulate gene expression is based on the changes of DNA methylation. Transposable elements (TEs) and other repetitive DNA sequences, the activation of which leads to the formation of epialleles, play the main role in this process. It is believed that the acquired epigenetic changes are inherited mainly by the mother line and their transmission is usually short-lived and disappears after several generations.

Stringent response in plant – the state of research

Grażyna B. Dąbrowska

Chair of Genetics, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: browsk@umk.pl

Developmental and reproductive success of plants strongly depends on the ability of those organisms to adapt to the diurnal and seasonal changes in their environment. Plant *RSH* (homologs of bacterial RelA/SpoT genes) are nuclei-encoded and function in chloroplasts, where alarmones ((p)ppGpp products) decrease transcription and translation and affect photosynthetic efficiency as well as plant growth and development (Dąbrowska et al., 2006, Boniecka et al., 2017). Guanosine tetra- and pentaphosphates – (p)ppGpp were identified in bacteria as a mechanism that orchestrates pleiotropic adaptations to nutritional deprivation and stress conditions (Dąbrowska et al., 2006a). Research results suggest that one of the mechanisms involved in the plant stress response and processes important for the plant growth and development is homologous to the bacterial stringent response. The plant *RSH* genes have been identified and characterized in a limited number of plant species, for example in *Arabidopsis thaliana*, *Ipomoea nil*, *Brassica napus, Nicotiana tabacum*, *Suaeda japonica, Oryza sativa* and *Capsicuum annuum* (Prusińska et al., 2019). *RSH* genes and alarmons as a signaling molecule play important function in response to environmental factors such as: heat shock, heavy metals, plant hormone treatment, wounding, drought, salinity, oxidative stresses and abrupt changes from light to dark (Prusińska et al., 2019; Szymańska et al., 2019). The expression of *RSH*, encoding enzymes responsible for metabolism of the effectors of the stringent response is downregulated in seeds of low vigour (Boniecka et al., 2019). Whether *RSH* gene expression and alarmons content are changed in plants grown under *in vitro* conditions is still not known.

ACKNOWLEDGEMENTS

The research was financed from the statutory fund of the Nicolaus Copernicus University in Toruń.

REFERENCES

Boniecka J, Kotowicz K, Skrzypek E, Dziurka K, Rewers M, Jedrzejczyk I, Wilmowicz E, Berdychowska J, Dąbrowska GB. 2019. Potential biochemical, genetic and molecular markers of deterioration advancement in seeds of oilseed rape (*Brassica napus* L.). Industrial Crops and Products 130: 478–490.

Dąbrowska G, Prusińska J, Goc A. 2006. Plant mechanism of an adaptive stress response homologous to bacterial stringent response. Postępy Biochemii 52: 94–100.

Dąbrowska G, Prusińska J, Goc A. 2006a. The stringent response bacterial mechanism of an adaptive stress response. Postępy Biochemii 52: 87–93.

Prusińska JM, Boniecka J, Dąbrowska GB, Goc A. 2019. Identification and characterization of the Ipomoea nil RelA/SpoT Homologs (InRSHs) and potential directions of their transcriptional regulation. Plant Science 284: 161-176.

Szymańska S, Dąbrowska GB, Tyburski J, Niedojadło K, Piernik A, Hrynkiewicz K. 2019. Boosting the *Brassica napus* L. tolerance to salinity by the halotolerant strain Pseudomonas stutzeri ISE12. Environmental and Experimental Botany 163: 55-68.

DNA methylation inhibitor (5-azacytidine) modulates ROS generation and antioxidant enzyme responses during androgenesis induction in rye (*Secale cereale* L.)

Ewa Dubas[1,2], Kamil Zieliński[1], Monika Krzewska[1], Ewa Surówka[1], Przemysław Kopeć[1], Sabina Malaga[1], Anna Nowicka[1], Agnieszka Janas[1,2], Iwona Żur[1]

[1] *The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: ifr@ifr-pan.edu.pl*
[2] *Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Gronostajowa 3, 30-387 Krakow, Poland*

Androgenesis is stress-induced sporophyte development from immature pollen grains (microspores). As a result, embryos capable of regenerating completely homozygous doubled haploid (DH) plants are formed. Since the DH production could possibly be the most important source of variability for rye (*Secale cereale* L.) breeding or selection, an effective method of androgenesis induction needs to be investigated. In this connection, an attempt was made to induce embryogenesis in anther cultures by using 5-azacitidine (AC). A positive outcome of DNA demethylation was observed in microspore cultures of triticale after four days of treating tillers with AC which significantly increased androgenic effectiveness (Nowicka et al., 2019).

To broaden our understanding of the mechanism of androgenesis initiation, the impact of the pre-treatment of tillers on reactive oxygen species (ROS) generation and antioxidant enzymatic responses was analyzed in Polish winter rye lines differing in embryogenic potential induced in anther cultures.

Anthers for analysis were sourced from control sample and tillers pre-treated with 5 μM AC for 4 days. Both treatments proceeded at low temperature (3 weeks at 4°C). The antioxidant enzyme responses (e.g. non-specific peroxidases POX, glutathione peroxidase GPX) were determined at the enzymatic activity and gene expression levels.

AC increased the effectiveness of androgenesis induction in the responsive line of rye due to the enhanced ROS production, associated with higher expression of some catalase (*CAT1b*), glutathione reductase (*GR*) and glutathione transferase (*GSTF2*) genes and higher activity of GPX that scavenges the hydrogen peroxide (H_2O_2). In the recalcitrant line, the excess of H_2O_2 increased the expression of *CAT1b*, glutathione peroxidase (*GlutPX2*), *GR* and *GSTF2* genes. Moreover, elevated *GlutPX2* expression coincided with increased enzymatic activity of POX and GPX.

The epigenetic effect of AC depends on the H_2O_2 level regulated by the activity of POX and GPX in anthers. Optimal H_2O_2 concentration in microspores might promote androgenesis induction in recalcitrant lines.

ACKNOWLEDGEMENTS

The research was supported by institutional funding of the Institute of Plant Physiology Polish Academy of Sciences, project no. T1Zb1/2018.

REFERENCES

Nowicka A, Juzoń K, Krzewska M, Dziurka M, Dubas E, Kopeć P, Zieliński K, Żur I. 2019. Chemically-induced DNA de-methylation alters the effectiveness of microspore embryogenesis in triticale. *Plant Science* 287: 110189.

Effect of phytosulfokine and putrescine on cell dedifferentiation in protoplast cultures of some cultivated and wild species

Ewa Grzebelus[1], Aneta Malec[1], Emilia Morańska[2], Tomasz Oleszkiewicz[1]

[1] *Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland, e-mail: e.grzebelus@urk.edu.pl*
[2] *Department of Plant Breeding, Physiology and Seed Science, Faculty of Agriculture and Economics, University of Agriculture in Krakow, Podłużna 3, 30-239 Krakow, Poland*

Protoplast technology with particular emphasis on protoplast fusion can be valuable tool in crop improvement. Nevertheless, application of that technique to breeding programs must be preceded by developing a highly efficient, universal, and dedicated to specific tissue, plant regeneration procedure for a given species. Although there are some known protocols to obtain plants from protoplasts for hundreds of species, especially for a number of cultivated species, the regeneration is occasional or its efficiency is low. Moreover, the protoplasts isolated from different species, or even from different tissues within the same species may have different requirements and, for this reason, the procedures useful in a cultivated species can fairly seldom be applied to its wild relatives, even of the same genus. Various support systems can be used to overcome so called cell recalcitrance status and to stimulate the process of cell dedifferentiation, including selection of an appropriate culture medium in which, in addition to typical plant growth regulators, mainly auxins and cytokinins, other compounds are used.

In the presented studies, the effect of phytosulfokine – peptide signaling molecule and putrescine – a growth regulator from the polyamine group on mitotic activity and plant regeneration in protoplast cultures of some cultivated and wild species will be discussed.

The results indicate that each of the compounds used, alone or in combination, can improve protoplast dedifferentiation and plant regeneration capacity of some studied species.

Cyathea delgadii as a model in the study of symplasmic communication during somatic embryogenesis

Małgorzata Grzyb[1], Justyna Wróbel-Marek[2], Ewa Kurczyńska[2], Anna Mikuła[1]

[1] *Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: m.grzyb@obpan.pl*
[2] *Department of Cell Biology, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland*

Somatic embryogenesis (SE) is one of the most widely analyzed methods of plants *in vitro* propagation. Although, until 2015 this regeneration path was not available to any Monilophyta representative. Using the tree fern *Cyathea delgadii* Sternb., we established an effective system of SE in which embryos can be induced directly from explants cultured on hormone-free medium (Mikuła et al., 2015). Depending on the type of initial explant used, the base of somatic embryo formation are the divisions of single epidermal cell or a group of neighboring cells including epidermis and cortex. Moreover, the path of embryo formation can be reversed from multi- to unicellular origin, by stress treatment with high sucrose solution (Grzyb and Mikuła, 2019). The unique course of somatic embryo formation in

C. delgadii allows for investigating these aspects of SE that were not available so far. Among them, the role of symplasmic communication, which is still poorly understood during embryogenic transition and explored only in the group of seed plants. Thus, the aim of the study was to analyze the correlation between changes in symplasmic communication and cell differentiation during SE of *C. delgadii*.

The research was carried out using etiolated sporophytes of *C. delgadii* as a source of initial explants (stipe and internode), that giving embryos of single- and multicellular origin, respectively. To show the dynamics of changes in symplasmic communication the low-molecular weight fluorochromes of symplasmic transport, such as 8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS) and fluorescein diacetate (FDA), were used. The confocal microscopy analyzes were performed on initial explants, and explants before and after embryogenic divisions.

Our studies showed that cell differentiation at different stages of SE in *C. delgadii* depend on the symplasmic continuity within explants. Both, the embryogenic transition and somatic embryo formation were preceded by the changes in the fluorochromes flow within the explant and the cells of embryo. The differences in symplasmic connection between SE induced by uni- and multicellular path will be discussed.

ACKNOWLEDGEMENTS

This research was supported by the Polish National Center for Science, no. 2017/27/N/NZ3/00434.

REFERENCES

Grzyb M, and Mikuła A. 2019. Explant type and stress treatment determine the uni- and multicellular origin of somatic embryos in the tree fern *Cyathea delgadii* Sternb. *Plant Cell, Tissue and Organ Culture* 136: 221–230.

Mikuła A, Pożoga M, Tomiczak K, Rybczyński JJ. 2015. Somatic embryogenesis in ferns: a new experimental system. *Plant Cell Reports* 34: 783–794.

3-D nucleus architecture in mono-, di-, and tetrasomic oat-maize addition lines

Dominika Idziak-Helmcke[1], Tomasz Warzecha[2], Edyta Skrzypek[3], Marta Sowa[1], Marzena Warchoł[3], Kinga Dziurka[3], Ilona Czyczyło-Mysza[3]

[1] *Institute of Biology, Biotechnology, and Environmental Sciences, University of Silesia in Katowice, Jagiellońska 28, 40-032 Katowice, Poland e-mail: dominika.helmcke@us.edu.pl*
[2] *Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, Podłużna 3, 30-239 Krakow, Poland*
[3] *Department of Biotechnology, Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Krakow, Poland*

It is widely believed that the internal architecture of the cell nucleus greatly impacts its functional response to the current needs of a cell. However, the mechanisms that link nucleus spatial organisation to its functionality remain unclear. Particularly, little is known about the 3-D arrangement of parental subgenomes in natural and artificial hybrid plants and its impact on intergenomic interactions.

To address this important topic, we applied a molecular cytogenetic approach to study nucleus architecture in oat-maize addition (OMA) lines. Oat and maize are among the most distantly related plant species that could be sexually hybridized and could produce stable fertile partial hybrids. In the wide crosses between oat and maize, a uniparental elimination of maize chromosomes results in euhaploid oat plants or, occasionally, aneuhaploid plants with one or more maize chromosomes added to the oat genomic background.

We have recently developed a series of novel, fertile OMA lines, with the added chromosome numbers varying from one to four. Using fluorescence in situ hybridisation with various probes, confocal laser scanning microscopy, and state-of-the-art image analysis to perform 3-D reconstruction of nuclei, we aim to address following questions: (1) is there a referential positioning of the alien chromosomes in the nuclei of the recipient? (2) what are the factors that determine the position of the alien chromosomes? and (3) whether and how the different number of added chromosomes affects the spatial distribution of specific chromosome domains in the recipient's nuclei.

ACKNOWLEDGEMENTS

Project supported by the National Science Centre Poland, grant no. 2018/02/X/NZ3/01545

Changes in hormonal profiles accompanying the induction of triticale microspore embryogenesis

Katarzyna Juzoń[1], Anna Nowicka[1,2], Lenka Plačková[3], Karel Doležal[3], Iwona Żur[1]

[1] *The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: k.juzon@ifr-pan.edu.pl; i.zur@ifr-pan.edu.pl*
[2] *Institute of Experimental Botany of the Czech Academy of Sciences v.v.i., Šlechtitelů 31, 78371 Olomouc, Czech Republic*
[3] *Laboratory of Growth Regulators and Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany AS CR and Faculty of Science of Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

One of the most deeply studied aspects of microspore embryogenesis (ME) is the role of plant growth regulators (PGRs). Among them, the most important role is played by auxins (Auxs) and cytokinins (CKs). In order to gain a better understanding of the mechanism controlling ME in triticale (x *Triticosecale* Wittm.), changes in endogenous level of Auxs and CKs associated with ME induction were analyzed.

Plant material consisted of two doubled haploid (DH) lines of winter triticale: DH19 and DH28 of low and high embryogenic potential, respectively. Beyond the standard cold tillers treatment (3 weeks at 4°C), the effect of exogenously applied PGRs (2,4-D and melatonin (MEL)) as well as PGR inhibitor (p-chlorophenoxyisobutyric acid (PCIB)) was also tested. Determination of endogenous hormones was performed using protocols by Pěnčík et al. (2018) and Svačinová et al. (2012) as described for Auxs and CKs, respectively.

After standard ME-inducing treatment, seven CKs and four Auxs were identified in triticale microspores. Microspores of DH28 were characterized by higher content of indole-3-acetic acid (IAA) and its derivatives, *cis*-zeatin ryboside (cZR), *cis*-zeatin-*O*-glucoside (cZOG) and isopentenyladenosine (iPR) in comparison with DH19. Moreover, only in DH28 the presence of 2-oxindole-3-acetic acid (oxIAA) and 6-benzylaminopurine (BAP) were detected. PCIB and MEL treatments increased the level of cZ and decreased the level of IAA, what was associated with increased number of green regenerated plants by ap. 35% and 40%, respectively. The obtained results suggest that effective ME induction and plant regeneration require a certain but various balance of PGRs.

ACKNOWLEDGEMENTS

The work was supported by International Visegrad Scholarship within International Visegrad Fund, application no: 51700770.

REFERENCES

Pěnčík A, Casanova-Sáez R, Pilařová V, et al. 2018. Ultra-rapid auxin metabolite profiling for high-throughput mutant screening in *Arabidopsis*. *Journal of Experimental Botany* 69: 2569–2579.

Svačinová J, Novák O, Plačková L, et al. 2012. A new approach for cytokinin isolation from *Arabidopsis* tissues using miniaturized purification: pipette tip solid-phase extraction. *Plant Methods* 8:17.

In vitro cultures of *Nasturtium officinale* as a valuable source of secondary metabolites

Marta Klimek-Szczykutowicz[1], Agnieszka Szopa[1], Michał Dziurka[2], Halina Ekiert[1]

[1] *Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: marta.klimek-szczykutowicz@doctoral.uj.edu.pl*
[2] *Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland*

Nasturtium officinale R. Br. is an aquatic, partially protected in Poland perennial plant species. Its herb is the valuable medicinal, cosmetic and culinary raw material. It possesses scientific proven activities e.g. antioxidant, hepatoprotective and anticancer, conditioned by a rich chemical composition, e.g. glucosinolates (Gls), phenolic acids (PhA) and flavonoids (Klimek-Szczykutowicz et al., 2018).

The aim of this work was the optimization of conditions for microshoot cultures of *N. officinale*. Different types of cultures (agar, agitated and RITA bioreactors) were studied. The MS medium variants supplemented with PGRs: cytokinins – BA, 2iP, KIN, Zea, and auxins – IAA, IBA, 2,4-D, IPA, NAA were tested. Cytokinins and auxins were tested at concentrations 1 mg/l each. The tested culture growth periods lased: 10, 20, 30-days for agar, and 10, 20-days for agitated and bioreactors cultures (3 series). Gls and PhA production, and antioxidant activities were evaluated. The contents of Gls and PhA in methanolic extracts were determined spectrophotometrically and by HPLC-DAD. The antioxidant activity was measured using the Folin-Ciocalteu (FC), CUPRAC, FRAP and DPPH methods (Klimek-Szczykutowicz et al., 2019).

The highest total Gls production (195.92 mg/100g DW) was confirmed for the bioreactors cultures (20 days on MS medium with BA and NAA). Out of 27 PhA analyzed, ten were identified: caffeic, o- and p-coumaric, ellagic, ferulic, gallic, isoferulic, protocatechuic, rosmarinic and syringic. The highest total PhA content (236.74 mg/100g DW) and the highest total polyphenol content measured by FC method (8.69 mmol trolox/100g DW) was obtained for agitated microshoots grown for 10 days on MS medium with Zea and NAA. The highest antioxidant potential for CUPRAC and FRAP methods was confirmed for agitated cultures grown for 20 days on MS medium with KIN and IAA, respectively 5.26 and 1.26 mmol trolox/100g DW. For DPPH, the highest potential was recorded for agar cultures grown for 30 days on MS medium with KIN and IAA (30.89 mmol trolox/100g DW).

REFERENCES

Klimek-Szczykutowicz M, Szopa A, Ekiert H. 2018. Chemical composition, traditional and professional use in medicine, application in environmental protection, position in food and cosmetics industries, and biotechnological studies of *Nasturtium officinale* (watercress) – a review. *Fitoterapia* 129: 283–292.

Klimek-Szczykutowicz M, Szopa A, Blicharska E, Dziurka M, Komsta Ł, Ekiert H. 2019. Bioaccumulation of selected macro- and microelements and their impact on antioxidant properties and accumulation of glucosinolates and phenolic acids in *in vitro* cultures of *Nasturtium officinale* (watercress) microshoots. *Food Chemistry* 300: 125184.

The use of *in vitro* cultures in the protection of rare species of the family Ranunculaceae on the example of *Aconitum bucovinense* Zapał. and *Ranunculus illyricus* L.

Dawid Kocot, Barbara Nowak, Ewa Sitek

Department of Botany, Physiology and Plant Protection, University of Agriculture in Krakow, Al. 29-Listopada 54, 31-425 Krakow, Poland, e-mail: dawid_2608@vp.pl

Currently observed climate change and human activity contribute to mass extinction of species. *In vitro* techniques of micropropagation of plants are very helpful in ex situ conservation of endangered species. In Poland, many species from the Ranunculaceae family are subject to legal protection. Studies conducted on two representatives of this family – *Aconitum bucovinense* Zapał. and *Ranunculus illyricus* L. – have shown that *in vitro* regenerated plants can be obtained by indirect organogenesis.

