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ABSTRACTS

X Conference
In Vitro Cultures In Plant Physiology

December 7–9, 2016
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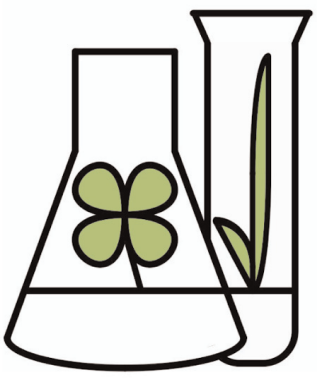

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ORAL PRESENTATIONS

Reaction of *Myrtus communis* L. to different LED spectra *in vitro*: the analysis of photosynthetic pigments and growth parameters

Monika Cioć[1], Marek Żupnik[2], Bożena Szewczyk-Taranek[1], Bożena Pawłowska[1]

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Myrtus communis is an evergreen shrub typical for Mediterranean region. The leaves, flowers and fruits of myrtle contain many beneficial components that are used in medical, food and cosmetic industries (Gardeli et al., 2008). In Europe, myrtle is also a valuable ornamental plant. Axillary bud development *in vitro* is an efficient way of myrtle propagation (Ruffoni et al., 2010).

The aim of this study was to investigate the influence of different LED light conditions and medium compositions on the growth and development of Myrtus communis propagated *in vitro*. Three different benzyladenin concentrations (1, 2.5 and 5 μM) were tested. Basic medium contained 100% Murashige and Skoog macro- and microelements (1962), 0.5 μM NAA, 3% sucrose and 0.5% BioAgar, pH 5.7. Three combinations of LED light spectra (100% red, 100% blue, mixture of 70% red and 30% blue LEDs) were tested. Fluorescent lamps (Philips TL-D 36W/54) were used as a control. Photosynthetic photon flux density in all combinations was 40 μmol m^{-2} s^{-1}. Biometrical observations were carried out after 6 weeks and the content of photosynthetic pigments was measured, according to the method by Lichtenthaler and Buschmann (2001).

The results of the study showed that the concentration of cytokinin in the medium had no effect on shoot multiplication as well as photosynthetic pigment contents in the leaves. The shortest shoots were observed on media containing 5 μM BA. In contrast, the applied light spectra affected shoot multiplication rate and pigment concentrations. Plants exposed to 100% red LED had the highest multiplication rate and the longest shoots. The highest content of photosynthetic pigments (chlorophyll *a*, *b*, *a* + *b* and carotenoids) was recorded in the leaves of plants cultured under fluorescent lamps, as opposed to the plants cultured under 100% blue LEDs, where the level of pigments was the lowest.

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Death hormone in the development of oat haploid embryos

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

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The "death hormone" hypothesis emerged in order to explain the phenomenon of monocarpic senescence. This supposed substance should be produced by generative parts of plant and transported to vegetative organs as a signal to senescence and redirection of nutrients to the developing seeds (Engvild, 1989). Chloroindole auxins and jasmonic acid were proposed by Engvild (1989) as a candidate for a death hormone.

4-Cloroindole-3-acetic acid (4-Cl-IAA) and jasmonic acid (JA) content in oat ovaries and haploid embryos produced by wide crossing was studied. Oat flowers were emasculated, pollinated with maize pollen and treated with 2,4-D. Twenty one days after pollination ovaries were collected, surface sterilized and haploid embryos were isolated. Analysis of hormones in ovaries and haploid embryos were done using UHPLC-MS/MS (Dziurka et al., 2016). Part of the haploid embryos were placed on 190-2 medium in order to germinate.

Conducted studies revealed significantly higher content of 4-Cl-IAA in empty ovaries (without developed embryos) comparing to ovaries in which embryos developed. It allows to suggest that this hormone could be involved in the process of senescence in oat florets. On the other hand, the presence of 4-Cl-IAA in germinating haploid embryos and not in haploid embryos directly isolated from ovaries implies its role in the early developmental stages of plant. There were no differences in JA content between ovaries with and without developed embryos. The mean amount of JA in ovaries was similar to that in haploid embryos directly after isolation and was about 2 μg/g DW. JA content in germinating haploid embryos were twenty times lower than in embryos directly after isolation. The role of JA in embryo formation and germination might depend on its content.

In conclusion 4-Cl-IAA could be involved in the process of oat florets senescence while the role of JA is ambiguous.

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Changes in gibberellin levels during winter wheat vernalization

Michał Dziurka[1], Kinga Dziurka[1], Barbara Jurczyk[2], Agnieszka Ostrowska[1], Franciszek Dubert[1], Anna Janeczko[1], Jolanta Biesaga-Kościelniak[1]

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Gibberellins (GAs) are a family of plant hormones controlling many aspects of plant growth and development. Although previous works showed contribution of GAs as a mobile signal transmitting flowering stimuli in grasses (King et al., 2006), they are not the sole factor in determining transition to flowering. Their concentrations are modulated by integrating various endogenous and external signals. The temperature is one of the most important environmental stimuli. An extended period of cold (vernalization) results in acquisition of competence to generative development. The mechanisms underlying the process are not fully understood. Plant regulators may partially substitute vernalization; zearalenone (ZEN) showed high activity shortening flowering induction period during vernalization of winter wheat (Biesaga-Kościelniak and Filek, 2010). The way of ZEN action remains unknown. Our aim was to verify the hypothesis: ZEN-induced stimulation of wheat generative development is *via* regulation of GA production.

During short vernalization of isolated wheat embryos in *in vitro* conditions (MS0 and medium with ZEN) levels of GA_1, GA_3, GA4, GA_5, GA_6, GA_7 were monitored by UHPLC-MS/MS (Dziurka et al., 2016). *GA3ox* transcript accumulation was analyzed by qPCR. Samples were collected after 0, 3, 6, 9, 12 and 15 days of vernalization at 5°C fallowed by 10 day acclimation at 10°C (9/15h photoperiod (d/n)).

ZEN significantly modifies GA levels which is accompanied by changes in accumulation of transcripts of genes coding enzymes involved in GA synthesis. Thus it can underlie the acceleration of wheat development induced by ZEN.

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The role of endogenous hormones and sugars in the acquisition of embryogenic competence in tree fern *Cyathea delgadii*

Małgorzata Grzyb[1], Agnieszka Kalandyk[2], Piotr Waligórski[2], Jan J. Rybczyński[1], Anna Mikuła[1]

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In *Cyathea delgadii* somatic embryogenesis (SE) is induced directly from the single epidermal cells of stipe explants. The first cell divisions starts at 10th day of the culture, and during the next 4–6 days, many somatic embryos at linear developmental stage are observed (Mikuła et al., 2015). By culturing explants on PGR-free medium we established a unique system, that is a good model for study hormonal regulation of SE. Thus, the aim of our research was to describe the role of endogenous hormones and sugars in the acquisition of embryogenic competence.

The SE was induced on stipes excised from the sporophytes maintained under 16/8 h photoperiod (non-etiolated; incapable of SE) or in constant darkness (etiolated; capable of SE). The plant material was cultured on 1/2 MS medium with 1% sucrose. Also, 1/2 MS medium supplemented with various hormone biosynthesis and transport inhibitors (HBTIs: TIBA, fluridone, salicylic acid, antipiryne and ancymidol) was used. The efficiency of SE was evaluated after 2 months of culture. For assessment of endogenous hormone (IAA, ABA, cytokinins) and sugar (glucose, fructose and sucrose) contents, the plant material was collected for HPLC analysis every 2 days during 14-day-long culture.

By comparing two types of initial explants, we found more than 10-times higher levels of ABA and sucrose, and several-times lower levels of IAA and cytokinins in non-etiolated than in etiolated explants. Because of explant excision all studied phytohormone levels were dramatically reduced. Cytokinins were found to be the predominant over the auxins throughout the 14 days of culture. An almost 12-fold increase in soluble sucrose concentration at day 6 seems to be an important point of SE induction. By using of HBTIs, we showed that the presence of high TIBA, fluridone or salicylic acid concentrations, totally inhibited induction of SE, while antipyrine and ancymidol disturbed the somatic embryo development. Those substances cause the disturbance of hormonal balance between ABA and auxin and cytokinins.

Our studies showed that the etiolation of initial sporophytes is critical for SE induction. The study showed that an appropriate hormonal balance between cytokinin and auxin plays the key role in acquisition of embryogenic competence. Moreover, the high level of ABA inhibits SE induction even when the auxin/cytokinin ratio is optimal.

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Plant growth stimulation by inoculation with rhizosphere microorganisms in soils with polymer materials

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The growing demand for film and plastic packaging used in many sectors of the economy is causing the increase in waste production or post-consumer waste. One of the methods for remediation of degraded areas is phytoremediation.

In vitro selected microorganisms are able to growth on media containing polymeric materials. Moreover, laboratory experiments conditions showed stimulating effect on germination and growth of rape for selected microorganisms.

In the pot experiment was used three plant species: winter oilseed rape (*Brassica napus* L.), willow (*Salix viminalis* L.) and giant miscanthus (*Miscanthus* × *giganteus*). Selected bacteria *Serratia* sp., *Arthrobacter* sp. and fungi *Clitocybe, Laccaria laccata was characterized by ability to grow on polymers (PLA* – polylactide, PET – polyethylene terephthalate). Those bacteria were a reason of changes in polymer degradation. Growing of plant in soils containing PLA and PET was stimulated by specified microorganisms.

Selected microorganisms have stimulated plant growth in soils containing PLA and PET. Biomass of willow inoculated by *Clitocybe* fungi was 25% greater than noninoculated plants. Presence of *L. laccata* in soils has positive effect on the growth of oilseed rape. *Serratia* sp. has same effect on miscanthus, increased biomass.

The number of microorganisms in soils inoculated and noninoculated soils were different. The presence of inoculum, polymer materials and plants had an influence on amount of soil bacteria and fungi.

Probably, obtained results will allow to develop a method of phytoremediation of polluted by polymer materials, which is a problem of urbanized regions of the world.

Role of steroids in regulation of winter wheat development

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Steroids are one of the most important group of compounds with multidirectional activity. They are present in all living organisms where serve among other as hormones. In plants are present brassinosteroids, but there are also discovered traces of mammalian steroids (i.e. androstenedione or progesterone) as well as typical for insects – ecdysteroids. Role of all this steroids in plants is not fully explained. This lecture will show current knowledge about activity of few steroid regulators (applied in *in vitro* culture) on plant physiological processes especially on generative development. Lecture will summarise the knowledge published in Steroids (Janeczko et al., 2015). In the article, manipulation of the content of few steroids using inhibitors of their biosynthesis and binding allowed to propose a model of steroid-induced regulation of the development of winter wheat, where brassinosteroids act as inhibitors of generative development, while progesterone as stimulator.

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Possible involvement of glucoside hydrolases in rye (*Secale cereale* L.) androgenesis induction

Katarzyna Juzoń[1], Zuzana Gregorová[2], Jana Moravčikova[2], Iwona Żur[1], Eva Boszorádová[2], Ildico Matusikova[3], Kamil Zieliński[1], Mária Zimová[2,4], Anna Nowicka[1], Przemysław Kopeć[1], Monika Krzewska[1], Ewa Dubas[1]

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The understanding of the molecular mechanism of androgenesis induction and the selection of markers identifying responsive/recalcitrant genotypes are crucial for wide exploitation of doubled haploid technology in crop improvement. Among various molecules, pathogenesis-related proteins (PRs, glucoside hydrolases) – involved in the plant defence (a)biotic responses and probably in cell differentiation and development – seem to be good candidates. Therefore, the presence of β-1,3-glucanases was screened in anthers of winter rye genotypes characterized by varying embryogenic potential, which were induced to androgenesis.

Anthers isolated from pre-treated tillers (3 weeks, 4°C) of two breeding lines of rye (*S. cereale* L.) were studied. Additionally, the effects of mannitol (0.3 M Mn) or/and reduced glutathione (0.3 M GSH) were tested. The β-l,3-glucanase activities were assayed spectrophotometrically and in the PAGE gels, with the laminarin as a substrate, after separation of proteins under native condition for acidic/neutral proteins.

We found that anthers of tested lines differed in the total β-l,3-glucanase activities. Combined, Mn and GSH treatment increased (>1.8-fold) the total glucanase activities in the line described as more responsive to androgenesis. In contrast, total glucanase activities in the less embryogenic line decreased. Mn treatment alone had no effect or decreased (>1.9-fold) the total glucanase activities, respectively. The results from the gel activity assays revealed at least four acidic isoforms with the β-l,3-glucanase activities in the responsive line and seven acidic isoforms in the less embryogenic one. Some isoforms could be linked to embryogenic potential, especially when associated with Mn or/and GSH treatment.

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Genome editing with CRISPR-Cas9 system

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Recent years have brought the rapid development of tools for targeted genome editing using programmable sequence-specific nucleases. Double-strand breaks (DSB) can be generated by cleavage domain of FokI endonuclease with combination of DNA-binding domains in zinc-finger nucleases (ZFNs) or transcription activator-like (TAL) effector nucleases (TALENs). However, construction of systems based on ZFNs and TALENs is technically difficult and time-consuming, and the efficiency of editing is relatively low (Voytas, 2013). Most recently, a CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 immune system has been described in bacteria *Streptococcus pyogenes*. The system is also capable of generating DSB at the protospacer adjacent motif (PAM; NGG) when Cas9 nuclease is targeted by two hybridized RNAs, a spacer-containing crRNA and trans-activating crRNA (tracrRNA) (Jinek et al., 2012). This three component native system in bacteria was engineered and reduced to two components composed of Cas9, a monomeric DNA endonuclease, that can be targeted to a specific genome sequence by a synthetic 20 nt guide RNA (gRNA, Mali et al., 2013). Cas9 generates DSB with blunt ends and DNA can be repaired by either nonhomologous end joining (NHEJ) or homology-directed repair (HDR) causing point or indel mutations. Several reports proved successful generation of DSB in bacteria, mammalian and plant genomes using this simplified system that makes CRISPR/Cas9 a versatile and relatively easy tool for genome editing (Bortesi and Fischer, 2015).

The advantage of the CRISPR/Cas9 system is that gRNA targeting a desired genome location is short and can be easily manipulated. Moreover, multiple gRNA can be co-delivered with Cas9 enabling genome editing at several locations simultaneously, including short deletion causing gene knock-outs (Song et al., 2016).

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The elaboration of propagation protocol for *Pulsatilla turczaninovii* Krylov & Sergievskaya under *in vivo* and *in vitro* conditions

Dawid Kocot, Ewa Hanus-Fajerska

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The experiments were undertaken with the aim to evaluate the propagation potential of rare, endangered *Pulsatilla* species, according to a classification of the zonal types of vegetation, native to the middle part of Eurasia. *Pulsatilla turczaninovii* Krylov & Serg. represent the mountain vegetation constituting a combination of true steppes with steppizated pine forests, which is called 'peristeppe form of contact' (Krivobokov and Nazimova, 2011; Vanteeva and Solodyankina, 2015).

Undertaken studies were focused on assessing the possibilities to obtain numerous true-to type plants under both *in vivo* and *in vitro* conditions. In order to start the experimental work fruits samples were taken from the *P. turczaninovi* specimens representing the natural population, growing on grassy slopes of Baikal Lake Basin. The obtained results let us to compare the effectiveness of generative and vegetative propagation in this important representative of Ranunculaceae family. Plants obtained as a result of seed sowing have been growing and developing in a very low rate. Additionally, under long-term storage seeds were characterized by low germination percentage (in long term stored fruit samples the seeds germinate below 50%. Therefore the development of an efficient, rapid *in vitro* clonal propagation method was proved essential with this plant material.

Aseptic seedlings, obtained after treatment of seeds with 20 mM of nitric oxide, were the source of explants The organ culture has been established on a set of modified WPM (Lloyd and McCown, 1981) and MS (Murashige and Skoog, 1962) media supplemented with different combinations and levels of plant growth regulators, whereas the control explants were cultivated on auxin-free medium. The cultures were placed in a growth chamber illuminated with white fluorescent lamps (80 μmol $m^{-2}s^{-1}$), with 16/9 h photoperiod. The temperature of the chamber was maintained at 24°C ± 2°C, and the relative humidity amounted to 70%. The influence of particular treatment on shoot growth was evaluated mainly biometrically. As a result of performed experiments it was ascertained that in order to conduct *in vitro* shoot culture of *P. turczaninovii* modified MS medium supplemented with 0,5 mg/l 2iP and 0,1 mg/l IAA can be optimal option. Currently further experiments are in progress to compare rooted plants removed from culture vessels and planted into commercial neutralized peat mixed with sand with those obtained as a result of generative propagation.

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Flowering of chamomile (*Matricaria chamomilla* L.) in *in vitro* cultures

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The aim of this study was to determine the effect of gibberellins: GA_3 and GA_{4+7} and BAP, KIN, IBA and IAA on the growth and flowering of chamomile (*Matricaria chamomilla* L.) in *in vitro* cultures. Shoots of chamomile (tetraploid varieties 'Golden Łan') propagated earlier *in vitro* cultures on MS media were used to set up the experiments.

The experiment was conducted in two stages: in the first of isolated fragments of shoots, with 1cm length, were put onto MS medium without the addition of plant growth regulator and supplemented with BAP, KIN, IBA, IAA and GA_3 at concentrations of 1, 2 and 3 $mg \cdot dm^{-3}$. In the second, the same explants were put onto MS medium with GA_3 or GA_{4+7} in the concentrations of 1, 2, 3, 4, 5, 7, and 10 $mg \cdot dm^{-3}$. Gibberellins added to the medium before the sterilization at a temperature of 121°C for 20 min.