Due to seed germination problems of the studied species, explants from vegetative organs were used to initiate *in vitro* cultures. After surface decontamination, the explants were places on medium with the addition of kinetin and picloram, on which callus tissue was obtained. Callus of *Aconitum bucovinense* was cultivated in the darkness on media supplemented with BAP and IBA, on which shoots also differentiated. After isolation and transfer to a medium with increased content of growth regulators and the addition of activated carbon they spontaneously rooted. Callus of *Ranunculus illyricus* was also differentiated on media supplemented with BAP and IBA, where rooted shoots appeared. The rooted shoots formed in this way were acclimated.

Plant material obtained *in vitro* could be used in active conservation activities, e.g. augmentation of existing population or creating of the secondary localities. Somaclonal variation of micropropagated plants can be observed as a consequence of cultivation conditions, particularly if indirect organogenesis is the way of regeneration. For this reason, *in vitro*-origin plants should be subjected to a detailed assessment, and usually they create collections in botanical gardens or are alternative plant material for industry. Despite somaclonal variation, it was speculated recently, if it would not be beneficial for a population with limited variability and restricted reproduction to be supplemented with plants from tissue culture.

Both, *A. bucovinense* and *R. illirycus*, are critically endangered species in Poland, and their national populations are very limited. The obtained plants will enable further studies on biology of species, maintaining plants in collections in botanical gardens or, in the long run, creating secondary localities.

Plant culture media enrichment with biosynthetic precursors as an effective method for increasing accumulation of phenolic acids in *in vitro* cultures of the aronia species

Paweł Kubica, Agnieszka Szopa, Aleksandra Walkowicz-Bożek, Augustyn Pietrzak, Halina Ekiert

Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Medyczna 9, 30-688 Cracow, Poland, e-mail: p.kubic@uj.edu.pl

Phenolic acids are very important group of plant secondary metabolites with many valuable activities important for human health.

Earlier studies from our Department confirmed high production of phenolic acids in *in vitro* cultures of *Aronia melanocarpa* (black aronia), *A. arbutifolia* (red aronia) and *A.* × *prunifolia* (purple aronia) (Szopa and Ekiert, 2014, Szopa et al., 2018).

The aim of the present studies was the investigation on influence of egzogenic biosynthetic precursors on the production of phenolic acids in agitated cultures of above- mentioned three aronias.

The cultures were maintained for 20 days (3 series) on Murashige & Skoog medium (BAP – 1 mg/l, NAA – 1 mg/l), without and with addition of phenylalanine, cinnamic acid, benzoic acid and caffeic acid (0,1; 0,5; 1; 5; 10 mM).

In the methanolic extracts from biomasses the quantitative analysis of 22 phenolic acids was performed using HPLC method (Ellnain-Wojtaszek, 1999).

The presence of seven compounds was confirmed. The maximal total content of phenolic acids ranged (depending on type and concentration of precursor) in *A. melanocarpa* from 503 to 855 mg%, in *A. arbutifolia* from 350 to 1098 mg% and in *A.* × *prunifolia* from 387 to 603 mg%. The highest production of phenolic acids in *A. melanocarpa* and *A.* × *prunifolia* was obtained after addition of 5 mM of cinnamic acid and for *A. arbutifolia* after addition of 5 mM of caffeic acid.

The results documented that the precursor feeding of culture media with biosynthetic precursors of phenolic acids could be an efficient way to increase production of phenolic acids in *in vitro* cultures of aronia species.

REFERENCES

Szopa A, and Ekiert H. 2014. Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott *in vitro* cultures cultivated on different variant of the Murashige and Skoog medium. *Plant Growth Regulation* 72: 51–58.

Szopa A, Kubica P, Snoch A, Ekiert H. 2018. High production of bioactive depsides in shoot and callus cultures of *Aronia arbutifolia* and *Aronia* × *prunifolia*. *Acta Physiologiae Plantarum* 40(3): 1–11.

Ellnain-Wojtaszek M, Zgórka G. 1999. High-performance liquid chromatography and thin-layer chromatography of phenolic acids from *Ginkgo biloba* L. leaves collected within vegetative period. *Journal of Liquid Chromatography and Related Technologies* 22: 1457–1471.

Influence of plant growth regulators on the *in vitro* development and biochemical activity of bleeding heart

Dariusz Kulus

Laboratory of Ornamental Plants and Vegetable Crops, UTP University of Science and Technology, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail:dkulus@gmail.com

Lamprocapnos spectabilis (L.) Fukuhara (bleeding heart) is a popular horticultural and medicinal plant species, originating from Asia. Despite its significance on the market, there is little information on the *in vitro* tissue culture systems in the species. The aim of this study was to analyse for the first time the influence of PGRs (plant growth regulators) on the development, quality and physiological state of *in-vitro*-grown *Lamprocapnos spectabilis*. For this purpose, the single-node explants were inoculated on the MS medium (Murashige and Skoog, 1962) fortified with different auxins; i.e. IAA (indole-3-acetic acid), NAA (1-naphthaleneacetic acid), PIC (picloram); and cytokinins; i.e. BA (6-benzyladenine), KIN (kinetin), TDZ (thidiazuron); at various concentrations. It was found that the morphogenetic response of the explants was cultivar-specific. KIN was preferable for the proliferation and development of shoots in 'Gold Heart'. On the other hand, none of the auxins or cytokinins improved the development of 'White Gold' explants compared with the PGRs-free control medium. NAA was most effective in stimulating rhizogenesis in both cultivars, although IAA favored regeneration of the longest roots. TDZ, NAA and PIC suppressed the development of shoots in both cultivars tested, stimulating abundant callus formation instead. Indirect regeneration of somatic embryos was found on the NAA- and PIC-fortified media. Especially the latter one stimulated regeneration of the highest number of somatic embryos per one inoculated nodal segment (up to 12), regardless of its concentration. Composition of the culture medium also affected the content of primary and secondary metabolites in shoots and calli of *L. spectabilis*. NAA (at 1.0 $mg \cdot dm^{-3}$) stimulated the synthesis of chlorophyll *a* and carotenoids in 'God Heart', while BA and KIN (at 0.5 $mg \cdot dm^{-3}$) had a negative impact on the concentration of chlorophyll *b* in the shoots of this cultivar. None of the PGRs increased the content of the analyzed pigments in the shoots of bleeding heart 'White Gold'. The concentration of chlorophylls and carotenoids in the callus of both cultivars tested was significantly lower compared to the shoots, however, it was abundant in flavanols. This study provided some basic knowledge on the *in vitro* development and physiological activity of bleeding heart cultivated in media with various PGRs, which can be useful in the *in vitro* reproduction of bleeding heart on a commercial scale. Further studies should focus on the synergistic effect of auxins and cytokinins on the *in vitro* development of bleeding heart.

ACKNOWLEDGEMENTS

The Author wishes to acknowledge Mr. Cihat Değirmenci for technical support in performing this research.

REFERENCES

Murashige T, and Skoog S. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Application of molecular markers (RAPD, ISSR) and High Performance Liquid Chromatography (HPLC) in the analysis of genetic stability and secondary metabolites content in *Echinacea purpurea* (L.) Moench. plants selected via somatic embryogenesis

Justyna Lema-Rumińska[1], Dariusz Kulus[1], Alicja Tymoszuk[1], Jorge M.T.B. Varejão[2], Kiril Bahcevandziev[2]

[1] *UTP University of Science and Technology, Faculty of Agriculture and Biotechnology, Laboratory of Ornamental Plants and Vegetable Crops, Bernardyńska 6, 85-029 Bydgoszcz, Poland e-mail: lem-rum@utp.edu.pl*

[2] *Polytechnic Institute of Coimbra, Agricultural College, IIA – Institute of Applied Research, CERNAS, Research Centre for Natural Resources, Environment and Society, 3000-316 Coimbra, Portugal, e-mail: jvarejao@esac.pt*

Purple coneflower (*Echinacea purpurea* (L.) Moench), belonging to the Asteraceae botanical family, is a species of high medicinal and ornamental value, and for these reasons, appreciated all over the world. Nowadays, there is a lack of standardized plant material with increased content of important components in purple coneflower. Selected lines of *E. purpurea* with high somatic embryogenesis (SE) capacity and with a higher content of valuable secondary metabolites can be desirable for the pharmaceutical and horticultural industries. The aim of this study was to analyze new lines of *E. purpurea*, selected and micropropagated via indirect somatic embryogenesis, on the molecular and biochemical levels. Genetic stability (within the line) and genetic diversity (between the lines) of the produced plants was studied with molecular markers: Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR). A higher mean polymorphism rate (>90%) was found with the RAPD technique, with a total of 1427 scorable bands produced (142.7 products per one primer). Unlike the RAPD analysis, ISSRs detected mostly monomorphic *loci* (63.4%), followed by polymorphic ones (36.6%), while there were no specific *loci* present. Cluster analysis of both marker systems showed that the tested specimens were grouped according to their respective lines. The main phenolic acids relative percentage composition was determined by the High Performance Liquid Chromatography (HPLC) method. Significant differences were found in the content of individual phenolic acids in the tested plant material. Among six selected lines of *E. purpurea*, three yielded higher cichoric acid content. The research has shown that the obtained lines can provide an enhanced content of secondary metabolites in purple coneflower. The present study confirmed also the applicability of SE in the selection and reproduction of genetically uniform *E. purpurea* plant material.

ACKNOWLEDGEMENTS

The research has been performed through the Polish-Portugal scientific cooperation between the UTP University of Science and Technology in Bydgoszcz, Poland and the Agricultural College, CERNAS, Polytechnic Institute of Coimbra, Portugal. The authors wish to acknowledge Magdalena Hajzer, Robert Nelke, Tajah Lewter, and Dominika Rymarz for their technical assistance in conducting the experiments.

New insights into somatic embryogenesis by *Cyathea delgadii* model system

Anna Mikuła, Małgorzata Grzyb, Wojciech Tomaszewicz, Jan J. Rybczyński

Conservation Biotechnology Research Group, Polish Academy of Sciences Botanical Garden – Center for Biological Diversity in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: a.mikula@obpan.pl

A clonal propagation process via somatic embryogenesis (SE), in which embryos are derived from somatic (non-sexual) cells of plant body under controlled *in vitro* culture conditions, has great potential for large scale production of different plants worldwide. For their commercial multiplication, the process offers opportunities to scale up for bulk production of desirable genotypes by using liquid culture medium and temporary immersion bioreactors. Moreover, the embryogenic tissue can be cryostored for many years, allowing long-term testing of clones in breeding programs. These systems currently provide the required conditions for both multiplication and maturation of somatic embryos, and also conservation of many economically important herbaceous and woody plants belonging to Spermatophyta. A few years ago, the first protocol of SE for fern production has also been developed (Mikuła et al., 2015). The discovery opens the way for both an effective fern micropropagation and research on the initiation of plant SE. Ferns, as sister to seed plants, can be important to understand evolutionary innovations specific to plant development and morphogenetic processes.

It is worth emphasizing that knowledge regarding the mechanism controlling the induction of the SE process is still limited. An experimental system that has been described for the tree fern *Cyathea delgadii* appears to be appropriate biotechnological approach in the study of some fundamental problems relating to somatic embryo initiation and development (Mikuła et al., 2018). In this system, the initiation of SE can occur independently or simultaneously along unicellular and multicellular pathways (Grzyb and Mikuła, 2019). Moreover, the somatic embryos are induced and produced on hormone-free medium, in a short time, and with high multiplication and replication rate. Insight into plant SE by this experimental system, and its structural and physiological circumstances will be presented during the lecture.

REFERENCES

Mikuła A, Pożoga M, Tomiczak K, Rybczyński JJ. 2015. Somatic embryogenesis in ferns: a new experimental system. *Plant Cell Reports* 34: 783–794.

Grzyb M, and Mikuła A. 2019. Explant type and stress treatment determine the uni- and multicellular origin of somatic embryos in the tree fern *Cyathea delgadii* Sternb. *Plant Cell Tissue and Organ Culture* 136: 221–230.

Mikuła A, Grzyb M, Tomiczak K, Rybczyński JJ. 2018. Experimental and practical application of fern somatic embryogenesis. In: Fernández H. (eds.) *Current Advances in Fern Research. Springer, Cham* pp 121–137.

In vitro regeneration from X-rays treated chrysanthemum ovaries

Natalia Miler

Laboratory of Ornamental Plants and Vegetable Crops, Faculty of Agriculture and Biotechnology, UTP University of Science and Technology, ul. Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: nmiler@utp.edu.pl

Chrysanthemum (*Chrysanthemum* × *grandiflorum* /Ramat./ Kitam.) ovaries are tiny, approximately 1 mm long, with a single anatropous ovule, located at the bottom of the ligulate and tubular florets. Isolated ovaries are commonly used in gynogenesis, however, in chrysanthemum they also served as a source of off-type shoots useful in breeding (Miler and Jędrzejczyk, 2018). The aim of this study was to evaluate the regeneration effect of X-ray treated ovaries for further application of chrysanthemum ovaries in mutation breeding programs.

In the experiments, two chrysanthemum cultivars bred in UTP University of Technology and Science, Bydgoszcz: 'Profesor Jerzy' and 'Karolina' were used. Whole inflorescences at the stage of full-flowering were cut off and put into the jar filled with distilled water. Pedicels were loaded with a pieces of plastic clay to keep the receptacles at the level of 2 cm under the water surface. A stream of photons (i.e. X-radiation) with an energy of 6 MeV was directed to the ovaries collected in the bottom of inflorescences, the dose rate was 3.18 Gy/min, doses of 5, 10 and 15 Gy were applied. Irradiation was carried out at the Bydgoszcz Oncology Center using the Vitalbeam particle accelerator. The control inflorescences were not treated with

X-rays. After the treatment with the mutagenic agent, the irradiated, as well as control florets were detached from the receptacle and disinfected, followed by the precise dissection of ovaries. Isolated explants (ovaries) were placed in Petri dishes on MS induction medium supplemented with 1 mg/L BAP and 1 mg/L 2,4-D, and then placed in a growth room. After 12 weeks, explants with the regenerated callus were transferred to MS regeneration medium supplemented with 2 mg/L kinetin, 1 mg/L IAA and 4 mg/L glycine. After another 12 weeks, regenerated shoots were counted and subcultured onto MS medium for further cultivation.

There were relevant differences in the ability of regeneration of shoots and callus from ovaries between cultivars. The callus diameter measured after the induction stage in 'Karolina' was 2 mm bigger than in 'Profesor Jerzy' (8.7 and 6,8 mm, respectively). In contrast, 'Karolina' ovaries produced only 5 shoots totally (two of them from the control explants), while 'Profesor Jerzy' produced 373 shoots. The number of regenerated shoots from 'Profesor Jerzy' decreased with the increase in the X-ray dose. The average number of regenerated shoots from control ovaries was 2.24, while in X-ray treated ovaries there were 1.78, 1.08 and 0.76 shoots per one explant for 5, 10 and 15 Gy, respectively. The apogeum of shoots regeneration in 15 Gy treated explants was delayed for 6 weeks in comparison with control explants.

REFERENCES

Miler N, and Jędrzejczyk I. 2018. Chrysanthemum plants regenerated from ovaries: a study on genetic and phenotypic variation. *Turkish Journal of Botany* 42: 289–297.

Two faces of DNA methylation inhibitors

Anna Nowicka[1,2], Barbara Tokarz[3], Katrzyna Juzoń[1], Monika Krzewska[1], Ewa Dubas[1], Iwona Żur[1], Ales Pecinka[2]

[1] *The Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland, email: a.nowicka@ifr-pan.edu.pl*
[2] *The Czech Academy of Sciences, Institute of Experimental Botany (IEB), Centre of the Region Haná for Biotechnological and Agricultural Research, 779 00 Olomouc, Czech Republic*
[3] *Unit of Botany and Plant Physiology, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland*

DNA methylation relies on the addition of a CH_3 group at the 5C position of deoxycytidine residues and is enzymatically established by DNA methyltransferases. Three functional DNA methylation sequence contexts: CG, CHG and CHH (where H = A, T or G) are recognized in plants. DNA methylation in CG context occurs exclusively in protein-coding genes and is associated with transcriptional activity. By contrast, accumulation of CG, CHG and CHH methylation in gene promoters and repetitive sequences suppresses transcription and leads to heterochromatinization. Modification of DNA methylation is possible by specific inhibitors. The most commonly used chemicals in the plant field are non-methylable cytidine analogs (e.g. 5-azacytidine, AC; 2'-deoxy-5-azacytidine, DAC and zebularine, Zeb) and the methyl group synthesis inhibitor 3-deazaneplanocin A (DZNep). There is emerging evidence that epigenetic inhibitors cause genome instability, but the nature of this damage and its repair remain unclear. On the other hand, manipulation of DNA methylation levels can alter the plant developmental program. First, we compared the cytotoxicity of a few cytidine analogs, and DZNep using Arabidopsis plants growing directly on the drug-containing media (direct treatment, DT) or on drug-free plates and then on drug-containing media (postponed treatment, PT). An intermediate growth reduction was observed after the treatments with AC and zebularine and a strong one with DAC and DZNep. However, DZNep strongly suppressed shoot growth over the root when compared to other drugs. We confirmed that all inhibitors induced DNA damage and this damage was repaired by multiple pathways. In another experiment, we checked, whether modification of DNA may affect the process of microspore embryogenesis in triticale. To test this, we used two DH lines (DH19 and DH28), significantly different with respect to embryogenic potential. We found that DNA demethylation caused chromatin relaxation and dysregulation of marker genes related to microspore embryogenesis. We noticed that DH19 (low embryogenic) after inhibitors treatment, showed higher microspore viability, but its recalcitrancy was not overcome. For highly embryogenic DH28, we observed significantly higher effectiveness of embryo-like structure production and plant regeneration. In summary, our study provides new insights into the role of DNA methylation in ME induction and show the link between the formation of DNA damage and cytidine-analogs induced DNA demethylation.

In vitro chromosome doubling of bilberry (*Vaccinium myrtillus* L.)

Małgorzata Podwyszyńska[1], Stanisław Pluta[2]

[1] *Department of Applied Biology, Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland, e-mail: malgorzata.podwyszynska@inhort.pl*
[2] *Department of Breeding, Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland, e-mail: stanislaw.pluta@inhort.pl*

The fruits of bilberry (*Vaccinium myrtillus* L.) are one of the richest sources of biologically active compounds, mainly phenolics, including anthocyanins. This is much higher content compared to other *Vaccinium* species. On the other hand, the cultivation of highbush blueberry (*Vaccinium corymbosum* L.) is the fastest growing direction of fruit production in Poland. Growing interest in the consumption of blueberry fruits because of their pro-health importance increased breeding attention to develop improved new cultivars. The introduction of genes responsible for several valuable traits (new bioactive compounds or drought and frost resistance) from bilberry to the blueberry genome is theoretically possible by obtaining interspecific hybrids and further breeding. However, due to the cross-breeding barrier, such hybrids have not been obtained so far. Cultivated species of blueberry are tetraploids and wild species – bilberry is diploid. In *Vaccinium*, there is a strong triploid block, a post-zygotic genetic crossing barrier between the interploid crosses. The solution to overcome interploid crossing barriers may be to produce bilberry tetraploids. However, such tetraploid plants are not available yet. The aim of study was to develop an *in vitro* method of bilberry polyploidisation using shoot explants of one of the accessions held in germplasm collection of the Research Institute of Horticulture in Skierniewice.