None of the plants growing on MS medium without the addition of growth regulators or on media supplemented with BAP, KIN, IAA and IBA, regardless of the concentration, not bloomed.

The addition of gibberellins to the media had a significant impact on the development of plants and their flowering. The addition of the two used in the experiment of gibberellins (GA_3 and GA_{4+7}) regardless of the concentration of induced flowering. Percentage of flowering plants grow with increasing concentration of GA_3 in the medium and reached a maximum value (90%) as chamomile propagated at the highest concentrations used (10 $mg \cdot dm^{-3}$). The highest number of inflorescences per plants were found for plants propagated on media 3, 4 and 5 $mg \cdot dm^{-3}$ GA_3. The addition of GA_{4+7} have less effect on flowering plants – bloomed from 3 to 21% chamomile, depending on the concentration used, and they have developed similar number of inflorescences per plant (1.0 to 2.2). Gibberellin GA_3 had a significant impact of elongation of shoots and increase the number of shoots and leaves and root length. No significant effect of the concentration of GA_3 on such morphological features as the number of roots, and the mass of plants. With identical concentrations of GA_{4+7} is not found to significantly affect the characteristics such as the length and number of shoots, number of leaves and roots, the plant weight. It was noted the inhibitory effect of GA_{4+7} on the development of root length.

Is it possible to reduce the toxic effect of cadmium on the elongation growth of plant cells?

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Nonessential metals such as cadmium (Cd) are toxic to plants even at low concentrations. One of the most spectacular symptoms of Cd toxicity is stunted growth (Kurtyka et al., 2008; Ahmad et al., 2015). The published data concerning cell extension during the elongation growth of plant organs have shown that the phytohormone auxin stimulates this process (Burdach et al., 2014; Falhof et al., 2016). It is well documented that auxin-mediated regulation of the plasma membrane (PM) H^+-ATPase activity appears to be required for cell elongation (for a review see Hager, 2003). According to the so-called "acid growth theory", by activating this enzyme, auxin causes both the cell wall acidification, which provides favorable conditions for cell wall loosening, and plasma membrane hyperpolarization, which promotes the uptake of osmotically active ions, especially K^+, which are required for cell elongation. It also established that the inhibition of the elongation of plant cells by Cd can be attributed in part to the inhibition of PM H^+-ATPase activity (Karcz and Kurtyka, 2007; Kurtyka et al., 2011; 2012). Therefore, it seems that a decrease in the toxicity of Cd on the elongation growth of plant cells can be obtained by stimulating the PM H^+-ATPase activity and changing the electrical properties of the PM.

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Engagement of light stress and osmotic stress on steviol glycosides biosynthesis and accumulation in hairy roots from *Stevia rebaudiana* Bertoni

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Stevia rebaudiana Bertoni produces and accumulates secondary metabolites known as steviol glycosides (SvG). They are several times sweeter than sucrose and not metabolized in human organism (Cramer and Ikan, 1986). Due to this property they are widely used in the food industry. The details concerning the regulation of SvG synthesis and accumulation are poorly investigated. It is postulated that reactive oxygen species (ROS) overproduces due to the influence of stress factors are important regulators of secondary metabolism in plants (Bulgakov, 2008). Hairy roots (HR) are unusual plant organs, which are a result of transformation induced by bacteria from the genus Agrobacterium. They are used for mass scale production of secondary metabolites in *in vitro* cultures (Wysokinska and Chmiel, 1997).

We investigated the influence of osmotic and light stress on SvG production and accumulation in HR acquired from Stevia explants cultured *in vitro*. HR were cultured in B5 liquid media in dark and light conditions or in the media supplemented with different osmotic regulators (sucrose, NaCl and PEG). The ability of HR to produce different SvG was investigated on the basis of some glucosyl transferase (UGT's) gene expression using RT-qPCR method, while the accumulation of selected SvG was measured using z UHPLC analysis.

On the basis of achieved results we can assume that HR cultured under light conditions possess the highest potential for SvG production. Although the accumulation of analyzed SvG was lower than in whole plant, the proportion of accumulated SvG were significantly different. Some SvG were specifically found only in HR cultured under stress conditions. The results could be exploited in further studies concerning the achievement of plant material exhibiting the optimal concentration and proportions of economically important SvG.

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The impact of culture conditions on plant cell viability in suspension culture after application of lead salt

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Soils naturally or anthropogenically contaminated by heavy metals are a significant problem worldwide. Lead causes several toxicity symptoms in plants (Sharma, Dubey, 2005). Species of several families: Asteraceae, Brassicaceae, Caryophyllaceae, Plumbaginaceae, Poaceae and Violaceae are well adapted to polluted with heavy metals soils. *Viola tricolor* L. is a metal-tolerant species colonizing old zinc-lead waste heaps with high concentrations of Zn, Pb, Cd in Southern Poland (Słomka et al., 2008, 2011). The impact of lead on cell viability was tested using cells in suspension culture of *V. tricolor*. It required stabilization of culture conditions because lead forms sparingly insoluble salts with components of medium what causes pH changes and turbidity in liquid medium influencing light intensity.

Cell suspension cultures were obtained from callus developed on *V. tricolor* leaf fragments cultured on MS medium supplemented with 2 mg l^{-1} 2,4-D + 2 mg l^{-1} BAP. Cell suspension was cultured on MS medium with addition of the same growth regulators and cultures were treated with $Pb(NO_3)_2$ in concentrations of 0 μM (control), 200 μM, 500 μM, 1000 μM, 2000 μM, for 24, 48 and 72 h. Lead salt, after adding to the medium, depending on its concentration, decreased pH level and changed the reflectance, measured using RAIRS method, due to medium turbidity. To confirm the impact of culture conditions on cell viability in suspension after lead treatment, two experiments were performed: no. 1 without stabilization of culture conditions (pH adjustment, darkness equalization) and no. 2 with the pH adjustment to 5.6 and darkness equalization by blackout of flask surfaces according to the treatment with 2000 μM $Pb(NO_3)_2$ (light absorption ~9.4%).

There were differences in the frequency of viable cells stained with Alamar Blue assay after lead salt addition depending on culture conditions. In experiment no. 1 negative impact of lead concentrations and timing of treatment on cell viability was not observed, the frequency of viable cells was very high (85-100%), whereas in experiment no. 2 the frequency of survived cells decreased and was correlated with concentrations of $Pb(NO_3)_2$ and timing of treatment (60 and 10% at 2000 μM $Pb(NO_3)_2$ after 24 and 72 h, respectively).

In conclusion, to test influence of lead and other heavy metals forming insoluble sediment with ions included into growth medium on plant cells in suspension culture, stability of culture conditions is absolutely required.

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Identification of oat x maize hybrids obtained by wide crossing method

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Oat (*Avena sativa* L.) is widely used cereal belonging to Poeaceae family. Global production of oats increased during the last years about 50% which results in necessity to improve the methods of new cultivars production. Doubled haploids (DH) allow to shorten production time of new cultivars comparing with traditional breeding methods. Oat DH lines are obtained by wide crossing with maize. In this method chromosomes from pollen donor usually are eliminated, but in oat in contrast to other cereals, it is possible to retain one or more maize chromosomes. Oat can form fertile hybrids with maize, called oat – maize addition lines (OMAs).

The aim of this research was to identify oat – maize addition lines by PCR and electrophoresis technique.

The experiment was performed on 91 oat DH lines obtaining by pollination with maize. In order to identify OMAs among obtained DH lines, oat genomic DNA was isolated from the 4 – 5 cm fragments of flag leaf using Murray and Thomphson (1980) method. DNA samples were analyzed by PCR method with 5'-AAA GAC CTC ACG AAA GGC CCA AGG-3' and 5'-AAA TGG TTC ATG CCG ATT GCA CG-3' primers to detect the presence of maize chromatin. Obtained products were separated by electrophoresis in 1 % agarose gel in TBE buffer. The length of amplified products was estimated using molecular marker (100 bp ladder). When the whole chromosomes or fragments of maize chromosomes were retained in oat DH line genome, genomic DNA of 500 bp (specific maize retrotransposon fragment, Grande 1) was amplified, and detected by electrophoresis. Genomic in situ hybridization for oat lines with maize chromosomes was performed.

Among 91 oat DH lines 30 lines with retained maize chromosomes was identified. Identified OMAs contained up to four maize chromosomes. Retained fragments or whole maize chromosomes in OMAs might result in morphological changes, affect the overall performance of the photosynthetic apparatus and yield.

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Cellular functions of *Arabidopsis thaliana* SMC5/6 complex

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The STRUCTURAL MAINTENANCE OF CHROMOSOMES (SMC) 5/6 complex, together with condensin and cohesin complexes, plays key functions in genome maintenance that include ensuring the faithful segregation of chromosomes at mitosis and facilitating critical DNA repair pathways. The SMC5/6 complex is essential for viability and therefore, recognition its contribution in diverse cellular processes and DNA damage repair has been challenging. The complex is conserved among eukaryotes and contains the core subunits SMC5 and SMC6, and six additional NON-SMC ELEMENTs 1 to 6 (NSE1-6). The *Arabidopsis thaliana* genome comprises functional homologs of all SMC5/6 complex components, including duplications of *SMC6* (*SMC6A* and *SMC6B*) and *NSE4* (*NSE4A* and *NSE4B*) genes. We focused on studies of *NSE2*, *NSE5* and *NSE6* genes, which are very weakly characterized in Arabidopsis. The *Arabidopsis* mutants *nse2-1*, *nse2-2*, *nse5* and *nse6* showed a reduction of heterochromatin due to dispersion of pericentromeric low-copy sequences away from heterochromatic chromocenters. In additional, strongly compact 45S rDNA and 5S rDNA heterochromatin loci were partially and fully dispersed, which resulted expression of silenced 5S rDNA loci. We also observed reduction of DNA and H3K9 methylation accompanying the reduction of the chromocenters size. Mutations in studied NSE genes caused greater endoreplication frequency on 16C, 32C and 64C levels and larger nuclei. Emerging data are expanding the range of processes in which components of SMC5/6 complex are known to participate and are enhancing our knowledge of how nucleus architecture is regulated to influence numerous cellular functions.

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Development of *in vitro* tetraploid induction method of *Ribes nigrum*

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The aim of the study was to develop an *in vitro* polyploidisation method for breeding of blackcurrant dessert type (*Ribes nigrum*) cultivars. Poland has been a world leader in the production and export of processed fruits of blackcurrants. A new direction of blackcurrant cultivation has recently been introduced – the production of dessert fruits for the fresh market and consumption. Obtaining genotypes with larger and tastier fruits might be possible by the classical hybridization and breeding of polyploids. Polyploidisation process is one of the sources of variation, which is important for selection of valuable genotypes. Polyploids are widely used in breeding programs of many plants. One of the most commonly observed feature of newly acquired polyploids compared to diploids is higher organ size, including fruit size. However, there is little information on blackcurrant polyploids (Chuvashina 1972; Stanys et al., 2004).

The Polish blackcurrant cultivar 'Gofert' was used for experiments. 'Gofert' was bred and registered in 2010 at the Research Institute of Horticulture in Skierniewice, Poland (Pluta and Żurawicz, 2014). In the first stage of the research, the procedure of efficient *in vitro* shoot multiplication was optimized. For polyploidisation, shoot explants were incubated for six days in darkness on multiplication medium containing one of the antimitotic agents, colchicine, trifluralin, oryzalin or amiprophos methyl (APM). The strongest phytotoxic effects of antimitotic agents were observed for trifluralin (all the explants died). Higher numbers of tetraploids, 5 and 11, were obtained after the application of colchicine (125-250 mg l^{-1}) and APM (5-10 mg l^{-1}), respectively. Besides, only 6 homogeneous tetraploids were selected directly. Instead, the high number of mixoploids (77) were selected. Based on flow cytometry analysis, 44 mixoploids were determined as ploidy chimeras with predominant diploid genome (2x+4x) and 33 mixoploids with prevailing tetraploid genome (4x+2x). All the mixoploids were multiplied and within the shoots derived from the 4x+2x chimeras, additional 15 homogeneous tetraploids were selected. The polyploidisation efficiency was on average 8.4%. Newly obtained tetraploids differ phenotypically from their diploid counterparts. Tetraploids were shorter, leaf shape was different and chlorophyll index was higher.

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Biosynthesis of galanthamine and lycorine in *in vitro* cultures of *Leucojum aestivum* L.

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Leucojum aestivum L. (Amaryllidaceae) is a source of alkaloids. Galanthamine is used for treatment of Alzheimer's disease while lycorine displays antiviral and antimalarial activities. Galanthamine production is obtained by chemical synthesis and by extraction from *L. aestivum* and *Narcissus* plants. Due to the growing demand and the limited availability of plant sources, the capacity of *in vitro* cultures of *L. aestivum* to accumulate alkaloids was studied (Diop et al., 2007; Ptak et al., 2009). The effect of the composition of the medium has been investigated (Ptak et al., 2010, 2013a, b; Tahchy et al., 2011). Elicitation with MeJA and SA and biotransformation using 4'-O-methylnorbelladine has been also studied (Saliba et al., 2016). Furthermore alkaloid production by *L. aestivum* cultures growth in bioreactor RITA® has proved to be a good basis for future scaling-up process (Ptak et al., 2013b).

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Effect of LEDs light color, cytokinin, and type of sugar on growth and shoot quality of serviceberry, rose and black currant in *in vitro* culture

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In recent years light-emitting diodes (LEDs) as alternative light source in tissue cultures technology have been used. Different plant species were investigated: banana (Nhut et al., 2000), lily (Lian et al., 2002), strawberry (Nhut et al., 2003).

Effect of light-emitting diodes (LEDs) on some growth parameters of rose (*Rosa pomifera* 'Karpatia'), serviceberry (*Amelanchier* × *grandiflora* 'Balerina') and black currant (*Ribes nigrum* 'Tiben') were investigated.

Light setting was as following: white fluorescent tubes as a control, 40% red LEDs+40% blue LEDs+20% white LEDs (I combination), 50% red LEDs+50% blue LEDs (II combination), 49% red LEDs+49% blue LEDs+2% far red LEDs (III combination). Combination of lights affected significantly: for rose- number of rosettes, dry matter and chlorophyll *a* and *b* content and for serviceberry- chlorophyll *a* content. Interaction between two factors (type of sugar and light combination) influenced significantly increased of fresh mass, number of leaves, dry matter content, chlorophyll and carotenoids content at serviceberry. Combination I improved the rose multiplication, while combination II enhanced significantly dry matter content, chlorophyll *a* and *b* content in rose shoots. For serviceberry sucrose increased significantly index of multiplication and dry matter content, on the other hand, glucose resulted in the highest increment of serviceberry shoot fresh mass.

Second light setting included: white fluorescent tubes as a control, 100% red LEDs (I combination), 100% blue LEDs (II combination), 50% red LEDs+50% blue LEDs (III combination). This light combination affected the increment of fresh mass (black currant), length of shoots and number of leaves, dry matter and chlorophyll b content (serviceberry). The highest increment of black currant fresh mass was observed in red light. Serviceberry shoots were shorter in red light, but at white and blue lights shoots exhibited greater number of leaves. Red and blue light in the ratio 1:1 enhanced significantly dry matter content in serviceberry shoots and leaves, while blue light increased chlorophyll *b* content at serviceberry.

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The influence of light quality on the accumulation of bioactive secondary metabolites in *in vitro* cultures of medicinal plant species

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Light conditions (both light intensity and photoperiod as well as light quality and also lack of light) belong to the most important physical factors which influence the biosynthesis and accumulation of secondary metabolites in plant *in vitro* cultures.

In the last two years, the studies in our biotechnological laboratory have concentrated on the influence of light quality: monochromatic light (far-red, red, blue, UV-A), darkness and white light on the accumulation of different groups of bioactive metabolites, including schisandra lignans, free phenolic acids, flavonoids and also verbascoside and isoverbascoside.

Methanol extracts of the biomasses from stationary *in vitro* cultures of *Schisandra chinensis* (Turcz.) Baill, *Aronia melanocarpa* (Michx.) Elliott, *Aronia arbutifolia* (L.) Pers., *Aronia* × *prunifolia* Marshall, *Scutellaria lateriflora* L. and *Verbena officinalis* L. were analyzed by HPLC methods (Zhang et al., 2009; Ellnain-Wojtaszek et al., 1999).

The applied light conditions influenced biomass growth and the accumulation of secondary metabolites. Blue light was found to be most effective for the production of a majority of the estimated metabolites: lignans and phenolic acids in *S. chinensis* (376.41 and 46.57 mg/100 g DW, respectively), (Szopa et al., 2016), phenolic acids (527.40; 543.27; 1615.18 mg/100 g DW) and flavonoids (144.61; 85.82; 220.65 mg/100 g DW) in three Aronia species, flavonoids and verbascoside (1204.33; 169.15 mg/100 g DW, respectively) in *S. lateriflora* and isoverbascoside in *V. officinalis* (1425.78 mg/100 g DW).

Our results documented an importance of light quality for the production of different groups of bioactive plant metabolites.

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The influence medium supplementation with permeabilizing agent and elicitors on accumulation of dibenzocyclooctadiene lignans in agitated microshoot cultures of *Schisandra chinensis*

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Dibenzocyclooctadiene lignans, also known as schisandra lignans, are determined as a specific group of secondary metabolites that occur in *Schisandra chinensis* (Turcz.) Baill. The scope of the pharmacological actions of the lignans ranges from: hepatoprotective, anticancer, immunostimulant, to adaptogenic properties (Szopa et al., 2016a). In our last work (Szopa et al., 2016b), well established agar and liquid stationary as well as agitated microshoot cultures of *S. chinensis*, were demonstrated to produce substantial amounts of these compounds. The major aim of the presented work was to examine the influence of permeabilizing agent and elicitors in the MS medium on the accumulation of lignans in agitated microshoot cultures of *S. chinensis*. The experiments included different concentrations of permeabilizing agent: dimethylsulfoxide – DMSO (0.2-2.0-4.0-8.0% v/v) and two elicitors: methyl jasmonate – MeJa (applied at 50-100-200 μM) and cadmium chloride – $CdCl_2$ (2.5-5.0-10.0-20.0 mM), which were added on the 23rd or 27th day of the growth cycle, and evaluated for their influence on growth parameters, and the accumulation of lignans. These compounds were estimated in the microshoot extracts and in the respective growth media samples by HPLC-DAD method (Zhang et al., 2009). All experiments were run for 30 days. DMSO caused the decrease in intracellular lignans content which was, however, not accompanied by the increase of lignan concentration in the growth medium. MeJa either decreased or did not affect the accumulation of lignans (max. total content 300-400 mg/100 g DW). The media samples showed no presence of lignans. $CdCl_2$ caused up to 2-fold increase in lignan content (ca. 600 mg/100 g DW for the addition of 2.5 or 20 mM $CdCl_2$ on the 23rd day). The related media samples showed only trace amounts of the examined compounds (< 5 mg/l).

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Control of somatic embryo differentiation process in selected fern species

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The process of somatic embryogenesis (SE) has been reported for the first time within the monilophyte clade in the tree fern Cyathea delgadii Sternb. (Mikuła et al., 2015a). At least 10 somatic embryos could be obtained from 2.5-mm-long stipe explant cultured for 2 months in dark on half-strength Murashige and Skoog agar medium without exogenous plant growth regulators. Subsequent studies revealed that, among other things, SE in C. delgadii can be effectively stimulated by the presence of sucrose or mineral salts in the medium and that the optimal sucrose concentration is only 1 % (Mikuła et al., 2015b).

Recently, SE has also been recognized in cultures of two herbaceous fern species, *Asplenium cuneifolium* Viv. and *Asplenium adiantum-nigrum* L. However, in spite of a high percentage of responding *Asplenium* explants (usually about 90 %), the number of somatic embryos per single stipe explant is far lower than in the case of *C. delgadii* (on average only 1-2 embryos). Thus, the aim of presented investigation was to find more favorable conditions for initiation of SE process in herbaceous ferns than those established for *C. delgadii*.

Fragments of stipe explants measuring 2.5 mm in length were excised from the first or second frond of young etiolated *Asplenium* sporophytes and cultured on basal agar medium containing half-strength Murashige and Skoog macro- and micro-nutrients and a full complement of vitamins. Cultures were kept in constant darkness at 22 ± 2°C and at a relative humidity of 35–55%. The influence of different pH and various concentrations of Fe, ammonium ions, and sucrose, as well as the effect of one-week osmotic shocks on the efficiency of somatic embryogenesis was evaluated after 1, 2, 3 and 4 months of culture.

The results obtained so far indicate that tested Fe concentrations, pH in range 5.8-6.8, and sucrose concentration between 1 and 5 % have no significant effect on the somatic embryo productivity in cultures of both Asplenium species. However, in the case of *A. cuneifolium*, the use of medium supplemented with 10% sucrose allowed to increase the average number of somatic embryos per explant from 2.6 to 9.8. On the other hand, a beneficial impact of the reduction of ammonium ions (from 825 to 206 mg/l) has been observed for *A. adiantum-nigrum*.

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In vitro culture as an effective method of reproduction *Hippeastrum hybridum*

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Hippeastrum hybridum L. plants are perennial bulbous plants that belong to the family *Amaryllidaceae*. *Hippeastrum* species have significant floricultural importance and are one of the major bulb crops in the commercial market (Ilczuk et al., 2005). In addition, these plants are able to phytoalexin biosynthesis and can be used as a bioreactor for the synthesis of phytoalexin (Świeżawska et al., 2015). Unfortunately, reproduction of this species by bulbs or seeds is relatively slow. The usage of *in vitro* cultures is an ideal solution to improve the efficiency of the process.

The objective of present work was to develop an efficient system for *Hippeastrum hybridum* regeneration. The experimental material comprised roots, single and twin scales, leaf blades, basal and middle parts of flower stalks (peduncles), ovaries , anthers, sepals and receptacle fragments. The explants were cultured on MS medium supplemented with 4 mg/dm^3 BAP and 0.1 mg/dm^3 NAA (Aslam et al., 2012). The morphogenetic response was best for receptacles (81.9%), basal part of peduncles (78.3%) and twin scales (79.8%). On average, 8.0 bulblets were formed per receptacles. For peduncles and twin scales 3.2 and 1.6 bulblets respectively per initial explants were induced. The yield of the technique is 15–25 shoots per bulb and 27-36 shoots per inflorescence. The obtained shoots were rooted on MS medium without plant regulators. Moreover, 100% of rooted shoots was able to acclimate to the *ex vitro* conditions and during this time the received bulbs increased several times their weight.

The regenerated plants was able to synthesis of phytoalexins , and the concentration of this substance in such bulbs was comparable to that noted in conventional breeding plants.

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Androgenesis induction of oat (*Avena sativa* L.) anther culture

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The production of doubled haploid (DH) plants through anther or microspore culture is one of the methods to generate homozygous lines in cereals. Oat (*Avena sativa* L.) is considered as one of the most recalcitrant cereal for haploidization. The objective of this study was to evaluate the influence of anther pretreatment on the production of oat doubled haploids.

Experiments were carried out on four oat cultivars: Akt, Bajka, Bingo and Chwat. Different pretreatments given to panicles of oat before anther culturing, and their effects on the frequency of embryo-like structures (ELS) were evaluated. Oat panicles were pretreated in liquid medium (Hoagland and Arnon, 1938) alone or supplemented with mannitol (0.3 M), glutathione (GSH, 0.3 mM) and hydroxynicotinic acid (HNA, 3.6 mM) for 14 days in 4°C. Than panicles were disinfected and anthers were cultured on solid C17 medium (Wang and Chen, 1986).

Anthers response was evaluated after 4 and 8 weeks of culture and number of ELS per 100 anthers were calculated. Anthers isolated from panicles pretreated in liquid medium with addition on GSH formed the highest number of ELS (1.6%) in comparison to the medium with mannitol or HNA. Regardless the applied pretreatment, the highest number of ELS was obtained from cv. Chwat (3.6%). Cultivars Bingo, Akt and Bajka formed lower number of ELS (1.6, 0.7, 0.5%, respectively).

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Antioxidative system activity as a determinant of the efficiency of microspore embryogenesis

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Microspore embryogenesis (ME), being the fastest route to total homozygosity, can be used as a highly advantageous tool in basic studies, biotechnology and breeding practice. However, to be used on a commercial scale the process should be highly effective and repeatable, which up to now has been achieved only for a limited number of plant species. Despite the considerable effort which has been invested to discover the mechanism underlying ME and to identify the factors determining its efficiency, the results are still not satisfactory.

On the basis of extensive studies on triticale (× *Triticosecale* Wittm.) with the use of anther culture method, it could be suggested that the first prerequisite for effective initiation of ME is highly efficient antioxidative defence system. It is probably due to the fact that ME is induced by a stress treatment usually accompanied by excessive generation of reactive oxygen species (ROS). High activity of ROS-decomposing enzymes, catalase and peroxidase seems to be crucial and can be used as a marker of embryogenic potential. Although low molecular weight (LMW) antioxidants contribute to cell antioxidative protection, in this case their endogenous level and activity seems to be of less importance. On the other hand, many published reports suggest that LMW antioxidants applied exogenously could positively affect the effectiveness of ME.

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POSTERS

The level of endogenous phenolic compounds and carbohydrates during the organogenesis of *Lachenalia* sp. cultured *in vitro* in various light conditions

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A native of South Africa *Lachenalia* sp., is one of the promising new species of bulbous plants, with large market opportunities. The aim of the research was to investigate the organogenesis of two lachenalia cultivars 'Ronina' (forming mainly shoots) and 'Rupert' (forming mainly bulblets) cultivated in various light conditions *in vitro* in terms of endogenous phenolic compounds and carbohydrates content. In the experiment, one-leaf shoot explants were cultivated on MS medium supplemented with 3% sucrose and growth regulators: BA and NAA (2.5 μM and 0.5 μM). The level of endogenous phenolic acids was estimated by HPLC while total phenolics and carbohydrates content was measured spectrophotometrically.

It was observed that a similar number of explants from both cultivars cultivated in the dark formed similar amount of adventitious bulbs while red, blue and white light strongly reduced the number of lachenalia 'Ronina' explants forming bulbs. The content of phenolic compounds was different in terms of quantity and quality in the newly formed lachenalia bulbs. A negative correlation has been shown between high amounts of phenolics and bulb regeneration ability. The total phenolics content in bulblets was ranging from about 0.5 mg/g DW ('Ronina' exposed to red light) to 2 mg/g DW (dark-grown 'Rupert'). Most of the examined conjugated phenolic acids (cinamic, p-coumaric, caffeic, ferulic, sinapic, chlorogenic) occurred in bulblets at a higher concentration in the white and blue light in comparison to the red light or in the darkness. It was observed that chlorogenic acid inhibited the process of swelling of new adventitious bulbs.It was observed that blue and white light stimulated shoot organogenesis, especially in 'Ronina'. At the same time this cultivar contained in the shoots more caffeic acid than 'Rupert' (respectively in the light white and blue). The highest concentration of starch was observed in 'Rupert' bulbs cultivated in the light white and blue and in shoots regenerated in darkness. Under these conditions 'Ronina' was characterized by higher content of soluble sugars in adventitious bulbs compared with 'Rupert'. No differences were found in the content of soluble carbohydrates in shoots of the cultivars tested. These investigations enhance the understanding of the bulb and shoot organogenesis of lachenalia in various light conditions in relation to the content of endogenous phenolic compounds and carbohydrates.

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Changes of biochemical parameters of wheat calli cells exposed to silver nanoparticles of various surface properties

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The impact of silver nanoparticles on calli cells of two varieties of wheat (*Triticum aestivum* L.): stress tolerant – Parabola and stress sensitive – Raweta was studied. Nanoparticles were synthesized by chemical reduction using $NaBH_4$. Surface properties of particles were modified by using various capping agents. Additionally, $HSCH_2CH_2NH_2 \cdot HCl$ was used to generate positive nanoparticle surface charge. Physico-chemical properties of silver nanoparticles were investigated using methods: dynamic light scattering (DLS), electrophoretic mobility measurements, electron microscopy (TEM), atomic absorption spectrometry (AAS) and surface enhanced Raman spectroscopy (SERS). The evaluation of changes of biochemical parameters of calli cells exposed to silver nanoparticles was based on the assessment of lipid peroxidation, proline content and antioxidant enzyme activity. The results revealed that the positively charged nanoparticles (SBATE) were more toxic, although they exhibited a similar ion release profile as the anionic nanoparticles obtained using sodium borohydride (SBNM). Both the positively and negatively charged silver nanoparticles caused increased peroxidation of membrane lipids in the cells of wheat. Stress tolerant Parabola proved to be more sensitive to the presence of SBATE, and Raweta showed an increased lipid peroxidation. The level of proline in the cells treated by silver nanoparticles increased nearly 100 fold in cell callus of Raweta variety, but only 2 fold in stress tolerant variety after contact with SBATE. For both wheat varieties the presence of silver nanoparticles resulted in a slight increase in the activity of superoxide dismutases. The presented results demonstrated that for plant cells positively charged silver nanoparticles caused grater changes of biochemical parameters than negatively charged ones.

Characterization of structural changes in the cells of wheat leaves under the direct and indirect action of zearalenone

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Zearalenone, a hormone controlling especially induction of generative development of winter plants (Biesaga-Koscielniak and Filek, 2010), when accumulated at highest level becoming the primary factor responsible for production losses and an increase in plant damage, as one of the dangerous mycotoxin. In presented experiments the changes of morphology of leaves cells exposed to direct action of zeralenone (by application of this substance on the leaf surface), and changes in plants grown from seeds infected by zearalenone, were examined. Two varieties of wheat (*Triticum aestivum* L.): stress tolerant – Parabola and stress sensitive – Raweta, were cultured in controlled, *in vitro*, conditions to obtain the phase of 3-leaves seedlings. Zearalenone, at 10 μM water solution, was used to infect both: the part of seeds and leaves of these plants which were grown from seeds no-treated by zearalenone. Observation of sections prepared from 2nd leaves and documentation of the results was performed with BX50 microscope (Olympus) with NIS Elements AR 3.00 NIKON software. After direct treatment of leaves with zearalenone the differences in the structure of their cells were observed in case of both varieties. These changes were related primarily to the presence of the central vacuole and to chloroplasts color pattern but were smaller for Parabola than for Raweta cells. In the mesophilic cells of Parabola, there were present chloroplasts with electron-opaque stroma, and with the faint visible thylakoid membranes. Changes in structure chloroplasts were observed only in individual cells. For Raweta, most of the cells were "flattened" – their cytoplasm was devoid of large vacuoles, indicating a loss of turgor and wilting of the leaf. In some cells, the chloroplasts without membranes and with the disintegrated tylakoid grains were found. Similar but smaller changes in anatomy were observed in leaves of plants grown from zearalenone-treated seeds. It may suggest, that independently on the way of zearalenone application, at higher concentration it can act toxically, influencing chloroplast structure, especially in plants sensitive to environmental stresses.

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Sulfur metabolism enzymes activity in *Brassica cretica* ssp. botrytis *in vitro* cultures

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Plants and microorganisms can assimilate inorganic sulfur as sulfate for reduction to sulfide leading in cascade of enzymatic steps to the synthesis of sulfur-containing amino-acids. In contrast humans and animals lack the capability to reduce sulfate. As a consequence humans and animals rely on their diet for provision of reduced sulfur in cysteine and methionine. Thus plants are the most important source of sulfur organic compounds like amino acids or others, lipoic acid for example. It constitutes economic interest of sulfur assimilation and sulfur amino acid biosynthesis in higher plants.

The aim of the present studies was to determine the effect of sulfate and cysteine concentrations on sulfur metabolism enzymes in *Brassica cretica* ssp. botrytis in *in vitro* cultures.

Brassica cretica ssp. *botrytis* experimental callus cultures were maintained on Murashige-Skoog (MS) medium containing two sets of plant growth regulators (2 mg/l BAP, 0.2 mg/l NAA, 1 mg/l 2,4-D and 2 mg/l BAP, 0.1 mg/l 2,4-D) supplemented with different amounts of sulfate (0, 0.5, 1.5, 3 and 5 mM) and cysteine (0 – 0.75 mM) under constant artificial light (ca. 4 W/m^2), at 24±2°C, and 4 weeks growing cycles. Activities of the following enzymes involved in thiol and sulfane sulfur metabolism were assayed, rhodanese, cystationase and β-cyanoalanine synthase.

Brassica cretica ssp. *botrytis in vitro* cultured callus tissue can assimilate cysteine and sulfate directly from medium. Both compounds affected sulfane sulfur level and activities of rhodanese and β-cyanoalanine synthase. Another sulfurtransferase – cystathionase was activated using only highest cysteine concentration and no influence of sulfate was observed.

In conclusion, sulfate is a good precursor of cysteine, utilized for glutathione biosynthesis, and leading to formation of sulfane sulfur-containing compounds. It could be good experimental model to modulate thiols and other sulfur compounds content in plants. Studies of the ubiquitous functions of sulfur in eatable plants provide integration in understanding of plant biology form molecular to cellular levels.

The use of *in vitro* cultures in obtaining the fertile interspecific hybrids

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Potato virus Y (PVY) is one of the most important pathogens of tobacco in Poland and in the world. The most effective method of preventing the crop loss caused by this virus is breeding for resistance. Since PVY is highly variable and capable of breaking existing sources of resistance, we made an attempt to combine va type of resistance originating form one of the two cultivars ('VAM' or 'Wiślica') with a complete immunity of a wild species *Nicotiana africana*. Interspecific crossings were performed in order to obtain hybrid offspring: *N. tabacum* 'Wiślica' × *N. africana* and *N. tabacum*' VAM' x *N. africana*.

Since genetic distance between parental species was large, seedlings of the acquired hybrid forms showed inviability caused by dying roots. In order to obtain viable hybrid forms *in vitro* cultures were used. Sterilized F1 seeds of hybrid forms *N. tabacum* 'Wiślica' × *N. africana* and *N. tabacum*' VAM' × *N. africana* were sown on LS medium (Linsmaier and Skoog, 1965), then the growth of seedlings was observed. At the first sign of root browning, cotyledons were cut out and their fragments were placed on medium prepared according to Lloyd (1975). After initial callus formation, shoot regeneration was observed. These shoots were cut out and placed on rooting medium. The growth and development of callus and the success of plant regeneration were variable and depended on maternal parent used for crossing with *N. africana*. Cotyledon explants of hybrids obtained from cv. 'VAM' regenerated significantly better, 88 amphihaploid forms were obtained. In contrast, from cv. 'Wiślica' only 27 viable hybrid forms were obtained.

Acquired amphihaploid plants were infertile, what made further breeding process impossible. In order to regain fertility, organogenesis from pith stems of amphihaploid plants was used in *in vitro* conditions. Pith stems of mature F_1 plants were sterilized and placed on Lloyd's medium. Initially these explants formed callus, then shoots, which were cut out and placed on rooting medium. Ploidy of acquired regenerants was determined by means of a flow cytometer. Results showed a high differentiation in the number and ploidy of acquired regenerants despite identical *in vitro* conditions for all experimental objects. Higher efficiency of forming amphihaploid forms was recorded for hybrids originating from crosses where maternal form was cv. 'VAM'.