In vitro shoot cultures of bilberry were established. The shoot explants, used for chromosome doubling, derived from standard five-week multiplication subculture. The shoots were incubated for six days on modified Anderson (1984) medium containing zeatin, gibberellic acid and indole-3-acetic acid, and one of the antimitotic agents, colchicine or amiprophos methyl (APM), at various concentrations. Subsequently, the explants were cultured on the medium without antimitotic agents and then subcultured on multiplication medium over a 16-h photoperiod. Tetraploids were detected using flow cytometry analysis. All tetraploids were then rooted and grown in a greenhouse. Early phenotype observation were performed after six months of growing plants *ex vitro*. The strongest phytotoxic effects of antimitotic agents were observed for colchicine. The highest tetraploid number (9), was obtained for treatment with APM. The tetraploids differed phenotypically from their diploid counterparts. Compared to diploids, tetraploid growth was very slow, tetraploids had shorter shoots and their chlorophyll index was higher. To our best knowledge, these are the first autotetraploids of bilberry obtained worldwide. These tetraploids will be further evaluated for their growth and development, especially their ability for producing viable pollen, which is critical for eventual hybridisation with other *Vaccinium* species.

The cell suspension culture of tree ferns and their biological potential

Jan J. Rybczyński

Polish Academy of Sciences, Botanical Garden Center for Biological Diversity Conservation, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: j.rybczynski@obpan.pl

The aim of this presentation is to demonstrate the structure and metabolic activity of cell suspensions of tree-ferns. In cell suspensions, the plant material is totally submerged in a liquid medium and depends on moderate aeration of the latter for its respiratory needs. Agitation of the suspension culture has some technical advantages such as improved uniformity of cell growth, easier control of the culture process and easier cultivation on a larger scale. Plants produce a wide range of low-molecular-weight, natural products *via* a network of typically complex metabolic pathways. These substances include: dyes, pigments, flavors, aromas, medicines and poisons but the following groups of chemical metabolites have also been listed in references relating to ferns: saccharides, polyketides, terpenoids, nitrogen-containing metabolites and phenolics. Fern cell suspensions were established using MS medium supplemented with various concentrations of 2,4-D and BAP. The optimal concentrations were 2.0 mgL^{-1} and 0.2 mgL^{-1}, respectively. For describing of biological processes happened inside of the cell various tools were employed; attention was paid for morphology of the cultured cells and confirmation of intense metabolite production. With the help of SEM numerous filaments on the surface of the cell wall were noticed. The second part of metabolic products was located inside of cell vacuole giving electron dense bodies tannosomes. With the help of autofluorescence excitation the high metabolic activity of the cytoplasm closely located to cell wall was improved. To induce morphogenic processes cell suspension were implanted on the media supplemented with various type of cytokinins in presence of NAA. The observations indicated various morphogenic response of the cell suspension in this type of the cultures. The processes of metabolism which occurred in suspension were showed with the help of post-culture medium analysis by means of LC/MS and GC/MS. LC/MS analysis was used to identify the chemical composition of the yellow coloration of the post-culture medium. This revealed that the color was due to flavonoids. Among found flovanoids we payed attention for kaempferol-3-O-rutinoside, a bitter-tasting flavonol glycoside and cynarin, the natural product having wide spectrum of biological activity including even anti HIV. It is recently established as a potent and highly selective class of HIV-1 integrase inhibitors. In addition, based on retention (GC/MS) time, the following sugars were identified: fructose, sorbose, mannose, glucose, threose, trehalose, and number of organic acids too, namely: lactic acid, acetic acid, glucuronic acid, hexadeconic acid and galacturonic acid, as well as phosphoric acid. On the basis of presented results numerous hypothesis for future work would be developed mainly with genome modification for example: somatic hybridization or transformation.

Improvement of seed germination and growth of *Stevia rebaudiana* Bertoni seedlings in *in vitro* conditions

Magdalena Simlat[1], Edyta Skrzypek[2], Marzena Warchoł[2], Emilia Morańska[1], Agnieszka Szewczyk[3], Agata Ptak[1]

[1] *Department of Plant Breeding, Physiology and Seed Science, University of Agriculture in Krakow, Podłużna 3, 30-239 Krakow, Poland, e-mail: m.simlat@ur.krakow.pl, emilia.moranska@gmail.com, mfptak@cyf-kr.edu.pl*

[2] *Department of Biotechnology, The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: e.skrzypek@ifr-pan.edu.pl, m.warchol@ifr-pan.edu.pl*

[3] *Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University, Medical College, Medyczna 9, 30-688 Krakow, Poland, e-mail: agnieszka.szewczyk@uj.edu.pl*

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb belonging to the Asteraceae family. In recent years, interest in this plant has increased due to steviol glycosides content. These compounds are more than 200 times sweeter than common sugar and resistant to high temperature. As natural sweetener, stevia is recommended for diabetes and phenylketonuria patients. Stevia is naturally propagated by seeds, however due to poor germination and a long period required for the development of seedlings suitable for planting in the field, the propagation by seeds is not the widespread method for stevia commercial production. There were already reported the possibilities of improving stevia seed germination by optimizing physical factors like light and temperature (Kumar and Sharma, 2012; Simlat et al., 2016). We have also investigate the effects of melatonin (Simlat et al., 2018), growth regulators (GA_3, thidiazuron, kinetin, BA) (Simlat et al., 2019) as well as endophytic bacteria on stevia seed germination and subsequent seedling development in *in vitro* conditions. We also assess the quality of stevia plantlets in and the content of steviol glycosides.

ACKNOWLEDGEMENTS

The research was financed by the Ministry of Science and Higher Education of the Republic of Poland. The authors wish to thank the POLAN Breeding and Seed Company, Poland for kindly providing the seeds of stevia.

REFERENCES

Kumar R, and Sharma S. 2012. Effect of light and temperature on seed germination of important medicinal and aromatic plants in north western Himalayas. *Journal of Medicinal and Aromatic Plants* 2: 468–475.

Simlat M, Ptak A, Skrzypek E, Warchoł M, Morańska E, Piórkowska E. 2018. Melatonin significantly influences seed germination and seedling growth of *Stevia rebaudiana* Bertoni. *PeerJ* 6: e5009.

Simlat M, Skrzypek E, Warchoł M, Maciaszek I, Ptak A. 2019. Evaluation on *Stevia rebaudiana* Bertoni seed germination and seedling development under phytohormones treatment. *Scientia Horticulturae* 257: 108717.

Simlat M, Ślęzak P, Moś M, Warchoł M, Skrzypek E, Ptak A. 2016. The effect of light quality on seed germination, seedling growth and selected biochemical properties of *Stevia rebaudiana* Bertoni. *Scientia Horticulturae* 211: 295–304.

The accumulation of secondary metabolites in *in vitro* cultures of some species of rue

Agnieszka Szewczyk, Aleksandra Mirek, Kamil Mateja, Sonja Bętkowska, Agnieszka Kwiecień, Halina Ekiert

Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: agnieszka.szewczyk@uj.edu.pl

Ruta graveolens L. is a plant species, which comes from the Mediterranean area. It is used in medicine for centuries, because of its high amounts of bioactive compounds like: coumarins, phenolic acids, alkaloids, flavonoids and essential oil (Ekiert and Czygan, 2007). Other species of rue are less explored.

The object of this study was to examine the influence of type of culture (agar, stationary liquid and agitated cultures), time of growth cycles (4–6 weeks) and concentration (0.1–3 mg/L) of plant growth regulators (auxins: NAA and 2,4-D, cytokinins: BAP and Kin) on dynamics of accumulation of secondary metabolites in shoot cultures of *Ruta chalepensis, Ruta corsica* and *Ruta macrophylla*. Cultures were supposed to grow on Linsmaier and Skoog medium (Linsmaier and Skoog, 1965). Five groups of secondary metabolites were analyzed by HPLC method: catechins (5 compounds), phenolic acids (24 compounds), flavonoids (29 compounds) alkaloids (2 compounds) and coumarins (26 compounds). Three groups of secondary metabolites were found: catechins, alkaloids, and coumarins. The presence of following metabolites was confirmed: catechin, γ-fagarin, isopentyloxy-γ-fagarin, bergapten, xanthotoxin, isoimperatorin, isopimpinellin, psoralen, osthol. Quantitatively dominated group of metabolites in all tested types of cultures were coumarins. Xanthotoxin was accumulated in the highest quantities.

The highest total contents of coumarins confirmed in biomass of stationary liquid cultures of investigated rue species were as follows: *R. chalepensis:* 1789.3 mg/100g DW (6-week growth cycle), *R. corsica:* 4935.4 mg/100g DW (6-week growth cycle) and *R. macrophylla:* 4727.7 mg/100g DW (6-week growth cycle). The highest total contents of coumarins confirmed in biomass of agar cultures were lower and were as follows: for *R. chalepensis*: 752.5 mg/100g DW (5-week growth cycle), for *R. corsica*: 921.4 mg/100g DW (5-week growth cycle), for *R. macrophylla*: 2120.7 mg/100g DW (5-week growth cycle). The contents of coumarins in the biomass of agitated cultures reached respectively: 1193.5 mg/100g DW (*R. chalepensis*, 5-week growth cycle), 1051.5 mg/100g DW (*R. corsica*, 6-week growth cycle), 850.2 mg/100g DW (*R. macrophylla*, 6-week growth cycle). All types of tested in vitro cultures of three species of rue could be a potential rich source of bioactive coumarins, especially of xanthotoxin.

REFERENCES

Ekiert H, and Czygan FC. 2007. Secondary metabolites in *in vitro* cultures of *Ruta graveolens* L. and *Ruta graveolens* ssp. *divaricata* (Tenore) Gams. In: Biotechnology – Secondary metabolites. *Plants and Microbes* (Ramawat KG. and Merillon JM. ed.), Science Publishers, Enfield, New Hampshire, USA, 445–482.

Linsmaier EM, and Skoog F. 1965. Organic growth factor requirements of tabacco tissue cultures. *Plant Physiology* 18(1): 100–127.

Research on the accumulation of selected groups of biologically active secondary metabolites in *in vitro* cultures of *Schisandra henryi*

Agnieszka Szopa[1], Karolina Jafernik[1], Magda Barnaś[1], Michał Dziurka[2], Halina Ekiert[1]

[1] *Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: a.szopa@uj.edu.pl, karolina.jafernik@doctoral.uj.edu.pl*
[2] *Polish Academy of Sciences The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland*

Our earlier studies biotechnological studies, confirmed the high utility of *in vitro* cultures of *Schisandra chinensis* as rich source of bioactive metabolites (Szopa et al., 2017). These results, encourage us to establish *in vitro* culture of endemic for Yunnan China province, less known species – *Schisandra henryi* C.B. Clarke (Szopa et al., 2019).

Under the studies, optimization of culture conditions for microshoot and callus cultures, were carried out. The agar microshoot cultures were maintained on 6 variants of MS medium differing in concentrations of the PGRs: BA, IBA and GA_3 in the range from 0 to 3 mg/l. Callus cultures were maintained on MS medium with 1 mg/l BA and 1 mg/l IBA. Different durations of growth cycles: 10, 20 and 30 days (3 series) were tested. In the methanolic extracts from biomasses, lignans, flavonoids and phenolic acids contents were evaluated by HPLC. Additionally the lignans identity was confirmed by UHPLC-MS/MS.

The maximal total contents of lignans for microshoot and callus cultures were equal: 873.71 mg/100 g DW and 43.18 mg/100 g DW, respectively. The main compounds were: schisantherin B (max. 622.59 mg/100 g DW) and schisantherin A (max. 143.74 mg/100 g DW). Additionally, other types of lignans – tetrahydrofuran and aryltetrahydrofuran lignans and also triterpenoids, were identified. The highest total contents of phenolic acids for microshoot and callus cultures were equal: 840.89 mg/100 g DW and 247.14 mg/100 g DW respectively. The main compounds were: neochlorogenic acid (max. 472.82 mg/100 g DW) and caftaric acid (max. 370.81 mg/100 g DW). The highest flavonoid total contents for both tested cultures were equal: 421.98 mg/100 g DW and 59.03 mg/100 g DW, respectively. The main compounds were: triofilin (max. 138.56 mg/100 g DW) and quercitirin (max. 122.54 mg/100 g DW).

This is the first report, documented the high biosynthetic potential of cells from *in vitro* cultures of *S. henryi*.

ACKNOWLEDGEMENTS

Funded by Polish Ministry of Science and Higher Education (K/DSC/004297)

REFERENCES

Szopa A, Ekiert R, Ekiert H. 2017. Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: a review on the bioactive components, pharmacological properties, analytical and biotechnological studies. *Phytochemistry Reviews* 16: 195–218.

Szopa A, Barnaś M, Ekiert H. 2019. Phytochemical studies and biological activity of three Chinese *Schisandra* species (*Schisandra sphenanthera, Schisandra henryi* and *Schisandra rubriflora*): current findings and future applications. *Phytochemistry Reviews* 18: 109–128.

Morpho-histology and physiology dependence of somatic embryo differentiation in the tree fern *Cyathea delgadii*

Wojciech Tomaszewicz[1], Michał Dziurka[2], Małgorzata Grzyb[1], Anna Mikuła[1]

[1] *Conservation Biotechnology Research Group, Polish Academy of Sciences Botanical Garden – Center for Biological Diversity in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: w.tomaszewicz@obpan.pl*

[2] *Department of Developmental Biology, The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: dziurka@ifr-pan.edu.pl*

The tree fern *Cyathea delgadii* is the species for which two pathways of somatic embryogenesis (SE) have been described. Somatic embryos initiated directly from stipe are the single-cell origin, while those induced on internodes are of multicellular origin. A well-known and fully controlled system of the somatic embryo initiation in this species allows us to better define the conditions underlying SE induction. The study aimed to indicate structural and physiological differences that could underlie a different way of somatic embryo production.

To compare stipe and internode initial explants 1) length of epidermal cells, 2) structure of cells, and 3) content of endogenous sugars, phytohormones, and phenolic acids were analysed. In experiments, etiolated sporophytes that had developed 4 or 5 leaves were used as a source of explants. The explants measuring about 2.5 mm were excised from the youngest frond of sporophytes. Plant material was cultured in ½ MS medium with 1% sucrose, without plant growth regulators, in constant darkness. The length of explant cells was measured by analysis of microscopic pictures using ImageJ. The content of sugars (i.e. fructose, glucose, 1-kestose, maltose, raffinose, starch, sucrose, trehalose, nystose), phytohormones (i.e. 9 auxins, 14 cytokinins, 9 gibberellins, abscisic acid and its glucosyl ester, jasmonic acid and its precursor and derivative, salicylic acid and its precursor) and 14 phenolic acids in control explants was analysed using UHPLC-MS/MS. To the structural analysis, explants were fixed, embedded in Epon-Spurr resin, cut in three-mictrometer-thick sections and observed using a light microscope.

Our studies showed that the average length of epidermal cells of stipes and internodes was equal to 232.9 μm and 149.1 μm, respectively. In stipe explants, about 90% of epidermal cells that formed somatic embryos were in the range of 160 to 500 μm, while internode explants were in the range of 44 to 160 μm. The content of 7 from 9 tested sugars was higher in stipes, and only the content of starch was higher in internodes. The high amount of amyloplasts in internodes was revealed by microscopic studies. The total concentrations of tested auxins, cytokinins, gibberellins, abscisic acid, jasmonic acid, and phenolic acids were significantly higher in stipe than internode explants. Among them, differences in the ABA and gibberellin contents were the most significant.

The hormonal relationships appear to be important in the trigger of embryogenic potential and the origin of somatic embryos.

Gentian somatic hybrids in the light of cytogenetic, molecular and proteomic studies

Karolina Tomiczak

Department of Conservation Biology of Plants, Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: k.tomiczak@obpan.pl

Research aimed at protoplast fusion and production of somatic hybrids within the genus *Gentiana* allowed to obtain several lines of hybrid calli and plants (Tomiczak et al., 2015). Among them somatic hybrids between two tetraploid species, i.e. Cross Gentian (*G. cruciata* L., $2n=52$) and Tibetan Gentian (*G. tibetica* King, $2n=52$) turned out to be the most promising in terms of vigorous growth and rooting *in vitro*, ease of being acclimatized to *ex vitro* conditions as well as stability of ploidy and nuclear DNA content. Cytogenetic analyses, especially genomic *in situ* hybridization, revealed that these hybrids possessed mostly $2n=88$ chromosomes, from which about 40 were inherited from *G. cruciata* and about 20 – from *G. tibetica*.

The rest of chromosomes showed a significant level of cross-hybridization, indicating a high degree of homeology between the genomes of parental species. According to the results of molecular AFLP and ISSR analyses, 31% of hybrid nuclear DNA sequences were inherited from *G. cruciata*, while 21% came from *G. tibetica*. The sequences unique for somatic hybrids comprised about 1% and those specific for both parental species – about 47%. In contrast to nuclear DNA, chloroplast DNA in all hybrids came from *G. tibetica*, as revealed by CAPS analysis (Tomiczak, 2019).

The genomic composition of somatic hybrids was juxtaposed with their proteomic profiles generated using 2D-PAGE. Taking into consideration both the presence/absence of protein spots and their profiles of intensity, hybrids also showed greater similarity to *G. cruciata* than to *G. tibetica*.

REFERENCES

Tomiczak K, Mikuła A, Rybczyński JJ. 2015. Protoplast culture and somatic cell hybridization of gentians. In: Rybczyński JJ, Davey MR, Mikuła A (eds) *The Gentianaceae – Volume 2: Biotechnology and Applications*. Springer-Verlag, Berlin Heidelberg, pp. 163–185.

Tomiczak K. 2019. Molecular and cytogenetic description of somatic hybrids between *Gentiana cruciata* L. and *G. tibetica* King. *Journal of Applied Genetics* (in press).

Evaluation of shoot development and terpenes production in *Lavandula angustifolia* plantlets *in vitro* cultured under red and blue light

Alina Trejgell, Jacek Kęsy

Department of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: trejgell@umk.pl

Lavandula angustifolia, belonging to the Lamiaceae family, is cultivated around the world. This is a significant perfume, cosmetic and pharmaceutical plant because of the high content and quality essential oils. The studies have documented antibacterial, antifungal, immunostimulatory and anticancer activity of oil compounds (Kraśniewska et al., 2017). The oil composition is determined mainly by the genotype of each cultivar (Cavanagh and Wilkinson, 2002), therefore micropropagation are an attractive way obtaining identical plantlets of cultivars with the high potential of metabolites biosynthesis. However, the accumulation of secondary metabolites in plants is influenced by various environmental factors and one of the key is light, especially spectral quality (Kopsell and Kopsell, 2008). Light is also one of the factors that influence the growth and development of different species of plants in vitro. It is essential for various physiological processes in plants, such as photosynthesis, hence influencing growth and development, as well as morphogenesis (Gupta and Jatothu, 2013). The aim of the study was to assess the influence of light quality on micropropagation of *L. angustifolia* and the ability to produce essential oils.

The experimental material were the single-node explants isolated from a 4-week-old culture, which were cultivated onto MS with 0,25 $mg \cdot dm^{-3}$ BAP, under white light and blue or red monochrome light. Continuous blue and red light growth conditions were obtained by placing explants in polystyrene culture vessels Phytatray II, which transmits light of wavelength 475 nm and 590 nm, respectively.