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Content of oenothein B and phytosterols in *Chamaenerion angustifolium* (L.) Scop. *in vitro* propagated plants

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Chamaenerion angustifolium (L.) Scop. (syn. *Epilobium angustifolium* L.) from Onagraceae family is one of the best known medicinal plant used in the treatment of urogenital disorders including BPH (Benign Prostatic Hypertrophy). The raw material (herba Epilobii) is a rich source of polyphenols: tannins, especially oenothein B, flavonoids and phenolic acids as well as steroids and triterpenoids. Oenothein B is a major constituent (2%–14% in dried weight) and has been regarded as main active compounds. The aim of this study was to determine the content of oenothein B and chosen steroids compounds in *in vitro* propagated plants. Multi-shoots cultures were initiated from root's explants according to Turker (Turker et al., 2008) in slight modification. Ten *C. angustifolium* genotypes originated from different individuals (lines) were obtained. Underground part of rooted plants were harvested at the age of 5 weeks and dried at room temperature. HPLC-DAD method was applied for quantitative and qualitative determination of oenothein B and phytosterols. Oenothein B was found in all samples and its concentration ranged between 1.7% and 3.2% in dried weight. Stigmasterol was the predominant steroid in raw material and its content ranged from 0.38% to 0.58% in d.w. Content of stigmasterol and campesterol was lower: 0.12%–0.29% and 0.07%–0.22% respectively. Intraspecific variability of detected compounds was observed.

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Improvement of plant regeneration from *Sorghum bicolor* (L.) Moench callus cultures

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Sorghum bicolor (L.) Moench is a drought-tolerant crop used as food for humans and animals as well as a raw material for the production of biofuel. Sorghum is considered to be the most recalcitrant crop for *in vitro* response due to the high degree of genotype dependence and browning of tissue as an effect of accumulation of phenolic compounds. The aim of this study was to develop an improved protocol for plant regeneration from callus cultures. Callus was induced from the shoot tips (cv. Rona 1) on MS (Murashige & Skoog 1962) medium supplemented with 2,4-D (2.0 mg/l), kinetin (0.2 mg/l), proline (1.0 g/l), vitamin C (0.1 g/l), sucrose (30 g/l) and agar (8.0 g/l) – a control medium. To test the effect of medium composition on the induction and regenerative potential of calli, the control medium was supplemented with casein hydrolysate, polyvinylpyrrolidone, honey and sucrose. Shoot regeneration was achieved on MS medium supplemented with BAP (2.0 mg/l), sucrose (30 g/l) as well as in medium with honey (15 g/l).

The best frequency of explants producing calli was found for medium with honey (80.0%) and for the control medium (79.8%). The highest percentage (40.8%) of calli regenerating shoots was noted for the media with addition of honey. The average number of shoots per explant was 4.23 (±2.50). *in vitro* rooting of shoots was achieved on one-half strength MS medium supplemented with IAA (0.5 mg/l) and vitamin C. Rooted plantlets were acclimatized with 92% survival rate. Addition of honey to the medium was found to have a positive effect both on callus induction and shoot regeneration.

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Effect of meta-topolin on micropropagation of selected species of fruit plants in *in vitro* cultures

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Recently the new endogenous cytokinin meta-topolin (mT), isolated from aspen is applied in micropropagation. In some cases mT had a positive impact on more efficient regeneration and multiplication of shoots geranium (Wojtania, 2010), on the initiation of culture, optimizing the prevention of various disorders physiological *in vitro* in many plant species (Aremu et al., 2012).

The aim of the study was to investigate the effect of mT on micropropagation of serviceberry (*Amelanchier* × *grandiflora* 'Ballerina') and black currant (*Ribes nigrum* 'Tiben') shoots. Murashige and Skoog (1962) medium with addition mT or BA, both at two concentration was used (0.7 $mg \cdot l^{-1}$; 1.5 $mg \cdot l^{-1}$). Irrespective of the mT concentration this cytokinin had a positive effect on the rate of proliferation and the length of the serviceberry shoots in comparison to BA. Type of cytokinins didn't affect significantly the number of shoots and leaves. Both cytokinins at the concentration of 1.5 $mg \cdot l^{-1}$ significantly enhanced the number of serviceberry leaves, while the shoots are longer only on media containing mT. Cytokinins at concentration of 1.5 $mg \cdot l^{-1}$ significantly affected the following black currant parameters: the number of rosettes, the content of chlorophyll *a* and *b*, and carotenoids, the ratio of chlorophyll *a*:*b*; the higher values of these parameters were observed for cytokinin BA. The proliferation of black currant and serviceberry on media with different concentration of mT affected the number of leaves and shoots of the species tested. The concentration of mT significantly affected the dry matter, chlorophyll *a* and *b* and the carotenoids content in the stems and leaves of black currant. Serviceberry produced a smaller number of shoots but with more number of leaves compared to black currant. However, mT concentration in the medium didn't affect significantly the number of shoots and leaves. Whereas for the black currant more rosettes with less number of leaves observed. Similarly as for serviceberry, mT concentration in the medium didn't affect the black currant parameters.

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Methyl jasmonate induced over production of secondary metabolites in *Taxus baccata* callus cultures

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Plant cell factories constitute an alternative source of high added value phytochemicals such as the anti-cancer drug taxol, biosynthesized in *Taxus* spp. This work is focused on recent advances in the production of taxol and related taxanes in *Taxus baccata*, the taxol-producing European yew, using *in vitro* culture technology and elicitor metyl-jasmonate. Elicitors have been used as an important means of enhancing the production of taxanes in cell cultures of Taxus species . It has been proposed that jasmonates are key signal transducers leading to the accumulation of secondary metabolites (Namdeo, 2007). The biosynthesis and accumulation of paclitaxel and related taxanes in *T. baccata* are strongly promoted by jasmonic acid or its methyl ester (Bentebibel et al., 2005). In this study, callus cultures from leaves and young shoots of Taxus baccata. Callus growth and taxane production were evaluated using two culture media: Woody Plant Medium (McCown and Lloyd, 1981) and Gamborg's B5 (1968) supplemented with picloram (2 mg/L), kinetin (0.1 mg/L) and gibberellic acid (0.5 mg/L). The effect of the inoculum size (50, 100 and 150 g FW/L) with and without the presence of methyl jasmonate (100 M) on *T. baccata* cell callus was assessed. Taxane analysis revealed that the callus in Gamborg's B5 produced taxol (50 g/g DW), baccatin III, 10-deacetyl baccatin III and 10-deacetyl taxol. WPM also induced the production of taxol, although to a lesser extent. The optimum inoculum size was 50 g FW/L. In cell callus cultures, both media had a significant effect on taxane production when supplemented with methyl jasmonate. In WPM, at day 14, a total concentration of 185.35 g/L of taxol, 172.98 g/L of baccatin III, 658.97 g/L of 10-deacetyl baccatin III and 259.75 g/L 10-deacetyl taxol were obtained, with total excretion of baccatin III and 10-deacetyl taxol to the culture medium. The best culture conditions for producing taxol were found to be WPM supplemented with MeJ. The taxol level achieved in these conditions was 3.4 higher than in the same medium without elicitation and over 9 times higher than in the cultures grown in B5, elicited or not.

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Changes in proliferative capacity and morphology of *Nicotiana tabacum* L., callus cultured *in vitro* in response to various 2,4-D concentrations

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Callus is dynamic, histologically heterogeneous system where tracheary elements and meristematic centers can be differentiated, giving the origin of important anatomical events as shoot and root primordia formation eventually leads to plant regeneration (Gavinlertvatana and Li, 1980; Gatz and Kowalski, 2011; Ikeuchi et al., 2013).

For the callus induction and proliferation it is necessary to use a suitable auxin added alone or with cytokinin. Among natural auxins, IAA is often used to stimulate callus proliferation in *Nicotiana tabacum*. The IAA application requires higher concentrations due to this auxin instability connected with light and high temperature. Stronger auxin than IAA in cell divisions stimulation by this means in callus proliferations is 2,4-D, being a herbicide. In opposite to IAA, this synthetic auxin characterized by lack of light sensitivity and it that does not undergo disintegration under autoclaving temperature although it may inhibit organogenesis and induce mutation (Pavlica et al., 1991). In this study the usefulness of 2,4-D for proliferation of *N. tabacum* callus with the following concentrations 0.1, 0.5, 1.0, 1.5 and 2.0 mg·dm^{-3} with constant level of KIN were examined. Callus was derived from the pith of stem and its proliferation took place on the MS medium through 5 subcultures in the darkness at 25°C. After each passage the fresh weigh and morphological callus features were determined.

Among 2,4-D concentrations, 0.5 mg·dm^{-3} appeared to be the most favourable with regard for the highest increase of fresh weight in each of five subcultures as well as proper morphology features for proliferation. Variant of 1.0 mg·dm^{-3} 2,4-D in comparison with 0.1 mg·dm^{-3} was more beneficial considering regular increases of fresh weight and better cell cohesion for callus growth. Decreasing tempo of callus proliferation in subsequent passages as well as less or more brown coloration which was characteristic for calluses from concentration variants 1.5 and 2 mg·dm^{-3} 2,4-D may testify about the occurrence within calli very early differentiation processes or cell necrosis.

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The influence of mineral fertilization on fungal communities inhabiting quinoa leaves (*Chenopodium quinoa* Willd.)

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When it comes to plant production not only the quantity of the harvested crops which is important but also their quality. Plant organs which are used for consumption must be microbiologically pure, especially free from toxin-producing fungi and micotoxins produced by them (Lugauskas et al., 2006). The amount of damage caused by fungi depends on the amount and the form of plant fertilization.

Currently quinoa gains a lot of popularity among crop plants. The lack of gluten and a good proteinaceous profile are the major reasons for quinoa to be an attractive diet ingredient for people who suffer from coeliac disease or food intolerances (Przetaczek-Rożnowska and Bubis, 2016). In Poland there are good conditions to grow edible cultivars of quinoa (Gęsiński and Gozdecka, 2006). Unfortunately, during vegetation quinoa is frequently harmed by numerous pathogens and destructed by insects, which has a significant influence on the quality of crops (Pańka et al., 2004).

The research aimed at studying the impact of mineral fertilization on quantitative and qualitative development of fungal communities present on quinoa leaves (*Chenopodium quinoa* Willd.). It studied the two varieties of quinoa, i.e. Bydgoszcz and Dutch types. The study revealed that the quinoa leaves were inhabited by 22 species of fungi classified to 12 genera. The most frequently isolated pathogenic species were *Botrytis cinerea* (42.2%), *Fusarium graminearum* (11.2%) and *Fusarium culmorum* (9.2%). *Rhizopus* genus of fungus was the most dominant among saprotrophic species (5.9%). The study showed that the type of pre-sowing mineral fertilization affects the number of fungi colonizing the quinoa leaves. The population of fungi isolated from the leaves which came from the areas fertilized with nitrogen is the largest (342 colonies) and the most diverse in terms of species composition (16 species). Regardless of the type of fertilization, the Bydgoszcz variety of quinoa leaves was inhabited by 19 species of fungi (500 colonies). On the other hand, the leaves of the Dutch variety were colonized by 16 fungal species (396 colonies). Furthermore, it has been established that the disinfection of leaf surface reduces the overall number of the pathogenic fungal colonies by 48%.

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The high efficient rhizogenesis obtained on the leaf blades of *Euphorbia milii*

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Euphorbia milii (Leafy spurge) is a native plant to Madagascar. Interesting property of the spurge is secretion white "milk" called latex. It is produced in special cells – laticifers that reach into the shoots, leaves and roots (Stocking and Heber, 1976). The latex of *E. milii* contains many medicinally valuable substances, recently discovered extremely stable serine protease called "Milin" (Yadav et al., 2006) and also can be used as promising biodegradable molluscicide which could help fight with neglected diseases in Africa (NTD) (Schall et. al., 1998). The purpose of this study was to evaluate the morphogenetic ability of selected types of explants. Next it can be used to make an efficient *in vitro* regeneration system of *E. milii*. Selected explants (leafs blades and stems) were placed on inducing media based on MS (Murashige and Skoog, 1962) in different combinations of auxin and cytokinin: Indole-3-acetic acid (IAA) with 6- Benzylaminopurine (BAP), Indole-3-butyric acid (IBA) with BAP, 1-Naphthaleneacetic acid (NAA) with 6-(y,y-Dimethylallyamino) purine (2iP) in various concentrations in the light conditions. The first on the seventh day of culture observed morphogenetic response was callus induction in most of the explants on media containing NAA and 2iP. In the course of the culture after seven days forming of adventitious shoots on the shoot explants was observed with the largest percentage – 50% on the medium which contains IBA and BAP (0,80 mg/l IBA, 0,25 mg/l BAP). The another way of morphogenetic response after two week of culture induced on the explants was rhizogenesis. Only leaf blades gave rise to roots. This process was obtained at 90 % explants on medium with 0,8 mg/l NAA and 2 mg/l 2iP. On average 32,2 roots per explant were formed. This is the first report of such a high efficient rhizogenesis on *E. milii* explants and what is more, the best response surprisingly was observed on the medium with the predominance of cytokinin. From this tissue culture the natural substances present in latex *E. milii* can be achieved. Histological analysis of plant materials confirmed mostly direct and rarely indirect rhizogenesis, also proved that this process proceeded asynchronously.

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Zinc absorption from extracts of *Cantharellus cibarius* biomass in HUVEC and A549 cells

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Edible mushroom *Cantharellus cibarius* is the most frequently harvested mushroom in Poland and Europe. It is appreciated due to its aromatic piper like taste and as a source of physiologically active compounds: carbohydrates, amino acids, unsaturated fatty acids, phenolic compounds, vitamins and minerals, including zinc.

Zinc ions play important immunoregulatory role and reduce oxidative stress in cells. Effectiveness of zinc properties strictly depend on maintaining the zinc concentration homeostasis. Easily accessible zinc source may have impact on the cells ability to survive and their response to inflammation processes in which the need for this metal increases.

The present study was undertaken to investigate the influence of zinc source on its absorption using extracts from cultured biomass of *C. cibarius* additionally enriched in zinc in HUVEC (Primary Human Umbilical Vein Endothelial Cells) and A549 (Human Lung Epithelial Carcinoma) cell models activated with lipopolysaccharide (LPS) to induce inflammation. *C. cibarius* biomass was obtained with the addition of zinc hydroaspartate and zinc sulfate. The applied concentration contained 20 mg/l zinc ions. The biomass after separation from the medium was used to prepare methanol extracts. HUVEC and A549 cells were incubated with 20 µl/ml extracts for 24 h. Then media and cell fraction were collected and prepared for determination of zinc content, which ranged from 0.2–1.1 mg/l.

We demonstrated LPS-induced inflammation in cells increased zinc absorption in HUVEC and A549 cells in comparison to non-inflammatory controls. Another finding was that zinc sulfate is better absorbed than zinc hydroaspartate and the presence of mushroom extracts in medium (obtained from *in vitro* mushroom and *in vitro* mushroom supplemented in zinc sulfate) promotes zinc absorption. Zinc from medium was effectively absorbed for samples with mushroom *in vitro* biomass extract and LPS induced inflammation whereby decreasing the content in the medium by half in comparison to control, similar effect was observed for samples with zinc sulfate and LPS.

Mushroom cultures with high ability to accumulate elements enabled the precise application of zinc compounds may influence their immuno-modulatory properties which can contribute to fight inflammation.

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Micropropagation of blueberry (*Vaccinium corymbosum* L.)

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Blueberry (*Vaccinium corymbosum* L.) is a very popular fruit crop gaining popularity all over the world. Its production is spreading in different countries and the plantation surface keeps increasing. A growing demand for blueberry fruits imposes on nurserymen a necessity to develop an efficient propagation method of this species. The conventional methods are not sufficiently efficient therefore nurserymen try the *in vitro* techniques.

The aim of the study was to evaluate the effect of the culture medium composition on shoot regeneration in two blueberry cultivars 'Bluecrop' and 'Duke'. A kind and concentration of cytokinins: N^6-benzyladenine (BA), N6-furfuryladenine (Kinetin) and 6-(γ,γ-dimethylallylamino)purine (2iP) as well as sucrose concentration were tested. Also the effect of auxins – indole-3-butyric acid (IBA) and 1-naphtalenacetic acid (NAA) – on the *in vitro* rooting of microcuttings was evaluated. Rooting *ex vitro* was studied as well: here the rooting powder Rhizopon AA (1% IBA), the water IBA solution (50 mg·dm^{-3}), salicylic acid (50 mg·dm^{-3}) and the mixture of two latter compounds (v/v 1:1) were used. Microshoots were sprayed with the above mentioned preparations while the control cuttings were treated with water.

Better regeneration on the initial medium occurred in the cultivar 'Duke' (93%) than in 'Bluecrop' (79%). In both cultivars the best regeneration was observed in presence of 3 mg·dm^{-3} or 5 mg·dm^{-3} 2iP. Supplementation of the medium with sucrose in conc. 10 g·dm^{-3} – 30 g·dm^{-3} resulted in an abundant shoot proliferation. Including 0,05 mg·dm^{-3} NAA into the W Woody Plant Medium (Lloyd and McCown, 1980) resulted in 42% rooting of the 'Bluecrop' microcuttings while 0,1 mg·dm^{-3} IBA stimulated root formation in 22% microshoots of 'Duke'. The constant presence of auxin in the medium caused callus formation at the bases of microshoots and the elongation of the latter.

Better results were obtained when microshoots were rooted ex vitro. In both cultivars microcuttings treated with the preparation Rhizopon AA rooted in 3 weeks in over 90%. In 'Duke' comparable results were obtained due to the foliar application of the water IBA solution or salicylic acid. The best formed root balls were found in microcuttings treated with the commercial rooting powder.

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Stevia rebaudiana Bertoni *in vitro* cultures for the production of steviosides

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Stevia rebaudiana Bertoni of Asteraceae family is cultivated as a natural sweetener source in many parts of the world. The plant comes from Paraguay and it is well known and been used by local societies for many centuries. Steviol glycosides (steviosides) are compounds responsible for the sweet taste of stevia and have been investigated also for their activity in regulation of glucose metabolism, e.g. the influence on glucose adsorption or insulin'secretion (Pande and Gupta, 2013, Chatsudthipong and Muanprasat, 2009). The high demand for natural sugar substitutes and the potential therapeutic properties have made S. rebaudiana also an object of various biotechnological investigation, where the intensification of steviosides production in *in vitro* cultures is one of the key directions (Bayraktar et al., 2016). In our experiments we have focused on *S. rebaudiana* shoot multiplication, natural and transformed roots formation, callus tissue development and HPLC investigation for stevioside presence in obtained plant tissues. Multiplication of shoots was performed on numerous plant culture media and showed the best growth, the best rooting (up to 95.6%) and the highest stevioside content (up to 1.46±0.22%) on modified NN and modified MS media, both with reduced salts and vitamin content, 3% of sucrose and without hormone presence. Establishing callus tissue was the most effective on MS medium supplemented with 1mg/l BAP and 1mg/l 2,4-D. Transformation of S. rebaudiana was performed with *Agrobacterium rhizogenes* ATCC 15834 strain. Preliminary HPLC DAD analysis of obtained tranformed roots showed the presence of stevioside in post-culture B5 and SH media (57.06 mg/l and 43.02 mg/l, respectively), while there was no stevioside in natural root cultures.

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Development of *Larrea tridentata* Coville hairy roots cultures and their preliminary phytochemical investigations

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Larrea tridentata is a small, evergreen, long-lived and very slow growing shrub belonging to Zygophyllaceae family. It occurs in desert areas in the Southeast United States and in Northern Mexico. It is known for its healing properties and is commonly used for a treatment of many diseases such as rheumatism, digestive problems, viral infections and certain cases of cancer Compounds identified in the plant belong to polyphenols and triterpenes classes. The lignans of dibenzylbutane type are the most common among the polyphenol compounds and among them the most widespread is nordihydroguaiaretic acid (NDGA) (Arteaga et al., 2005). Previous studies have shown that extracts of *L. tridentata* contain biologically active compounds with antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal and antiviral activities (Gnabre et al., 2015). The *L. tridentata* has not been introduced to *in vitro* culture yet.

Objective of this project was to elaborate the optimal medium for biomass growth and secondary metabolites biosynthesis in hairy roots cultures of *L. tridentata*. The identification and quantification of NDGA in extracts obtained from hairy roots using HPLC-DAD was performed.

The *L. tridentata* hairy roots cultures have been achieved for the first time. Young stems of *in vitro* germinated plants were transformed with *Agrobacterium rhizogenes* ATCC 15834 and the transformation has been successfully confirmed using PCR method. The obtained clones of transformed roots have exhibited distinctive morphology features and fast growth.

Up to date we have selected SH and DKW medium for the highest biomass production. Various rhizoclones have differed in their morphology and growth ratio determined after 4-week-lasting passages. Polyphenolic glycosides extracted from lyophilised tissues of transformed roots were hydrolysed with almond β-glucosidase and subjected to HPLC analysis. In transformed roots extracts we confirmed the presence of NDGA among other polyphenols.

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Accumulation of secondary metabolites in agitated shoot cultures of *Scutellaria lateriflora* L

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Scutellaria baicalensis Georgi (Lamiaceae), known for a long time as a medicinal plant species in Traditional Chinese medicine, was introduced into official European phytotherapy in 2011(European Pharmacopoeia, 2011). Another species of *Scutellaria* genus, with other pharmacological properties is *Scutellaria lateriflora* L. The overground parts of this plant species are used in traditional North American therapy, because of their documented sedative and anticonvulsant properties. This raw material posses also an antioxidant and DNA protecting effects. The main groups of bioactive compounds of this plant include specific flavonoids, phenolic acids and the phenol glycoside – verbascoside (Barnes et al., 2007).

The aim of the present studies was to determine the influence of different concentrations of plant growth regulators (PGRs) in the medium on accumulation of bioactive compounds in agitated, shoot cultures of *Scutellaria lateriflora* L. and to propose this cultures as a potential, biotechnological, rich source of these bioactive components.

The agitated cultures were maintained to grow four weeks on six different variants of the Murashige and Skoog (MS) medium variants (Murashige et al., 1962) supplemented with PGRs: 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA) in range from 0.5 to 3.0 mg/l. The compounds found in the methanolic extracts from biomass were analised by HPLC method (Ellnain-Wojtaszek et al., 1999).

The influence of PGRs on accumulation of estimated compounds was confirmed. The presence of five flavonoids (baicalein, baicalin, scutellarin, wogonin, wogonoside), 3,4-dihydroxyphenylacetic acid and verbascoside were estimated. Baicalin was the main metabolite that accumulated in the highest amounts on medium variant with 1 mg/l BAP and 0,5 mg/l NAA (491,9 mg%). For verbascoside the best "productive" medium was MS variant supplemented with 1 mg/l BAP and 1 mg/l NAA (84,5 mg%).

The best "growth-promoting" medium and parallel the best total flavonoid-productive medium was MS variant supplemented with 1 mg/l BAP and 0,5 mg/l NAA. For the total content of flavonoids (659,5 mg%) the best "growing" and "productive" medium variant was also a MS5 supplemented with 1 mg/l BAP and 0,5 mg/l NAA.

The great role of varied supplementation with plant growth regulators on accumulation of bioactive compounds was documented in agitated cultures model.

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The influence of different concentrations of plant growth regulators on accumulation of secondary metabolites in *in vitro* cultures of *Scutellaria lateriflora* L.

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Scutellaria lateriflora L. (Lamiaceae) is also known as American skullcap and it is used in traditional North American therapy. The herb of this plant species has been used to treat nervousness, irritability, and neuralgia, as well as for its anticonvulsant and antioxidant properties and it posses also DNA protecting effects. *Scutellaria lateriflora* L. contains some specific flavonoids, phenolic acids and the phenol glycoside – verbascoside (Barnes et al., 2007).

The aim of this study was to establish agar, shoot cultures of *Scutellaria lateriflora* L. on Linsmaier – Skoog (LS) medium (Linsmaier et al., 1965) and to determine the influence of combined concentrations of plant growth regulators (PGRs), cytokinin – BAP and an auxin – NAA (ranging from 0.5 to 3.0 mg l^{-1}) in the medium on the accumulation of flavonoids, phenolic acids and verbascoside.

The established shoot cultures of *Scutellaria lateriflora* L. were maintained on six different Linsmaier – Skoog (LS) medium variants. Methanolic extracts from the biomass collected after 4-week growth cycles (3 series) of *Scutellaria lateriflora* L. were tested for the amounts of above mentioned groups of metabolites using DAD-HPLC method (Ellnain-Wojtaszek et al., 1999).

The presence of five specific flavonoids (baicalein, baicalin, scutellarin, wogonin, wogonoside), 3,4-dihydroxyphenylacetic and verbascoside was confirmed in the methanolic extracts from *in vitro* biomass. The great influence of PGRs in the culture media on production of all metabolites were documented. The highest total content of flavonoids was obtained on LS medium variant supplemented with 1 mg/l BAP and 0,5 mg/l NAA (2,93 g%). This medium variant is also the best "growth-promoting" and "productive" medium for the main flavonoid, baicalin (1,84 g%). The best productive" medium for verbascoside (543 mg%) and 3,4-dihydroxyphenylacetic acid (70,5 mg%) was LS medium variant with 2 mg/l BAP and 2mg/l NAA.

The results of the studies have shown that the concentration of plant growth regulators affects the production of estimated bioactive secondary metabolites, especially flavonoid glycosides.

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The influence of precursor of ethylene (ACC) and silver nitrate (AgNO3) on accumulation of free proline in adventitious bulbs and roots of *Lilium martagon* L. (Liliaceae) in *in vitro* cultures

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Accumulation of proline in plants is an indication of physiological disorders, induced by biotic or abiotic stress conditions. Determination of free proline content is a useful to monitor physiological status and to assess stress tolerance of higher plants (Abraham et al., 2010).

The aim of the present experiments was to determine the effect of precursor of ethylene biosynthesis – 1-aminocyclopropane-l-carboxylic acid (ACC) and ethylene inhibitor ($AgNO_3$) on the organogenesis and the content of free proline in Turk's cup lily (*Lilium martagon* L.) in *in vitro* cultures. Lilium bulblet scales explants were multiplied on the medium with basic composition described by Murashige and Skoog (1962), contains 0–1.0 μM BA and 0–1.0 μM IAA. Media were supplemented with ACC (1.0 and 2.0 mg l^{-1}). On the other hand the silver nitrate ($AgNO_3$) was used which is inhibitor of ethylene action. The proline analysis was made in the scales of adventitious bulbs and roots with the method of Bates (1973).

The content of free proline was found depended by type of regenerating organ and medium composition. It was ranged from 1.36 to 71.94 mg 100 g^{-1} f.m. In the bulbs with a diameter of over 5 mm, the proline level was higher than in the bulbs with diameter smaller than 5 mm. The level of free proline in de novo formed bulbs was higher than in the roots.

Cytokinin in the medium affected the reduction in the level of proline in the bulbous scales and roots. The proline content in the tissues examined regenerants Turk's cap lily (*Lilium martagon* L.) grown on the control media (without the addition of ACC and $AgNO_3$ containing 1.0 μM l^{-1} BA and 0.1 μM l^{-1} IAA) was lower than for lilium origin from other tested media. In contrast it was observed that ACC at a dose of 2.0 mg l^{-1} stimulated accumulation of proline in bulbous scales and roots.

The results will help to optimize the process of forming adventitious bulbs at the Turk's cap lily propagated using *in vitro* techniques.

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Preliminary studies on the response of immature endosperm in ovules cultures of sunflower

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Endosperm occurs in angiosperms after the process of double fertilization and is used as a source of nutrients by the embryo during its development. The short life-cycle of this tissue determines its extremely specialized role in plant life. However endosperm can be stimulated to the proliferation and differentiation under *in vitro* conditions (Thomas and Chaturvedi, 2008).

Helianthus annuus L. which is known as a sunflower is an annual plant of the *Asteraceae* family, commercially used in the industry due to the nutritional value of the edible seeds and their high content of unsaturated fatty acids. So far there is no scientific reports of proliferation and differentiation of sunflowers endosperm in tissue culture. Interestingly, the untypical growth and activity of endosperm tissue was observed in culture of isolated embryo sacs (Popielarska and Przywara, 2003) and ovules (Popielarska, 2005) sporadically.

In the following study sunflower cv. LG 55.25 (Lima Grain Central Europe) was used as source of pollinated ovules. Histological sections of explants at inoculation showed embryo sacs with the first events of the fertilization. Explants (intact ovules or with small micropylar part dissected) were placed on solid modified MS media, in a darkness. During the culture explants were collected and fixed for histological analysis. The most promising response on AEM medium (Góralski et al., 2005) was noticed. Histological sections revealed that after 3 weeks of the culture endosperm tissue was still visible, simultaneously with or without embryo. However strong callogenesis of ovule tissues, especially from vascular bundles were also observed. Next modifications, like plant growth regulators composition of medium and the ovules culture combined with isolated embryo sacs technique should be tested.

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The problem of explants darkening during tissue culture of *Miscanthus* × *giganteus*

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The perennial grass, *Miscanthus* × *giganteus* is a sterile natural interspecific triploid hybrid. The large biomass production and the low input requirements makes this plant an interesting non-food crop. Giant miscanthus can only be propagated vegetatively by rhizome division or by *in vitro* propagation. The major problem in tissue culture of *Miscanthus* × *giganteus* is the phenomenon of darkening of explants. Tissue is very sensitive to technique of micromanipulation and intensively produces phenolic compounds which products of oxidation reduce callus induction.

This study was aimed to increase efficiency of callus induction and minimize the intensity of darkening of explants in anther, ovary, flower bud and immature inflorescences cultures. To reduce the darkening, callus-inducing media (CIM) was supplemented L-proline (2,88 $mg \cdot dm^{-3}$), L-cysteine (50 $mg \cdot dm^{-3}$), reduced glutathione (GSH; 2,88 $mg \cdot dm^{-3}$) or honey (30 $g \cdot dm^{-3}$; instead of sucrose/maltose). CIM were modified MS medium.

Extensive explants tissue darkening were observed in all cultures regardless of the type of medium. All tested substances were increased efficiency of embryogenic callus induction in immature inflorescence culture. The best induction rate was observed on medium supplemented with honey – 87,0 % explants formed calli. In other media, the frequencies of embryogenic callus formation were 76,0% on CIM + GSH, 67,7% on CIM + L-proline, 61,7% on CIM + L-cysteine and 44,3% on control CIM. All embryogenic calli regenerated green plants on regeneration medium. Flower buds produced several calli only on control medium, while anthers and ovaries desiccated and degenerated.

Accumulation of phenylpropanoid glycosides – verbascoside and isoverbascoside in callus cultures of *Verbena officinalis* L. cultivated under different light conditions

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Verbena officinalis L. (vervain) it's known medicinal plant species. One group of the main constituents of the herb plant raw material are phenylpropanoid glycosides: verbascoside and isoverbascoside. These compounds show valuable biological properties: anti-inflammatory, antimicrobial, cytoprotective, chemopreventive, and UV-radiation protective activities (Alipieva et al., 2014).

Our earlier research confirmed the high production of verbascoside in extracts from callus cultures of *V. officinalis* cultivated on Murashige-Skoog (MS) (1962) medium under constant artificial white light and in darkness (Kubica et al., 2016).

The aim of our current study was to investigate the accumulation of verbascoside and additionally it's isomer – isoverbascoside under different light conditions: blue, red, far-red light, UV-A irradiation, in darkness and in the presence of white light as control conditions. The callus cultures were maintained on MS medium with 1mg/l of BA and 1mg/l of IBA and were collected after 2, 3 and 4-weeks of growth cycles. The estimations of both compounds were performed in methanolic extracts by DAD-HPLC method (Ellnain-Wojtaszek and Zgórka, 1999).

The biomass increments and the accumulation of investigated compounds were associated with the light conditions and duration of growth cycles. The dry biomass increments were high, ranged from 11- to 20-fold. The accumulation of verbascoside was high, ranged from 0.72 to 6.35 g/100g DW. The highest amount of this compound was detected in extracts from biomass cultured in darkness for 3 weeks. The accumulation of isoverbascoside was also high, ranged from 0.06 to 1.43 g/100g DW with the highest amount in extracts from biomass cultured under blue light for 2 weeks.

The estimated amounts of both glycosides are interesting from practical point of view.

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Preliminary study on micropropagation of wild beets from *Patellifolia* section

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Wild beets from *Patellifolia* section are considered as a valuable source of resistance genes for beet breeding. These plants are characterized by a morphologic and genotypic diversity and with the physiology in the large measure dependent on particular conditions of their habitats (Asher at al., 2001). The regeneration capacity of whole plants of the wild beet species by tissue culture has not been well-known. Most of previous research have concentrated mainly on sugar beet (*Beta vulgaris* L.).The main aim of this study was to detect the ability to plant regeneration of autotetraploid species – *Patellifolia patellaris* (Moq.) and the diploid species – *Patellifolia procumbens* (Chr. Sm.) in *in vitro* cultures. The plant material was originated from the glasshouse gene bank collection of the Plant Breeding and Acclimatization Institute in Bydgoszcz belonging to National Centre for Plant Genetic Resources in Radzików. The investigation was carried out in a growth chamber at 23°C under artificial daylight conditions with 16-h photoperiod. The initial explants were the 0,1–0,3 cm long tips and lateral buds of flowering shoots – organs of beet appear to be genetically stable and have high morphogenetic potential. Sterilized pieces were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with 0,2–1 mg l^{-1} BAP (6-benzyloaminopurine). After about four weeks explants of wild beets started to regenerate shoots or shoot like structures. Inflorescence tips produced more adventitious shoots than lateral buds. Coefficient of reproduction was high and dependent on plant genotype organ and components of used medium. For both species the highest multiplication rate was achieved on MS medium supplemented with 0,2 mg l^{-1} BAP. Of the investigated combinations of root-inducing medium full MS with 1,0 or 3,0 mg l^{-1} NAA (1-naftalenoacetic acid) with addition of vitamins and on full MS with fructose and 1,0 or 3,0 mg l^{-1} NAA in combination with 0,4 mg l-1BAP were the best for rooting *P. patellaris* species. *P. procumbens* showed very closely to sugar beet a high ability to developed roots on 1/2 MS medium with 3,0 mg l^{-1} IBA (indole-3-butric acid). In *in vitro* produced plantlets were successfully removed to the soil. The percentage of survival was between 25–85% and dependent on plant genotype. Both wild beets from *Patellifolia* section exhibited the tendency to shortening of the vegetative phase in *in vitro* cultures.