Analysis of the shoot rate multiplication, the microshoots quality and the ability to essential oils production were made after 4 weeks of culture. In cultures conducted in white light the highest multiplication ratio was recorded, in addition, the shoots had also the best quality. Both the microshoots during *in vitro* culture and transferred to *ex vitro* conditions were able to produce essential oils, however, the ratio between the amounts of the components were different in comparison to plants from traditional cultivation.

REFERENCES

Cavanagh HMA, and Wilkinson JM. 2002. Biological Activities of Lavender Essential Oil. *Phytotheraphy. Research* 16: 301–308.

Gupta SD, and Jatothu B. 2013. Fundamentals and applications of light-emitting diodes LEDs in in vitro plant growth and morphogenesis. *Plant Biotechnology Reports* 7: 211–220.

Kraśniewska K, Gniewosz M, Kosakowska O, Pobiega K. 2017. Chemical composition and antimicrobial properties of essential oil from lavender (*Lavandula angustifolia* L.) in commercial available preparation. *Postępy Fitoterapii* 18: 13–118.

Kopsell DA, and Kopsell DE. 2008. Genetic and environmental factors affecting plant lutein/zeaxanthin. *Agro Food Industry Hi-Tech* 19: 44–46.

Adventitious organogenesis in *in vitro* cultures of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. (Asteraceae), *Gerbera jamesonii* H. Bol. (Asteraceae) and *Streptocarpus* × *hybridus* Voss (Gesneriaceae) after application of silver and gold nanoparticles

Alicja Tymoszuk, Natalia Miler

Laboratory of Ornamental Plants and Vegetables Crops, Faculty of Agriculture and Biotechnology, UTP University of Science and Technology in Bydgoszcz, Bernardyńska 6/8, 85-029 Bydgoszcz, Poland, e-mail: alicja.tymoszuk@utp.edu.pl, nmiler@utp.edu.pl

Plants are constantly exposed to naturally occurring nanoparticles. However, exposure to engineered nanoparticles is new and requires sufficient consideration (Dietz and Herth, 2011; Khot at al., 2012). The aim of this study was to evaluate the effect of silver and gold nanoparticles (20 nm-in-size) at the concentration of 10 and 30 ppm, on the adventitious roots regeneration in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Bydgoszczanka' and *Gerbera jamesonii* H. Bol. 'Suri', as well as adventitious shoots regeneration in *Streptocarpus* × *hybridus* Voss. As for shoot formation in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Lilac Wonder' and 'Richmond', silver nanoparticles at the concentration of 50 and 100 were applied. Chrysanthemum 'Bydgoszczanka' shoot fragments and gerbera single rosettes were polarly inoculated on the modified MS medium with 2 mg L^{-1} indole-3-acetic acid for rooting. Leaf explants of *Streptocarpus* were horizontally inoculated on the modified MS medium with 0.1 mg L^{-1} 1-naphthaleneacetic acid. Chrysanthemum 'Lilac Wonder' and 'Richmond' internodes were horizontally put onto the modified MS medium with 0.6 mg L^{-1} benzylaminopurine and 2 mg L^{-1} indole-3-acetic acid. Nanoparticles were poured on the culture medium immediately after explants inoculation. Chrysanthemum shoot explants treated with silver nanoparticles regenerated less adventitious roots than control explants. These roots were also of the smallest area and diameter, but the length of the longest root was the highest in the experiment. Roots parameters after gold nanoparticles treatment were similar to those reported for the control object, expect for one parameter – the biggest values of root diameter. As for gerbera, the efficiency of adventitious roots regeneration was also reduced by silver nanoparticles, but the addition of 10 ppm gold nanoparticles stimulated rhizogenesis. Similarly to chrysanthemum, the greatest length of the longest root, the smallest root system area and root diameter were also observed under the influence of silver nanoparticles. The best effects in the stimulation of the rosulate and phyllomorphs regeneration on *Streptocarpus* leaf explants were reported for both silver and gold nanoparticles at the concentration of 10 ppm. Formation of adventitious shoots on chrysanthemum internodes was inhibited as a result of silver nanoparticles application.

REFERENCES

Dietz KJ, and Herth S. 2011. Plant nanotoxicology. *Trends in Plant Science* 16(1): 582–589.

Khot LR, Sankaran S, Maja JM, Ehsanie R, Schuster EW. 2012. Application of nanomaterials in agricultural production and crop protection: A review. *Crop Protection* 35: 64–70.

Media for oat (*Avena sativa* L.) haploid embryo rescue

Marzena Warchoł, Kinga Dziurka, Ilona Czyczyło-Mysza, Katarzyna Juzoń, Kamila Kapłoniak, Izabela Marcińska, Edyta Skrzypek

The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences, Niezapominajek 21, 30-239 Kraków, Poland, e-mail: m.warchol@ifr-pan.edu.pl

Biotechnological methods of new cultivars production are based on the formation of DH lines with completely homozygous plants. In oat (*Avena sativa* L.) the most efficient method for DH lines production is wide crossing with maize (*Zea mays* L var. *saccharata*). In this method embryo abortion is often observed and therefore embryo rescue is required. Abortion of oat haploid embryos is usually a consequence of failed development of endosperm. The culture medium replaces the endosperm and provides the nutrients to the developing embryo. The younger the embryos are removed from the ovaries, the more complex are the steps involved in rescuing them and the medium requirements.

In our study developed embryos were transferred on the following media composition: MS (Murashige and Skoog, 1962) and 190-2 medium (Zhuang and Xu, 1983) supplemented with 3% (w/v) sucrose, 6 and 9% (w/v) maltose. The pH of the tested media was adjusted to 5.5 and 6.0. Analysis of variance showed significant differences in the efficiency of haploid embryo germination dependently on the type of media, plant growth regulators, sugars and media pH (Noga et al., 2016; Warchoł et al., 2018). The highest percentage of germinated haploid embryos was observed on the 190-2 medium containing 0.5 mg L^{-1} KIN, 0.5 mg L^{-1} NAA and 9% maltose. The efficiency of oat haploid embryo germination ranged from 3.25% to 19.0%. In total, we obtained 591 haploid embryos and 48 fertile DH plants which produced a total of 4,878 seeds.

ACKNOWLEDGEMENT

The research was funded by the Ministry of Agriculture and Rural Development, grant No HORhn-801-4/12.

REFERENCES

Murashige T, and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Noga A, Skrzypek E, Warchoł M, Czyczyło-Mysza I, Dziurka K, Marcińska I, Juzoń K, Warzecha T, Sutkowska A, Nita Z, Werwińska K. 2016. Conversion of oat (*Avena sativa* L.) haploid embryos into plants in relation to embryo developmental stage and regeneration media. *In Vitro Cellular and Developmental Biology – Plant* 52: 590–597.

Warchoł M, Czyczyło-Mysza I, Marcińska I, Dziurka K, Noga A, Skrzypek E. 2018. The effect of genotype, media composition, pH and sugar concentrations on oat (*Avena sativa* L.) doubled haploid production through oat × maize crosses. *Acta Physiologiae Plantarum* 40: 93.

Zhuang JJ, and Xu J. 1983. Increasing differentiation frequencies in wheat pollen callus. In: Cell and Tissue Culture Techniques for Cereal Crop Improvement. (eds: Hu H., Vega M.R.) *Science Press, Beijing*, p 431.

DH lines application in studies of barley (*Hordeum vulgare* L.) resistance to *Fusarium culmorum* infection

Tomasz Warzecha[1], Edyta Skrzypek[2], Agnieszka Sutkowska[1]

[1] *University of Agriculture in Kraków, Department of Plant Breeding, Physiology and Seed Science, Podłużna 3, 30-239 Krakow, Poland, e-mail: rrwarzec@cyf-kr.edu.pl, asutkowska@ar.krakow.pl*
[2] *Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: e.skrzypek@ifr-pan.edu.pl*

Fusarium seedling blight (FSB) and Fusarium head blight (FHB) caused by *Fusarium* genera are the most devastating diseases in barely worldwide (Warzecha et al., 2019). The genotype influence the susceptibility which is reflected in the various levels of seedling growth reduction (Buerstmayr et al., 2004). The plant material for the study consisted of 32 spring barley DH lines developed by the *Hordeum bulbosum* technique and *in vitro* culture of immature embryos (Pickering and Devaux, 1992). In plate assay for resistance direct assessment (disease rating (DR) and fresh weight of seedlings) and selected physiological parameters (phenolic compounds, soluble sugars, chlorophyll *a* and *b*, and carotenoids) as well as chlorophyll fluorescence parameters (Fv/Fm – maximum PSII photosystem efficiency, P.I. – overall performance of PSII photosystem) were determined in control and *F. culmorum* infected plants. The objective of the studies were to test association of direct assessment with physiological parameters to facilitate the most resistant DH lines, and to check biochemical changes in plants caused by infection. The hulled lines revealed less roots susceptibility to infection of *F. culmorum* expressed in DR and in fresh weight. In roots infection caused significant increase of phenolics content, in contrast soluble sugars and pigment content in leaves (carotenoids, chlorophyll *a*, chlorophyll *b*) significantly decrease comparing with control. Inoculation affected significantly overall performance index of PSII (P.I.) photochemistry but not the maximum photochemical efficiency (Fv/Fm). Positive correlation coefficients were found between DR of roots and following physiological parameters: phenolics in leaves, soluble sugars in roots, and between fresh weight of roots and following physiological parameters: chlorophyll *a*, *b*, carotenoids and P.I. Negative correlation coefficients were found between DR of roots and following physiological parameters: soluble sugars in leaves, chlorophyll *a*, *b*, carotenoids, maximum photochemical efficiency (Fv/Fm).

REFERENCES

Buerstmayr H, Legzdina L, Steiner B, Lemmens M. 2004. Variation for resistance to Fusarium head blight in spring barley. *Euphytica* 137: 279–290.

Pickering Ra, and Devaux P. 1992. Haploid production: Approaches and use in plant breeding. In: *Barley: Genetics. Biochemistry. Molecular biology and biotechnology*. CAB International. Wallingford. UK, 519–547.

Warzecha T, Skrzypek E, Adamski T, Surma M, Kaczmarek Z, Sutkowska A. 2019. Chlorophyll a fluorescence parameters of hulled and hull-less barley (*Hordeum vulgare* L.) DH lines inoculated with *Fusarium culmorum*. *The Plant Pathology Journal* 35(2): 112–124.

LEAFY COTYLEDON2 gene – a central regulator of the auxin-induced somatic embryogenesis in *Arabidopsis*

Barbara Wójcikowska, Małgorzata Danuta Gaj

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Jagiellońska 28, 40-032 Katowice, Poland, e-mail: barbara.wojcikowska@us.edu.pl; malgorzata.gaj@us.edu.pl

The process of somatic embryogenesis (SE) leads to the development of embryos from somatic plant cells and is widely used in plant biotechnology for *in vitro* plant regeneration. Genes encoding transcription factors (TFs) are the most frequently represented among the identified SE regulators and among them, *LEAFY COTYLEDON2* (*LEC2*), a master regulator of zygotic embryogenesis was indicated to be essential for SE induction in Arabidopsis. In support, overexpression of the *LEC2* was indicated to promote SE induction *in planta* and in *in vitro* cultured explants, while the *lec2* mutant showed inhibited embryogenic potential. The relationship of *LEC2* and hormones during SE induction was indicated. Accordingly, auxin treatment was found to promote *LEC2* expression in the embryogenic explant culture and overexpression of *LEC2* significantly modified the hormone content in the seedlings including the increased auxin and salicylic acid level and decreased content of abscisic acid and most of cytokinins. Conclusively, LEC2 has been postulated to promote the SE induction by controlling auxin biosynthesis via regulation of three of the *YUCCA* genes (*YUC1, 4, 10*) encoding the key enzymes of the tryptophan-dependent pathway of IAA biosynthesis. In support, a decrease in *YUCs* expression was observed in the *lec2* mutant and a reduced embryogenic potential was found in the *yuc* mutant culture.

The multi-level control of *LEC2* activity was postulated in SE including miRNA-mediated and epigenetic processes. AUXIN RESPONSE FACTORs (ARF10, 16) and PHABULOSA, PHAVOLUTA TF that are repressed by miR160 and miR165/166, respectively, are suggested to positively regulate *LEC2* during embryogenic transition (Wójcik et al., 2017). Moreover, *LEC2* is assumed to be under DNA methylation and histone acetylation control as explant treatment with 5-AzaC (5-Aza cytosine) and TSA (an inhibitor of histone deacetylases) of SE-induction activity resulted in down- and up-regulated expression of *LEC2*, respectively (Grzybkowska et al., 2018). In addition, the RNAseq analysis of the TSA-induced SE indicated intensive de-regulation of *LEC2* and numerous genes related with auxin and other hormones.

ACKNOWLEDGEMENT

This work was supported by a research grant from the National Science Centre, Poland (OPUS13 2017/25/B/NZ1/01615).

REFERENCES

Grzybkowska D, Morończyk J, Wójcikowska B, Gaj MD. 2018. Azacitidine (5-AzaC)-treatment and mutations in DNA methylase genes affect embryogenic response and expression of the genes that are involved in somatic embryogenesis in *Arabidopsis*. *Plant Growth Regulation* 85: 243–256.

Wójcik AM, Nodine MD, Gaj MD. 2017. miR160 and miR166/165 contribute to the LEC2-mediated auxin response involved in the somatic embryogenesis induction in *Arabidopsis*. *Frontiers in Plant Science* 8: 2024.

Can we affect the appearance of albino, androgenic rye regenerants?

Janusz Zimny, Sylwia Oleszczuk, Aleksandra Zimny, Andrzej Czaplicki, Sławomir Sowa

Plant Biotechnology and Cytogenetics Department, Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland, e-mail: j.zimny@ihar.edu.pl

Nowadays, all strategies for heterosis breeding of cereals include the use of double haploids (DH) at various stages of the breeding process. DH allow the use of homozygous lines in basic research, as well as in experimental plant breeding. The progeny of such lines does not segregate in subsequent generations. The progress observed in plant breeding, which is made thanks to homozygous lines, leads to the conclusion that *in vitro* induced androgenesis is currently the most effective biotechnological method used in breeding practice. Despite many efforts to develop an effective method for producing DH of cereal plants, many problems remain unsolved. One of them is that the genotype still remains the main factor that determines the efficiency of androgenesis in *in vitro* cultures. An indispensable condition for initiating microspore divisions leading to embryo formation and regeneration of the plant, is to subject microspores to stress. Applying the right stress at the proper stage not only stops the natural development of microspores, but also reprograms these cells to initiate embryo development. Moreover, the type of stress is related to the number of regenerated green and albino plants.

The phenomenon of albinism is inherent in the production of DHs and it applies to all cereals. Albino plants, due to their inability to form chloroplasts in cells, become heterotrophs and as such are only able to reach early developmental stages on nutrient media in *in vitro* culture, hence they are useless for breeding. It used to be once associated with the growth temperature of the initial plants and the culture conditions and to this day the causes of this phenomenon are not recognized, although one can partially influence the reduction of the number of regenerating albino plants. Despite many years of research on reducing albinism in androgenic cultures, a satisfactory remedy for them has not yet been invented. The present research focuses on minimizing its occurrence during haploid embryogenesis. The presented data indicate that albinism can be reduced by selecting the appropriate *in vitro* culture conditions, in particular the type of microspore reprogramming stress. It seems that the best stress in the context of minimizing this phenomenon is cooling the ears at 4°C for 21 days, and then incubating isolated anthers in mannitol solution for another 7 days at 4°C. But we have observed exceptions to this rule.

Dual role of glutathione in microspore embryogenesis of triticale (× *Triticosecale* Witttm.)

Iwona Żur[1], Ewa Dubas[1], Franciszek Janowiak[1], Monika Krzewska[1], Przemysław Kopeć[1], Ewa Surówka[1], Anna Nowicka[1], Kamil Zieliński[1], Katarzyna Gawrońska[2], Gabriela Gołębiowska-Pikania[2], Jozsef Fodor[3], Balazs Barna[3]

[1] *The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: i.zur@ifr-pan.edu.pl*
[2] *Department of Cell Biology and Genetics, Institute of Biology, Pedagogical University, Podchorążych 2, 31-054 Krakow, Poland, e-mail: ibiol@op.krakow.pl*
[3] *Plant Protection Institute, Hungarian Academy of Sciences, Herman Ottó út 15, Budapest 1022, Hungary, e-mail: atk@agrar.mta.hu*

Glutathione is the most abundant low molecular weight (LMW) thiol compound found in almost all eukaryotic cells. It occurs predominantly in its reduced form (GSH), continuously oxidized in redox reactions into glutathione disulphide (GSSG). The GSH/GSSG ratio regulates cell redox homeostasis and could be used as a parameter of oxidative stress intensity. Apart from being the most potent intracellular scavenger of reactive oxygen species (ROS), glutathione plays an important role in many physiological processes connected with plant growth, development and stress defence.

In the present study, the effect of GSH on the process of microspore embryogenesis (ME) was studied using several DH lines of winter triticale significantly different in their embryogenic potential. Tiller treatment with low temperature (3 weeks at 4°C) and 0.3 mM GSH significantly increased the effectiveness of ME initiation in the majority of studied DH lines. The parameters influencing ME effectiveness (microspore viability, embryo-like structure (ELS) production and their regeneration potential) were associated with endogenous GSH and GSSG levels measured by the method of Knörzer et al. (1996), glutathione redox status (GSH/GSH+GSSG) and LMW antioxidative activity (Brand-Williams et al., 1995). It was revealed that glutathione plays a dual role in the process of ME, protecting cells from oxidative stress and stimulating ELS differentiation. However, further study revealed that the effect of GSH strongly depends on the physiological status of microspores and the activity of antioxidative enzymes. Our results suggest that ROS act as signalling molecules in microspore reprogramming, which is why its effective elimination increases microspore viability, but at the same time it can decrease the efficiency of ME initiation.

ACKNOWLEDGEMENTS

The research was supported by the National Science Centre, Poland (project 2015/18/M/NZ3/00348).

REFERENCES

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free-radical method to evaluate antioxidant activity. *Food Science and Technology* 28: 25–30.

Knörzer OC, Burner J, Boger P. 1996. Alterations in the antioxidative system of suspension-cultured soybean cells (*Glycine max*) induced by oxidative stress. *Physiology Plantarum* 97: 388–396.

POSTERS

Toxicological effect of metal oxide nanoparticles: ZnO, TiO_2, Al_2O_3, ZrO_2 on wheat callus (*Triticum aestivum* L.)

Anna Barbasz, Agnieszka Czyżowska

Department of Biochemistry and Biophysics, Institute of Biology, Pedagogical University of Krakow, Podchorążych 2, 30-084 Krakow, Poland, e-mail: anna.barbasz@up.krakow.pl

Metal oxide nanoparticles are constantly gaining in popularity due to their characteristic properties which allow for their use, among others, for microbial protection (e.g. in food packaging, textiles) and as sunscreens. Due to the high risk of these nanomaterials getting into the environment, there is a real risk of exposure both aquatic and terrestrial plants to them.

This work investigates the cytotoxic effect of the most commonly used metal oxide nanoparticles: zinc oxide (ZnO-NPs), aluminum oxide (Al_2O_3-NPs), titanium oxide (TiO_2-NPs) and zirconium oxide (ZrO_2-NPs) on callus cells of two wheat (*Triticum aestivum* L.) varieties – Parabola (stress tolerant) and Raweta (stress sensitive). The evaluation of nanoparticle activity was made on the basis of determining the degree of lipid peroxidation, the amount of lactate dehydrogenase (LDH) released into the cell environment and the activity of antioxidant enzymes: superoxide dismutase (SOD) and peroxidases (POX).