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Thiols and sulfane sulfur levels in agitated shoot cultures of *Brassica cretica* ssp. botrytis

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Sulfur is an essential macronutrient for the majority of organisms. It is a component of many compounds such as amino acids – cysteine, cystine, methionine, glutathione, enzymes and vitamins. The biggest content of active sulfur metabolites can be found in plant families like Brassicaceae and Alliaceae. Plants and microorganisms can assimilate inorganic sulfur as a sulfate. The reduction of this compound to sulfide is leading to cascade of enzymatic steps of the sulfur-containing amino acids synthesis. In contrast humans and animals lack the capability to reduce sulfate. As a consequence humans and animals rely on their diet for provision of reduced sulfur in cysteine and methionine. Biological activity of sulfur compounds makes them essential for human diet, so their deficiency may cause many health complications. Thus plants are the most important source of sulfur organic compounds like cysteine or lipoic acid for example. It constitutes economic interest of sulfur assimilation and sulfur amino acid biosynthesis in higher plants. Additionally the sulfide/disulfide redox system was retained as detoxification mechanisms for reactive oxygen species and signal transduction mechanisms in plant.

The aim of the presented studies was to conduct a comprehensive study on the effect of sulfate supplementation on level of active sulfur compounds and their metabolism in agitated shoot cultures of *Brassica cretica* ssp. *botrytis*.

Agitated shoot cultures were maintained on Murashige-Skoog (MS) medium supplemented with different amounts of sulfate (0–5 mM) under constant artificial light (ca. 4 W/m^2), at 24±2°C, during 4 weeks. The levels of the following compounds were measured: non-protein sulfhydryl groups, glutathione, cysteine, cystine, sulfane sulfur, reactive oxygen species and malondialdehyde (a product of lipid peroxidation).

The presented studies demonstrated that supplementation of sulfur compounds has a significant impact on the development of the cultures. Sulfate ions are taken from the medium by the *in vitro* cultures and efficiently metabolized to thiols and sulfane sulfur containing compounds. In higher concentrations NPSH, glutathione, cysteine and sulfane sulfur levels were significantly elevated. Medium sulfate supplementation protects cells against reactive oxygen species, too. We demonstrated the effect of different composition of growth regulators on tested parameters.

In conclusion, sulfate is a good precursor of cysteine, utilized for glutathione biosynthesis, which was indicated by its capability to elevate non-protein sulfhydryl groups level and leading to formation of sulfane sulfur-containing compounds.

Application of *in vitro* culture in order to evaluate the efficiency of sorghum transgenesis via microprojectile bombardment

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Sorghum (*Sorghum bicolor* L. Moench) is an important grain and forage crop that is uniquely adapted to semi-arid environments. Advances in biotechnology are now beginning to be used to augment traditional approach for crop improvement. Recently, owing to fast development of molecular biology and the availability of genetic engineering tools, a wider range of genetically modified organisms (GMO) has emerged. The development of plant transformation methods is very dynamic, and the methods have been commercialised very quickly. Microprojectile bombardment employs high velocity metal particles to deliver biologically active DNA into plant cells. This method is especially useful for monocot plants such as sorghum.

A DNA construct containing a marker gene β-glucuronidase (GUS) under the control of a promoter S35 was used for optimization of the method of DNA introduction into sorghum's tissues. Microprojectile bombardment was performed to two types of tissue, i.e. the tip of the shoot and callus. Shot parameters differed in the speed of gold coated DNA particles and the distance from the source of particles and the tissue. After 24 hours of bombardment the tips of shoot were transferred into induction medium and the culture was carried out in the dark, while the callus tissues were transferred into a regeneration medium and grown under a photoperiod (16/8). In the next step the explants were subjected to staining.

Microprojectile of DNA into the callus tissue did not produce the expected results. Only one case of GUS gene expression in sorghum callus tissue was detected and only in one root of 17 adventitious organs tested glucuronidase gene activity was showed. However, bombardment of DNA into top of shoots achieved positive effect in all cases. The highest efficiency of the process was obtained using the smallest energy shot (pressure 1100 psi) and the smallest distance between the gold particles and the tissue. The results indicate the possibility of obtaining transgenic sorghum organs by the means of microprojectile bombardment but the process needs further investigation.

Synthesis of pharmacologically active compounds of plants from the Droseraceae family in response to light stress in *in vitro* conditions

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Carnivorous plants from the Droseraceae family have the ability to produce secondary metabolites, ie. naphthoquinones, which have strong antibacterial and antifungal properties. One of the possible methods of increasing the content of secondary compounds in *in vitro* conditions can be elicitation by biotic or abiotic factors (Babula et al., 2006).

Changes in the spectral composition and the intensity of light can be a stress factor for plants. Response to radiation stress may lead to changes in the intensity of secondary compound production.

The aim of the study was to examine the response of *Drosera peltata* (Sundew) and *Dionaea muscipula* (Venus flytrap) plants to modified conditions of light quality and quantity, at the level of secondary metabolite synthesis and the activity of antioxidant enzymes.

Tissue cultures of both species were cultivated on 1/2 MS medium with no growth regulators in 24±1°C, and in various light conditions: blue-red and white LED light, fluorescence light (all three with 120 [µmol (quantum) × m^{-2} × s^{-1}] intensity, photoperiod 16/8 (light/dark)) and darkness. Control objects were grown in fluorescent light conditions with 50 [µmol (quantum) × m^{-2} × s^{-1}] intensity. Plants were tested for plumbagin concentration, total phenolic content and the peroxidase and catalase activity.

The results showed that concentration of phenolic compounds and plumbagin in *D. muscipula* increased (in a positive correlation) after four weeks in high light intensity conditions, independently of the light spectral composition, and decreased in darkness. The most effective accumulation of naphthoquinone in *D. peltata* was observed in the darkness. Plumbagin production intensity in Sundew in various light conditions was depend on the spectral composition. Differences observed in the secondary compounds synthesis may arise from the different acclimatization strategies of particular genes to modification of light conditions. The study also showed the differences in activity of antioxidant enzymes between the examined objects.

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Production of *Narcissus* L. 'Carlton' somatic embryos using a temporary immersion bioreactor

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Propagation by conventional tissue culture based on solid medium is cost-intensive due to high labor inputs and low degree of automation. Temporary immersion bioreactor is effective technology for increasing the efficiency of production and quality of a resulting plant material. Temporary immersion (RITA® bioreactor), liquid (rotary shaker) and solid culture systems were compared to evaluate *in vitro* production of somatic embryos in *Narcissus* L. 'Carlton' embryogenic callus. The following immersion frequencies: 15 min every 24 or 8 hours were tested. The clusters of embryogenic callus were cultured on MS liquid medium with 3% sucrose and growth regulators: 25 μM Picloram + and 5 μM BA for 7 weeks and then for the next 12 weeks on solid medium with 0.5 μM NAA and 5 μM BA. After 7 weeks the biomass growth and somatic embryos of different stages, and after 19 weeks somatic embryos converted into plants, plants with malformations and number of roots were calculated. Results showed that the culture system did not affect biomass growth but it influenced somatic embryos formation. The highest number of somatic embryos (48,6–50 embryos per 1 g of callus) in bioreactor was obtained and the number was independent on immersion frequency. However, of the different immersion frequencies, 1 time per day accelerated embryo maturation. Generally, liquid culture systems allowed to obtain high number of somatic embryos but also delayed their maturation and conversion. Rotary shaker cultures inhibited somatic embryo formation and stimulated roots development. In turn, solid medium cultures promoted embryo conversion. Plant malformations did not depend on culture system.

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Effect of chilling *in vitro* on maturation of somatic embryos of tulip 'Apeldoorn'

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In natural conditions, an zygotic embryo of tulip requires low temperature to break dormancy and also for further embryo development (Niimi, 1978; De Hertogh and Le Nard, 1993). According to that information, the authors decided to analyze if chilling period enhance maturation of somatic embryos of tulip. To that end, the somatic embryos of tulip 'Apeldoorn', at torpedo stage, 5–10 mm length, were placed on MS solid medium containing 6% saccharose, 5 μM picloram and 1 μM 6-benzylaminopurine (BAP). The embryos were maintained in darkness at 5°C or 25°C for ten weeks. After that period biometrical observation were done.

The obtained results have revealed that chilling significantly affected maturation and development of the tulip somatic embryos. Low temperature treatment decreased over five times the growth value indicator (GV) in fresh weight (FW) of the embryos, which amounted 0.8. GV = (FW final in g – FW initial in g)/ FW initial in g. The embryos increase in length, equal 1.6 mm, was nearly six times lower in comparison with the increase in length of the embryos from 25°C. Dry weight (DW) of the chilled embryos was also significantly lower (about 23%) than DW of non-chilled embryos. The embryos maintained at 5°C have not malformed, what was positive impact of chilling. About 40% of the somatic embryos, cultivated at 25°C underwent malformation. During maturation, both chilled and non-chilled somatic embryos have not formed any shoots. The obtained results allow to optimize production process of the somatic embryos of tulip.

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The impact of silver nanoparticles on photosynthesis and carbohydrates metabolism in *Triticum aestivum* L.

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Several kinds of silver nanoparticles are considered as a good material for use in cultures *in vitro* as a bacteriostatic component. The mechanism of interaction of silver nanoparticles on plants has not been well recognized yet. Silver nanoparticles impact on plant physiology is very important in utility point of view. The aim of the study was to evaluate the impact of three types of silver nanoparticles suspensions with different surface properties on the process of photosynthesis and carbohydrates metabolism in common wheat (*Triticum aestivum*). A silver nitrate was used as references of pure ionic form of silver.

The experiment was carried out on the spring common wheat, cv. Tybalt. The seeds surface was sterilized in a 5% sodium hypochlorite solution for 5 min, then rinsed thoroughly several times with deionized water. The experiment was performed in seed trays containing 100 grains. The trays were placed in four-chamber plastic containers filled with the tested suspensions or water (a control group). Four trays (one as a repetition) with 100 seeds per treatment were used. The plants were cultured in a greenhouse at 18°C. The natural light was supplemented to 250 μmol $m^{-2} \cdot s^{-1}$ PPFD (AGRO Philips sodium lamps) up to a 12 h photoperiod. The plants were treated with the silver nanoparticles suspensions and silver nitrate solution (concentration of silver 10 mg L^{-1}) in an amount of 100 mL per each 100-seeds tray. After a two-week growth in the hydroponic culture with silver suspensions (nano and ionic forms) and next after transplanting into pots, ie. in the 3rd and 4th weeks of growth the activity of RuBisCO, the contents of the most important carbohydrates and enzymes of their metabolism were analyzed.

It was found that the applied of silver nanoparticles colloids and $AgNO_3$ solution affect significantly the physiological processes. Silver nanoparticles suspensions and $AgNO_3$ solution caused significant increase in sucrose and decrease in fructose but not affected glucose as comparing to control in all treatments after 3 weeks from the beginning of the experiment. Applying nanosilver suspension reduced using sodium hexametaphosphate (SHSH) resulted in increased activity of RuBisCO while suspension reduced with tannic acid (TA) and sodium citrate (SBTC) caused a decrease of this parameter. The lowest activity of sucrose synthase (SUS) had occured in the case of treatment of seedlings of wheat with $AgNO_3$ solution after 3and 4 weeks from the beginning of the experiment. In turn SHSH suspension stimulated activity of sucrose phosphate synthase (SPS) after 3 weeks.

To summarize the impact of silver on the plant is very dependent on the form (nano or ionic), and consequently (for nanoform) on the type of reduction used in the production of silver nanoparticles, ie. the surface properties of the nanoparticles. The physiological reactions of plants can be triggered by silver nanoparticles.

In vitro culture of lateral roots of *Iris pseudacorus* and influence of sucrose concentration on root growth

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Irises of the *Iridaceae* family are mostly known as an ornamental flowers. In traditional medicine they were used as expectorant, analgesic and disinfectant agents (Grieve, 1971). Nowadays they are used in the perfume and cosmetic industry (Braton et al., 2001)

Some iris species produce numerous biologically active secondary metabolites such as isoflavones, chinons, flavonoids and triterpenoids, which exhibit cytotoxic and antimicrobial activity, and therefore have enormous pharmaceutical potential. These compounds are produced in plants cultured *in vivo* mostly in underground tissues: rhizomes and roots. Production of secondary metabolites in irises varies between tissue and species (Kaasak, 2012).

One of the wild type species, *Iris pseudacorus*, produces secondary metabolites mostly in rhizomes in in vivo conditions. Unfortunately production of rhizomes in all iris species is too extended in time to be suitable for *in vitro* production of valuable secondary metabolites. Our preliminary research on this species show that *I. pseudacorus* is also able to produce secondary metabolites in roots in in vivo conditions. Because this tissue type grows much faster, we focus on it as a possible source of metabolites in *in vitro* culture.

During our research we initiated stable culture of lateral and adventitious roots in Murashige and Skoog (MS) liquid medium (2% sucrose; 0.3% polyvinylpyrrolidone; pH 5.8) medium. We tested different culture conditions such as sucrose concentration: (2, 3 and 4%), addition of plant growth regulators (PGRs): auxin and cytokinin (0.5 mg/ml NAA and 0.5 mg/ml BAP), solid (0.7% agar) and liquid MS medium, standard photoperiod (16/8h) and constant darkness, to find the best conditions for high biomass production. The best results were obtained for 4% sucrose in liquid MS medium without PGRs in standard photoperiod. We also established that 4% sucrose concentration in liquid medium increases the rate of adventitious root growth in whole plants of *I. pseudacorus*.

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Lipid staining as an indicator of the embryonic identity of structures regenerated *in vitro* in *Arabidopsis*

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Regeneration of plants under *in vitro* culture is accomplished via two alternative morphogenic pathways, somatic embryogenesis (SE) or shoot organogenesis (ORG). SE is manifested by the formation of bipolar structures resembling zygotic embryos and thus termed as somatic embryos while ORG proceeds through development of shoot primordia. Besides extensive use of SE and ORG in micropropagation of plants, these processes provide the model systems in studies on molecular determination of developmental plasticity of plant somatic cells. Hence, it is of importance to define a mode of morphogenetic pathway that is induced in the regeneration system studied. However, morphological observations are often not sufficient and thus laborious histological analysis are used to distinguish somatic embryos from shoot primordia.

In the present study a simple method that enables identification of the embryogenic tissue and somatic embryos is proposed. Explants (immature zygotic embryos) of Arabidopsis thaliana, a model plant in molecular studies, were cultured *in vitro* on media with different plant growth regulators to induce SE and ORG (Kraut et al., 2011). To distinguish between embryogenic and non-embryogenic (organogenic) structures a Fat Red dye that specifically stains neutral lipids, including triacylglycerols, was used. A rationale to use the Fat Red staining as an indicator of the embryogenic structures was a high accumulation of triacylglycerols observed in zygotic embryos and seeds and lipid disappearance in vegetative organs of Arabidopsis (Ogas et al., 1997).

Explants cultured on SE- and ORG-induction media were stained overnight in filtered Fat Red solution, rinsed with distilled water and examined under a stereomicroscope. The results indicated that in contrast to the unstained shoot primordia formed on ORG-medium, the structures that developed on SE-medium were intensively stained. In addition, Fat Red method was used to examine a type of morphogenic response induced on hormone-free medium supplemented with trichostatin A (TSA), a chemical that causes deacetylation of histones. Analysis show a strong Fat Red staining of TSA-induced regenerative structures that indicated their embryogenic character.

Results of the study indicated that the Fat Red staining can be recommended as a simple, low-cost and reliable method to distinguish between embryogenic and non-embryogenic structures regenerated *in vitro* on the cultured plant explants.

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Cell wall regeneration in cultured protoplasts of *Brassica oleracea* var. capitata

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The important step in the process of obtaining complete plants from single protoplasts is a undisturbed and overall cell wall regeneration. The reconstruction of cell wall is a precondition for cell division. The Fluorescent Brightener 28 (Calcofluor White M2R, Sigma) is a fluorescent dye used for visualization of cell wall in higher plants (Hahne et al., 1983). Calcofluor White M2R added to the medium for culturing protoplasts allow observing cell wall formation in time.

In this study we used mesophyll protoplasts enzymatically isolated from *in vitro* cultured plants of *Brassica oleracea* var. *capitata* cultivar 'Kilaton F1' according to the protocol of Kiełkowska and Adamus (2014). The isolated protoplasts were purified by filtering, centrifugation and washing and after determining density the protoplasts were embedded in a calcium alginate layers. Immobilized protoplasts were placed in liquid on Kao and Michayluk (1975) based medium and with the addition of calcofluor. The suitable dye concentration in culture medium was chosen experimentally and finally 4,375 µl of calcofluor per ml of protoplast suspension allowed for necessary observations.

Process of regeneration of the protoplast cell wall was observed over 10 days of culture, using an invert fluorescence microscope. The rate of cell wall rebuild and irregularities in this process was observed. Also on the day of isolation frequency of protoplasts with improperly digested cell walls we observed, as the remnants of undigested cell wall can interfere with subsequent proper cell wall regeneration of protoplasts.

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The study on an accumulation and release of Pb and Cd from *Imleria badia* (Boletaceae) *biomass* to artificial digestive juices

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Imleria badia a good-known edible mushroom was the object in presented work. Mushrooms are characterized by a much greater ability to accumulate the elements compared to green plants. Thus, in some cases they can be harmful to humans. The ability to accumulate heavy metals by the fruiting bodies of mushrooms can be considered in two ways; using their predisposition for an accumulation they can be considered as bioindicators of environmental pollution in order to assess its contamination. On the other hand, more significant from the consumer point of view, an evaluation of heavy metals content is important for human health protection (Zhu et al., 2011).

The aim of the study was to determine the contents of cadmium and lead ions in the biomass from *in vitro* cultures of *I. badia* on the media enriched with cadmium and lead, in order to determine the relationship of increased metals content in the culture medium and the degree of their accumulation in the resulting biomass. The analysis of metals content was performed using the method of differential pulse anodic stripping voltammetry. In order to determine the rate of metals release into artificial digestive juices, the samples were subjected to extraction processes using artificial gastric juice and artificial intestinal juice.

Based on the determined contents of cadmium and lead, an antagonism between them at the stage of accumulation can be observed. The examined mushrooms include one of the metals in a higher concentration and the amount of the another one is substantially lower. The average concentrations of heavy metals in *I. badia* biomass on media with cadmium and lead ions addition at a concentration of $5 \cdot 10^{-5}$ mol/L are 12.3 mg/kg d.w. for cadmium, and

36.7 mg/kg d.w. for lead. This means that *I. badia* exhibits a substantially greater potential for accumulation of lead than cadmium. Gastric juice contained 2.9 mg/kg d.w. cadmium, and nearly 8 mg/kg, d.w. lead. In turn, intestinal juice contained 0.04 mg/kg d.w. cadmium, and 0.58 lead. The results concerning the content of cadmium and lead are well below the allowable standards for human consumption. That is also proved by the fact that both metals are highly accumulated in mushrooms, and to a small extent are released into artificial digestive juices, which emphasizes their role as a soil remediation factor.

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The physiological response to lead and cadmium ions of micropropagated *Alyssum montanum* ecotype

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The aim of undertaken experiments was to evaluate the potential of calamine species to grow and develop in the presence of heavy metals ions, using as a model the culture of *Alyssum montanum* proliferating shoots. The organ culture has been established by placing 5 mm long shoot explants on modified WPM medium (Lloyd and McCown, 1981) supplemented with cadmium chloride and lead (II) nitrate. The following combinations of mentioned salts were tested: (1) 0.5 μM $CdCl_2$ + 0.1 mM $Pb(NO_3)_2$ and (2) 5.0 μM $CdCl_2$ + 0.5 mM $Pb(NO_3)_2$. As the control medium was used modified WPM without any addition of $CdCl_2$ or $Pb(NO_3)_2$.

The influence of particular treatment on plant growth parameters was evaluated biometrically. The phenolic profile was assessed according the methodology of Swain and Hillis (1959) and Fukumoto and Maza (2000), whereas DPPH free radical scavenging activity following Pekarinen et al. (1999) and photosynthetic pigments content following Welburn (1994). The obtained values were compared between cultures growing on medium supplemented with lead and cadmium for long or short period of time (i.e. 36- and 6 month, respectively). In long-term cultures treated with Cd^{2+} and Pb^{2+} ions, the propagation coefficient increased by 16.3–27.6% in comparison to the control one, and on the other hand by 30.4–50.0% in comparison to shoots grown for six month on medium enriched with 0.5 μM Cd^{2+} + 0.1 mM Pb^{2+} and 5.0 μM Cd^{2+} + 0.5 mM Pb^{2+}, respectively. Moreover, the content of total chlorophylls in leaves of long-term cultures was proved to be much higher, than those in leaves of short-term one, in which the highest concentration of phenolic compounds was ascertained at the same time. The obtained results clearly indicate that *A. montanum* lines with increased resistance to cadmium and lead have been obtained. Further experiments are in progress, and further data will be carefully evaluated in order to assess more precisely the currently observed impact.

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Application of the tissue culture techniques in propagation of endangered species

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Endangered plant species are protected ex situ, for example in botanic gardens or gene banks and this protection should aim to restore them in their natural environment. It is necessary as in case of numerous species preservation only *in situ* proves insufficient.

There are several factors which speak for use of tissue cultures in plant preservation: inefficiency of the traditional propagation methods and a high productivity of the *in vitro* methods. The latter is especially important in case of very small plant populations where it may be possible to produce a large number of new plants starting from just a few specimen (Krogstrup et al., 1992; Fay, 1994). Tissue cultures have been broadly used in plant preservation since the end of the last century. In 1999 Pence reported their application in preservation of over 170 endangered plant species from 60 families, occurring in different countries.

Micropropagation used to preserve rare and endangered species is important for orchids (Fakouri Ghaziani et al., 2014). In natural conditions a developmental cycle of certain rare species such as *Cypripedium calceolus* or *Orchis morio* lasts 5–10 years until plants reach a flowering stage and are able to set seeds (Zaniecka and Łojkowska, 2004) therefore the *in vitro* propagation often offers a unique solution being as well much faster. In such cases the solution used in biotechnology – the *in vitro* – is applied. The leading institutions using this technique include The Center of Research and Preservation of Alpine Plants in Zakopane where propagation of 12 species such as for example *Pulsatilla slavica*, *Erigeron hungaricus*, *Senecio umbrosus* and *Carex pulicaris* are under study Also The Karkonosze National Park uses this technique in cooperation with The Wroclaw Botanic Garden in order to propagate the endangered species from that region (Kuś, 2007).

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Changes in carotenoid rich carrot callus at transcriptional, histological and ultrastructural levels

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Cultivated carrot is one of a few plant species synthesizing carotenoids in an underground storage organ that are sequestered in large quantities in chromoplasts mainly of crystalline type well visible using light microscopy (Rodriguez-Conception and Stange, 2013; Li et al., 2016). To reveal genetic mechanisms of carotenoid biosynthesis in carrot root we used a model system utilizing callus tissue *in vitro*. A root derived callus was pale yellow but after six months of culture orange cell clumps appeared in one clone DH1/7. These clumps were selected and cultured separately for the next months resulting in establishment of an orange coloured DH1/7-P callus clone.

Both callus clones were compared at histological and ultrastructural levels with the use of different light microscope techniques and a High Resolution Electron Microscope. Carotenoid crystals were abundant in orange callus composed mainly of embryogenic-like cells characterized by dense cytoplasm, large nucleus with prominent nucleoli and small starch grains. Meristematic cells were also present. In contrast, light yellow DH1/7 callus was almost free of carotenoid crystals, had loosely connected cells with abundant intercellular spaces. Cells were large, rounded with large central vacuole. Many cells showed signs of programmed cell death. At the ultrastructural level the differences between both callus types were seen in the intensity of the exocytosis, plasmodesmata presence, and in the occurrence and diversity of plastids.

Expression of 24 carotenoid pathway genes was assessed using a qPCR approach. Some genes had elevated expression in an orange callus while other were expressed at the same level as in yellow callus or repressed. The most overexpressed were ε-lycopene cyclase (LCYE) and phytoene synthase (PSY) but PSY paralogs were expressed differentially.

The results indicate that the obtained callus clones had different embryogenic and morphogenic potential that coincided with increased expression of some essential carotenoid genes.

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Enhancement of *in vitro* regeneration potential of Golden Grape Hyacinth (*Muscari macrocarpum* Sweet) with different light qualities

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Muscari macrocarpum Sweet is an ornamental geophyte, native to Western part of Turkey (Aegean region) and south-eastern Greece. It grows in the mountains, on rocky slopes. Flowers (blooming in the spring) are yellowish and fragrant, clustered in 15-cm-high cones. An intensive flower scent is a distinguishing trait and explains its English name "the Golden Grape Hyacinth". Due to the decorative and sensory values, it is recommended as a new ornamental perennial for gardens and green urban areas (Spence, 2003). It is propagated by seeds, bulb scales or lately with the use of *in vitro* cultures (Ozel et al,. 2007, 2009). In this study, we investigated the effect of light qualities on the regeneration potential of *M. macrocarpum* bulb scales, collected form *in vitro* plants after 12 weeks of cold exposure (+4°C). Explants (fragments of bulb scales) were cultured on MS medium (Murashige and Skoog 1962), supplemented with 5 μM BA and 0.5 μM NAA, 3% sucrose and 0.5% BioAgar, pH 5.7. Three different types of LED light were tested: 100% red (R); 100% blue (B); mixture of 70% red and 30% blue (RB). The control plants were placed either under fluorescent lamps (Philips TL-D 36W/54) or in the dark. Photosynthetic photon flux density in all light combinations was 40 μmol $m^{-2} \cdot s^{-1}$.

After 6 weeks of exposure to the tested light and dark conditions, the formation of adventitious bulbs, development of shoots and roots were observed in all treatments. The quality of light affected *Muscari* adventitious bulb formation. The highest number of bulblets was obtained under RB light (29.3 per explant) compared to red light treatment and control plants (21.4–12.2, respectively). Blue light stimulated bulbs to develop shoots, but none of the light treatments significantly affected the shoot height. Dark conditions increased the adventitious root formation (6.7 per explant), while the longest roots were observed in plants developing under fluorescent lamps.

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Growth characteristics of plants *Lemna minor* L. cultivated *in vitro* in laboratory conditions, supplemented by leachate coming from the process of methane fermentation

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Pollution of the environment by waste from methane fermentation is one of the most serious problems of the modern world. This forces the development and use of new, low-cost and environmentally friendly technologies of their management. An effective tool for removing pollutants from the environment appears to be the use of plants *Lemna minor* L. High efficiency of removal of harmful substances exhibit plants because of their ability to effectively retrieve and degradation of harmful compounds. Neutralization of polluting substances is made possible by the enzymes produced by these plants and their incorporation into their own cells.

In the experiment, the *in vitro* plant cultures were carried out in the Department of Ecophysiology and Plant Development at the University of Lodz. The study was conducted in laboratory conditions using leachate coming from the anaerobic digestion. The evaluation of metabolic activity of *Lemna minor* L. plants was carried out on the basis of parameters such as plant growth, index of chlorophyll content in the leaves, activity of net photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration and measurements of fresh and dry matter. These tests are widely recognized as useful markers of metabolic activity and plant responses to external stimuli, which were found in previous studies and world literature (Badek et al., 2014).

The study showed a positive effect of effluent from the process of anaerobic digestion technology on *Lemna minor* L. plant development. These plants treated with this effluent, in the appropriate concentration, were characterized by a greater growth increase, in comparison with the control. Obtained results of the study indicate a possibility of supplementing the media cultures *in vitro* plants of the family *Lemnaceae*. The use of *Lemna minor* L, can help to reduce environmental pollution with unfavorable organic compounds.

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The lead tolerance selection of kidney vetch (*Anthyllis vulneraria* L.) in *in vitro* culture condition

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Kidney vetch (*Anthyllis vulneraria* L.) is leguminous plant known from its medicinal properties. A. vulneraria, widely distributed in Europe, occurs commonly in the lowlands and foothills in Poland. Furthermore, calamine ecotype of *A. vulneraria* was located on the post-mining waste dumps of Olkusz region (Muszyńska et al., 2015). Therefore, it seems desirable to carry out research on the usefulness of this plant for the biological reconstruction of habitats destroyed by metallurgical industry as well as for phytoremediation of substrates contaminated with heavy metals.

The aim of presented research was assessment of the regeneration abilities and changes in physiological activity of kidney vetch plants cultivated *in vitro* on the media containing lead ions.

Fragments of shoots were laid on the basal medium (MS medium with supplements) with the addition of lead nitrate ($Pb(NO_3)_2$) in concentration: 0.0; 0.1; 0.5; 1.0 and 1.5 mM Pb. Shoot multiplication rate, plant height and rooting rate were evaluated after 4 weeks of culture. Moreover, a number of physiological parameters (activity of antioxidant enzymes, content of phenolic compounds and photosynthetic pigments) were estimated in newly regenerated shoots and roots.

After 4 weeks of cultivation, shoot multiplication rate ranged from 2.7 to 4.3 (shoots/explants) on the media containing 1.5 and 0.5 mM Pb, respectively. However, any of used Pb concentration caused significance reduction of *Anthyllis* regeneration abilities in comparison to control. Similarly, Pb addition had no influence on plant height (4.7–5.3 cm), percentage of rooting (60–80%) and average root number per explant (6–9.2).

We noted higher activity of antioxidant enzymes (catalase and peroxidase) in roots than in shoots but higher fenolic compounds content, including anthocyanins, in shoots than in roots. Enzymatic activity and phenolics accumulation were altered in plants cultivated on the media with Pb ions. Moreover, higher Pb concentration used (1.0 and 1.5 mM) increased chlorophyll *b* and carotenoids content in *Anthyllis* shoots.

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Strain of *Chlorella* sp. proposed for cultivation in biorefinery as a part of waste treatment

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There are a great possibilities of obtaining valuable chemicals from algae biomass and thus they can play a crucial role in economy, including the fuel production. Biomass of microalgae contains a large quantity of lipids, carbohydrates and many valuable components that could be refined and converted into biofuels. It is also predicted that algae can be useful in utilizing the liquid and gaseous wastes generated from biogas production as a growth substrates, as it is planned in the experimental biorefinery located in Piaszczyna (Poland) that converts plant biomass into biofuel. The selection of strains that utilize wastes as a source of macro and micronutrients for algae growth, and their activity are not restricted in environment containing high concentrations of various organic compounds and microorganisms from biogas fermentation is very complicated. In a series of screening experiments we found the strain of *Chlorella* sp may meet all the above mentioned conditions.

In the presented experiments strain of *Chlorella* sp. was cultivated *in vitro* in 300 ml Erlenmeyer's flasks on medium composed of raw biogas wastes and water. Cultures were incubated at 25°C for 30 days with photoperiod 16 hours light 8 hours dark. Media with for different concentration of wastes and standard growth medium were tested. The growth of *Chlorella* sp. was determined by optical density of cultures at 680 nm and 720 nm as well as cell counting. Morphology of cells was inspected under the light microscope. Chlorophyll fluorescence, phosphatases (pH=7,5 and pH=6,0) activity, COD and dry mass were measured. The obtained results indicated that algae can download the nitrogen and phosphorus from waste biogas and contribute to their purification. This method is promising, and in the future may be more effective than conventional sewage treatment plants.

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Microalgae species *Scenedesmus obliquus* as a potential candidate for utilization of wastes from biogas production

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The energy production from renewable sources is one of the means to decrease the anthropogenic emissions of greenhouse gases. One of such sources is biogas – a gaseous biofuel produced by microorganism from biomass. The production of biogas often creates a small carbon footprint, but always generates solid and liquid wastes that contains significant biogenic amounts of biogenic elements. These wastes are potentially dangerous for water environments if they are treated and stored improperly. One of the proposed methods of these waste utilization is their use in cultivation of algae as the biofertilizer included in media in which microalgae are produced. The selection of the algae strain which develop and grow on the biogas wastes gives an opportunity for cheaper production of algae biomass. In a series of screening experiments we found the strain of *Scenedesmus obliquus* that grows *in vitro* on liquid fraction of biogas wastes can be the source of nutrients needed for this species growth.

The green algae of *Scenedesmus obliquus* was cultivated in 125 ml Erlenmeyer's flasks on biogas wastes diluted with water. Cultures were incubated at 27°C for 30 days with 16 hours light 8 hours dark photoperiod. Five different concentrations of wastes were tested in this experiment. The Z8 medium was used in control cultivation. The *Scenedesmus obliquus* cells growth was determined by measuring optical density at 680 nm and 720 nm. Morphology of cells was evaluated under the light microscope. Chlorophyll fluorescence, phosphatases (pH=7,5 and pH=6,0) activity, cells count and dry mass were measured. The obtained results show that green algae can be useful for utilization of wastes from biogas production.

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Protective effect of selenium on zearalenone treated wheat calli

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Zearalenone is one of the mycotoxins, which are natural food and feed contaminants, produced by Fusarium. The infection of the grains by mycotoxin is a considerable problem due the reducing of yields but more important is the pollution of grains (Schwake-Anduschus et al., 2015). These metabolites can enter the food chain through the ingestion of contaminated products, leading to a number of major health problems, including immunosuppression and carcinogenesis. In the plants exposed to stress conditions protective effect of the selenium were reported i.e. by Filek et al. (2010) and Sieprawska et al. (2015). The aim of this study was testing the influence of selenium as a potential protector of wheat genotypes under zearalenone stress conditions. The experiments were performed on calli obtained from two spring wheat (*Triticum aestivum* L.) varieties: tolerant (cv. Parabola) and sensitive (cv. Raweta). Callus cultures were checking to observe the direct effects of both investigated substances on the cells. Zearalenone (ZEA) was added to Murashige-Skooge media at 0 (control), 30 μM and in mixture with selenium (30 μM ZEA + 15 μM Na_2SeO_4). The stresogenic activity of zaralenone and effectivity of Se (as a protector against zearalenone treatment) was assessed on the basis the changes of the increase of the weight of callus and on the analysis of lipid peroxidation (MDA), polyphenols content and antioxidative enzymes activities, after 10 days of culture. It was observed that the presence of zearalenone caused a reduction of the growth of the fresh mass of both wheat calli, in comparison to control. Moreover, the increase of lipid membranes peroxidation and rise of polyphenols content was registered, especially in the case of the sensitive genotype. The enhancement of superoxide dismutases and peroxidases activities was indicated only for tolerant wheat calli. For sensitive one, zearalenone application resulted in the decrease of both antioxidative enzymes activities, in comparison to control. Selenium presence in culture media diminished all the studied effects of zearalenone treatment. It was particularly visible in the case of tolerant wheat. It can be suggested that selenium ions can partially reduce the oxidative stress action of zearalenone on wheat cells, especially for more tolerant genotypes.