The studied nanoparticles caused oxidative damage to membranes of the Raweta variety. A significant increase in MDA production was noted as a result of the action of ZnO-NPs and Al_2O_3-NPs at the lowest tested concentration (3 mg/L). Other nanoparticles increased lipid peroxidation at a concentration of 6 mg/L. The concentration-dependent rise in LDH leakage from the cells was observed as a result of treatment with all nanoparticles. The degree of membrane damage caused by contact with nanoparticles enhance in the following series: TiO_2-NPs, Al_2O_3NPs, ZrO_2-NPs and ZnO-NPs. For the sensitive variety, an increase in antioxidant enzymes activity was also observed as a result of the tested nanoparticles acting (with the exception of ZnO-NPs, which caused a significant decrease in SOD activity). No noticeable toxicity of these metal oxide nanoparticles was observed for the Parabola variety. ZnO-NPs caused an approximately 10% rise in LDH leakage only at a concentration of 12 mg/L. The action of the other NPs did not cause membrane damage that would result in uncontrolled outflow of LDH from callus cells. The increase in the level of MDA produced in stress tolerant cells was slight.

The cytotoxicity of metal oxide nanoparticles is highly dependent on the sensitivity of the cells which they interact with. The tested nanoparticles can damage membranes and generate the production of reactive oxygen species, leading to oxidative stress in the cells. Environmental pollution with engineered nanomaterials can significantly affect crop productivity.

Physicochemical characterization of structure changes in *in vitro* cells and model membranes determined by the direct action of reactive oxygen species

Barbara Dyba, Elżbieta Rudolphi-Szydło, Maria Filek

Department of Biochemistry and Biophysics, Institute of Biology, Pedagogical University of Cracow, Podchorążych 2, 30-084 Krakow, Poland, e-mail: elzbieta.rudolphi-szydlo@up.krakow.pl

The mechanism of oxidative stress resulting from the action of many abiotic and biotic factors has still not been fully explained. The factors that initiate this stress in the cell are reactive oxygen species (ROS), which, generated in excess, change redox conditions, lead to disturbances in the course of important metabolic processes. *In vitro* methods, and studies of membranes in model systems in particular, may contribute to determine the initial stages of oxidative stress.

In presented experiments, *in vitro* cultures of two wheat varieties (resistant and sensitive to oxidative stress) were directly exposed to ROS action for 30 min. Membranes of these cells were extracted and lipid fractions were separated by column chromatography. Physicochemical properties of phospholipids (PL) and monogalactolipids (MGDG) were characterized according to Langmuir technic. In this technic, monolayers formed from lipids are characterized by measuring surface pressure isotherms i.e. the dependence of surface pressure on area per molecule. On this basis, parameters such as A_{lim} (area/molecule in condensed monolayer), π_{coll} (surface pressure corresponding to this state) and C_s^{-1} (parameter describing the layer compressibility). These factors allow to quantify subtle changes in the monolayers' structure.

It was shown that the ROS action modified the composition of membrane lipids particularly that of the sensitive variety. Generally, the percentage of PL fraction in membranes increased at the expense of the content of the MGDG fraction. The physicochemical parameters of monolayers formed from both lipid fractions were modified to a different degree. For the PL fraction, ROS leads to a reduction of the A_{lim} and π_{coll} values only for lipids obtained from the sensitive variety. The change of these parameters for monolayers formed from MGDG fractions was noticed for both varieties.

These results allow to conclude that in the mechanisms of protecting cells against the action of ROS, the changes of physicochemical properties of lipid membranes such as compressibility and elasticity are important factors. Modification within the MGDG fraction seems to be more important for membrane protection processes than in the PL fraction.

Physiological and histological aspects in preliminary polyploidization studies of *Plantago major* L. in *in vitro* culture conditions

Maciej Gustab[1], Magdalena Kędra[2], Małgorzata Kozieradzka-Kiszkurno [3], Agata Maczuga[1], Przemysław Malec[2], Monika Tuleja[1]

[1] *Department of Plant Cytology and Embryology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland, e-mail: monika.tuleja@uj.edu.pl*
[2] *Department of Plant Physiology and Biochemistry, Jagiellonian University, Gronostajowa 7, 30-387, Krakow, Poland*
[3] *Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdansk, Poland*

Plantago major L. is a common plant species occurring in Poland with potential of medical use (Gonçalves and Romano, 2016; Samuelsen, 2000). Nevertheless, it is not widely used, due to its low efficiency of harvesting compared to other plants with similar properties. Increasing the ploidy level may be one of the possibilities to obtain specimens with desirable characteristics (Sabzehzari et al., 2019). For this explants obtained from *P. major* leaves and seedlings were cultured on MS + 0.9 μM TDZ + 1.0 μM IBA and MS + 4.7 μM KIN + 4.5 μM 2,4-D media respectively for callus induction and MS + 18.6 μM KIN + 0.05 μM NAA for morphogenesis. Observed callus was characterized by the presence of extracellular matrix (ECM). Multiplication of the chromosomes was induced by oryzalin addition in concentrations: 1 μM, 5 μM and 10 μM. (with the exposition 7 days). The morphogenetic response obtained on callus differed from the assumed one, mainly by roots developing instead of adventitious shoots formation. As a result, 14 adventitious shoots were obtained from only one explant. The roots forming on callus were characterized by a green color and surprisingly were proved to be photosynthetically active. For some of them, the chromosome number was analyzed to assess the level of ploidy, which did not differ with the original roots. Adventitious shoots were rooted without success on MS + 2.7 μM NAA and ½ MS + 4.9 μM IBA and they produced deformed inflorescences. Histological analyzes revealed differences in structure between natural and in vitro roots, as well as the abnormalities in the achieved inflorescences with the presence of an embryo sac in it. In applied conditions the ploidy level was not changed, but the effect of oryzalin could be visible on the physiological level as the clear photosynthetic activity of the roots. The intended effect of regenerated plants with confirmed polyploidy was not obtained, however, this results have a big value for the future research of *P. major* polyploidization and its biology at all.

REFERENCES

Goncalves S, and Romano A. 2016. The medicinal potential of plants from the genus *Plantago* (Plantaginaceae). *Industrial Crops and Products* 83: 213–226.

Sabzehzari M, ET AL. 2019. Morphological, anatomical, physiological, and cytological studies in diploid and tetraploid plants of *Plantago psyllium*. *Plant Cell, Tissue and Organ Culture* 139: 131–137.

Samuelsen AB. 2000. The traditional uses, chemical constituents and biological activities of *Plantago major* L. – a review. *Journal of Ethnopharmacology* 71: 1–21.

Changes in *Linum usitatissimum* CHS genes expression after low temperature stress treatment

Agata Hnitecka, Magdalena Żuk

Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Przybyszewskiego 63-77, 51-148 Wroclaw, Poland
e-mail:agata.hnitecka@uwr.edu.pl, magdalena.zuk@uwr.edu.pl

Chalcone synthase (CHS) is a key enzyme of the flavonoids biosynthesis pathway. This secondary metabolites function in plants as protectives against unfavorable environmental conditions. In flax, seven CHS genes where recognized, but their role in stress defense is still poorly determined. In this study, six time points of low temperature (12°C) stress treatment were examined: before stress treatment, 24h, 48h, and 1 week after introducing stress conditions, 24h and 1 week after stress removal. Real Time PCR analysis of six CHS genes: LuCHS3, LuCHS4, LuCHS6, LuCHS7, LuCHS10, and LuCHS11 indicate, that they differ in expression levels and in time of expression induction by low temperatures. These results suggest, that some of them act as primary stress protection and others as protection from prolonged stress. Moreover, differences between three flax plant materials were examined: Linola – the oily flax type and two transgenic flax derived from Linola – one with introduced antisense fragment of LuCHS6 – named aCHS6, and one with antisense fragment of LuCHS4 – named aCHS4. LuCHS6 and LuCHS4 were chosen for transformation because they differ in expression patterns in flax stress responses.

Does the trophic stress cause defective female gametophyte development and weak seed set of common buckwheat? – *in vitro* and *in vivo* model of experiments

Marta Hornyák[1], Agnieszka Płażek[1], Aneta Słomka[2], Przemysław Kopeć[3], Anna Szczerba[1], Jakub Pastuszak[1], Franciszek Dubert[3]

[1] *University of Agriculture in Krakow, Faculty of Agriculture and Economics, Department of Plant Physiology, Podłużna 3, 30-239 Krakow, Poland, e-mail: Marta.golebiewska1990@gmail.com*
[2] *Institute of Botany, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*
[3] *Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland*

Common buckwheat (*Fagopyrum esculentum* Moench) belongs to Polygonaceae and has many advantages, such as well-balanced seed composition and high ecological value. However, cultivation area of buckwheat is limited due to low seed yield and the heterogeneity of its maturation. Seed set is insufficient and it amounts to 15-53%, depending on the genotype and growth conditions. One plant develops from 500 to 2000 flowers, but only a few percent of flowers produce seeds. Often, even if the flower is pollinated young embryos and fruits degenerate, probably for trophic reasons. Our experiments studied the impact of the trophic stress in *in vivo* and *in vitro* conditions on the embryological processes and seed yield. The studies were carried out on common buckwheat plants of Polish cultivar 'Panda' and strain PA15 which show significant variation in term of the degeneration of the embryo sacs as well as yield productivity. The goal of first experiment was to study the trophic stress effect on megasporo- and megagametophytogenesis performed on isolated flower buds grown on different variants of medium (optimal, reduced to the half and third part of nutritional components) in *in vitro* conditions. The objective of second experiment was to investigate the trophic stress effect on seed yield *in planta* by reducing proper amount of flowers (50 and 75%) compared to that of the control. The both of studied genotypes demonstrated different responses to applied treatments. In the experiment conducted *in vitro* conditions strain PA15 seems to be more sensitive to trophic stress than cv. 'Panda'. However, the most 'poor' medium increased embryo sacs degeneration in both genotypes. In the experiment performed *in planta*, 50% reduction of PA15 flowers increased seed yield, while cv. 'Panda' showed the highest yield only in control plants.

ACKNOWLEDGEMENTS

This study was supported by funding of the National Centre of Knowledge of Poland (No. 2017/25/B/NZ9/00148)

REFERENCES

Cawoy V, Ledent J-F, Kinet J-M, Jacquemart A-L. 2009. Floral biology of common buckwheat (*Fagopyrum esculentum* Moench.) *European Journal of Plant Science and Biotechnology* 3: 1–9.

Halbrecq B, Rommedenne P, Ledent JF. 2005. Evolution of flowering, ripening and seed set in buckwheat (*Fagopyrum esculentum* Moench.): quantitative analysis. *European Journal of Agronomy* 23: 209–224.

Słomka A, Michno K, Dubert F, Dziurka M, Kopeć P, Płażek A. 2017. Embryological background of low seed set in distylous common buckwheat (*Fagopyrum esculentum* Moench) with biased morph ratios, and biostimulant-induced improvement of it. *Crop and Pasture Science* 68: 680–690.

Accumulation of flavonoids, verbascoside and phenolic acids after elicitation by methyl jasmonate in *in vitro* cultures of *Scutellaria lateriflora* L.

Beata Kawka, Inga Kwiecień, Halina Ekiert

Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Medyczna 9, 30-688 Krakow, Poland, email: inga.kwiecien@uj.edu.pl, beata.kawka@doctoral.uj.edu.pl

Scutellaria lateriflora L. – also known as American skullcap is well famous medicinal plant species in traditional North American therapy. Herb of this plant species contains as main compounds flavonoids typical for genus *Scutellaria* – baicalein, scutellarein, wogonin, and their glucuronides. That metabolites have a lot of pharmacological properties like hepatoprotective, sedative, antioxidative and antiinflamatory (Barnes et al., 2007).

The aim of the research was to evaluate the effect of elicitation of agitated *in vitro* cultures of *Scutellaria lateriflora* L. on accumulation of flavonoids, verbascoside and phenolic acids.

Agitated cultures were grown on Linsmaier and Skoog medium containing 1.0 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l 1-naphthaleneacetic acid (NAA). After two weeks of the growth cycle, methyl jasmonate (ME) was added to each flask in such amounts as to obtain the final concentrations of 10, 50 and 100 μM. The methanolic extracts from biomasses collected after 3 and 7 days of growth cycles were analysed with DAD-HPLC method (Ellnain-Wojtaszek, 1999).

The presence of 5 flavonoids (baicalin, scutellarin, wogonin, wogonoside and oroxylin A), verbascoside and 3,4-dihydroxyphenylacetic acid was confirmed.

The main metabolite in both cases was verbascoside. The maximum amounts of this compound (after 3 and 7 days) riched 381.5 mg/100 g DW and 121.6 mg/100 g DW (both by 10 μM of ME) respectively and they are 10-, and 4-time higher than in control culture. Baicalin was accumulated also in high amount. The maximum amount was documented after 3 days (152.4 mg/100 g DW) by 10μM of ME. The total amounts of the estimated flavonoids ranged from 176.9 to 308. mg/100 g DW after 3 days of culture, and from 26.7 to 259.0 mg/100 g DW after 7 days. Higher levels of flavonoids were obtained after using lower concentrations of ME.

The testing of elicitation conditions led to increased accumulation of verbascoside and some flavonoids specific for the *Scutellaria* genus.

REFERENCES

Barnes J, Anderson La, Philipson J. 2007. Herbal Medicines, 3rd Edition. The Pharmaceutical Press, London, 530–532.

Ellnain-Wojtaszek M, and Zgórka G. 1999. High-performance liquid chromatography and thin-layer chromatography of phenolic acids from *Ginkgo biloba* L. leaves collected within vegetative period. *Journal of Liquid Chromatography & Related Technologies* 22: 1457–1471.

The effect of selected plant growth regulators on glucosinolate production in agar, agitated and bioreactor cultures of *Nasturtium officinale*

Marta Klimek-Szczykutowicz[1], Agnieszka Szopa[1], Michał Dziurka[2], Halina Ekiert[1]

[1] *Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: marta.klimek-szczykutowicz@doctoral.uj.edu.pl*
[2] *Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland*

Metabolites characteristic for Brassicaceae family – glucosinolates (Gls), are recommended in the prevention of cancers, heart diseases and diabetes. The rich source of Gls is *Nasturtium officinale* R. Br. (watercress), plant of scientific proven activities e.g. antioxidant, hepatoprotective and anticancer (Klimek-Szczykutowicz et al., 2018).

The aim of the study was to investigate the effect of selected plant growth regulators (PGRs) on Gls production in agar, agitated, as well as in RITA-bioreactors microshoot cultures of *N. officinale*. Under the experiment six variants of MS medium (Murashige and Skoog., 1962) supplemented with different PGRs were studied. In the agar and agitated cultures the following PGRs were tested: BA and NAA, 2iP and NAA, KIN and IAA, KIN and IBA, Zea and IBA, Zea and NAA, in concentration of 1 mg/l cytokinin and 1 mg/l auxin. The bioreactor cultures were maintained on MS medium with 1 mg/l BA and 1 mg/l NAA. As control were assumed agar cultures cultivated on MS medium without PGRs. The different culture growth periods: 10, 20 and 30-days for agar and 10 and 20-days, for agitated and bioreactor cultures, were tested (3 series). The estimation of total Gls content was performed spectrophotometrically (Gallaher et al., 2012).

In the control microshoot cultures the Gls content was equal (mg/100 g DW): 116.19 (10 days), 89.51 (20 days) and 78.71 (30 days). In experimental agar microshoot cultures the Gls content ranged from 100.23 (MS with Zea and IBA, 10 days) to 194.77 mg/100 g DW (MS with KIN and IAA, 30 days). In agitated microshoot cultures the Gls content ranged from 78.09 (MS with Zea and IBA, 20 days) to 182.80 mg/100 g DW (MS with BA and NAA, 10 days). The highest total Gls content (195.92 mg/100 g DW) was confirmed for extracts of microshoots collected from RITA bioreactors after 20 days of growth period.

REFERENCES

Klimek-Szczykutowicz M, Szopa A, Ekiert H. 2018. Chemical composition, traditional and professional use in medicine, application in environmental protection, position in food and cosmetics industries, and biotechnological studies of *Nasturtium officinale* (watercress) – a review. *Fitoterapia* 129: 283–292.

Murashige T, and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Gallaher CM, Gallaher DD, Peterson S. 2012. Development and validation of a spectrophotometric method for quantification of total glucosinolates in cruciferous vegetables. *Journal of Agricultural and Food Chemistry* 60: 1358–1362.

5-azacytidine modulates ROS generation and antioxidant enzyme responses during microspore embryogenesis induction in triticale (× *Triticosecale* Wittm.)

Monika Krzewska, Ewa Dubas, Ewa Surówka, Przemysław Kopeć, Sabina Malaga, Anna Nowicka, Kamil Zieliński, Agnieszka Janas, Iwona Żur

The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland, e-mail: m.krzewska@ifr-pan.edu.pl

Microspore embryogenesis (ME) starts with stress-induced reprogramming of male gametophytic cells (microspores) towards sporophytic developmental pathway. As a result haploid embryos are formed, which can be regenerated into doubled haploid plants (DHs) after the chromosome-doubling process. It is known that ME effectiveness is controlled by numerous factors, but the knowledge concerning the molecular and physiological background behind this process is still fragmentary.

Epigenetic modifications, such as DNA methylation, are involved in many developmental and physiological processes. A positive effect of DNA demethylation was observed in microspore and anther cultures of triticale, where four days of tillers treatment with DNA methylation inhibitor (5-azacytidine, AC) significantly increased ME effectiveness (Nowicka et al., 2019). To widen our understanding of the mechanism of ME initiation, the effect on reactive oxygen species (ROS) generation and antioxidant enzymatic responses were analyzed in two DH lines of triticale significantly differing in embryogenic potential.

ME effectiveness was evaluated in anther cultures. Anthers were excised from control and 5 μM AC pre-treated tillers (the last 4 days). Both treatments proceeded at low temperature (3 weeks at 4°C). The antioxidant enzyme responses (non-specific peroxidases POX, glutathione peroxidase GPX) were determined at the enzymatic activity and gene expression levels.

AC significantly increased ME induction effectiveness of the highly responsive DH line (DH28) due to the decreased production of ROS in parallel with the alteration of endogenous H_2O_2 content. However, the state of ROS was not maintained through POX and GPX. Additionally, RT-PCR analysis showed that expression level of *GSTF2*, a putative gene encoding glutathione transferase, was elevated.

To summarize, DNA demethylation, by modulation of ROS generation, helps to improve ME effectiveness in the highly responsive genotype. Probably, this process in triticale is maintained through ROS – scavenging enzyme, such as glutathione transferase F2 (EC:2.5.1.18) known as enzyme taking part in auxin-activated signalling pathway. Therefore, further examination of this complex phenomenon is still necessary to overcome the recalcitrance in the low responsive genotypes.

ACKNOWLEDGEMENTS

The research was supported by institutional funding of the Institute of Plant Physiology Polish Academy of Sciences, project no. T1Zb1/2018.

REFERENCES

Nowicka A, Juzoń K, Krzewska M, Dziurka M, Dubas E, Kopeć P, Zieliński K, Żur I. 2019. Chemically-induced DNA demethylation alters the effectiveness of microspore embryogenesis in triticale. *Plant Science* 287: 110189.