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The influence of precursor (L-phenylalanine) on phenolic acids accumulation in mycelial cultures of *Ganoderma applanatum* (Ganodermataceae, Basidiomycota)

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Arboreal fungi constitute a significant source of compounds with multidirectional therapeutic activity. Convenient alternative for efficient production of biomass from medicinal mushrooms and their metabolites with biological activity are mycelial cultures of arboreal species. One of the most important group of secondary metabolites with multidirectional therapeutic activity (e.g. antioxidant, antiseptic) are phenolic acids. The object of this study was the mycelial cultures of *Ganoderma applanatum* (Pers.) Pat. (Ganodermataceae, Basidiomycota) – common arboreal species of native mycoflora (Jong et al., 1992). The aim of this study was to investigate the influence of precursor (L-phenylalanine) on phenolic acids accumulation in biomass from mycelial cultures.

Agitated mycelial cultures were maintained in 500 ml Erlenmeyer flasks with 250 ml Oddoux medium (Oddoux, 1957). After two weeks L-phenylalanine in two concentrations (75 mg and 200 mg) have been added. Biomass was collected after 5 and 10 days as well as control samples. Phenolic acids were assayed in methanolic extract and after acid hydrolysis (2M HCl, 30 min). Quantitative and qualitative analysis of compounds in extracts from biomass were made by DAD-HPLC method.

In all analyzed extracts (methanolic and after acid hydrolysis), either with the precursor or without (control), were revealed the presence of three phenolic acids: gallic acid, protocatechuic and *p*-hydroxybenzoic acid. Among the determined phenolic acids in the largest amount occurred gallic acid (11.55 mg/100 g DW) marked in biomass harvested after 10 days from the medium enriched in 200 mg of precursor.

Analysis of the results in the described experiment showed that it is possible to increase the accumulation of phenolic acids in mycelia cultures of *Ganoderma applanatum* by adding the precursor L-phenylalanine.

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Mycelial cultures of *Piptoporus betulinus* (Fomitopsidaceae, Basidiomycota) as a potential source of metabolites with multidirectional therapeutic effects

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Mycelial culture of Basidiomycota species is a convenient alternative for efficient production of biomass from medicinal mushrooms and their active metabolites. Potential advantages of *in vitro* culture include a higher production of mycelial biomass in a compact space and over a shorter time period.

The aim of the study was qualitative and quantitative analysis of the extracts obtained from mycelial cultures of *Piptoporus betulinus* (Bull) P. Karst. (birch bracket), arboreal, parasitic fungus for birch commonly found in Poland (Lemieszek et al., 2009).

Mycelial cultures were established from fruiting bodies derived from natural state. The submerged cultures were maintained on the Linsmaier-Skoog liquid medium (Linsmaier and Skoog, 1965) at temp. ±22°C and pH 5 for 3 weeks. In obtained biomass the presence of following group of compounds were detected i.e. sterols, fatty acids, phenolic acids, indole derivatives, betulin and betulinic acid. High performance reverse phase liquid chromatography (RP-HPLC) and gas chromatography (GC) were used for this purpose.

In the extracts three sterols were determined ergosterol, hexestrol and ergosterol peroxide. Quantitatively, the dominant compound was ergosterol. Eleven fatty acids were estimated, including unsaturated ones as oleic and α-linolenic acid. Analysis of phenolic acids composition showed the presence of *p*-hydroxybenzoic, protocatechuic and syringic acids. Among the indole derivatives L-tryptophan, tryptamine and serotonin were determined. Additionally, betulin and betulinic acid were found in the biomass.

The study indicates, that mycelial culture of *Piptoporus betulinus* may be a potential source of the tested groups of compounds and could be interesting object for future chemical analysis.

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The influence of phenylalanine on the accumulation of phenolic acids in *Ginkgo biloba in vitro* cultures

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Maidenhair-tree – *Ginkgo biloba* L. has been used for many years to treat disorders of cerebral or peripheral circulation. Its therapeutical properties result from the presence of compounds such as terpenoids, flavonoids, as well as phenolic acids. (Van Beek, 2000; Huh and Staba, 1992).

This work concerns the effect of the addition of the precursor – phenylalanine on biomass growth and biosynthesis of phenolic acids in suspension cultures of *Ginkgo biloba*. The agitated suspension cultures of *Ginkgo biloba* were maintained on the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing BAP (2 mg/l) and Picloram (4 mg/l). Phenylalanine, in concentrations 25, 50, 75, 100, 150, 200 mg/150 ml was added after 2 and 3 weeks of inoculation. Biomass from experimental and control cultures was collected every 24 h for 4 days. HPLC method (Ellnain-Wojtaszek, 1997) was used for analysis of phenolic acids in the methanol extracts from biomass of *Ginkgo biloba* cultures and its hydrolysates.

The addition of phenylalanine did not inhibit the biomass growth. The presence of cinnamic acid and 10 phenolic acids: chlorogenic, ferulic, gallic, caffeic, *o*-coumaric, protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, syringic and vanillic acid was revealed. The free phenolic acids were revealed for the first time. There was observed an increase in the production of phenolic acids after the precursor addition. The highest total content of free phenolic acids and cinnamic acid was 95.37 mg/100 g d. m. The highest total content of bound phenolic acids was 232.92 mg/100 g d. m.

In vitro cultures of *Ginkgo biloba* can be an important source for obtaining phenolic acids, especially chlorogenic acid (the highest content in this study was 57.75 mg/100 g d. m.), protocatechuic acid (the highest content in this study was 46.1 mg/100 g d. m.) and syringic acid (the highest content in this study was 129.09 mg/100 g d. m.).

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The influence of phenylalanine on the accumulation of secondary metabolites in shoot agitated cultures of *Ruta graveolens* L.

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

The object of this study was to examine the influence of concentration of plant growth regulators (auxin – NAA and cytokinin – BAP) and addition of precursor (phenylalanine) on accumulation of the secondary metabolites in agitated, shoot cultures of *Ruta graveolens*. Cultures were supposed to grow on two different variants of medium, based on Linsmaier and Skoog medium (Linsmaier and Skoog, 1965) – LS 0.1/0.1 (0.1 mg/l NAA and 0.1 mg/l BAP) and LS 3/1 (3 mg/l NAA and 1 mg/l BAP). Phenylalanine was added into the cultures in a concentration of 1.25 g/l after 4, 5 and 6 weeks of growth cycles. To control probes sterile water was added. Biomass was collected 2, 3, 4 and 7 days after addition of precursor.

The presence of coumarins: xanthotoxin, bergapten, imperatorin, isopimpinellin and scopoletin was identified and quantified by HPLC method in the methanolic extracts, whereas in hydrolysates phenolic acids such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid and ferulic acid were estimated. Additionally, the presence of epigallocatechin gallate and coumarins: xanthotoxin, bergapten, imperatorin, isopimpinellin and marmesin was indicated in hydrolysates as well. The highest total content of phenolic acids (1.97 mg/g d.m.) was obtained on the second day after addition of phenylalanine (the fourth week of growth cycles) on LS 0.1/0.1 medium – in the test sample, whereas the highest total content of coumarins (98.06 mg/g d.m.) on the second day after addition of phenylalanine (the fifth week of growth cycles) on LS 0.1/0.1 medium – in the control sample. The highest total content of epigallocatechin gallate (0.28 mg/g d.m.) was obtained on the third day after addition of phenylalanine (the fourth week of growth cycles) on LS 0.1/0.1 medium – in the test sample. The addition of precursor significantly increases the production of phenolic acids (almost 2-fold) and epigallocatechin gallate (16-fold). There was no significant effect of the addition of phenylalanine for the production of coumarins.

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The influence of phenylalanine feeding on polyphenols production in agitated cultures of *Aronia* × *prunifolia* Marsh. – preliminary results

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The fruit of *Aronia melanocarpa* (black chokeberry) finds common application not only in phytotherapy and cosmetology, but also in the food industry, especially in functional food. Numerous scientific studies have demonstrated high antioxidant properties of extracts from this fruit. This activity is attributed to polyphenolic compounds, mainly anthocyanins, flavonoids, phenolic acids and catechins (Kokotkiewicz et al., 2010).

A. × *prunifolia* (purple chokeberry) is recognised as a less common aronia – a hybrid of *A. melanocarpa* and *A. arbutifolia*. Our previous studies proved high contents of polyphenol compounds in fruit, leaf and *in vitro* cultured biomass extracts (Szopa et al., 2016a; 2016b). These results testify about competitiveness of this hybrid towards to *A. melanocarpa*.

Under current studies the preliminary results on increasing of phenolic compounds production on the way of precursor feeding (L-phenylalanine – Phe) are presented.

Agitated shoot cultures of *A.* × *prunifolia* were maintained on Murashiege and Skoog (1962) medium with 1 mg/l BA and 1 mg/l NAA. The different concentrations of Phe were applied at the first day of cultivation: 1, 5, 10 and 15 mmol Phe /l medium. The cultures without Phe were treated as control conditions. The cultures were maintained for 2, 3 and 4-week growth periods. The phenolic acids, flavonoids and catechins were estimated in methanolic extracts from biomasses and the respective growth media samples by DAD-HPLC method (Ellnain-Wojtaszek and Zgórka, 1999).

The influences of the addition of Phe for the production of the investigated compounds were showed only for the samples harvested after 3 and 4 weeks. The highest total amounts of investigated polyphenols were confirmed after 3-week growth period and addition of 5 mmol Phe to the medium. The total amounts increased up to: 1.65 times for phenolic acids, 1.45 times for flavonoids and 3.50 times for catechins.

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Effectiveness of polyploid induction using pith stem tissue culture of *Nicotiana tabacum*

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The production of doubled haploids of tobacco via another culture and then chromosome doubling is a well established technique. Doubled haploids generate breeding lines that can be used directly as new cultivars or as parental forms for the hybrid cultivar production. Chromosome doubling in tobacco was induced by colchicine (Berbeć and Laskowska, 2006) or by plant regeneration during *in vitro* cultures. Callus cultures from midvein explants of leaves have been initiated (Walker and Aycock, 1994). The percentage of regenerated polyploids was quite low and varied depending on the tobacco genotype. In our experiment, the effectiveness of plant regeneration as well as doubled haploid induction during pith stem tissue culture were estimated. Six different F1 hybrid combinations (BPA × WGLA, WGLA × BPA, WABPA × WGLA, WGLA × WABPA, WGLA × PW 900, PW 900 × WGLA) were used as an initial plant material. Stem fragments excised from mature (full flowering) plants were surface-sterilized. Then pith stem explants were cultured on Lloyd (1975) medium to generate callus and micropropagated shoots. Calli-derived plants were verified for ploidy level. For each sample, around 5000 nuclei were analyzed using flow cytometer Partec Cube 8. The frequency of explants forming shoots ranged from 72.7 to 100% depending on tobacco genotype. Flow cytometric analysis revealed that there were three types of regenerants: haploids, doubled haploids and polyploids. The highest number of the induced doubled haploids (51.9%) was obtained for WGLA × WABPA hybrids. The technique of plant regeneration from pith stem fragments can be utilized for obtaining doubled haploids.

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Physiological status of *Daphne* shoots multiplicated *in vitro* on medium supplemented with biochar

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Biochar is the solid product from pyrolysis of waste biomass residues from agricultural and forestry production. It is known as excellent soil amendment that increases soil productivity and alleviates toxic effects of soil contaminants (Zhang et al., 2013). In recent years there is an interest in introducing biochar into agrobiotechnological practice, as a cost-effective medium supplement that can be used in plant micropropagation. The aim of this study was to evaluate the suitability of biochar for replacing activated charcoal in *in vitro* culture of woody *Daphne* plants. For this purpose we have compared multiplication efficiency and physiological status of shoots cultured in the presence of either biochar or activated charcoal.

Shoots of *Daphne jasminea* and *D. tangutica* (Thymelaeaceae) were cultured *in vitro* on medium supplemented with 0.6% either activated charcoal (Sigma) or biochar (Fluid S.A.). In all treatments medium contained phytohormones: 12.3 μM N6-[2-isopentyl]adenine (2iP) and 5.37 μM 1-naphthaleneacetic acid (NAA), as well as additional organic compounds in optimal concentrations (Hanus-Fajerska et al., 2012). After 8 weeks of culture biometrical measurements were conducted and micropropagation coefficient was calculated. Plant samples were collected and analyzed for pigment content as well as for endogenous phytohormones and stress-related compounds.

The replacement of activated charcoal by biochar stimulated formation of new shoots in both *Daphne* species. The micropropagation coefficient increased in *D. tangutica* from 1.2 to 1.7, and in *D. jasminea* from 2.1 to 4.1. The height of microshoots was comparable in both charcoal treatments. In the presence of biochar *D. jasminea* shoots more frequently formed flower buds and flowers, as well as adventitious roots.

However, accelerated growth of *D. tangutica* in biochar-supplemented medium led to decrease in content of pigments, both chlorophylls and carotenoids. Cultured shoots were pale green, in contrast to dark-green shoots developed on activated charcoal.

In both species the concentration of endogenous cytokinins increased in the presence of biochar. Also, elevated level of jasmonic acid and proline was detected, in comparison with plants cultured on activated charcoal. Specific reaction of *D. tangutica* was increased concentration of endogenous auxins and abscisic acid, and decreased level of gibberellins. In *D. jasminea* the content of ethylene precursor was elevated, while the concentrations of benzoic and salicylic acid were substantially reduced.

Our study revealed that commercially produced biochar can successfully replace activated charcoal in *Daphne in vitro* culture. Together with its stimulatory influence on micropropagation efficiency, biochar seems to affect physiological status of cultured shoots, especially in relation to the level of endogenous phytohormones. This property may be used to modulate organogenic response of cultured explants. Moreover, broader exploitation biochar in tissue culture technology would allow more efficient utilization of waste biomass in sustainable agriculture.

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In vitro propagation of *Rosa* 'Konstancin' (*R. rugosa* × *R. beggeriana*), a plant with high nutritional and pro-health value

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Rosa 'Konstancin' is an interspecific hybrid between *R. rugosa* and *R. beggeriana*, indroduced by J. Milewski in 1971. Among rose hip species and cultivars, rose 'Konstancin' shows relatively high content of ascorbic acid (3000–3500 mg/100 g FM) and carotenoids (Milewski 1974). A commercial rosehip production using selected cultivars has recently been the subject of great interest because of an improved understanding of the important role of dietary fruit in maintaining human health. A low rooting ability of stem cutting is major limiting factor in conventional propagation of *Rosa* 'Konstancin' and implementation in horticultural production.

The aim of the study was to develop an efficient micropropagation system for *Rosa* 'Konstancin' using axillary buds of mature 15 years-old plant derived from the Genebank of *Rosa* at Research Institute of Horticulture. The shoot buds were collected in May and August. The effect and interaction of different concentrations of phytohormones, carbohydrates and mineral salts were studied on *in vitro* shoot initiation, multiplication and rooting.

The term of explant collecting from the mother plants significantly affected the initiation of shoot culture of *Rosa* 'Konstancin'. Considerable higher frequency of bud break (100%) was obtained in explants isolated in August as compared to those collected at the end of May (30%). All buds developed into single shoots in 2–4 weeks growing on the MS medium containing 0.5 mg l^{-1} BAP, 0.1 GA_3 and 30 g l^{-1} of sucrose. After transferring to the fresh medium, the explants started to form a new shoots. The most effective axillary multiplication (3.9 shoots/explant) in a 5-week cycle and high quality of shoots were obtained on MS medium containing 50% of nitrogen salts, 0.5 mg l^{-1} BAP, 0.3 mg l^{-1} GA_3 and 20 g l^{-1} sucrose. The well developed shoots of *Rosa* 'Konstancin' rooted in 95.2% on medium containing 0.1 mg l^{-1} IBA. The addition of activated charcoal decreased the rooting efficiency by 20%. Plantlets were successfully transferred to pots and acclimatized in the greenhouse conditions.

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Chitinase activity changes under androgenesis-inducing spike pre-treatment in winter rye (*Secale cereale* L.)

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The process of androgenesis is induced by a stress treatment and accompanied by a number of physiological and molecular changes. Several genes associated with the early embryo induction from immature pollen grains (microspores) were identified in some crops. Among them, there are genes encoding chitinases (EC 3.2.1.14), which belong to pathogenesis-related (PR) proteins. Their hypothetical involvement in androgenesis induction has been tested in generally *in vitro* recalcitrant rye (*S. cereale* L.) genotypes.

Anthers isolated from cold pre-treated tillers (3 weeks, 4°C) of two breeding lines of rye were studied. Additionally, the effects of mannitol (0.3 M Mn) or/and reduced glutathione (0.3 M GSH) were tested in relation to the androgenesis induction effectiveness and chitinases gene and protein expression.

Total chitinase activities were measured fluorimetrically using 4-methylumbelliferyl-β-D-N,N´,N´´-triacetylchitotrioside. The chitinase activities in the gel were detected after renaturation of proteins which were separated in the SDS-PAGE with incorporated glycol chitin as a substrate. Total RNAs were isolated using the RNease Plant Mini Kit (Qiagen). The cDNA was made using the Maxima H Minus First Strand cDNA Synthesis Kit and OligodT primers (Thermo Scientific). RT-PCR and qPCR analyses were performed with primers designed to amplify a 102 bp internal fragment of the chitinase gene AF280438.1 (*S. cereale* class II endochitinase-antifreeze protein). β-tubulin gene (U76895) was used as an internal standard.

The two rye lines differed both in their embryogenic potential and total chitinase activities. Spikes pre-treatments with Mn and/or GSH increased chitinase activities in anthers of the less responsive line. In contrast, chitinase activities in the more embryogenic line decreased (> 2-fold). At least six isoforms with the chitinase activity were in the gels.

The transcript of AF280438.1 gene was detected in anthers from all treatments. The identity of the PCR product was confirmed by sequencing. The qPCR analysis performed on the responsive line revealed that in spikes treated with Mn and/or GSH the transcript was 4-fold up-regulated.

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