Optimization of verbascoside and isoverbascoside production in different types of shoot cultures of *Verbena officinalis* – preliminary results

Paweł Kubica[1], Adam Kokotkiewicz[2], Maria Łuczkiewicz[2], Agnieszka Szopa[1], Halina Ekiert[1]

[1] *Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Medyczna 9, 30-688 Krakow, Poland, e-mail: p.kubica@uj.edu.pl*
[2] *Chair and Department of Pharmacognosy, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416, Gdansk, Poland*

Verbena officinalis L. is a medicinal plant with many valuable biological activities, e.g. anti-inflammatory, antimicrobial and secretolytic. For these properties of investigated plant species, among others, phenylpropanoid glycosides such as: verbascoside and isoverbascoside are responsible (Kubica et al., 2018). Our earlier studies confirmed high production of these compounds in *in vitro* cultures of *V. officinalis* (Kubica et al., 2017).

Our recent research is concentrated on optimization of *V. officinalis* shoot cultures conditions for production of both glycosides. The agar cultures, stationary liquid cultures, agitated cultures and cultures grown in Rita bioreactors were maintained on two basal media – Murashige and Skoog (MS) medium and Schenk and Hildebrandt medium (SH). We tested different compositions of plant growth regulators (6-benzylaminopurine – BAP, indole-3-butyric acid – IBA, tidiazurone – TDZ and 2-izopentyloadenine – 2IP) and adenine. The content of metabolites was determined in methanolic extracts from biomasses with HPLC-DAD method (Schönbichler et al., 2013).

The tested *in vitro* conditions showed a strong influence on the content of analyzed phenylpropanoid glycosides. The amounts of verbascoside accumulated in shoot cultures varied from 707 to 4956 mg%. The highest contents of this compound (4956 mg%) was documented in agitated cultures maintained on SH medium with addition of 2 mg/L 2IP and 0.22 mg/L TDZ. The highest amount of isoverbascoside was confirmed in the same conditions but amounts of this compounds in biomass were lower – from 63 to 287 mg%.

The obtained maximal total amounts of verbascoside and isoverbascoside are interesting from practical point of view.

REFERENCES

Kubica P, Szopa A, Dominiak J, Łuczkiewicz M, Ekiert H. 2018. Common vervain (*Verbena officinalis* L.) – botanical characteristics, chemical composition, therapeutic significance, studies on the biological activity and biotechnology researches. *Postępy Fitoterapii* 19(3): 183–194.

Kubica P, Szopa A, Ekiert H. 2017. Production of verbascoside and phenolic acids in biomass of *Verbena officinalis* L. (vervain) cultured under different *in vitro* conditions. *Natural Product Research*, 31(14): 1663–1668.

Schönbichlera SA, Bittner LKH, Pallua JD, Popp M, Abel G, Bonn GK, Huck CW. 2013. Simultaneous quantification of verbenalin and verbascoside in Verbena officinalis by ATR-IR and NIR spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* 84: 97–102.

Agitated and bioreactor's cultures of aronia species – investigations on accumulation dynamics of bioactive phenolic acids during the growth cycles

Paweł Kubica, Agnieszka Szopa, Joanna Żywko, Adrianna Pałka, Halina Ekiert

Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Medyczna 9, 30-688 Krakow, Poland, e-mail: halina.ekiert@uj.edu.pl

Aronia species are a rich source of phenolic acids (PhAs) – very important antioxidants in pharmacy and cosmetology.

Our earlier studies with solid and agitated cultures of *Aronia melanocarpa* (black aronia) and *A.* × *prunifolia* (purple aronia) documented their ability to high production of (PhAs) (Szopa and Ekiert, 2014; Szopa et al., 2018).

The aim of the present studies was the investigations on accumulation dynamics of (PhAs) in agitated cultures of both aronias. Additionally, the establishment of bioreactors cultures (RITA and PlantForm bioreactors) was performed for the first time.

Both types of cultures were maintained on Murashige and Skoog medium with BAP (1 mg/l) and NAA (1 mg/l). In methanolic extracts of biomasses collected in 1-week intervals during 8-weeks growth cycles from agitated culture and after 4-weeks and 8- or 6-weeks growth cycles from bioreactors 26 (PhAs) were estimated by HPLC method (Ellnain-Wojtaszek, 1999).

The presence of the same 11 (PhAs) were documented in biomass extracts of both aronias. The main metabolites in both type of cultures of black aronia were 3,4-dihydroxyphenylacetic acid (3,4-DPhAcA), 3-phenylacetic acid (3-PhAcA), cryptochlorogenic acid (CChlA) and isochlorogenic acid (IChlA). The main metabolites in purple aronia cultures were (3,4-DPhAcA), (3-PhAcA), (IChlA) and chlorogenic acid.

In agitated cultures of black and purple aronias the total amounts of metabolites riched the maximum after 5th-weeks (1238 mg%) and after 4-weeks growth cycles (1139 mg%), respectively. In black aronia bioreactors culture, the higher amounts of (PhAs) were confirmed after 4-weeks growth cycles (787 mg% – RITA and 693 mg% – PlantForm). In purple aronia culture maintained in RITA bioreactor the total amounts of (PhAs) estimated after 4 and 8-weeks were almost the same (693 mg% and 698 mg%, respectively), in PlantForm bioreactor were higher after 4-weeks (869 mg%).

The obtained maximal total amounts of (PhAs) are interesting from practical point of view.

REFERENCES

Szopa A, and Ekiert H. 2014. Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott *in vitro* cultures cultivated on different variant of the Murashige and Skoog medium. *Plant Growth Regulation* 72: 51–58.

Szopa A, Kubica P, Ekiert H. 2018. Agitated shoot cultures of *Aronia arbutifolia* and *Aronia* × *prunifolia*: biotechnological studies on the accumulation of phenolic compounds and biotransformation capability. *Plant Cell, Tissue and Organ Culture* 134: 467–479.

Ellnain-Wojtaszek M, and Zgórka G. 1999. High-performance liquid chromatography and thin-layer chromatography of phenolic acids from *Ginkgo biloba* L. leaves collected within vegetative period. *Journal of Liquid Chromatography and Related Technologies* 22: 1457–1471.

Cell wall composition analysis of transgenic flax (*Linum usitatissimum* L.) plants with increased tocopherol content

Dzmitry Lysh, Karolina Hasiewicz-Derkacz, Anna Kulma

Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland, e-mail: dzmitry.lysh@uwr.edu.pl

Over the past few decades, there has been a growing interest in the use of flax plants thanks to its nutritional and healing properties. The use-value of flax can be improved by increasing the content of compounds influencing both their of survival in the environment, and with a pro-health significance for humans. Examples of such compounds are anitoxidants, such as tocopherols.

The main point of this work is to estimate of the effect of genetic modification increasing the level of tocopherols on the composition of the cell wall of high-linolenic acid flax (*Linum usitatissimum* L.) of line *Opal*, obtained from field cultivation as phenotypic changes were observed in those plants.

Modification of the above-mentioned plants was performed *in vitro* cultures and consisted of the introduction of the *vte-2* gene encoding the key enzyme of tocopherol biosynthesis pathway – homogentisate phytyltransferase from *Arabidopsis thaliana*.

First, the content of components involved in the cell wall of transgenic flax plants (lignin, cellulose and pectin) was determined. Subsequently the effect of transformation on the content of biologically active compounds derived from the terpene and phenylpropanoid biosynthesis pathways was estimated. The chemical analysis of cell wall structure of transgenic plants was confirmed by FT-IR spectroscopy.

The study revealed that a given modification does not significantly affect the structure and composition of the cell wall, but causes changes in the content of phenolic and terpene compounds in transgenic plants in relation to the control.

In vitro germination of *Lachenalia doleritica* seeds

Małgorzata Maślanka, Anna Kapczyńska

Department of Ornamental Plants and Garden Art, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Krakow, Poland, e-mail: m.maslanka@urk.edu.pl

Lachenalia doleritica belongs to ornamental geophytes from Asparagaceae family, naturally grown as endemic species in South Africa. It is highly valued plant amounted to 12–24 cm high with light green and pinkish flowers (Duncan, 2012).

The presented study focused on germination of the *L. doleritica* seeds by means of *in vitro* cultures, as an alternative to traditional propagation methods.

Disinfected, chilled (1 week at 5°C) and non-chilled seeds were sown on 100% MS media containing 3% sucrose. Cultures were maintained at 23 ± 2°C, in darkness and then (after development of cotyledon) under 16h light condition. Gradual germination was observed within 20 weeks. All germinating seeds developed radicle, stolon and cotyledon, respectively. First radicles were seen two weeks after sawing, in the case of chilled seeds. The none-chilled seeds started to germinate five days later. Cotyledons appeared approximately three weeks after sawing. Mean length of the radicles, stolons and cotyledons of 8-week seedlings (from chilled seeds) amounted to: 66 mm, 6.5 mm and 42 mm, respectively. Seedlings from non- chilled seeds, in the same phase of development, were smaller. Mean length of the particular organs amounted to: 58 mm, 5.5 mm and 33.5 mm.

Chilling accelerated and enhanced germination (100%) in comparison with non-chilled seeds (80%) of *L. doleritica*.

REFERENCES

DUNCAN GD. 2012. Botanical Magazine Monograph: The Genus *Lachenalia*. *Kew Publishing, Royal Botanic Gardens, Kew, UK.*

The influence of elicitors for total phenolic content in tissues of *Iris pseudacorus* plants cultured *in vitro*

Angelika Michalak, Aleksandra Królicka

University of Gdansk, Intercollegiate Faculty of Biotechnology UG and MUG, Laboratory of Biologically Active Compounds, Abrahama 58, 80-307 Gdansk, Poland, e-mail: angelika.michalak1@gmail.com

Iris pseudacorus, also known as yellow flag iris, is a fast growing and widely spread representative of the Iridaceae family, native to Europe, Western Asia and North Africa. It is an invasive species growing mostly on wetlands and banks of water reservoirs. It developed mechanisms of resistance to stressors like anoxia (due to submersion of the underground parts of the plants); pollutants of water and soil like heavy metals, pharmaceuticals, organic matter; as well as to herbivores and competitors.

Such comprehensive adaptations are often connected with complex production of secondary metabolites in plant tissue. It is already known that *I. pseudacorus* synthesizes phenolic compounds (mangiferin, genistein, quercetin, kaempferol or daidzein). Extracts can modulate differentiation of osteoblasts and osteoclasts and contain compounds responsible for estrogenic activity observed both *in vitro* on cell lines and *in vivo* in rats. Polyphenols isolated from roots also can inhibit spontaneous colony formation of colon carcinoma cells HT-29.

Taking under consideration high biomass production and easily accessible source of the tissues, *I. pseudacorus* is a perfect candidate to look for biologically active compounds desirable in industry. Having in mind care for biological diversity in nature and limited resources of culture place, micropropagation in *in vitro* conditions is a suitable alternative. This brought us to ask two questions. Is it possible to increase the level of polyphenolic compounds in *in vitro* culture of *I. pseudacorus* plants in comparison to plants growing *in vivo*? Which stressors or factors can increase level of polyphenolic compounds in its tissues?

To answer these questions, two different approaches of the *in vitro* culture and six elicitors were used. *I. pseudacorus* was successfully introduced to *in vitro* from seeds. Plants cultured in liquid Murashige and Skoog medium in Erlenmeyer flasks with continuous shaking or in temporary immersion system – Plantform, were treated with stress factors. As biotic elicitors: bacterial lysates of *Cronobacter sakazakii* and *Dickeya dadanti*, also methanolic extract from *Aphid* sp., were used. As abiotic elicitors: jasmonic acid, phenylalanine and reduction of nitrogen source in medium were examined. Elicitors were added to the culture medium to stimulate phenolic production. Methanolic extracts were prepared from roots and stems and examined with the colorimetric Folin-Ciocialteu modified method. Metabolic profiles were also analyzed with the use of High-Performance Liquid Chromatography and compared with extracts from untreated plants.

Preliminary results suggest that jasmonic acid increased the most phenolic production in *I. pseudacorus* tissue.

ACKNOWLEDGEMENTS

The scientific work is funded by Ministry of Science and Higher Education – Diamond Grant 0145/DIA/2015/44.

Tolerance to zinc and lead of the suspended cells, derived from metallophytes of the calamine soils

Szymon Miszczak[1], Klaudia Sychta[1], Ewa Gregoraszczuk[2], Aneta Słomka[1]

[1] *Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University in Kraków, Gronostajowa 9, 30-387 Krakow, Poland,*
e-mail: szym.miszczak@gmail.com, klaudia.michno@doctoral.uj.edu.pl, aneta.slomka@uj.edu.pl
[2] *Department of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Science, Jagiellonian University in Kraków, Gronostajowa 9, 30-387 Krakow, Poland,*
e-mail: ewa.gregoraszczuk@uj.edu.pl

Several metallophytes *e.g. Viola tricolor, Silene vulgaris* ssp. *humilis, Arabidopsis hallerii, Armeria maritima* occur abundantly on calamine soils polluted with high concentrations of Zn, Pb, Cd in Southern Poland (Nowak et al., 2011). So far, their tolerance to these metals has been tested mostly at the seedling and adult plant stage development (Wierzbicka, 2015). Here, we applied fairly new approach based on establishing cell suspension cultures from the callus tissue of these metallophytes obtained from their leaf fragments cultured on solidified half-strength MS medium supplemented with 2 mg L^{-1} 2,4-D and 2 mg L^{-1} BAP. Viability of cells in suspensions treated with 0, 200, 500 and 1000 μM of $ZnSO_4 \times 7H_2O$ or $Pb(NO_3)_2$ for 24, 48 and 72h was measured using alamarBlue assay as described by Sychta et al. (2018). Metallophytes differed in their level of tolerance to Zn and Pb. *A. hallerii* and *S. vulgaris* ssp. *humilis* were tolerant to Zn, but poorly tolerant to Pb, contrary to *A. maritima* which, taking into account high Pb toxicity, revealed unexpected tolerance to Pb (75% of living cells after 72h). All three investigated species revealed overall lower tolerance than the violets, facultative metallophyte, *V. tricolor* and obligate metallophyte, *V. lutea* ssp. *westfalica* (Sychta et al., 2018). Higher cell tolerance levels of *Viola* metallophytes than the metallophytes of the other genera, suggest the development of specific adaptations in *Viola.*

REFERENCES

Nowak T, Kapusta P, Jędrzejczyk-Korycińska M, Szarek-Łukaszewska G, Godzik B. 2011. The vascular plants of the Olkusz ore-bearing region. *W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.*

Sychta K, Słomka A, Suski Sz, Fiedor E, Gregoraszczuk E, Kuta E. 2018. Suspended cells of metallicolous and non-metallicolous *Viola* species tolerate, accumulate and detoxify zinc and lead. *Plant Physiology and Biochemistry* 132: 666–674.

Wierzbicka M (ed.). 2015. *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa.

Morphogenetic response of *Lilium candidum* L. bulb scales to different light qualities *in vitro*

Piotr Pałka, Bożena Pawłowska

Department of Ornamental Plants and Garden Art, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland, e-mail: piotr.palka@student.urk.edu.pl

Lilium candidum L. is valuable medicinal and ornament species in Liliaceae family. It is native to Mediterranean and Minor Asia region and it is treated as an endangered species in Israel. It is early flowering perennial with pure white and fragrant flowers that are popular in flower arrangements and garden art in Europe. Madonna lily contains biologically active compounds that are the objectives of pharmaceutical studies and harvesting in its natural environment. Traditional methods of both vegetative and generative propagation are not sufficient enough to meet the market demand. All these makes *L. candidum* L. an important subject of micropropagation attempts. LED light systems are energetically efficient and give an opportunity of spectrum selection which influences organogenesis *in vitro*.

Bulb scales used in the study have been obtained from the *in vitro* bulbs stored at 6°C for twelve months. Inner and outer scales were maintained separately on Murashige and Skoog solid medium without plant growth regulators. The aim of the study was to determine the effect of light quality on adventitious organogenesis. Different light wavelengths were tested: white LEDs; monochromatic blue (B); monochromatic red (R); a mixture of red and blue LEDs in the 7:3 ratio (RB); these mixture improved with far red (RBfR), yellow (RBY), UV (RBUV) and green (RBG). Fluorescent light served as a control and one part of bulb scales was cultivated in darkness.

After 6 weeks of culture we observed bulbs, stems and roots formation. Percent of the regenerating scales range from 73.8% in darkness to 94.3% under fluorescent lamp. Outer scales regenerate in higher percent (87.1%) comparing to inner scales (77.9%).

Our results showed that under RBfR light outer scales gave the best results in most examined parameters of adventitious organogenesis. Percent of scales regenerating bulbs was 90.8%, single scale formed 1.3 bulb in average that gave 14.4 new bulbs form the single initial bulbs.

The highest number of stems (2.5) developed under white LEDs with the longest ones (21.1mm) under RBfR light. The highest root formation occurred (0.6 per one scale) under RBG and fluorescent light, the longest roots developed under the same type of light.

Identification of genes involved in the metabolism of cyanogenic glycosides (CGs) and examination of their expression during flax (*Linum usitatissimum*) development

Katarzyna Pelc[1], Jakub Szperlik[2], Magdalena Żuk[1]

[1] *University of Wroclaw, Faculty of Biotechnology, Department of Genetic Biochemistry, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland, e-mail: pelc.katarzyna@yahoo.com*
[2] *Wroclaw University of Environmental and Life Sciences, Faculty of Life Sciences and Technology, Department of Plant Breeding and Seed Production, pl. Grunwaldzki 24A, 50-363 Wroclaw, Poland*

Cyanogenic glycosides (CGs) have been identified in approximately 2,600 plant species, which is almost 1% of all plant species described to date. There were identified in almond (amygdalin), manioc, sorghum, acacia, plum, apple, yew or flax. They are the main source of hydrogen cyanide in plants. By far, 4 cyanogenic glycosides have been demonstrated in flax: two L-valine derivatives (linamarin and linustatin) and two L-isoleucine derivatives (neolinustatin and lotaustralin). The main function of these compounds is protection against pathogens and herbivores, hence their highest concentrations are observed in seeds and seedlings. The content of CGs changes significantly during plant growth, it also depends on the mineral economy (sulfur content) and many environmental factors.

Cyanogenic monoglycosides are formed in a several-step process starting with L-amino acids. Then monoglycosides can be glycosylated to diglycosides, and both groups, under the influence of beta-glycosidases, are hydrolyzed to cyanohydrins. Cyanohydrins dissociate to carbonyl compounds to release hydrogen cyanide, which can be neutralized by conjugation with glutathione or incorporation into cysteine to form asparagine. If HCN is not neutralized, it can lead to blockage of the mitochondrial respiratory chain in a potential consumer.

Using IT tools, based on homology to the sequence of known genes from other cyanogenic plants, potential sequences encoding genes of CGs metabolism were identified in the flax genome. *In silico* analysis has been experimentally verified by checking the presence of transcripts in genetic material isolated from Linola variety flax. An analysis of the expression of selected genes associated with the cyanogenic glycoside pathway in development stages was performed. The tissue was harvested at the following time points: 2 days, 6 days (seedlings), 10 days, 20 days, 30 days, 40 days (plants at the stage of vegetative growth), in flowers, young seed baskets and seeds. Gene expression was verified using Real-time PCR. Literature premises and own data indicate the significant importance of 3 enzymes: valine monooxygenase, hydroxynitrilase and linamarase.

REFERENCES

Niedźwiedź-Siegień I. 1998. Cyanogenic Glucosides In *Linum usitatissimum*. *Phytochemistry* 49: 59–63.

The effect of salt stress on the growth and development of Lemnaceae aquatic plants

Krzysztof Piotrowski[1], Zdzisława Romanowska-Duda[1], Maria Skłodowska[2], Marcin Naliwajski[2]

[1] *Department of Plant Ecophysiology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 92-237 Lodz, Poland, e-mail: zdzislawa.romanowska@biol.uni.lodz.pl*

[2] *Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 92-237 Lodz, Poland*

The increasing salinity of surface waters and soils leads to environmental degradation and serious economic problems. The negative effects of salt stress have increased in recent years due to the significant reduction in fresh water resources, the use of more and more salt in industry and agriculture. The studies have confirmed that a high concentration of salt has a destructive effect on fresh surface waters and causes disturbance of hydrobiological balance.

The aim of the study was to assess the effect of salt stress on the growth and development of macrophytes from the Lemnaceae family. The experiment was conducted in laboratory conditions on the model *Spirodela polyrrhiza* plant grown *in vitro* at the Department of Plant Ecophysiology at the University of Lodz. The plants were grown on a diversified culture medium including: standard "Z" medium, tap water, postferment from biogas plant in Piaszczyna, which were supplemented with various NaCl concentrations ranging from 25 to 100 nM. Plant cultivation was carried out under phytotron conditions at 24°C. After 10 days of culture, analyses of plant growth, fresh and dry biomass, as well as physicochemical parameters, i.e. chlorophyll content index, gas exchange parameters (net photosynthesis, transpiration, stomata conductivity and intercellular CO_2 concentration), chlorophyll fluorescence measurement were performed. On the last day of the experiment, the percentage of starch was determined in *Spirodela* shoots using the Starch Assay Kit from Sigma-Aldrich, product Code STA20. The performed analyses and tests are commonly used indicators of the metabolic activity of plants (Badek et al., 2014).

The obtained results showed that among the tested NaCl concentration variants, the one with tap water supplemented with 50 mM NaCl most effectively promoted high starch production by the macrophytes. The analysis of individual parameters of Lemnaceae plant growth and development indicates new biotechnological possibilities of this group of plants (in addition to the so-far confirmed: phytoremediation of waters, bioindication properties, animal feed supplementation) and confirms that these macrophytes can be successfully used in the production of biofuels (bioethanol).

ACKNOWLEDGEMENTS

The research was financed by National Center for Research and Development in Poland, Grant BIOSTRATEG2/296369/5/NCBR/2016

REFERENCES

Badek B, Romanowska-Duda Z, Van Duijn B, Grzesik M. 2014. Rapid evaluation of germinability of primed china aster (*Callistephus chinesis* Ness.) seeds with physiological and biochemical markers. *Journal of Horticultural Research* 22: 2.

Potential of the *Leucojum aestivum* L. endophytic bacteria to promote Amaryllidaceae alkaloids biosynthesis

Agata Ptak[1], Magdalena Simlat[1], Emilia Morańska[1], Rosella Spina[2], Francois Dupire[2,3], Sonia Kozaczka[1], Dominique Laurain-Mattar[2]

[1] *Department of Plant Breeding, Physiology and Seed Science, University of Agriculture in Krakow, Podłużna 3, 30-239 Krakow, Poland, e-mail: mfptak@cyf-kr.edu.pl, magdalena.simlat@urk.edu.pl, emilia.moranska@gmail.com*

[2] *Université de Lorraine, CNRS, L2CM, F-54000 Nancy, France, e-mail: rosella.spina@univ-lorraine.fr, dominique.mattar@univ-lorraine.fr*

[3] *Service Commun de Spectrométrie de Masse, F-54000 Nancy, France*

Leucojum aestivum L. is a natural source of Amaryllidaceae alkaloids, especially galanthamine and lycorine. Galanthamine is an acetylocholinesterase inhibitor used for the treatment of Alzheimer's disease, while lycorine may inhibit cell division. Several research works indicated that biotechnological methods using *L. aestivum in vitro* cultures could be alternative sources of Amaryllidaceae alkaloids for total chemical synthesis and extraction from field-grown plants (Laurain-Mattar and Ptak, 2018; Ptak et al., 2019). Exploitation of that endophytic microorganism as a secondary metabolite-promoting agent is a very important strategy in the biotechnological production of bioactive compounds.

We isolated the endophytic bacterium from plant cultures of *L. aestivum*, and basing on the data from the 16S rRNA gene sequence we identified it as *Paenibacillus lautus*. Subsequently, we investigated the effects of dead endophytic bacterial elicitors, used at concentrations of 0 (control), 0.01, 0.02 and 0.04% for 7, 14 and 28 days, on biomass and Amaryllidaceae alkaloid production in *L. aestivum in vitro* plants. Elicitation with 0.04% extract of *P. lautus* for 28 days stimulated the growth of *L. aestivum* plants. Capillary GC-MS led to the identification of five alkaloids in obtained plant materials. Their number depended on the concentration and duration of the elicitor action. The highest galanthamine (44.47 μg/g DW) and lycorine (235.73 μg/g DW) contents were observed in plants treated with 0.02% bacterial extract for 14 days.

This is the first report on the isolation of *P. lautus* from *in vitro* culture of *L. aestivum* and the use of that endophytic bacterium as an elicitor of Amaryllidaceae alkaloid biosynthesis.

ACKNOWLEDGEMENTS

The research has been performed within the framework of the international Polonium programme (DWM.WKE.183.10.2018/AM, 2018–2019).

REFERENCES

Laurain-Mattar D, and Ptak A. 2018. Amaryllidaceae alkaloid accumulation by plant *in vitro* systems. In: Pavlov A, Bley T (eds). *Bioprocessing of plant in vitro systems.* Springer International Publishing AG, part of Springer Nature, Switzerland, pp 203–220.

Ptak A, Simlat M, Morańska E, Skrzypek E, Warchoł M, Tarakemeh A, Laurain-Mattar D. 2019. Exogenous melatonin stimulated Amaryllidaceae alkaloid biosynthesis in *in vitro* cultures of *Leucojum aestivum* L. *Industrial Crops and Products* 138: 111458.

The model of sustainable aquatic plant biomass production for biofuels

Zdzisława Romanowska-Duda, Agata Pszczółkowska, Krzysztof Piotrowski, Wiktor Pszczółkowski, Andrzej Stępiński

Department of Plant Ecophysiology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha Str. 12/16, 92-237 Lodz, Poland, e-mail: zdzislawa.romanowska@biol.uni.lodz.pl

Currently, the share of renewable energy sources in the EU's energy balance is estimated at 18%. By 2020, the World Commission of the Energy Council predicts an increase in the share of renewable energy to 21.3%, and the optimistic scenario assumes an increase of up to 29.6%. Such a high share of renewable carriers in the energy balance requires the activation and application of mechanisms supporting the development of renewable energy sources, including the development of biomass from plants, which can be used for direct combustion, production of biogas or liquid fuels (biodiesel and bioethanol) (Grzybek, 2011).

The research determined the effect of the addition of post-fermentation effluents from biogas plants (post-fermentation effluents, PFE) on the growth and development of aquatic plants belonging to the Lemnaceae family (*Spirodela polyrrhiza* and *Lemna minor* L.) and microalgae (*Chlorella* sp.). *Lemna minor* L. and *Spirodela polyrhiza* cultures were carried out in the media containing 2.5% PFE and microalgae ranging 0-20%. All experimental variants indicated the possibility of using PFE in breeding media, and its content can be increased up to 20%.

The results of analyzed parameters: plant growth, chlorophyll index, fresh plant weight, and parameters of the photosynthesis process (net photosynthesis, stomatal conductance, transpiration and concentration of intercellular CO_2) indicated a beneficial effect of PFE on the growth of *Lemna minor* L. and *Spirodela polyrhiza.* The results obtained for *Chlorella sp*. in terms of cell density and measurement of chlorophyll fluorescence also indicated the potential for growing microalgae in the presence of PFE.

Conclusion: Lemnaceae plants and microalgae can be grown on the basis of PFE obtained from biogas plants, causing at the same time reduction of this waste and its utilization as part of the circular economy. Plants of the Lemnaceae family and microalgae can be useful in the production of bioethanol.

ACKNOWLEDGEMENTS

The research was financed by National Center for Research and Development in Poland, Grant BIOSTRATEG2/296369/5/NCBR/2016

REFERENCES

Grzybek A. 2011. *Alternatywne źródła energii i ich zastosowanie*. Polskie Towarzystwo Biomasy.

Stimulation of tuber induction in *Drosera gigantea*

Michał Rzeszotarski, Aleksandra Królicka

University of Gdansk, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Laboratory of Biologically Active Compounds, Abrahama 58, 80-307 Gdansk, Poland, e-mail: m.rzeszotarski45@gmail.com

Drosera gigantea is an endemic carnivorous plant, native to Western Australia, belonging to the perennial, tuberous genus *Drosera* (family Droseraceae) which is known from its ability to catch and digest small animals. Plants from Droseraceae family were used in traditional medicine, for example for their antitussive properties. Plants from *Drosera* species are rich in secondary metabolites like naphthoquinones and flavonoids. Plumbagin which is main secondary metabolite in carnivorous plants possess antibacterial, antifungal and cytotoxic activity. The propagation can be difficult because most of the species do not produce seed in cultivation and they are difficult to grow from leaf cuttings (Kim and Jang, 2004). *D. gigantea* produces tubers which have high potential in micropropagation. Each tuber can regenerate to mature plant. Because the production of secondary tubers is a slow process (Dixon and Pate, 1978), a method to increase tuber production could lead to more efficient micropropagation and receiving more plants from *in vitro* culture. The aim of the presented research was to increase tuber production in *in vitro* grown *D. gigantea*.

During our research we initiated cultures of *D. gigantea* on solid (0.75% agar), half strength Murashige and Skoog (½ MS) medium with 2% of sucrose and 0.1% MES, pH 5.5 at a temperature of 20°C, light 30-35 μmol/m^2×s and photoperiod 16/8 h. For tuberization process various combinations of growth regulators [6-benzylaminopurine (BAP), abscisic acid (ABA)] and different concentrations of sucrose were tested.

We analyzed the effect of different culture conditions on tuberization process: sucrose concentration (4 and 8%) and plant growth regulators (1.5 mg/L ABA; 1.5 mg/L ABA + 5.0 mg/L BAP; 1.5 mg/L ABA + 0.5 mg/L BAP). After eight weeks of growth, the number of tubers in each medium was counted and the tubers were weighted.

The best results were obtained for medium with 4% sucrose and 1.5 mg/L ABA + 5.0 mg/L BAP, where addition of plant growth regulators increased number of tubers by 259% in comparison to control medium (½ MS + 4% sucrose, but without plant growth regulators). Increasing medium sucrose concentration from 4% to 8% resulted in reduced plant viability and fitness as well as in lower average tuber mass.

ACKNOWLEDGEMENTS

The scientific work is funded by the Polish Ministry of Science and Higher Education [grant number DS 530-M035-D673-18].

REFERENCES

Kim KS, and Jang GW. 2004. Micropropagation of *Drosera peltata*, a tuberous sundew, by shoot tip culture. *Plant Cell, Tissue and Organ Culture* 77: 211–214.

Dixon KW, and Pate JS. 1978. Phenology, morphology and reproductive biology of the tuberous sundew, *Drosera erythrorhiza* Lindl. *Australian Journal of Botany* 26: 441–454.

Cyanogenic glycosides in flax (*Linum usitatissimum*) – metabolism and biologic activity assay

Agnieszka Sawuła

Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland, e-mail: agnieszka.sawula2@uwr.edu.pl

Flax (*Linum usitatissimum*) is a plant commonly used as a food and in industry. Significant losses of crop is caused by *Fusarium oxysporum* infections. One of defense mechanism evolved by flax is synthesis of cyanogenic glycosides: linamarin, linustatin, neolinustatin and lotaustralin. Cyanide – cyanogenic glycosides degradation product is reassimilated with sulphuric aminoacids use. Influence of sulphur content in medium and *Fusarium* infection were investigated. Cyanogenic glicosydes synthese and gene involved in its metabolism expression were examined. Transgenic CD flax line accumulating sulphuric aminoacids showed reduced cyanogenic glycosides content in compare to Linola line. It was proved also that changes in cyanogenic glycosides synthesis genes are connected with changes in expression of genes responsible for their reassimilation. It is especially pronounced in CD line in *Fusarium* infection response.

Protective effect of manganese ions on zearalenone and its metabolites stress in wheat

Magdalena Skórka, Apolonia Sieprawska

Institute of Biology, Pedagogical University, Podchorążych 2, 30-084 Krakow, Poland, e-mail: apolonia.sieprawska@up.krakow.pl

Zearalenone, mycotoxin produced by *Fusarium* fungi, with its structure reminds an estrogen molecule, may displays a hormone-like effect in cells. Accumulated in the tissues of plants that constitute the basics of human and animal diet usually act carcinogens and initiate pathogenic changes in various organs (Ismaiel and Papenbrock, 2015) Thus, many methods are searched for prevent the absorption of this substance. The mechanism of toxic effect of zearalenone in plants is based on the concept of involving the oxidative stress, as the final stage of a series of reactions occurring in cells.

The aim of the study was to check whether the addition of Mn ions may protect plants against ZEN diminishing the effects of oxidative stress action. The presence of Mn ions in antioxidant enzymes (e.g. superoxide dismutase), may suggest their direct involving in cell protection. Since ZEN after penetration into cells, undergoes biotransformation into derivatives such as α-zearalenol and α-zearalanol, the toxicity of ZEN versus its derivatives was also examined.

The experiments were performed in the in vitro conditions for calli cells of two wheat cultivars (sensitive and tolerant to oxidative stress) where ZEN and its derivatives were applicated to culture media at stressogenic concentration (30 μM) and Mn ions at non-stressogenic amounts. It was found that the introduction of especially ZEN and α-zearalenol to the culture media reduced the growth of calli in the sensitive wheat and this effect was dependent on the kind of toxin. Mn presence partly reduced the negative action of tested mycotoxins on growth of calli. The changes of antioxidative enzymes activity in what cells confirmed the stimulation of oxidative stress under these mycotoxins' absorption. The lowered activity of SOD, POX, APX and CAT in the presence of Mn ions, in comparison to the increase observed under mycotoxins application, confirmed the Mn role in defense mechanisms, especially against ZEN and α-zearalenol induced stress. On the basis of this experiment the toxicity of tested mycotoxins was established as: ZEN ≥ α-zearalenol > α-zearalanol. For the media supplemented with Mn and ZEN or its derivatives it was noticed a significant protecting effect vs. the media with toxins alone.

ACKNOWLEDGEMENTS

This work was supported of a project of the National Center of Science (NCN) Poland No. 2014/15/B/NZ9/02192.

REFERENCES

Ismaiel A, and Papenbrock J. 2015. Mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture* 5: 492–537.

Studies on the accumulation of selected secondary metabolites in agitated shoot cultures of *Centella asiatica* L. Urban

Ewa Skrzypczak-Pietraszek, Aleksandra Twardowska, Paweł Żmudzki, Agnieszka Szewczyk

Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: ewa.skrzypczak-pietraszek@uj.edu.pl

Centella asiatica L. Urban (Apiaceae) is an important pharmacopoeial plant used not only in medicine but also in cosmetology (European Pharmacopoeia 9th Edition, 2017).

The aim of the experiment was to study the influence of different concentrations of plant growth regulators (BAP, NAA, GA_3) and standard (3%) or increased (4.5%) saccharose content in the medium on the biomass growth and plant secondary metabolites accumulation (phenolic acids, flavonoids, triterpene saponins). *Centella asiatica* agitated shoot cultures were established on Murashige and Skoog (MS) (Murashige and Skoog, 1962) liquid medium, and maintained for 4 weeks. Then the biomass was collected and dried. Methanol and ethanol extracts were prepared from the dried biomass. Qualitative and quantitative HPLC analysis of phenolic acids and flavonoids and LC/MS analysis of triterpene saponins were carried out.

Increased to 4.5% saccharose content in the medium stimulates the biomass growth. The following variants are the best growth media: A1(MS BAP – 1 mg/l, NAA – 0.5 mg/l, GA_3 – 0.25 mg/l, saccharose 4.5%), C1(MS BAP – 2 mg/l, NAA – 2 mg/l, saccharose 4.5%). The presence of the following secondary metabolites was confirmed in the biomass extracts: 5 phenolic acids (chlorogenic acid, rosmarinic acid, neochlorogenic acid, *p*-coumaric acid, *o*-coumaric acid), cinnamic acid, 6 flavonoids (cinnaroside, rutoside, luteolin, kaempherol, quercitrin, myricetin) and two triterpene saponins (asiaticoside, madecassoside). The highest metabolite contents in the biomass were: 296.92 mg/100 g DW for the total sum of phenolic acids and cinnamic acid (variant B – MS BAP – 2 mg/l, NAA – 0.2 mg/l); 1009.03 mg/100 g DW for the total sum of flavonoids (variant B); 16.62 mg/ 100 g DW for the total sum of triterpene saponins (variant C – MS BAP – 2 mg/l, NAA- 2 mg/l). The highest productivity of biotechnology process (mg of the compound/culture flask) was observed on medium variant A for the most of the metabolites: 6.79 mg/ flask for the total sum of phenolic acids and cinnamic acid; 25.85 mg/flask for the total sum of flavonoids; 0.42 mg/flask for the total sum of triterpene saponins.

The highest, maximum contents of the analyzed compounds in the *in vitro* culture biomass usually exceed their concentration in the mother greenhouse plant. *Centella asiatica* agitated shoot cultures could be a potential source of biologically active secondary metabolites.

REFERENCES

Centellae asiaticae herba. 2017. In: *European Pharmacopoeia 9th Edition, European Directorate for the Quality of Medicines, Council of Europe* 1311–1311.

Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15(3): 473–497.

Accumulation of flavonoids in biomass of *Schisandra henryi in vitro* cultures

Agnieszka Szopa[1], Karolina Jafernik[1], Magda Barnaś[1], Michał Dziurka[2], Halina Ekiert[1]

[1] *Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Krakow, Poland, e-mail: a.szopa@uj.edu.pl, karolina.jafernik@doctoral.uj.edu.pl*
[2] *Polish Academy of Sciences The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Krakow, Poland*

The object of our study was endemic for Yunnan province in China, species – *Schisandra henryi* C.B. Clarke. This plant species has not been fully researched yet, but some available studies show similarity of its biological activity with *Schisandra chinensis* (Turcz.) Baill. *S. chinensis* is a pharmacopoeial plant species which shows e.g. hepatoprotective, antioxidant and anticancer activity (Szopa et al., 2019*)*.

The aim of the study was the analysis of flavonoids in extracts of agar microshoot and callus cultures, as well as leaves of parent plant, using the DAD-HPLC method (Ellnain-Wojtaszek and Zgorka, 1999).

The microshoot cultures were maintained on 6 MS medium variants, differing in concentrations of the PGRs: BA, IBA and GA_3 in the range from 0 to 3 mg/l. The callus cultures were cultivated on MS medium with 1 mg/l BA and 1 mg/l IBA. The cultures were maintained over 30 days growth periods (3 series). The leaves were harvested from the parent plant in May and September 2016 ("Clematis", Poland).

In methanolic extracts from biomasses of *in vitro* cultures as well as of parent plant leaves, the presence of 6 flavonoids, out of 14 tested, was confirmed: hyperoside, rutoside, trifolin, quercitirin, querctein and kaempferol. Their contents were dependent on culture type and MS medium variants. The highest individual, and total (421.98 mg/100 g DW) flavonoid amounts were reached in microshoots cultivated on MS medium with 1 mg/l BA, 1 mg/l IBA and 0.25 mg/l GA_3. The main compounds were: triofilin (max. 138.56 mg/100 g DW) and quercitirin (max. 122.54 mg/ 100 g DW).

In the leaf extracts the total flavonoid amounts were respectively 1.44- and 2.22-times lower than their maximal amount in *in vitro* cultures. The main compounds were also triofolin and quercitirin.

Our study proved the high utility of biomass of *S. henryi in vitro* cultures as rich source of flavonoids. This is the first report on flavonoids accumulation in *S. henryi in vitro* cultures.

ACKNOWLEDGEMENTS

Funded by Polish Ministry of Science and Higher Education (K/DSC/004297).

REFERENCES

Szopa A, Barnaś M, Ekiert H. 2019. Phytochemical studies and biological activity of three Chinese *Schisandra* species (*Schisandra sphenanthera*, *Schisandra henryi* and *Schisandra rubriflora*): current findings and future applications. *Phytochemistry Reviews* 18: 109–128.

Ellnain-Wojtaszek M, and Zgorka G. 1999. High-performance liquid chromatography and thin-layer chromatographyof phenolic acids from *Ginko biloba* L. leaves collected within vegetative period. *Journal of Liquid Chromatography and related Technologies* 22: 1457–1471.

Comparison the efficiency of obtaining haploids of winter wheat at the *In Vitro* Laboratory in Poznan Plant Breeding

Magdalena Taciak, Maria Zdziechowska-Dudek, Justyna Patelska, Michał Rokicki

Poznan Plant Breeding Sp. z o.o., Wiatrowo Plant Breeding Branch, Wiatrowo 16, 62-100 Wągrowiec, Poland, e-mail: magdalena.taciak@phr.pl

Two major methods for producing wheat doubled haploids (DH) are androgenesis (anther culture) and embryo culture using wide hybridization between wheat and maize. The aim of the study was to compare the efficiency of obtaining haploids of winter wheat. Greenhouse-germinated seedlings were vernalized for eight weeks then grown under greenhouse conditions for four to five weeks.

Androgenic cultures of winter wheat were led in accordance with the methodology used in the In Vitro Laboratory of Poznan Plant Breeding. Spikes with microspores in the late uninucleate stage were collected and cold pre-treated (4°C) to stimulate androgenetic development. Anthers were isolated aseptically and transferred onto Petri dishes containing modified induction media: C17 (Wang and Chen, 1983) or 190-2 (Wang and Hu, 1984). In all experiments, cultures were grown in the dark at 26°C. Androgenic structures (calli and embryos) at approximately 1 mm in diameter were successively transferred onto the modified 190-2 regeneration medium (Wang and Hu, 1984).

In experiments using *in vitro* culture of embryos, spikes of wheat were given castrated, pollinated and treated by 2,4-D solution in glasshouse conditions. Embryos were isolated 20 days after pollination and next were placed on the B5 medium (Gamborg et al., 1968), cultures were grown in the dark at 26°C.

Our results showed a significant difference in the number of regenerants of winter wheat obtained, depending on the methods used. Embryo culture using wide hybridization between wheat and maize is an effective and handy tool among available methods for haploid induction in wheat. Its superiority over other methods includes higher efficacy, simple and less genotype dependent response.

ACKNOWLEDGEMENT

The research is carried out within the project entitled “New trends and technologies in breeding of cereals and legumes for sustainable agriculture”, co-financed by the National Centre for Research and Development from the European Regional Development Fund, grant agreement no. POIR.01.01.01-00-0449/18-01.

REFERENCES

Gamborg OL, Miller RA, Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* 50: 151–158.

Wang P, and Chen Y. 1983. Preliminary study on prediction of height of pollen H2 generation in winter wheat grown in the field. *Acta Agronomica Sinica* 9: 283–284.

Wang XZ, and Hu H. 1984. The effect of potato II medium for triticale anther culture. *Plant Science Letters* 36: 237–239.

Morphogenetic potential of *Hypericum perforatum* L. under *in vitro* conditions and its secondary metabolites level estimation from differential natural sites

Monika Tuleja[1], Aleksandra Brankiewicz[1], Maciej Gustab[1], Diana Saja[2], Elżbieta Szostak[3]

[1] *Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University in Kraków, Gronostajowa 9, 30-387 Krakow, Poland, e-mail: monika.tuleja@uj.edu.pl*
[2] *Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Krakow, Poland*
[3] *Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland*

Considered an antidepressant and anti-anxiety agent, *Hypericum perforatum* L. owns this properties to several bioactive constituents e.g. flavonol derivatives (quercetrin) as well as naphthodiathrones such as hypericin. What is more, hypericin showed to be cytotoxic, and it seems to be a chemopreventive agent and a good candidate for antineoplastic drug development (Mirmalek et al., 2016). Because of another pharmacological properties: wound healing, antiflammatory, antiviral, antimicrobal and antioxidant effect (Sevastre-Berghian et al., 2018) *H. perforatum* presently is one of the most consumed medicinal plant all over the world. Southwell and Bourke (2001) revealed that the concentration of this active substances can be seasonal dependent. Taking into account this variability, it seems interesting whether, depending on the place of growth, the content of active substances changes or not, and whether the *in vitro* conditions also affect their content. Therefore the protocol for *H. perforatum* regeneration *in vitro* based on indirect organogenesis was established. The best influence among used phytohormones (NAA+BAP, 2,4-D+KIN and TDZ) on the internode culture maintained in the light, had thidiazuron (TDZ). In this conditions the organogenesis efficiency was 13.63 with 71.43% reacting explants and here callus was inducted with the biggest intensity. Second type of used explants, parts of leaf blades reacted better in the darkness, were they produced callus in the higher intensity than in the light. *H. perforatum* was collected from several places (meadows: 1-Pychowice, 2-Bieszczady, 3-Skotniki, 4- Norymberska Kraków antropogenic site) and their leaves and internodes were tested for bioactive substances content using FT-RS and FT-IR spectroscopy. The analysis of FT-Raman spectroscopy of the chemical composition of St. John's wort leaves showed that the plants differed from each other depending on the places of growth. Most differences were observed in carotenoids and flavonoid compounds. The highest content was observed in plants which growing in the first site, and the lowest in the third, what coincides with FT-IR data considering "C-O" content. Comparison of chemical compounds in leaves and internodes showed first of all that there was a decrease in their content in the internode in relation to leaves.

REFERENCES

Mirmalek SA, et al. 2016. *Cancer Cell International 16: 3.*

Southwell IA, and Bourke CA. 2001. *Phytochemistry* 56: 437–41.

Sevastre-Berghian AC, et al. 2018. *Journal of Physiology and Pharmacology* 69: 789–800.

In vitro culture of carnivorous plants with antioxidant activity

Weronika Wojciechowska[1], Angelika Michalak[1], Wojciech Makowski[2], Michał Rzeszotarski[1], Marta Krychowiak-Maśnicka[1], Aleksandra Królicka[1]

[1] *University of Gdansk, Intercollegiate Faculty of Biotechnology UG and MUG, Laboratory of Biologically Active Compounds, Abrahama 58, 80-307 Gdansk, Poland, e-mail: aleksandra.krolicka@biotech.ug.edu.pl*

[2] *Unit of Botany and Plant Physiology, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Krakow, Poland, e-mail: wojtek.makowski.1305@gmail.com*

The *Drosera* genus is a natural source of pharmacologically important secondary metabolites. *Droserae herba* is a source of cyanogens, flavonoids, naphthoquinones, proteolytic enzymes, anthocyanins and phenolic compounds. Due to the fact that antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of free-radical-mediated diseases, the aim of our study was to examine the antioxidant potential of extracts from 28 plants from *Drosera* species.

Plants from *Drosera* sp. were grown *in vitro* on ½ Murashige and Skoog medium with 2% sucrose and 1.5% active carbon, pH 5.5. Plant tissues were extracted with two methods: ultrasonic assisted extraction in methanol and microwave-assisted extraction in distilled water. The obtained extracts were tested for antioxidant properties with the use of the DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) assay. DPPH is a free radical which undergoes decolorization when it binds electron. Methanol extract at concentrations from 10 to 0.15 mg/ml fresh weight (FW) and aqueous extracts at 30 to 0.5 mg/ml FW were added to DPPH solution (final concentration 0.1 mM). After 30 min of incubation at room temperature decolorization of solution was measured at 517 nm wavelength. The decrease in absorbance of DPPH radical was calculated according to the formula: inhibition (%) = {[Abs (C) – Abs (S)]/Abs (C)} × 100, where Abs (C) is the absorbance of control (solution DPPH radical alone) and Abs (S) is the absorbance of sample with added extract. Then to compare obtained results IC_{50} (the half maximal inhibitory concentration) values were determined. All analyses were performed in triplicate.

In case of DPPH assay, aqueous extracts from *Drosera aliciae*, *Drosera ramentaceone* and *Drosera capensis* with IC_{50} values 0.881 mg FW/mL; 0.887 mg FW/mL; 0.959 mg FW/mL respectively, proved to be a very potent antioxidant. The best results and the highest antioxidative activity of methanol extracts were obtained for *Drosera anglica* and *Drosera spatulata* extracts with IC_{50} values 0.418 mg FW/mL and 0.483 mg FW/mL respectively. The lowest radical scavenging activity were identified in extracts from: *Drosera gigantea*, *Drosera adelae* and *Drosera binata*, where IC_{50} values were from 3 mg FW/mL to 14 mg FW/mL.

ACKNOWLEDGEMENTS

The scientific work is funded by the Polish Ministry of Science and Higher Education [grant number DS 530-M035-D673-18].

The analysis of the *in vitro* flax plant with tocopherol-pathway gene *VTE2* overexpression

Iwan Zalewski, Karolina Hasiewicz-Derkacz, Aleksandra Boba, Anna Kulma

Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wrocław, Przybyszewskiego 63, 50-148 Wroclaw, Poland, e-mail: iwan.zalewski@uwr.edu.pl

The flax is a unique cultivated plant, it is one of the oldest plants domesticated by human. It provides fibers for the textile industry and the oil from flax seeds is used in the cosmetic, chemical, food and pharmaceutical industries. Flax seed oil contains a large number of biologically active components. One of these components is vitamin E, which is synthesized exclusively by plants and have to be supplied with food. In humans, this vitamin plays an important role in the prevention of neurodegenerative, cardiovascular diseases and cancer. In plants, vitamin E functions to protect cell membranes from oxidative stress. The antioxidant properties come from the ability to reduce free radicals, before their interaction with the biological membrane. In this wok we overexpressed the *vte2* gene, which encode homogentisate phytyl transferase from *Arabidopsis thaliana*, the enzyme produces the first substrate in the tocopherol pathway. Which is considered as a key enzyme in the synthesis of tocopherols.

This report shows the analysis of the transgene plants. The introduced gene was confirmed by PCR, the increase level of tocopherol level of tocopherol was determined by high performance liquid chromatography. The terpenoid pathways activity was checked by real-time PCR and, and the content of the main metabolites formed in these pathways checked by HPLC. Also, the impact of the observed changes in metabolites were verified on the antioxidant potential of plant extracts. Affection of the improvement of the resistance against fungal infections using Fusarium fungi family was also examined.

Index of Authors

INSTRUCTIONS

ACTA BIOLOGICA CRACOVIENSIA Series Botanica is an English-language journal founded in 1958, devoted to plant anatomy and morphology, cytology, genetics, karyology, embryology, tissue culture, physiology, biochemistry, biosystematics, molecular phylogenetics and phylogeography, as well as phytochemistry. It is published twice a year.

1. ACTA BIOLOGICA CRACOVIENSIA Series Botanica publishes **original papers** embodying the results of experimental or theoretical research, **invited reviews**, and **brief communications**. Manuscripts will be considered only on the understanding that they have not been published and are not being considered for publication elsewhere, that all authors agree on the content of the manuscript, and that laws on nature protection were not violated during the study. Authors have to indicate their specific contributions to the published work in **Authors' Contributions** and the sources of financial support of their research in **Acknowledgements**. They should clearly describe the following in their cover letter: (1) the aims and hypothesis of the paper; (2) the novelty of the paper – new achievements or innovations contained in the paper; and (3) the general significance of their paper.
   Articles should be written in English (American spelling). Authors whose native language is not English are strongly advised to have their manuscripts checked by a professional translator or a native speaker prior to submission. Manuscripts should be written concisely. Purely descriptive studies, karyological notes on plants outside of central Europe, papers on economic botany as well as manuscripts of restricted interest generally are not considered for publication. *In vitro* studies which only describe protocols for plant regeneration without providing relevant biological information will not be considered for publication. A manuscript in the field of plant cell culture, physiology, biochemistry and phytochemistry must contain new insights that lead to a better understanding of some aspect of fundamental plant biology. They should be of interest to a wide audience and/or the methods employed should contribute to the advancement of established techniques and approaches.
   Authors are charged a fee for publication of their articles. The bill for publication will be sent with the galley proof. The fee, which is calculated after all articles are accepted, will not exceed 20 USD per printed page for foreign authors and 70 PLZ per printed page for Polish authors. For the standard fee, color illustrations will appear only in the online version of the Journal. At authors' request and for an extra fee, color illustrations may also appear in the printed version. While sending the manuscript, in the letter to the Editor, the authors should declare their contribution towards the extra costs and enumerate the illustrations which are to be printed in color.
2. Manuscripts should be submitted via the editorial manager: **www.editorialmanager.com/abcbot/**
   Editor:
   Prof. Dr. ANDRZEJ JOACHIMIAK
   Department of Plant Cytology and Embryology
   Jagiellonian University
   ul. Gronostajowa 9, 30-387 Kraków, Poland
   e-mail: a.joachimiak@uj.edu.pl
   Manuscripts will be examined by at least two anonymous and independent referees who have declared that they have no conflict of interest with the author(s). Invited referees evaluate the manuscript according to the following criteria: (1) formal aspects, (2) originality, (3) importance in its field, (4) theoretical background, (5) adequacy of methodology, (6) results and interpretation, and (7) overall quality.
3. To shorten the review process, authors are asked to indicate 3 or 4 names of specialists working in the same scientific discipline outside of their institution (including the name of their institution and e-mail addresses) who could serve as reviewers of the manuscript. Manuscripts should be double-spaced, with lines numbered. On all points of **style regarding text and tables**, follow a current copy of the journal. Words to be italicized (scientific names of genus and species only) should be typed in italics.
4. Original papers should not exceed 8 printed pages (approx. 24 manuscript pages including tables and figures).
5. Original papers should be headed by the title of the paper, author's name, institution, address, e-mail address of corresponding author(s) and short title (no more than 50 characters), and should be preceded by 5–10 **Key words** and a short **Abstract**. Original research papers should be divided into the following sections: **Introduction**, **Materials and Methods**, **Results**, **Discussion**, **Conclusion, Authors' Contributions**, **Acknowledgements** and **References**.
6. Invited reviews are mostly of limited scope on timely subjects written for a general, well-informed audience. Invited reviews are solicited by the Editor. Ideas for unsolicited reviews should be discussed with the Editor. They are subject to the usual review procedure.

7. Brief communications are short papers (1–4 printed pages) reporting new findings that do not need a standard full-length treatment with the usual main headings. Brief communications are subject to normal review.
8. **References** in the text should be cited in the following form: Newton (1990) or Newton and Berrie (1982) or (Ward, 1950; Hiroshi and Ohta, 1970). For three or more authors, use the form Zinkowski et al. (1991) or (Zinkowski et al., 1991).
   Examples of style for references:
   a) citations of journal papers:
   PALMER TP. 1962. Population structure, breeding system, interspecific hybridization and alloploidy. *Heredity* 17: 278–283.
   CHEN BY, HENEEN WK, SIMONSEN V. 1989. Comparative and genetic studies of isozymes in resynthesized and cultivated *Brassica napus* L., *Brassica campestris* L., and *B. alboglabra* Baitey. *Theoretical and Applied Genetics* 77: 673–679.
   b) citations of books, congress proceedings, theses:
   BERGRREN DJ. 1981. *Atlas of Seeds*, part 3. Swedish Museum of Natural History, Stockholm.
   BING D, DOWNEY RK, RAKOW GFW. 1991. Potential of gene transfer among oilseed *Brassica* and their weedy relatives. *Proceedings of the GCTRC Eighth International Rapeseed Congress*, 9–11 July 1991, 1022-1027. Saskatoon, Saskatchewan.
   ROMEO JT. 1973. A chemotaxonomic study of the genus *Erythrina* (Leguminosae). Ph.D. disseration, University of Texas, Austin, TX.
   c) citations of articles and chapters from books:
   PHILLIPS RL. 1981. Pollen and pollen tubes. In: Clark G [ed.], *Staining Procedures*, 61-366. Williams and Wilkins, Baltimore, MD.
   Authors' names in References should be written in small caps.
9. **Tables** must be numbered consecutively with Arabic numerals and submitted separately from the text at the end of the paper. The title should be brief and written in the upper part of the table. Footnotes to tables should be indicated by lower-case letters.
10. **Illustrations** must be restricted to the minimum needed to clarify the text. Previously published illustrations are not accepted. All figures (photographs, graphs, diagrams) must be mentioned in the text. All figures are to be numbered consecutively throughout and submitted separately. Figure captions should be given on a separate page.
    Photographs should be submitted the same size as they are to appear in the journal. If reduction is absolutely necessary, the scale desired should be indicated. The publisher reserves the right to reduce or enlarge illustrations. Photographs should match either the column width (83 mm) or the printing area (170 x 225 mm). Whenever possible, several photos should be grouped in a plate. The photos should be sharp, and each one should be marked with a lower-case letter on the plate. For photographs without an integral scale the magnification of photographs must be stated in the legend. Color illustrations will be accepted; however, the author will be expected to contribute towards the extra costs. The charge will not exceed 150 USD per printed page for foreign authors and 500 PLZ per printed page for Polish authors.
11. **Manuscripts** resubmitted after revision: Submit your text written in a standard program (Microsoft Word). Bitmap graphics files should be written in TIFF, PCX, EPS or BMP, and vector graphics in AI or CDR (curves). Illustrations written in MS Word or PowerPoint will not be accepted. Submit the text, tables and each figure (plate) as separate files. Every paper will be checked for style and grammar.
    The Editor reserves the right to introduce corrections suggested by the journal's line editor.
12. **Proof** will be sent directly to the authors in electronic form as a pdf file. Authors' corrections have to be inserted in the printout of the *PDF proof*. The corrected proofs must be returned to the Editor within six days via Editorial Manager or by e-mail. Proofs not returned promptly by authors will be corrected by the Editor.
13. **Copyright**. Exclusive copyright in all papers accepted for publication must be assigned to the Polish Academy of Sciences, but the Academy will not restrict the authors' freedom to use material contained in the paper in other works by the authors (with reference where they were first published).
14. **Offprints**. A pdf of each paper is supplied to the authors free of charge.